Anatolian Journal of Botany

Ant J Bot

e-ISSN 2602-2818

5(1) (2021) - Anatolian Journal of Botany

Anatolian Journal of Botany

Anatolian Journal of Botany

e-ISSN 2602-2818
Volume 5, Issue 1, Year 2021

Published Biannually

Owner
Prof. Dr. Abdullah KAYA

Corresponding Address
Gazi University, Science Faculty, Department of Biology, 06500, Ankara – Turkey
Phone: (+90 312) 2021235
E-mail: anatolianjbot@gmail.com
Web: http://dergipark.gov.tr/ajb

Anatolian Journal of Botany is Abstracted/Indexed in Directory of Research Journal Indexing (DRJI), Eurasian Scientific Journal Index (ESJI), Google Scholar, International Institute of Organized Research (I2OR), Scientific Indexing Services (SIS) and TR Dizin

Anatolian Journal of Botany

Anatolian Journal of Botany
e-ISSN 2602-2818
Volume 5, Issue 1, Year 2021

Contents / İçindekiler

Research articles

Anatolian Journal of Botany
5(1): 1-5 (2021)
Research article *doi:10.30616/ajb.800992*

Floristic studies of the pteridophytes of district Tor Ghar KP, Pakistan

Humaira BIBI[1], Ahmad ZADA[2*], Jan ALAM[3], Adil ALTAF[4]
[1,3]*Hazara University, Department of Botany, Mansehra, KP, Pakistan*
[2]*Yangzhou University, College of Bioscience and Biotechnology, Yangzhou, China*
[4]*Yangzhou University, College of Agriculture, Key Laboratory of Crop Genetics and Physiology of Jiangsu Province/Wheat Research Center, Yangzhou, China*
**dh19025@yzu.edu.cn, [1]humairatanoli93@gmail.com, [3]bibih2695@gmail.com, [4]er.altafadil@outlook.com*

Received : 12.10.2020
Accepted : 10.12.2020
Online : 09.01.2021

Tor Ghar KP, Pakistan bölgesinin pteridofitleri üzerine floristik çalışmalar

Abstract: This study was aimed to document the floristic attributes of the pteridophytes of district Tor Ghar; Khyber Pakhtunkhwa, Pakistan. Total 41 species distributed in 20 genera and 10 families were recognized. *Dryopteridaceae* was the largest family with 12 species (29.26%). Second and third large family is *Pteridaceae* and *Thelypteridaceae* with 10 (24.39%) and 5 (12.19%) species respectively. Like-wise, *Dryopteris* was the largest genus with 6 (14.63%) species. The main families were *Aspleniaceae, Blechnaceae, Dennstaedtiaceae, Dryopteridaceae, Equisetaceae, Lygodiaceae, Marseliaceae, Pteridaceae, Thelypteridaceae,* and *Woodsiaceae. Polystichum integrilobium* (Ching ex Y.T. Hsieh) W.M. Chu ex H.S. Kung is reported for the first time for Pakistan and 33 species are new records for the Tor Ghar. These identified species were collected from different localities i.e. Shumli Bandi, Bandi, Pabal, Tilli, Panja Gali, Kotkay, Ganga , Judba, Seri Kohani, Tagram, Nabori, Tor Kandow and Kandar (Hussanzai).

Key words: District Tor Ghar; Pakistan; taxonomy; pteridophytes; distribution pattern

Özet: Bu çalışma, Tor Ghar; Khyber Pakhtunkhwa, Pakistan Bölgesi pteridofitlerinin floristik özelliklerini belgelemeyi amaçlamıştır. On familya ve 20 cins içinde dağılım gösteren toplam 41 tür tanımlanmıştır. *Dryopteridaceae,* 12 türle (%29.26) en büyük familyadır. İkinci ve üçüncü büyük aile sırasıyla 10 (%24.39) ve 5 (%12.19) tür ile *Pteridaceae* ve *Thelypteridaceae*'dir. Aynı şekilde *Dryopteris,* 6 (%14.63) türle en büyük cins olmuştur. Asıl familyalar *Aspleniaceae, Blechnaceae, Dennstaedtiaceae, Dryopteridaceae, Equisetaceae, Lygodiaceae, Marseliaceae, Pteridaceae, Thelypteridaceae* ve *Woodsiaceae'*dir. *Polystichum integrilobium* (Ching ex Y.T. Hsieh) W.M. Chu ex H.S. Kung, Pakistan için ilk kez rapor edilmiştir ve 33 tür Tor Ghar için yeni kayıt durumundadır. Teşhis edilen bu türler, Shumli Bandi, Bandi, Pabal, Tilli, Panja Gali, Kotkay, Ganga, Judba, Seri Kohani, Tagram, Nabori, Tor Kandow ve Kandar (Hussanzai) gibi farklı yerlerden toplanmıştır.

Anahtar Kelimeler: Tor Ghar bölgesi, Pakistan, taksonomi, pteridofitler, dağılım modeli

Citation: Bibi H, Zada A, Alam J, Altaf A (2021). Floristic studies of the pteridophytes of district Tor Ghar KP, Pakistan. Anatolian Journal of Botany 5(1): 1-5.

1. Introduction

The term Pteridophytes is a fusion of two Greek words (*pteron*= feather, *phyton*= plants) means the plants having feathers like shape of their fronds. These are also known as 'vascular cryptogams' and Carolus Linnaeus (1754) categorized them in the class Cryptogamia. Pteridophytes is a group of spore producing plants, designed by two lineages, one is Lycophytes (club mosses) fronds and Monilophytes or ferns (Pryer et al., 2004; Smith et al., 2006). Presently, pteridophytes comprises of approximately about 300 genera and 9600 ferns and 1400 fern allies around world (Smith et al., 2006). However according to Chapman (2006) about 15% of all fern and lycophyte species may not yet be known to science, while according to Moran (2008) there are approximately 13.600 species in the tropics with the richest diversity (Kornas, 1993; Linder, 2001). Nearly an updated checklist of Pakistan suggests that there are 206 taxa (194 species; 4 forma; 8 hybrids) distributed in 62 genera and 19 families (Gul et al., 2017).

Systematic studies of pteridophyte flora of Pakistan are not fully done. Previously, few workers have contributed in the exploration of the fern flora of Pakistan. Pteridophyte flora of Kashmir Valley resulted in the discovery of only 90 species and 4 varieties of ferns from Kashmir as their collective contribution (Dar et al., 2002). Stewart (1972) enlisted about 133 taxa from Pakistan and Kashmir in his catalogue "An Annotated Catalogue of The Vascular Plants of West Pakistan and Kashmir". Later Hope (1903) and Beddome (1892) during their work on the pteridophytes documented 27 species from Chitral and 25 species from British India respectively. Nakaike and Malik (1992) prepared a list of pteridophytes including 82 species of ferns belonging to 30 genera and 18 families of Pakistan. Murtaza et al. (2008) investigated *Schizaea dichotoma* (L.) Smith and were collected specimens from Neelum Valley. Khan (2011) investigated the pteridophytes of district upper Dir and documented 37 ferns species from the area. Wani et al. (2012) reported 106 taxa of fern and fern allies of Kashmir Valley, Gores and Ladakh. Sundas et al. (2012) documented 36 fern species belongs to 18 genera and distributed in 13 families. Fraser-Jenkins (2012) worked on the pteridophytes of political India and documented a revised list of 337 pteridophytes according to the six higher IUCN categories. Gul et al. (2016) studied traditional uses of 60 taxa of fern and fern allies distributed in 16 families, and 26 genera from district Mansehra KPK, Pakistan. Gul et al. (2017) recorded 178 pteridophyte taxa distributed in 43 genera and 17 families from district Mansehra KPK, Pakistan. In the present study an attempt has been made to analyze the magnitude of the taxa under consideration by using previous literature and field based observations.

2. Materials and Method

2.1. Study area

Tor Ghar is a combination of two Pashto words (Tor = Black, Ghar = Mountain) which means black mountain, previously Tor Ghar was known by hindko word Kala Dhaka which is the part of Western Himalayan province (Takhtajan et al., 1986). It lies between, 72º48'-72º58'E and 34º32'-34º50'N. It is an uneven, mountainous area of about 800 km, surrounded at south by Tanawal and Agror valley, on the east by Tikuari and Nandiar, District Buner to the west and Indus River on north (Mehmood et al., 2015). The single highway passes from Darband to Thakot is 85 km. Tor Garh is part of the Western Himalayan Province of Irano-Turanian Region (Takhtajan et al., 1986). District is elevated to a range from 450 to 3.500 m (Mehmood et al., 2015).

2.2. Study Plan

At initial stage, a detailed literature survey was carried out. Accordingly, a comprehensive field plan was designed, extensive field surveys were arranged in suitable seasons of the year from January-October 2017 in order to study and collect the specimens of ferns and fern allies from the various localities of Tor Garh. During field survey all data was collected related to the habit, type of habitat(s), geographic range, altitudinal range, life form, distribution range and ethnobotanical uses was studied in the natural habitat. The different localities visited were Shumli bandi, Panja gali, Pabal, Tilli, Seri kuhani, Ganga muchcot, Judba, Nabori, Tor kandow, Kandar, Bandagai etc.

2.3. Identification and Laboratory work

For identification purposes authentic literature and Flora of China and Flora of North America were consulted. However, the morphological features were studied and found out (length, pinnule size, pinnule number, pinna size, pinna number, hairs, and scale on rachis costae and indusia present or absent). Scales, lens, and stereoscope and high resolution compound microscope were used for identification purposes. For anatomy, cross sections of the material were used. Slides were made for each specimen like spore, sporangia, and rachis. Manual methods and microtome (if available) were also used for this purpose. Digital camera was used for anatomical images.

3. Results

In total one hundred twenty plants specimens were collected from various localities of the study area. Based on collected materials, 41 species were recognized having 20 genera and distributed in 10 families. For families, *Dryopteridaceae* was the largest family with 12 species (29.26%), followed by *Pteridaceae* having 10 species (24.39%). In the remaining 8 families 19 species (46.34%) were documented as shown in (Table 1, Fig. 1). Genus-wise, *Dryopteris* Adans. was the largest genus with 6 species (14.63%) (Table 2, Fig. 2) followed by *Polystichum* Roth. with 5 species (12.19%) of the total plant species. Amongst these taxa 33 were the new records for the study area, while 7 species have been previously reported in the studied area. Habitat wise 13 species are terrestrial which is 31.70%, 26 species are lithophytic which is 63.41% and 2 species are aquatic which represents 4.87% of total known species from District Tor ghar (Fig. 3). As for altitude is concerned, 25 species (60.97%) are reported from 700-1900. Thirteen species (31.70%) are reported from 1901- 2300, 3 species (7.31%) are reported from 2300-2800 (Fig. 4). Ten species (24.39%) of total recorded species from the area are traditionally used to cure ailments.

Table 1. Family wise distribution of pteridophytes in district Tor Ghar

Family	Genera	Species	% of taxa in total
Aspleniaceae	1	3	7.31
Blechnaceae	1	1	2.43
Dennstatidiaceae	1	1	2.43
Dryopteridaceae	3	12	29.26
Equisetaceae	1	3	7.31
Lygodiaceae	1	1	2.43
Marseliaceae	1	2	4.87
Pteridaceae	5	10	24.39
Thelypteridaceae	4	5	12.19
Woodsiaceae	2	3	7.31

Table 2. Ten largest genera in the pteridophyte flora Tor Ghar

Genus	No. Species	% in total
Dryopteris	6	14.63
Polysticum	5	12.19
Adiantum	4	9.75
Equisetum	3	7.31
Asplenium	3	7.31
Thelypteris	2	4.87
Pteris	2	4.87
Marselia	2	4.87
Onychium	2	4.87
Diplazium	2	4.87

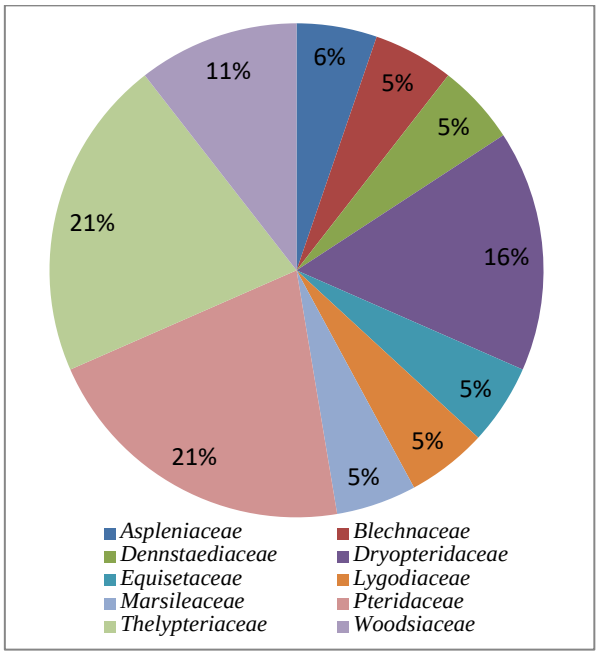

Figure 1. Family-wise distribution of the taxa in district Tor Ghar

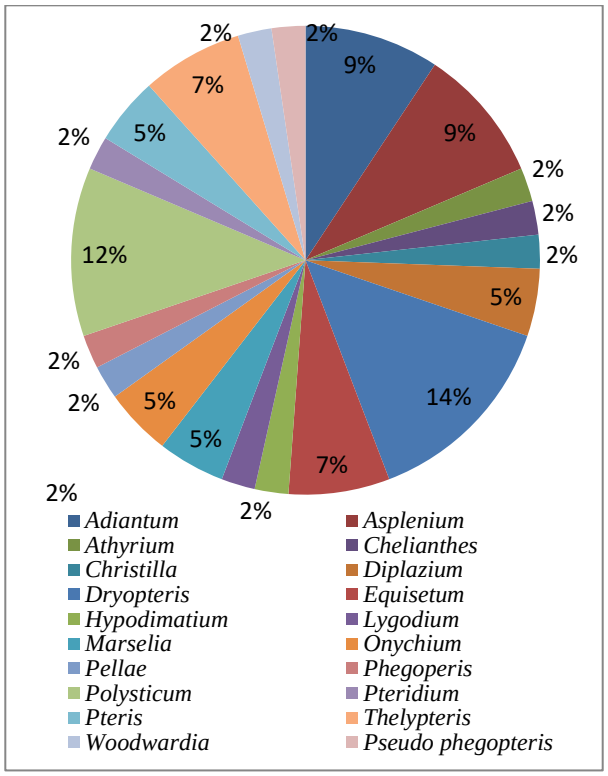

Figure 2. Genus-wise distribution of the taxa in district Tor Ghar

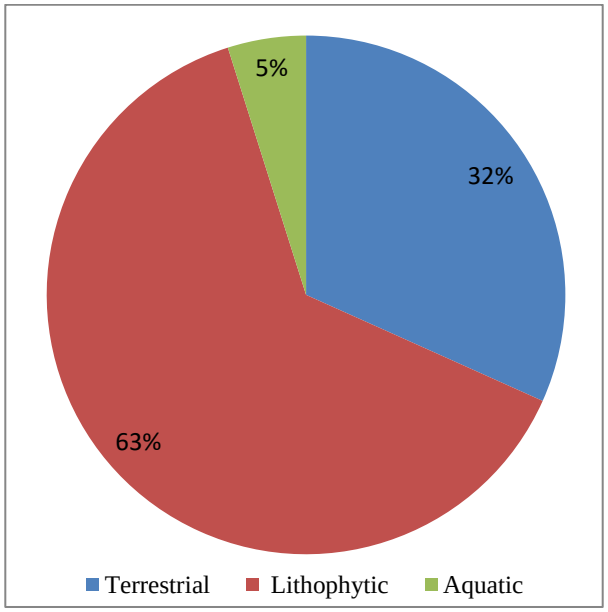

Figure 3. Habitat-wise distribution of the taxa in district Tor Ghar

3.1. New Generic Record

Polystichum integrilobium (Ching ex Y.T. Hsieh) W.M. Chu ex H.S. Kung Sin. 36: 244. 1998) (Fig 5)

Plants summer-green. Rhizome erect thickly covered with lanceolate golden to brown scales. Fronds 16 cm; stipe stramineous, 4 cm, ca. 2 mm in diameter at base scaly, scales dense, narrowly ovate, linear yellow-brownish. Lamina bi-pinnatifid, lanceolate, gradually becomes narrower at base, acuminate; rachis deprived of proliferous bulbils, dense scales both surfaces; stramineous, lanceolate and linear. Pinnae 18 pairs, alternate,

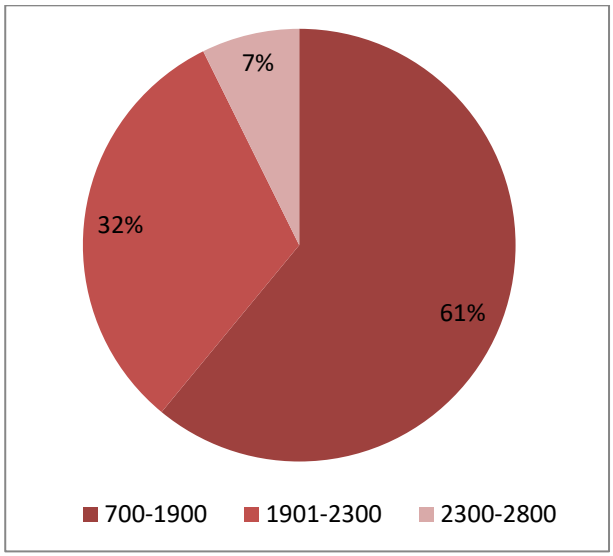

Figure 4. Altitude-wise distribution of the taxa in district Tor ghar

sessile, deltoid to lanceolate, middle pairs 18 × 7 mm, bases oblique, broadly cuneate, macroscopically auriculate, pinnatipartite, apices obtuse. Lobes 5 pairs, nearly opposite, slightly ascendant, approximate, oblong, entire; many lanceolate yellowish brown microscales on abaxial surface, subglabrous adaxially; frond texture papery; venation pinnate on lobes, inarticulate on both surfaces. Sori in a single row on each side of midrib; indusia serrulate.

Specimens Examined: Tor Ghar; Tilli, 1790 m, 12-07-2017, Humaira Bibi, Muhammad Irfan 38 (HUP).

Habitat: Near moist and shady place.

General Distribution: North Western Yunnan (China).

4. Discussions

District Tor Gar falls in the Irano-Turanian phyto-geographical region of Pakistan (Takhtadzhian and Cronquist, 1986). In terms of pteridophyte exploration this region remains neglected. Therefore, an attempt has been made to analyze the magnitude of the taxa under consideration.

In Pakistan, the floristic studies of pteridophytes are still not completed. Most of the work has been done on angiosperms but only a little work is presented on lower plants particularly cryptogams. Recently Tor Gar is little explored in terms of higher plants i.e. angiosperms and gymnosperms (Mehmood et al., 2015). Extensive review of literature revealed that only 16 pteridophytes species are known to the district. However, in current investigation 41 taxa are recorded and these belongs to 20 genera and 10 families. One species is reported for the first time for Pakistan and 33 species are new records for the study area. However, these figures represent about 19% (species), 30.64% (genera) and 52.63% of the total known (families) for Pakistan. Of these, 33 species are new records for the study area. The scientists contributed in exploration of flora of pteridophytes were Ching (1940), Fraser-Jenkins (2012), Sundas et al. (2012), Shah et al. (2019), Nakaike and Malik (1992), Zaman et al. (2019) and Gul et al. (2016). The largest family is *Dryopteridaceae* having 12 species and 3 genera

recognized i-e. *Dryopteris*, *Polystichum* and *Hypodematium* Kunze. *Dryopteris* has 6 species, *Polystichum* has 5 species and *Hypodematium* has 1. The same work was done by Gul et al. (2017) and 18 genera were documented. In the current study *Thelypteridaceae* with 4 genera i.e., *Thelypteris* Schmidel with 2 species, *Pseudophegopteris* Ching, *Christella* H.Lév. and *Phegopteris* (C.Presl) Fée have 1 species each, and similar research was done by Ching (1940) who documented 12 genera in *Thelypteridaceae*. *Pteridaceae* with 9 species and 5 genera i.e. *Pteris* has 2 species, *Adiantum* L. has 4 species, *Cheilanthes* Sw. has 1 species, while *Onychium* Kaulf. has 2 species and *Pellea* Link. has 1 species. A similar result was obtained with this work and in general agreement with the work of Fraser-Jenkins (2012) who revised the list of 337 pteridophytes from India. *Asplaniaceae* has 3 species and 1 genus i.e. *Asplenium* L. Similarly, Zaman et al. (2012) documented a total of about 36 fern taxa belonging to 18 genera and 13 families. Three species are identified for family *Equisetaceae* with 1 genus *Equisetum* L. Shah et al. (2019) documented 8 genera belonged to 4 families of pteridophytes from Chakesar valley at district Shangla Pakistan. Three species are identified for family *Woodsiaceae* with 2 genera i-e, *Athyrium* Roth has 1 taxon and *Diplizium* Swartz has 2 taxa. Nakaike and Malik (1992) reported 82 species of pteridophytes belonged to 30 genera, 18 families and distribution pattern from Pakistan. *Dennsteadaceae* have 1 genus *Pteridium* Gleditsch ex Scop. and 1 species. The same work was done by Mir et al. (2015) who reported 4 species of pteridophytes for the first time from Kashmir Valley. Two species were identified for family *Marsilaceae* having 1 genus *Marsilea* L. The same work was done by Fazal et al. (2014) who collected 25 species of ferns and ferns allies belonged to 13 genera and 8 families for Maidan valley Dir lower district at Pakistan. *Lygodiaceae* has 1 species and 1 genus i.e. *Lygodium* Sw. The same work was done by Gul et al. (2016) a checklist of 130 species of ferns and lycophytes distributed in 34 genera and 17 families from district Mansehra, KP, Pakistan were documented. *Blechnaceae* has 1 species and 1 genus i-e *Woodwardia* Sm.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

Figure 5. *Polystichum integrilobium***:** A, Close up of frond;B, Closeup of pinna and rachis abaxially; C, Habit; D, Closeup of stipe with scales adaxially; E, Section of scale

References

Beddome R (1892). Handbook to the ferns of British India, Ceylon and the Malay Peninsula with supplement. Calcutta: Thacker, Spink, and Co.

Chapman AD (2006). Numbers of Living Species in Australia and the world. Report for the department of the environment and heritage. Canberra: Australian Biolical Resources Study.

Ching RC (1940). On natural classification of the family *Polypodiaceae*. Sunyatsenia 5: 201-268.

Dar GH, Bhagat R, Khan MA (2002). Biodiversity of the Kashmir Himalaya. Srinagar: Valley Book House.

Fazal U, Asad U, Ali U (2014). Pteridophytic flora of Maidan valley Dir (L) Khyber Pakhtunkhwa, Pakistan. International Journal of Biology Biotechnology 11(4): 649-653.

Fraser-Jenkins CR (2012). Rare and threatened Pteridophytes of Asia 2. Endangered species of India-the higher IUCN categories. Bulletin of National Museum of Nature and Science Series B 38(4): 153-181.

Gul A, Alam J, Ahamd H, Irfan M (2016). An updated checklist of Pteridophytes of district Mansehra, Khyber Pakhtunkhwa, Pakistan. Plant Science Today 3(2): 237-247.

Gul A, Alam J, Majid A, Ahmad H, Qaiser M (2017). Diversity and distribution patterns in the Pteridophyte flora of Pakistan and Azad Kashmir. Pakistan Journal of Botany 42: 83-88.

Hope CW (1903). The Ferns of North-western India including Afghanistan Trans-Indus protected areas & Kashmir. Journal of the Bombay Natural History Society 14: 720-749.

Khan MS (2011). Diversity of vascular plants, ethnobotany and their conservation status in ushairy valley, distt. Dir (upper) nwfp; northern pakistan. Islamabad: Quaid-i-Azam University.

Kornas J (1993). The significance of historical factors and ecological preference in the distribution of African pteridophytes. Journal of Biogeography: 281-286.

Linder H (2001). Plant diversity and endemism in sub-Saharan tropical Africa. Journal of Biogeography 28(2): 169-182.

Linnaeus C (1754). Genera plantarum eorumque characteres naturales secundum numerum, figuram, situm, & proportionem omnium fructificationis partium. Leiden: Wishoff.

Mehmood A, Khan SM, Shah AH, Shah AH, Ahmad H (2015). First floristic exploration of the District Torghar, Khyber Pakhtunkhwa, Pakistan. Pakistan Journal of Botany 47: 57-70.

Mir SA, Mishra AK, PALA SA, Reshi ZA, Sharma MP (2015). Ferns and fern allies of district Shopian, Kashmir Valley, India. Biodiversitas Journal of Biological Diversity 16(1): 27-43.

Moran R (2008). Diversity, biogeography, and floristics. Biology and Evolution of Ferns and Lycophytes: 367-394.

Murtaza G, Asghar R, Majid SA, Malik ZH (2008). Morphopalynological and anatomical studies on fan fern Schizaea dichotoma (L.) Smith from Neelum Valley, Azad Kashmir. Pakistan Journal of Botany 40(1): 59-63.

Nakaike T, Malik S (1992). A list of pteridophytes collected from Pakistan in 1990. Cryptogamic flora of Pakistan National Science Museum, Tokyo 1: 261-316.

Pryer KM, Schuettpelz E, Wolf PG, Schneider H, Smith AR, Cranfill R (2004). Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. American Journal of Botany 91(10): 1582-1598.

Shah SN, Ahmad M, Zafar M, Ullah F, Zaman W, Mazumdar J, Khuram I, Khan SM (2019). Leaf micromorphological adaptations of resurrection ferns in Northern Pakistan. Flora 255: 1-10.

Smith AR, Pryer KM, Schuettpelz E, Korall P, Schneider H, Wolf PG (2006). A classification for extant ferns. Taxon 55(3): 705-731.

Stewart R (1972).An annotated catalogue of the vascular plants of West Pakistan and Kashmir In: Nasir E and Ali SI (eds.) Flora of West Pakistan. Karachi: Fakhri Press.

Sundas I, Zaheer-ud-Din K, Noreen R (2012). A contribution to the taxonomic study of fern flora of Punjab, Pakistan. Pakistan Journal of Botany 44(Suppl.1): 315-322.

Takhtadzhian AL, Cronquist A (1986). Floristic Regions of the World. Berkeley: University of California Press.

Takhtajan A, Crovello TJ, Cronquist A (1986). Floristic regions of the world. Berkeley: University of California press.

Wani M, Shah M, Naqshi A (2012). The ferns of Kashmir-an update account. Indian Fern Journal 29(1-2): 100-136.

Zaman W, Shah SN, Ullah F, Ayaz A, Ahmad M, Ali A (2019). Systematic approach to the correct identification of *Asplenium dalhousiae* (*Aspleniaceae*) with their medicinal uses. Microscopy Research and Technique 82(4): 459-465.

Anatolian Journal of Botany
5(1): 6-18 (2021)
Research article *doi:10.30616/ajb.791872*

Taxonomic contributions to genus *Gypsophila* in Turkey and a new taxon from Erzurum: *G. venusta* subsp. *staminea*

Hasan ÖZÇELİK[1], Fevzi ÖZGÖKÇE[2*]
[1] *Süleyman Demirel University, Science & Arts Faculty, Department of Biology, Isparta, Turkey*
[2] *Yüzüncü Yıl University, Science Faculty, Department of Molecular Biology and Genetics, Van, Turkey*
**fevziozgokce@yyu.edu.tr, [1]hasanozcelik@sdu.edu.tr*

Received : 08.09.2020
Accepted : 13.12.2020
Online : 09.01.2021

Türkiye'deki *Gypsophila* cinsine taksonomik katkılar ve Erzurum'dan yeni bir takson: *G. venusta* subsp. *staminea*

Abstract: *Gypsophila* species in Flora of Turkey had been divided into 4 groups as A, B, C and D. Annuals are in group A. The current key for identification of annuals (A Group) is not able to identify species and subspecies. Moreover, there are new species records in this group. Some *Gypsophila* taxa such as *G. bitlisensis, G. elegans* and subspecies of *G. heteropoda* are confused to each other. In this study, annual *Gypsophila* taxa and confused subspecies were revised, up to date identification keys were prepared and *G. venusta* Fenzl subsp. *staminea* Özçelik and Özgökçe is described as a new taxon from Erzurum (Turkey). The list of Turkey's *Gypsophila* taxa was also updated. Though the existance of *G. gracilescens* and *G. erikii* seem to be doubtfull due to the unavailability of new samples from the determined localities, 63 *Gypsophila* species currently exist in Turkey. Some observations related to taxonomic and geographical characters of the taxa are provided.

Key words: *Gypsophila venusta* subsp. *staminea, G. bitlisensis, G. elegans,* current list.

Özet: Türkiye Florası'ndaki *Gypsophila* türleri A, B, C ve D olmak üzere 4 gruba ayrılmıştır. Tek yıllıklar A grubundadır. Tek yıllıkların (A Grubu) mevcut teşhis anahtarı türleri ve alt türleri ayırt edememektedir. Dahası, bu grupta yeni tür kayıtları vardır. *Gypsophila bitlisensis, G. elegans* türleri ve *G. heteropoda*'nın alt türleri gibi bazı *Gypsophila* taksonları birbiriyle karıştırılmaktadır. Bu çalışmada, tek yıllık *Gypsophila* taksonları ve karışık alt türler gözden geçirilmiş, güncel teşhis anahtarları hazırlanmış ve *G. venusta* Fenzl subsp. *staminea* Özçelik ve Özgökçe Erzurum (Türkiye)'dan yeni bir takson olarak tanımlanmıştır. Türkiye'nin *Gypsophila* takson listesi de güncellenmiştir. Belirlendikleri lokalitelerden yeni örneklerin temin edilememesi nedeniyle *G. gracilescens* ve *G. erikii*'nin varlığı şüpheli olmasına karşın, halihazırda Türkiye'de 63 *Gypsophila* türü vardır. Taksonların taksonomik ve coğrafi karakterlerine ilişkin bazı gözlemler verilmiştir.

Anahtar Kelimeler: *Gypsophila venusta* subsp. *staminea, G. bitlisensis, G. elegans,* güncel liste.

Citation: Özçelik H, Özgökçe F (2020). Taxonomic contributions to genus *Gypsophila* in Turkey and a new taxon from Erzurum: *G. venusta* subsp. *staminea*. Anatolian Journal of Botany 5(1): 6-18.

1. Introduction

The genus *Gypsophila* L. was first described by Linnaeus (1753) and has more than 150 species (Anonymous, 2020a). Sixty three *Gypsophila* species (41 endemic) are currently known in Turkey (Barkoudah 1962; Huber-Morath et al., 1967; Davis et al., 1988; Ataşlar, 2000; Ekim, 2012; Armağan, 2016). Huber-Morath's revision of *Gypsophila* L. in 'Flora of Turkey and the East Aegean Islands'(1967) provides a useful basis for the identification of the genus, but it has some serious shortcomings due to insufficiently studied specimens. Previously, Barkoudah (1962) made a revision of the genus and allied genera i.e. *Phyrana* Pax et Hoff., *Ankyropetalum* Fenzl, *Bolanthus* (Ser.) Rchb. which were separated from *Gypsophila* L. This revision is also important and served as a basis for the revision of Huber-Morath et al. (1967).

Gypsophila includes annuals, biennials and perennial herbaceous or semishrubs. Taxonomically it is related to *Bolanthus* (Ser.) Rchb., *Ankyropetalum* Fenzl and *Acanthophyllum* C.A. Mey. (Davis, 1967; Davis et al., 1988).

More than 50 % of the total *Gypsophila* species currently exist in Turkey. Azerbaijan and Iran, respectively, follow Turkey in terms of *Gypsophila* species diversity (Schischkin, 1936; Rechinger, 1988). According to the Flora of Turkey (Davis, 1967; Davis et al,. 1988; Güner et al., 2000), 60 *Gypsophila* taxa belonging to 56 species had been reported. Thirty five of them are considered to be endemic. The genus is also among the genera to which maximum number of new species have been added after the publication of Flora of Turkey (Davis, 1967; Davis et al., 1988), and most of the *Gypsophila* samples are kept in GUL and VANF Herbarium. New *Gypsophila* taxa have been presented by subsequent studies, increasing the current species number of the genus in Turkey to 63 (Karagüzel and Altan, 1999; Ataşlar, 2000; Korkmaz and Özçelik, 2011a; Budak, 2012; Koç, 2013; Armağan, 2016; Armağan et al., 2017; Anonymous, 2020a).

Since new specimens of *Gypsophila gracilescens* Schischk. and *G. erikii* Yıld. could not be found from the specified localities, the existance of these two species seem to be doubtfull. Though four species, *G. pilosa* Hudson, *G. perfoliata* L., *G. sphaerocephala* Fenzl ex Tchihat., *G. viscosa* Murr., of the genus are quite common, the others are rare or endemics.

Gypsophila is an economically important genus and the members having economic importance are named as "Çöven" by public, and seven species, *G. bicolor* (Freyn. & Sint.) Grossh. (Van çöveni, Tarla çöveni), *Gypsophila arrostii* Guss. subsp. *nebulosa* (Boiss. & Heldr.) Greuter & Burdet (Beyşehir çöveni, Konya çöveni, Helvacı çöveni, Şekerci çöveni), *G. paniculata* L. (Bahar yıldızı), *G. eriocalyx* Boiss. (Çorum çöveni, Yozgat çöveni), *G.*

bitlisensis Barkoudah (Bitlis çöveni), *G. perfoliata* L. (Niğde çöveni), *G. elegans* M. Bieb. (Bebek nefesi), are of economic impotance. Some members are used to make "Tahini Halvah", "Foam Halvah", "Turkish Delight", "Herbal Cheese" and "Çöven Bread". Some are used for the production of detergents, fire extinguisher, liquor and soap. *Gypsophila arrostii* var. *nebulosa* is used for commercial saponin production. *Gypsophila elegans* and *G. paniculata* are produced for floristry purposes (Korkmaz and Özçelik, 2011b). Beside the use of *Gypsophila* species for some health purposes such as diuretic, expectorant, acne remover, they are also used to polish gold at jewellery sector (Özçelik and Yıldırım, 2011).

Though its economic importace, *Gypsophila* is not known sufficiently in Turkish Flora. Thirty five of the 63 taxa were presented from East Anatolian region. Beside being endemic or rare, most of them are known only from type localities or from very few localities (Table 1). It is also the third largest genus in family *Caryophyllaceae* after *Silene* L. (c. 125 species) and *Dianthus* L. (c. 70 species) in Turkey.

Many studies (Özçelik and Özgökçe, 1995; 1999; Korkmaz and Özçelik, 2011a) have been carried out about the *Gypsophila* members of Turkey, and some new species and, new square records and new materials were presented from different regions. Although it is among the most complex genera of Turkish Flora taxonomically, a detailed revisional study related to *Gypsophila* hasn't been conducted.

The study aims to to clarify the problems in the taxonomy *Gypsophila* in Turkey and reveal information about the genus we have obtained during our work in different regions of Turkey.

2. Materials and Method

Research materials were collected from different regions of Turkey between 1988 and 2018, from February to September, both in the flowering and fruiting periods. Specimens were collected from as many different parts of the existing distribution area of the genus as possible to be able to study the variation patterns. The study area included East Anatolian vilayets, such as Van, Bitlis, Muş, Ağrı, Iğdır, Siirt, Şırnak, Bingöl, Erzurum and Hakkari provinces. Type specimens and the collections of herbaria ANK, ATA, E, EGE, FUH, GAZI, GUL, HUB, ISTF, KNYA and VANF were also examined. A range of characteristics that were considered to be taxonomically important in the genus was investigated.

Almost 150 collections from 75 localities were identified by using the second and supplementary volumes of Flora of Turkey and the East Aegean Islands (Davis, 1967; Davis et al., 1988). Twenty five of them belong to *G. bitlisensis* and *G. elegans*. The details about the collection sites of these plants; their direction, distance, biometric measurements, distribution patterns additional characters which were not given in previous revisions were also investigated. The differences from other publications were also noted (Table 1). Recent publications (Barrera and Arenas, 1999; Güner, 2012; Armağan, 2016; Armağan et al., 2017; Anonymous, 2020b) were taken into account in the spelling of the taxa authorities. Except for Table 2 and descriptions of taxa in results section, the authorities of the taxa were not given. Given authors are based on Huber-Morath et al. (1967), Rechinger (1988), Güner et al. (2000) and Güner (2012) with new publications (Budak, 2012; Hamzaoğlu, 2012; Yıldırımlı, 2012; Koç, 2013; Armağan, 2016; Armağan et al., 2017; Anonymous, 2020c).

All species of the genus were investigated by grouping them in four. Annual *Gypsophila* taxa (Group A) and perennials (Group B, C and D).

New identification keys and descriptions were prepared for subspecies and varieties of *G. heteropoda, G. venusta, G. bitlisensis* and *G. elegans*. All hesitant populations between *G. elegans* and *G. bitlisensis* were examined, after their species and populations were distinguished. A revised diagnostic key was prepared for annual members of the genus. A revision was made on Davis' (1967) key. Diagnostic features such as calyx shape, inflorescence and number of flowers, capsule shape and length, number of main stems in the plant, habitus of the plant, body length, leaf shape, size and indumentum, the shape and size of the brackets and the ratio to the calyx, pedicel length, thickness and indumentum, number of ovules in the ovary, indumentum in the calyx, shape, length, and structure of edges of the teeth, type and thickness of the underground organs, were used respectively while preparing the identification keys for Turkish *Gypsophila* members.

Abbreviations in the text and for table 2 are as follows:

*: Only known from type locality, rare; Mt: Mountain, el: Element, Euro-Sib.: Euro-Siberian, Medit.: Mediterranean, Hb: Herbarium/Herbaria; ±: more or less; N: North, S: South; E: East, W: West; Prov: Province (vilayet in Turkish).

Collectors and researchers in the text: Altan: Yasin Altan, Behçet: Lütfi Behçet, Tatlı: Âdem Tatlı, MK: Mustafa Korkmaz, Özgökçe: Fevzi Özgökçe, A.Özçelik: Adnan Özçelik, Özçelik: Hasan Özçelik, A.Ç.: Ali Çelik, Muca: Belkıs Muca Yiğit; K.Aydınşakir: Köksal Aydınşakir.

3. Results

3.1. Taxonomic contributions to some members of *Gypsophila* in Turkey

3.1.1. *Gypsophila venusta* Fenzl subsp. ***staminea*** Özçelik and Özgökçe, subsp. nov.

Differt a subsp. *venusta* floribus minoribus; stamina in serie 2 disposita; flamenta 0.8-1.2 mm vel 2-3.2 mm longa, antherae 0.1-0.2 mm longae, in calyce inclusa, petala breve 5-6(-9) mm.

Type: B8 Prov. Erzurum: Aşkale-Erzincan highway, about 40 km. from Aşkale, steppe, 2200 m, 23 vii 1993, Özçelik 6225 (HOLO in Hb. GUL and ISO in Hb. VANF).

Description: Plant 70-85 cm tall, strong, clearly swollen at nodes, whitish stemmed. Leaves lanceolate, acute to acuminate, 3-5 subveined, 10-60 x 1-10 mm, thin; papillose at margin; Inflorescence large, dense, many flowered paniculate-dichasial. Pedicels capillary, up to 25 mm. Calyx 3-3.5 (-4) mm; petals 5-6(-9) mm; petals milk white, 2-2.5 x longer than calyx; widened cuneate and emarginate -retuse to truncate at the top. Calyx teeth about half of the tube with large scarious intervals. It has two different stamen groups (5 longer + 5 shorter) which are never visible. Filament length of the short stamens 0.8-1.2 mm, the others 2-2.5 (-3.2) mm; anthers 0.1-0.2 mm and style 3-3.5 (-4) mm. long, visible.

Notes: Features related to stamens and petal length bewildered us and provide the most important diagnostic characters. This new subspecies is based on differences in flower size, calyx and petal lengths, ratio of petals to calyx; stamen disposition, filament and anther sizes. At the same time, the region where it spreads, habitat and altitude are very different.

The two subspecies may be distinguished as below:

1. Stamens included in calyx, arranged in two groups as short and long; petals 5-6 (-9) mm, calyx 3-3.5 (-4) mm ..... subsp. *staminea*

1. Stamens visible, apparently longer than calyx, similar one to other; petals 8-12 mm, calyx 3-5 mm …… ..... …… subsp. *venusta*

Subsp. *staminea* is only known from the locus classicus. This taxon is apparently endemic (may probably occur in some areas of Ir.-Tur. region in Turkey) and is geographically isolated from subsp. *venusta*. According to the key in Turkish Flora (Davis, 1967), general characters of the type specimen resemble to *G. venusta.* But only petal length different to *G. silenoides* Rupr. We therefore propose to treat it as subspecies of *G. venusta*. Flower structure is somewhat anomalous, it has both short and long stamens. Thus, it differs from all other Turkish *Gypsophila'*s and cannot be identified by the key given in Flora of Turkey (Fig. 1,2). The anatomical, ecological and palynological features of the species have been studied by us in comparison with other species. In addition, revision of the species and other ones in the Hagenia section were revised and a identification key was prepared (Fidan and Özgökçe, 2016).

Examination and comparison of *Gypsophila venusta* Fenzl. subsp. *staminea* Özçelik & Özgökçe, subsp. nov. showed that it is not only merely an aberrant form of *G. venusta* with a smaller calyx and petals, but also features related to stamens and petal length bewildered us and provide the most important diagnostic characters. This new subspecies is based on differences in flower size, calyx and petal lengths, ratio of petals to calyx; stamen disposition, filament and anther sizes. At the same time, the region where it spreads, habitat and altitude are very different.

Subsp. *venusta* Fenzl subsp. *venusta* is known as "Konya Çöveni in Turkish". There are halva producers in Konya. They mix roots of *G. arrostii* Guss., *G. perfoliata* and *G. venusta* subsp. *venusta* uand also use for halva production (Koyuncu et al., 2008). *G. venusta* subsp. *staminea* is an endemic taxon with local distribution in Eastern Anatolia. There is no information about the use of subsp. *staminea* Özçelik and Özgökçe (Özçelik and Özgökçe, 1995).

Specimens examined: A.Özçelik & K.Aydınşakir Ç.G.G. 140(GUL 13/24/46-1); A.Özçelik & K. Aydınşakir, Ç.G.G. 93(GUL 13/24/46-2); A.Özçelik & K.Aydınşakir Ç.G.G. 20(GUL 13/24/46-3); Özçelik & A.Ç. 13 (GUL 13/24/46-5-13); Özçelik & A.Ç.13(GUL 13/24/46/14-21); A.Özçelik & K.Aydınşakir ÇGG 28(GUL 13/24/46-22); A.Özçelik & K.Aydınşakir 11(GUL 13/24/46-23); A.Özçelik & K.Aydınşakir 22(GUL 13/24/46-24); A.Özçelik & K.Aydınşakir 88(13/24/46-25); Özçelik 12776 (GUL 13/24/46-26).

3.1.2. *Gypsophila elegans* M. Bieb.

A8 Prov. Bayburt: Kop Mountains, steppe, about 2300 m, 23 vii 1993, Özçelik 6229. Erzurum: Erzurum to İspir, between Rizekent and Çıkrıklı villages, steppe, on sandy places, 2100 m, 22 vii 1976, Tatlı 4914. Erzurum to Tortum; 6 km N of Karagöbek, 2200 m, 27 vii 1973, EGE 13595.

Figure 1. *Gypsophila venusta* subsp. *staminea* Özçelik and Özgökçe (a-c: habit; d1: flower; d2: dissected calyx; d3:petal and stamens d4: gynoecium) Özçelik 6225 (HOLO in Hb. GUL

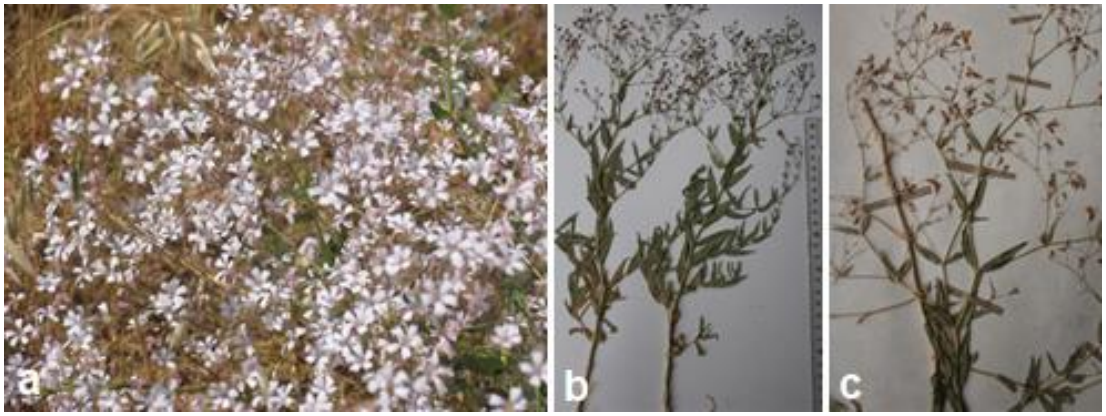

Figure 2. *Gypsophila venusta* subsp. *venusta* (a-c: habit)

A9 Prov. Erzurum: Şenkaya, Gülveren village, Acısu locality, steppe, 2500 m, 26 ix 1984, FUH (Fırat Univ.) Altan 3903.

B9 Prov. Bitlis: Adilcevaz, Aydınlar village, 2200 m, alpinic steppe; Adilcevaz (Bitlis)-Erciş (Van) road, 40-45 km, meadow, 21.05.1995, Özgökçe 2240(13/24/35-09); Özgökçe 2242 (GUL 13/24/35-10); Özgökçe 2243 (GUL 13/24/35-11); Özgökçe 2246 (GUL13/24/35-12); Özgökçe 2247(GUL 13/24/35-13); Özgökçe 2248 (GUL 13/24/35-14); Özgökçe 2249(GUL 13/24/35-15); Özgökçe 2250 (GUL 13/24/35-16); Özgökçe 2252 (GUL 13/24/35-17); Özgökçe 2253 (GUL 13/24/35-18): Özgökçe 2272 (GUL 13/24/35-19); Özgökçe 2273 (GUL 13/24/35-20); Özgökçe 2274 (GUL 13/24/35-21); Özgökçe 2276 (GUL 13/24/35-22); Özgökçe 2278, 2279 (GUL 13/24/35-23); Özgökçe 2280, 2281 (GUL 13/24/35-24); Özgökçe 4148 (GUL 13/24/35-25); Özgökçe 4158 (GUL 13/24/35-26); Özgökçe 2257, 2272, 2276, 2278, 2279, 2280, 2281, 2282(GUL 13/24/35-27).

Notes: Specimens vouchered as Özgökçe 2255, 2256 (GUL 13/24/35-28) are taxonomically problematic. Specimen with the voucher number MK. G. 51 is bottom branched and like biennial. MK. G. 83 seem like perennial.

B9 Prov. Van: Bahçesaray, Kavuşşahap Mountains, around Çatbayır village, field sides, 7 vii 1988, Özçelik 2245. Gürpınar, slopes of Başet Mountain, steppe, 2200 m, 4 vii 1993, Altan & Özçelik 5288. Güzeldere Pass, 2200 m, 21 vi 1986, EGE 33213. Bitlis: Süphan Mountain, around Aydınlar village (Adilcevaz), steppe, 2200 m, 9 vi 1987, Behçet 255; Özgökçe 2240 (in VANF); ATA 620. The distribution area, in Turkey, is Eastern Anatolian region. Therefore, Tatlı 4914: Özçelik 2245, ATA 620, K. Aydınşakir Ç.G.G. 150 (GUL 13/24/35-2).

Notes: Specimens numbered as Tatlı 4914 (GUL 13/24/35/03-05) are hybrids with *G. bitlisensis*.

Specimen examined: A. Özçelik & K. Aydınşakir Ç.G.G. 121 (GUL 13/24/35-1); A. Özçelik & K. Aydınşakir Ç.G.G. 150 (GUL 13/24/35-2); Tatlı 4914-a (GUL 13/24/35/03-05); Özçelik 2245 (GUL 13/24/35-06); A. Özçelik Ç.G.G. 94 (GUL 13/24/35-08); Özgökçe 2240, 2242, 2243, 2246, 2247, 2248, 2249, 2250, 2252, 2253, 2255, 2256, 2257, 2272, 2273, 2274 2276, 2278, 2279, 2280, 2281, 2282; 2249 4148, 4158, (GUL 13/24/35-10-28); MK. G.3, 5, 51, 200, 203, 296, 302, 330, 334, 339, 342, 352, 336, 397; ATA 620.

Notes: The distribution areas of the species mainly fall in Eastern Anatolian region and also spread in Eastern Black Sea region close to this region. Also, in Lakes region etc. Steppe, meadow and arid meadows are important habitats for it. Özgökçe 4158: Inflorescence lax, bract and bracteoles similar in shape, linear and pink. Rare and interesting specimen. Özgökçe 4148: Inflorescence congested, flowers small, bract and bracteoles similar to leaves in colour not pink, scarious (Figure 3).

Gypsophila elegans and *G. bitlisensis* are widely distributed in xerophytic and partially mesophotic habitats in the region. Our field observations indicated that identification of this group have many difficulties. Descriptions of these species have been given using few specimens. They are distinguished in the key as follows:

1. Inflorescence loosely dichasium; petals 2-5 x longer than calyx; calyx 3-5 mm, main stem dominant, seeds bulging and tubers pronounced..... *elegans*

1. Inflorescence densely dichasium, petals c. 1.5-2 x longer than calyx, calyx 2-3 mm, main stem many, seeds long and flat tuberred ..... *bitlisensis*

According to the above key, many specimens of *G. elegans* deviate in qualitative and quantitative characters as well as general appearance, branching, stem number belonging to the same root, petal length and the ratio of petals to calyx. Our results show that even in the same population one can find plants with linear-oblong to linear bract shapes, flowers can be a few or many in number, arranged in dense or loose dichasiums and branched from base or in upper half.

3.1.3. *Gypsophila bitlisensis* Barkoudah

A8 Prov. Erzurum: Tortum, above salt pans, roadsides, 2160 m, 5 vii 1975, Tatlı 2101.

B9 Prov. Bitlis: Süphan Mountain between Ahlat-Adilcevaz cities, steppe, 2200-2800 m, 28 vii 1988, Özçelik 1711; Behçet 1215; Tatvan, Nemrut Mountain, steppe, 1700-2400 m, Aug. 1991, Özçelik 2620; W and N slopes of Yumurtatepe locality, 2250-2350 m, alpinic sandy steppe. 5 vii 1972, Tatlı 717. Por stream, near the centrum; ca. 1500 m, 23 vi 1983, Hb. FUH 7547 (Fırat Univ., Elazığ).

Three types of *G. bitlisensis* occur in the mountains of East Anatolian region. The identification key of these types is prepared as follows:

1. Inflorescence dense and many flowered, only branched from base

   2. Number of main stem many and stems short …… ..... Group suphanis

   2. Number of main stem one or a few and stems long …… ..... Group nemrutis

1. Inflorescence lax or little flowered, branched from base, near base or upper parts ..... Group sarkis

Group.suphanis Özçelik and Özgökçe: Only grow on Suphan Mountain of Bitlis. Naming is new.

Group.nemrutis Özçelik and Özgökçe: Only grow on Nemrut Mountain of Bitlis. Naming is new.

Group.sarkis Özçelik and Özgökçe: 1-main stemmed, stems long or short (found in several localities of Van, Ağrı, Erzurum). Naming is new.

Specimen examined: Özgökçe 1971(GUL 13/24/37-1-2); Özçelik 5173(GUL 13/24/37-3); Özgökçe 1711 (GUL 13/24/37-04-05); Tatlı 717(GUL 13/24/37-06); Tatlı 2101(GUL 13/24/37-07); A.Özçelik 80 (GUL 13/24/37-08); A.Özçelik 74(GUL 13/24/37-09); Özçelik 2264(GUL 13/24/37/10-11); Özçelik 7206(GUL 13/24/37/12-17); MK.G. 6(10), 19, 32, 34, 88, 150, 162, A.Özçelik (GUL 13/24/37-39); A.Ç., Muca & Özçelik 03(GUL 13/24/37/18-37); Özçelik 12773(GUL 13/24/37-b/01-03); Tatlı 4914, (GUL 13/24/37-38); MK. G. 83(GUL 13/24/37-39); MK. G.296, 318(GUL 13/24/37-38); A. Özçelik ÇGG.108(GUL 13/24/37-38); Özçelik 6487(GUL 13/24/37-39); A. Özçelik Ç.G.G. 106 (GUL 13/24/37-40).

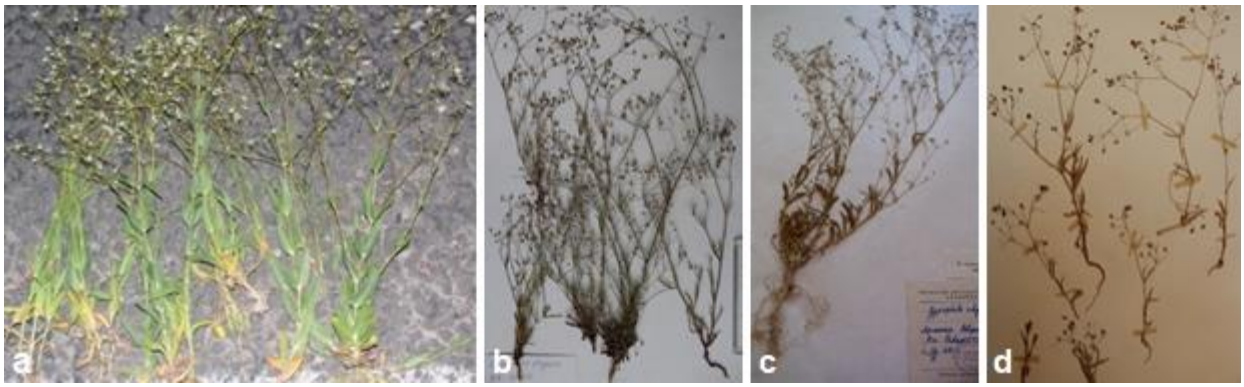

Figure 3. *Gypsophila elegans* (a: habit; b:MK. G. 339; c and d: Tatlı 4914).

Only Group Sarkis of *G. bitlisensis* can't be distinguished clearly from *G. elegans.* It may be a hybrid of *G. elegans* and *G. bitlisensis.* The Lake Van basin is a difference center of these species. For this reason, variation is very high in the species. Though each of the above mentioned types can taxonomically be thought as a variety, such a process has no contribution to systematics. These differences arise from the ecological features of the geography in which it is located. Distributed in Van, Ağrı, Bayburt, Erzurum, Artvin, Ardahan.

Gypsophila bitlisensis and *G. elegans* show a significant distribution. *Gypsophila bitlisensis* often occupies large areas in the Lake Van basin and dominantly grows in the area. A comparison of the two species is given in Table 1.

Among our collections, a large number of the samples belonging to both *G. elegans* and *G. bitlisensis* were observed to grow in the region. These specimens are:

A8 Prov. Erzurum: Tortum, 4 km to Aksu, roadsides, 2250 m, 5 vii 1975, Tatlı 2076. 5 km. from Karakurt to Horasan, roadsides and on moving rough stones, 1600 m., 10 vi 1983, Tatlı 6923. Near Horasan borderland from Ağrı, roadsides and steppe, ±1950 m, 21 vii 1990, Özçelik 2264.

A9 Prov. Artvin: Artvin to Ardahan, 7 km from Yalnızçam pass, 2250 m, 4 viii 1973, EGE 13600. Erzurum: Şenkaya, Gülveren village, steppe, 2500 m, 20 viii 1982, Fırat Univ. 2894. Kars: 8 km from Kars to Ardahan, steppe, roadsides, 1800 m, 10 vii 1975, Tatlı 2680; Ardahan: 32 km from Göle to Ardahan, pastures, 2060 m, 8 vii 1975, Tatlı 2451.

B9 Prov. Ağrı: Eleşkirt, Tahir Mountains, S of Naziktepe, stony steppe, 1940 m, 10 vii 1994, Özgökçe 1970; Özçelik 3151; 3153. Bitlis: Tatvan Nemrut Lake, volcanic rocky places, ca. 2250 m, 5 vii 1986, EGE 33216. Van: W slope of Büyük Erek Mountain, steppe, 2100-2200 m, 6 viii 1989, Özçelik 295; 1113, 1299, EGE 32327. N of Beyüzümü village, sandy-stony steppe, 1750 m, 31 vii 1994, Özgökçe 1971. Özalp, Muhammed valley, steppe and roadsides, 1800 m, 2 viii 1994, Özgökçe 1972.

Gyprosphila bitlisensis: Erzurum-Ağrı; Bitlis/Tatvan, Erzurum/ Aşkale Sivas /Zara Erzurum/Horasan Refahiye, Erzincan; Its roots are perennial; Özçelik 5173 (GUL 13/24/37-3); Bitlis/Ahlat-Adilcevaz. Identification of it is problematic and suspicious. Mixed with *G. elegans.* MK. G. 83 (GUL 13/24/37-39). An interesting example, like perennial and multi-branched from the base. MK. G.296, 318; Erzurum-Ağrı: A. Özçelik (GUL 13/24/37-38); A. Özçelik ÇGG.108(GUL 13/24/37-38); Özçelik 6487(GUL 13/24/37-39); A. Özçelik Ç.G.G.106 (GUL 13/24/37-40); Refahiye, 50 km to Erzincan, 13.7.2007; Bitlis/Ahlat-Adilcevaz; Bitlis/Tatvan, Erzurum/Aşkale.

Notes: Specimens, collected from Sivas/Zara Erzurum/Horasan and vouchered as MK.G.162 had very thin branches and sparsely flowered, a weak plant, a new population. Lower part of the plant is thickened, like a biennial or perennial (Fig. 4).

Table 1. A taxonomic comparison of *G. elegans* and *G. bitlisensis* (Özçelik and Özgökçe, 1999)

Characters	*G. elegans*	*G. bitlisensis*
Habit	Up to 80 cm tall, branched from upper part or near it, rarely unbranched; an or a few main stemmed	Up to 50 cm tall, always branched from the base, Often many stemmed
Leaves	10 - 60 x 1-15 mm	10-40 x 1-8 mm
Branching	Often clearly dichotomously branched	Many branched, weakly dichotomously branched
Inflorescence	Often diffuse, lax, less-flowered dichasium finer branched and sparse flowering	Large, dense, many-flowered dichasium Thicker branched and many-flowered
Bracts	Linear-oblong to ovate-triangular	Ovate-triangular
Pedicels	5-20 (-35) mm often longer than *G. bitlisensis*	5-25 mm
Calyx	3-4 mm long	2-3.5 (-4) mm long
Petals	4-8(-10) mm; broadly oblong to cuneate, emarginate	3.5-6 mm; linear-oblong
Seeds	With obtuse tubercles, a little	With minute obtuse tubercles, very much
Habitat	Slopes, steppe, gravel banks, roadsides, open woodland	Steppe, slopes, rarely stream sides
Distribution	East and North parts of east Anatolia in Turkey from sea level to 650-2600 m	Endemic to Van Lake basin and its environs, from sea level to 1650-1800 m

3.1.4. *Gypsophila heteropoda* Freyn & Sint.

MK.G.314 (GUL 13/24/32-1); MK. 49 (GUL 13/24/33/2-09).

Two varieties of this species exist in Turkey. However, in Flora of Turkey (Davis 1967), the key is inadequate to identify these two varieties. Korkmaz (2011a) made a revision of annual *Gypsophila* species in Turkey, but the taxonomic problems of this species could not be adequately resolved, and a detailed study is necessary about this species. These variants can be distinguished with the key given below:

1. Plant densely branched and stems viscose with sessile glands .....var. *heteropoda*

1. Plant delicate, sparsely branched and glandular hairy, never viscose with sessile glands .....var *minutiflora*

Gyprosphila heteropoda Freyn & Sint subsp. ***heteropoda***: A9 Prov. Iğdır and B10 Prov. Ağrı: This taxon was collected by us from many localities. Taxonomically confused with *G. parva.*

Gyprosphila heteropoda Freyn & Sint subsp. ***minutiflora*** Barkoudah: It is known only from the collections made from Prov. Sivas. It is seen in rocky, arid areas. In the Flora of Turkey (Davis, 1967), it recorded as subsp. *minutiflora* Bark. Obviously, it is rare endemic and Ir.-Tur. el. (Figure 5).

3.2. Revision of *Gypsophila* in the Group A of Turkey's Flora

Group A comprises only annuals. During preparation of the illustrated Flora of Turkey, this grouping might very important. However, for most species, some important diagnostic characters such as inflorescence type, fruit shape, and number of ovules are still missing. Some of these shortcomings have been completed in this study. The definition of the sections will remain weak without removing these deficiencies. After that, species identification keys related to the sections should be made and the group key in Flora of Turkey (Davis, 1967) should be abandoned.

3.2.1. Revised grouping of *Gypsophila* members in Turkey

1.Annual herbaceous, without woody roots and vegetative stems..... Group **A**

1.Biennial or perennial herbaceous, with woody roots and vegetative stems..... Groups **B, C, D**

Figure 4. Variations in *Gypsophila bitlisensis* (a-c: habit)

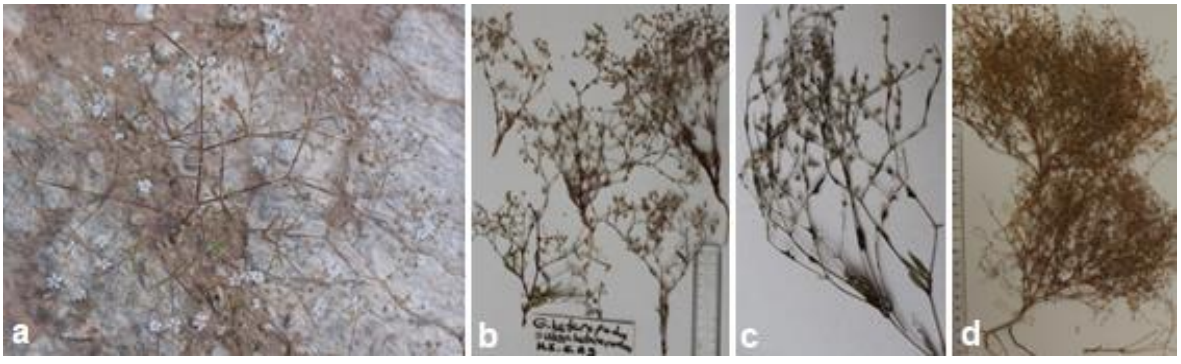

Figure 5. *Gypsophila heteropoda* (a: subsp. *minutiflora*; b and c: subsp. *heteropoda*; d: *G. parva*)

3.2.2. Revised identification key for annual *Gypsophila* members (Group A) in Turkey by taking advantage of Davis (1967).

1. Stem (at least at the base) and calyx hairy

2. Calyx pilose hairy (stem and leaves densely pilose-villose), leaves lanceolat, 10-30(-40) mm wide..... ***pilosa***

2. Calyx pilose not hairy, leaves linear, oblanceolate, up to 4 mm wide

3. Calyx tubulate, 4-8 mm, petals 5-10(-12) mm long

4. Pedicels 5.0-15.0 mm, inflorescence lax, bracts not leaf like ..... ***tubulosa***

4. Pedicels 1.0-2.0 mm, inflorescence densely globose, bracts leaf like ..... ***confertifolia***

3. Calyx campanulate, up to 4.0 mm, petals 2-5(-6) mm long

5. Bracts foliaceus, linear or oblanceolate, petals linear, cuneate, emarginate to bilobed

6. Pedicels 5-10 mm, petals 3-6 mm, cuneate, whitish or pink.....***torulensis***

6. Pedicels 10-20 mm, petals 2-3.5 mm, linear, white.....***linearifolia***

5. Bracts scarious, triangular, petals oblanceolate, acute to obtuse

7. Seeds obtus to acute tubercles; bracts and calyx glandular pubescent ..... …… ..... ***heteropoda*** subsp. ***minutiflora***

7. Seeds sharply echinate (prickly), bracts and calyx glabrous.....***parva***

1. Stem (at the base) and calyx never hairy or viscous

8. Pedicels not capillary, leaves ± oblanceolate rarely linear-lanceolate,

9. Whole plant viscous, leaves 3-5 non-apparent veined .....***viscosa***

9. The plant never viscous, leaves 1-3 distinctly grained

10. Petals oblanceolate to cuneate, bracts triangular, acute ..... ***heteropoda*** subsp. ***heteropoda***

10. Petaller linear-oblong, bracts ovate-triangular, obtuse

11. Seeds obtuse bulging prominent tubercles, main stem dominant, 1 (-2), inflorescence loose dichasium .....***elegans***

11. Seeds long flat tubercles, main stem usually numerous, inflorescence frequent dichasium ..... ***bitlisensis***

8. Pedicels capillary, leaves linear to lanceolate

12. Bracts linear to lanceolate, leafy, calyx 2.5-4.8 mm

13. Seeds flat tubercles, petals cuneate, calyx 2.5-4.0 mm

14. Pedicels 5-10 mm (show only distributed in Tekirdağ for Turkey ..... ***muralis***

14. Pedicel 10-25 mm (only shows distribution in Şanlıurfa in Turkey..... ***antari***

13. Seeds acute tubercles, petals linear-oblong, calyx 3.0-4.8 mm .....***munzurensis***

12. Bracts triangular, scarious, calyx 1.5-2.5 mm…..... ……***heteropoda*** subsp. ***heteropoda***

4. Conclusions and Suggestions

In this study, it has been tried to solve taxonomic problems of some Turkish *Gypsophila* members.

1. Actual list of Turkey *Gypsophila* taxa and their sections have determined and correctly named taxa and sections (Table 2). While preparing this list in the light of observations and literature, contributions to solve of taxonomic problems, examined voucher specimens and important results are mentioned. In addition, all *Gypsophila* taxa have ranked on sections on the basis of kinship. Table 2 will not only be a check list of studies on *Gypsophila* taxa, but also it will be a scientific infrastructure showing the systematic location and correct naming of taxa.

2. In the all Floras books, large genera mainly are classified into sections. In the Flora of Turkey (Davis, 1967; Davis et al., 1988) too, this is the case. However, this principle was not applied in *Gypsophila* genus. Because the sections were not defined at the time of writing the Flora of Turkey (1967), the taxa could not be placed in the sections. Many new taxa have been recorded since then. These taxa had to be placed in their sections before starting the revision. For this reason, all *Gypsophila* taxa are classified in 4 large groups. It is an artificial but practical classification. Annual *Gypsophila* taxa were collected in Group A. B, C and D Groups include the perennial taxa.

3. We have been studying on the *Gypsophila* revision since 1993 (Özçelik and Özgökçe, 1995; 1999; Korkmaz and Özçelik, 2011a; Özgökçe et al., 2012; Fidan and Özgökçe, 2014; Armağan et al., 2017; Armağan and Özgökçe, 2018). We started from annual *Gypsophila* which a group of clear boundaries, and in this study, we prepared an identification key for annual members of the genus. To make this key, we were identified firstly problematic species, after their taxonomic problems were solved. Then species identification key was made.

4. There are two subspecies of *G. heteropoda*. These are *G. heteropoda* subsp. *heteropoda* and *G. heteropoda* subsp. *minutiflora. G. heteropoda* subsp. *minutiflora* appears to be a taxon far from the other taxon (subsp. *heteropoda*). For this reason, it should be increased to the statu of species category. However, its status has not been changed. Because it mixes with *G. parva*. If more detailed studies are conducted, if the difference can be clearly distinguished from *G. parva,* it can be promoted to the species category.

5. Another problem group is complex of *G. elegans* with *G. bitlisensis.* This complex group is partially mixed with *G. viscosa.* This confusion was fixed with the new key. All hesitant populations between *G. elegans* and *G. bitlisensis* were examined, after their species and populations were distinguished. Three groups of *G. bitlisensis* have been appeared. These groups might be called varieties or they might be called new species. But for now it has been called the group and the diagnostic key has been made. *G. munzurensis* is close to *G. elegans*, not to *G. pilosa*. The reason for the author to make this mistake is that *G. elegans* is not clearly defined.

6. Although a large number of *Gypsophila* taxa have been studied, revision of annuals is given in this study. A new subspecies of *G. venusta* species (subsp. *staminea* Özçelik and Özgökçe) have been added from perennial *Gypsophila* members.

7. The *Gypsophila* list of Turkey was updated. It has 63 *Gypsophila* species.

8. *Gypsophila erikii* Yild. (Yıldırımlı, 2012) and *G. gracilescens* Schischke's presence (Davis 1967) in Turkey is doubtful. The existence of *G. laricina* Schreb. (Sin.: *Gypsophila sphaerocephala* Fenzl ex Tchihat. Asie Min., Bot. 1: 205 (1860)) was confirmed. Some taxa of it are still known from type collection and some are relict. Some observations about taxonomic and geographical characters of all taxa are stated in a list of them.

Taxonomical and distributional data are unsatisfactory for Turkey. For floristic studies, few collections (of which some are new species or records) have been made from some mountains in the region by other botanists (Güner, 1983; Vural and Tan, 1983; Tan, 1984; Alpınar, 1994; Barrera et al., 1999; Yıldırımlı, 2012; Armağan, 2016;

Table 2. Updated *Gypsophila* species of Turkey and their investigated collections and some characteristics

Taxon	Endemism	Distribution area	Phytogeogra-phical region	Taxonomic situation, distribution and investigated collections
1. Sect. **Excapae** Williams				
*1. ***G. serpylloides*** Boiss. & Heldr.	Endemic	C4 Antalya	E. Medit.Mt. el	It is easily distinguished by its short creeping stems. Özgökçe 3173, 3175.
2. G. peshmenii Güner	Endemic	B9 Bitlis	Ir.-Tur el.	-
3.*G. adenophylla* Barkoudah	Endemic	B7 Erzincan ?; B9 Bitlis, Van; C9 Hakkari	Ir.-Tur el.	Recorded from Erzincan (Kandemir and Türkmen, 2008) suspect.
4. *G. pulvinaris* Rech. f.		A10 Ağrı		-
5. G. hakkiarica Kit Tan	Endemic ?	C10 Hakkari	Ir.-Tur el.	-
6. *G. briquetiana* Schischk.	Endemic	B7 Erzincan, Tunceli; B8 Erzurum	Ir.-Tur el.	-
2. Sect. **Gypsophila**				
7. G. davisii Barkoudah	Endemic	C2 Muğla	E. Medit.Mt. el.	-
3. Sect. **Ensifoliae** Bark.				
8. G. graminifolia Barkoudah	Endemic	A8 Erzurum; B9 Van	Ir.-Tur. el.	Local endemic Başkale (Van) city and its environs. Erzurum record of it is new.
4. Sect. **Corymbosae** Barkoudah				
9. G. patrinii Seringe		B10 Ağrı	Ir.-Tur. el.	It is written as a new record for Turkey. *G. patrinii* is the synonym of this species (Armağan et al., 2017).
10. G. brachypetala Trautvetter	Endemic	A9 Kars	Euro.-Sib. el.	It was written as Blacksea Mt. el. (Güner 2012) and *G. brachypetala* Trautv. (Davis, 1967). Özçelik & A.Çelik 23 (GUL 13/24/6/1-4); Özçelik & A.Ç. 27 (GUL 13/24/6-5).
11. *G. guvengorkii* Armağan, Özgökçe & Çelik	Endemic	A4 Karabük	Euxine (Mt.) el	-
12. *G. yusufeliensis* Budak	Endemic	A8 Artvin	Ir.-Tur. el.	-
13. *G. transcaucasica* Barkoudah		B9 Ağrı	Ir.-Tur el.	Only recorded in Doğubeyazıt (Ağrı). Detailed research is required to deduct taxonomic status.
14. *G. tenuifolia* M.Bieb.		A8 Artvin, A9 Ardahan	Euxine (Mt.) el	It's a rare species. Taxonomically very problematic.
5. Sect. **Capituliformes** Williams.				
15. *G. glomerata* Pall. ex Adams		A1 Tekirdağ	Euxine (Mt.) el.	It was written as *G. glomerata* Adams by Güner (2012). It is rare in Turkey. *G. glomerata* Pall. ex Adams is considered as a valid name by the international websites (Anonymous, 2020a,b). New record is a species, very narrow range in Turkey, rare. Edirne, from Süloğlu to Lalapaşa, Süloğlu exit, MK. 1971, 1978; A.Ç. 88.
16. *G. syriaca* Schischk.	Endemic	C6 Adana	E.Medit.Mt. el.	It was a variety of *G. sphaerocephala*. It had been removed from the synonym, but the synonym made valid again as the species. It was more appropriate to have a subspecies. According to Davis (1967), it is an element of Ir.-Tur. region.
17. G. pilulifera Boiss. & Heldr.	Endemic	B5 Kırşehir, Nevşehir; B7 Erzincan; C3/ C4 Antalya	E. Medit.Mt. el.	The distribution area of this species tends to expand over time. Özçelik 1002, 1003(GUL 13/24/9/3-4); A.Özçelik (GUL 13/24/9/1-2; Özçelik 1001(GUL 13/24/9-5); Özçelik & Muca 2013(GUL 13/24/9-6); Dönmez 13754 B. Mutlu T. Ağar (İnönü Univ. Hb. 728); A. Özçelik (GUL 13/24/9/1-2; Özçelik 1002, 1003(GUL 13/24/9/3-4; Özçelik 1001(GUL 13/24/9-5; Özçelik & Muca 2013(GUL 13/24/9-6); A. Özçelik ÇG.18 (GUL 13/24/9-7); MK. 2104(GUL 13/24/9/8-10); Muca 15(GUL 13/24/9/11-12; A.Ç.01 (GUL 13/24/9/13-20).
18. G. olympica Boiss.	Endemic	A2 Bursa	E. Medit.Mt. el	-
19. *G. pinifolia* Boiss. & Hausskn.	Endemic	B6 Malatya, K. Maraş; B7 Malatya, Elazığ; B8 Erzurum	Ir.-Tur. el.	It can be distinguished by its leaves being pointed and stinging.
20. G. leucochlaena Hub.-Mor.	Endemic	B6 Malatya, Sivas	Ir.-Tur. el.	Between Gürün and Darende is the most important habitat area of the species.

				Özçelik 12209 (GUL 13/24/12/ 1-5).
21. *G. osmangaziensis* Ataşlar & Ocak	Endemic	B2 Kütahya, B3Eskişehir	Ir.-Tur. el.	-
22. *G. laricina* Schreb.	Endemic	B6 Tokat, B8 Erzurum	Ir.-Tur. el.	*G. sphaerocephala* Fenzl ex Tchihat. var. *cappadocica* Boiss. was made synonym to this species. Detailed taxonomic study required. The spread of var. *cappadocica* is also spreading area of this species.
6. Sect. **Rokejeka** (Forssk.) A. Braun.				
23a. *G. paniculata* L. var. ***araratica*** Hub.-Mor.	Endemic	A9/A10 Iğdır, Ağrı	Ir.-Tur. el.	Endemic to Mount Ağrı. A rare species.
23b. *G. paniculata* L. var. ***paniculata***		Culture form	-	This subspecies is being recorded for the first time for Turkey. It is produced by florists in Izmir, Istanbul, Yalova and Antalya for ornamental and landscape purposes. Its origine is unknown.
24. *G. bicolor* (Freyn. & Sint. Grossh.		A8 Artvin; B8 Erzurum; B9 Van, Bitlis, Iğdır, Kars	Ir.-Tur. el.	The population has weakened due to excessive collection. Van lake Basin is an important spread area. It is abundant in parts of Azerbaijan near Iran and Turkey.
25. *G. arrostii* Guss. var. ***nebulosa*** (Boiss. & Heldr.) Greuter & Burdet	Endemic	B2 Manisa; B3 Afyonkarahisar; B4 Ankara; C2 Burdur, Uşak, Denizli; C3 Konya/ Isparta/ Burdur	Ir.-Tur. el.	It is endemic to the Lakes Region. It is endemic to the Lakes Region (Isparta, Burdur, Konya, Afyonkarahisar, Denizli).
26. *G. simulatrix* Bornm. & Woron	Endemic	A8 Erzurum; A8/A9 Artvin; A9 Kars/Ardahan; B5 Niğde; B6 Sivas; B6/B7 Malatya C4 Konya; C3 Afyonkarahisar	Ir.-Tur. el.	The phytogeographic region is specified for the first time. It is the endemic of the Eastern Black Sea Region and its environs. Its spread in Central Anatolia is interesting. Thus, the area of soaking has expanded. These examples and their land should be examined in detail.
27. *G. baytopiorum* Kit Tan	Endemic	C9 Hakkari	Ir.-Tur. el.	-
28a. *G. perfoliata* L. var. ***perfoliata***		B3 Afyonkarahisar; B4 Ankara/ Konya, Kayseri; B5 Kayseri; B6 Sivas; B7 Erzincan; C2 Denizli; C4 Konya	Ir.-Tur. el.	This taxon was newly created for Turkey. Before, there was only *G. p.* var. *araratica*. Its habitus is variable. It is a very polymorphic species. Their forms could be seen between Afyonkarahisar, Eskişehir and Ankara. It is easily distinguished by its large layers of flowers and strong plant. It is also produced by florists in Antalya, Izmir, Manisa vilayets etc. There are culture and wild forms.
28b. *G. perfoliata* L. var. ***araratica*** Kit Tan	Endemic ?	A9 Erzurum-Ağrı B9/10 Iğdır	Ir.-Tur. el.	It is endemic to the Ağrı mountain Its endemicity to Turkey section of Mount Ağrı is controversial. It is unknown presence in the part that does not belong to Turkey of the mount. The accuracy of the taxon is questionable. It purchased from the Real Market/flower section.
29. *G. simonii Hub-Mor.	Endemic	A4 Çankırı; B5 Yozgat/ Kayseri/ Ankara; B6 Sivas; B7 Erzincan/ Malatya; B9 Van/ Kars/ Iğdır; C4 Konya.	Ir.-Tur. el.	It spreads depending on gypsum rock. It is one of the indicator plants of gypsum rocks. It is an endemic specific to salty, gypseous areas around Çankırı. Rare endemic. A.Özçelik ÇGG. 61(GUL 13/24/18-1); A.Özçelik ÇGG.46 (GUL 13/24/18-2); A.Özçelik ÇGG.99(GUL 13/24/18-94); MK.48 (GUL 13/24/18/95-96.
30. *G. oblanceolata* Barkoudah	Endemic	B4 Niğde/Aksaray/ Konya	Ir.-Tur. el.	The fleshy structure and oblanceolat shape of the leaves is distinctive. It is peculiar to salty marshes in the Middle Anatolian region. It mixes with *G. germanicopolitana* in the identification key. Only the leaves differed from *G. germanicopolitana* it may be distribution. M.K. 67 (GUL 13/24/18/01-02).
31. *G. germanicopolitana* Hub.-Mor.	Endemic	A4 Çankırı; B5 Yozgat, Kırşehir; B5/B6 Kayseri; Sivas	Ir.-Tur. el.	It is grown on stony, loamy, sandy soils. It was a local endemic known only from Çankırı. The distribution area has been extended with new records. A.Özçelik 116(GUL 13/24/20-1); A.Özçelik 207(GUL 13/24/20-2); A.Özçelik 43(GUL 13/24/20-3); A.Özçelik & K.Aydınşakir 03(GUL 13/24/20-4).
32. *G. nabelekii* Schischk.		B10 Iğdır; C9/10 Hakkari	Ir.-Tur. el.	Endemicity of it is controversial. Its spread can also be found in Iraq. Its type specimen from Turkey.
33. *G. curvifolia* Fenzl	Endemic	C3 Antalya, Isparta, Burdur;	E. Medit. Mt. el.	It grows in swamps and wetlands or on their edges. It is an endemic to Lakes region.

		C4 Antalya, Konya		Özçelik 7335(GUL 13/24/22-1-5); MK. 897(GUL 13/24/20-6); Özçelik 8038 (GUL 13/24/22/7-8)
34. *G. festucifolia* Hub.-Mor.	Endemic	B6 Sivas, Kayseri	Ir.-Tur. el.	-
35. *G. turcica* Hamzaoğlu	Endemic	B6 Sivas	Ir.-Tur. el.	It is a new recorded species known from type gathering (Hamzaoğlu, 2012).
36. *G. libanotica* Boiss.		B6 Niğde; C5 Konya, Niğde; C6 K. Maraş, Osmaniye	E. Medit. (Mt.) el.	-
37. *G. ruscifolia* Boiss.		B7 Elazığ, Tunceli; B8 Erzurum, Muş; B9 Van, Bitlis, Ağrı; C6 Gaziantep; C8 Diyarbakır, Mardin	Ir.-Tur. el.	It is easily distinguished by the perfoliate leaves. It is common in East and South East Anatolian regions. Its rhizomes are very flexible.
38. *G. pallida* Stapf.		B6 Kahramanmaraş, Malatya, Elazığ; B9 Van; C9 Hakkari	Ir.-Tur. el.	_
39. *G. tuberculosa Hub.-Mor.	Endemic	B7 Erzincan	Ir.-Tur. el.	Its identification is very difficult to make from the current key. It mixes with *Bolanthus*, but it is annual.
40. *G. aucheri* Boiss.	Endemic	B7Sivas/Erzincan/ Tunceli, Malatya, Adıaman; B8 Erzurum	Ir.-Tur. el.	It spreads in environment peculiar to rock.
41. *G. eriocalyx* Boiss.	Endemic	A4 Çankırı; A9 Kars/Ardahan; B3 Eskişehir; B4 Ankara; B5 Kayseri/ Çorum; B6 Sivas; C5 Niğde	Ir.-Tur. el.	Hair features in the stem, leaves, and calyx provide important diagnostic characters.
42. *G. lepidioides Boiss.	Endemic	B7 Erzincan	Ir.-Tur. el.	It develops depending on gypsum rock, it shows local distribution. It is endemic to Erzincan environment. It is close to *G. eriocalyx*. is easily distinguished by its inflorescence stalk and indumentum characters. Özçelik 12876 (GUL 13/24/30/1-13).
7. Sect. Heterochroa (Bunge) Fenzl.				
43. *G. glandulosa* (Boiss.) Walp.	Endemic ?	A7 Trabzon; A8 Erzurum, Rize; A9 Artvin	Euxine Mt. el.	It may be not endemic. Type specimen of it from Turkey (Güner, 2012).
8. Sect. **Dichoglottis** (Fisch. & Mey.) Fenzl				
44a. *G. heteropoda* Freyn & Sint. subsp. ***heteropoda***		A9 Kars; B10 Ağrı	Ir.-Tur. el.	In identification, 2 subspecies seems to be impossible with the existing key. A new key was made by us. Subsp. *heteropoda* can be easily distinguished by presence of viscous structures in stems and inflorescences. But subsp. *minutiflora* is very difficult to define. Detailed studies are needed. Subsp. *minutiflora* may be a separate species. The taxon is mixed with *G. parva*. The plant is completely hairless and not viscos, it should be studied in detail, it does not go from 1st to 1st, not from 2nd to 1st. Some have viscosity, some do not.
44b. *G. heteropoda* Freyn & Sint. subsp. ***minutiflora*** Barkoudah	Endemic	B6 Sivas	Ir.-Tur. el.	
45. *G. parva* Barkoudah	Endemic	A4 Çankırı; A5 Çorum	Ir.-Tur. el.	-
46. *G. linearifolia* (Fisch. & C.A. Mey.) Boiss.		B5 Nevşehir and its environs	Ir.-Tur. el.	Taxonomic features are not safe. It is particularly confused with *G. elegans.*
47. *G. bitlisensis* Barkoudah	Endemic	B6/7 Sivas; B7 Erzincan; B8 Erzurum; B9 Bitlis, Van	Ir.-Tur. el.	-
48. *G. viscosa* Murr.		A9 Kars; Iğdır; B3 Eskişehir; B4 Konya, Ankara; B5 Kayseri; B6 Sivas; C3 Konya, Isparta; C6 Şanlıurfa	Ir.-Tur. el.	-
49. *G. elegans* M. Bieb.		A7 Gümüşhane, Bayburt; A8 Erzurum; B7 Erzincan, Diyarbakır; B9 Van, Bitlis; B10 Kars, Iğdır, Ağrı; C3/C4 Konya	Ir.-Tur. el.	The distribution area of the species is mainly in the Eastern Anatolia region and it also spreads in the Eastern Black Sea region close to this region. Steppe, meadow and arid meadows are important habitats for it.
50. *G. silenoides* Rupr.		A7 Giresun, Gümüşhane, Trabzon; A8 Trabzon, Rize, Artvin; A9 Artvin; Ardahan	Euxin el.	It is usually biennial, rarely perennial. In the first year, rosette leaves are formed, in the 2nd year there is flowering. If this condition is unknown, its identification is difficult. The spreading area of the species is essentially the Eastern Black Sea region. It is

				the Kashgar mountains between Trabzon and Rize. It grows abundantly on gravelly slopes.
51. *G. polyclada* Fenzl. ex Boiss.		C10 Hakkari	Ir.-Tur. el.	It is rare species.
52. *G. antari* Post. & Beauverd.		C7 Şanlıurfa	Sahara Arabian el.	It is rare species. Especially in Akçakale, which is the border of Syria, it has spread.
9. Sect. **Marcrorhizaea** Boiss.				
53. *G. muralis* L.		A1 Edirne	Euro.-Sib. el.	Its general appearance is similar to *Arenaria* genus. It was recorded from Çörekköy (Davis 1967). Today, Çörekköy is within the borders of Greece, 13 km away from the Customs Gate of Pazarkule. It has been revealed with the subsequent research that it has spread in Tekirdağ / Silivri-Çorlu. It's a rare species. Sammel 02.284(EGE); A.Özçelik (GUL 13/24/40-2
54. *G. torulensis M.Koç		A7 Gümüşhane	Euro.-Sib. el. ?	It resembles to *G. muralis* (Koç 2013)
55. *G. tubulosa* (Jaub. & Spach) Boiss.	Endemic	B1 İzmir; B2 Uşak; C1/C2 Aydın	E. Medit. el.	It leaves from genus *Bolanthus* as it is annual. There is also an example in IZEF Hb. (İzmir). It is a difficult species to identification.
56. *G. confertifolia* Hub.-Mor.	Endemic	C2 Muğla, Burdur	E. Medit. el.	This kind of short, dense flowered inflorescence looks like *Velezia*. MK. 16 (GUL13/24/42-1)
57. *G. hispida* Boiss.		A8 Gümüşhane, Erzurum; A9 Kars, Iğdır; B7 Erzincan, Tunceli; B8 Erzurum; B10 Iğdır, Kars	Ir.-Tur. el.	Type sample from Turkey. This species has not been seen by us.
10. Sect. **Hagenia** A. Braun.				
58. *G. pilosa* Hudson		A2 İstanbul, Bilecik; A9 Kars; B2 Kütahya; B3 Afyonkarahisar; B4 Ankara; B5 Kayseri; B7 Elazığ; C2Antalya; C3 Isparta; C4 Konya; C5 Niğde; C6 Şanlıurfa	Ir.-Tur. el. ?	It is a species widespread throughout Turkey. It doesn't choose many habitats. It must be a cosmopolitan species. Özçelik & Muca 05(GUL 13/24/44/02-09); Özçelik & Muca 12(GUL 13/24/44-10); Özçelik & Muca 04(GUL 13/24/44/11-16); Özçelik 12877(GUL 13/24/44-17); Özçelik 12877(GUL 13/24/44-18); MK. 704(GUL 13/24/44-19); MK. 700.
59. *G. munzurensis* Armağan	Endemic	B7 Tunceli	Ir.-Tur. el.	It is seem like *G. elegans* not *G. pilosa*. (Armağan 2016)
60. *G. nodiflora* (Boiss.) Barkoudah	Endemic	B7 Elazığ; C6 Malatya	Ir.-Tur. el.	This species has not been seen by us.
61a. *G. venusta* Fenzl subsp. ***venusta***		A4 Çankırı; A7 Sivas; B3 Afyonkarahisar; B4 Ankara, Konya; B5 Yozgat, Kayseri; B6 Sivas; B7 Malatya; B8 Erzurum, Erzincan; C3 Isparta, Konya, Karaman; C5 Niğde; C6 Gaziantep, Adana; C7 Şanlıurfa	Ir.-Tur. el.	The most distinctive feature of the length of the petals and the high ratio of calyx. Subsp. *venusta* was named for the first time. Although it is seen in the same region with subsp. *staminea*, its main spreading area is the Lakes Region and Central Anatolia Region. It is very rare in Eastern Anatolia.
61b. *G. venusta* Fenzl subsp. ***staminea*** Özçelik and Özgökçe	Endemic	Erzurum	Ir.-Tur. el.	It is a rare endemic known from type gathering. Its stamen characters is distinguished from subsp. *venusta*.
62. *G. gracilescens* Schischk. (Davis 1967) and **63. *G. erikii*** Yild. (Yıldırımlı, 2012) are suspected of being in Turkey. Their samples are not seen.				

Armağan et al., 2017). General literatures (Rechinger, 1988; Karagüzel et al., 1992; Kandemir and Türkmen, 2008; Özçelik and Yıldırım, 2012; Anonymous, 2020c) which are still available are those primarily meant for the general systematic and taxonomy. For most of the plants described appears to us as invalid. In view of this, we have started investigation on the genus based on personal observations and wider collections on population basis.

Most of the investigated collections are dated after the publication of the Flora of Turkey, and some of them could not be distinguished from each other with the help of the Flora (Davis, 1967). Those taxa included in Güner (2012) were prepared more accurately. However, there is no identification key and also their Turkish names and geographical regions are dream, does not meet to the reality of Turkey in general. Some members of the genus show a great variation in indumentum, branching and flower number, due to polymorphism, hybridisation, polyploidy and habitat differences. As such, their taxonomical status has not been revealed fully.

While visiting the area, we came to conclusion that the region between Ağrı and Van, Bitlis especially Tahir, Tendürek, Süphan, Nemrut and Ağrı Mountains, Başkale environs appear to us as center of great diversity for the genus. The mountains and their environs are very rich in *G. bitlisensis* and *G. elegans*. The area should therefore be investigated in detail.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

Acknowledgments

The examined materials in this study were collected by us within the scope of the following projects: TÜBİTAK, TBAG 107T147 numbered Project; TÜBİTAK-DPT, TBAG. Ç. Sek. No:4; in 2006-2008 years, by TOVAG, titled Cut Flower Variety Development Project-Creating Gene Pools (Phase I); Süleyman Demirel Univ. Rectorate Research Fune Project 44; Yüzüncü Yıl University Rectorate, Research Fune, 2009-FBE- YL 011; 2011-FBE-D- 030; VET-2957. We thank to the relevant institutions and work teams. Also, we would like to thank the researchers and herbarium officers whose examples have been reviewed in the article.

References

Alpınar K (1994). Some Contributions to the Turkish Flora. Edinburgh Journal of Botany 51(1): 65-73.

Anonymous (2020a). http://www.theplantlist.org/tpl1.1/record/kew-2836684 [accessed 14 March 2020].

Anonymous (2020b). http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:154479-1 [accessed 05 April 2020].

Anonymous (2020c). https://www.gbif.org/species/155862787 [accessed 05 April 2020].

Armağan M (2016). *Gypsophila munzurensis* (Caryophyllaceae), a new species from Tunceli (Turkey). Phytotaxa 275(2): 175-180.

Armağan M, Özgökçe F, Çelik A (2017). Notes on the genus *Gypsophila* (Caryophyllaceae) in Turkey, with a description of *G. guvengorkii* sp. Nov. Phytotaxa 295(3): 271-275.

Armağan M, Özgökçe F (2018). Anatomical, Palynological, Morphological, Karyological, and Ecological Investigations on *Gypsophila davisii*. Anatolian Journal of Botany 2(1): 39-45.

Ataşlar E, Ocak A (2005). *Gypsophila osmangaziensis* (Caryophyllaceae), a new species from Central Anatolia, Turkey. Annales Botanici Fennici 42: 57-60.

Ataşlar E (2000). in edts: Güner A, Özhatay N, Ekim T, Başer KHC 2000. Flora of Turkey and the East Aegean Islands, Vol. 11. Edinburgh: Edinburgh University Press.

Barkoudah YI (1962). A Revision of *Gypsophila, Bolanthus, Ankyropetalum* and *Phryna*. Wentia 9: 1-203.

Barrera AC, Arenas G (1999). Cutting-time effect during harvest cycle on postharvest behavior of three *Gypsophila paniculata* cv. perfecta clones. Proc of the Int Symp on Cut Flowers in the Tropics. Acta Horticulturae 482: 71-76.

Budak Ü (2012). A new species of *Gypsophila* (*Caryophyllaceae*): *Gypsophila yusufeliensis* Budak, from Turkey. Annales Botanici Fennici 49: 425-427.

Davis PH (1967). Flora of Turkey and the East Aegean Islands, Vol. 2. Edinburgh: Edinburgh University Press.

Davis PH, Mill RR, Tan K (1988). Flora of Turkey and the East Aegean Islands, Vol. 10. Edinburgh: Edinburgh University Press.

Ekim T (2012). *Gypsophila*. Bizimbitkiler (2013). http://www.bizimbitkiler.org.tr [accessed 12 November 2020].

Fidan M, Özgökçe F (2014). Türkiye *Gypsophila* L. (*Caryophyllaceae*) cinsine iit Hagenia A. Braun., seksiyonunun polen ve tohum morfolojisi. 22. Ulusal Biyoloji Kongresi Eskişehir Osmangazi Üniversitesi, 537, 23-27 Haziran 2014.

Fidan M, Özgökçe F (2016). Türkiye *Gypsophila* L. (*Caryophyllaceae*) cinsine iit Hagenia A. Braun. seksiyonunun revizyonu. Turkish Journal of Life Sciences 1(2): 75-85.

Güner A (1983). New records for the Flora of Turkey and two new species from Anatolia. Notes from the Royal Botanic Garden 41(2): 283-288.

Güner A (2012). Türkiye bitkileri listesi (Damarlı Bitkiler). ANG Vakfı Nezahat Gökyiğit Botanik Bahçesi Yayınları Flora Dizisi 1. İstanbul: NAMAŞ Nurtan Ambalaj ve Matbaacılık San ve Tic AŞ.

Güner A, Özhatay N, Ekim T, Başer KHC (2000). Flora of Turkey and the East Aegean Islands, Vol. 11. Edinburgh: Edinburgh University Press.

Hamzaoğlu E (2012). A new species of *Gypsophila* and a new name for *Silene* (*Caryophyllaceae*) from Turkey. Turkish Journal of Botany 36: 135-139.

Huber-Morath A, McNeill J, Reeve H (1967). Materials for a Flora of Turkey. XIV: *Caryophyllaceae*, Notes from the Royal Botanic Garden 27(1): 17-23.

Kandemir A, Türkmen Z (2008). The flora of Üzümlü-Sakaltutan (Erzincan-Gümüşhane). Turkish Journal of Botany 32: 265-304.

Karagüzel O, Altan S (1999). *Gypsophila paniculata* L. "Perfecta"nın büyüme ve çiçeklenmesi üzerine dikim zamanı ve gün uzunluğunun etkileri. Turkish Journal of Agriculture and Forestry 23(Ek Sayı 2): 257- 280.

Koç M (2013). *Gypsophila torulensis* (*Caryophyllaceae*) a New Species from Turkey. Annales Botanici Fennici 50: 149-150.

Korkmaz M, Özçelik H (2011a). Systematical and morphological characteristics of annual *Gypsophila* L. (Caryophyllaceae) taxa of Turkey. Biological Diversity and Conservation 4(1): 79- 98.

Korkmaz M, Özçelik H (2011b). Economic importances of *Gypsophila* L., *Ankyropetalum* Fenzl and *Saponaria* L. (*Caryophyllaceae*) taxa of Turkey. African journal of Biotechnology 10(47): 9533-9541.

Koyuncu M, Kılıç CS, Güvenç A (2008). Soaproot yielding plants of East Anatolia and their potential in nature. Turkish Journal of Botany 32: 489-494.

Linnaeus C (1753). Species plantarum: exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. Stockholm: Laurentius Salvius.

Özçelik H, Özgökçe F (1995). Taxonomic contributions to genus *Gypsophila* L. (*Caryophyllaceae*) from East Anatolia (Turkey). IV th Plant Life of South West Asia Symposium. 195-209, May 23-25 1995 İzmir Turkey.

Özçelik H, Özgökçe F (1999). *Gypsophila bitlisensis* Bark. ve *Gypsophila elegans* M. Bieb. üzerinde morfolojik, taksonomik ve ekolojik araştırmalar. 1st International Symposium on Protection of Natural Environment and Ehrami Karaçam 23-25th September 1999, Kütahya/Turkey 295- 313.

Özçelik H, Yıldırım B (2011). Türkiye çövenlerinin (*Gypsophila* L. ve *Ankyropetalum* Fenzl spp.) ekonomik önemi, kullanım olanakları ve korunması üzerine düşünceler. SDÜ Orman Fakültesi Dergisi 12: 57-61.

Özgökçe F, Fidan M, Armağan M (2012). *Gypsophila nodiflora* (Boiss.) Barkoudah (Caryophyllaceae) üzerine bazı araştırmalar, 21. Ulusal Biyoloji Kongresi. 14-22, 3-7 Eylül 2012 Ege Üniversitesi İzmir Türkiye.

Rechinger KH (1988). *Gypsophila* L., in Rechinger KH (ed.), Flora Iranica 163. Akademische Druck und Verlagsanstalt, Graz: 206–246.

Schischkin BK (1936). *Gypsophila* L., in Komarov VL (ed.), Flora of the U.S.S.R., Vol. 6. Leningrad: Izdatel' stvo Akademii Nauk SSSRR.

Tan K (1984). More new taxa from South and East Anatolia. Notes from the Royal Botanic Garden 42(1): 61-67.

Vural M, Tan K (1983). New taxa and records from Turkey. Notes from the Royal Botanic Garden 41(1): 65-67.

Yıldırımlı Ş (2012). Nine new species from Kaz dağları, Munzur dağları, Bolkar dağları and Karçal dağları Turkey. Ot Sistematik Botanik Dergisi 19(1): 1-34.

Anatolian Journal of Botany
5(1): 19-22 (2021)
Researh article *doi:10.30616/ajb.826640*

Hymenoscyphus caudatus, a new ascomycete record for the mycobiota of Turkey

Ahmet ÇETİNKAYA[1], Yasin UZUN[2*]
[1]*Ayrancı Social Assistance and Solidarity Foundation, 70100 Karaman, Turkey*
[2]*Karamanoğlu Mehmetbey University, Science Faculty, Department of Biology, Karaman, Turkey*
**yuclathrus@gmail.com, [1]ahmet_cetinkayaaa@hotmail.com*

Received : 16.11.2020
Accepted : 17.12.2020
Online : 09.01.2021

Hymenoscyphus caudatus, Türkiye mikobiyotası için yeni bir askomiset kaydı

Abstract: *Hymenoscyphus caudatus* (P. Karst.) Dennis is given as new record for the mycobiota of Turkey. The macro and micromorphological characters of the species are provided together with the localities of collection, collector numbers and the photographs related to its macro and micromorphologies.

Key words: Biodiversity, macrofungi, new record, *Helotiaceae*

Özet: *Hymenoscyphus caudatus* (P. Karst.) Dennis Türkiye için yeni kayıt olarak verilmiştir. Türün makro ve mikromorfolojik karakterleri, toplanma lokaliteleri, toplayıcı numaraları ve makro ve mikromorfolojilerine ait fotoğrafları ile birlikte verilmiştir.

Anahtar Kelimeler: Biyoçeşitlilik, makromantarlar, yeni kayıt, *Helotiaceae*

Citation: Çetinkaya A, Uzun Y (2021). *Hymenoscyphus caudatus*, a new ascomycete record for the mycobiota of Turkey. Anatolian Journal of Botany 5(1): 19-22.

1. Introduction

Hymenoscyphus Gray is a widespread genus within the family *Helotiaceae* (*Ascomycota*) with more than 800 species (Kirk et al., 2008). The members of the genus are generally characterized by their stipitate to sessile discoid apothecia; white to yellowish hymenial surface and subellipsoid, fusoid, or scutuloid ascospores. Species of the genus are normally saprophytic on plant debris, such as wood, twigs, fruits, leaves, and herbaceous stems. Though the known species are mainly reported from America, Asia and Europe, it is a cosmopolitan one.

Hymenoscyphus fructigenus (Bull.) Gray was the first species of the genus *Hymenoscyphus* to be reported in Turkey (Aktaş et al., 2006). Between the years 2009 and 2019, twelve members of the genus (*H. calyculus* (Fr.) W. Phillips, *H. epiphyllus* (Pers.) Rehm ex Kauffman, *H. fagineus* (Pers.) Dennis, *H. herbarum* (Pers.) Dennis, *H. immutabilis* (Fuckel) Dennis, *H. kathiae* (Korf) Baral, *H. lepismoides* Baral & Bemmann, *H. lutescens* (Hedw.) W. Phillips, *H. robustior* (P. Karst.) Dennis, *H. scutula* (Pers.) W. Phillips, *H. serotinus* (Pers.) W. Phillips, *H. umbilicatus* (Le Gal) Dumont) have also been presented (Kaya, 2009; Kaya et al., 2009; Doğan and Aktaş, 2010; Öztürk et al., 2010, 2016; Uzun et al., 2010, 2014; Akata et al., 2014; Işık and Türkekul, 2018; Keleş, 2019a), increasing the current taxa number of the genus to 14 in Turkey.

During routine field trips in Yeşildere district of Karaman, some stipitate discoid ascomycete samples were collected. As a result of field and laboratory investigation they were identified as *H. caudatus*. Tracing the current checklists on Turkish macromycota (Sesli and Denchev, 2014; Solak et al., 2015) and the latest contributions (Berber et al., 2019; Kaya et al., 2019; Keleş, 2019b; Sesli, 2019; Türkekul and Işık, 2019; Yıldız et al., 2019; Acar et al., 2020; Akçay, 2020; Çelik et al., 2020; İleri et al., 2020), it was noticed that the taxon has not been recorded from Turkey before.

The study aims to make a contribution to the determination of the macrofungal biodiversity of the Karaman and Turkey.

2. Materials and Method

Hymenoscyphus samples were collected from Yeşildere village of Karaman province. The fruit bodies were photographed at their natural habitat and notes were taken about the morphological and ecological characteristics of them. Then the specimens were carried to the fungarium and dried in an air conditioned room. Microscopic studies were performed on dried specimens under a Nikon Eclipse Ci-S trinocular light microscope. The specimens were mounted in water and Melzer reagent. The samples were identified with the help of Kimbrough and Atkinson (1972), Dumort and Carpenter (1982), Zhuang and Korf (1989), Zhuang (1995), Ellis and Ellis (1997). The collected specimens are kept at Karamanoğlu Mehmetbey University, Kamil Özdağ Science Faculty, Department of Biology, Karaman, Turkey.

3. Results

Fungi R.T. Moore

Ascomycota Caval.-Sm.

Helotiales Nannf. ex Korf & Lizoň

Helotiaceae Rehm

Hymenoscyphus caudatus (P. Karst.) Dennis, Persoonia 3(1): 76 (1964)

Syn: [*Helotium caudatum* (P. Karst.) Velen.; *Helotium scutula* var. *caudatum* (P. Karst.) P. Karst., *Peziza caudata* P. Karst.**]**

Macroscopic and microscopic features: Apothecia 0.5-2 mm in diameter, scutellate, whitish-cream, disc flat to concave, hymenium white to pale yellow when young, straw-yellow to pale yellow-orange when dry; stipe

cylindrical, broader above and tapering slightly toward the base, concolorous with the outer surface of the receptacle, some hairy at the base (Fig. 1). Asci 100-120 × 9-12.5 µm, cylindrical to clavate, eight-spored, walls outlined blue in Melzer's reagent, especially at the apex. Paraphyses filiform, equal or slightly exceeding the asci, some branched at the base, septate (Fig. 2a). Ascospores 15-24 × 4-5.5 µm, ellipsoid, subfusoid to ovoid, hyaline, smooth, aseptate or rarely l-septate, generally with two large, irregular guttules and several smaller guttules (Fig. 2b).

Figure 1. Ascocarps of *Hymenoscyphus caudatus*

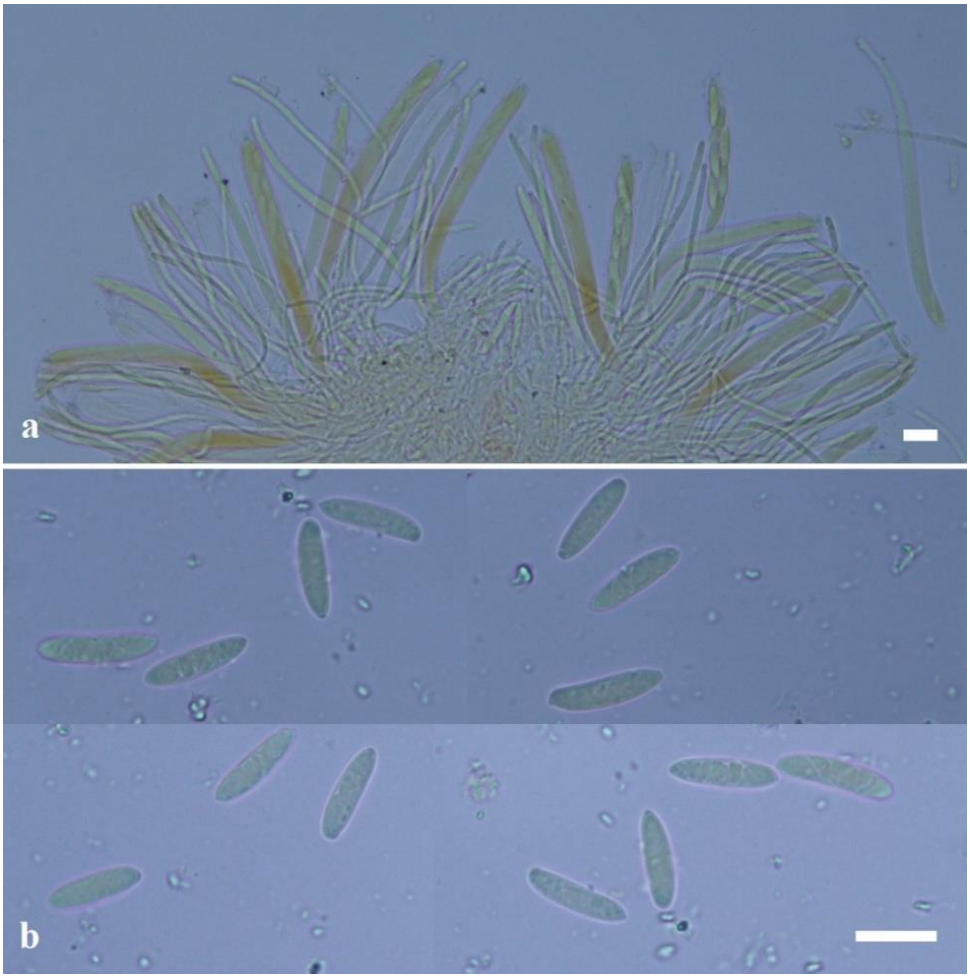

Figure 2. Asci and paraphyses (a), and ascospores of *Hymenoscyphus caudatus* (b) (bars: 10 µm) (a: Melzer; b: water)

Hymenoscyphus caudatus grows on decaying leaves and leaf parts of many trees such as the members of *Acer*, *Aesculus*, *Alnus*, *Betula*, *Castanea*, *Carpinus*, *Corylus*, *Crataegus*, *Fagus*, *Fraxinus*, *Pinus*, *Platanus*, *Populus*, *Prunus*, *Quercus*, *Robinia*, *Salix*, *Tilia*, *Ulmus*, and rarely on herbaceous stem (Kimbrough and Atkinson, 1972; Ellis and Ellis, 1997; Zhuang, 1995).

Specimens examined: Karaman, Yeşildere village, on decaying leaves and twigs of *Populus* sp., 37°09′N-33°29′E, 1160 m, 07.05.2015, AÇK. 223.

4. Discussions

Hymenoscyphus caudatus was given as new record for Turkish mycobiota as the fourteenth member of the genus *Hymenoscyphus*. In general, macro and micromorphology are in agreement with those given in literature.

Hymenoscyphus caudatus was reported to be common on leaf litter and grow on decaying leaves and leaf parts of deciduous trees (Kimbrough and Atkinson, 1972; Ellis and Ellis, 1997; Zhuang, 1995). Zhuang and Korf (1989) reported on roots of a grass. Beside rotting leaf litter, our samples were also collected on rotting *Populus* twigs.

Hymenoscyphus caudatus may easily be confused with *H. hyaloexcipulus* H.D. Zheng & W.Y. Zhuang in terms of morphology, but it differs from the latter species in having much narrower ectal excipular cells, and narrower ascospores (Zheng and Zhuang, 2013).

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

Acknowledgments

The authors would like to thank Karamanoğlu Mehmetbey University Research Fund (09-YL-15) for its financial support.

References

Acar İ, Uzun Y, Akata I (2020). Some macrofungi determined in Şemdinli and Yüksekova districts (Hakkari-Turkey). KSU Journal of Agriculture and Nature 23(1): 157-167.

Akata I, Uzun Y, Kaya A (2014). Macromycetes determined in Yomra (Trabzon) district. Turkish Journal of Botany 38(5): 999-1012.

Akçay ME (2020). A new record for the mycota of Turkey. Anatolian Journal of Botany 4(1): 8-10.

Aktaş S, Kaşık G, Doğan HH, Öztürk C (2006). Two new taxa records for the macrofungi of Turkey. Turkish Journal of Botany 30(3): 209-212.

Berber O, Uzun Y, Kaya A (2019). *Genea lobulata*, A new hypogeous ascomycete record for Turkish mycobiota. Süleyman Demirel University Journal of Natural and Applied Sciences 23(3): 922-924.

Çelik A, Uzun Y, Kaya A (2020). Macrofungal biodiversity of Güneysınır district (Konya-Turkey). The Journal of Fungus 11(1): 75-83.

Doğan HH, Aktaş S (2010). Two new ascomycetes records from Mediterranean part of Turkey. Biological Diversity and Conservation 3(1): 83-86.

Dumort KP, Carpenter SE (1982). Los Hongos de Colombia-VII: Leotiaceae IV. *Hymenoscyphus caudatus* and related species from Colombia and adjacent regions. Caldasia 13(64): 567-602.

Ellis BM, Ellis PJ (1997). Microfungi on land plants, an identification handbook. Slough-UK: The Richmond publishing Co. Ltd.

Işık H, Türkekul İ (2018). A new addition to Turkish *Helotiaceae*. Süleyman Demirel University Journal of Natural and Applied Sciences 22(2): 595-597.

İleri R, Uzun Y, Kaya A (2020). Macromycetes of Karadağ (Karaman) and its environs. The Journal of Fungus 11(1): 57-63.

Kaya A (2009). Macrofungi of Huzurlu high plateau (Gaziantep-Turkey). Turkish Journal of Botany 33(6): 429-437.

Kaya A, Uzun Y, Karacan İH (2009). Macrofungi of Göksun (Kahramanmaraş) district. Turkish Journal of Botany 33(2): 131-139.

Kaya A, Uzun Y, Karacan İH, Yakar S (2019). Contributions to the macromycota of Gaziantep province. Kastamonu University Journal of Forestry Faculty 19(3): 329-341.

Keleş A (2019a). New records of *Hymenoscyphus, Parascutellinia*, and *Scutellinia* for Turkey. Mycotaxon 134(1): 169-175.

Keleş A (2019b). New records of macrofungi from Trabzon province (Turkey). Applied Ecology and Environmental Research 17(1): 1061-1069.

Kimbrough JW, Atkinson M (1972). Cultural features and imperfect stage of *Hymenoscyphus caudatus*. American Journal of Botany 59(2): 165-171.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). Dictionary of the fungi. 10th ed. Wallingford, UK: CAB International.

Öztürk Ö, Doğan HH, Şanda MA (2016). Some new additions to Turkish mycobiota from Sakarya region. Biological Diversity and Conservation 9(1): 97-100.

Öztürk Ö, Doğan HH, Yıldırımlı Ş (2010). Macrofungi of Eldivan dağı (Çankırı). The Herb Journal of Systematic Botany 17(2): 141-154.

Sesli E (2019). *Lacrymaria hypertropicalis* (Guzmán, Bandala & Montoya) Cortez (Psathyrellaceae): Türkiye mikotası için yeni bir kayıt. Bağbahçe Bilim Dergisi 6(2): 99-102.

Sesli E, Denchev CM (2014). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. 6th ed. Mycotaxon Checklists Online. 136 p. (http://www.mycotaxon.com/resources/checklists/sesli-v106-checklist.pdf)

Solak MH, Işıloğlu M, Kalmış E, Allı H (2015). Macrofungi of Turkey, Checklist, Vol. 2. İzmir, Turkey: Üniversiteliler Ofset.

Türkekul İ, Işık H (2019). Macrofungal biodiversity of Reşadiye (Tokat) district. Acta Biologica Turcica 32(2): 95-101.

Uzun Y, Acar İ, Akçay ME, Akata I (2014). Additions to the Turkish Discomycetes. Turkish Journal of Botany 38(3): 617-622.

Uzun Y, Kaya A, Akçay ME, Demirel K (2010). New additions to the Turkish macromycota from Bingöl province (Turkey). Turkish Journal of Botany 34(1): 63-66.

Yıldız MS, Türkekul İ, Işık H (2019). Macrofungal biodiversity of Pazar (Tokat) district. BEÜ Journal of Science 8(2): 387-395.

Zheng H, Zhuang W (2013). Four new species of the genus *Hymenoscyphus* (fungi) based on morphology and molecular data. Science China Life Sciences 56(1): 90-100.

Zhuang WY (1995). Some new species and new records of Discomycetes in China. V. Mycotaxon 56: 31-40.

Zhuang WY, Korf RP (1989). Some new species and new records of Discomycetes in China. III. Mycotaxon 35(2): 297-312.

Anatolian Journal of Botany
5(1): 23-28 (2021)
Research article *doi:10.30616/ajb.846497*

Macrofungal biodiversity of Gürpınar (Van) district

Ezelhan ŞELEM[1], Ali KELEŞ[2*], Sinan İŞLER[3]
[1]*Yüzüncü Yıl University, Agriculture Faculty, Department of Field Crops, Van, Turkey*
[2,3]*Yüzüncü Yıl University, Education Faculty, Department of Science and Mathematics Education, Van, Turkey*
alikeles61@yahoo.com, [1]ezelhanselem@hotmail.com, [3]sinan_1970@hotmail.com

Received : 24.12.2020
Accepted : 19.01.2021
Online : 24.01.2021

Gürpınar (Van) yöresinin makromantar biyoçeşitliliği

Abstract: The study was based on macrofungi samples collected from Gürpınar district of Van province between 2015 and 2017. As a result of field and laboratory studies 94 macrofungi species belonging to 49 genera, 27 families, seven orders and three classes within Ascomycota and Basidiomycota were determined. The list of the determined taxa were presented together with their habitats, substrates, collection localities and personel voucher numbers.

Key words: Biodiversity, macrofungi, mycota, Turkey

Özet: Çalışma 2015 ve 2017 yıllarında Van'ın Gürpınar ilçesinden toplanan makromantar örnekleri üzerinde gerçekleştirilmiştir. Arazi ve laboratuvar çalışmaları sonucunda *Ascomycota* ve *Basidiomycota* bölümleri içinde yer alan üç sınıf, yedi takım, 27 familya ve 49 cinse ait 94 tür belirlenmiştir. Belirlenen taksonlar, habitatları, substratları, toplanma yerleri ve toplayıcı numaraları ile birlikte listelenmiştir.

Anahtar Kelimeler: Biyoçeşitlilik, makromantar, mikota, Türkiye

Citation: Şelem E, Keleş A, İşler S (2021). Macrofungal biodiversity of Gürpınar (Van) district. Anatolian Journal of Botany 5(1): 23-28.

1. Introduction

The kingdom Fungi constitutes the second most diverse living group in the world with about more than 1.5 million species (Hawksworth et al., 1995). They can grow almost everywhere in the world as saprophytes, parasites and symbionts, and those with fruiting bodies that can be seen by naked eye are known as macrofungi. Besides being consumed as food, macrofungi are used in cosmetics and pharmacology and have high economic value (Adanacıoğlu et al., 2016; Süfer et al., 2016).

Studies on the macrofungal biodiversity of Turkey have been started in the first quarter of the 19th century, and lots of works have been conducted by many researchers (Alkan et al., 2010; Demirel et al., 2010; Doğan et al., 2012; Türkekul and Işık, 2016; Uzun et al., 2017; Yıldız et al., 2019; Acar et al., 2020). Sesli and Denchev (2014) present about 2.400 macrofungi species in Turkey, and Kaya and Uzun (2018) introduces this number as 2.500. Considering, the 15.000 macromycete taxa (Lukić, 2008) determined in Europe, it becomes clear that there is a lot of work to be done in Turkey.

Gürpınar is the largest district of Turkey with a surface area of 4.063 km^2 within the boundaries of Van province (Fig. 1), and located between 37°44′-28°29′ north latitudes and 43°07′-44°07′ east longitudes. The district lies within the IranoTuranean phytogeographical flora sector. The climate of the research area is Mediterranean with an annual rainfall of 281 mm and an annual average temperature of 8.1 °C (Bani and Adıgüzel, 2008). Though the list of naturally growing edible mushrooms was presented by Şelem et al. (2019), there isn′t a detailed study on the overall macrofungal biodiversity of Gürpınar district.

The study aims to determine naturally growing macrofungi of the district and make a contribution to the mycobiota of Turkey.

2. Materials and Method

Macrofungi samples were collected from the region within the boundaries of Gürpınar districts of Van province. During field studies, first of all the fruit bodies were photographed at their natural habitats. Then necessary notes about the ecological and morphological characteristics and the geographical positions of the the samples were recorded. The collected samples were in paper boxes and transferred to the fungarium. They were dried in an air conditioned room and kept as fungarium materials in polyethylene bags. Further investigations were carried out in the fungarium on dried samples. Microscopic investigations were performed under a compound microscope. The specimens were identified by comparing the obtained data with the relevant literature (Moser, 1983; Breitenbach and Kränzlin, 1984, 1986, 1991, 1995, 2000; Buczacki, 1989; Bresinsky and Besl, 1990; Jordan, 1995; Pegler et al., 1995; Philips, 1991; Dähncke, 2004; Hausknecht, 2009; Uzun, 2010; Kuo and Methven, 2014). The determined macrofungi samples are kept in the fungarium of Biology Department, Science Faculty, Van Yüzüncü Yıl Üniversity(VANF).

3. Results

The determined taxa are listed in alphabetical order. Index Fungorum (accessed on 20 December 2020) were followed for the systematics of taxa. Previously reported taxa were given with the citation.

Ascomycota Whittaker

Leotiomycetes O.E. Erikss. & Winka

Helotiales Nannf.

Helotiaceae Rehm

1. ***Hymenoscyphus calyculus*** (Fr.) W. Phillips: On decaying *Populus* sp., twigs, locality 17, 05.10.2016, Şelem 276.

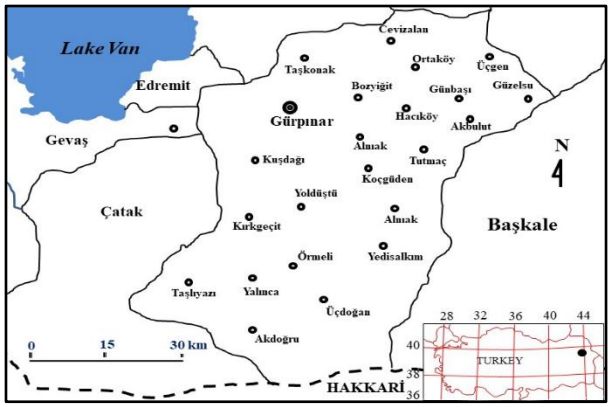

Figure 1. Map of the research area

Lachnaceae Raitv.

2. ***Lachnum bicolor*** (Bull.) P. Karst.: On dead *Salix* sp. branches, locality 17, 05.10.2016, Şelem 263.

Pezizomycetes O.E.Erikss. & Winka

Pezizales J.Schröt.

Helvellaceae **Fr.**

3. ***Helvella acetabulum*** (L.) Quél: (Şelem et al., 2019).

4. ***Helvella crispa*** (Scop.) Fr.: On soil under *Populus* sp., locality 17, 05.10.2016, Şelem 276.

5. ***Helvella lacunosa*** Afzel.: (Şelem et al., 2019).

6. ***Helvella leucopus*** Pers.: (Şelem et al., 2019).

7. ***Paxina queletii*** (Bres.) Stangl: (Şelem et al., 2019).

Table 1. Collection localities of the macrofungi samples

Loc. No	Locality	Coordinates	Altitude (m)
1	Akbulut village	38°18′06″N; 43°41′02″E	2186
2	Albenek village	38°36′01″N; 43°17′01″E	2022
3	Alnıak village	38°13′12″N; 43°42′48″E	2502
4	Bozyiğit Village	38°22′57″N; 43°34′08″E	1845
5	Cevizalan village	38°24′19″N; 43°47′56″E	2172
6	Çörekli village	38°21′53″N; 43°47′54″E	2001
7	Cevizalan village	38°22′16″N; 43°47′34″E	2025
8	Erkaldı quarter	38°21′26″N; 43°33′04″E	1809
9	Giyimli village	38°12′16″N; 43°47′05″E	2322
10	Günbaşı village	38°18′46″N; 43°43′45″E	2168
11	Gürpınar (entrance)	38°19′27″N; 43°24′06″E	1745
12	Gürpınar centre	38°19′33″N; 43°24′24″E	1751
13	Gürpınar-Hakkari highway 14th km	38°21′59″N; 43°34′02″E	2010
14	Güzelsu village	38°18′58″N; 43°48′04″E	1982
15	Güzelsu village	38°18′52″N; 43°48′25″E	1980
16	Güzelsu village	38°19′11″N; 43°48′07″E	1996
17	Güzelsu village	38°19′00″N; 43°47′58″E	1977
18	Güzelsu village	38°19′02″N; 43°48′12″E	1986
19	Güzelsu village	38°18′57″N; 43°48′00″E	1972
20	Hacıköy village	38°18′22″N; 43°38′23″E	2205
21	Hamurkesen village	38°20′38″N; 43°37′29″E	1948
22	Işıkpınar village	38°19′16″N; 43°37′01″E	2134
23	Kırgeçit village	38°08′17″N; 43°29′43″E	2105
24	Kırgeçit village	38°08′35″N; 43°31′27″E	2147
25	Kırgeçit village	38°08′08″N; 43°31′17″E	2165
26	Koçgüden village	38°09′20″N; 43°38′33″E	2570
27	Kuşdağı village	38°14′35″N; 43°27′29″E	1905
28	Murataldı village	38°15′17″N; 43°51′08″E	2136
29	Ortaköy village	38°22′02″N; 43°38′04″E	1910
30	Ortaköy village	38°21′50″N; 43°37′55″E	1868
31	Örmeli village	38°07′05″N; 43°30′52″E	2231
32	Sapakonak village	38°12′46″N; 43°36′35″E	2537
33	Sevindik village	38°18′11″N; 43°52′51″E	2098
34	Sevindik village	38°18′22″N; 43°52′27″E	2038
35	Taşdöndüren village	38°16′01″N; 43°48′53″E	2031
36	Tepegören village	38°21′35″N; 43°53′13″E	2107
37	Tutmaç village	38°15′08″N; 43°42′34″E	2401
38	Üçgen village	38°22′31″N; 43°44′46″E	2141

39	Üçgen village	38°23′05″N; 43°44′34″E	2135
40	Yedisalkım village	38°11′08″N; 43°42′11″E	2441
41	Yoldüştü village	38°09′15″N; 43°33′12″E	2187
42	Yurtbaşı village	38°14′08″N; 43°47′59″E	2108
43	Zernek village	38°21′26″N; 43°39′25″E	1934
44	Zernek village	38°21′24″N; 43°39′26″E	1900

Morchellaceae Rchb.

8. ***Mitrophora semilibera*** (DC.) Lév.: (Şelem et al., 2019).

9. ***Morchella elata*** Fr.: (Şelem et al., 2019).

10. ***Morchella esculenta*** (L.) Pers.: (Şelem et al., 2019).

11. ***Morchella esculentoides*** M. Kuo, Dewsbury, Moncalvo & S.L. Stephenson: (Şelem et al., 2019).

12. ***Morchella prava*** Dewsbury, Moncalvo, J.D. Moore & M. Kuo**.:** (Şelem et al., 2019).

13. ***Verpa conica*** (O.F. Müll.) Sw.: On soil under *Populus* and *Salix* sp., locality 13, 03.06.2016, Şelem 34.

14. ***Geopora arenicola*** (Lév.) Kers: In soil under *Populus* sp., locality 12, 05.10.2016, Şelem 101; locality 15, 05.10.2016, Şelem 310; locality 23, 03.06.2016, Şelem 254.

15. ***Geopora arenosa*** (Fuckel) S. Ahmad: On soil under *Populus* sp., locality 16, 18.05.2015, Şelem 23.

16. ***Geopora sepulta*** (Fr.) Korf & Burds.: On soil under *Populus* sp., locality 29, 18.05.2015, Şelem 74.

17. ***Pulvinula convexella*** (P. Karst.) Pfister.: On burned ground, locality 15, 05.10.2016, Şelem 289.

18. ***Scutellinia scutellata*** (L.) Lambotte: On damp soil among leaf litter, locality 15, 05.10.2016, Şelem 290.

19. ***Tricharina praecox*** (P. Karst.) Dennis: On damp soil, locality 17, 05.10.2016, Şelem 273.

20. ***Trichophaea pseudogregaria*** (Rick) Boud.: (Keleş and Şelem, 2017).

Pezizaceae Dumort.

21. ***Peziza succosa*** Berk.: On burned ground, locality 18, 18.05.2015, Şelem 30.

Basidiomycota R.T.Moore

Agaricomycetes Doweld

Agaricales Underw.

Agaricaceae Chevall.

22. ***Agaricus bisporus*** (J.E. Lange) Imbach: (Şelem et al., 2019).

23. ***Agaricus campestris*** L.: (Şelem et al., 2019).

24. ***Agaricus urinascens*** (Jul. Schäff. & F.H. Møller) Singer: (Şelem et al., 2019).

25. ***Bovista plumbea*** Pers.: (Şelem et al., 2019).

26. ***Coprinus comatus*** (O.F. Müll.) Pers.: (Şelem et al., 2019).

27. ***Cyathus olla*** (Batsch) Pers.: On soil near woody debris, locality 17, 18.05.2015, Şelem 59.

28. ***Lepiota cristata*** (Bolton) P. Kumm: On soil among leaf litter, locality 8, 05.06.2015, Şelem 171.

29. ***Lepiota subincarnata*** J.E. Lange: On soil under *Salix* sp., locality 15, 05.10.2016, Şelem 299.

Bolbitiaceae Singer

30. ***Conocybe aporos*** Kits van Wav.: On soil under *Salix* sp., locality 17, 05.06.2015, Şelem 137.

31. ***Conocybe fuscimarginata*** (Murrill) Singer: On soil under *Salix* sp., locality 14, 18.05.2015, Şelem 43; locality 17, 05.06.2015, Şelem 145.

32. ***Conocybe pygmaeoaffinis*** (Fr.) Kühner: On soil under *Salix* sp., locality 17, 05.06.2015, Şelem 146.

33. ***Conocybe rickeniana*** P.D. Orton: On soil among grass, locality 17, 05.06.2015, Şelem 149.

34. ***Conocybe tenera*** (Schaeff.) Fayod: On soil among grass, locality 17,18.05.2015, Şelem 43.

35. ***Conocybe vestita*** (Fr.) Kühner: On soil among grass, locality 11, 18.05.2015, Şelem 27.

Cortinariaceae R. Heim ex Pouzar

36. ***Cortinarius decipiens*** (Pers.) Fr.: On soil under *Salix* sp., locality 8, 05.06.2015, Şelem 175.

37. ***Cortinarius vernus*** H. Lindstr. & Melot: On soil under *Populus* sp., locality 15, 05.10.2016, Şelem 297.

Cyphellaceae Lotsy

38. ***Chondrostereum purpureum*** (Pers.) Pouzar: On *Populus* sp. stump, locality 11, 18.11.2015, Şelem 297; locality 8, 05.06.2015, Şelem 167.

Entolomataceae Kotl. & Pouzar

39. ***Entoloma caccabus*** (Kühner) Noordel.: On soil under *Salix* sp., locality 11, 19.05.2016, Şelem 343.

40. ***Entoloma rusticoides*** (Gillet) Noordel: On soil under *Malus* sp., locality 11, 18.05.2015, Şelem 108.

Hymenogastraceae Vittad.

41. ***Hebeloma mesophaeum*** (Pers.) Quél.: On soil under *Salix* sp., locality 17, 05.06.2015, Şelem 139.

42. ***Hebeloma pusillum*** J.E. Lange: On soil under *Salix* sp., locality 17, 18.05.2015, Şelem 53; on soil under *Populus* sp., locality 17, 05.10.2016, Şelem 256.

43. ***Hypholoma fasciculare*** (Huds.) P. Kumm.: On *Populus* sp. stump, locality 17, 05.06.2015, Şelem 165.

44. ***Psilocybe coronilla*** (Bull.) Noordel.: (Şelem et al., 2019).

Incertae sedis

45. ***Panaeolus fimicola*** (Pers.) Gillet: On decaying cow dung, locality 4, 18.11.2015, Şelem 201.

Inocybaceae Jülich

46. ***Crepidotus vulgaris*** Hesler & A.H. Sm.: On decaying *Salix* sp. stump, locality 13, 03.06.2016, Şelem 222.

47. ***Inosperma maculatum*** (Boud.) Matheny & Esteve-Rav.: On soil under *Salix* sp., locality 28, 08.11.2015, Şelem 280.

48. ***Inocybe cincinnata*** (Fr.) Quél.: On soil under *Populus* sp., locality 30, 18.06.2016, Şelem 79.

49. ***Inocybe dulcamara*** (Pers.) P. Kumm.: On soil under *Populus* sp., locality 13, 18.05.2015, Şelem 81; on soil under *Salix* sp., locality 14, 05.06.2015, Şelem 119; locality 17, 18.11.2015, Şelem 235.

50. ***Inocybe flocculosa*** Sacc.: On soil under *Populus* sp., locality 14, 18.05.2015, Şelem 37.

51. ***Inocybe fuscomarginata*** Kühner: On soil under *Salix* sp., locality 17, 05.06.2015, Şelem 145.

52. ***Inocybe perbrevis*** (Weinm.) Gillet: On soil under *Salix* sp., locality 17, 05.10.2016, Şelem 121; on soil under *Populus* sp., locality 16, 05.06.2015, Şelem 278.

53. ***Pseudosperma rimosum*** (Bull.) Matheny & Esteve-Rav.: On soil under *Populus* sp., locality 8, 05.06.2015, Şelem 169; on soil under *Salix* sp., locality 4, 18.11.2015, Şelem 197.

Pleurotaceae Kühner

54. ***Pleurotus eryngii*** (DC.) Quél.: (Şelem et al., 2019).

55. ***Pleurotus ostreatus*** (Jacq.) P. Kumm.: (Şelem et al., 2019).

56. ***Pleurotus populinus*** O. Hilber & O.K. Mill.: (Şelem et al., 2019).

Pluteaceae Kotl. & Pouzar

57. ***Pluteus aurantiorugosus*** (Trog) Sacc.: (Şelem et al., 2019).

58. ***Pluteus romellii*** (Britzelm.) Sacc.: (Şelem et al., 2019).

59. ***Volvopluteus gloiocephalus*** (DC.) Vizzini, Contu & Justo: (Şelem et al., 2019).

Psathyrellaceae Vilgalys, Moncalvo & Redhead

60. ***Coprinellus disseminatus*** (Pers.) J.E. Lange: (Şelem et al., 2019).

61. ***Coprinellus domesticus*** (Bolton) Vilgalys, Hopple & Jacq. Johnson: On soil around *Populus* sp. stump, locality 44, 18.05.2015, Şelem 68.

62. ***Coprinellus micaceus*** (Bull.) Vilgalys, Hopple & Jacq. Johnson: (Şelem et al., 2019).

63. ***Coprinopsis acuminata*** (Romagn.) Redhead, Vilgalys & Moncalvo: On soil aronud *Populus* sp. stump, locality 12, 18.11.2015, Şelem 213.

64. ***Coprinopsis atramentaria*** (Bull.) Redhead, Vilgalys & Moncalvo: (Şelem et al., 2019).

65. ***Parasola auricoma*** (Pat.) Redhead, Vilgalys & Hopple: On soil among grass, locality 17, 05.06.2015, Şelem 164.

66. ***Parasola hemerobia*** (Fr.) Redhead, Vilgalys & Hopple: On soil under *Salix* sp., locality 17, 05.06.2015, Şelem 152.

67. ***Parasola kuehneri*** (Uljé & Bas) Redhead, Vilgalys & Hopple: On soil under *Salix* sp., locality 17, 05.06.2015, Şelem 138.

68. ***Parasola lactea*** (A.H. Sm.) Redhead, Vilgalys & Hopple: On soil under *Salix* sp., locality 17, 05.06.2015, Şelem 142.

69. ***Parasola plicatilis*** (Curtis) Redhead, Vilgalys & Hopple: On soil among grass, locality 15, 05.10.2016, Şelem 294.

70. ***Psathyrella candolleana*** (Fr.) Maire: (Şelem et al., 2019).

71. ***Psathyrella fatua*** (Fr.) P. Kumm. *Populus* sp. ağaçları altı, 17, 18.05.2015, Şelem 56.

72. ***Psathyrella panaeoloides*** (Maire) Arnolds: On soil under *Salix* sp., locality 17, 05.06.2015, Şelem 162; locality 15, 05.10.2016, Şelem 302.

73. ***Psathyrella potteri*** A.H. Sm.: On soil under *Populus* and Salix sp., locality 13, 18.05.2015, Şelem 103.

74. ***Psathyrella prona*** (Fr.) Gillet: On soil under *Populus* and Salix sp., locality 13, 18.05.2015, Şelem 204; locality 17, 05.10.2015, Şelem 263; locality 15, 05.10.2016, Şelem 301.

75. ***Psathyrella pseudogracilis*** (Romagn.) M.M. Moser: On decaying stump, locality 17, 18.05.2015. Şelem 58.

Strophariaceae Singer & A.H. Sm.

76. ***Agrocybe dura*** (Bolton) Singer: (Şelem et al., 2019).

77. ***Agrocybe paludosa*** (J.E. Lange) Kühner & Romagn. ex Bon: On soil among grass, locality 17, 05.06.2015. Şelem 148.

78. ***Agrocybe pediades*** (Fr.) Fayod: (Şelem et al., 2019).

79. ***Agrocybe praecox*** (Pers.) Fayod: (Şelem et al., 2019).

80. ***Cyclocybe cylindracea*** (DC.) Vizzini & Angelini: (Şelem et al., 2019). On *Populus* sp. stump, locality 13, 05.10.2016, Şelem 268.

81. ***Pholiota aurivella*** (Batsch) P. Kumm.: (Şelem et al., 2019).

Tricholomataceae Lotsy

82. ***Melanoleuca angelesiana*** A.H. Sm.: On soil among needle litter under *Pinus* sp., locality 29, 05.06.2015, Şelem 179.

83. ***Melanoleuca brevipes*** (Bull.) Pat.: (Şelem et al., 2019).

84. ***Melanoleuca cognata*** (Fr.) Konrad & Maubl.: (Şelem et al., 2019).

85. ***Pseudoclitocybe cyathiformis*** (Bull.) Singer: (Şelem et al., 2019).

86. ***Lepista personata*** (Fr.) Cooke: (Şelem et al., 2019).

Tubariaceae Vizzini

87. ***Tubaria conspersa*** (Pers.) Fayod: On damp soil among leaf litter under *Salix* sp., 17, 05.06.2015, Şelem 122.

88. ***Tubaria furfuracea*** (Pers.) Gillet: On woody debris, locality 30, 18.11.2015, Şelem 195

Boletales E.-J. Girbert

Suillaceae Besl & Bresinsky

89. ***Suillus collinitus*** (Fr.) Kuntze: (Şelem et al., 2019).

Hymenochaetales Oberw.

Hymenochaetaceae Donk

90. ***Phellinus igniarius*** (L.) Quél.: On *Malus* sp. stump, locality 29, 18.11.2015, Şelem 189.

Polyporales Gäum.

Polyporaceae Fr. ex Corda

91. ***Fomes fomentarius*** (L.) Fr.: On *Populus* sp. stump, locality 20, 28.06.2017, Şelem 41.

92. ***Cerioporus squamosus*** (Huds.) Quél.: (Şelem et al., 2019).

93. ***Trametes trogii*** Berk.: On *Populus* sp. stump, locality 17, 05.06.2015, Şelem 118; locality 18, 19.05.2016, Şelem 332; locality 26, 13.06.2017, Şelem 375; locality 5, 13.11.2016, Şelem 381.

94. ***Trametes versicolor*** (L.) Lloyd: On *Populus* sp. stump, locality 19, 19.05.2016, Şelem 362.

4. Discussions

Ninety four macrofungi species belonging to 49 genera, 27 families, seven orders and three classes were determined from Gürpınar district. Twenty one of the determined taxa belong to *Ascomycota* (*Leotiomycetes* 2, *Pezizomycetes* 19) while 73 belong to *Basidiomycota* (*Agaricomycetes* 73). Except previously reported 36 edible species (Şelem et al., 2019), all the taxa are new for the region.

The taxa are distributed in 7 orders (Fig. 2) and 27 families. *Psathyrellaceae* and *Inocybaceae* were found to the most crowded first two families with 16 and 7 taxa respectively. *Agaricaceae*, *Bolbitiaceae*, *Incertae sedis*, *Morchellaceae* and *Strophariaceae*, are the third crowded families each with 6 taxa. Then *Helvellaceae* and *Pyronemataceae* come each with 5 taxa. Two of the families (*Hymenogasteraceae*, *Polyporaceae*) comprise 4, Three of them (*Pleurotaceae*, *Pluteaceae*, *Tubariaceae*) comprises 3, three of them (*Cortinariaceae*, *Entolomataceae*, *Pulvinulaceae*) comprises 2 taxa, and the rest of the 10 families comprises 1 taxon.

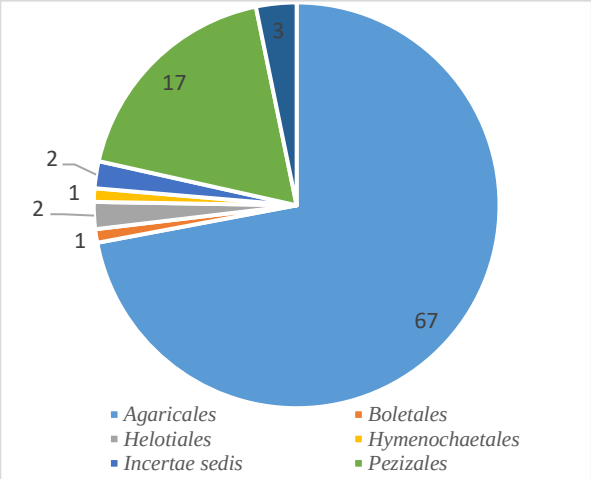

Figure 2. Ditribution of the determined taxa within orders.

The determined taxa are distributed in 49 genera. The most crowded genera are *Conocybe* and *Psathyrella* each with 6 taxa. *Inocybe* and *Parasola* were found to be the second crowded two genera each with 5 taxa. Three of of the genera (*Agrocybe*, *Helvella*, *Morchella*) comprise 4 taxa, five of them (*Agaricus*, *Coprinellus*, *Geopora*, *Melanoleuca*, *Pleurotus*) comprise 3 taxa, eight of them (*Coprinopsis*, *Cortinarius*, *Entoloma*, *Hebeloma*, *Lepiota*, *Pluteus*, *Trametes*, *Tubaria*) comprise 2 taxa, and the rest of the 29 taxa comprise one taxon.

Coprinus comatus, *Coprinellus disseminatus*, *C. micaceus*, *Coprinopsis atramentaria*, *Psathyrella candolleana*, *Pleurotus ostreatus*, *P. eryngii*, *Inocybe dulcamara* and *Pholiota aurivella* were found to be the most widespread species in the region.

Thirty six (38.30%) of the determined taxa are edible. Among them *Pleurotus eryngii* is collected and consumed in all villages of the district. This mushroom is known with the Turkish name “heliz mantarı” and also has regional economic importance. *Agaricus bisporus*, *A. campestris*, *A. urinascens* and *P. ostreatus* are regionally known in research area and they are also collected and consumed by some locals. Forty four (46.81%) of them are inedible and 14 (14.89%) of them are more or less poisonous.

The taxa determined in Gürpınar district were compared with the findings of the studies carried out in neighbouring regions and some similarities were observed. These studies and the similarity percentages are given in Table 2.

Table 2. Similarity percentages of neighbouring studies with Gürpınar district.

Neighbouring study	# of identical taxa	Total taxa	Similarity (%)
Bitlis (Kaya, 2001)	17	60	28.33
Ağrı (Demirel et al., 2002)	20	45	44.44
Erzurum (Demirel et al., 2003)	17	114	14.91
Malazgirt (Akçay et al., 2010)	16	50	32.00
Van (Demirel et al., 2015)	40	122	32.79
Bingöl (Uzun et al., 2017)	16	112	14.29
Şemdinli and Yüksekova (Acar et al., 2020)	40	197	20.30
Muradiye (Çağli and Öztürk, 2020)	38	86	44.19
Karz Dağı (Sadullahoğlu and Uzun, 2020)	25	95	26.32

Conflict of Interest

Authors have declared no conflict of interest.

Authors’ Contributions

The authors contributed equally.

Acknowledgments

The authors would like to thank Van Yüzüncü Yıl University Research Project Unit (Project No: FYL-2016-5213) for its financial support.

References

Acar İ, Uzun Y, Akata I (2020). Some macrofungi determined in Şemdinli and Yüksekova districts (Hakkari-Turkey). Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi 23(1): 157-167.

Adanacıoğlu N, Yıldız Ü, Erdinç O, Aykas L, Tan A, Taylan T (2016). Türkiye makromantarı genetik kaynakları I. Ege Bölgesi. Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi 26(1): 46-61.

Akçay ME, Uzun Y, Kaya A (2010). Malazgirt (Muş) yöresi makrofunguslarına katkılar. Mantar Dergisi 1(1): 14-20.

Alkan S, Kaşık G, Aktaş S 2010. Macrofungi of Derebucak district (Konya, Turkey). Turkish Journal of Botany 34(4): 335-350.

Bani B, Adıgüzel N (2008). Flora of an important plant area: Çatak valley I-(Çatak-Pervari) Van Turkey. Flora Mediterranea 18: 11-63.

Breitenbach J, Kränzlin F (1984). Fungi of Switzerland, Vol.1. Lucerne: Verlag Mykologia.

Breitenbach J, Kränzlin F (1986). Fungi of Switzerland, Vol.2. Lucerne: Verlag Mykologia.

Breitenbach J, Kränzlin F (1991). Fungi of Switzerland, Vol.3. Lucerne: Verlag Mykologia.

Breitenbach J, Kränzlin F (1995). Fungi of Switzerland, Vol.4. Lucerne: Verlag Mykologia.

Breitenbach J, Kränzlin F (2000). Fungi of Switzerland, Vol.5. Lucerne: Verlag Mykologia.

Bresnsky A, Besl H (1990). A color atlas of poisonous fungi. London: Wolfe Publishing.

Buczacki S (1989). Mushrooms and toadstools of Britain and Europe. Glasgow: Harper Collins Publishers.

Çağli G, Öztürk A (2020). Macromycetes determined in Muradiye (Van) district. Anatolian Journal of Botany 4(1): 57-64.

Dähncke MR (2004). 1200 Pilze in Farbfotos. Sweden: Verlag.

Demirel K, Erdem Ö, Uzun Y, Kaya A (2010). Macrofungi of Hatila Valley National Park (Artvin-Turkey). Turkish Journal of Botany 34(5): 457-465.

Demirel K, Kaya A, Uzun Y (2003). Macrofungi of Erzurum province. Turkish Journal of Botany 27(1): 29-36.

Demirel K, Uzun Y, Akçay ME, Keleş A, Acar İ, Efe V (2015). Van yöresi makromantarlarına katkılar. Mantar Dergisi 6(2): 12-23.

Demirel K, Uzun Y, Kaya A (2002). Macrofungi of Ağrı Province. Turkish Journal of Botany 26: 291-295.

Doğan HH, Aktaş S, Öztürk C, Kaşık G (2012). Macrofungi distribution of Cocakdere valley (Arslanköy, Mersin). Turkish Journal of Botany 36(1): 83-94.

Hausknecht A (2009). Fungi Europaei. A monograph of the genera Conocybe Fayod, Pholiotina Fayod in Europe. Italia: Massimo condusso.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995). Ainsworth and Biby's dictionary of the fungi (8th Ed.). Wallingford: CAB International.

Index Fungorum. (2020). http://www.indexfungorum.org/names/Names.asp / [20 December 2020].

Jordan M (1995). The encyclopedia of fungi on Britain and Europe. Devon: David & Charles Book.

Kaya A (2001). Contributions to the macrofungi flora of Bitlis province. Turkish Journal of Botany 25: 379-383.

Kaya A, Uzun Y (2018). New contributions to the Turkish Ascomycota. Turkish Journal of Botany 42: 644-652.

Keleş A, Şelem E (2017). Türkiye mikobiyotası için yeni bir kayıt (*Trichophaea pseudogregaria* (Rick) Boud.). Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi 22(2): 142-145.

Kuo M, Methven SA (2014). Mushrooms of the Midwest. Urbana: University of Illinois Press.

Lukić M (2008). The distribution and diversity of *Boletus* genus in central Serbia. Kragujevac Journal of Science 31: 59-68.

Moser M (1983). Keys to agarics and boleti (*Polyporales, Boletales, Agaricales, Russulales*). Stuttgart: Gustav Fischer Verlag.

Pegler DN, Læssøe T, Spooner BM (1995). British Puffballs, Earthstars, and Stinkhorns. Kew: Royal Botanic Gardens.

Phillips R (1981). Mushrooms and other fungi of Great Britain, Europe. London: Pan Books.

Sadullahoğlu C, Uzun Y (2020). Karz Dağı (Tatvan-Bitlis) ve çevresinde belirlenen makrofunguslar. Mantar Dergisi 11(1): 1-11.

Sesli E, Denchev CM (2014). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. 6th edn. *Mycotaxon* Checklists Online (http,//www.mycotaxon.com/resources/checklists/sesli-v106-checklist.pdf): 1-136.

Süfer Ö, Bozok F, Demir HG (2016). Usage of edible mushrooms in various food products. Turkish Journal of Agriculture-Food Science and Technology 4(3): 144-149.

Şelem E, Keleş A, Acar İ, Demirel K (2019). Edible macrofungi determined in Gürpınar (Van) district. Anatolian Journal of Botany 3(1): 7-12.

Türkekul İ, Işık H (2016). Bozatalan (Tokat) Yöresi Makrofungusları. Kafkas Üniversitesi Fen Bilimleri Enstitüsü Dergisi 9(1): 5-11.

Uzun Y (2010). Macrofungal diversity of Ardahan and Iğdır province. International Journal of Botany 6(1): 11-20.

Uzun Y, Acar İ, Akçay ME, Kaya A (2017). Contributions to the macrofungi of Bingöl, Turkey. Turkish Journal of Botany 41(5): 516-534.

Yıldız MS, Türkekul İ, Işık H (2019). Macrofungal Biodiversity of Pazar (Tokat) District. Bitlis Eren University Journal of Science 8(2): 387-395.

Anatolian Journal of Botany
5(1): 29-31 (2021)
Researh article *doi:10.30616/ajb.857752*

Elaphomyces anthracinus, a new hypogeous ascomycete record for Turkish mycota

Yasin UZUN
Karamanoğlu Mehmetbey University, Ermenek Uysal & Hasan Kalan Health Services Vocational School, Department of Pharmacy Services, Karaman, Turkey
yuclathrus@gmail.com

Received : 10.01.2021
Accepted : 28.01.2021
Online : 01.02.2021

Elaphomyces anthracinus, Türkiye mikotası için yeni bir toprakaltı askomiset kaydı

Abstract: *Elaphomyces anthracinus* Vittad. is given as new record for the mycobiota of Turkey. The macro and micromorphological characters of the species are provided together with the collection locality, voucher number and the photographs related to its macro and micromorphologies.

Key words: Ascomycota, mycodiversity, hypogeous macrofungi, new record

Özet: *Elaphomyces anthracinus* Vittad. Türkiye için yeni kayıt olarak verilmiştir. Türün makro ve mikromorfolojik karakterleri, toplanma lokalitesi, toplayıcı numarası ve makro ve mikromorfolojilerine ait fotoğrafları ile birlikte verilmiştir.

Anahtar Kelimeler: Askomikota, mikoçeşitlilik, toprakaltı makromantarlar, yeni kayıt

Citation: Uzun Y (2021). *Elaphomyces anthracinus*, a new hypogeous ascomycete record for Turkish mycota. Anatolian Journal of Botany 5(1): 29-31.

1. Introduction

Elaphomyces T. Nees is a widespread genus within the family *Elaphomycetaceae* (*Ascomycota*). Members of the genus generally bear the following characteristics: subglobose to globose spores, globose to subglobose 1-8 spored asci, a more or less powdery spore mass, fleshy to leathery peridium, a single chambered gleba, and globose to subglobose or irregular hypogeous ascomata (Castellano et al., 2016, 2018). They generally form ectomycorrhizal association, and widespread in temperate and subtropical forests.

Index Fungorum (2020) lists 73 conformed *Elaphomyces* taxa, six of which are also known to exist in Turkey. *Elaphomyces leucocarpus* Vittad. and *Elaphomyces muricatus* Fr. were the first two species of the genus to be reported in Turkey (Türkoğlu et al., 2015). Later on, four members of the genus (*E. citrinus* Vittad.; *E. cyanosporus* Tul. & C. Tul.; *E. granulatus* Fr. and *E. septatus* Vittad.) have also been presented from Turkey (Uzun and Kaya, 2019a,b; Uzun and Kaya, 2020).

In this paper *Elaphomyces anthracinus* is presented as the seventh member of the genus in Turkey, based on the collection from Tonya district of Trabzon province.

The current checklists on Turkish macromycota (Sesli and Denchev, 2014; Solak et al., 2015) and the last decade contributions to the hypogeous ascomycota of Turkey (Castellano and Türkoğlu, 2012; Türkoğlu and Castellano, 2014; Elliot et al., 2016; Alkan et al., 2018; Doğan et al., 2018; Kaygusuz et al., 2018; Uzun and Kaya, 2018; Uzun and Kaya, 2019c,d; Allı and Doğan, 2019; Berber et al., 2019; Yakar et al., 2019; Akata et al., 2020), indicate that this taxon has not been recorded from Turkey before.

The study aims to make a contribution to the macrofungal biodiversity of the Trabzon and Turkey.

2. Materials and Method

Fruit bodies of *E. anthracinus* were collected from Tonya district of Trabzon province. They were photographed at natural habitats, and notes were taken about the morphological and ecological characteristics. Then the specimen was carried to the fungarium, and dried in an air conditioned room. Microscopic studies were performed on dried specimen under a Nikon Eclipse Ci-S trinocular light microscope. The specimens were mounted in water and Melzer reagent. The samples were identified with the help of Hawker (1954), Moreno et al. (1991), Pegler et al. (1993), Arroyo et al. (2005), Montecchi and Sarasini (2000), Paz et al. (2017). The collected specimen are kept at Karamanoğlu Mehmetbey University, Science Faculty, Department of Biology, Karaman, Turkey.

3. Results

Ascomycota Caval.-Sm.

Eurotiomycetes O.E. Erikss. & Winka

Eurotiales G.W. Martin ex Benny & Kimbr.

Elaphomycetaceae Tul. ex Paol.

Elaphomyces anthracinus Vittad., Monogr. Tuberac. (Milano): 66 (1831).

Syn: [*Elaphomyces anthracinus* f. *talosporus* A. Paz & Lavoise; *Lycoperdastrum anthracinum* (Vittad.) Kuntze**]**

Macroscopic and microscopic features: Ascoma hypogeous, 19.5 mm in diameter, subglobose to globose, with carbonaceous exterior, umbilicate, lacking a true sterile base, minutely verrucose, or somewhat covered with binding soil particles, dark brown to black. Peridium 2-3 mm thick and composed of two layers. Thinner cortex carbonaceous, black, hard and slightly grainy. Inner peridium thick, whitish to pale grey or light brownish,

composed of subhyaline, filamentous hyphae. Gleba whitish or greyish at first, cottony, at maturity filled with a powdery mass of spores (Fig. 1). Glebal hyphae narrow and branched. Asci 30-50 µm diam., subglobose, thin-walled, usually with randomly 8-spored, evanescent. Ascospores 13-19 µm in diameter, globose, hyaline at first, pale to dark brown-blackish, nearly black when mature, and almost opaque at maturity, ornamented with numerous shallow alveoli (Fig. 2).

Elaphomyces anthracinus grows in soil and decaying leaves of many trees in deciduous or coniferous forest, commonly under *Fagus* L. and *Quercus* L. species (Moreno et al., 1991; Pegler et al., 1993; Montecchi and Sarasini, 2000; Arroyo et al., 2005).

Specimen examined: Trabzon, Tonya, İskenderli village, in soil and decaying leaves under *Fagus orientalis* Lipsky, *Rhododendron ponticum* L. and *Quercus* sp. 40°55′N-39°14′E, 760 m, 12.11.2016, Yuzun 5463.

4. Discussions

Elaphomyces anthracinus was given as new record for Turkish mycobiota as the seventh member of the genus *Elaphomyces*. In general, macro and micromorphology are in agreement with those given in literature.

Figure 1. Ascocarp of *Elaphomyces anthracinus*

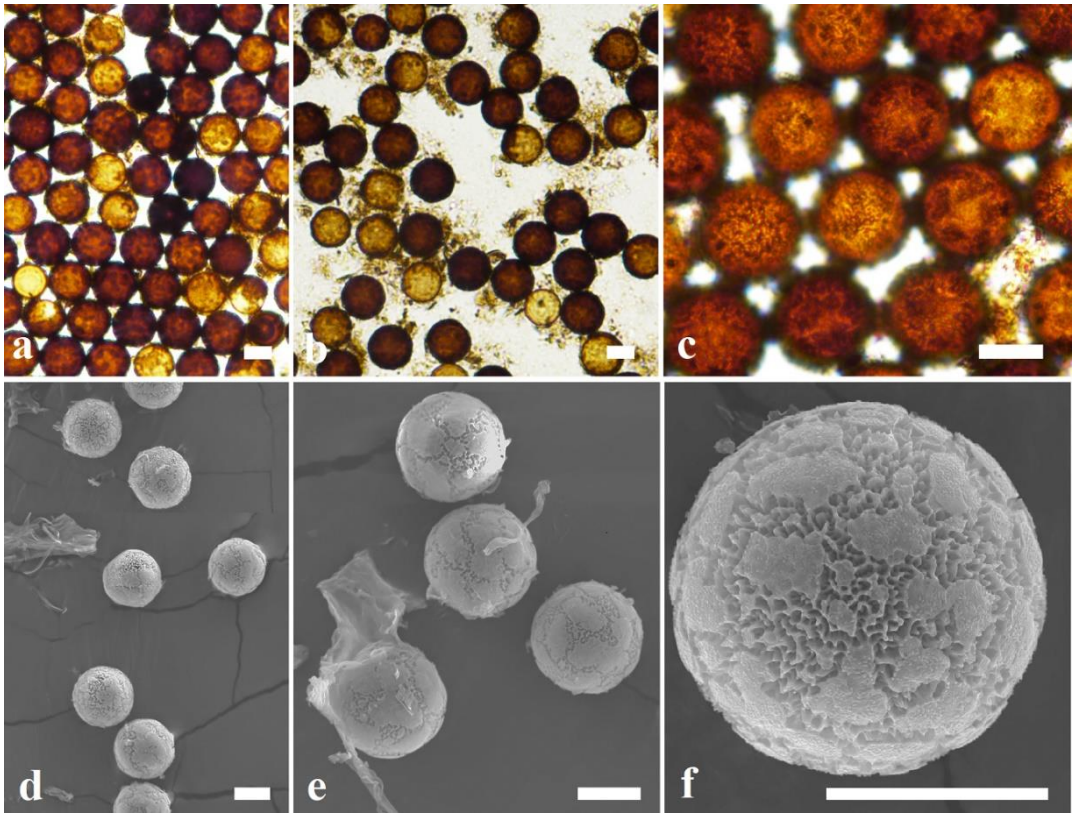

Figure 2. Light microscope (a-c) and SEM (d-f) images of the ascospores of *Elaphomyces anthracinus* (bars: 10 µm)

Since the fruit bodies of *E. anthracinus* recall the traditional charcoal balls, macroscopical identification does not offer any difficulty. The very small and blackish spores also distinguish *E. anthracinus* from the other *Elaphomyces* species with blackish and almost smooth surface.

Regarding the previous Turkish collections of *Elaphomyces*, five of them, *E. cyanosporus, E. granulatus, E. leucocarpus, E. muricatus* and *E. septatus*, have notably larger spores (with a minimum size of 19 µm), and can easily be distinguished from *E. anthracinus*. On the other hand, the sixth one, *E. citrinus*, has much more smaller spores (10.5-12.5 µm) compared to *E. anthracinus*.

Acknowledgments

The author would like to thank Karamanoğlu Mehmetbey University Research Fund (02-M-15) for its financial support.

References

Akata I, Sevindik M, Şahin E (2020). *Tuber fulgens* Quél. A new record for Turkish truffles. Turkish Journal of Agriculture- Food Science and Technology 8(11): 2472-2475.

Alkan S, Aktaş S, Kaşık G (2018). Türkiye'deki *Tuber* türleri ve *Tuber aestivum* için yeni bir lokalite. Selçuk Üniversitesi Fen Fakültesi Fen Dergisi 44(1): 25-29.

Allı H, Doğan HH (2019). A new genus (*Balsamia*) addition for Turkish mycota. Mantar Dergisi 10(1): 23-25.

Arroyo BM, Fernández JG, Calmaestra EP (2005). Tesoros de nuestro montes. Trufas de Andalucía. Consejería de Medio Ambiente, Junta de Andalucía, Córdoba.

Berber O, Uzun Y, Kaya A (2019). *Genea lobulata*, A new hypogeous ascomycete record for Turkish Mycobiota. Süleyman Demirel University Journal of Natural and Applied Sciences 23(3): 922-924.

Castellano MA, Dentinger BTM, Séné O, Elliott TF, Truong C, Henkel TW (2016). New species of *Elaphomyces* (*Elaphomycetaceae, Eurotiales, Ascomycota*) from tropical rainforests of Cameroon and Guyana. IMA Fungus 7(1): 59-73.

Castellano MA, Elliott TF, Trappe JM (2018). Three new black *Elaphomyces* species (*Elaphomycetaceae, Eurotiales, Ascomycota*) from eastern North America with notes on selected European species. Fungal Systematics and Evolution 1: 1-12.

Castellano MA, Türkoğlu A (2012). New records of truffle taxa in *Tuber* and *Terfezia* from Turkey. Turkish Journal of Botany 36(3): 295-298.

Doğan HH, Bozok F, Taşkın H (2018). A new species of *Barssia* (*Ascomycota, Helvellaceae*) from Turkey. Turkish Journal of Botany 42(5): 636-643.

Elliot TF, Türkoğlu A, Trappe JM, Yaratanakul Güngör M (2016). Turkish truffles 2: eight new records from Anatolia. Mycotaxon 131(2): 439-453.

Hawker LE (1954). British hypogeous fungi. Philosophical Transactions of the Royal Society B 237: 429-546.

Index Fungorum (2020). http://www.indexfungorum.org/Names/Names.asp / [accessed 15 December 2020].

Kaygusuz O, Çolak ÖF, Matočec N, Kušan I (2018). New data on Turkish hypogeous fungi. Natura Croatica 27(2): 257-269.

Montecchi A, Sarasini M (2000). Fungi Ipogei D'Europa. Vicenza: Fondazione Centro Studi Micologici dell'AMB.

Moreno G, Galan R, Montecchi A (1991). Hypogeous fungi from peninsular Spain II. Mycotaxon 42: 231-238.

Paz A, Bellanger JM, Lavoise C, Molia A, Ławrynowicz M, Larsson E, Ibarguren IO, Jeppson M, Læssøe T, Sauve M, Richard F, Moreau PA (2017). The genus *Elaphomyces* (*Ascomycota, Eurotiales*): a ribosomal DNA-based phylogeny and revised systematics of European 'deer truffles'. Persoonia 38: 197-239.

Pegler DN, Spooner BM, Young TWK (1993). British truffles: a revision of British hypogeous fungi. Royal Botanic Gardens, Kew.

Sesli E, Denchev CM (2014). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. 6th ed. Mycotaxon Checklists Online. 136 p. (http://www.mycotaxon.com/resources/checklists/sesli-v106-checklist.pdf)

Solak MH, Işıloğlu M, Kalmış E, Allı H (2015). Macrofungi of Turkey, Checklist, Vol. 2. İzmir, Turkey: Üniversiteliler Ofset.

Türkoğlu A, Castellano MA (2014). New records of some Ascomycete truffle fungi from Turkey. Turkish Journal of Botany 38(2): 406-416.

Türkoğlu A, Castellano MA, Trappe JM, Güngör Yaratanakul M (2015). Turkish truffles I: 18 new records for Turkey. Turkish Journal of Botany 39(2): 359-376.

Uzun Y, Kaya A (2018). First record of *Hydnobolites* and *Pachyphlodes* from Turkey. Mycotaxon 133(3): 415-421.

Uzun Y, Kaya A (2019a). *Elaphomyces granulatus*, A new hypogeous ascomycete record for Turkey. KSÜ Tarım ve Doğa Dergisi 22(1): 85-88.

Uzun Y, Kaya A (2019b). A new *Elaphomyces* record for Turkey. Mantar Dergisi 10(1): 40-43.

Uzun Y, Kaya A (2019c). New additions to Turkish *Pezizales* from the eastern Black Sea region. Turkish Journal of Botany 43(2): 262-270.

Uzun Y, Kaya A (2019d). *Geopora clausa*, a new hypogeous ascomycete record for Turkey. Biological Diversity and Conservation 12(2): 193-196.

Uzun Y, Kaya A (2020). *Elaphomyces citrinus* and *E. cyanosporus*, new for Turkey. Mycotaxon 135(2): 339-344.

Yakar S, Uzun Y, Çevik FT (2019). New locality records for two hypogeous basidiomycete species in Turkey. Anatolian Journal of Botany 3(1): 28-33.

Anatolian Journal of Botany
5(1): 32-36 (2021)
Research article *doi:10.30616/ajb. 866427*

In vitro effectiveness of some plant species as green manure for the control of stem and bulb nematode (*Ditylenchus dipsaci*)

Elif YAVUZASLANOĞLU[1*], Gamze AKSAY[2], Ahmet ÇETİNKAYA[3]
[1]*Karamanoğlu Mehmetbey University, Technical Sciences Vocational School, Department of Plant and Animal Production, Karaman, Turkey*
[2,3]*Karamanoğlu Mehmetbey University, Institute of Science, Karaman, Turkey*
**eyavuzaslanoglu@kmu.edu.tr, [2]gggamze.21@gmail.com, [3]ahmet_cetinkayaaa@hotmail.com*

Received :22.01.2021
Accepted :19.02.2021
Online : 24.02.2021

Bazı bitki türlerinin yeşil gübre olarak soğan sak nematodunun (*Ditylenchus dipsaci*) mücadelesi için in vitro etkinliği

Abstract: Stem and bulb nematode, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, is a plant parasitic nematode that causes significant losses in plant production in the world. One of the promising methods for control of soil-borne diseases and pests that do not accumulate in nature is green manure application. Studies on the effectiveness of green manure on stem and bulb nematode are limited. In this study, the activity of 4 plant species from *Brassicaceae* family and one *Tagetes patula* L. variety on stem and bulb nematode was investigated in vitro. In the study, rates of motionless nematodes were recorded in the water and sand medium in 4 days and 11 days in five plant species. The efficiency of the plant species was increased over time. In all treatments in the study, the highest rate of motionless nematodes was obtained with arugula. On the 4th and 11th days in the water medium, 84.1% and 95.7% of motionless nematodes were obtained, respectively, while in the sand medium, the rate of motionless nematodes was 60.2% and 86.1%. Following the arugula, *Tagetes patula* showed activity at the rates of 72.9% and 98.3% in the water medium and 40.9% and 81.9% in the sand medium on the 4th and 11th days, respectively. Radish was also found promising with 62.5% and 94.2% in water medium, 59.2% and 80.9 in the sand medium, on the 4th and 11th days, respectively. The data obtained in the study provided preliminary data for green manure applications under field conditions.

Key words: Biofumigation, *Brassicaceae*, cultural management, nematode suppression, *Tagetes* spp.

Özet: Soğan sak nematodu, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, dünyada bitkisel üretimde önemli kayıplara neden olan bitki paraziti bir nematoddur. Doğada birikim yapmayan ve toprak kökenli hastalık ve zararlıların kontrolünde ümitvar mücadele yöntemlerinden biri yeşil gübrelemedir. Yeşil gübrelemenin soğan sak nematodu üzerine etkinliği ile ilgili çalışmalar sınırlıdır. Bu çalışmada, *Brassicaceae* familyasından 4 bitki türü ve bir *Tagetes patula* L. çeşidinin soğan sak nematodu üzerindeki etkinliği in vitro olarak araştırılmıştır. Çalışmada su ve kum ortamında 4. ve 11. günde 5 bitki türünde nematodlardaki hareketsizlik oranları kayıt edilmiştir. Bitki türlerinin etkinliği zamanla artmıştır. Çalışmada bütün uygulamalarda en yüksek hareketsiz nematod oranı roka ile elde edilmiştir. Su ortamında 4. ve 11. günlerde sırasıyla %84.1 ve %95.7 oranlarında hareketsiz nematod elde edilirken kum ortamında, hareketsiz nematod oranı %60.2 ve %86.1 olarak kayıt edilmiştir. Rokayı takiben *Tagetes patula* 4. ve 11. günlerde sırasıyla su ortamında %72.9 ve %98.3 oranlarında, kum ortamında ise %40.9 ve %81.9 oranlarında etkinlik göstermiştir. Turpta sırasıyla 4. ve 11. günlerde su ortamında %62.5 ve %94.2, kum ortamında %59.2 ve %80.9 hareketsiz nematod oranları ile ümitvar bulunmuştur. Çalışmada elde edilen veriler tarla koşullarında yeşil gübre uygulamaları için ön veri oluşturmuştur.

Anahtar Kelimeler: Biyofumigasyon, *Brassicaceae*, kültürel mücadele, nematod baskılanması, *Tagetes* spp.

Citation: Yavuzaslanoğlu E, Aksay G, Çetinkaya A (2021). In vitro effectiveness of some plant species as green manure for the control of stem and bulb nematode (*Ditylenchus dipsaci*). Anatolian Journal of Botany 5(1): 32-36.

1. Introduction

Nematoda filum of the animal kingdom include plant parasitic microscopic round worms. The plant parasitic nematode species that causes the most economic losses in the 4th place in the world is stem and bulb nematode (*Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Abd-Elgawad and Askary, 2015). Stem and bulb nematode is distributed all over the world, parasites more than 500 plant species and causes yield losses economically (Sturhan and Brzeski, 1991).

The economic damage threshold of the stem and bulb nematode is very low. It has been reported that serious damages occur on plant production when 10 nematodes are found in 500 g soil (Seinhorst, 1956). It is necessary to control the nematode by applying an integrated control strategy in order to continue plant production economically. It is very important for soil sustainability to prioritize cultural and biological control practices such as rotation practices with non-host plant species, growing resistant varieties, use of nematode suppressive plant species and green manure practices in integrated control practices.

Stem and bulb nematode lives in the intercellular parts on the above-ground organs of the plant, damaging the plant. Systemically effective chemicals which are necessary for killing the nematode in plant tissue are very toxic to humans and other organisms in the environment. Their use under field conditions is not economical. For this reason, it is recommended to use of chemicals as soil fumigation for chemical control of nematodes. However, since the synthetic chemicals used are not natural, it is possible that they cause biological accumulation in biological organisms and chronic poisoning. For this reason, plant species with biofumigant effect that naturally decompose and do not leave residues in the soil constitute an important alternative source for the control of nematodes. Effectiveness of plant species such as radish (*Raphanus sativus* L.), arugula (*Eruca sativa* Mill.), broccoli (*Brassica oleraceae* L.), canola (*Brassica napus* L.) and

mustard (*Brassica juncea* Czern.), from the *Brassicaceae* family, on nematode control have been found to be high, mostly for *Meloidogyne* spp. (Johnson et al., 1992; Mojtahedi et al., 1993; Al-Rehiayani and Hafez, 1998; Potter et al., 1998; Stapleton and Duncan, 1998; McLeod and Steel, 1999; Melakeberhan et al., 2006; Kaşkavalcı et al., 2009; Youssef and Lashein, 2013; Kruger et al., 2015).

Out of the *Brassicaceae* family, *Tagetes* spp., has become a highly emphasized plant as green manure to plant parasitic nematodes, especially for the suppressive effects on *Meloidogyne* spp. (Kimpinski et al., 2000; Evenhuis et al., 2004; Seigies and Pritts, 2006; Kaşkavalcı et al., 2009).

Among the plant parasitic nematodes, the most studied nematode species are root knot nematodes (*Meloidogyne* spp.), which are the most economically important nematodes in the world. There are limited studies on the control of stem and bulb nematode using biofumigant plant species in the world. No study was found in Turkey. With this purpose, it is aimed to determine the in vitro activities of different plant species for the usage as green manure in plant production areas where stem and bulb nematode is infested.

2. Materials and method

2.1. Plant materials

Totally 4 plant species of broccoli (*Brassica oleraceae* var. *botrytis* L. cv. Standard, Intfa Seed, Konya),cabbage (*B. oleraceae* var. *capitata* L. cv. Standard, Intfa Seed, Konya), radish (*Raphanus sativus* L. cv. Standard, Intfa Seed, Konya) and arugula (*Eruca sativa* Mill. cv. Standard, Intfa Seed, Konya) from *Brassicaceae* family and a marigold species (*Tagetes patula* L. cv. Bonanza, Harmony, Tasaco Agriculture, Antalya) were investigated for in vitro effectiveness as green manure against *D. dipsaci*.

2.2. Nematode inoculums

Pure culture of nematode was used in the study. Nematodes were originally isolated from garlic samples in Karaman Province, Central District in Central Anatolian Plateau (N: 37.10351 E: 33.117). Nematode culture was maintained on sterile carrot discs at 20 ℃ in dark (Yavuzaslanoglu and Aksay, 2021). Nematodes for the experiment were extracted from two months old sterile carrot disc cultures by washing the surface of the carrot discs with sterile tap water. The nematode suspension was then concentrated to the volume including 400 nematodes in 10 µl to use in each individual replication.

2.3. Experimental design and treatments

Sterile plastic petri dishes (60 mm diameter) were used in the study. The experiment was established according to the factorial completely randomized plot design. There were three factors in the experiment: experiment medium, incubation time and plant species. In sand medium treatments, petri dishes included 10 g sterile sand moistened with 2 ml of sterile water. However, in water medium treatments, the petri dishes included 4 ml sterile tap water (Oka et al., 2012). Petri dishes were incubated for 4 days and 11 days.

The leaves of the plant materials were frozen at -80 ℃ overnight, grinded by hand in plastic bag and 2 g of powdered plant materials was added to the petri dishes (Oka et al., 2012). Immediately afterwards 400 nematodes in 10 µl water were inoculated to all petri dishes. A negative control without plant material was included in each time and medium treatments. Each treatment had 5 replicates and experiment was repeated once again in same conditions. Petri dishes were incubated at 20 ℃ at dark during experiment.

The mobiled nematodes were extracted from all petri dishes using "Modified Baermann Funnel" technique for 24 hours (Hooper et al., 2005). Obtained nematode suspension was then concentrated to 1 ml. The nematode numbers were counted in whole sample under light microscope at 10x magnification. Living nematodes were obtained with extraction technique (Hooper et al., 2005). The number of motionless nematodes was determined by subtracting the number of motile nematodes obtained in each petri dish from the initial number of 400 nematodes. The motionless nematodes per treatment was calculated and presented as percent (%) (Aydınlı et al., 2019).

2.4. Statistical analysis

Data of percent motionless nematodes per petri dish was analyzed according to fit model of completely randomized plot design with four factors. In addition to three factors investigated in the experiment, repetition of the experiment was included as a factor to the statistical analysis. Effect of factors and their interactions were investigated. Analysis of variance (ANOVA), Tukey HSD test and Student's t test were used for determination of statistically significant differences among treatments and their interactions. Statistical analyses were performed using JMP© 5.0 software (JMP, 2020).

3. Results

The number of motionless nematodes (%) obtained was not statistically different in the two experiments performed. Therefore, the data obtained from two experiments were combined and evaluated. While the number of nematodes in the two different media examined in the experiment did not differ statistically, the number of motionless nematodes on the 4^{th} and 11^{th} days in the experiment showed a statistically significant difference ($P < 0.05$).

In the experiment, no statistically significant interaction was found between the experiment medium and the incubation time. The rates of motionless nematodes at plant species treatments in the experiment in sand and water medium as green fertilizer were statistically different ($P<0.05$). The interaction of plant species with experiment medium and the incubation time was also found to be statistically significant ($P < 0.05$).

On 4^{th} day in the water medium, the lowest number of immobile nematodes was obtained in negative control (9.6%). The rate of motionless nematodes obtained in the all plant species was found to be statistically higher than the negative control ($P < 0.05$). Among the plant treatments, the lowest rates of motionless nematodes were recorded in cabbage and broccoli plant species at 38.5% and 46.9%, respectively. These plant species were found to have statistically significantly lower motionless nematode rates than of the radish, arugula and marigold in the experiment. The highest rate of motionless nematodes

was obtained with arugula (84.1%). Subsequently, 72.9% in marigold and 62.5% in radish were obtained (Table 1).

The rate of motionless nematodes on the 11th day in the water medium did not show a statistically significant difference between plant species, while the control treatment was significantly lower (24.9%) than all plant species treatments. The highest rates of motionless nematodes on the 11th day in the water medium, as on the 4th day, were obtained in marigold (98.3%), arugula (95.7%) and radish (94.2%) treatments. It was recorded as 90.2% and 92.6% in cabbage and broccoli treatments, respectively (Table 1).

The rates of motionless nematodes obtained on the 11th day in the water medium were significantly higher than the 4th day in all plant treatments but not negative control treatment.

Table 1. Percentage of motionless nematodes (*Ditylenchus dipsaci*) in plant treatments at water medium with 4 and 11 days incubation times.

Plant Treatments	4 days incubation	11 days incubation
Broccoli (*Brassica oleraceae* var. *botrytis* L. cv. Standard)	46.9±2.9 cB	92.6±3.1 aA
Cabbage (*B. oleraceae* var. *capitata* L. cv. Standard)	38.5±2.9 cB	90.2±3.1 aA
Radish (*Raphanus sativus* L. cv. Standard)	62.5±3.1 bB	94.2±3.1 aA
Arugula (*Eruca sativa* Mill. cv. Standard)	84.1±2.9 aB	95.7±3.1 aA
Marigold (*Tagetes patula* L. cv. Bonanza, Harmony)	72.9±2.9 abB	98.3±3.4 aA
Negative Control	9.6±3.1 dA	24.9±3.9 bA

Values are means ± standart error of 10 replicates. Means within a column followed by different lower case letters for each plant treatment are significantly different according to Tukey HSD test at P <0.05. Means within a row followed by different capital letters for each incubation time are significantly different according to Student's t test at P <0.05.

No statistically significant difference was found among the plant and negative control treatments on the 4th day in sand medium. While the rate of motionless nematodes in negative control was 39.1%, it was recorded as 40.9, 52.0, 59.2, 60.2 and 64.4% in marigold, broccoli, radish, arugula and cabbage plant species, respectively (Table 2).

On the 11th day of the experiment in the sand medium, the lowest statistically significant rate of motionless nematodes was obtained in negative control treatment (62.1%). Although there is no statistical difference among plant treatments, the highest rate of motionless nematodes was obtained in arugula plant species (86.1%), followed by marigold and radish plant species at 81.9% and 80.9%, respectively. On the other hand, 80.6% and 77.3% motionless nematodes were obtained in cabbage and broccoli treatments, respectively (Table 2).

The rate of motionless nematodes in all plant treatments and negative control in the sand medium was found to be statistically significantly higher on the 11th day than on the 4th day (Table 2).

4. Discussions

In vitro suppression efficacy of potential green manure plants, which is a promising tool for controlling soil-borne pathogens and nematodes without accumulation in nature, for the control of stem and bulb nematode, which is common in onion and garlic growing areas in our country was investigated. The study provided preliminary data useful for determining the plant species for field experiments.

Table 2. Percentage of motionless nematodes (*Ditylenchus dipsaci*) in plant treatments at sand medium with 4 and 11 days incubation times.

Plant Treatments	4 days incubation	11 days incubation
Broccoli (*Brassica oleraceae* var. *botrytis* L. cv. Standard)	52.0±6.1 aB	77.3±3.4 abA
Cabbage (*B. oleraceae* var. *capitata* L. cv. Standard)	64.4±61 aB	80.6±3.4 aA
Radish (*Raphanus sativus* L. cv. Standard)	59.2±6.1 aB	80.9±3.4 aA
Arugula (*Eruca sativa* Mill. cv. Standard)	60.2±6.1 aB	86.1±3.6 aA
Marigold (*Tagetes patula* L. cv. Bonanza, Harmony)	40.9±6.1 aB	81.9±3.4aA
Negative Control	39.1±7.2 aB	62.1±3.8bA

Values are means ± standart error of 10 replicates. Means within a column followed by different lower case letters for each plant treatment are significantly different according to Tukey HSD test at P < 0.05. Means within a row followed by different capital letters for each incubation time are significantly different according to Student's t test at P < 0.05.

It is stated that one of the most important environmental factors for the high suppression efficiency of plants under field conditions is the moisture content in the soil during application. Water plays a critical role in the activity of myrosinase enzyme, which is necessary for the formation of suppressive isothiocyanate compounds (Matthiessen and Kirkegaard, 2006). In the study carried out, obtaining suppression efficiency higher in water medium, even it was not statistically significant, once again revealed that the water is important factor for myrosinase enzyme activity and biofumigation action.

In the experiment, arugula was the plant species with the highest efficiency in both water and sand mediums. In a study conducted under greenhouse conditions, arugula as applied green manure in soil reduced the populations of *Meloidogyne hapla* Chitwood 1949 and *M. chitwoodi* Golden et al. 1980 and *Paratrichodorus allius* Jensen, 1963 up to 99% (Riga, 2011). Similar effects have been recorded against root knot nematodes by Kruger et al. (2015).

Radish, which caused a high rate of motionless nematodes in the current experiment, has also been shown to be highly effective on other plant parasitic nematodes. It has been determined by different studies that the potato cyst nematode *Globodera pallida* Stone, 1973 second-stage larvae and cyst populations had been decreased by radish green manure application (Lord et al., 2011; Ngala et al., 2015). Similarly, higher effects of this plant as green manure has been found on *M. citwoodi* nematode population (Hafez and Sundararaj, 2000).

Other than the *Brassicaceae* family, one of the most promising plant species with the suppression effect is the *Tagetes* species that was shown in our study as well. Seigies and Pritts (2006) reported that after rotation with *T. erecta* in strawberry plantations, the number of nematodes decreased, the roots of the plants became

stronger and fruit yield increased. In a study conducted in Canada, it was determined that tuber yield increased and *P. penetrans* population density decreased in potato cultivation after rotation using *Tagetes* species (Kimpinski et al., 2000). In organic tomato cultivation, it was determined that there is a decrease in galls formed on the roots in infested soil with *M. incognita* during the autumn period in the co-cultivation with *T.erecta* (Kaşkavalcı et al., 2009). It is recommended to wait 7-10 days until the cultivation after the incorporation of suppressive plant species under field conditions in order to fully realize the biofumigation activity (Youssef, 2015). In our study, high efficiency rates with plant species in water and sand medium were obtained on the 11th day of the experiment and it was found that it increased depending on time.

A significant contribution has been made to the literature on the control of stem and bulb nematode using green manure application. In the next stage of the study, we will focus on the efficacy of promising plant species identified in the current study under field conditions.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

References

Abd-Elgawad MMM, Askary TH (2015). Impact of phytonematodes on agriculture ecology. In: Askary TH, Martinelli PRP (eds.). Biocontrol Agents of Phytonematodes. London: CAB International.

Al-Rehiayani S, Hafez S (1998). Host status and greend manure effect of selected crops on *Meloidogne chitwodi* race 2 and *Pratylenchus neglectus*. Nematropica 28: 213-230.

Aydınlı G, Şen F, Mennan S (2019). Bazı bitki ekstraktlarının kök-ur nematodu *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: *Meloidogynidae*)'nın kontrolünde kullanılabilme potansiyeli. KSÜ Tarım ve Doğa Dergisi 22(3): 414-420.

Evenhuis A, Korthals GV, Molendijk LPG (2004). *Tagetes patula* as an effective catch crop for long-term control of *Pratylenchus penetrans.* Nematology 6: 877-881.

Johnson AW, Golden AM, Auld DL, Summer DR (1992). Effects of rapeseed and vetch as green manure crops and fallow on nematodes and soilborne pathogens. Journal of Nematology 24: 117-126.

JMP (2020). Statistics Website: https://www.jmp.com [Accessed: 19 January 2021].

Hafez SL, Sundararaj P (2000). Evaluation of chemical strategies along with cultural practices for the management of *Meloidogyne chitwoodi* on potato. International Journal of Nematology 10: 89-93.

Hooper DJ, Hallmann J, Subbotin SA (2005). Methods for Extraction, Processing and Detection of Plant and Soil Nematodes. In: Luc M, Sikora RA, Bridge J (eds.). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. London: CABI Publishing. pp. 87-119.

Kaşkavalcı G, Tüzel Y, Dura O, Öztekin GB (2009). Effects of alternative control methods against *Meloidogyne incognita* in organic tomato production. Ekoloji 72: 23-31.

Kimpinski JW, Arsenault J, Gallant CE, Sanderson JB (2000). The effect of marigolds (*Tagetes* spp.) and other cover crops on *Pratylenchus penetrans* and on following potato crops. Supplemental Journal of Nematology 32: 531-536.

Kruger DHM, Fourie JC, Malan A (2015). Control potential of *Brassicaceae* cover crops as green manure and their host status for *Meloidogyne javanica* and *Criconemoides xenoplax*. South African Journal for Enology and Viticulture 36(1): 165-174.

Lord JS, Lazzeri L, Atkinson HJ, Urwin PE (2011). Biofumigation for control of pale potato cyst nematodes: activity of *Brassica* leaf extracts and green manures on *Globodera pallida* in vitro and in soil. Journal of Agricultural and Food Chemistry 59(14): 7882-7890.

Matthiessen JN, Kirkegaard JA (2006). Biofumigation and enhanced biodegradation: Oppotununity and challenge in soilborne pest and disease management. Critical Reviews in Plant Sciences 25: 235-265.

McLeod RW, Steel CC (1999). Effects of brassica-leaf gren manures and crops on activity and reproduction of *Meloidogyne javanica.* Nematology 1: 613-624.

Melakeberhan H, Xu A, Kravchenko A, Mennan S, Riga E (2006). Potential use of arugula (*Eruca sativa* L.) as a trap crop for *Meloidogyne hapla*. Nematology 8(5):793-799.

Mojtahedi H, Santo GS, Wilson JH, Hang AN (1993). Managing *Meloidogyne chitwoodi* on potato with rapeseed as gren manure. Plant Disease 77: 42-46.

Ngala BM, Haydock PPJ, Woods S, Back MA (2015). Biofumigation with *Brassica juncea*, *Raphanus sativus* and *Eruca sativa* for the management of field populations of the potato cyst nematode *Globodera pallid*a. Pest Management Science 71(5): 759-769.

Oka Y, Ben-Daniel B, Cohen Y (2012). Nematicidal activity of the leaf powder and extracts of *Myrtus communis* to the root knot nematode *Meloidogyne javanica*. Plant Pathology 61: 1012-1020.

Potter MJ, Davies AJ, Rathjen AJ (1998). Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematode *Pratylenchus neglectus.* Journal of Chemical Ecology 24: 67-80.

Riga E (2011). The effects of *Brassica* green manures on plant parasitic and living nematodes used in combination with reduced rates of synthetic nematisides. Journal of Nematology 43(2): 119-121.

Seigies AT, Pritts M (2006). Cover crop rotations alter soil microbiology and reduce replant disorders in strawberry. Hortscience 41: 1303-1308.

Seinhorst JW (1956). Population studies on stem eelworm *Ditylenchus dipsaci*. Nematologica 1: 159-164.

Stapleton JJ, Duncan RA (1998). Soil disinfestation with cruciferous amendments and sublethal heating: effects on *Meloidogyne incognita*, *Sclerotium rotfsii* and *Pythium ultimum*. Plant Pathology 47: 737-742.

Sturhan D, Brzeski MW (1991). Stem and bulb nematodes, *Ditylenchus* spp.. In: Nickle WR (ed.). Manual of agricultural nematology. New York: Marcel Dekker Publications.

Yavuzaslanoglu E, Aksay G (2021). Susceptibility of different plant species to two populations of *Ditylenchus dipsaci* Kühn, 1857 (Tylenchida: Anguinidae) from Turkey. Turkish Journal of Entomology 45(1): 77-86.

Youssef MMA (2015). Biofumigation as a promising tool for managing plant parasitic nematodes, a review. Scientia Agriculturae 10(3): 115-118.

Youssef MMA, Lashein AMS (2013). Effect of cabbage (*Brassica oleraceae*) leaf residue as a biofumigant, on root-knot nematode *Meloidogyne incognita* infecting tomato. Journal of Plant Protection Resources 53(3): 271-274.

Anatolian Journal of Botany
5(1): 37-43 (2021)
Research article *doi:10.30616/ajb.869240*

Effects of Panax ginseng on cisplatin-induced testicular damage of rats

Esra ASLAN[1], Kübra KUMALAR[2], Hilal GÜZEL[3], Hasan Hüseyin DEMİREL[4], Sefa ÇELİK[5], Mehmet Bilgehan PEKTAŞ[6*]

[1,2]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Histology and Embryology, 03200, Afyonkarahisar, Turkey*
[3]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Anatomy, 03200, Afyonkarahisar, Turkey*
[4]*Afyon Kocatepe University, Bayat Vocational School, 03782, Afyonkarahisar, Turkey*
[5]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Biochemistry, 03200, Afyonkarahisar, Turkey*
[6]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Pharmacology, 03200, Afyonkarahisar, Turkey*
**mbpektas@gmail.com, [1]dr_esragul@hotmail.com, [2]k.kumalar@gmail.com, [3]hilalgzl@hotmail.com, [4]hdemirel@aku.edu.tr, [5]sefa.celik@afsu.edu.tr*

Received : 27.01.2021
Accepted : 02.03.2021
Online : 25.03.2021

Panax ginseng'in sıçanlarda cisplatin ile oluşturulan testis hasarı üzerine etkileri

Abstract: Infertility has become an increasingly important health problem due to genetic, familial, hormonal, and congenital abnormalities, environmental and chemical reasons. This study aimed to investigate the effects of Panax ginseng (Pnx) root extract on cisplatin (CP) induced testicular damage of rats. Four animal groups were applied with different protocols as control, Pnx (200 mg / kg), CP (7 mg / kg), and CP + Pnx (200 mg / kg). At the end of the experiment, the body and testicular weights of the rats were measured. While free/total testosterone, total antioxidant capacity (TAC), and total oxidative species (TOS) levels were analyzed in blood samples, apoptotic cells were marked by TUNEL staining in testicular samples of rats. According to the results, free/total testosterone and TAC levels were decreased while TOS levels increased in injured rats' plasma. On the other hand, seminiferous tubule diameters widened, and the number of apoptotic cells increased in rats' testis. These variables were significantly improved with the consumption of Pnx. As a result, Pnx has a significant protective effect on testicular tissue; however, further studies are needed to elucidate its action mechanism.

Keywords: Apoptosis, Cisplatin, Panax ginseng, TAC, TOS

Özet: İnfertilite genetik, ailevi, hormonal nedenler, doğumsal anormallikler, çevresel ve kimyasal nedenlerle günümüzde artmakta olan önemli bir sağlık sorunu haline gelmiştir. Bu çalışma ile sıçanlarda sisplatin (CP) ile indüklenen testis hasarı üzerine Panax ginseng (Pnx) kök ekstraktının etkilerinin incelenmesi amaçlanmıştır. Sıçanlar kontrol, Pnx (200 mg/kg), CP (7 mg/kg) ve CP+Pnx (200 mg/kg) olmak üzere 4 gruba ayrılmıştır. Deney sonunda sıçanların vücut ve testis ağırlıkları ölçülmüştür. Sıçanların kan örneklerinde serbest/total testosteron, toplam antioksidan kapasite (TAK) ve toplam oksidan durum (TOD) seviyeleri analiz edilirken, testis örneklerinde TUNEL boyaması ile apoptotik hücreler işaretlenmiştir. Sonuçlara göre hasar oluşturulmuş sıçanların plazma örneklerinde serbest/total testosteron ve TAK düzeyleri azalırken, TOD miktarı artmıştır. Testis dokularında seminifer tübül çapları genişlemiş ve apoptotik hücre sayısı artmıştır. Pnx uygulaması ile bu değişkenlerin ciddi oranda düzeldiği saptanmıştır. Sonuç olarak, Pnx'in testiküler dokuda belirgin koruyucu etkinliği bulunmaktadır; fakat etki mekanizmasının aydınlatılabilmesi için ileri çalışmalara gereksinim vardır.

Anahtar Kelimeler: Apoptoz, Cisplatin, Panax ginseng, TAK, TOD

Citation: Aslan E, Kumalar K, Güzel H, Demirel HH, Çelik S, Pektaş MB (2021). Effects of Panax ginseng on cisplatin-induced testicular damage of rats. Anatolian Journal of Botany 5(1): 37-43.

1. Introduction

Cisplatin (CP) is an alkylating chemotherapeutic agent used alone or in combination with antineoplastic agents in mouth, head, neck, endometrium, lung, ovarian, and testicular cancers (Dasari et al., 2014). The therapeutic effect of CP is dose-dependent and cumulative (Hanigan et al., 2003). The most important known side effect of CP, which is frequently used in the clinic, is nephrotoxicity. Therefore, kidney functions are commonly followed in cancer chemotherapy, depending on CP (Barabas et al., 2008). The copper transporter Ctr1 mediates CP absorption and transport in mammals (Dasari et al., 2014). CP is known to induce DNA damage by binding to the N7 reagent center on purine residues and rising apoptotic cell death by blocking cell division in cancer cells. (Aly et al., 2020). Cancer cells cause more oxidation than normal cells with increased metabolic activity, oncogenic activation, and corruption of mitochondrial functions. One of the most critical contraptions is oxidative stress that plays a role in CP toxicity (Wang et al., 2020). Recent studies showed that CP induced genotoxicity and reproductive toxicity accompanied by severe oxidative stress (Aksu et al., 2016; Sadeghi et al., 2018). Similarly, CP reduced catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) expressions; however, it increased malondialdehyde (MDA) levels in the testicular tissues of mice (Zhang et al., 2020). On the other hand, CP caused lower testosterone levels and induced apoptosis via enhanced Bcl-2-associated X protein (Bax) and decreased B-cell lymphoma 2 (Bcl-2) levels in the testis of rats (Azab et al., 2020). Generally, CP-induced animals also exhibited lower sperm count and motility as well as higher abnormal sperm; however, testicular injury adversely affects spermatogenesis, sperm quality, and oxidative

stress parameters in the related studies (Ceylan et al., 2020; Kohsaka et al., 2020; Saad et al., 2020; Wang et al., 2020). These findings showed the CP-induced genotoxicity and reproductive toxicity, which might be closely related to oxidative stress. Until now, there is no effective treatment agent for CP-induced genotoxicity and reproductive toxicity, and finding new protective and therapeutic strategies against CP-induced adverse changes is mandatory.

Panax ginseng (Pnx) is an important medicinal herb and root extract, and has antioxidant effects with predominant metabolic, neurological, and urological activities. The use of Pnx in traditional Chinese medicine dates back to about 5000 years ago. Researches on Pnx have been increasing in recent years due to the biotransformation of ginsenosides in the roots or extracts of Pnx in the intestine and its high pharmacological activity and pharmacokinetics (Mancuso et al., 2017). Pnx consumption might reduce prenatal stress in rats (Kim et al., 2015). In another study, Korean Ginseng increased C-21 steroid metabolism in the testis' interstitial Leydig cells (Kim et al., 2011). However, many studies suggested that Pnx decreases oxidative stress and increases antioxidant capacity in various tissues (De Freitas et al., 2019; Kopalli et al., 2016; Kumar et al., 2003).

The purpose of this study is to examine the effects of Pnx, which has been used as a chemotherapeutic drug for centuries, on possible damages on the testis, and to develop an alternative treatment method in male infertility, which is a significant health problem today.

2. Materials and method

2.1. Reagents

Pnx pure 99% (2018040200019) was obtained from Aksu Vital Doğal Ürünler Gıda Sanayi ve Tic. A.Ş. CP (LRAB7778) and other reagents were obtained from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Animals and treatments

The standard rodent chow diet and living conditions have been applied to the ten-week-old and approximately 200–300 g of 28 male Wistar rats. The rats were acclimated one week for the optimization, and then four groups were formed for different protocols: Control (n=7), Pnx (n=7), CP (n=7), and CP plus Pnx (n=7). Pnx (200 mg/kg) was given in 2 mL of saline by gastric gavage once a day for four weeks to the Pnx group; however, after this, single dose of CP (7 mg/kg) was administered intraperitoneally (i.p.) to CP and CP plus Pnx groups. Three days after, rats were anesthetized with ketamine (100 mg/kg) and xylazine 10 mg/kg) at the end of the experiment, blood and testis samples were collected rapidly. The Ethical Animal Research Committee of Afyon Kocatepe University (AKUHADYEK 268-17) approved this study's animal procedures.

2.3. Determination of the TAC, TOS, and total/free testosterone levels in the plasma

Non-fasted rats' cardiac blood was centrifuged immediately at +4°C and 1,000*g* for 30 min. The resulting supernatant is utilized and stored at −85 °C until the total antioxidant capacity (TAC) (Erel, 2004) and total oxidant status (TOS) (Erel, 2005) and testosterone levels are measured. TAC and TOS Assay Kits (Rel Assay Diagnostics, TR) were used for the measurements. The TAC results were presented as mmol Trolox Eq/L; however, TOS results were given as μmol H_2O_2 Eq/L for plasma. The oxidative stress index (OSI) was calculated according to the formula; OSI = [(TOS, μmol H_2O_2 Eq/L) / (TAC, (mmol Trolox Eq / L) x 100]. Free and total testosterone levels were determined by an ELISA (eBioscience, USA) kit according to the manufacturer's instructions,

2.4. Histopathological evaluations

Tissue samples taken from sacrificed rats were fixed in Bouin's fixative and then histologically processed and embedded in paraffin blocks. Five μm sections were taken on polylyzed slides which were stained with Hematoxylin & Eosin (HE) for the general histomorphological appearance. Besides, 25 different tubules were selected from each section, and Johnsen scoring was applied. According to this scoring; 10: Tubules perfect and complete spermatogenesis, 9: Numerous spermatozoa, irregular spermatogenesis, 8: There are only a few sperm, 7: There are no spermatozoa, but many spermatids, 6: Just a few spermatids, 5: No spermatozoa or spermatids, but many spermatocytes, 4: Only a few spermatocytes are present, 3: Only spermatogonia are present, 2: No germ cells, 1: Neither germ cells nor Sertoli cells are present. Then, the data were compared statistically. Testicular injury and spermatogenesis in the sections were evaluated histopathologically using Johnsen's mean testicular biopsy score (MTBS) criteria (Johnsen, 1970).

2.5. Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling Assay (TUNEL)

Testicular tissues were detected by TUNEL assay according to the instructions of ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, Millipore, Billerica, MA, USA). 5 μm sections were taken from paraffin blocks and stained according to the manufacturer's protocol. Dark brown stained cells were considered positive. TUNEL-positive cells were counted in 6 randomly chosen areas (400X).

2.6. Statistical analysis

All data is represented as mean ± standard error of the mean (SEM) throughout the study and compared for differences using the Prism 6.01 GraphPad software. Student's t-test for unpaired data or one-way ANOVA followed by the *Bonferroni* post hoc analysis were used to compare different groups. Johnsen's scoring was made using the Chi-Square test. All statistical tests were performed at a p-value less than 0.05.

3. Results

3.1. The effects of Pnx and CP on body and testis weight

As shown in Table 1, both Pnx and CP did not affect the bodyweights of rats. Similarly, there is no change in the ratio of right and left testis weight to body weight in the Pnx-treated healthy rats. However, CP significantly decreased the ratio of right testis weight to body weight, and there was a tendency toward reduction in left testis weights, but it was not significant. This reduction remained at the levels of Control rats with Pnx-treatment given before CP administration. In this respect, Pnx-treatment significantly prevented the decrease in CP-dependent testicular weights (Table 1).

Table 1. The initial and terminal body weights, right and left testicular weights, and their ratio to the bodyweight of Control, Pnx, CP, and CP plus Pnx groups.

Groups	Control	Pnx	CP	CP+Pnx
Initial body weight (g)	254.9±11.5	227,1±14.3	291.6±14.6	267.9±14.2
Terminal body weight (g)	297±19.3	299.6±15	355±14.2	307.7±15.4
Right testis absolute weight (g)	3.44±0.21	3.41±0.05	3.57±0.18	3.79±0.18
Left testis absolute weight (g)	3.24±0.23	3.72±0.27	3.76±0.14	4.04±0.23
Right testis weight to body weight (g/100g BW)	1.16±0.07	1.14±0.01	1.01±0.05*	1.23±0.06#
Left testis weight to body weight (g/100g BW)	1.19±0.07	1.24±0.09	1.06±0.04	1.31±0.08#

Values are expresses as mean ± SEM, n = 7; * $p < 0.05$, significantly different from control; #$p < 0.05$, significantly different from CP.

3.2. The effects of Pnx and CP on endocrine parameters, TAC, TOS, and OSI levels in the plasma of rats

The levels of total and free testosterone in the plasma samples were established by using ELISA kits. Accordingly, there was no change between Pnx and control groups on the total and free testosterone levels (Fig. 1a,b). Giving CP to the healthy rats significantly reduced total and free testosterone levels compared to controls. On the other hand, as shown in Figures 1a and b, there were preventive effects with Pnx against CP-administration, achieving a significance level. Plasma TAC and OSI levels did not change, but TOS levels decreased with Pnx-feeding (Fig. 1c,d,e). Furthermore, reduced TAC and increased TOS and OSI levels were found after CP-injection in healthy rats' plasma compared to controls. As shown in Figures 1c, d, and e, there was specific prevention with Pnx-treatment to impaired TAC, TOS, and OSI levels of CP injected rats.

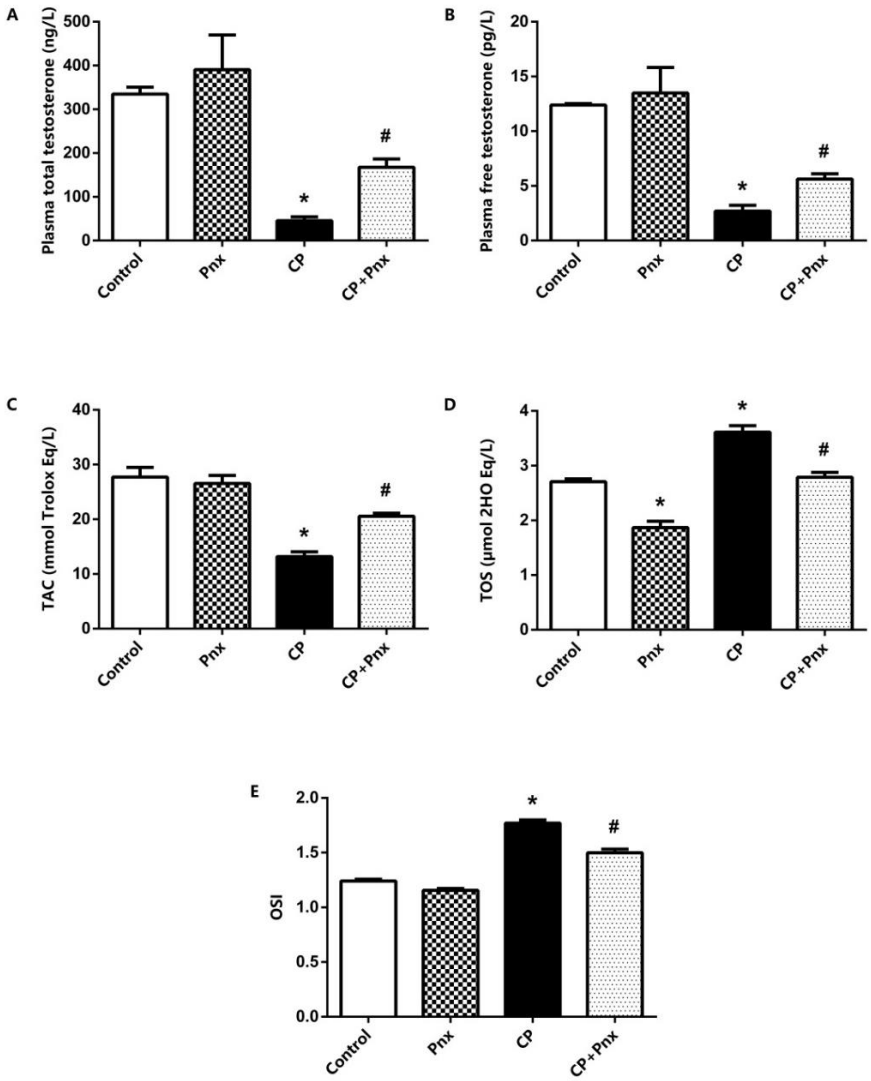

Figure 1. Changes in total testosterone (A), free testosterone (B), TAC (C), TOS (D), and OSI (E) levels in the plasma of the rats. Values are expressed as mean ± SEM, and each bar represents the means from at least six rats. *P<0.05, significantly different from the Control; #P<0.05, significantly different from the CP-treated rats.

3.3. The effects of Pnx and CP on testis histology; results of TUNELassay and Johnsen's score

Dietary Pnx did not change TUNEL (+) spermatogenic and Leydig cells and Johnsen's scores in healthy rats' testicular tissues compared to controls (Table 2; Fig. 2a,b). There was a significant increment in TUNEL (+) spermatogenic and Leydig cells; however a reduction in Johnsen's scores with CP-administration in the testis of healthy rats. On the other hand, Pnx-feeding prevented these changes in the CP groups. Seminiferous tubules showed normal histological appearance in control and Pnx groups. Testes of cisplatin-treated rats showed loss of sperm production and lots of degenerated cells. Pnx improved the histopathological morphology of testes in animals. Testes showed typical histological structure in the seminiferous tubules with complete spermatogenesis.

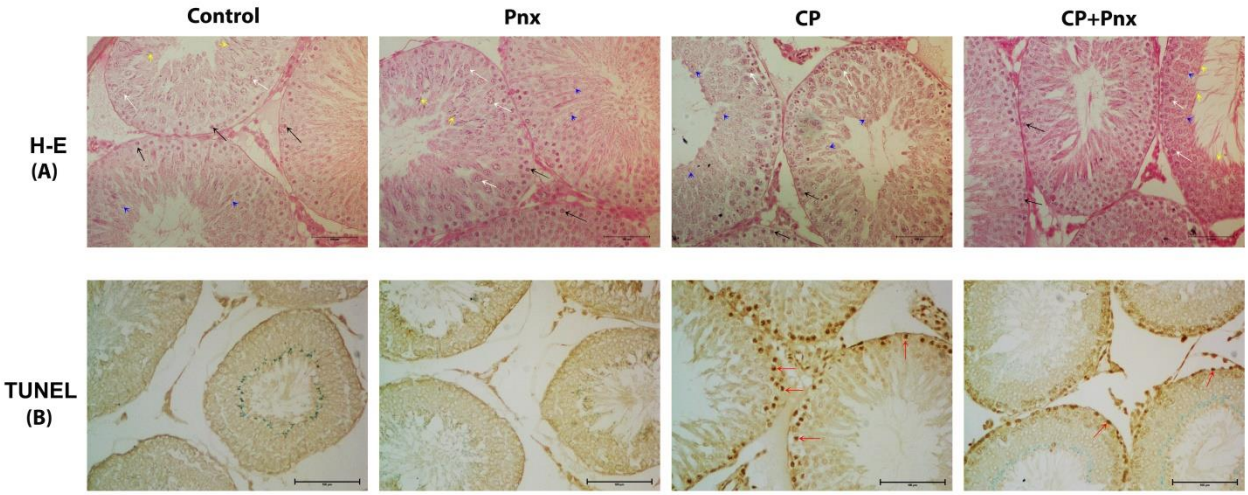

Figure 2. (A) Histological features of testis sections from Control, Pnx, CP, and CP+Pnx groups. In all groups, spermatogonia are indicated by the black arrow. The white arrow indicates primary spermatocytes, the blue arrow indicates spermatids, and many spermatozoa are indicated by the yellow arrow (200X magnifications, H-E). (B) Spermatogenic and Leydig cells apoptosis detected via TUNEL assay (200X magnifications). TUNEL (+) cells have dark Brown nuclei and are indicated by the red arrow.

Table 2. Johnsen's score of tubules and number of TUNEL (+) spermatogenic and Leydig cells of Control, Pnx, CP, and CP plus Pnx groups.

Groups	Control	Pnx	CP	CP+Pnx
TUNEL (+) spermatogenic cells	8.7±1	8.4±0.4	463±22*	159±8#
TUNEL (+) Leydig cells	3.4±0.2	3.3±0.2	32.6±1.4*	12.9±1#
Johnsen's score	10±0.1	10±0.1	7±0.3*	9±0.3#

Values are expressed as mean ± SEM, n = 7; *p < 0.05, significantly different from control; #P < 0.05, significantly different from CP.

4. Discussions

Herein, we demonstrated dietary Pnx prevented oxidative stress and dysmorphology in the CP-induced degeneration of testis via rising plasma TAC, total & free testosterone, and testicular Johnsen's scores, and reducing plasma TOS levels, testicular TUNEL (+) spermatogenic cells, and TUNEL (+) Leydig cells.

CP has been considered the most frequently used chemotherapeutic agent against various solid tumors because of its significant therapeutic effects (Barabas et al., 2008). However, it also damages many tissues such as the kidney, brain, liver, and testis due to its intense cytotoxic activity (Dasari et al., 2014; Hanigan et al., 2003). Many studies examining its effects on the testis have been repeatedly shown that CP causes mitochondria-mediated oxidative stress, apoptosis, inflammation, and gonadotoxicity (Aly et al., 2020; Ceylan et al., 2020; De Freitas et al., 2019). Recently, research investigating Ginger juice's effects on CP-induced testicular damage showed that rats' body weights did not change at the end of the fifth day after CP administration. However, testicular weights significantly decreased (Famurewa et al., 2020). A similar study found a decrease in testicular tissue weight at the end of the fifth day after CP injection in the hamsters (Wang et al., 2020). In this study, the ratio of right testis weight to body weight decreased at the end of the third day after CP application, but body weight did not change. Moreover, left testis weights were reduced, but they could not reach the significance level because, unlike other studies in our study, animals were decapitated at the end of the third day after CP application. Several studies also showed that the reason for CP-mediated lower testis weight is related to decreased sperm count and epididymal weight (Wang et al., 2020; Yucel et al., 2019). On the other hand, Pnx-feeding increased testicular weights to body weights in the CP-administered rats. This finding is the indicator of the protective efficacy of Pnx against CP-mediated testicular damage. Recent studies showing the antioxidant, anti-inflammatory, and cytoprotective effects of Pnx also support our results. (Kim et al., 2017; Kopalli et al., 2016; Zhu et al., 2020). It is known that CP used for therapeutic purposes causes the increment of reactive oxygen species and the suppression of antioxidant structures in the blood or tissues (Rashid et al., 2013). Besides, the studies showed that CP-administration causes an increase in testicular MDA, 4-hydroxynonenal, and caspase-3 levels while reducing superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) levels of rats (Saad et al., 2020; Majd et al., 2021). In another study, CP caused a

reduction in TAC and an increment in TOS in rats' plasma samples (Geyikoglu et al., 2017). We determined lower TAC and higher TOS levels in CP-treated rats' plasma in the present study. Herein, it was shown that CP increases reactiCP-treated species and reduces the antioxidants' impact (Saad et al., 2004). Moreover, the unconfronted reactive oxygen species enhance DNA and mitochondrial membrane damage via lipid peroxidation (Sadi and Sadi, 2010). Therefore, these changes due to CP have already been expected. However, Pnx-feeding significantly increased plasma TAC and reduced TOS levels in the CP-induced testicular damage. De Freitas et al. showed that Pnx metabolite (GIM-1) prevented oxidative stress and apoptosis in human Sertoli cells via increased SOD, CAT, GPx, and caspase-3 levels (De Freitas et al., 2019). In another study, in GC-2 sperm cells, Pnx prevented hydrogen peroxide-induced oxidative stress via increased GPx expression (Kopalli et al., 2016). These results are consistent with our current study and point to the cytoprotective efficacy of Pnx.

One of the leading causes of reproductive dysfunction is oxidative stress (Lonare et al., 2016). Reactive oxygen species may lead to testosterone synthesis disorder via impaired cyclic adenosine monophosphate (cAMP) in Leydig cells. Kong et al. expressed that testosterone synthesis is a complex process that involved many served enzymes. (Kong et al., 2017). When gonadotropin luteinizing hormone is released, it results in cAMP release and protein kinase A activation, which initiates a series of enzyme cascade reactions (Liu et al., 2015). In various studies examining the effects of CP on testicular and reproductive function, it has been shown that plasma or testicular total and free testosterone levels are also reduced; these results have often been associated with oxidative stress (Azab et al., 2020; Ceylan et al., 2020; Famurewa et al., 2020; Jourabi et al., 2020; Kohsaka et al., 2020; Majd et al., 2021; Saad et al., 2020; Wang et al., 2020). In the present study, we also demonstrated that CP-induced oxidative stress significantly decreased total and free testosterone levels in rats' plasma. However, the antioxidant effects of Pnx increased both total and free testosterone productions. This result indicated that Pnx might promote the testosterone secretion of Leydig cells via suppressing oxidative stress which was induced by CP in rat testes. Kohsaka et al. reported that CP causes an increase in TUNEL (+) spermatogenic and Leydig cells apoptotic index; however, it reduces Johnsen's score in rats' testes (Kohsaka et al., 2020). Research on the protective effect of caffeic acid phenethyl ester on testicular damage caused by CP showed that CP decreased Johnsen's score and damaged spermatogenic cells (Ceylan et al., 2020). Sperm count, viability, and motility were reduced by CP-administration, while sperm dysmorphology increased in rats' testes. CP-administration decreased sperm count, viability, and motility, while sperm dysmorphology increased in rats' testes (Jourabi et al., 2020). Our results suggest augmented TUNEL (+) spermatogenic and Leydig cells in CP-induced damaged rats. A significant pathological effect that included a reduced Johnsen's score was revealed in rats treated with CP. However, treatment for four weeks with Pnx prevented TUNEL (+) spermatogenic and Leydig cells and Johnsen's score. These results indicated that the rat model of testicular lesion induced by CP was successfully established, and Pnx had protective effects on CP-induced basic toxicity.

In conclusion, an animal model of CP-induced testicular damage and Pnx intervention was established in the current study. Results indicated that CP might lead to testicular toxicity via oxidative stress and inhibiting testosterone synthesis in rats. Moreover, Pnx has preventive effects on CP-induced modulations. Further research is required to clarify its preventive effects and mechanism.

Conflict of interest

The authors report no conflicts of interest related to this study.

Authors' Contributions

EA, KK, HG, HHD, SÇ, and MBP performed the research. EA, KK, and MBP helped during the experimental work, statistical analysis and in writing the manuscript. MBP drafted the manuscript. EA, HG, and MBP conceived and designed the study and critically revised the manuscript.

Acknowledgments

The Afyon Kocatepe University Research Fund supported this work (17-SAG.BIL-25).

References

Aksu EH, Kandemir FM, Altun S, Küçükler S, Çomaklı S, Ömür AD (2016). Ameliorative effect of carvacrol on cisplatin-induced reproductive damage in male rats. Journal of Biochemical and Molecular Toxicology 30(10): 513-520.

Aly HAA, Eid BG (2020). Cisplatin-induced testicular damage through mitochondria-mediated apoptosis, inflammation and oxidative stress in rats: Impact of resveratrol. Endocrine Journal 67(9): 969-980.

Azab SS, Kamel I, Ismai, NN, El Din Hosni H, El Fatah MA (2020). The defensive role of taurine against gonadotoxicity and testicular apoptosis effects induced by cisplatin in rats. Journal of Infection and Chemotherapy 26(1): 51-57.

Barabas K, Milner R, Lurie D, Adin C (2008). Cisplatin: A review of toxicities and therapeutic applications. Veterinary and Comparative Oncology 6(1): 1-18.

Ceylan T, Kaymak E, Cantürk F, Yakan B (2020). Research on the protective effect of caffeic acid phenethyl ester on testicular damage caused by cisplatin. Turkish Journal of Medical Sciences 50(8): 2032-2039.

Dasari S, Bernard TP (2014). Cisplatin in cancer therapy: Molecular mechanisms of action. European Journal of Pharmacology 740: 364-378.

De Freitas AT, Figueiredo PC, de Aquino AM, Fernandes AH, Fantin DR, Justulin LA, Scarano WR (2019). Panax ginseng metabolite (GIM-1) prevents oxidative stress and apoptosis in human Sertoli cells exposed to Monobutyl-phthalate (MBP). Reproductive Toxicology 86: 68-75.

Erel O (2004). A novel automated method to measure total antioxidant response against potent free radical reactions. Clinical Biochemistry 37(2): 112-119.

Erel O (2005). A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry 38(12): 1103-1111.

Famurewa AC, Ekeleme-Egedigwe CA, Onwe CS, Egedigwe UO, Okoro CO, Egedigwe UJ, Asogwa NT (2020). Ginger juice prevents cisplatin-induced oxidative stress, endocrine imbalance and NO/iNOS/NF-κB signalling via modulating testicular redox-inflammatory mechanism in rats. Andrologia 52(10): 1-10.

Geyikoğlu F, Çolak S, Türkez H, Bakır M, Koç K, Hosseinigouzdagani MK, Çerig S, Sönmez M (2017). Oleuropein ameliorates cisplatin-induced hematological damages via restraining oxidative stress and DNA injury. Indian Journal of Hematology and Blood Transfusion 33(3): 348-354.

Hanigan MH, Devarajan P (2003). Cisplatin nephrotoxicity: molecular mechanisms. Cancer Therapy 1: 47-61.

Johnsen SG (1970). Testicular biopsy score count – A method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormone Research in Paediatrics 1(1): 2-25.

Jourabi F, Yari S, Amiri P, Heidarianpour A, Hashemi H (2020). The ameliorative effects of methylene blue on testicular damage induced by cisplatin in rats. Andrologia 53(1): e13850.

Kim IH, Kim SK, Kim EH, Kim SW, Sohn SH, Lee SC, Choi SD, Pyo SN, Rhee DK (2011). Korean red ginseng up-regulates C21-steroid hormone metabolism via Cyp11a1 gene in senescent rat testes. Journal of Ginseng Research 35(3): 272-282.

Kim MK, Cha KM, Hwang SY, Park UK, Seo SK, Lee SH, Jeong MS, Cho S, Kopalli SR, Kim SK (2017). Pectinase-treated Panax ginseng protects heat stress-induced testicular damage in rats. Reproduction 153(6): 737-747.

Kim YO, Lee HY, Won H, Nah SS, Lee HY, Kim HK, Kwon JT, Kim HJ (2015). Influence of Panax ginseng on the offspring of adult rats exposed to prenatal stress. International Journal of Molecular Medicine 35(1): 103-109.

Kohsaka T, Minagawa I, Morimoto M, Yoshida T, Sasanami T, Yoneda Y, Ikegaya N, Sasada H (2020). Efficacy of relaxin for cisplatin-induced testicular dysfunction and epididymal spermatotoxicity. Basic and Clinical Andrology 30(1): 1-13.

Kong D, Zhang J, Hou X, Zhang S, Tan J, Chen Y, Yang W, Zeng J, Han Y, Liu X, Xu D, Cai R (2017). Acetamiprid inhibits testosterone synthesis by affecting the mitochondrial function and cytoplasmic adenosine triphosphate production in rat Leydig cells†. Biology of Reproduction 96(1): 254-265.

Kopalli SR, Cha KM, Jeong MS, Lee SH, Sung JH, Seo SK, Kim SK (2016). Pectinase-treated Panax ginseng ameliorates hydrogen peroxide-induced oxidative stress in GC-2 sperm cells and modulates testicular gene expression in aged rats. Journal of Ginseng Research 40(2): 185-195.

Kumar M, Sharma MK, Saxena PS, Kumar A (2003). Radioprotective effect of Panax ginseng on the phosphatases and lipid peroxidation level in testes of Swiss albino mice. Biological and Pharmaceutical Bulletin 26(3): 308-312.

Liu HC, Zhu D, Wang C, Guan H, Li S, Hu C, Chen Z, Hu Y, Lin H, Lian QQ, Ge RS (2015). Effects of etomidate on the steroidogenesis of rat immature Leydig cells. PLoS One 10(11): e0139311.

Lonare M, Kumar M, Raut, S, More A, Doltade S, Badgujar P, Telang A (2016). Evaluation of ameliorative effect of curcumin on imidacloprid-induced male reproductive toxicity in Wistar rats. Environmental Toxicology 31(10): 1250-1263.

Majd EN, Hajirahimi A, Tabandeh MR, Molaei R (2021). Protective effects of green and chemical zinc oxide nanoparticles on testis histology, sperm parameters, oxidative stress markers and androgen production in rats treated with cisplatin. Cell and Tissue Research (online 12 Jan 2021).

Mancuso C, Santangelo R (2017). Panax ginseng and Panax quinquefolius : From pharmacology to toxicology. Food and Chemical Toxicology 107: 362-372.

Rashid K, Sinha K, Sil PC (2013). An update on oxidative stress-mediated organ pathophysiology. Food and Chemical Toxicology 62: 584-600.

Saad KM, Abdelrahman RS, Said E (2020). Mechanistic perspective of protective effects of nilotinib against cisplatin-induced testicular injury in rats: Role of JNK/caspase-3 signaling inhibition. Environmental Toxicology and Pharmacology 76: 103334.

Saad SY, Najjar TA, Alashari M (2004). Role of non-selective adenosine receptor blockade and phosphodiesterase inhibition in cisplatin-induced nephrogonadal toxicity in rats. Clinical and Experimental Pharmacology and Physiology 31(12): 862-867.

Sadeghi F, Etebari M, Habibi-Roudkenar M, Jahanian-Najafabadi A (2018). Lipocalin2 protects human embryonic kidney cells against cisplatin-induced genotoxicity. Iranian Journal of Pharmaceutical Research : IJPR 17(1): 147-154.

Sadi G, Sadi Ö (2010). Antioxidants and regulation of antioxidant enzymes by cellular redox status. Turkish Journal and Scientific Reviews 3(2): 95-107.

Wang L, He Y, Li Y, Pei C, Olatunji OJ, Tang J, Famurewa AC, Wang H, Yan B (2020). Protective effects of nucleosides-rich extract from cordyceps cicadae against cisplatin induced testicular damage. Chemistry and Biodiversity 17(11): e2000671.

Wang PT, Sudirman S, Hsieh MC, Hu JY, Kong ZL (2020). Oral supplementation of fucoxanthin-rich brown algae extract ameliorates cisplatin-induced testicular damage in hamsters. Biomedicine and Pharmacotherapy 125: 109992.

Yucel C, Arslan FD, Ekmekci S, Ulker V, Kisa E, Erdogan-Yucel E, Ucar M, Ilbey YO, Celik O, Basok BI, Kozacioglu Z (2019). Protective effect of all-trans retinoic acid in cisplatin-induced testicular damage in rats. The World Journal of Men's Health 37(2): 249-256.

Zhang K, Weng H, Yang J, Wu C (2020). Protective effect of Liuwei Dihuang Pill on cisplatin-induced reproductive toxicity and genotoxicity in male mice. Journal of Ethnopharmacology 247: 112269.

Zhu D, Fang X, Chen Y, Shan M, Jiang R, Qiu Z, Luo H (2020). Structure-activity relationship analysis of Panax ginseng glycoproteins with cytoprotective effects using LC-MS/MS and bioinformatics. International Journal of Biological Macromolecules 158: 1352-1361.

Anatolian Journal of Botany
5(1): 44-50 (2021)
Research article *doi:10.30616/ajb.873328*

The relationships between environmental and disturbance factors in temperate deciduous forest ecosystem (Amasya/Turkey)

Dudu Duygu KILIÇ[1], Burak SURMEN[2]*, Hamdi Güray KUTBAY[3]
[1]*Amasya University, Science and Arts Faculty, Department of Biology, Amasya, Turkey*
[2]*Karamanoğlu Mehmetbey University, Kamil Özdağ Science Faculty, Department of Biology, Karaman, Turkey*
[3]*Ondokuz Mayıs University, Science and Arts Faculty, Department of Biology, Samsun, Turkey*
**buraksurmen@gmail.com, [1]drduygukilic@gmail.com, [3]hguray@omu.edu.tr*

Received :02.02.2021
Accepted :24.03.2021
Online :05.04.2021

Ilıman yaprak döken ormanlarda (Amasya/Türkiye) tahribat ve çevresel faktörler arasındaki ilişkiler

Abstract: Deciduous forests face many disturbance factors. Grazing and cutting are the leading factors in this disturbance. The study area's vegetation was analyzed using numerical methods to identify plant communities and determine the relationship between environmental gradients and disturbance factors. The species diversity was calculated using alpha and beta diversity indexes. As a result, four different communities were identified in the study area. One of the communities was under grazing pressure while the other community was under cutting pressure. No disturbance factors were found in the remaining two communities. Elevation and soil moisture were found to be important in the distribution of plant communities. pH, soil moisture, soil % N content and canopy factors were found to be important. The highest Shannon-Wienner diversity index values were found in non-cutting and non-grazing forest communities. The lowest Shannon-Wienner diversity index values were found in grazing and cutting forest communities. Unlike the Shannon-Wienner diversity index, the highest beta index values were found in grazing and cutting forest communities. The lowest beta index values were found in non-cutting and non-grazing forest communities.

Key words: Plant ecology, plant diversity, numerical method

Özet: Yaprak döken ormanlar birçok tahribat faktörüyle karşı karşıyadır. Özellikle otlatma ve ağaç kesimi bu faktörlerin başında gelmektedir. Çalışma alanının vejetasyonu, bitki komünitelerinin tespiti ve çevresel faktörler ile tahribat faktörleri arasındaki ilişkiyi belirlemek için nümerik metotlar kullanılarak analiz edilmiştir. Tür çeşitililiği alfa ve beta çeşitlilik indeksleri kullanılarak hesaplanmıştır. Sonuç olarak, çalışma alanında 4 farklı komünite tespit edilmiştir. Bu komünitelerden biri otlatma baskısı altındayken diğer komünite ağaç kesimi baskısı altındadır. Diğer komüntelerde ise tahribat faktörleri bulunmamıştır. Rakım ve toprak neminin bitki komünitelerinin dağılımında önemli olduğu bulunmuştur. pH, toprak nemi, toprak N içeriği ve kanopi faktörleri önemli bulunmuştur. En yüksek Shannon-Wienner indeks değerleri ağaç kesimi ve otlatma olmayan orman komünitelerinde bulunmuştur. En düşük Shannon-Wienner çeşitlilik değerleri ise otlatma ve ağaç kesimi olan orman komüntelerinde bulunmuştur. Shannon-Wienner çeşitlilik indekslerinin aksine, en yüksek beta çeşitlilik indeks değerleri otlatma ve ağaç kesimi olan orman komünitelerinde bulunmuştur. En düşük beta index değerleri ağaç kesimi ve otlatma olmayan orman komünitelerinde bulunmuştur.

Anahtar Kelimeler: Bitki ekolojisi, bitki çeşitliliği, numerik metod

Citation: Kılıç DD, Sürmen B, Kutbay HG (2021). The relationships between environmental and disturbance factors in temperate deciduous forest ecosystem (Amasya/Turkey). Anatolian Journal of Botany 5(1): 44-50.

1. Introduction

There are significant relationships between plant species and environmental factors in terrestrial ecosystems. Topography, soil characteristics and climatic conditions are determinants factors affecting plant diversity (Davies et al., 2007; Korkmaz et al., 2016). For example, soil pH (Borchsenius et al., 2004; Hofmeister et al., 2009), nutrient availability (Small and McCarthy, 2005; van Calster et al., 2008), soil moisture (Qian et al., 1997, Lenière and Houle, 2006), the mass of litter layer (Gazol and Ibánez, 2009; Kooijman, 2010), light availability (Härdtle et al., 2003; Tinya et al., 2009) and distance to forest edge (Harper et al., 2005; Gonzalez et al., 2010) are among the most critical environmental factors (Vockenhuber et al., 2011).

In temperate deciduous forests, approximately the 90% of vegetation consists of vascular plant diversity (Whigham, 2004; Gilliam, 2007). The composition and diversity of the ground flora in temperate deciduous forests are affected by the composition of the canopy species and soil and climate characteristics (Hunter, 1999; Augusto et al., 2003; Gilliam, 2007; Barbier et al., 2008). While underground vegetation contributes significantly to total biodiversity in temperate forests, it contributes less to total forest biomass (Gilliam, 2007).

There are many disturbance factors in temperate forests. Among these, grazing and tree cutting are among the most important. Grazing and tree cutting cause complexity and instability in species interactions (Fakhireh et al., 2012; Hüseyinova et al., 2013; Xu et al., 2016; Kılıç et al., 2018). The intensity of disturbance allows some species to establish, grow, and reproduce (Pierce et al., 2007; Duru et al., 2010; Frenette-Dussault et al., 2012; Kılıç et al., 2018).

In this study, we examined relationships among disturbance (grazing and tree cutting), environment factors (soil pH, soil nitrogen, soil moisture and light availability) and biodiversity in the temperate deciduous forest.

2. Materials and Method

The study area is located in the Yeşilırmak basin in the central region of Turkey. The study area is located

between 400 m and 1100 m in altitude (Fig. 1). The study area has between oceanic and continental climates. The mean annual temperature and the mean annual precipitation are 13.9°C and 397.5 mm, respectively. The maximum mean temperature is 31.7 °C (August), while the lowest mean temperature is − 0.6 °C (January). The vegetation consists of Irano-Turanian and Mediterranean species. Natural flora has been affected by grazing and tree cutting.

Taxonomic nomenclature followed was that of Davis (1965-1985) and Davis et al. (1988), Tutin and Heywood (1964-1980), Güner et al. (2000) and Güner et al. (2012). Four plots were selected from floristically and structurally homogeneous places according to the goal of study. Ten relevés was established for each plot, and the size of plots was determined according to the minimal area method (Westhoff and van Der Maarel, 1978). A cover-abundance value for each species in each relevés was determined using the Braun-Blanquet (1964) scale.

Soil samples for each relevés were taken at a depth of 35 cm. Soil pH values were measured using deionized water (1:1) by pH meter (Kacar, 2012). Soil nitrogen was determined by the way of micro-Kjeldahl method (Bradstreet, 1954). Water content was determined by the gravimetric method (Bayrakli, 1987, Kutbay and Ok, 2003). Light availability was determined using a Lutron Light Meter LX-1102 (Schuster and Diekmann, 2005).

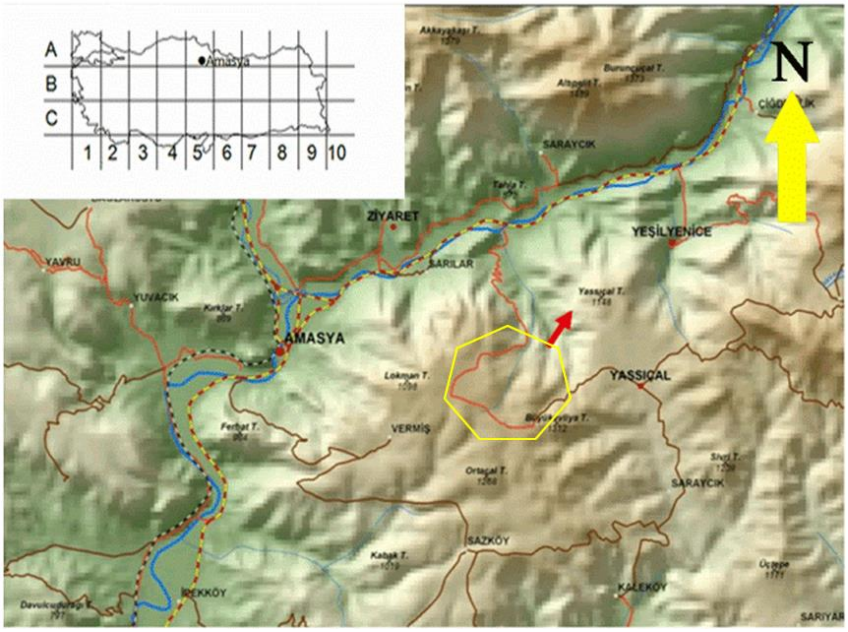

Figure 1. Map of the study area

The Shannon – Wienner diversity indices of the plant communities were calculated using the following formula (Magurran, 2004).

$$H = \sum_{i=1}^{s} pi \; x \; lnpi$$

"s" is the total number of recorded species, "pi" is the proportion of percentage cover of the "i"th species to the sum of the percentage cover of all species and ln is the natural logarithm.

Evenness was quantified using Shannon's indices. Indices of the plant communities were calculated using the following formula (Magurran, 2004).

$$J = H'/Hmax$$

where Hmax is maximum species diversity and calculated as $\log_2$ Pi.

Beta diversity is defined as spatial heterogeneity or pattern diversity was calculated using the Whittaker formula (Whittaker, 1960; Gulsoy and Ozkan, 2008).

$$\beta = S/\alpha - 1$$

where S is the total number of species, α is the mean species richness.

Plant communities according to disturbance factors were separated by using TWINSPAN procedure. To determine what environmental factors were significant, we also treated our data with Detrended Correspondence Analysis (DCA) and Principal Component Analysis (PCA). Numerical methods were performed by using the "Community Analysis Package 4 version" software (Seaby and Henderson, 2007).

Statistical analysis was performed by using a SPSS (25.0 version) software. The differences among plant communities were investigated by one-way ANOVA. The biodiversity parameters were assessed by Tukey's significant difference (HSD) test to rank the means.

3. Results

TWINSPAN analysis revealed four plant communities. They are grazing, non-grazing, cutting and non-cutting plant communities. Diagnostic species of the grazed area are *Acantholimon acerosum* (Willd.) Boiss. var. *acerosum*, *Achillea setacea* Waldst. et Kit, *Carduus pycnocephalus* L. subsp. *albidus* (Bieb.) Kazmi, *Globularia trichosantha* Fisch, and *Juniperus foetidissima* Wild., while the ungrazed areas are characterized by *Avena sterilis* L., *Calepina irregularis* (Asso) Thell., *Capsella bursa-pastoris* (L.) Medik., *Hordeum vulgare* L., *Taraxacum officinale* (L.) Weber ex F.H.Wigg., and *Urtica dioica* L.

diagnostic species. Diagnostic species of tree cutting areas are *Cerasus mahaleb* (L.) Miller var. *mahaleb* (L.) Miller, *Cistus creticus* L., *Colutea arborescens* L, *Cruciata taurica* (Pallas ex Willd.) Ehrend, *Jasminum fruticans* L., *Pistacia terebinthus* L. subsp. *palaestina* (Boiss.) Engler, *Polygala pruinosa* Boiss. subsp. *pruinosa* Boiss., and *Vicia narbonensis* L., while uncutted areas are characterized by *Amelanchier rotundifolia* (Lam). Dum.-Courset, *Arbutus andrachne* L., *Globularia trichosantha* Fisch, *Juniperus oxycedrus* L. subsp. *oxycedrus*, *Phillyrea latifolia* L., *Quercus hartwissiana* Steven, and *Q. petraea* (Mattuschka) Liebl. diagnostic species (Fig. 2).

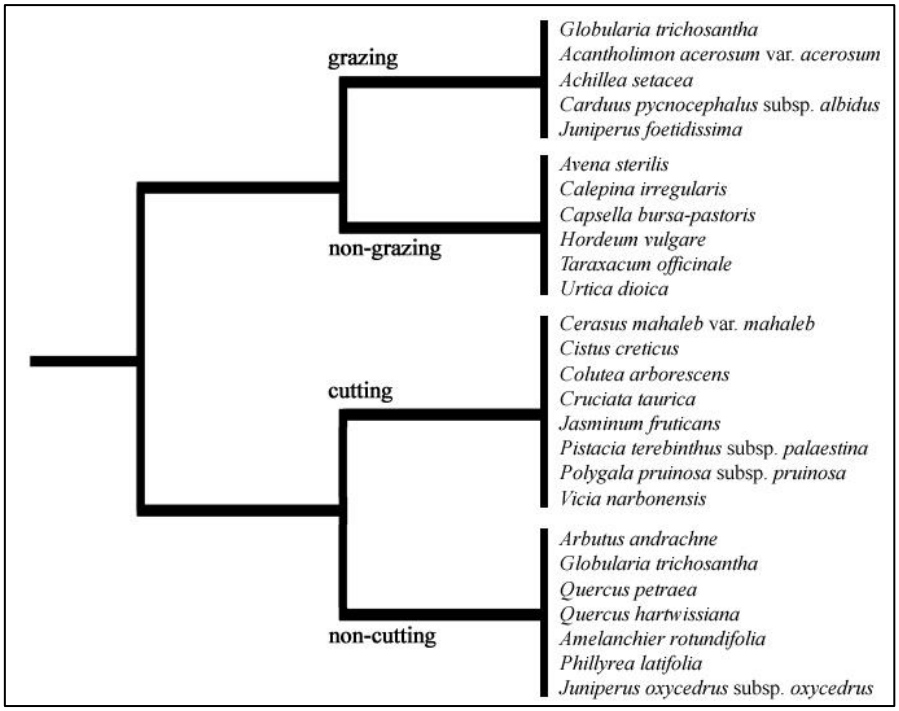

Figure 2. Plant communities considering disturbance factors in the study area resulting from the TWINSPAN analysis.

Detrended correspondence analysis (DCA) diagram showed the existence of the gradient considering the first axis (Eigenvalue of axis 1 is 0.96). It is an elevation gradient. Plant communities have spread depending on the elevation. Plant communities in non-grazing and non-cutting areas grouped at the left of ordination plot, whereas plant communities in cutting and grazing areas grouped at the right of the ordination plot (Fig. 3).

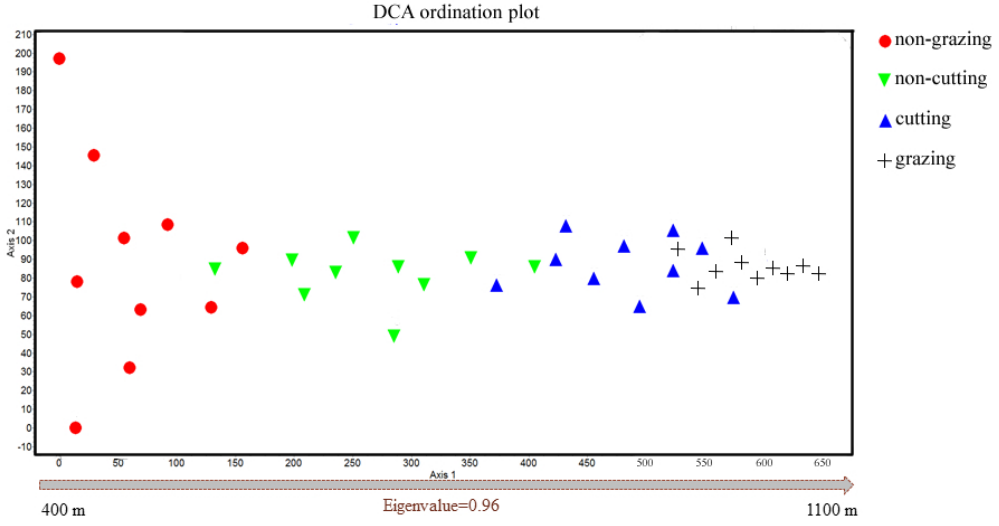

Figure 3. Result of the detrended correspondence analysis (DCA) withthe ordination diagram showing plant communities' position.

The first two axes explained 81.79 % total variance of the Principal Component Analysis (PCA). PCA revealed that pH, soil nitrogen content (%), soil water content were found to be significant in axis 1, while light availability was found to be significant in axis 2. Soil nitrogen (%) and soil water content were negative in axis 1, while soil pH and light availability were positive in axis 1 and 2, respectively (Table 1).

Light availability was positively correlated with plant communities in cutting areas, While soil nitrogen content and water content were negatively correlated plant communities in grazing areas. pH was positively correlated with plant communities in non-grazing areas (Fig. 4).

Table 1. Eigenvalues for studied environmental factors (Significant values were marked in bold).

	Axis 1	Axis 2
Soil pH	**0.508**	-0.266
Soil nitrogen content (%)	**-0.568**	-0.450
Soil moisture	**-0.646**	0.229
Light availability	0.033	**0.820**

Species diversity indices (H and J) were high in non-cutting and non-grazing plant communities compared to the other areas. Beta diversity was high in cutting and grazing plant communities as compared to the other areas. Statistically significant differences were found among the beta diversities with respect to plant communities (Table 2).

4. Discussions

The effects of environmental and disturbance factors on plant communities in terrestrial ecosystems are significant (Davies et al., 2007; Pausas and Austin, 2001). These factors affect the establishment, growth and reproduction of species (Pierce et al., 2007; Duru et al., 2010; Frenette-Dussault et al., 2012). According to TWINSPAN and DCA analysis, we found the main four plant communities: grazing, cutting, non-cutting and non-grazing. These communities are distributed according to altitude. Because topographic factors (altitude, geographical aspect, and slope) are primary factors of vegetation distribution (Mark et al., 2000) and affecting plant diversity (Vujnovic et al., 2002).

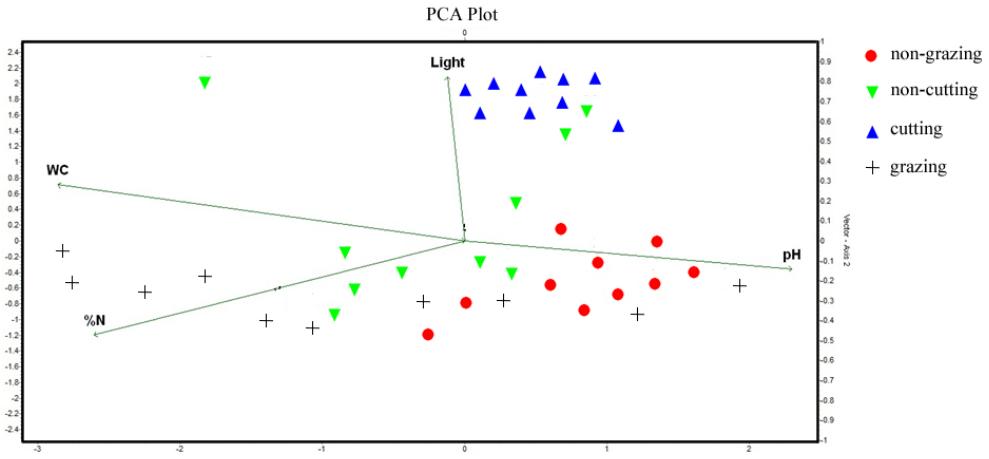

Figure 4. PCA analysis of the among environmental factors and plant communities.

PCA analysis showed that pH, soil nitrogen content (%), soil water content and pH were determining factors of vegetation distribution. In the cutting area, light availability is the main determining factor on species composition, while in the non-cutting area, soil pH and nitrogen content are determining factors (Chai et al., 2016; Tardella et al., 2016). Additionally, unpalatable and thorny species are dominant in grazing vegetation, and fast-growing species are dominant in non-grazing vegetation (Tardella et al., 2016; Kılıç et al., 2018).

Table 2. Diversity indices of plant communities (Different lowercase letters indicate significant differences)

	Grazing	Non cutting	Non grazing	Cutting	*Sig.*
Shannon–Wienner H indice	0.882±0.024**a**	0.973±0.012**a**	0.921±0.008**a**	0.890±0.011**a**	0.352**ns**
Shannon J indice	1.016±0.031**a**	1.062±0.027**a**	1.229±0.019**a**	1.235±0.009**a**	0.244**ns**
β indice	3.481±0.240**c**	0.367±0.004**a**	0.215±0.003**a**	1.137±0.021**b**	0.022*****

Overgrazing in meadows and pastures damages the ground flora and prevents the regeneration of dominant species (Malik et al., 2016). However, Pettit et al. (1995) stated that overgrazing increases the proportion of unrelated species.

When evaluated results obtained, Shannon-Wienner diversity indexes of grazing vegetation were lower than the other vegetation types (Zhao et al., 2007; García et al., 2009; Tälle et al., 2016; Faria et al., 2018).

It has been found that grazing has a significant effect on species richness and diversity and that the number of species and diversity indexes are lower in these areas (Lu et al., 2017; Tälle et al., 2016). Besides, it has been shown that overgrazing negatively affects bush and tree species and thus decreases species richness (Roder et al., 2002; Kumar and Shahabuddin, 2005).

In cutting vegetation, light availability is the main factor (Tardella et al., 2016). Cutting causes permanent grazing gaps, and grassland species are recolonized (Dzwonko and Loster, 1998). These areas are called wood-pastures. If regeneration fails, wood-pastures become permanent. (Bergmeier et al., 2010). In non-cutting vegetation, canopy species have an excellent availability to take light as compared to subcanopy species. Besides, soil pH and nitrogen content affect ground flora formations (Augusto et al., 2003; Chai et al., 2016).

Species diversity indices (H and J) were high in non-cutting vegetation compared to the cutting vegetation. The ground flora diversity and composition is influenced by the species composition of the canopy species (Barbier et al., 2008; Gilliam, 2007; Hunter, 1999). Beta diversity was higher in cutting and grazing plant communities than the other plant communities.

Overgrazing harms the ground flora in meadows and pastures, preventing the regeneration of dominant species (Malik et al., 2016). Also, the disappearance of shrub and tree species causes the species richness to decrease

gradually (Roder et al., 2002; Kumar and Shahabuddin, 2005). Considering the results obtained, it was consistent with previous studies (Faria et al., 2018). Tree cutting and forestry studies increase habitat heterogeneity (Bergmeier et al., 2010).

Paying attention to the protection of biological diversity in forestry activities should be the main goal of sustainable forest management. In this study, we revealed that disturbance and environmental factors affect vegetation types and species composition.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

References

Augusto L, Dupouey JL, Ranger J (2003). Effects of tree species on understory vegetation and environmental conditions in temperate forests. Annals of Forest Science 60: 823-831.

Barbier S, Gosselin F, Balandier P (2008). Influence of tree species on understory vegetation diversity and mechanisms involved – a critical review for temperate and boreal forests. Forest Ecology and Management 254: 1-15.

Bayrakli F (1987). Plant and soil analysis. Samsun: OMU Faculty of Agriculture.

Bergmeier E, Petermann J, Schroder E(2010). Geobotanical survey of wood-pasture habitats in Europe: Diversity, threats and conservation. Biodiversity and Conservation 19: 2995-3014.

Borchsenius F, Nielsen PK, Lawesson JE (2004). Vegetation structure and diversity of an ancient temperate deciduous forest in SW Denmark. Plant Ecology 175: 121-135.

Bradstreet RB (1954). Kjeldahl method for organic nitrogen. Analytical Chemistry 26(1): 185-187.

Braun-Blanquet J (1964). Pflanzensoziologie-Grundzüge der vegetationskunde. Springer Verlag, Wien and New York.

Chai Y, Yue M, Wang M, Xu J, Liu X, Zhang R, Wan P (2016) Plant functional traits suggest a change in novel ecological strategies for dominant species in the stages of forest succession. Oecologia 80:771-783.

Davies KW, Bates JD, Miller RF (2007). Environmental and vegetation relationships of the *Artemisia tridentata* spp. *wyomingensis* alliance. Journal of Arid Environments 70: 478-494.

Davis PH (1965-1985). Flora of Turkey and the East Aegean Islands, Vol. 1-9. Edinburgh: Edinburgh Univ. Press.

Davis PH, Mill RR, Tan K (1988). Flora of Turkey and the East Aegean Islands, Vol. 10 (Suppl.). Edinburgh: Edinburgh Univ. Press.

Duru M, Ansquer P, Jouany C, Theau JP, Cruz P (2010). Comparison of methods for assessing the impact of different disturbances and nutrient conditions upon functional characteristics of grassland communities. Annals of Botany 106:823-831.

Dzwonko Z, Loster S (1998). Dynamics of species richness and composition in a limestone grassland restored after tree cutted. Journal of Vegetation Science 9:387-394.

Fakhireh A, Ajorlo M, Shahryari A (2012). The autecological characteristics of *Desmostachya bipinnata* in hyper-arid regions. Turkish Journal of Botany 36:690-696.

Faria N, Peco B, Carmona CP (2018). Effects of haying on vegetation communities, taxonomic diversity and sward properties in mediterranean dry grasslands: A preliminary assessment. Agriculture, Ecosystems & Environment 251: 48-58.

Frenette-Dussault C, Shipley B, Léger JF, Meziane D, Hingrat Y (2012). Functional structure of an arid steppe plant community reveals similarities with Grime's C-S-R theory. Journal of Vegetation Science 23:208-222.

García RR, Jáureguı BM, García U, Osoro K, Celaya R (2009). Effects of livestock breed and grazing pressure on ground-dwelling arthropods in Cantabrian heathlands. Ecological Entomology 34(4): 466-475.

Gazol A, Ibánez R (2009). Different response to environmental factors and spatial variables of two attributes (cover and diversity) of the understorey layers. Forest Ecology and Management 258: 1267-1274.

Gilliam FS (2007). The ecological significance of the herbaceous layer in temperate forest ecosystems. Bioscience 57: 845-858.

Gonzalez M, Ladet S, Deconchat M, Cabanettes A, Alard D, Balent G (2010). Relative contribution of edge and interior zones to patch size effect on species richness: an example for woody plants. Forest Ecology and Management 259: 266-274.

Guner A, Aslan S, Ekim T, Vural M, Babaç MT (2012). Türkiye bitkileri listesi (damarlı bitkiler). İstanbul: Flora Araştırmaları Derneği ve Nezahat Gökyiğit Botanik Bahçesi Yayınları.

Guner A, Ozhatay N, Ekim T, Başer KHC (2000). Flora of Turkey and the Aegean Islands, Vol. 11 (Suppl. 2). Edinburgh: Edinburgh Univ. Press.

Gülsoy S, Özkan K (2008). Tür çeşitliliğinin ekolojik açıdan önemi ve kullanılan bazı indisler. Turkish Journal of Forestry 9(1): 168-178.

Härdtle W, von Oheimb G, Westphal C (2003). The effects of light and soil conditions on the species richness of the ground vegetation of deciduous forests in northern Germany (Schleswig-Holstein). Forest Ecology and Management 182: 327-338.

Harper KA, Macdonald SE, Burton PJ, Chen JQ, Brosofske KD, Saunders SC, Euskirchen ES, Roberts D, Jaiteh MS, Esseen PA (2005). Edge influence on forest structure and composition in fragmented landscapes. Conservation Biology 19: 768-782.

Hofmeister J, Hosek J, Modry M, Rolecek J (2009). The influence of light and nutrient availability on herb layer species richness in oak-dominated forests in central Bohemia. Plant Ecology 205: 57-75.

Hunter ML (1999). Maintaining biodiversity in forest ecosystems. Cambridge: Cambridge University Press.

Hüseyinova R, Kılınc M, Kutbay HG, Kılıc DD, Bilgin A (2013) The comparison of Grime's strategies of plant taxa in Hacı Osman Forest and Bafra Fish Lakes in the central Black Sea region of Turkey. Turkish Journal of Botany 37:725-734.

Kacar B (2012). Toprak analizleri, 3rd ed. Ankara: Nobel Ltd.

Kılıç DD, Kutbay HG, Sürmen B, Hüseyinoğlu R (2018). The classification of some plants subjected to disturbance factors (grazing and cutting) based on ecological strategies in Turkey. Rendiconti Lincei. Scienze Fisiche e Naturali 29(1): 87-102.

Kooijman A (2010). Litter quality effects of beech and hornbeam on undergrowth species diversity in Luxembourg forests on limestone and decalcified marl. Journal of Vegetation Science 21: 248-261.

Korkmaz H, Yalcin E, Kutbay HG, Yildirim C (2016). The influence of environmental factors on the distribution and composition of plant communities in Kizilirmak Valley-Black Sea region, Turkey. Revue d'écologie 71(1): 21-34.

Kumar R, Shahabuddın G (2005). Effects of biomass extraction on vegetation structure, diversity and composition of forests in Sariska Tiger Reserve, India. Environmental Conservation 32(3): 248-259.

Kutbay HG, Ok T (2003). Foliar N and P resorption and nutrient levels along an elevational gradient in *Juniperus oxycedrus* L. subsp. *macrocarpa* (Sibth. & Sm.) Ball. Annals of Forest Science 60:449-454.

Leniere A, Houle G (2006). Response of herbaceous plant diversity to reduced structural diversity in maple-dominated (Acer saccharum Marsh.) forests managed for sap extraction. Forest Ecology and Management 231: 94-104.

Lu X, Kelsey KC, Yan Y, Sun J, Wang X, Cheng G, Neff JC (2017). Effects of grazing on ecosystem structure and function of alpine grasslands in Qinghai-Tibetan Plateau: a synthesis. Ecosphere 8(1): e01656.

Magurran AE (2004). Measuring biological diversity. Oxford: Wiley-Blackwell Publishing.

Malik ZA, Pandey R, Bhatt AB (2016). Anthropogenic disturbances and their impact on vegetation in Western Himalaya, India. Journal of Mountain Science 13(1): 69-82.

Mark AF, Dickinson KJ, Hofstde RG (2000). Alpine vegetation, plantdistribution, life form, and environments a humid New Zealandregion. Arctic, Antarctic, and Alpine Research 32:240-254.

Pausas JG, Austın M (2001). Patterns of plant species richness in relation to different environments: An appraisal. Journal of Vegetation Science 12: 153-166.

Pettit NE, Froend RH, Ladd PG (1995). Grazing in remnant woodland vegetation: changes in species composition and life form groups. Journal of Vegetation Science 6(1): 121-130.

Pierce S, Luzzaro A, Caccianiga M, Ceriani RM, Cerabolini B (2007). Disturbance is the principal α-scale filter determining niche differentiation, coexistence and biodiversity in an alpine community. Journal of Ecology 95:698-706.

Qian H, Klinka K, Sivak B (1997). Diversity of the understory vascular vegetation in 40 year-old and old-growth forest stands on Vancouver Island, British Columbia. Journal of Vegetation Science 8: 773-780.

Roder W, Gratzer G, Wangdi K (2002). Cattle grazing in the conifer forests of Bhutan. Mountain Research and Development 22(4): 1-7.

Schuster B, Diekmann M (2005). Species richness and environmental correlates in deciduous forests of Northwest Germany. Forest Ecology and Management 206:197–205.

Seaby RM, Henderson PA (2007).Community analysis package (4.1.3). Lymington: Pisces Conservation Ltd.

Small CJ, McCarthy BC (2005). Relationship of understory diversity to soil nitrogen, topographic variation, and stand age in an eastern oak forest, USA. Forest Ecology and Management 217: 229-243.

Tälle M, Deák B, Poschlod P, Valkó O, Westerberg L, Milberg P (2016). Grazing vs. mowing: A meta-analysis ofbiodiversity benefits for grassland management. Agriculture, Ecosystems & Environment 222: 200-212.

Tardella FM, Piermarteri K, Malatesta L, Catorci A (2016). Environmental gradients and grassland trait variation: insight into the effects of climate change. Acta Oecologica 76:47-60.

Tinya F, Marialigeti S, Kiraly I, Nemeth B, Odor P (2009). The effect of light conditions on herbs, bryophytes and seedlings of temperate mixed forests in O″ rseg, Western Hungary. Plant Ecology 204: 69-81.

Tutin TG, Heywood VH (1964-1980). Flora Europaea, Vol. 1-5. Cambridge: Cambridge Univ. Press.

van Calster H, Baeten L, Verheyen K, De Keersmaeker L, Dekeyser S, Rogister JE, Hermy M (2008). Diverging effects of overstorey conversion scenarios on the understorey vegetation in a former coppice-with-standards forest. Forest Ecology and Management 256: 519-528.

Vockenhuber EA, Scherber C, Langenbruch, C, Meißner M, Seidel D, Tscharntke T (2011). Tree diversity and environmental context predict herb species richness and cover in Germany's largest connected deciduous forest. Perspectives in Plant Ecology, Evolution and Systematics 13(2): 111-119.

Vujnovic K, Wein RW, Dale MRT (2002). Predicting plant species diversity in response to disturbance magnitude in grassland remnants of central Alberta. Canadian Journal of Botany 80: 504-511.

Westhoff V, Van Der Maarel E (1978). The Braun-Blanquet approach. In Classification of plant communities (pp. 287-399). Dordrecht: Springer.

Whigham DE (2004). Ecology of woodland herbs in temperate deciduous forests. Annual Review of Ecology, Evolution, and Systematics 35: 583-621.

Whittaker RH (1960). Vegetation of the Siskiyou Mountains, Oregon and California. Ecological Monographs 30:279-338.

Xu F, Li M, Zhou D, Liu X, Wang R, Guo W (2016). The response of wetland plant communities to disturbance: alleviation through symmetric disturbance and facilitation. Polish Journal of Ecology 64:327-338.

Zhao WY, Li JL, Qi JG (2007). Changes in vegetation diversity and structure in response to heavy grazing pressure in the northern Tianshan Mountains, China. Journal of Arid Environments 68(3): 465-479.

Anatolian Journal of Botany
5(1): 51-57 (2021)
Research article *doi:10.30616/ajb.893690*

Effects of resveratrol and 1,3-bis(2-chloroethyl)-1-nitrosurea combination on YKG1 glioblastoma cells

Gökhan PEKTAŞ[1*], Esra ASLAN[2], Hilal GÜZEL[3], Betül DEMİRCİLER YAVAŞ[4], Sefa ÇELİK[5]
[1]*Muğla Sıtkı Koçman University, Faculty of Medicine, Department of Hematology, 48000, Mugla, Turkey*
[2]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Histology and Embryology, 03200, Afyonkarahisar, Turkey*
[3]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Anatomy, 03200, Afyonkarahisar, Turkey*
[4]*Private Practice, Traditional and Complementary Treatment Center, 03200, Afyonkarahisar, Turkey*
[5]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Biochemistry, 03200, Afyonkarahisar, Turkey*
**gokhanpektas@gmail.com, [2]dr_esragul@hotmail.com, [3]hilalgzl@hotmail.com, [4]betuldy@gmail.com, [5]sefa_celik@hotmail.com*

Received : 09.03.2021
Accepted : 05.04.2021
Online : 30.04.2021

Resveratrol ve 1,3-bis(2-kloroetil)-1-nitrozüre kombinasyonunun YKG1 glioblastoma hücreleri üzerine etkileri

Abstract: Glioblastoma is a primary malignant brain tumor that can be treated with 1,3-bis(2-chloroethyl)-1-nitrosurea (BiCNU/carmustine). Resveratrol is a natural phenol that can interfere with apoptosis. This study aims to investigate how the combination of BiCNU and resveratrol affects glioblastoma cells in vitro. Accordingly, YKG1 glioblastoma cells were treated with different amounts of resveratrol (50 and 100 µM) and BiCNU (10 and 20 µM) either alone or in combination. Cell viability tests and immunochemical studies were conducted on these cells. According to results, increasing the amount of resveratrol and BiCNU decreased cell viability. Additionally, when these maximal doses of resveratrol and BiCNU (100 µM resveratrol plus 20 µM BiCNU) were applied, viability decreased to the highest cytotoxicity levels. Immunohistochemical analysis also revealed the significantly upregulated H scores of beclin-1 and caspase-3 in treated groups with the highest value in maximally combined concentration. These results indicated the cumulative effects of concurrent administration of BiCNU and resveratrol on the cytotoxicity of malignant human YKG1 glioblastoma cells in vitro.

Keywords: Apoptosis, carmustine, glioblastoma, resveratrol

Özet: Glioblastoma, 1,3-bis (2-kloroetil) -1-nitrosüre (BiCNU / carmustin) ile tedavi edilebilen birincil kötü huylu beyin tümörüdür. Resveratrol, apoptozu engelleyebilen doğal bir fenoldür. Bu çalışma, BiCNU ve resveratrol kombinasyonunun glioblastoma hücrelerini in vitro nasıl etkilediğini araştırmayı amaçlamaktadır. Çalışma kapsamında glioblastoma YKG1 hücreleri, tek başına veya kombinasyon halinde farklı miktarlarda resveratrol (50 ve 100 uM) ve BiCNU (10 ve 20 uM) ile muamele edildi. Bu hücreler üzerinde hücre canlılığı testleri ve immünokimyasal çalışmalar yapıldı. Sonuçlara göre, resveratrol ve BiCNU miktarının artırılması hücre canlılığını kademeli olarak azalttı. Ek olarak, maksimum resveratrol ve BiCNU dozu (100 µM resveratrol artı 20 µM BiCNU) verildiğinde, hücre canlılıkları oldukça azaldı. İmmünohistokimyasal analizler, maksimum kombine konsantrasyonda muamele edilen gruplarda beklin-1 ve kaspaz-3 H skorları düzeylerini önemli ölçüde arttırdığını ortaya çıkarmıştır. Bu sonuçlar, BiCNU ve resveratrolün aynı anda uygulanmasının, malign insan YKG1 glioblastoma hücrelerinin in vitro sitotoksisitesi üzerindeki kümülatif etkilerini göstermiştir.

Anahtar Kelimeler: Apoptozis, karmustin, glioblastoma, resveratrol

Citation: Pektaş G, Aslan E, Güzel H, Demirciler Yavaş B, Çelik S (2021). Effects of resveratrol and 1,3-bis(2-chloroethyl)-1-nitrosurea combination on YKG1 glioblastoma cells. Anatolian Journal of Botany 5(1): 51-57.

1. Introduction

The brain's primary malignant tumor, glioblastoma (GB), is considered the second most frequently encountered brain tumor following the meningioma (Omuro and De Angelis, 2013). It has been reported that GB makes up 12% to 15% of all intracranial tumors and 50% to 60% of astrocytic tumors. This tumor occurs in 3 out of 100.000 individuals per year (Williams, 2014). It has been stated that GB often starts at around 64 years of age, and incidence in males was more common than females (Tian et al., 2018).

GB presents non-specific symptoms, including headache, nausea, vomiting, seizures, changes in personality, and symptoms resembling those of a stroke initially. These symptoms might become worsen rapidly and give rise to unconsciousness (Alexander and Cloughesy, 2017). The causes of GB is unknown in most cases. However, genetic disorders such as neurofibromatosis, Li Fraumeni syndrome and previous radiotherapy might pose risk factors for GB, which might originate from normal neurons or develop from an existing low-grade astrocytoma (Alifieris and Trafalis, 2015). Its diagnosis is based on computational tomography, magnetic resonance imaging, and tissue biopsy (Omuro and De Angelis, 2013; Batash et al., 2017).

Glioblastoma is a neurological pathology with high morbidity and mortality; therefore, its treatment is challenging for clinicians (Adamson et al., 2009). The first reason for this challenge is the susceptibility of brain damage from conventional treatments, and the second is the brain's limited capacity for repairing itself. The third reason is the resistance of the tumor cells to the traditional treatment methods, and the last is the inability of chemotherapeutics to cross the blood-brain barrier (Adamson et al., 2009; Batash et al., 2017).

The cure for GB refers to palliative treatment and other methods that intend to improve patients' survival. These methods consist of surgery, chemotherapy, radiotherapy, and immunotherapy (Alifieris and Trafalis, 2015). Thus, treatment of GB usually involves surgery followed by chemotherapy and radiotherapy. It is well known that more extensive surgical removal is associated with more prolonged survival (Villà et al., 2014). Chemotherapy for GB indicates the administration of vincristine, hydroxyurea, temozolomide, 6-thioguanine, 5-fluorouracil, and 1, 3-bis (2-chloroethyl)-1-nitrosurea (BiCNU) (Desai et al., 2019). BiCNU has been used both for the initial diagnosis of glioma and tumor recurrence via intravenous administration (Xiao et al., 2020). Amongst these, BiCNU which is an alkylating agent called carmustine, has been well established but may cause hepatotoxicity and pulmonary fibrosis (Desai et al., 2019). However, some studies have suggested that BiCNU provides a survival advantage for GB patients (Spiegel et al., 2007). Despite the overall treatment methods, GB usually remits, and the typical survival length following diagnosis is 12 to 15 months. Fewer than 7% of people might survive longer than five years, and without any treatment, the survival period is typically three months (Stoyanov et al., 2018; Witthayanuwat et al., 2018).

Apoptosis is a form of programmed cell death that occurs in multicellular organisms. In contrast to necrosis, which addresses traumatic cell death associated with acute cellular injury, apoptosis is an extremely controlled and regulated process that confers advantages during an organism's life cycle. These advantages especially appear during embryogenesis (Kaczanowski, 2016). Its high rate causes atrophy, but reduced apoptosis might cause uncontrolled cell proliferation, leading to carcinogenesis (Lopez and Tait, 2015; Kaczanowski, 2016).

A natural polyphenol, resveratrol (3, 5, 4'-trihydroxy-trans-stilbene), is produced by grapes, blueberries, raspberries, mulberries, and peanuts naturally (Kataria and Khatkar, 2019). It acts through signaling pathways related to growth factors and receptor tyrosine kinases and interferes with signal transduction by the growth factor β. Experimental studies have demonstrated that resveratrol might impact apoptosis by regulating molecules that comprise caspase-3, p53, peroxisome proliferator-activated receptor, NfκB, Bax, Bcl-2, and apoptotic protease activating factor-1 (Emsen and Turkez, 2017; Rauf et al., 2018).

Chemoprevention is defined as the administration of a pharmacological agent to prevent infection or disease. In malignancy, chemoprevention is initiated to avoid the spread of an existing condition, help differentiate molecular targets, and increase chemotherapeutics' bioavailability (Crusz and Balkwill, 2015). The use of chemoprophylaxis is limited primarily by two factors which are biological risk and financial cost. Since all medications can cause side effects, chemoprevention should only be considered when treatment benefits outweigh the risks. The cost associated with chemoprevention may be prohibitive, mainly when the cost of treatment is high or the target disease's incidence is low (Huang and Mellor, 2014). Resveratrol appears as an appropriate pharmacological for the chemoprevention due to its mild adverse effects and relatively low cost. Its adverse impacts are usually related to long-term use and/or ingestion of higher doses. Nausea, stomach pain, flatulence, and diarrhea are the side effects of resveratrol treatment (Vervandeur-Fasser and Latruffe, 2014). The treatment process is very limited in GB, and there is no effective treatment. Therefore, this study aims to compare the effectiveness of resveratrol as an alternative to BiCNU treatment in the YKG1 glioblastoma cell line and to determine whether it potentiates the effects of each other depending on their possible combination.

2. Materials and Method

YKG1 human glioblastoma cells were obtained commercially (RIKEN BioResource Center, Japan), whereas analytical grade BiCNU (15493-8, SigmaAldrich, USA) and trans-resveratrol (CAS 501-36-0, Santa Cruz, USA) were used for pharmacological treatment.

2.1. Cell Culture

Cells were incubated in culture media, including Dulbecco's Modified Medium with 10% Fetal Calf Serum, 1mM sodium pyruvate, and 2 mM L-glutamine in an incubator with 5% CO_2 and 95% humidity at 37°C. Cells were grown in flasks of 75 cm^2 at an approximate density of $4x10^4/cm^2$. After trypsinization, the cell suspension was transferred to sterile capped tubes and centrifuged at 400g and 25°C for 5 minutes. The cell pellet was mixed with new media that had a volume corresponding to 1/3 of the tube. Twenty µl of this suspension was mixed with 90 µl buffer solution (PBS + %1 FCS) and 100 µl trypan blue in an eppendorf tube. White-colored living cells were counted by a hemocytometer in microscopic examination. When the cell count became sufficient, they were passaged at a ratio of 1:4, allocated into new flasks, and allowed to replicate under convenient incubation conditions. Cell viability was assessed in samples obtained from these culture media.

2.2. Experimental Design

Malignant YKG1 human glioblastoma cells that were passaged and replicated in the laboratory were treated by different doses of BiCNU and resveratrol for 48-h. DMSO (0.1%) was the solvent for the chemicals, and all treatments are summarized in Table 1. All groups contained at least triple biological replicates.

Table 1. Different experimental applications on glioblastoma cells

Group 1	50 µM resveratrol
Group 2	100 µM resveratrol
Group 3	10 µM BiCNU
Group 4	20 µM BiCNU
Group 5	50µM resveratrol + 10 µM BiCNU
Group 6	50µM resveratrol + 20 µM BiCNU
Group 7	100 µM resveratrol + 10 µM BiCNU
Group 8	100 µM resveratrol + 20 µM BiCNU
Group 9	Control (0.1% DMSO)

2.3. Cell Viability Measurement

MTT (3-(4,5-methylthiazol-2-yl)-2,5-diphenyl tetrazolium -bromide) is a colorimetric agent used to access cell viability in vitro. Living cells degrades MMT enzymatically, leading to a color change. This experiment aims to determine the percentage of cells that keep their viability with respect to the control after pharmacologic

agents' treatment. According to the method, after 48 hours of incubation with different amounts of resveratrol and BiCNU alone or in combination, the cells were treated with 20 µl MTT dye (5 mg/ml) for 2 hours. Afterward, MTT was eliminated, and 200 µl DMSO was added to each well. Following an incubation period of 10 minutes, the color change was assessed at a wavelength of 540 nm. Cell viability was accepted as 100% in the control group.

2.4. Immunocytochemistry

Human glioblastoma cells were cultured in 12-well chamber slides, and these cultures were treated by pharmacological agents. At the 48th hour of pharmacological treatment, the growing media were removed, and the cells were fixed by 4% paraformaldehyde. After washing by phosphate buffer saline (PBS), they were put on ice floating in a 0.1% Triton-X100 solution for 15 minutes, and endogenous activation of peroxidation was provided by 3% H_2O_2 for 10 minutes. The cells were washed in PBS three times in 5 minutes and then kept in a blocking solution for 10 minutes. Later, the cells were incubated with primary antibodies of Beclin-1 (ab62472, 1/200) ve Caspase-3 (Sc-56053, 1/200) at room temperature for 1 hour, and the suspensions were washed by PBS three times. After the cells were treated with secondary antibodies, they were incubated with biotin for 30 minutes. Then, the cells were washed by PBS three times in 5 minutes and incubated with avidin for 30 minutes. After the cells were washed by PBS three times in 5 minutes, they were colored by 3-amino-9-ethyl carbazole. Finally, they were washed with distilled water and stained with Mayer's hematoxylin. The cells were counted by a light microscopy under X20 magnification by the Image Analysis Program (NIS, Japan). The percentage of stained cells was specified semi-quantitatively depending on the following score formula.

Score = (Staining scale+1) x (Percentage of cells stained at each scale)

Staining was designated as 0 (no staining), +1 (weak staining), +2 (moderate staining), and +3 (strong staining).

2.5. Statistical Analysis

Data were analyzed by SPSS (Statistical Package for Social Sciences) software version 22.0 (IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test analyzed the distribution of data. Continuous variables were expressed as mean ± standard deviation, and categorical variables were denoted as numbers or percentages where appropriate. Student t-test was used, and P-values less than 0.05 were accepted as significant.

3. Results

3.1. Viability Test Results

Figure 1 indicates the viability test results obtained after 48 hours of pharmacological treatment. Accordingly, cell cytotoxicity was significantly higher in Group 2 (cells treated with 100 µM resveratrol) than Group 1 (cells treated with 50 µM resveratrol). Similarly, cytotoxicity was significantly increased in Group 4 (cells treated with 20 µM BiCNU) than Group 3 (cells treated with 10 µM BiCNU). Compared to other groups, cytotoxicity was significantly higher in Group 8 (cells treated with 100 µM resveratrol + 20 µM BiCNU).

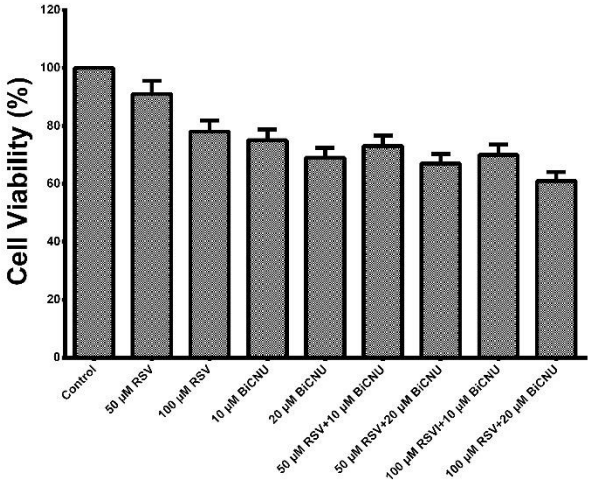

Figure 1. Effects of resveratrol (RSV) and 1,3-bis(2-chloroethyl)-1-nitrosurea (BiCNU) treatment on YKG1 glioblastoma cells' viability after 48-hours of treatment.

3.2. Immunocytochemistry Results

Expression levels of Beclin-1 and Caspase-3 proteins were analysed with immunohistochemical staining, and the results are summarised in Figure 2.

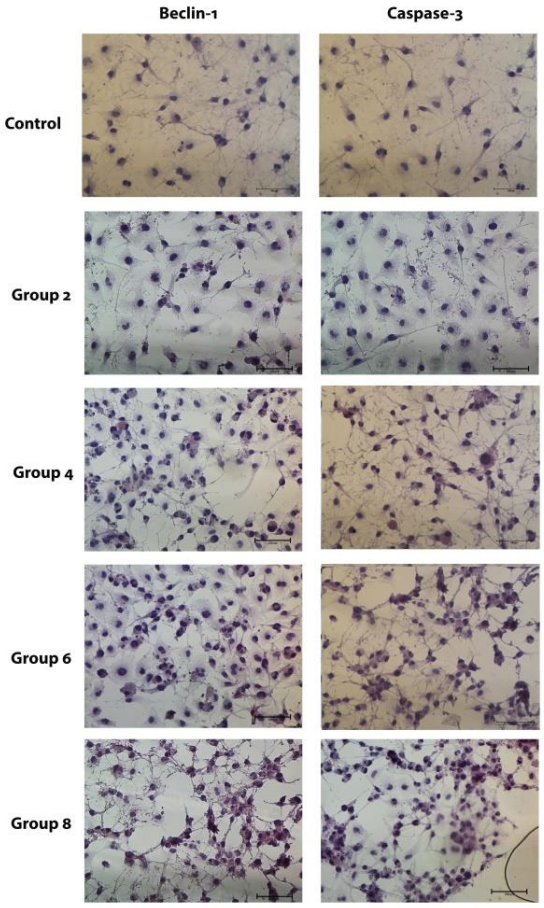

Figure 2. Images of the immunostained YKG1cells, which were treated with different amounts of resveratrol and BiCNU. The cells were stained with Beclin-1, and Caspase-3 antibodies and images were taken by a light microscopy at 20X magnification.

The images of immunostained slides were analysed semi-quantitatively, and Hscore results are summarized in Figure 3.

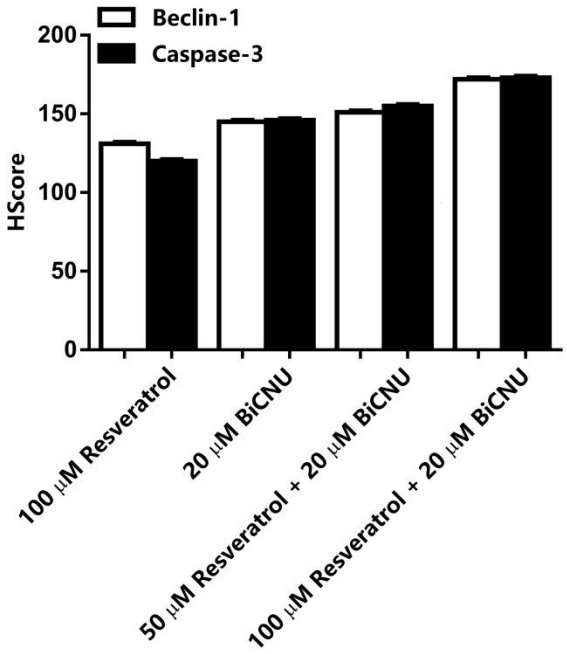

Figure 3. Beclin-1 and Caspase-3 HScore results obtained as a result of immunocytochemical staining.

According to results, the expression of Beclin-1 was significantly higher in Group 8 (cells treated with 100 μM resveratrol + 20μM BiCNU) as compared to Group 2, Group 4, Group 6 respectively ($P < 0.0001$, $P = 0.001$, $P = 0.002$). The expression of Caspase-3 was significantly higher in Group 8 as compared to Group 2, Group 4, and Group 6 ($P < 0.001$, $P < 0.001$ and $P = 0.004$). All groups except Group 8 showed +1 immunostaining while there was both +1 and +2 immunostaining in Group 8 (cells treated with 100 μM resveratrol + 20 μM BiCNU).

4. Discussions

Glioblastoma is the most invasive tumor of the central nervous system with the worst prognosis among the other cancer types. The typical survival duration following diagnosis is around 12 to 15 months (Omuro and DeAngelis, 2013; Alexander and Cloughesy, 2017). The treatment of GB usually involves surgery followed by chemotherapy and radiotherapy. It is well known that larger surgical removal is associated with more prolonged survival (Villà et al., 2014; Alifieris and Trafalis, 2015). However, complete excision of GB is not possible in most cases, and the role of chemotherapy is often limited in preventing recurrences (Anjum et al., 2017). Therefore, an integrated approach should be developed to prolong the patients' survival span diagnosed with this malignancy.

Nitrosoureas, including BiCNU (carmustine), was first introduced to treat gliomas and their effectiveness was modest, but doses that produced response rates up to 50% leaded severe systemic side effects (Nagpal, 2012). Afterward, polymers were developed to maintain the efficacy of BiCNU beyond the blood-brain barrier so that, subsequently, biodegradable polymers might permit more constant drug delivery. Such BiCNU polymers were used to make up carmustine wafer which is also named as BiCNU wafer or Gliadel wafer (Nagpal, 2012; Zhang et al., 2014).

The beneficial effects of carmustine wafer on patients' survival with GB have been highlighted in various studies. A meta-analysis found that treatment with carmustine wafer and temozolomide was significantly more effective than avoiding chemotherapy in improving survival. It was also remarked that the carmustine wafer's clinical efficiency extended up to 24 months (Spiegel et al., 2007). Another systematic review also aimed to examine the potency of carmustine wafer in malignant glioma treatment and included three randomized controlled trials and one prospective cohort study. This systematic review came up with the conclusion that the patients who received the diagnosis of GB for the first time and who were treated with carmustine wafer had a significantly longer survival span than the controls (Perry et al., 2007). Based on the findings of two randomized controlled trials, a Cochrane review concluded that carmustine wafer improved GB patients' survival without significantly contributing to the increase in adverse effects (Hart et al., 2011).

Resveratrol demonstrates many anti-carcinogenic effects on various cancer cells in vitro (Le Corre et al., 2005; Kundu and Surh, 2008; Shukla and Singh, 2011). When evidence from in vitro studies was analyzed, Jang et al. (1997) were the first showing resveratrol as a chemopreventive agent. It was found that topical application of resveratrol could inhibit tumor formation in an animal model of skin cancer. Later studies also confirmed that resveratrol's topical application prevented tumor formation by regulating the cell cycle and endorsing apoptosis, reducing COX activity and prostaglandin production in a skin cancer mouse model (Afaq et al., 2003; Reagan-Shaw et al., 2004). The findings related to in vitro use and efficacy of resveratrol for other types of cancer that require its oral ingestion or intraperitoneal injection has been more controversial. This discrepancy has been attributed to the poor bioavailability of trans-resveratrol. Wenzel & Somoza (2005) clarify the bioavailability and metabolism of resveratrol. Accordingly, as resveratrol is consumed orally in rodents and humans, 70–80% is quickly absorbed via passive diffusion in the intestines (Kaldas et al., 2003; Walle et al., 2004). After absorption, resveratrol is conjugated into glucuronides and sulfates so that trans-resveratrol levels in peripheral circulation reach their peak 30–60 minutes after oral administration (Soleas et al., 2001; Yu et al., 2002).

In humans, circulating unmodified trans-resveratrol levels make up only about 2% of the peak serum concentration of total free resveratrol and conjugates after a single dose of 25 mg/70 kg body weight (Goldberg et al., 2003). Another study has ended up with the conclusion that at least 70% of resveratrol is absorbed after a single 25 mg dose, and there is a peak serum concentration of 2 μM (approximately 490 ng/ml) for resveratrol and all of its metabolites (Walle et al., 2004). After administering multiple oral doses (5 g daily for 29 days), plasma concentrations of trans-resveratrol are as high as 4 μM (4.29 nmol/ml). However, it should be noted that resveratrol at this high dose was also associated with gastrointestinal side effects (Brown et al., 2010). On the contrary, in human colon tissue, resveratrol levels and its

metabolite resveratrol-3-O-glucuronide have been detected in relatively higher concentrations (674 and 86 nmol/g, respectively) when 0.5–1.0 g of resveratrol was taken orally once per day. In the study mentioned above, resveratrol supplementation decreased cellular proliferation by 5% in colorectal cancer tissue, as assessed by Ki67 staining (Patel et al., 2010). Since there are such rapid conjugation and low bioavailability of resveratrol, the in vitro use of resveratrol for cancer prevention and treatment has been a matter of debate.

As for brain cancer, Xu and coworkers were the first reporting the combination of resveratrol, and temozolomide significantly down-regulates the expression of matrix metalloproteinase-9, enhances the production of reactive oxygen species, and inhibits the anti-apoptotic protein Bcl-2. Thus, this combination has been considered to suppress cell proliferation in malignant U87MG glioma cell line. The significant pro-apoptotic effect of resveratrol has been observed through the increase in Bax expression, the decrease in the expression of Bcl-2, and the cleavage of caspase-3 (Xu et al., 2005).

Filippi-Chiela and colleagues were the first to determine that resveratrol induced autophagy formation by the up-regulation of autophagy proteins such as Atg5, Beclin-1, and LC3-II in three human glioblastoma cell lines. Correspondingly, the authors have hypothesized that resveratrol accelerates autophagy inhibition, which results in apoptosis in turn (Filippi-Chiela et al., 2011). Firouzi and coworkers found that methoxyamine and resveratrol can significantly reduce colony numbers and induce DNA damage of glioblastoma spheroid cells. This result reflected the promise of resveratrol at a 20-μM concentration in cancer-treatment therapy when used together with radiation and radiosensitizer (Firouzi et al., 2015). Other researchers demonstrated that resveratrol might reduce the expression and activity of the POK erythroid ontogenic factor (Pokemon) in glioma cells, suppress the Sp1 DNA binding activity, and enhance the recruitment of HDAC1 (Yang et al., 2016). Later, Song et al. showed that administration of 20 μM resveratrol decreases cell viability to a lesser extent and the administration of 40 μM resveratrol decreases cell viability significantly in malignant human LN18 and U87 glioblastoma cell lines (Song et al., 2019). Another study by Cilibrasi et al. found out that resveratrol at a dose of 100 μM had the highest efficiency for cytotoxicity for human glioma stem cells. In that study, cytotoxicity was observed at the minimum resveratrol dose of 50 μM but, interestingly, the resveratrol dose of 200 μM was found to have significantly lower cytotoxicity than the resveratrol dose of 100 μM (Cilibrasi et al., 2017).

In this study, cytotoxicity was significantly higher in the cells treated with 100 μM resveratrol than the cells treated with 50 μM resveratrol. Likely, toxic effects was significantly increased in the cells treated with 20 μM BiCNU than the cells treated with 10 μM BiCNU. Compared to other groups, cell viability was significantly higher in the cells treated with 100 μM resveratrol + 20 μM BiCNU. Moreover, the expression of Beclin-1 and Caspase-3 were significantly higher in cells treated with 100 μM resveratrol + 20μM BiCNU than in the other groups. All groups except those treated with 100 μM resveratrol + 20 μM BiCNU showed +1 immunostaining while there was both +1 and +2 immunostaining in cells treated with 100 μM resveratrol + 20 μM BiCNU. The present study's findings suggest that the concurrent administration of BiCNU and resveratrol has cumulative effects on the cytotoxicity of malignant human YKG1 glioblastoma cells in cell culture. This cytotoxicity is related to both autophagy and apoptosis as it is reflected at least enhanced expression of Beclin-1 and Caspase-3. The present study's findings should be interpreted carefully as their power is limited by the in vitro design of the research and the administration of relatively lower doses of BiCNU and resveratrol to the cell culture. A clinical implication of these findings might be the utilization of resveratrol as a chemoprevention agent in patients receiving BiCNU to treat malignant glioma. To verify this implication, clinical studies should be undertaken to specify the efficacy and safety of concurrent BiCNU and resveratrol treatment in patients diagnosed with malignant glioma. Hence, further research is warranted to clarify the effects of the BiCNU and resveratrol combination for the glioblastoma cells in vitro and the GB patients in vitro.

Conflict of interest

The authors state no conflict of interest.

Author Contribution

GP, EA, HG, BDY, and SÇ performed the research. EA and HG helped during the experimental study and statistical analysis. GP wrote the manuscript. GP and EA drafted the manuscript. GP and EA conceived and designed the study and critically revised the manuscript.

Acknowledgments

The Scientific Research Projects Unit of Afyon Kocatepe University (Grant no: 17.KARİYER.171) funded this study.

References

Adamson C, Kanu OO, Mehta AI, Di C, Lin N, Mattox AK, Bigner DD (2009). Glioblastoma multiforme: a review of where we have been and where we are going. Expert Opinion of Investig Drugs 18(8): 1061-1083.

Afaq F, Adhami VM, Ahmad N (2003). Prevention of short-term ultraviolet B radiation-mediated damages by resveratrol in SKH-1 hairless mice. Toxicology and Applied Pharmacology 186(1): 28-37.

Alexander BM, Cloughesy TF (2017). Adult Glioblastoma. Journal of Clinical Oncology 35(21): 2402-2409.

Alifieris C, Trafalis DT (2015). Glioblastoma multiforme: Pathogenesis and treatment. Pharmacology and Therapeutics 152: 63-82.

Anjum K, Shagufta BI, Abbas SQ, Patel S, Khan I, Shah SAA, Akhter N, Hassan SSU (2017). Current status and future therapeutic perspectives of glioblastoma multiforme (GB) therapy: A review. Biomedicine and Pharmacotherapy 92: 681-689.

Batash R, Asna N, Schaffer P, Francis N, Schaffer M (2017). Glioblastoma Multiforme, Diagnosis and Treatment; Recent Literature Review. Current Medicinal Chemistry 24(27): 3002-3009.

Brown VA, Patel KR, Viskaduraki M, Crowell JA, Perloff M, Booth TD, Vasilinin G, Sen A, Schinas AM, Piccirilli G, Brown K, Steward WP, Gescher AJ, Brenner DE (2010). Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. Cancer Research 70(22): 9003-9011.

Cilibrasi C, Riva G, Romano G, Cadamuro M, Bazzoni R, Butta V, Paoletta L, Dalprá L, Strazzabosco M, Lavitrano M, Giavannonni R, Bentivegna A (2017). Resveratrol impairs glioma stem cells proliferation and motility by modulating the wnt signaling pathway. PLoS One 12(1): e0169854.

Crusz SM, Balkwill FR (2015). Inflammation and cancer: advances and new agents. Natural Reviews Clinical Oncology 12(10): 584-596.

Desai K, Hubben A, Ahluwalia M (2019). The Role of Checkpoint Inhibitors in Glioblastoma. Targeted Oncology 14(4): 375-394.

Emsen B, Turkez H (2017). The protective role of resveratrol against zinc oxide induced nanotoxicity. Anatolian Journal of Botany 1(2): 21-25.

Filippi-Chiela EC, Villodre ES, Zamin LL, Lenz G (2011). Autophagy interplay with apoptosis and cell cycle regulation in the growth-inhibiting effect of resveratrol in glioma cells. PLoS One 6(6): e20849.

Firouzi F, Khoei S, Mirzaei HR (2015). Role of resveratrol on the cytotoxic effects and DNA damages of iododeoxyuridine and megavoltage radiation in spheroid culture of U87MG glioblastoma cell line. General Physiology and Biophysics 34(1): 43-50.

Goldberg DM, Yan J, Soleas GJ (2003). Absorption of three wine-related polyphenols in three different matrices by healthy subjects. Clinical Biochemistry 36(1): 79-87.

Hart MG, Grant R, Garside R, Rogers G, Somerville M, Stein K (2011). Chemotherapy wafers for high grade glioma. Cochrane Database Systematic Review 1(3): CD007294.

Huang L, Mellor AL (2014). Metabolic control of tumour progression and antitumour immunity. Current Opinion in Oncology 26(1): 92-99.

Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275(5297): 218-220.

Kaczanowski S (2016). Apoptosis: its origin, history, maintenance and the medical implications for cancer and aging. Physical Biology 13(3): 031001.

Kaldas MI, Walle UK, Walle T (2003). Resveratrol transport and metabolism by human intestinal Caco-2 cells. Journal of Pharmacy and Pharmacology 55(3): 307-312.

Kataria R, Khatkar A (2019). Resveratrol in Various Pockets: A Review. Current Topics in Medicinal Chemistry 19(2): 116-22.

Kundu JK, Surh YJ (2008). Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. Cancer Letters 269(2): 243-261.

Le Corre L, Chalabi N, Delort L, Bignon YJ, Bernard-Gallon DJ (2005). Resveratrol and breast cancer chemoprevention: molecular mechanisms. Mol Nutrition and Food Research 49(5): 462-471.

Lopez J, Tait SW (2015). Mitochondrial apoptosis: killing cancer using the enemy within. British Journal of Cancer 112(6): 957-962.

Nagpal S (2102). The role of BCNU polymer wafers (Gliadel) in the treatment of malignant glioma. Neurosurgery Clinics of North America 23: 289-295.

Omuro A, DeAngelis LM (2013). Glioblastoma and other malignant gliomas: A clinical review. Journal of American Medical Association 310(17): 1842-1850.

Patel KR, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, West KP, Booth TD, Perloff M, Crowell JA, Brenner DE, Steward WP, Gescher AJ, Brown K (2010). Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. Cancer Research 70(19): 7392-7399.

Perry J, Chambers A, Spithof K, Laperriere N (2007). Gliadel wafers in the treatment of malignant glioma: A systematic review. Current Oncology 14: 189-194.

Rauf A, Imran M, Butt MS, Nadeem M, Peters DG, Mubarak MS (2018). Resveratrol as an anti-cancer agent: A review. Critical Reviews in Food Science and Nutrition 58(9): 1428-1447.

Reagan-Shaw S, Afaq F, Aziz MH, Ahmad N (2004). Modulations of critical cell cycle regulatory events during chemoprevention of ultraviolet B-mediated responses by resveratrol in SKH-1 hairless mouse skin. Oncogene 23(30): 5151-5160.

Shukla Y, Singh R (2011). Resveratrol and cellular mechanisms of cancer prevention. Annals of the New York Academy of Sciences 1215: 1-8.

Soleas GJ, Angelini M, Grass L, Diamandis EP, Goldberg DM (2001). Absorption of trans-resveratrol in rats. Methods in Enzymology 335: 145-154.

Song Y, Chen Y, Li Y, Lyu X, Cui J, Cheng Y, Zheng T, Zhao L, Zhao G (2019). Resveratrol Suppresses Epithelial-Mesenchymal Transition in GB by Regulating Smad-Dependent Signaling. Biomed Research International 2019: 1321973.

Spiegel BM, Esrailian E, Laine L, Chamberlain MC (2007). Clinical impact of adjuvant chemotherapy in glioblastoma multiforme: A meta-analysis. Central Nervous System Drugs 21: 775-787.

Stoyanov GS, Dzhenkov D, Ghenev P, Iliev B, Enchev Y, Tonchev AB (2018). Cell biology of glioblastoma multiforme: from basic science to diagnosis and treatment. Medical Oncology 35(3): 27.

Tian M, Ma W, Chen Y, Yu Y, Zhu D, Shi J, Zhang Y (2018). Impact of gender on the survival of patients with glioblastoma. Bioscience Reports 38(6): BSR20180752.

Vervandier-Fasseur D, Latruffe N (2019). The Potential Use of Resveratrol for Cancer Prevention. Molecules 24(24): 4506.

Villà S, Balañà C, Comas S (2014). Radiation and concomitant chemotherapy for patients with glioblastoma multiforme. Chinese Journal of Cancer 33(1): 25-31.

Walle T, Hsieh F, DeLegge MH, Oatis JE Jr, Walle UK (2004). High absorption but very low bioavailability of oral resveratrol in humans. Drug Metabolism and Disposition 32(12): 1377-1382.

Wenzel E, Somoza V (2005). Metabolism and bioavailability of trans-resveratrol. Molecular Nutrition and Food Research 49(5): 472-481.

Williams DS (2014). Glioblastoma multiforme. Journal of Insurance Medicine 44(1): 62-64.

Witthayanuwat S, Pesee M, Supaadirek C, Supakalin N, Thamronganantasakul K, Krusun S (2018). Survival Analysis of Glioblastoma Multiforme. Asian Pacific Journal of Cancer Prevention 19(9): 2613-2617.

Xiao ZZ, Wang ZF, Lan T, Huang WH, Zhao YH, Ma C, Li ZQ (2020). Carmustine as a Supplementary Therapeutic Option for Glioblastoma: A Systematic Review and Meta-Analysis. In Frontiers in Neurology 11: 1036.

Xu GW, Mymryk JS, Cairncross JG (2005). Pharmaceutical-mediated inactivation of p53 sensitizes U87MG glioma cells to BCNU and temozolomide. International Journal of Cancer 116(2): 187-192.

Yang Y, Cui J, Xue F, Overstreet AM, Zhan Y, Shan D, Li H, Li H, Wang Y, Zhang M, Yu C, Xu ZD (2016). Resveratrol represses pokemon expression in human glioma cells. Molecular Neurobiology 53(2): 1266-1278.

Yu C, Shin YG, Chow A, Li Y, Kosmeder JW, Lee YS, Hirschelman WH, Pezzuto JM, Mehta RG, van Breemen RB (2002). Human, rat, and mouse metabolism of resveratrol. Pharmacological Research 19(12): 1907-1914.

Zhang YD, Dai RY, Chen Z, Zhang YH, He XZ, Zhou J (2014). Efficacy and safety of carmustine wafers in the treatment of glioblastoma multiforme: a systematic review. Turkish Neurosurgery 24(5): 639-645.

Anatolian Journal of Botany
5(1): 58-64 (2021)
Research article *doi:10.30616/ajb.890955*

Some minerals and fatty acid compositions of five different wild edible mushrooms species collected in Tokat and Yozgat provinces in Turkey

Aydın Şükrü BENGÜ[1*], Hakan IŞIK[2], İbrahim TÜRKEKUL[3], Handan ÇINAR YILMAZ[4]
[1,4]*Bingöl University, Health Services Vocational School, Dept. of Medical Services and Techniques, Medical Laboratory Techniques Program, 12000, Bingöl, Turkey*
[2]*Tokat M. Emin Saraç Anatolian Religious High School, 60030, Tokat, Turkey*
[3]*Gaziosmanpaşa University, Science Faculty, Department of Biology, 60250, Tokat, Turkey*
[*]*abengu@bingol.edu.tr,* [2]*hakanbiyoloji@gmail.com,* [3]*ibrahim.turkekul@gop.edu.tr,* [4]*h.platanus@gmail.com*

Received : 04.03.2021
Accepted : 27.04.2021
Online : 01.05.2021

Türkiye'de Tokat ve Yozgat illerinde toplanan beş farklı yenilebilir mantar türünün bazı mineralleri ve yağ asidi bileşimleri

Abstract: The present study was made to determine the fatty acids composition and some minerals of five wild edible mushrooms species (*Agaricus benesii* (Pilát) Pilát, *Amanita vaginata* (Bull.) Lam., *Leccinum aurantiacum* (Bull.) Gray, *Macrolepiota phaeodisca* Bellù, *Sarcodon imbricatus* (L.) P. Karst.). Mushroom specimens, which were our research material, were collected from different localities in Tokat and Yozgat provinces. The minerals were examined in atomic absorption spectrophotometric (AAS) and fatty acids were detected by Gas chromatographic-mass spectrometry system (GC-MS) on dried mushrooms samples. In the result of analyses, six different minerals (Cu, Mn, Zn, Ni, Fe, Al) and six different fatty acids (pentadecanoic, palmitic, palmitoleic, stearic, oleic and linoleic acid) have been identified. The dominant fatty acid in basidiocarps of *A. benesii* and *M. phaeodisca* was linoleic acid (C18:2), and was determined as 62.58%, 45.02%, respectively. The dominant fatty acid in basidiocarps of *A. vaginata*, *L. aurantiacum* and *S. imbricatus* was oleic acid (C18:1), and was determined as 54.32%, 46.98% and 48.67%, respectively. The most abundant mineral in basidiocarp of *S. imbricatus* was Zinc (Zn) with 112.29 mg/kg. Also it was found that aluminium (Al) was the most abundant mineral in other ones with quantities ranging from 93.77–3349.02 mg/kg.

Key words: AAS, GC-MS, nutrition, wild edible mushrooms, Turkey

Özet: Bu çalışma, beş yabani yenilebilir mantar türünün (*Agaricus benesii* (Pilát) Pilát, *Amanita vaginata* (Bull.) Lam., *Leccinum aurantiacum* (Bull.) Gray, *Macrolepiota phaeodisca* Bellù, *Sarcodon imbricatus* (L.) P. Karst.)'un yağ asitleri bileşimini ve bazı minerallerini belirlemek için yapılmıştır. Araştırma materyalimiz olan mantar örnekleri Tokat ve Yozgat illerinin farklı yörelerinden toplanmıştır. Kuru mantar örneklerinde mineraller atomik absorpsiyon spektrofotometrik (AAS) ve yağ asitleri Gaz kromatografik-kütle spektrometri sistemi (GC-MS) ile tespit edilmiştir. Analizler sonucunda altı farklı mineral (Cu, Mn, Zn, Ni, Fe, Al) ve altı farklı yağ asidi (pentadekanoik, palmitik, palmitoleik, stearik, oleik ve linoleik asit) tanımlanmıştır. *Agaricus benesii* ve *M. phaeodisca*'nın basidiokarplarında dominant yağ asidi linoleik asit (C18: 2) olup, sırasıyla %62.58, %45.02 olarak belirlenmiştir. *A. vaginata*, *L. aurantiacum* ve *S. imbricatus*'un bazidiyokarplarında dominant yağ asidi oleik asit (C18: 1) olup, sırasıyla %54.32, %46.98 ve %48.67 olarak belirlenmiştir. *S. imbricatus*'un basidiocarp'ında en bol bulunan mineral 112.29 mg/kg ile Çinko (Zn) idi. Ayrıca 93.77–3349.02 mg/kg arasında değişen miktarlarda alüminyumun (Al) diğerlerinde en bol bulunan mineral olduğu bulunmuştur.

Anahtar Kelimeler: AAS, GC-MS, beslenme, yabani yenilebilir mantar, Türkiye

Citation: Bengü AŞ, Işık H, Türkekul İ, Çınar Yılmaz H (2021). Some minerals and fatty acid compositions of five different wild edible mushrooms species collected in Tokat and Yozgat provinces in Turkey. Anatolian Journal of Botany 5(1): 58-64.

1. Introduction

Fungi are one of organisms having the most diverse in the world. Although it is estimated that there are 1.5 million fungi species in worldwide, 70 thousand of them are in the literature. About 10 thousand species of these recorded fungi are macrofungi including 5020 edible, 1250 inedible, 1010 poisonous, 1820 medicinal (Hawksworth, 1996, 2001; Pekşen, 2013).

Mushrooms are used in medicine as support for the treatment and prevention of many diseases, such as cardiovascular and inflammatory diseases. The nutrients used for these purposes are called nutraceutical. They have antioxidant, antitumor and antimicrobial properties. Mushrooms, one of these nutraceutical foods, attract attention with their low cholesterol, fat and carbohydrate content as well as basic nutritional elements. It has been recommended for people who diet with food having with high protein and low lipid content. Many studies have shown that mushrooms are excellent sources of essential unsaturated fatty acids. The unsaturated fatty acids are precursors for the synthesis of eicosanoids such as prostaglandins that are important for cardiovascular health. Oleic acid from the omega-9 family is a monounsaturated fatty acid, and is produced in our body. It is known as blood cholesterol lowering. Linoleic acid from the omega-6 can not be synthesized by the human body. This fatty acid is very important for human health, especially to regulate blood lipid profiles. In addition, linoleic acid is used in the production of arachidonic acid, which is the polyunsaturated fatty acid from Omega-6 group, in our body (Barros, 2008; Riberio, 2009; Orsine, 2012; Ravikrishnan, 2015; Doğan, 2016).

The elements are inorganic substances that are not produced in the human body and must be taken from

outside with liquid or solid foods. Minerals such as zinc, copper, iron and manganese are found in the human body in small amounts (approximately 0.02% of the total body weight or required in amounts <100 mg/day), and that's why they are called microelements (trace elements). These minerals must be taken from outside with nutrients even if their requirements are low. Zinc (Zn) involved in the structure of some important metabolic enzymes such as polymerase, carbonic anhydrase, peptidase, alkaline phosphatase has important task in the metabolic functions of our body (wound healing, improved resistance against the infections, synthesis of nucleic acids and proteins etc.), and at protection of children against certain diseases. Copper (Cu) is an important trace element involved in multiple enzyme systems such as ascorbic acid oxidase, cytochrome oxidase, monoamine oxidase, superoxide dismutase and lactase. It is also an essential element for the neurologic and hematologic systems. Manganese (Mn) takes part in the activation of enzymes such as superoxide dismutase, glycosyltransferase, pyruvate carboxylase. It is also involved in blood sugar regulation, fat and carbohydrate metabolism, normal brain and nerve functions, and calcium absorption. Iron (Fe) is used in metabolic processes such as DNA synthesis, electron and oxygen transport, production of hemoglobin and myoglobin, synthesis of connective tissue and some hormones. Nickel (Ni) is also accepted as ultra-trace nutrient. The functions of nickel in the human body have not been fully revealed. However, it is thought that it is a cofactor of some enzymes involved in the metabolism of glucose. Also it is a toxic mineral for many systems in human body. Contact with nickel can cause the allergic reactions on skin such as contact dermatitis. Some studies have shown that it causes respiratory cancers (Wada, 2004; Duda-Chodak, 2008; Al-Fartusie, 2017). Aluminum (Al) is the toxic microelement for human body, in spite of the fact that it is the most abundant metal in the earth's crust. It has an inhibitory effect for many biological functions (Kawahara, 2007).

The aim of this study was to examine the composition of some mineral and fatty acids of five edible mushroom species, namely *Agaricus benesii, Amanita vaginata, Leccinum aurantiacum, Macrolepiota phaeodisca,* and *Sarcodon imbricatus.*

2. Materials and Method

2.1. Collection and Identification of Mushroom Samples

During regular field trips, mushroom samples were collected from different regions in Tokat and Yozgat provinces and fatty acid and mineral analysis were made. The habitats, localities and families of the samples are given in Table 1.

The fresh specimens were photographed in the field, and their macroscopic and ecological features were noted. They were brought to the laboratory and the collection numbers were given to each. Afterly the specimens were dried and put into polyethylene bags for later studies. Characteristics of microscopical structures were investigated under a light microscope by mounting them in some reagents (lactofenol stain, Melzer's reagent, congo red, KOH 5%, distilled water etc). The specimens were identified based on their ecological, macroscopic and microscopic features with the literature such as Philips (1981), Moser (1983), Bon (1987), Jordan (1995) and Breitenbach and Kränzlin (1995). The examined specimens were deposited in the Fungarium of Biology Department, Gaziosmanpaşa University, Tokat, Turkey.

2.2. Fatty acid analysis

The fatty acids were detected by gas chromatographic-mass spectrometry instrument (GC-MS, Agilent 7890 GC/5970 MS Series-Santa Clara, CA, USA) using the conditions in Table 2, and a high polarity capillary column (HP-88, 100 m × 0.25 mm, 0.20 um film (Part no: 112-88A7, Agilent, Santa Clara, CA, USA). Dried and ground mushroom samples were used in chemical analyzes. The method of Hara and Radin (1978), for lipid extraction and Christie (1990), Christie (1998) and Wretensjö (1990), process to obtain methyl esters was revised and used. For this purpose, 5 g of dried mushroom samples were taken and broken in 10 mL of hexane/isopropanol (3:2) in homogenizer at 10.000 rpm for 30 seconds. After then, the mixture was centrifuged at 5000 rpm for 10 minutes, the upper part was taken, and filtered and then it was put into test tubes. The lipid extract was transferred to cap tubes (30 mL) to prepare the methyl ester. 5 mL of 2% methanolic sulfuric acid was added into the extract and it was vortexed. This mixture was held for methylation in the incubator at 50˚C for 15 hours. After then, the tubes were removed from the incubator and cooled to room temperature and vortexed with the addition of 5 mL of 5% NaCl. The methyl esters of the fatty acids formed in the tubes were extracted with 5 mL of hexane. The hexane phase was taken from the top with a Pasteur pipette and treated with 5 mL of 2% $KHCO_3$ and kept for 1-2 hours to separate the phases. The solvent of the mixture containing the methyl esters was evaporated under nitrogen at 45˚C. Fatty acids at the under of the test tubes were dissolved with 1 mL of hexane and analyzed with GC-MS by transferring to dark GC vials. SGE Analytical (BP×90 100 m × 0.25 mm × 0.25 um) column (Australia) and Agilent brand GC-MS instrument were used in our study. The temperature was gradually increased from 120˚C to 250˚C within 45 minutes and kept at this temperature during the analysis. In the analysis of the samples, Helium (He) was selected as the carrier gas. The system was calibrated with the standard fatty acid samples and natural fatty acids in the samples were determined. All analyzes were carried out in triplicate and average of the results were taken. The results of fatty acids methyl ester were reported as percentage (Bengü, 2019).

2.3. Mineral analysis

The minerals were examined in atomic absorption spectrophotometric instrument (AAS, Perkin Elmer brand AAS 800 Model, USA). In the preparation of the samples, approximately 0.5 g of the dried mushroom samples were weighed and transferred to the microwave oven teflon containers, and 10 mL nitric acid was added to each sample and burned in the microwave. The samples were read with each element wavelength, specific lamp, and standard graphics in AAS studies. The studies had been made in the form of three repetitions and were averaged. The data of mineral analysis were reported as mg/kg.

3. Results

The results of fatty acid analysis of the five wild edible macrofungi species and the amounts of total saturated (ΣSFAs), unsaturated (ΣUFAs), monounsaturated (ΣMUFAs) and polyunsaturated fatty acids (ΣPUFAs) in the analyzed samples were given in Table 3.

According to the results, six different saturated (pentadecanoic, palmitic and stearic acid) and unsaturated fatty acids (palmitoleic, oleic and linoleic acid), which carbon chain lengths ranging from 14-24, have been detected in quantities ranging from 1.61% to 62.58% from five wild edible mushrooms (*A. benesii*, *A. vaginata*, *L. aurantiacum*, *M. phaeodisca*, *S. imbricatus*) collected from different localities in Tokat and Yozgat provinces in Turkey. As a result of the analysis of the samples, short chain fatty acids could not be detected due to the destruction and loss of fatty acids as a result of the temperature applied during the preparation and methylation of the extraction. It has been reported that short-chain fatty acids, which are liquid at room temperature, evaporate easily at high temperatures (Woldegiorgis, 2015). The highest rate of total saturated fatty acid was found in *M. phaeodisca* with 43.11% due to the high levels of palmitic acid (30.70%), while the lowest of total saturated fatty acid was found in *L. aurantiacum* with 21.65%. The highest rate of total unsaturated fatty acid was determined in *L. aurantiacum* with 78.35% due to the high levels of oleic acid (46.98%) and linoleic acid (31.37%), while the lowest of total unsaturated fatty acid was determined in *M. phaeodisca* with 56.89%. Also it was determined that the unsaturated fatty acid levels (ΣMUFAs+ΣPUFAs) in all of the analyzed mushroom species samples was higher than the saturated fatty acid levels (Table 3). This result is consistent with previous studies such as Ribeiro et al. (2009), Ravikrishnan et al. (2015), Yılmaz et al. (2006), Ergönül et al. (2012), Doğan and Akbaş (2013), Goyal et al. (2015), Pietrzak-Fiećko et al. (2016), Türkekul et al. (2017), Bengü (2019) and Bengü et al. (2019). ΣPUFAs amount was higher in *A. benesii* and *M. phaeodisca* samples, and ΣMUFAs was higher in *A. vaginata* and *S. imbricatus*. Because of the major fatty acid was linoleic acid, which is the precursor of mushroom alcohol (1-octen-3-ol), was found in the samples of *A. benesii* and *M. phaeodisca* with 62.58%¬45.02%, respectively. The major fatty acid was oleic acid in the samples of *A. vaginata*, *L. aurantiacum* and *S. imbricatus* with 54.32%, 46.98% and 48.67%, respectively. While pentadecanoic acid with 1.83% was observed only in *L. aurantiacum* species, palmitoleic acid was observed only in *M. phaeodisca* species. Palmitic, stearic, oleic and linoleic acid were determined in different amounts in all mushroom samples (Table 3). The main fatty acid in mushrooms was found to be linoleic acid, followed by oleic acid and palmitic acid as were in many studies, such as Akyüz (2011) Goyal (2015), Pietrzak (2016) and Bengü et al. (2019). Our results are consistent with the results of these studies.

In literature review, any work on the fatty acid profiles of *A. benesii* could not detected. In our studies made on this mushroom; palmitic, stearic, oleic and linoleic acids were detected with proportions of 19.95%, 12.59%, 4.88% and 62.58%, respectively (Table 3). In a similar study made with *Agaricus bisporus* and *Pleurotus sajor caju* by Goyal et al. (2015), seven different fatty acids have been identified including palmitic, stearic, oleic and linoleic acid. Arachidic acid, which we could not detect in our study, was found in studies made with *Agaricus bisporus* and *A. campestris* by Yılmaz et al. (2006). In a study made on natural specimens of *A. bisporus* by Bengü et al. (2019), nine different fatty acids (myristic, penthadecanoic, palmitic, heptadecanoic, stearic, oleic, linoleic, eicosenoic, behenic acid) have been identified. Unlike this result, myristic, penthadecanoic, heptadecanoic, eicosenoic, behenic acid could not be detected in *A. benesii* samples which we used in our studies.

In our studies made with *Amanita vaginata*, four different fatty acids (palmitic, stearic, oleic and linoleic acid) were found with proportions of 20.68%, 4.81%, 54.32% and 20.19%, respectively (Table 3). The fatty acids of *A. rubescens* has been analyzed in a similar study made by Ribeiro et al. (2009). Twenty one different fatty acids have been detected in the result of this study. The rates of palmitic, stearic, oleic and linoleic acid which we were identified in our work were higher than the others. In a study made by Karliński et al. (2007), *A. muscaria* and *A. rubescens* were analyzed for the determination of fatty acids content. The dominant fatty acid in basidiocarps of *A. muscaria* was linoleic acid. In addition tridesilic acid (C13:0) was found to be the dominant fatty acid in *A. rubescens.*

In the chemical analysis of *L. aurantiacum*, five different fatty acids (pentadecanoic, palmitic, stearic, oleic and linoleic acid) were found with proportions of 1.83%, 14.93%, 4.89%, 46.98% and 31.37%, respectively (Table 3). Pentadecanoic acid could only be detected in this mushroom among the fungi which we analyzed. In this study made to determine fatty acid composition of *L. aurantiacum* species by Pedneault et al. (2006), the major fatty acid was linoleic acid. In our studies, we found that the highest amount of fatty acid is oleic acid. In the study made with *L. scabrum* by Karliński et al. (2007), linoleic acid was identified to be the most abundant fatty acid with proportions of 72.6%. In addition, in this study made on *L. scabrum* by Dembitsky et al. (2010), oleic acid and linoleic acid was found to be the most abundant fatty acids with proportions of 31.7% and 45.8%, respectively.

In literature searches, any work made on the fatty acid profiles of *M. phaeodisca* could not detected. However, there are studies made with similar species such as *Macrolepiota procera*, *M. mastoidea*. Palmitic, palmitoleic, stearic, oleic and linoleic acid were found with proportions of 30.70%, 2.01%, 12.41%, 9.86% and 45.02%, respectively in analysis that we made with the test samples of *M. phaeodisca* (Table 3). In the result of study made by Barros et al. (2008), it was detected that the proportion of palmitic, oleic and linoleic acid was higher than others in the dry samples of *Macrolepiota procera* and *Macrolepiota mastoidea*. In this study made on *Macrolepiota procera* by Fernandesa et al. (2013), linoleic acid was detected as the major fatty acid compared to other fatty acids.

In the chemical analysis of *S. imbricatus*, four different fatty acids (palmitic, stearic, oleic and linoleic acid) were found with proportions of 15.95%, 8.93%, 48.67% and 26.45%, respectively (Table 3). Pentadecanoic and palmitoleic acid were not found in our samples. Oleic and

linoleic acid were detected in our study as the major fatty acids. The studies made on dry samples of *S. imbricatus* by Barros et al. (2007) were showed that the major fatty acids were palmitic, stearic, oleic and linoleic acids as in our results.

According to the results of mineral analysis, six different minerals (Cu, Mn, Zn, Ni, Fe, Al) have been detected in quantities ranging from 4.81 mg/kg to 3349.02 mg/kg from five wild edible mushrooms (*A. benesii*, *A. vaginata*, *L. aurantiacum*, *M. phaeodisca*, *S. imbricatus*) collected from different localities in Tokat and Yozgat provinces in Turkey (Table 4, Figure 1).

While copper (Cu) was found at the highest level in *S. imbricatus* with 66.16 mg/kg, it was found at the lowest level in *L. aurantiacum* with 41.11 mg/kg. Manganese (Mn) was found at most in *A. vaginata* with 69.90 mg/kg, it was found at lowest in *S. imbricatus* with 7.18 mg/kg. The lowest and highest levels of Zinc (Zn) were found in *M. phaeodisca* (with 53.68 mg/kg) and *S. imbricatus* (with 112.29 mg/kg), respectively. In our research, the amount of nickel (Ni) was found to be higher in *A. vaginata* with 15.29 mg/kg than the others, and the lowest value of this mineral was measured in *M. phaeodisca* with 4.81 mg/kg. Iron (Fe) mineral was measured at the most level in *A. vaginata* with 1631.86 mg/kg, and the least level of it was observed in *S. imbricatus* with 35.45 mg/kg was observed at the least amount in this mushroom. In addition, the analysis showed that aluminum (Al) was at the highest level in *A. vaginata* with 3349.02 mg/kg and was at the lowest level in *M. phaeodisca* with 93.77 mg/kg. In result the highest concentration of Mn, Ni, Fe and Al was observed in *A. vaginata* with 69.90, 15.29, 1631.86, 3349.02 mg/kg, respectively, and the the highest concentration of Cu and Zn was observed in *S. imbricatus* with 66.16–112.29 mg/kg, respectively.

In the present study, Cu, Mn, Zn, Ni, Fe, Al contents were 58.96, 15.74, 97.43, 9.62, 85.95 and 309.10 mg/kg in *Agaricus benesii* and 60.25, 69.90, 104.61, 15.29, 1631.86 and 3349.02 mg/kg in *Amanita vaginata*, respectively (Table 4). In study made for *Agaricus xanthodermus* by Jonnalagadda et al. (2006), Fe and Mn have been detected to be 306 mg/kg and 30 mg/kg, respectively. *Agaricus bisporu*s from *Agaricus* genus is one of the most studied fungi in the world. In study made on *A. bisporu*s by Işıldak et al. (2004), they found that Fe was the dominant element followed by Cu and Zn. *Amanita vaginata* is an edible macrofungi that naturally collected and consumed in many regions in Turkey. Cu, Mn, Zn, Ni, Fe, Al were determined in quantities ranging from 15.29 mg/kg to 3349.02 mg/kg in our *A. vaginata* samples collected from different area of Tokat province. The study made on *A. vaginata* by Radulescu et al. (2010). Cu, Mn, Zn, Ni, Fe were determined in quantities ranging from 0.7 mg/kg to 112.10 mg/kg. Zn content was higher in this study than our results. But Cu, Mn, Ni, Fe amounts in our study were higher than the results of the study made by Radulescu et al. (2010).

In addition Cu, Mn, Zn, Ni, Fe, Al contents were 41.11, 19.30, 77.33, 9.10, 227.38 and 480.28 mg/kg in *L. aurantiacum* and 61.18, 10.33, 53.68, 4.81, 53.92 and 93.77 mg/kg in *M. phaeodisca*, respectively (Table 4). Trace minerals (Cu, Mn, Zn, Fe) that we found in the studies were detected by Brzezicha-Cirocka et al. (2016) from *L. aurantiacum* samples with 37, 17, 100 and 150 mg/kg, respectively. According to this, our results are higher in terms of these minerals than the results of Brzezicha-Cirocka et al. (2016). In literature searches, any work made on the mineral content of *Macrolepiota phaeodisca* could not detect. But in a study made on a close species (*M. procera*) by Keleş et al. (2017), Cu, Mn, Zn, Fe and Ni were determined in quantities ranging from 6.98 to 151.5 mg/kg. In our studies copper content was found to be higher according to other study, and iron content was higher at the study made by Keleş et al. (2017).

In analysis of *S. imbricatus* samples; the amounts of Cu, Mn, Zn, Ni, Fe and Al were found as 66.16, 7.18, 112.29, 5.38, 35.45, 94.49 mg/kg, respectively. Among mushroom species analyzed, the greatest concentrations of Cu an Zn were obtained in *S. imbricatus* with 66.16 mg/kg and 112.29 mg/kg, respectively. In a study made to determine the amount of Zn, Mn, Fe and Cu on dry samples of the same macrofungi by Çolak et al. (2009), it was determined that Zn was the highest amount mineral compared to other minerals like our study. Although the amount of copper at our studies is higher compared to the study made by Çolak et al. (2009). The amont of Mn detected by Çolak et al. (2009) is higher than our study.

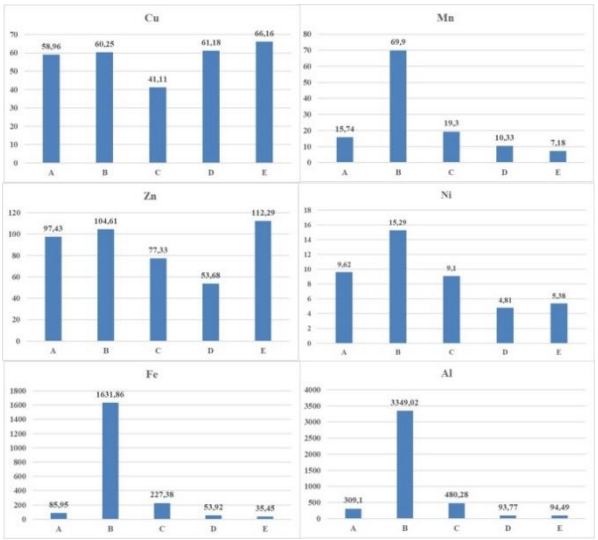

Figure 1. Microminerals levels of mushroom samples (mg/kg)

Table 1. Some features of the mushroom species

Species	Family	Localities	Habitat
Agaricus benesii	*Agaricaceae*	Yozgat-Akdağmadeni	Under deciduous trees
Amanita vaginata	*Amanitaceae*	Tokat center	In broad-leaf woods
Leccinum aurantiacum	*Boletaceae*	Tokat center	On soil under poplar
Macrolepiota phaeodisca	*Agaricaceae*	Yozgat-Kadışehri	On soil under oak
Sarcodon imbricatus	*Bankeraceae*	Yozgat-Akdağmadeni	In coniferous woods

Table 2. GC-MS analytical conditions for fatty acid analysis

Parameter/Component	Description / Value
GC-MS instruments	Agilent- Santa Clara, CA, USA
Series	7890 GC/5970 MS
Column	SGE Analytical BP×90 100m × 0.25 mm × 0.25 um (Australia)
Detector	FID for GC, Triple-axis for MS
Auto sampler	CTC- PAL
Temperature program	120°C to 250°C, 5°C/min. with temperature rise rate
Total time	45 min.
Split ratio	10:1
Injection volume	1 µL
Solvent delay	12 min.
Dry air flow	350 mL/min.
H_2 flow mode	35mL/min.
N_2 flow mode	20,227 mL/min.
Carrier gas	He
He flow mode	1 mL/min, constant flow mode

4. Discussions

The edible macrofungi, which are rich in terms of minerals, proteins, fiber, vitamins, and are low in terms of calories and cholesterol, are becoming increasingly important as a food source. Both the results of this study and the previously reported studies have shown that macrofungi are rich in terms of mono- and polyunsaturated fatty acids too. In addition, the studies have shown that macrofungi are an important nutrient sources in terms of minerals too. Wild and cultivated mushrooms species can be an important sources of food to meet the nutritional needs of the growing world population. Besides the wild edible macrofungi are an important source of income both nutrient and economically in many places around the world. However, studies made on the diagnosis of macrofungi were shown that very few of the edible macrofungi are known and consumed by the local people.

Table 3. Fatty acid profile of five wild edible mushrooms (%)

Fatty Acid Type	*A. benesii*	*A. vaginata*	*L. aurantiacum*	*M. phaeodisca*	*S. imbricatus*
Pentadecanoic acid (C15:0)	ND	ND	1.83	ND	ND
Palmitic acid (C16:0)	19.95	20.68	14.93	30.70	15.95
Palmitoleic acid (C16:1)	ND	ND	ND	2.01	ND
Stearic acid (C18:0)	12.59	4.81	4.89	12.41	8.93
Oleic acid (C18:1)	4.88	54.32	46.98	9.86	48.67
Linoleic acid (C18:2)	62.58	20.19	31.37	45.02	26.45
ΣSFAs	32.54	25.49	21.65	43.11	24.88
ΣUFAs	67.46	74.51	78.35	56.89	75.12
ΣMUFAs	4.88	54.32	46.98	11.87	48.67
ΣPUFAs	62.58	20.19	31.37	45.02	26.45

Table 4. Mineral levels of mushroom samples (mg/kg)

Species	Cu	Mn	Zn	Ni	Fe	Al
Agaricus benesii	58.96	15.74	97.43	9.62	85.95	309.10
Amanita vaginata	60.25	69.90	104.61	15.29	1631.86	3349.02
Leccinum aurantiacum	41.11	19.30	77.33	9.10	227.38	480.28
Macrolepiota phaeodisca	61.18	10.33	53.68	4.81	53.92	93.77
Sarcodon imbricatus	66.16	7.18	112.29	5.38	35.45	94.49

The results of this study provide us with information on the fatty acid and some mineral contents of five different wild edible macrofungi species that are *A. benesii*, *A. vaginata*, *L. aurantiacum*, *M. phaeodisca*, *S. imbricatus*. For some of these mushroom species, no studies can be found on the analysis of fatty acid or mineral content. According to results of the present study, the analyzed macrofungi can be sorted according to total unsaturated fatty acid content like *L. aurantiacum* > *S. imbricatus* > *A. vaginata* > *A. benesii* > *M. phaeodisca*. Evaluation of mineral analysis results show that *A. vaginata* is richer in Mn, Ni, Fe and Al while *S. imbricatus* is in Cu, Zn than the other mushroom specimens under discussion. This reported study will contribute to the studies on this subject.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

Acknowledgments

The authors would like to thank the experts of Bingol University Central Research Laboratory for assistance at chemical analysis.

References

Akyüz M, Kırbağ S, Karatepe M, Güvenç M, Zengin F (2011). Vitamin and fatty acid composition of *P. eryngii var. eryngii*. Bitlis Eren University Journal of Science and Technology 1: 16-20.

Al-Fartusie FS, Mohssan SN (2017). Essential trace elements and their vital roles in human body. Indian Journal of Advances in Chemical Science 5(3): 127-136.

Barros L, Baptista P, Correia DM, Morais JS, Ferreira IC (2007). Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. Journal of Agricultural and Food Chemistry 55(12): 4781-4788.

Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira ICFR (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food and Chemical Toxicology 46: 2742-2747.

Bengü AŞ (2019). Some elements and fatty acid profiles of three different wild edible mushrooms from Tokat province in Turkey. Progress in Nutrition 21(1): 189-193.

Bengü AŞ, Çınar Yılmaz H, Türkekul İ, Işık H (2019). Doğadan toplanan ve kültürü yapılan *Pleurotus ostreatus* ve *Agaricus bisporus* mantarlarının toplam protein, vitamin ve yağ asidi içeriklerinin belirlenmesi. Turkish Journal of Agricultural and Natural Sciences 6(2): 222-229.

Bon M (1987). The Mushrooms and Toadstools of Britain and North-Western Europe. London: Hodder-Stoughton.

Breitenbach J, Kränzlin F (1995). Fungi of Switzerland. Vol: 4, Agarics 2. Luzern: Verlag Mykologia.

Brzezicha-Cirocka J, Mędyk M, Falandysz J, Szefer P (2016). Bio- and toxic elements in edible wild mushrooms from two regions of potentially different environmental conditions in eastern Poland. Environmental Science and Pollution Research 23: 21517-21522.

Christie WW (1990). The analysis of fatty acids, in gas chromatography and lipids. Glaskow: The Oil Press Ltd.

Christie WW (1998). Gas chromatography–mass spectrometry methods for structural analysis of fatty acids. Lipids 33: 343-353.

Çolak A, Faiz Ö, Sesli E (2009). Nutritional composition of some wild edible mushrooms. Turkish Journal of Biochemistry 34(1): 25-31.

Dembitsky VM, Terent'ev AO, Levitsky DO (2010). Amino and fatty acids of wild edible mushrooms of the genus *Boletus*. Records of Natural Products 4(4): 218-223.

Doğan HH, Akbaş G (2013). Biological activity and fatty acid composition of Caesar's mushroom. Pharmaceutical Biology 51(7): 863-871.

Doğan HH (2016). Fatty acid compositions of two mushrooms in Turkey. International Journal of Recent Scientific Research 7(4): 863-871.

Duda-Chodak A, Błaszczyk U (2008). The impact of nickel on human health. Journal of Elementology 13(4): 685-696.

Ergönül PG, Ergönül B, Kalyoncu F, Akata I (2012). Fatty acid compositions of five wild edible mushroom species collected from Turkey. International Journal of Pharmacology 8(5): 463-466.

Fernandesa Â, Barros L, Barreira JCM, Antonio AL, Oliveira MBPP, Martins A, Ferreira ICFR (2013). Effects of different processing technologies on chemical and antioxidant parameters of *Macrolepiota procera* wild mushroom. Food Science and Technology 54(2): 493-499.

Goyal R, Grewal RB, Goyal RK (2015). Fatty acid composition and dietary fibre constituents of mushrooms of North India. Emirates Journal of Food and Agriculture 27(12): 927-930.

Hara A, Radin NS (1978). Lipid extraction of tissues with a low-toxicity solvent. Analytical Biochemistry 90: 420-426.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1996). Ainsworth and Bisby's Dictionary of the Fungi, 8th edition, Wallingford: CAB International.

Hawksworth DL (2001). The magnitude of fungal diversity: the 1.5 million specie estimate revisited. Mycological Research 105(12): 1422-1432.

Işıldak Ö, Türkekul İ, Elmastaş M, Tüzen M (2004). Analysis of heavy metals in some wild-grown edible mushrooms from the middle black sea region, Turkey. Food Chemistry 86: 547-552.

Jonnalagadda SB, Pienaar DH, Haripersad K (2006). Elemental distribution in selected *Agaricus* and *Rhizina* mushrooms in South Africa. Journal of Environmental Science and Health Part A 41(3): 507-514.

Jordan M (1995). The Encyclopedia of Fungi of Britain and Europe. London: Frances Lincoln.

Karliński L, Ravnskov S, Kieliszewska-Rokicka B, Larsen J (2007). Fatty acid composition of various ectomycorrhizal fungi and ectomycorrhizas of Norway spruce. Soil Biology and Biochemistry 39: 854-866.

Kawahara M, Konoha K, Nagata T, Sadakane Y (2007). Aluminum and human health: Its ıntake, bioavailability and neurotoxicity. Biomedical Research on Trace Elements 18(3): 211-220.

Keleş A, Gençcelep H, Demirel K (2017). Elemental composition of naturally growing wild edible mushroom. Journal of Natural Product and Plant Resources 7(4): 37-44.

Moser M (1983). Keys to Agarics and Boleti. Stuttgart: Gustav Fischer Verlag,

Orsine JVC, Novaes MRCG, Asquieri ER (2012). Nutritional value of Agaricus sylvaticus; mushroom grown in Brazil. Nutrición Hospitalaria 27(2): 449-455.

Pedneault K, Angers P, Gosselın A, Tweddell RJ (2006). Fatty acid composition of lipids from mushrooms belonging to the family *Boletaceae*. Mycological research 110: 1179-1183.

Pekşen A (2013). Mantarların insan hayatı ve sağlığındaki yeri. Bahçe Haber 2: 10-14.

Phillips R (1981). Mushrooms and Other Fungi of Great Britain & Europe, London: Pan Books Ltd.

Pietrzak-Fiećko R, Gałgowska M, Bakuła S (2016). Fatty Acid Composition in Wild *Boletus edulis* from Poland. Italian Journal of Food Science 28(3): 402-411.

Radulescu C, Stihi C, Busuioc G, Popescu IV, Gheboianu AI, Cimpoca VGH (2010). Evaluation of essential elements and heavy metal levels in fruiting bodies of wild mushrooms and their substrate by EDXRF spectrometry and FAA spectrometry. Romanian Biotechnological Letters 15(4): 5444-5456.

Ribeiro B, Guedes de Pinho P, Andrade PB, Baptista P, Valentão P (2009). Fatty acid composition of wild edible mushrooms species: A comparative study. Microchemical Journal 93: 29-35.

Türkekul İ, Çetin F, Elmastaş M (2017). Fatty acid composition and antioxidant capacity of some medicinal mushrooms in Turkey. Journal of Applied Biological Chemistry 60(1): 35-39.

Ravikrishnan V, Naik P, Ganesh S, Rajashekhar M (2015). Amino acid, fatty acid and mineral profile of mushroom *Lentinus polychrous* lév. from Western Ghats, Southern India. The International Journal of Plant, Animal and Environmental Sciences 5(1): 278-281.

Wada O (2004). What are trace elements? Their deficiency and excess states. Journal of the Japan Medical Association 47(8): 351-358.

Wretensjö I, Svensson L, Christie WW (1990). Gas chromatographic—mass spectrometric identification of the fatty acids in borage oil using the picolinyl ester derivatives. Journal of Chromatography A 521: 89-97.

Woldegiorgis AZ, Abate D, Haki GD, Ziegler GR, Harvatine KJ (2015). Fatty acid profile of wild and cultivated edible mushrooms collected from Ethiopia. Journal of Nutrition & Food Sciences 5(3): 360.

Yılmaz N, Solmaz M, Türkekul İ, Elmastaş M (2006). Fatty acid composition in some wild edible mushrooms growing in the middle Black Sea region of Turkey. Food Chemistry 99: 168-174.

Anatolian Journal of Botany
5(1):65-77 (2021)
Research article *doi:10.30616/ajb.907233*

Root-based characterization of intergeneric hybrids with *Triticum* and *Aegilops* species in early vegetative and stem elongation growth stages

Hayati AKMAN
Selçuk University, Sarayönü Vacational School, Department of Plant and Animal Production, Konya, Turkey
hayatiakman@selcuk.edu.tr

Received : 31.03.2021
Accepted : 27.04.2021
Online : 12.05.2021

Erken vejetatif ve sapa kalkma gelişme dönemlerinde *Triticum* ve *Aegilops* türleriyle intergenerik hibritlerin kök bazlı karakterizayonu

Abstract: Cultivated and wild species of wheat are valuable breeding resources used for the development of new cultivars with superior root traits suited to drought and non-stressed conditions. The present study aimed to determine genotypes with superior root traits and phenotypic variability among intergeneric hybrids with *Triticum* and *Aegilops* species in the early vegetative (Z11) and stem elongation (Z31) growth stages. Results indicated that phenotypic variability in rooting depth was 3.2- and 3.4 fold among the genotypes in Z11 and Z31, and it was as great as 20- and 23.8 fold for root biomass, respectively. Hierarchical clustering among 35 genotypes for root traits in both growth stages identified four major clusters, grouping the six deep-rooted genotypes in cluster 2 and three genotypes with high root biomass in cluster 1. In both growth stages, significant associations were found among the root traits. Also, the relationship was stronger between the root and shoot biomass in Z11 (r^2=0.83) than in Z31 (r^2=0.44). As an overall assessment, the suggested genotypes with superior root characteristics such as deep roots and/or high root biomass sustained in both growth stages might be used for the development of new cultivars.

Key words: Intergeneric hybrids, phenotypic variability, root traits, *Triticum* species, wheat wild relatives

Özet: Buğdayın kültüre alınmış ve yabani türleri, kurak ve stres içermeyen şartlara uygun üstün kök özelliklerine sahip yeni çeşitlerin geliştirilmesi için kullanılabilecek değerli ıslah materyalleridir. Bu çalışma, erken vejetatif (Z11) ve sapa kalkma (Z31) gelişme dönemlerinde *Triticum* ve *Aegilops* türleriyle birlikte intergenerik hibritler arasındaki fenotipik farklılığı ve üstün kök özelliklerine sahip genotipleri belirlemeyi amaçlamaktadır. Araştırma sonuçları, genotipler arasında kök derinliği bakımından Z11 ve Z31 gelişme dönemlerinde sırasıyla 3.2 ve 3.4 kat, kök biyoması bakımından ise 20 ve 23.8 kat önemli bir fenotipik farklılığın olduğunu göstermiştir. Hiyerarşik kümelemede her iki gelişme döneminde kök özellikleri değerlendirildiğinde 35 genotip dört farklı gruba ayrılmıştır, gurup 1'de yüksek kök biyomasına sahip 3 genotip ve grup 2'de ise derin köklü altı genotip yer almıştır. Her iki gelişme döneminde de kök özellikleri arasında önemli ilişkiler tespit edilmiştir. Bununla birlikte, Z11'de (r^2=0.83) kök ve sürgün biyoması arasındaki ilişki, Z31'den (r^2=0.44) daha güçlü bulunmuştur. Genel bir değerlendirme olarak her iki gelişme döneminde de derin köklere ve/veya yüksek kök biyoması gibi üstün kök özelliklerine sahip genotipler yeni çeşitlerin geliştirilmesinde kullanılabilir.

Anahtar Kelimeler: İntergenerik hibritler, fenotipik çeşitlilik, kök özellikleri, *Triticum* türleri, yabani buğday akrabaları

Citation: Akman H (2021). Root-based characterization of intergeneric hybrids with Triticum and Aegilops species in early vegetative and stem elongation growth stages. Anatolian Journal of Botany 5(1): 65-77.

1. Introduction

Wheat is a staple crop of historical economic importance that is widely grown worldwide. It was first domesticated 10-12 thousand years ago in the Fertile Crescent, an area including Southeastern Turkey (Maron, 2019). With evolution over 300 thousand years, wild tetraploid wheat, *Triticum dicoccoides*, was derived from *T. urartu* (genome A) and most likely from *Aegilops speltoides* (genome B) (Dvorak and Akhunov, 2005; Nevo, 2011). Cultivated hulled emmer wheat was created by plant selection of wild emmer and then evolved into the free-threshing ears of *T. turgidum*, *T. polonicum*, *T. turanicum*, and *T. carthlicum* by natural mutation (Peng et al., 2011). Another wild diploid wheat used in this study, *T. boeoticum*, has been described as the wild progenitor of *T. monococcum* (Özkan et al., 2010). The D genome donor of the hexaploid wheat species *T. aestivum* and *T. spelta* has been regarded as *Ae. tauschii*. The oldest cultivated and hulled wheat genotypes, spelt wheat (*T. spelta*), einkorn wheat (*T. monococcum*), emmer wheat (*T. dicoccum*), and Vavilov wheat (*T. vavilovii*), are found among the wild and modern wheats (Adu et al., 2011). Different cultivated and wild species of wheat, landraces, and wheat hybrids offer biotic and abiotic stress tolerance, high biochemical contents, and quality in the improvement of new cultivars (Mathre et al., 1985; Arzani and Ashraf, 2017; Li et al., 2018; Ullah et al., 2018; Kishii, 2019).

Little is known about the root systems of wheat genetic resources, and root studies have not traditionally been a common objective of breeding programs (Friedli et al., 2019). Recently, however, the importance of root studies has been well understood. Selection of genotypes with superior roots through phenotyping of root traits may contribute to the improvement of promising cultivars with desirable root traits. Breeders have recently given priority to the improvement of higher adaptation capability and climate-resilient cultivars to avoid yield losses (Banga and Kang, 2014). For this purpose, cultivars with deep root systems may explore deep soil profile under water deficit, while a dense root system may improve nutrient uptake and support higher yield and above-ground biomass (Manschadi et al., 2006; Sayar et al., 2007; Prasad et al., 2008; Bengough et al., 2011; Heřmanská et al., 2015).

Considering the evaluation of root traits towards the use of genetic resources in breeding programs, the present study aimed to i) determine phenotypic variability for root and shoot features and their relationships, and ii) selection of genotypes with superior root traits for the rain-fed and irrigated conditions in a set of *Triticum* and *Aegilops* species together with their hybrids in early vegetative and stem elongation growth stages.

2. Materials and Method

This study was conducted to characterize root and shoot parameters and their relationships in early vegetative growth stage (Z11 on the Zadoks scale of cereal growth) for two weeks after germination in controlled conditions in first experiment and at stem elongation growth stage (Z31) during the nodal root growth stage in second experiment in a glasshouse.

Table 1. Taxa, accession number, country of origin, local/GRIN and common names of the studied material.

Taxa	Accession No.	PL	Local name or GRIN name	Common name/origin
Triticum taxa and intergeneric hybrids				
Agrotriticum ssp.	PI 550715	8×	Agrotana	*Agropyron* × *Triticum*, USA
Elytritilops ssp.	PI 605347	-	Sando Selection 538	*Elymus* × *Triticum* x *Aegilops*, USA
T. aestivum L. ssp. *aestivum*	-	6×	5924[a]	Common wheat, line, Australia
T. aestivum L. ssp. *aestivum*	-	6×	Ahmetağa[b]	Common wheat, cultivar, Konya, Turkey
T. aestivum L. ssp. *aestivum*	-	6×	Ak 702[c]	Common wheat, old cultivar, Eskişehir, Turkey
T. aestivum L. ssp. *aestivum*	PI 660669	6×	Daws High PPO	Common wheat/NIL, USA
T. aestivum L. ssp. *aestivum*	-	6×	Tir[d]	Common wheat, landrace, Van, Turkey
T. aestivum L. ssp. *compactum* (Host) Mac Key	PI 159101	6×	Spitskop	South Africa
T. aestivum L. ssp. *spelta* (L.) Thell.	PI 295064	6×	Weisser Granenspeltz	Spelt wheat, Bulgaria
T. aestivum L. ssp. *sphaerococcum* (Percival) Mac Key	PI 277142	6×	Acarp	India
T. boeoticum Boiss.	PI 352270	2×	Baydaricum	Wild einkorn, Germany
T. ispahanicum Heslot	PI 330548	4×	184	England
T. monococcum L. ssp. *monococcum*	PI 192063	2×	Escanha Menor	Einkorn, Portugal
T. petropavlovskyi Udacz. et Migusch.	PI 585015	6×	Maik	*T. aestivum* × *T. polonicum*, China
T. soveticum ssp. *fungicidum* (Zhuk.)	PI 251015	8×	-	*T. carthlicum* × *T. timopheevii*, Russia
T. timopheevii (Zhuk.) Zhuk. ssp. *armeniacum* (Jakubz.) Slageren	PI 538522	4×	G3217	Wild form of *T. timopheevii*, Iraq
T. turgidum L. ssp. *carthlicum* (Nevski) Á. Löve & D. Löve	PI 70738	4×	22	Persian Wheat, Iraq
T. turgidum L. ssp. *dicoccoides* (Körn. ex Asch. & Graebn.) Thell.	PI 346783	4×	Nakhichevan	Wild wheat, Hungary
T. turgidum L. ssp. *dicoccum* (Schrank ex Schübl.) Thell.	-	4×	Kavılca[d]	Emmer, Kars, Turkey
T. turgidum L. ssp. *durum* (Desf.) van Slageren	-	4×	Altın 40/98[e]	Durum wheat, cultivar, Ankara, Turkey
T. turgidum L. ssp. *durum* (Desf.) van Slageren	-	4×	Berkmen 469[e]	Durum wheat, cultivar, Ankara, Turkey
T. turgidum L. ssp. *durum* (Desf.) van Slageren	-	4×	Gır[a]	Durum wheat, landrace, Turkey
T. turgidum L. ssp. *durum* (Desf.) van Slageren	-	4×	Meram 2002[b]	Durum wheat, cultivar, Konya, Turkey
T. turgidum L. ssp. *durum* (Desf.) van Slageren	-	4×	Yılmaz 98[e]	Durum wheat, cultivar, Ankara, Turkey
T. turgidum L. ssp. *polonicum* (L.) Thell.	PI 185309	4×	Polonicum	Polish wheat, Argentina
T. turgidum L. ssp. *turanicum* (Jakubz.) Á. Löve & D. Löve	PI 68293	4×	351	Khorasan wheat, Azerbaijan
T. turgidum L. ssp. *turgidum*	PI 134953	4×	Lusitanicum	Portugal
T. urartu Tumanian ex Gandilyan	PI 662281	2×	IG 117911	Wild einkorn, Syria
T. vavilovii Jakubz.	PI 428342	6×	-	Vavilov's wheat, Sweeden
Aegilops taxa				
Ae. biuncialis Vis.	Clae 67	4×	2215a	Turkey
Ae. caudata L.	PI 277000	2×	M10	Unknown
Ae. columnaris Zhuk.	Clea 34	4×	No. 1	Unknown
Ae. comosa Sm.	PI 542174	2×	84TK154-018	Turkey
Ae. crassa Boiss.	PI 219863	-	72	Iraq, Arbil
Ae. geniculata Roth	Clae 65	4×	Sando 253	Unknown
Ae. juvenalis (Thell.) Eig	PI 276693	6×	19	Unknown
Ae. kotschyii Boiss.	Clae 36	4×	No. 4	Unknown
Ae. neglecta Req. ex Bertol.	PI 170198	4×	2640	Turkey
Ae. peregrina (Hack.) Maire & Weiller var. *brachyathera* (Boiss.) Maire & Weiller	PI 542236	4×	84TK075-030	Turkey
Ae. speltodies Tausch var. *speltoides*	PI 542259	2×	84TK109-078	Turkey
Ae. tauschii Coss.	Clae 1	2×	2001	Pakistan

USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network (GRIN). (Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland, USA. Available: http.//www.ars-grin.gov. Note: Accessions with PI and Clae numbers were supplied from the USDA ARS. PL indicates ploidy level of the studied material.

[a] Department of Field Crops, Selçuk University, [b] Bahri Dağdaş International Agricultural Research Institute, [c]Transitional Zone Agricultural Research Institute, [d] Local farmer, [e] Field Crops Central Research Institute

Germplasm samples of 10 *Triticum* species or 19 taxa and 12 *Aegilops* species with 2 intergeneric hybrids were selected to represent a wide range of diversity in the two separate experiments (Table 1). Genotypes of non-Turkish and Turkish origin were obtained from the US Department of Agriculture's National Plant Germplasm System (https://www.ars-grin.gov). Also, materials of Turkish origin, including landrace, old, and modern cultivars were supplied from institutes and local farmers in different regions of the country (Table 1).

2.1. Controlled condition experiment

The first experiment was carried out under controlled conditions to evaluate coleoptile length with root and shoot traits. Three seeds of each genotype as three replications were placed in the middle of a wet germination towel 5 cm apart. The samples were rolled loosely and secured with a rubber band. They were then placed vertically in plastic bags in a dark room at 15-16 °C for 15 days. The room humidity was between 50-60%. Coleoptile length was determined with a ruler as the distance from the scutellum to the tip of the coleoptile. The seminal root depth was measured, and the number of seminal roots was manually counted. Root and shoot biomasses were recorded after dehydrated thoroughly with an absorbent towel. Root proportion of total biomass and root biomass-to-shoot biomass ratio were calculated as root-to-total biomass ratio and root-to-shoot ratio, respectively.

2.2. Glasshouse experiment

The second experiment was conducted under glasshouse conditions from April 11 to May 18. Seeds were sown into long columns (100 cm in depth and 12 cm in diameter), which were filled with field soil. The climate of the glasshouse was measured with a data logger (T & D Corporation/TR-74Ui) recording data in five-minute intervals. The glasshouse temperature in April and May was 22.2 °C day / 10 °C night and 24.0 °C day / 13.4 °C night, respectively. The mean relative humidity in April and May was respectively approximately 39.7% day / 73.4% night and 37.8% day / 66.5% night with photosynthetic photon flux densities of 249.9 µmol m^{-2} s^{-1} and 216.7 µmol m^{-2} s^{-1} during the day. The soil used in the experiment, taken from the field (0-40 cm), was clay-loam with low organic matter (1.7%) and a high level of $CaCO_3$ (23.7%) and Ca (5491 mg/kg). EC was 0.6 mmhos/cm. Soil pH was 7.7 and no salinity problems were observed. Levels of P_2O_5 (4.58 mg/kg), Zn (0.67 mg/kg), and Mn (8.13 mg/kg) were low, while K_2O (1265 kg/ha), Mg (464.4 mg/kg), Fe (5.1 mg/kg), and Cu (3.1 mg/kg) were found to be adequate.

All measurements were conducted for a single plant grown individually in a column (Fig. 1). Leaf chlorophyll content was measured with a Minolta SPAD-502. As morphological characteristics shoot height was measured and number of tillers was counted. Washed and cleaned roots were measured for the longest rooting depth. The number of nodal roots was counted manually. Root and shoot biomasses were recorded after drying at 80 °C for three days.

Figure 1. *Triticum* and *Aegilops* species with hybrids grown in 100 cm columns (12 cm in diameter) under glasshouse conditions.

2.3. Statistical analysis

The experiments were arranged in a completely randomized design with three replications. Analysis of variance was performed with the MSTAT-C statistical package and significant differences between means were tested by the Least Significant Difference (LSD) test. Regression analysis was performed in a Microsoft Excel (Excel version in Microsoft Office 2016 for Windows) for significantly correlated traits. The root morphological data (root biomass, rooting depth, root-to-shoot ratio, root-to total biomass ratio, numbers of seminal and nodal roots) for both growth stages were converted to text format and imported to DARwin 6 software (Perrier and Jacquemoud-Collet, 2006). The groups were determined by hierarchical clustering for root data. A dendrogram was constructed using the Euclidean distance and unweighted pair group linkage methods (UPGMA).

3. Results

Significant differences were found among the genotypes in terms of investigated traits and their relationships in two experiments (Tables 2 and 3).

3.1. Phenotypic variability in root system

Results indicated significant variations among the genotypes in terms of rooting depth, root biomass, root-to-shoot ratio, root-to-total biomass ratio, and numbers of seminal and nodal roots in the early vegetative and at stem elongation growth stages (P<0.01; Tables 2 and 3).

The lowest and highest rooting depths among *Aegilops* species were obtained from *Ae. caudata* (6.6 cm) and *Ae. geniculata* (13 cm) in the early growth stage (Z11) and from *Ae. crassa* (35.7 cm) and *Ae. neglecta* (88.3) in the stem elongation stage (Z31). Shallow- and deep-rooted genotypes among the wheat species and wheat hybrids were respectively observed in *T. urartu* (9 cm) and *T. turanicum* (21.2 cm) in Z11 and in *T. urartu* (34.8 cm) and *T. aestivum* 'Ak 702' (116.7 cm) in Z31. The rooting depth

Table 2. Shoot height (SH), coleoptile length (CL) shoot biomass (SB), number of seminal roots (SRN), rooting depth (RD), root biomass (RB), root-to-shoot ratio (R/S), and root-to-total biomass ratio (R/TB) of *Triticum* species, *Aegilops* species and hybrids in the Z11 growth stage.

Taxa	SH (cm)	CL (cm)	SB (g)	SRN plant^{-1}	RD (cm)	RB (g)	R/S plant^{-1}	R/TB (%)
Ae. biuncialis	15.3^{e-l}	5.5^{k-n}	0.13^{k-o}	2.5^{j-n}	8.3^{lm}	0.02^{kl}	0.16^{ij}	14.0^{mn}
Ae. brachyathera	17.3^{b-g}	6.6^{g-k}	0.06^{opq}	2.7^{i-m}	10.8^{kl}	0.03^{jkl}	0.46^{a-f}	31.4^{a-g}
Ae. caudata	10.3^{n-q}	4.6^{nop}	0.14^{k-n}	2.4^{k-n}	6.6^{m}	0.03^{jkl}	0.26^{f-j}	20.6^{i-n}
Ae. columnaris	13.1^{h-o}	5.2^{l-o}	0.08^{m-q}	2.9^{h-l}	9.7^{l}	0.02^{kl}	0.26^{f-j}	20.6^{i-n}
Ae. comosa	11.6^{l-p}	5.5^{k-n}	0.05^{pq}	2.2^{mn}	9.2^{lm}	0.01^{l}	0.33^{c-j}	24.5^{d-l}
Ae. geniculata	16.7^{c-i}	6.3^{h-l}	0.07^{n-q}	3.0^{h-k}	13.0^{jk}	0.02^{kl}	0.25^{g-j}	20.1^{i-n}
Ae. juvenalis	14.7^{f-m}	6.0^{i-m}	0.07^{n-q}	2.7^{i-m}	11.3^{kl}	0.02^{kl}	0.24^{g-j}	19.2^{j-n}
Ae. kotschyi	13.9^{g-n}	5.6^{j-n}	0.20^{f-k}	2.3^{lmn}	10.8^{kl}	0.05^{h-k}	0.28^{d-j}	21.7^{h-m}
Ae. neglecta	6.9^{q}	3.2^{qr}	0.05^{pq}	1.9^{n}	8.8^{lm}	0.01^{l}	0.21^{hij}	17.2^{lmn}
Ae. tauschii	12.7^{i-p}	6.8^{f-j}	0.06^{opq}	3.0^{h-k}	10.7^{kl}	0.01^{l}	0.14^{j}	11.8^{n}
'Agrotriticum'	8.8^{pq}	3.7^{pqr}	0.05^{pq}	2.3^{lmn}	16.5^{b-h}	0.03^{jkl}	0.54^{ab}	34.1^{a-d}
'Elytritilops'	17.3^{b-g}	8.3^{bcd}	0.27^{def}	3.0^{h-k}	14.7 $^{g-j}$	0.10^{def}	0.37^{b-h}	26.9^{b-k}
T. aestivum '5924'	11.3^{l-p}	5.2^{l-o}	0.28^{cde}	3.5^{e-h}	18.2^{a-d}	0.07^{f-i}	0.30^{d-j}	22.5^{f-m}
T. aestivum 'Ahmetağa'	10.8^{m-q}	4.4^{n-q}	0.26^{d-g}	3.0^{h-k}	18.2^{a-d}	0.12^{de}	0.48^{a-d}	32.1^{a-f}
T. aestivum 'Ak 702'	17.0^{b-h}	5.3^{l-o}	0.36^{ab}	2.9^{h-l}	15.8^{c-j}	0.18^{ab}	0.52^{abc}	33.5^{a-d}
T. aestivum 'Daws High PPO'	9.7^{opq}	3.1^{r}	0.32^{bcd}	3.0^{h-k}	14.9^{f-j}	0.19^{ab}	0.64^{a}	37.7^{a}
T. aestivum 'Tir'	19.3^{a-e}	9.3^{b}	0.23^{e-i}	4.7^{ab}	18.6^{abc}	0.10^{def}	0.42^{b-g}	29.2^{a-I}
T. armeniacum	16.7^{c-i}	7.0^{e-i}	0.12^{l-p}	3.0^{h-k}	13.5^{h-k}	0.04^{i-l}	0.31^{d-j}	23.3^{e-m}
T. boeoticum	11.3^{l-p}	3.8^{pqr}	0.06^{opq}	3.0^{h-k}	10.9^{kl}	0.03^{jkl}	0.40^{b-h}	28.4^{a-k}
T. carthlicum	14.3^{f-n}	7.0^{e-i}	0.19^{g-l}	4.0^{cde}	19.5^{ab}	0.08^{fgh}	0.42^{b-g}	29.5^{a-i}
T. compactum	19.6^{a-d}	7.1^{d-i}	0.14^{k-n}	3.8^{def}	16.4^{c-h}	0.07^{f-i}	0.48^{a-d}	32.2^{a-e}
T. dicoccoides	16.3^{d-i}	8.0^{c-f}	0.09^{m-q}	3.0^{h-k}	15.2^{d-j}	0.03^{jkl}	0.30^{d-j}	23.3^{e-m}
T. dicoccum	20.5^{abc}	6.6^{g-k}	0.13^{k-o}	4.5^{abc}	17.9^{b-f}	0.06^{g-j}	0.47^{a-e}	31.8^{a-g}
T. durum 'Altın 40/98'	11.7^{k-p}	4.8^{m-p}	0.18^{h-l}	5.0^{a}	18.0^{b-e}	0.05^{h-k}	0.26^{f-j}	20.8^{i-n}
T. durum 'Berkmen 469'	15.9^{d-j}	6.6^{g-k}	0.42^{a}	3.0^{h-k}	16.4^{c-h}	0.16^{bc}	0.38^{b-h}	27.3^{b-k}
T. durum 'Gır'	15.3^{e-l}	7.2^{d-i}	0.22^{e-j}	3.5^{e-h}	18.8^{abc}	0.05^{h-k}	0.23^{g-j}	18.8^{k-n}
T. durum 'Meram 2002'	12.8^{i-p}	4.8^{m-p}	0.35^{abc}	3.2^{f-i}	16.3^{c-i}	0.13^{cd}	0.36^{b-i}	26.4^{b-l}
T. durum 'Yılmaz 98'	11.3^{l-p}	4.1^{o-r}	0.39^{ab}	4.2^{bcd}	15.3^{d-j}	0.20^{a}	0.52^{abc}	34.3^{abc}
T. fungicidum	20.7^{abc}	9.0^{bc}	0.24^{e-i}	4.7^{ab}	15.0^{e-j}	0.07^{f-i}	0.29^{d-j}	22.3^{g-m}
T. ispahanicum	21.7^{a}	11.3^{a}	0.17^{i-l}	5.0^{a}	15.3^{d-j}	0.08^{fgh}	0.46^{a-f}	31.3^{a-h}
T. monococum	12.0^{j-p}	4.7^{nop}	0.07^{n-q}	3.0^{h-k}	13.3^{ijk}	0.04^{i-l}	0.55^{ab}	35.0^{ab}
T. petropavlovskyi	19.7^{a-d}	7.4^{d-h}	0.25^{d-h}	3.1^{g-j}	17.9^{b-f}	0.08^{fgl}	0.33^{c-j}	25.1^{c-l}
T. polonicum	16.3^{d-i}	7.5^{d-h}	0.14^{k-n}	3.0^{h-k}	13.7^{h-k}	0.06^{g-j}	0.41^{b-h}	28.7^{a-j}
T. spelta	15.8^{d-k}	5.6^{j-m}	0.09^{m-q}	2.7^{i-m}	12.9^{jk}	0.03^{jkl}	0.39^{b-h}	28.0^{b-k}
T. sphaerococcum	16.0^{d-j}	6.1^{l-k}	0.15^{j-m}	3.0^{h-k}	15.0^{e-j}	0.06^{g-j}	0.41^{b-h}	28.8^{a-j}
T. turanicum	21.0^{ab}	8.2^{c-e}	0.24^{e-i}	3.0^{h-k}	21.2^{a}	0.09^{efg}	0.35^{b-i}	25.9^{b-l}
T. turgidum	19.0^{a-e}	7.5^{d-h}	0.19^{g-l}	4.0^{cde}	17.8^{b-f}	0.08^{fgl}	0.40^{b-h}	28.4^{a-k}
T. urartu	11.7^{k-p}	5.2^{l-o}	0.04^{q}	3.0^{h-k}	9.0^{lm}	0.01^{l}	0.27^{e-j}	21.4^{i-n}
T. vavilovii	18.3^{a-f}	7.7^{d-g}	0.25^{d-h}	3.7^{d-g}	17.7^{b-g}	0.07^{f-i}	0.30^{d-j}	23.1^{e-m}
Mean	15.0	6.2	0.17	3.2	14.4	0.07	0.36	25.9
LSD (0.01)	4.19	1.27	0.075	0.69	3.1	0.034	0.21	9.64
CV (%)	13.0	9.5	19.9	9.8	9.8	23.7	26.4	17.3

in the great majority of genotypes was 12.8-18.9 cm in Z11, while in Z31 the rooting depth of genotypes was almost equally distributed across a range of 34.8-116.7 cm (Fig. 3). In Z11 and Z31, the phenotypic variability in rooting depth was 3.2- fold and 3.4-fold, respectively.

According to the mean values, the rooting depth of *Aegilops* species was shallower than that of *Triticum* species and the hybrids in both Z11 and Z31 (Fig. 4). Furthermore, rooting depth was linked with number of seminal roots, number of nodal roots, and root biomass in both Z11 and Z31 (Fig. 5).

High variation of root biomass was observed as a 20-fold difference in a range between 0.01 g and 0.20 g in Z11 and a 23.8-fold difference ranging between 0.11 g and 2.62 g in Z31 among the genotypes (Tables 2 and 3). Most of the genotypes possessed values between 0.01 g and 0.11 g in Z11 and 0.1 g and 1.5 g in Z31 (Fig. 3). The mean root biomasses of *Triticum* species and hybrids were higher in Z11 (4-fold) and Z31 (3.1-fold) than that of the *Aegilops* species (Fig. 4). In Z11, D genome progenitor *Ae. tauschii* (0.01 g), *Ae. comosa* (0.01 g), and *Ae. neglecta* (0.01 g) had the lowest root biomass and *Ae. kotschyi* (0.05 g) had the highest value among the *Aegilops* species. *T. durum* 'Yılmaz 98' (0.20 g) and *T. urartu* (0.01 g) showed the highest and lowest values, respectively, among the *Triticum* species and hybrids. The lowest and highest root biomass values in Z31 were obtained from *Ae. crassa* (0.15 g) and a wheat ancestor, *Ae. speltoides* (0.77 g), among the *Aegilops* species and from *T. boeoticum* (0.11 g) and *T. dicoccum* (2.62 g) among the *Triticum* species and hybrids (Tables 2 and 3).

Significant variation was observed in the seminal root number of genotypes, ranging from 1.9 to 5 in Z11, and in number of nodal roots, ranging from 4 to 21 in Z31

Table 3. SPAD chlorophyll (CHL), number of tillers (TN), shoot height, (SH), shoot biomass (SB), number of nodal roots (NRN), rooting depth (RD), root biomass (RB), root-to-shoot ratio (R/S), and root-to-total biomass ratio (R/TB) of *Triticum* species, *Aegilops* species and wheat hybrids in the Z31 growth stage.

Taxa	CHL (µmol m^{-2})	TN $plant^{-1}$	SH (cm)	SB (g)	NRN $plant^{-1}$	RD (cm)	RB (g)	R/S $plant^{-1}$	R/TB (%)
Ae. biuncialis	43.0^{e-i}	7.3^{cd}	21.3^{nop}	0.48^{q-r}	9.3^{c-h}	68.8^{h-m}	0.52^{j-m}	1.08^{d-k}	51.9^{g-l}
Ae. brachyathera	43.3^{e-i}	6.0^{cde}	24.0^{m-p}	0.28^{qr}	6.7^{h-k}	76.2^{g-k}	0.42^{j-n}	1.53^{d-g}	60.1^{cd}
Ae. caudata	37.7^{i}	12.0^{b}	21.7^{nop}	0.61^{nop}	8.0^{f-j}	46.3^{n-q}	0.51^{j-m}	0.88^{g-p}	46.1^{m-p}
Ae. comosa	49.9^{b-g}	8.3^{c}	22.3^{nop}	0.42^{q-r}	8.0^{f-j}	57.7^{j-p}	0.31^{lmn}	0.75^{j-q}	42.1^{pq}
Ae. crassa	41.0^{hi}	8.0^{c}	21.7^{nop}	0.86^{i-n}	7.0^{h-k}	35.7^{pq}	0.15^{lmn}	0.18^{q}	15.4^{t}
Ae. geniculata	53.5^{bcd}	11.0^{b}	18.3^{p}	0.46^{q-r}	12.0^{bcd}	51.3^{l-q}	0.53^{jkl}	1.18^{d-l}	53.1^{g-k}
Ae. juvenalis	-	14.7^{a}	21.0^{nop}	0.66^{mno}	5.3^{ijk}	68.0^{h-n}	0.16^{lmn}	0.25^{pq}	19.4^{t}
Ae. kotschyii	41.2^{ghi}	7.0^{cd}	19.7^{op}	0.34^{a}	9.0^{d-h}	71.0^{h-l}	0.37^{k-n}	1.11^{d-k}	52.5^{g-l}
Ae. neglecta	48.7^{b-h}	13.0^{ab}	19.0^{op}	0.69^{l-o}	7.3^{g-k}	88.3^{b-h}	0.42^{j-n}	0.63^{k-q}	38.0^{qr}
Ae. speltoides	83.7^{a}	7.0^{cd}	22.0^{nop}	0.46^{q-r}	9.7^{c-h}	60.0^{i-o}	0.77^{ij}	1.69^{cde}	62.6^{c}
Ae. tauschii	40.8^{hi}	4.3^{efg}	26.3^{k-n}	0.34^{pqr}	7.3^{g-k}	85.3^{d-h}	0.21^{lmn}	0.62^{k-q}	38.1^{qr}
'Agrotriticum'	41.6^{f-i}	3.3^{fg}	19.0^{op}	0.20^{r}	4.7^{jk}	38.2^{opq}	0.23^{lmn}	1.16^{d-l}	53.3^{f-j}
'Elytritilops'	44.3^{e-i}	4.3^{efg}	59.0^{a}	1.46^{cde}	9.3^{c-h}	75.7^{g-k}	0.53^{jkl}	0.36^{n-q}	26.6^{s}
T. aestivum '5924'	46.1^{b-f}	2.7^{g}	49.7^{cd}	1.37^{d-g}	9.0^{d-h}	73.7^{h-k}	0.44^{j-n}	0.34^{opq}	24.8^{s}
T. aestivum 'Ahmetağa'	50.8^{b-e}	4.3^{efg}	37.3^{ij}	1.14^{f-i}	12.3^{bcd}	86.0^{c-h}	1.89^{c}	1.66^{c-f}	62.2^{c}
T. aestivum 'Ak 702'	49.3^{b-h}	8.0^{c}	43.3^{e-h}	2.02^{b}	18.7^{a}	116.7^{a}	1.92^{c}	0.96^{g-o}	48.7^{j-o}
T. aestivum 'Daws High PPO'	50.8^{b-e}	7.0^{cd}	24.5^{l-o}	0.56^{n-q}	11.7^{b-e}	73.7^{h-k}	0.73^{ijk}	1.31^{d-j}	56.6^{d-g}
T. aestivum 'Tir'	50.3^{bc}	7.7^{cd}	44.7^{d-g}	1.38^{d-g}	11.7^{b-e}	106.8^{a-d}	1.27^{fgh}	0.93^{g-o}	48.0^{k-o}
T. armeniacum	44.4^{e-i}	5.3^{def}	20.3^{op}	0.54^{opq}	7.0^{h-k}	88.0^{b-h}	1.24^{fgh}	2.32^{bc}	69.3^{b}
T. boeoticum	43.6^{e-i}	6.0^{cde}	21.0^{nop}	0.28^{qr}	4.0^{k}	49.7^{l-q}	0.11^{n}	0.40^{m-q}	28.4^{s}
T. compactum	43.7^{e-i}	3.3^{fg}	40.7^{f-i}	0.70^{k-o}	6.7^{h-k}	100.7^{a-e}	1.02^{ghi}	1.46^{d-h}	59.2^{cde}
T. dicoccoides	44.7^{e-i}	6.0^{cde}	41.0^{f-i}	1.52^{cd}	11.7^{b-e}	108.0^{abc}	1.37^{d-g}	0.93^{g-o}	47.8^{l-o}
T. dicoccum	43.8^{e-i}	8.3^{c}	55.0^{bc}	2.16^{b}	19.0^{a}	109.5^{ab}	2.62^{a}	1.21^{d-l}	54.8^{e-I}
T. durum 'Altın 40/98'	55.8^{b}	8.0^{c}	30.0^{kl}	1.00^{h-l}	9.0^{d-h}	96.3^{a-g}	2.35^{ab}	2.49^{b}	70.0^{b}
T. durum 'Berkmen 469'	50.6^{b-e}	8.3^{c}	38.3^{hi}	1.61^{cd}	10.0^{c-h}	101.7^{a-e}	1.70^{cde}	1.05^{e-m}	51.2^{h-m}
T. durum 'Yılmaz 98'	54.8^{bcd}	7.0^{cd}	29.7^{klm}	0.42^{o-r}	11.0^{b-f}	80.7^{e-I}	1.98^{bc}	4.75^{a}	82.3^{a}
T. fungicidum	40.6^{hi}	2.0^{g}	43.3^{e-h}	0.73^{j-o}	9.0^{d-h}	86.3^{c-h}	1.02^{ghi}	1.43^{d-i}	58.3^{c-f}
T. ispahanicum	44.0^{e-i}	4.0^{efg}	44.7^{d-g}	0.95^{h-m}	6.7^{h-k}	55.3^{k-q}	0.54^{jkl}	0.57^{l-q}	36.4^{r}
T. monococcum	43.6^{e-i}	7.0^{cd}	39.0^{ghi}	1.04^{hij}	14.0^{b}	78.0^{f-j}	1.32^{e-h}	1.25^{d-k}	55.4^{d-h}
T. petropavlovskyi	54.8^{bc}	3.3^{fg}	48.0^{b}	1.46^{cde}	12.7^{bc}	101.3^{a-e}	1.37^{d-g}	0.95^{g-o}	48.0^{k-o}
T. polonicum	49.2^{b-h}	2.0^{g}	65.0^{a}	1.45^{c-f}	12.0^{bcd}	103.8^{a-d}	1.43^{def}	0.99^{f-o}	49.7^{i-n}
T. spelta	42.4^{e-i}	7.0^{cd}	32.0^{jk}	1.13^{ghi}	21.0^{a}	60.8^{i-n}	0.94^{hi}	0.85^{h-q}	45.6^{nop}
T. sphaerococcum	41.5^{ghi}	3.0^{fg}	39.3^{f-i}	1.11^{ghi}	8.3^{e-i}	85.3^{d-h}	1.20^{fgh}	1.10^{d-k}	51.5^{g-l}
T. turanicum	49.0^{b-h}	3.0^{fg}	58.7^{b}	1.70^{c}	10.7^{b-g}	99.7^{a-f}	1.37^{d-g}	0.83^{h-q}	44.5^{op}
T. turgidum	45.4^{d-i}	2.7^{g}	45.0^{def}	1.01^{h-k}	7.3^{g-k}	47.7^{m-q}	1.75^{cd}	1.75^{cd}	63.3^{c}
T. urartu	44.7^{e-i}	4.3^{efg}	18.7^{p}	0.17^{r}	4.3^{k}	34.8^{q}	0.13^{mn}	0.78^{i-q}	41.3^{pqr}
T. vavilovii	53.9^{e-i}	4.0^{efg}	42.0^{f-i}	1.18^{e-h}	10.7^{b-g}	50.0^{l-q}	1.18^{fgh}	1.02^{e-n}	49.9^{i-n}
Mean	47.4	6.2	34.2	0.92	9.8	76.1	0.97	1.16	48.8
LSD (0.01)	8.78	2.47	5.72	0.31	3.44	22.33	0.40	0.68	5.14
CV (%)	8.60	18.3	7.70	15.7	16.3	13.6	18.8	27.1	11.6

(Tables 2 and 3). *Triticum* species and hybrids had more seminal and nodal roots than *Aegilops* species (Fig. 4). Most genotypes exhibited 2.5-3.0 seminal roots/plant in Z11 and 6.6-11.7 nodal roots/plant in Z31 (Fig. 4). This study further revealed that the number of nodal roots in Z31 was linked to rooting depth (r^2=0.22) and root biomass (r^2=0.36) (Fig. 5).

With respect to the root-to-shoot and root-to-total biomass ratios, significant differences were observed in Z11, ranging from 0.14 to 0.64% and from 11.8 to 37.7%, respectively (Table 2). They also varied from 0.18 to 4.75 for the root-to-shoot ratio and 15.4 to 82.3% for root-to-total biomass ratio in Z31 (Table 3). The mean values in *Aegilops* species were lower than those of the *Triticum* species and hybrids in both Z11 and Z31 (Fig. 4). The genotypes mostly had root-to-shoot ratios of 0.25-0.50 in Z11 and there were three genotypes with values above 1.81 for the root-to-shoot ratio in Z31 (Fig. 3). The lowest and highest values for root-to-shoot and root-to-total biomass ratios were obtained from *Ae. tauschii* and *Ae. brachyathera* among *Aegilops* species and *T. durum* 'Gır' and *T. aestivum* 'Daws High PPO' among *Triticum* species and hybrids in Z11, respectively. The lowest and highest values were obtained from *Ae. crassa* and *Ae. speltoides* among *Aegilops* species and *T. aestivum* '5924' and *T. durum* 'Yılmaz 98' among wheat species and hybrids in Z31.

3.2. Phenotypic variability in shoot traits

This study proved that significant variations exist among the genotypes in terms of coleoptile length, shoot height, number of tillers, shoot biomass, and SPAD chlorophyll in the early vegetative and stem elongation growth stages (P<0.01; Tables 2 and 3). Coleoptile length varied from 3.1 to 11.3 cm in studied genotypes (Table 2). The lowest coleoptile length was obtained from the 'Daws High PPO' line, while *T. ispahanicum* had the highest coleoptile length among genotypes. The mean coleoptile length of *Aegilops* species (5.5 cm) was lower than that of *Triticum* species and hybrids (6.4 cm) (Table 2). Moreover, the progenitor of the wheat D genome, *A. tauschii*, had the highest coleoptile length compared to other *Aegilops* species. Shoot height in the early growth and stem elongation stages of the genotypes ranged from 6.9 to

Table 4. *Triticum* species and hybrids paired in 10 genotypes for the lowest and the highest rooting depth and root biomass in both Z11 and Z31 growth stages.

High rooting depth (cm)				High root biomass (g)			
Genotypes	Z11	Genotypes	Z31	Genotypes	Z11	Genotypes	Z31
T. turanicum	21.2	Ak 702	116.7	Yılmaz 98	0.20	T. *dicoccum*	2.62
*T. carthlicum**	19.5	*T. dicoccum*	109.5	Daws High PPO	0.19	Altın 40/98	2.35
Gır	18.8	*T. dicoccoides*	108.0	Ak 702	0.18	Yılmaz 98	1.98
Tir	18.6	Tir	106.8	Berkmen 469	0.16	Ak 702	1.92
Altın 40/98	18.0	*T. petropavlovskyi*	101.3	Ahmetağa	0.12	Ahmetağa	1.89
T. dicoccum	17.9	*T. turanicum*	99.7	*T. turanicum*	0.09	*T. turgidum*	1.75
T. petropavlovskyi	17.9	Altın 40/98	96.3	*T. turgidum*	0.08	Berkmen 469	1.70
						T. turanicum	1.37
Low rooting depth (cm)				Low root biomass (g)			
Genotypes	Z11	Genotypes	Z31	Genotypes	Z11	Genotypes	Z31
T. urartu	9.0	*T. urartu*	34.8	*T. urartu*	0.01	*T. boeoticum*	0.11
T. boeoticum	10.9	*T. boeoticum*	49.7	*T. dicoccoides**	0.03	*T. urartu*	0.13
T. spelta	12.9	*T. spelta*	60.8	*T. boeoticum*	0.03	Agrotriticum	0.23
Elytritilops	14.7	Daws High PPO	73.7	Agrotriticum	0.03	*T. spelta*	0.94
Daws High PPO	14.9	Elytritilops	75.7	*T. spelta*	0.03		

21.7 cm and 18.3 to 65.0 cm, respectively. The mean shoot height of *Triticum* species and hybrids was higher than that of *Aegilops* species in both growth stages and the mean shoot biomass was approximately 2-fold higher in both growth stages compared to *Aegilops* species. Minimum and maximum shoot biomass values were obtained in a range from 0.04 to 0.42 g and from 0.17 to 2.16 g in the Z11 and Z31 growth stages, respectively, among the genotypes (Tables 2 and 3). The shoot biomass was highest for *Ae. kotschyi* in Z11 and *Ae. crassa* in Z31 among the *Aegilops* species. Among the *Triticum* species and hybrids, *T. aestivum* 'Ak 702' and *T. durum* 'Berkmen 469' had higher shoot biomass in both growth stages while *T. urartu*, 'Agrotriticum', and *T. boeoticum* possessed the lowest shoot biomasses. Table 3 shows significant variations among the genotypes regarding number of tillers, ranging from 2 to 14.7. The mean number of tillers in the *Aegilops* species (9) was higher than that of *Triticum* species and hybrids (5.1). SPAD chlorophyll ranged from 37.7 to 83.7 µmol m^{-2} among the genotypes in Z31. According to the results, *Ae. speltoides* had the highest mean SPAD chlorophyll values among the mean values of genotypes (47.4 µmol m^{-2}).

3.3. Hierarchical clustering

Hierarchical cluster analysis revealed the relationship among the genotypes using a dendrogram constructed from the dissimilarity matrix. The results proved that all 35 genotypes were grouped into four main clusters in terms of rooting depth, number of seminal roots, number of nodal roots, root biomass, root-to-shoot ratio, and root-to-total biomass ratio in both Z11 and Z31. Groups were formed in clusters 1, 2, 3, and 4, including 9 genotypes in cluster 1, 9 genotypes in cluster 2, 5 genotypes in cluster 3, and 12 genotypes in cluster 4 (Fig. 2). *Aegilops* species were grouped in clusters 3 and 4 with the except for *Ae. brachyathera.* The two wheat progenitors *T. urartu* and *T. boeoticum*, with low root biomass, as well as the hulled genotypes *T. spelta, T. vavilovii, T. ispahanicum*, and Agrotriticum were grouped in cluster 1. Cluster 3 contained D genome progenitor *Ae. tauschii* together with *Ae. neglecta*. The genotypes with shallow rooting and low biomass in both the Z11 and Z31 growth stages were included in clusters 3 and 4 (Fig. 2). The deep-rooted genotypes for both growth stages, *T. dicoccum*, *T. dicoccoides*, *T. petropavlovskyi*, *T. turanicum*, and *T. aestivum* 'Ak 702' and 'Tir' were placed in cluster 2. Furthermore, the cultivars with high root biomass for both growth stages ('Altın 40/98', 'Yılmaz 98', and 'Ahmetağa') constituted cluster 1 (Fig. 2). The wild form of *T. timopheevii, T. armeniacum*, was in the same cluster as *T. fungicidum* (*T. carthlicum* × *T. timopheevii*). Moreover, a wild form of *T. dicoccum, T. dicoccoides*, was in the same cluster as *T. dicoccum*. This study further indicated that wheat hybrids with genotypes of different genera such as 'Elytritilops' (*Elymus* × *Triticum* × *Aegilops*) and 'Agrotriticum' (*Agropyron* × *Triticum*) had low root biomass and shallow rooting depth, appearing in clusters 3 and 4.

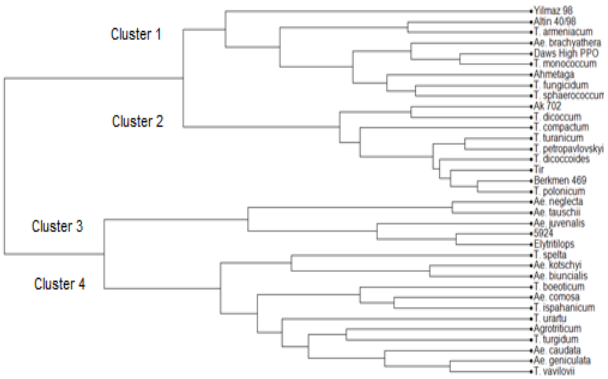

Figure 2. Hierarchical clustering of *Triticum* and *Aegilops* taxa, and hybrids for root traits such as rooting depth, number of seminal roots, number of nodal roots, root biomass, root-to-shoot ratio, and root-to-total biomass ratio in both Z11 and Z31 growth stages.

4. Discussions

4.1. Root traits of genotypes

4.1.1. Deep-rooted genotypes in drought conditions

This study showed that in the Z11 growth stage, genotypes including *T. turanicum, T. carthlicum, T. durum* genotypes 'Gır' and 'Altın 40/98', *T. aestivum* genotypes 'Tir', line '5924', 'Ahmetağa', *T. dicoccum*, and *T. petropavlovskyi* had deep roots, while *T. aestivum* genotypes 'Ak 702',

'Tir', *T. durum* 'Berkmen 469', *T. dicoccum*, *T. dicoccoides*, *T. polonicum*, *T. petropavlovskyi*, and *T. compactum* were found to have rooting depths of more than 100 cm among the *Triticum* species in Z31. Root traits in the early growth stage can be used as a secondary selection criterion in breeding programs (Manschadi et al., 2006). In this context, *T. boeoticum* and *T. urartu* with 14 chromosomes as diploid wild forms of wheat possessed shallow rooting patterns in both growth stages. In Z11 and Z31, *Triticum* species and wheat hybrids had deeper rooting than *Aegilops* species (Fig. 4). The rooting pattern in wheat species and in hybrids was affected by high shoot biomass, which was remobilized from higher leaf photosynthesis reserves to roots, important for meeting carbon requirements. A previous study of wheat cultivars grown in a mixture of peat and perlite found greater rooting depths in Z31, ranging from 189 to 216.6 cm (mean of two years), in comparison to values of 34.8-116.7 cm in the present study (Akman et al., 2017b). However, the present study used field soil in the rooting zone, which was reported to pose mechanical impedance to root growth, determining the root elongation and proliferation within a soil profile (Bengough and Mullins, 1990). The selected deep-rooted genotypes could be utilized in breeding programs to access the water from deep soil in water-limited conditions (Sayar et al., 2007).

4.1.2. Genotypes with high root biomass may be evaluated as breeding materials under non-stressed conditions

As a significant feature of plants, root biomass is an indicator of the size of a root system (Ehdaie et al., 2010). Large and shallow root systems can take up water from the upper layers of the soil during vegetative growth when rainfall is abundant in the winter (Manschadi et al., 2006). A small root system can be useful in rain-fed field areas with water-use efficiency (Passioura, 1983). In contrast, cultivars with large root systems had greater grain yields than cultivars with small root systems in rain-fed experiments in Central Europe (Středa et al., 2012). Contrasting results are due to the variable rainfall in dryland farming systems in various growth stages (Palta et al., 2011). Our results in Z31 agreed with those obtained in our previous study of field-grown wheat, suggesting that *T. aestivum* 'Ahmetağa', 'Ak 702', *T. durum* 'Berkmen 469', and *T. turgidum* had greater root biomass, while *T. aestivum* '5924' (line), *T. boeoticum*, and *Ae. biuncialis* possessed lower root biomass among genotypes (Akman et al., 2017a). A greater root system contributes to yield stability because during drought periods the root system can access water in deeper soil layers (Středa et al., 2012). The present study also revealed that *T. aestivum* 'Ak 702', 'Ahmetağa', 'Yılmaz 98', *T. durum* 'Berkmen 469', *T. turgidum*, and *T. turanicum* had the highest root biomass in both Z11 and Z31 while *T. urartu*, *T. boeoticum*, 'Agrotriticum', and *T. spelta* constituted the group of genotypes with the lowest root biomass. Moreover, among the genotypes studied, *T. dicoccum* and *T. durum* 'Altın 40/98' had higher root biomass in only Z31. Small amounts of shoot biomass contributing to low assimilate allocation in the *Aegilops* species led to smaller root biomass than in the *Triticum* species and hybrids.

4.1.3. Number of seminal and nodal roots

Two types of roots occur in wheat: seminal roots emerge directly from the embryo, while the later nodal roots come from the lower tiller nodes (Manske and Vlek, 2002). A higher number of seminal roots in wheat has been associated with more intensive root branching with at greater depths (Manschadi et al., 2008). Previous studies found that number of seminal roots varied from 3.2 to 5 in bread wheat genotypes, which falls between range of the number of seminal roots (2.7-5) in this study for wheat species and hybrids (Manschadi et al., 2008; Richard et al., 2015; Bektaş and Waines, 2020). In this study, the numbers of seminal root were not more than three per plant among *Aegilops* species. However, tetraploid wheat species *T. dicoccum* (4.5), *T. fungicidum* (4.7), *T. durum* 'Altın 40/98' (5), and *T. ispahanicum* (5) and the hexaploid landrace 'Tir' (4.7) possessed higher numbers of seminal roots than the other genotypes studied. In Z31, *T. aestivum* 'Ak 702' (18.7), *T. dicoccum* (19), and *T. spelta* (21) had the highest nodal root numbers among the studied genotypes. Thus, this study has further indicated that *T. dicoccum* has the highest numbers of seminal and nodal roots among other genotypes.

4.1.4. Root-to-shoot and root-to-total biomass ratios

The root-to-shoot ratio has been used to describe assimilate allocation to roots (Nakhforoosh et al., 2014). The root-to-total biomass ratio represents the assimilate proportion to roots into total biomass. Root-to-shoot and root-to-total biomass ratios were higher in Z31 than in Z11 as the growth stage. The results reported by Siddique et al. (1990) of a root-to-shoot ratio of 1.1 to 1.8 and a root-to-total biomass ratio of 52.1% to 64.4% in the 62 days after sowing were within the ranges of values found in the present study in Z31. This study indicated that an increase in root biomass generally enhanced the root-to-shoot and root-to-total biomass ratios; however, this was not always observed. This can be expressed as better representing assimilate accumulation into root growth up to the Z31 growth stage rather than that into shoot growth. The wild wheat ancestors *T. urartu* and *T. dicoccoides* and line 5924 were found to have low root-to-shoot and root-to-total biomass ratios in both Z11 and Z31 among the studied wheat species and hybrids, while *T. aestivum* 'Daws High PPO', 'Ahmetağa', *T. durum* 'Yılmaz 98', and *T. monococcum* had high ratios. Evaluating the *Aegilops* species, *Ae. brachyathera* had high root-to-shoot and root-to-total biomass ratios in both growth stages; however, *A. tauschii* had low values. *Ae. brachyathera* was a located *Aegilops* species in cluster 1 of the dendrogram with *Triticum* species and hybrids because it had relatively high rooting depth, root biomass, and root-to-shoot and root-to-total biomass ratios among the *Aegilops* species in both growth stages (Fig. 4). Although *T. aestivum* 'Ak 702' was among the genotypes with high root biomass in both growth stages, it was not included in the group with high root-to-shoot and root-to-total biomass ratios in Z31. This can be explained by the fact that the assimilating allocation into root biomass was lower than that into shoot biomass up to Z31.

4.2. Variability in shoot traits of genotypes

4.2.1. Shoot biomass

Plant breeders have primarily concentrated on grain yield rather than root growth; however, there is a critical

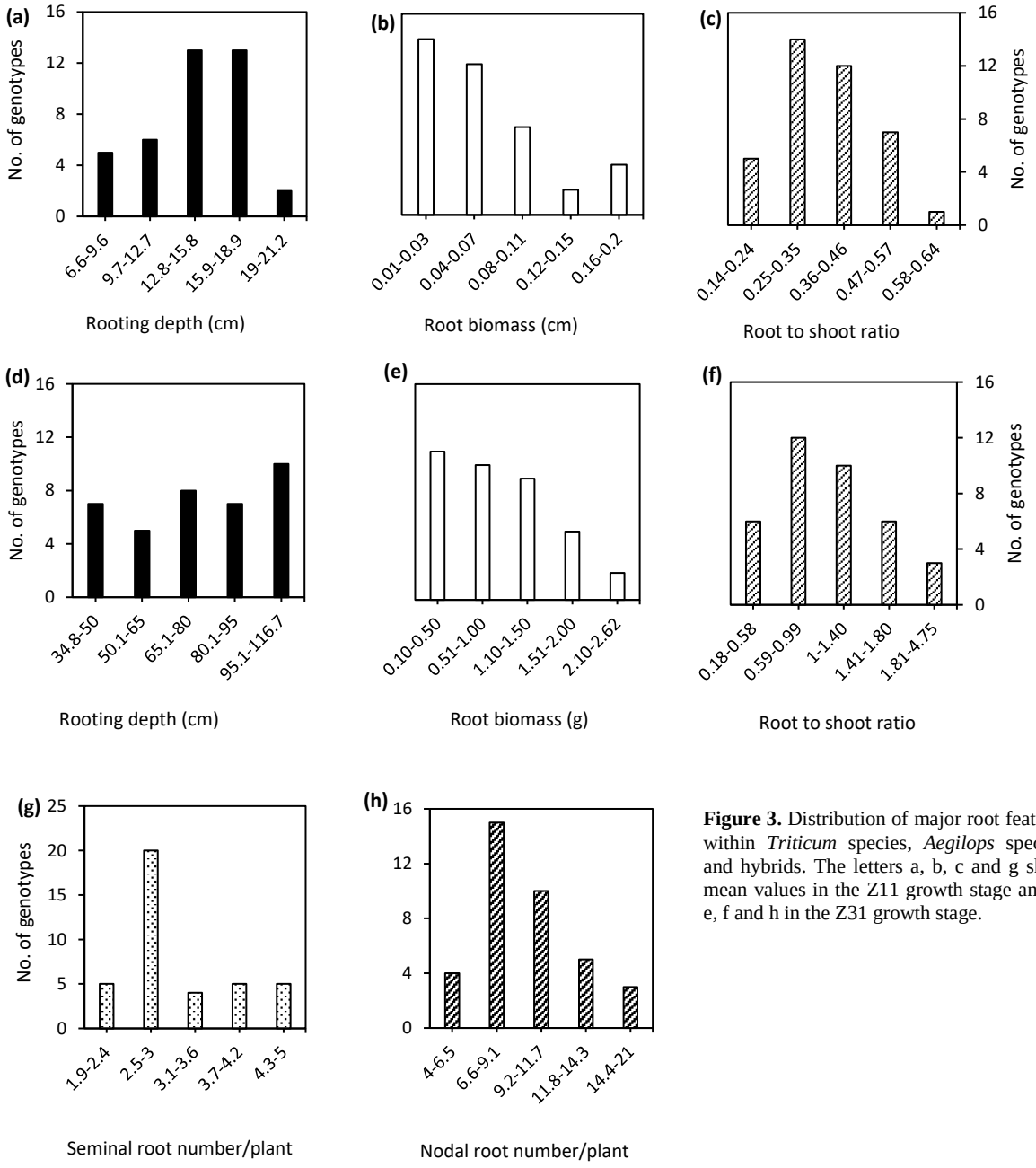

Figure 3. Distribution of major root features within *Triticum* species, *Aegilops* species, and hybrids. The letters a, b, c and g show mean values in the Z11 growth stage and d, e, f and h in the Z31 growth stage.

association between the root and shoot growth for high yield. In this study, shoot biomass was linked to root biomass more strongly in Z11 (r^2=0.83) than Z31 (r^2=0.44) (Fig. 5). The results were consistent with the findings of previous studies indicating a significant association between the root and shoot biomass (Sharma, 1993; Atta et al., 2013; Akman et al., 2017a). There was also a link between shoot biomass and rooting depth (r^2=0.41). Significant differences in shoot biomass were found among the genotypes. Compared to other wheat species and hybrids, *T. durum* 'Berkmen 469', and *T. aestivum* '5924' (line), 'Ak 702' maintained higher shoot biomass in both growth stages, while *T. urartu*, *T. boeoticum*, and 'Agrotriticum' had the lowest shoot biomass in both growth stages. Among the *Aegilops* species, *Ae. brachyathera* and *Ae. tauschii* had lower shoot biomass in both growth stages. *T. durum* 'Yılmaz 98', *T. aestivum* 'Daws High PPO', and *Ae. kotschyi* were ranked as genotypes with higher shoot biomass in Z11; however, they were among the bottom of genotypes with low shoot biomass in Z31. *T. durum* 'Yılmaz 98' had higher root biomass in Z31, when assimilates may be transported more into the roots. *T. aestivum* 'Daws High PPO' and *Ae. kotschyi* simultaneously had reduced root biomass and shoot biomass as their genotypic characteristics.

4.2.2. Coleoptile length

Selecting wheat cultivars with long coleoptiles is a significant target for sustaining emergence, weed competition, and grain yield in water-deficient regions of the world (Singh and Khanna-Chopra, 2010). Coleoptile length was reported to be affected by both genetic background and environmental factors (Allan et al., 1962;

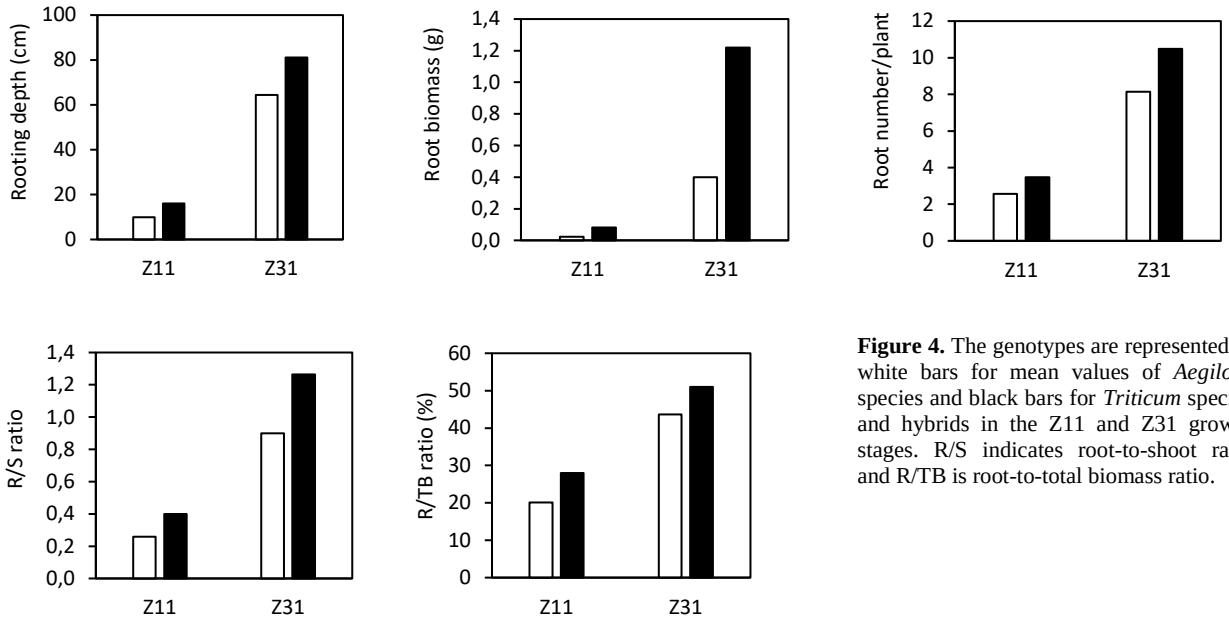

Figure 4. The genotypes are represented in white bars for mean values of *Aegilops* species and black bars for *Triticum* species and hybrids in the Z11 and Z31 growth stages. R/S indicates root-to-shoot ratio and R/TB is root-to-total biomass ratio.

Murray and Kuiper, 1988; Botwright et al., 2001). Wheat with long coleoptiles has greater emergence in deep planting than wheat with short coleoptiles (Rebetzke et al., 2007). However, Mohan et al. (2013) showed that coleoptiles longer than 9 cm had no advantage for emergence in deep planting. They also indicated that coleoptile length ranged from 3.4 to 11.4 cm in 662 studied wheat cultivars. Liatukas and Ruzgas (2011) showed that none of 564 winter wheat cultivars possessed a coleoptile length longer than 10 cm. In the present study, *T. ispahanicum* (11.3 cm), *T. aestivum* 'Tir' (9.3 cm), *T. fungicidum (*9 cm), *T. turanicum* (8.3 cm), and *T. dicoccoides* (8 cm) had the longest coleoptiles among the *Triticum* genotypes and wheat hybrids. *T. monococcum* (4.7 cm) and its wild form, *T. boeoticum* (3.8 cm), had shorter coleoptiles. Wheat progenitors *T. dicoccoides* and *T. urartu* had lengths of 8 cm and 5.2 cm, respectively. The D genome progenitor of bread wheat, *Ae. tauschii* (6.8 cm), possessed the longest coleoptile length among the *Aegilops* species.

4.2.3. Shoot height

Wheat grain yield was increased due to reduced height by controlled *Rht* alleles to improve semi-dwarf cultivars (Chapman et al., 2007). The present study revealed that modern wheat cultivars had lower shoot heights in both Z11 and Z31. However, different wheat species, landraces, and wheat hybrids were tall shoot height in both Z11 and Z31 (Tables 2 and 3). This is most likely due to the presence and/or absence of *Rht* alleles in the genotypes. An earlier study showed that semi-dwarf wheat cultivars had shorter root systems than tall cultivars in field experiments (Subbiah et al., 1968). Figure 4 indicates a relationship between shoot height and rooting depth (r^2=0.30). As for the *Aegilops* species, in both growth stages, *Ae. brachyathera* and *Ae. neglecta* exhibited taller and shorter shoot heights, respectively.

4.2.4. Number of Tillers

The set of genotypes used in this study exhibited significant variation for number of tillers. High tillering genotypes may compensate for lower numbers of plants caused by late drought or early frost (Acevedo et al., 2002; Elhani et al., 2007). In this study, both maximum and minimum number of tillers were observed in tetraploid wheat genotypes among the wheat species and hybrids in Z31. *T. durum* 'Berkmen 469', 'Altın 40/98', *T. dicoccum* (landrace), *T. aestivum* 'Ak 702' (old cultivar), 'Tir' (landrace) were found to be high tillering genotypes and are grown with superiority in regions of Turkey prone to early frost damage and late drought stress. The genotype with the lowest tillering, *T. polonicum*, was reported to have a high grain weight by Bienkowska et al. (2020) (57.9 mg) and Wang et al. (2002) (80 mg). This result was confirmed by Dreccer et al. (2013), who indicated that low tillering wheat lines accumulated more water-soluble carbohydrates in the stems, which supplied a higher grain number per spike and heavier kernels with minimal yield increase.

4.3. SPAD chlorophyll readings

SPAD chlorophyll meter readings allow for rapid assessment of chlorophyll density in plants (Puangbut et al., 2017). The chlorophyll content is a good indicator of the "stay-green" trait of photosynthetic tissue (Fotovat et al., 2007). It has been recommended that high chlorophyll content be used to represent a low degree of photoinhibition (Farquhar and Richards, 1984). Chlorophyll readings of the genotypes were similar among the *Aegilops* species, wheat species, and hybrids with one exception. An ancestor of wheat, *Ae. speltoides*, had exceptionally high chlorophyll (83.7 µmol m^{-2}) compared to the mean values of the other studied genotypes.

4.4. Selection of genotypes with superior root features for the target environment

Root traits have been neglected in the development of new cultivars, and more efforts are being taken in the measurement and explanation of below-ground traits (Richards, 2006; Bektaş et al., 2020). The root biomass and depth are valuable selection criteria in breeding programs. Also, measurements of these traits are

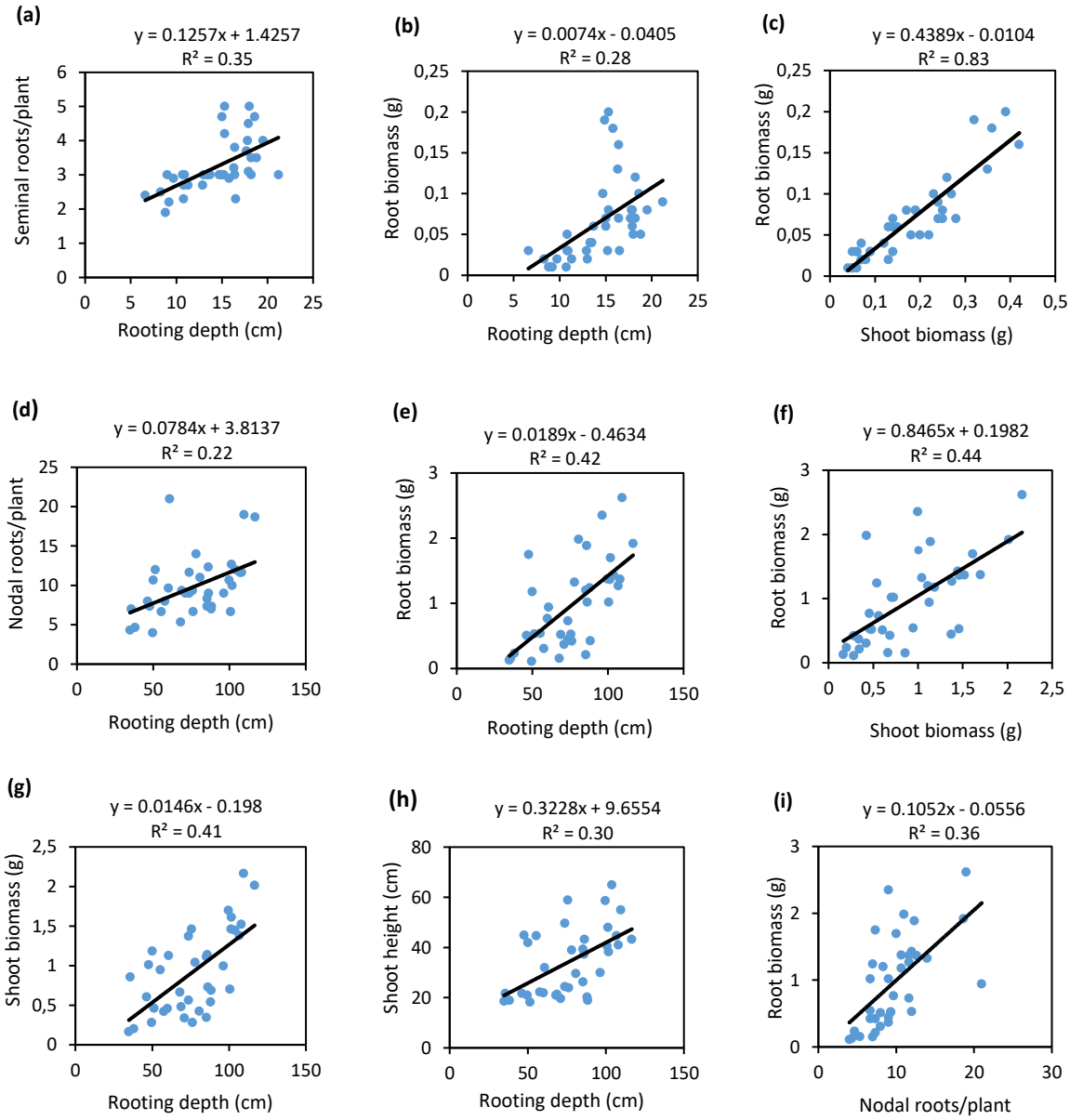

Figure 5. Relationships between/within root and shoot traits in the Z11 (a, b, and c) and Z31 (d, e, f, g, h, and i).

simple and inexpensive. The root traits should be phenotypically screened in both early vegetative and later growth stages for variability in drought-adaptive and non-stressed traits. The rooting depth and root biomass seem to be the primary selection features, substantially affecting the root system's architecture and therefore providing more water and nutrient uptake in the soil profile. In the present study, both deep-rooted and shallow-rooted genotypes were selected, as were genotypes with both low and high root biomass in the early vegetative and stem elongation stages. Deep rooting has been reported to increase wheat yield considerably by extracting more water from the deep soil profile under water deficit (Fang et al., 2017; El Hassouni et al., 2018). The genotypes *T. turanicum*, *T. aestivum* 'Tir', *T. durum* 'Altın 40/98', *T. dicoccum*, and *T. petropavlovskyi* were identified as having the deepest rooting in both Z11 and Z31 (Table 4).

Our previous findings demonstrated that root biomass was positively and significantly correlated with grain yield in field-grown genotypes under well-watered conditions (Akman et al., 2017a). Qi et al. (2019) showed positive and significant relations between grain yield and root weight density of maize in topsoil (0-40 cm) in which a significant rate of root biomass accumulated. Kanbar et al. (2009) revealed that root biomass in rice had a significant effect on grain yield in well-watered conditions, but rooting depth was important for improving the grain yield in low-moisture conditions. There is no consensus on whether the improvement of wheat with a large root system for rain-fed conditions is the best scheme in breeding programs (Palta et al., 2011), and Passioura (1983) proved that a smaller root system may be beneficial in water-insufficient conditions. Our earlier findings in mature field-grown plants also identified the same genotypes as having high biomass, such as *T. aestivum* 'Ahmetağa', 'Ak 702', and *T. durum* 'Berkmen 469' (Akman et al., 2017a). As a result, the genotypes *T. durum* 'Yılmaz 98', 'Berkmen 469', *T. aestivum* 'Ak 702', 'Ahmetağa', *T. turgidum*, and *T. turanicum*, which maintained high root biomass consistently in both Z11 and Z31, can be evaluated for the cultivar improvement with

desirable roots in both target environments. Moreover, the *T. turanicum*, *T. dicoccum*, *T. durum* 'Altın 40/98', and *T. aestivum* 'Ak 702' genotypes not only had high root biomass values but also deep-rooting features in both growth stages among the genotypes studied (Table 4). This study further supports that these genotypes should be evaluated for both water-deficit and non-stressed conditions with a view to using them in breeding programs to develop promising cultivars with desirable root systems.

References

Acevedo E, Silva P, Silva H (2002). Wheat growth and physiology. Bread Wheat, Improvement and Production 30: 39-70.

Adu MO, Sparkes DL, Parmar A, Yawson DO (2011). Stay green in wheat: Comparative study of modern bread wheat and ancient wheat cultivars. Journal of Agricultural and Biological Science 6: 16-24.

Akman H, Akgun N, Tamkoc A (2017a). Comparison of root and shoot traits of different wheat species and wild wheat relatives: Does feature of shoot biomass have positive and significant relationships with grain yield and root traits?. Revista de la Facultad de Agronomia de la Universidad del Zulia 34: 428-447.

Akman H, Akgun N, Tamkoc A (2017b). Screening for root and shoot traits in different wheat species and wild wheat relatives. Botanical Sciences 95: 147-154.

Allan R, Vogel O, Burleigh J (1962). Length and estimated number of coleoptile parenchyma cells of six wheat selections grown at two temperatues 1. Crop Science 2: 522-524.

Arzani A, Ashraf M (2017). Cultivated ancient wheats (*Triticum* spp.): A potential source of health-beneficial food products. Comprehensive Reviews in Food Science and Food Safety 16: 477-488.

Atta BM, Mahmood T, Trethowan TM (2013). Relationship between root morphology and grain yield of wheat in North-Western NSW, Australia. Australian Journal of Crop Science 7: 2108-2115.

Banga SS, Kang MS (2014). Developing climate-resilient crops. Journal of Crop Improvement 28: 57-87.

Bektaş H, Waines JG (2020). Effect of grain size on the root system architecture of bread wheat (*Triticum aestivum* L.). Turkish Journal of Agricultural Research 7: 78-84.

Bektaş H, Hohn CE, Waines JG (2020). Dissection of quantitative trait loci for root characters and day length sensitivity in SynOpDH wheat (*Triticum aestivum* L.) bi-parental mapping population. Plant Genetic Resources, 18(3): 130-142.

Bengough AG, McKenzie B, Hallett P, Valentine T (2011). Root elongation, water stress, and mechanical impedance: A review of limiting stresses and beneficial root tip traits. Journal of Experimental Botany 62: 59-68.

Bengough AG, Mullins CE (1990). Mechanical impedance to root growth: A review of experimental techniques and root growth responses. Journal of Soil Science 41: 341-358.

Bienkowska T, Suchowilska E, Wiwart M (2020). *Triticum polonicum* L. As promising source material for breeding new wheat cultivars. Journal of Elementology 25: 237-248.

Botwright T, Rebetzke G, Condon A, Richards R (2001). Influence of variety, seed position and seed source on screening for coleoptile length in bread wheat (T*riticum aestivum* L.). Euphytica 119: 349-356.

Chapman S, Mathews K, Trethowan R, Singh R (2007). Relationships between height and yield in near-isogenic spring wheats that contrast for major reduced height genes. Euphytica 157: 391-397.

Dreccer MF, Chapman SC, Rattey AR, Neal J, Song Y, Christopher JT, Reynolds M (2013). Developmental and growth controls of tillering and water-soluble carbohydrate accumulation in contrasting wheat (*Triticum aestivum* L.) genotypes: Can we dissect them?. Journal of Experimental Botany 64: 143-160.

Dvorak J, Akhunov ED (2005). Tempos of gene locus deletions and duplications and their relationship to recombination rate during diploid and polyploid evolution in the *Aegilops-Triticum* alliance. Genetics 171: 323-332.

Ehdaie B, Merhaut D, Ahmadian S, Hoops A, Khuong T, Layne A, Waines J (2010). Root system size influences water-nutrient uptake and nitrate leaching potential in wheat. Journal of Agronomy and Crop Science 196: 455-466.

El Hassouni K, Alahmad S, Belkadi B, Filali-Maltouf A, Hickey L, Bassi F (2018). Root system architecture and its association with yield under different water regimes in durum wheat. Crop Science 58: 2331-2346.

Elhani S, Martos V, Rharrabti Y, Royo C, del Moral LG (2007). Contribution of main stem and tillers to durum wheat (*Triticum turgidum* L. var. *durum*) grain yield and its components grown in mediterranean environments. Field Crops Research 103: 25-35.

Fang Y, Du Y, Wang J, Wu A, Qiao S, Xu B, Zhang S, Siddique KH, Chen Y (2017). Moderate drought stress affected root growth and grain yield in old, modern and newly released cultivars of winter wheat. Frontiers in Plant Science 8: 1-14.

Farquhar G, Richards R (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Functional Plant Biology 11: 539-552.

Fotovat R, Valizadeh M, Toorchi M (2007). Association between water-use efficiency components and total chlorophyll content (spad) in wheat (*Triticum aestivum* L.) under well-watered and drought stress conditions. Journal of Food Agriculture & Environment 5: 225-227.

Friedli CN, Abiven S, Fossati D, Hund A (2019). Modern wheat semi-dwarfs root deep on demand: Response of rooting depth to drought in a set of Swiss era wheats covering 100 years of breeding. Euphytica 215: 85.

Heřmanská A, Středa T, Chloupek O (2015). Improved wheat grain yield by a new method of root selection. Agronomy for Sustainable Development 35: 195-202.

Kanbar A, Toorchi M, Shashidhar H (2009). Relationship between root and yield morphological characters in rainfed low land rice (*Oryza sativa* L.). Cereal Research Communications 37: 261-268.

Kishii M (2019). An update of recent use of *Aegilops* species in wheat breeding. Frontiers in Plant Science 10: 585.

Li D, Long D, Li T, Wu Y, Wang Y, Zeng J, Xu L, Fan X, Sha L, Zhang H (2018). Cytogenetics and stripe rust resistance of wheat–*Thinopyrum elongatum* hybrid derivatives. Molecular Cytogenetics 11: 1-16.

Liatukas Z, Ruzgas V (2011). Relationship of coleoptile length and plant height in winter wheat accessions. Pakistan Journal of Botany 43: 1535-1540.

Manschadi AM, Christopher J, deVoil P, Hammer GL (2006). The role of root architectural traits in adaptation of wheat to water-limited environments. Functional Plant Biology 33: 823-837.

Manschadi AM, Hammer GL, Christopher JT, Devoil P (2008). Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). Plant and Soil 303: 115-129.

Manske GG, Vlek PL (2002). Root architecture–wheat as a model plant. Plant Roots: The Hidden Half 3: 249-259.

Maron L (2019). Sequencing of ancient wheat genomes opens a window into the past. The Plant Journal 99: 199-200.

Mathre D, Johnston R, Martin J (1985). Sources of resistance to *Cephalosporium gramineum* in *Triticum* and *Agropyron* species. Euphytica 34: 419-424.

Mohan A, Schillinger WF, Gill KS (2013). Wheat seedling emergence from deep planting depths and its relationship with coleoptile length. PLoS One 8: 1-9.

Murray G, Kuiper J (1988). Emergence of wheat may be reduced by seed weather damage and azole fungicides and is related to coleoptile length. Australian Journal of Experimental Agriculture 28: 253-261.

Nakhforoosh A, Grausgruber H, Kaul H-P, Bodner G (2014). Wheat root diversity and root functional characterization. Plant and Soil 380: 211-229.

Nevo E (2011). Cereals. Wild Crop Relatives: Genomic and Breeding Resources. Springer.

Özkan H, Tuna M, Kilian B, Mori N, Ohta S (2010). Genome size variation in diploid and tetraploid wild wheats. AoB Plants 2010: 1-11.

Palta JA, Chen X, Milroy SP, Rebetzke GJ, Dreccer MF, Watt M (2011). Large root systems: Are they useful in adapting wheat to dry environments? Functional Plant Biology 38: 347-354.

Passioura J (1983). Roots and drought resistance. Agricultural Water Management 7: 265-280.

Peng J, Sun D, Nevo E (2011). Wild emmer wheat,'*Triticum dicoccoides*', occupies a pivotal position in wheat domestication process. Australian Journal of Crop Science 5: 1127-1143.

Perrier X, Jacquemoud-Collet JP (2006). DARwin software. Available from http://www.darwin.cirad.fr/darwin/Home.php / [accessed 25 December 2020].

Prasad P, Staggenborg S, Ristic Z (2008). Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes 1: 301-355.

Puangbut D, Jogloy S, Vorasoot N (2017). Association of photosynthetic traits with water use efficiency and spad chlorophyll meter reading of *Jerusalem artichoke* under drought conditions. Agricultural Water Management 188: 29-35.

Richards RA (2006). Physiological traits used in the breeding of new cultivars for water-scarce environments. Agricultural Water Management 80: 197-211.

Qi D, Hu T, Song X, Zhang M (2019). Effect of nitrogen supply method on root growth and grain yield of maize under alternate partial root-zone irrigation. Scientific Reports 9: 1-10.

Rebetzke G, Ellis M, Bonnett D, Richards R (2007). Molecular mapping of genes for coleoptile growth in bread wheat (T*riticum aestivum* L.). Theoretical and Applied Genetics 114: 1173-1183.

Richard CA, Hickey LT, Fletcher S, Jennings R, Chenu K, Christopher JT (2015). High-throughput phenotyping of seminal root traits in wheat. Plant Methods 11: 1-11.

Sayar R, Khemira H, Kharrat M (2007). Inheritance of deeper root length and grain yield in half-diallel durum wheat (*Triticum durum*) crosses. Annals of Applied Biology 151: 213-220.

Sharma R (1993). Selection for biomass yield in wheat. Euphytica 70: 35-42.

Siddique K, Belford R, Tennant D (1990). Root: Shoot ratios of old and modern, tall and semi-dwarf wheats in a mediterranean environment. Plant and Soil 121: 89-98.

Singh K, Khanna-Chopra R (2010). Physiology and QTL analysis of coleoptile length, a trait for drought tolerance in wheat. Journal of Plant Biology 37: 1-9.

Středa T, Dostál V, Horáková V, Chloupek O (2012). Effective use of water by wheat varieties with different root system sizes in rain-fed experiments in central europe. Agricultural Water Management 104: 203-209.

Subbiah B, Katyal J, Narasimham R, Dakshinamurti C (1968). Preliminary investigations on root distribution of high yielding wheat varieties. The International Journal of Applied Radiation and Isotopes 19: 385-390.

Ullah S, Bramley H, Daetwyler H, He S, Mahmood T, Thistlethwaite R, Trethowan R (2018). Genetic contribution of emmer wheat (T*riticum dicoccon* Schrank) to heat tolerance of bread wheat. Frontiers in Plant Science 9: 1-11.

Wang H-J, Huang X-Q, Röder M, Börner A (2002). Genetic mapping of loci determining long glumes in the genus *Triticum*. Euphytica 123: 287-293.