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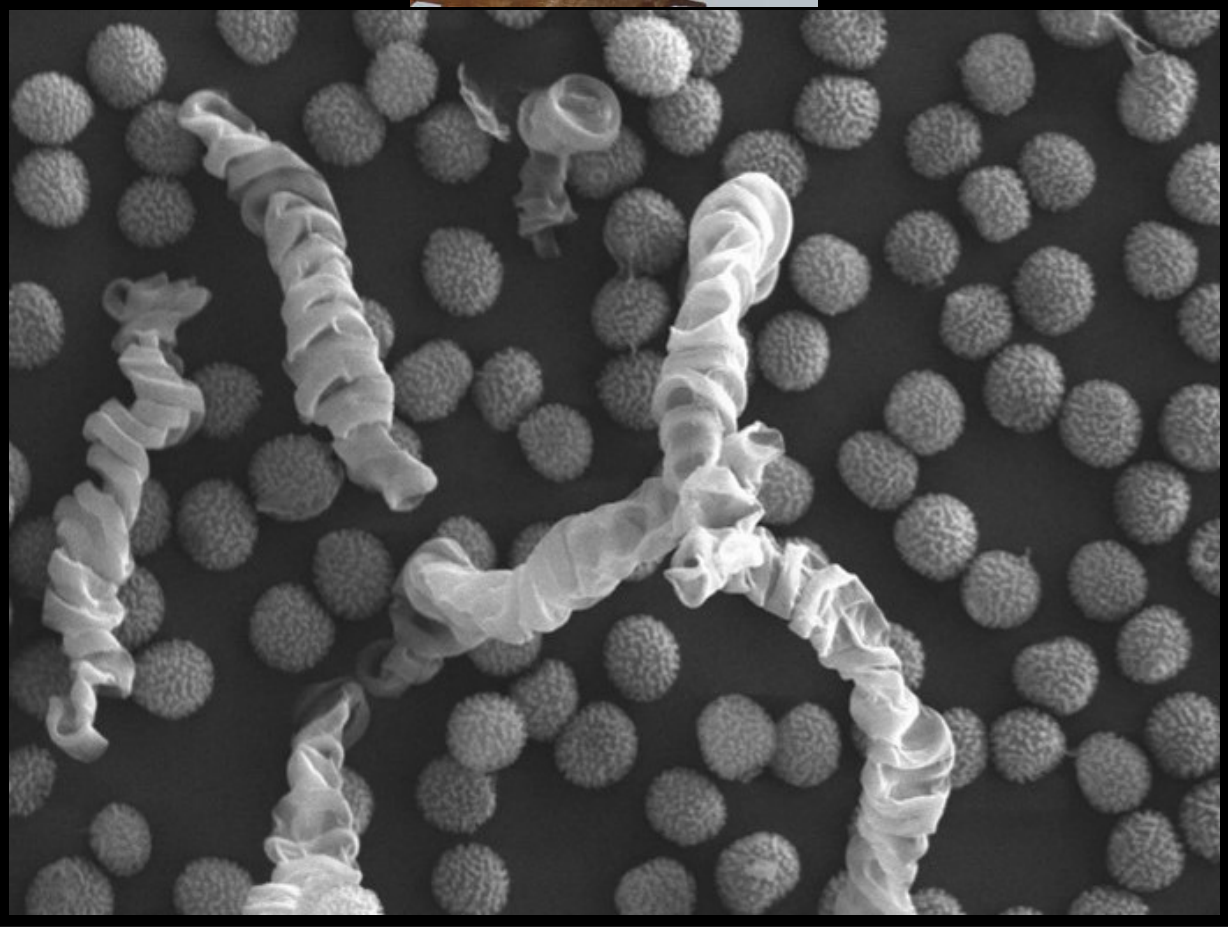
Battarrea phalloides



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S.Ü.
Mantarcılık Uygulama ve Araştırma Merkezi Müdürlüğü
Alaaddin Keykubat Yerleşkesi, Fen Fakültesi B Blok,
Zemin Kat-42079/Selçuklu-KONYA

Tel:(+90)0 332 2233998/ Fax: (+90)0 332 241 24 99

Web: http://mantarcilik.selcuk.edu.tr
http://dergipark.gov.tr/mantar

E-Posta:mantarcilik@gmail.com

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A Note on *Battarrea phalloides* in Turkey

Ilgaz AKATA[1*], Deniz ALTUNTAŞ[2], Ergin ŞAHİN[1],
Hakan ALLI[3], ŞANLI KABAKTEPE[4]

**Corresponding author: akata@science.ankara.edu.tr*

[1] Ankara University, Faculty of Science, Department of Biology, Ankara, *Turkey*
Orcid ID: 0000-0002-1731-1302/ akata@science.ankara.edu.tr
Orcid ID: 0000-0003-1711-738X/ erginsahin@ankara.edu.tr
[2]Ankara University, Graduate School of Natural and Applied Sciences, Ankara, Turkey
Orcid ID: 0000-0003-0142-6188/ denizaltuntas91@gmail.com
[3]Muğla Sıtkı Koçman University, Faculty of Science, Department of Biology, Muğla, Turkey
Orcid ID: 0000-0001-8781-7089/ hakanalli@gmail.com
[4]Malatya Turgut Ozal University, Battalgazi Vocat Sch., Battalgazi, Malatya, Turkey
Orcid ID: 0000-0001-8286-9225/skabaktepe@gmail.com

Abstract: The current study was conducted based on a *Battarrea* sample obtained from Muğla province (Turkey). The sample was identified based on both conventional methods and ITS rDNA region-based molecular phylogeny. By taking into account the high sequence similarity between the sample (ANK Akata & Altuntaş 690) and *Battarrea phalloides* the relevant specimen was considered to be *B. Phalloides* and the morphological data also strengthen this finding. In this study, photos of macro and microscopic structures, a short description, scanning electron microscope (SEM) images of spores and elaters, and the ITS rDNA region-based molecular phylogeny of the samples were given. Also, the distribution of *B. phalloides* specimens identified thus far from Turkey was revealed for the first time in this study.

Key words: Fungal diversity, gasteroid fungi, Turkey

Türkiye'deki *Battarrea phalloides* Üzerine Bir Not

Öz: Bu çalışma, Muğla yöresinden (Türkiye) elde edilen *Battarrea* örneğine dayanılarak yapılmıştır. Örnek, hem konvansiyonel yöntemlere hem de ITS rDNA bölgesi bazlı moleküler filogeniye dayalı olarak tanımlanmıştır. Örnek (ANK Akata & Altuntaş 690) ile *Battarrea phalloides* arasındaki yüksek sekans benzerliği dikkate alınarak ilgili örnek *B. phalloides* olarak kabul edilmiş ve morfolojik veriler de bu bulguyu güçlendirmiştir. Bu çalışmada, makro ve mikroskobik yapıların fotoğrafları, kısa bir betimleme, sporların ve elaterlerin taramalı elektron mikroskobu (SEM) görüntüleri ve numunelerin ITS rDNA bölgesi bazlı moleküler filogenisi verilmiştir. Ayrıca Türkiye'den bugüne kadar tespit edilen *B. phalloides* örneklerinin dağılımı ilk kez bu çalışmada ortaya konmuştur.

Anahtar kelimeler: Mantar çeşitliliği, gasteroid mantarlar, Türkiye

Introduction

The gasteroid genus of fungi, *Battarrea* Pers. is used to be placed in the families *Battarreaceae* Corda or *Tulostomataceae* E. Fisch. (*Tulostomatales* Demoulin.). According to molecular phylogenetics, the genus is placed within the order *Agaricales* Underw. (Ivančević et al, 2016).

Battarrea phalloides (Dicks.) Pers., the type species of the genus, was firstly described in 1785 by Dickson as *Lycoperdon phalloides* Dicks , however, it was later transferred to the genus *Battarrea* by Persoon in 1801. More than sixteen species involved in the genus since 1801 but most of them are currently considered as synonyms of *B. phalloides* (Shepherd and Cooper, 2017).

B. phalloides, also known as tall stiltball, scaley-stalked puffball, mallee drumstick, desert stalked puffball or sandy stiltball, is reported from all continents except Antarctica and red-listed in nine Europen countries. The species is mainly characterized with convex to hemispherical spore sac; fibrous to scaly stipe with volva; globose, subglobose to broadly ellipsoid spores with warty ornamentation and spirally thickened elaters (Calonge, 1998; Pegler et al., 1995).

Considering the literature on Turkish mycobiota, *Battarrea phalloides* have thus far been reported from four locations in Turkey (Sesli and Denchev, 2008; Adanacıoğlu et al, 2016). In the current study, a new location was added to the distribution of Turkish *B. phalloides* along with the details of its macro and micromorphology, ITS rDNA region-based molecular phylogeny and SEM images of spores and capillitium. The aim of this study is to reveal a new locality and distribution of *B. phalloides* in Turkey.

Material and metod

Morphological study: The materials used in this study originates from both a research trip and fungarium samples kept in Biology Department of Muğla Sıtkı Koçman University. In the field, necessary macroscopic features of the specimens were noted; in the laboratory, microscopic structures were scrutinized using both simple light microscope and scanning electron microscope (SEM). Averagely 30 measurements were done using Euromex Oxion Trinocular microscope, 100X magnification rates were utilized for each structure and the compiled data were statisitically analyzed. For SEM studies, pieces of spore mass reside inside the gleba were fixed on stubs using double-sided sticky tape, coated with gold particles, and examined using an EVO 40XVP (LEO Ltd., Cambridge, UK) scanning electron microscope with an accelerating voltage of 20 kV. Identification of the samples was performed in accordance with the relevant literature (Pegler et al., 1995; Hansen and Knudsen, 1997; Calonge, 1998). The exsiccatae were deposited in the Ankara University Herbarium (ANK).

Determination of the ITS rDNA sequences: The genomic DNA was isolated from ANK Akata & Altuntaş 690 using the CTAB method as described before (Rogers and Bendich, 1994). After the spectrophotometric verification of the quality and quantity of the extracted genomic DNA, it was used as a template in polymerase chain reaction for the amplification of the Internal Transcribed Spacer (ITS) rDNA regions. The ITS rDNA regions were amplified by PCR using the universal ITS1 forward and ITS4 reverse oligonucleotides as described before (Stielow et al., 2015). After confirming the presence of amplification product as single, distinct band on agarose gel, the amplicon was cleaned-up with PCR purification kit (QIAquick PCR Purification kit, QIAGEN) and its sequence was determined by Sanger sequencing method. The sequencing PCR was executed with the same ITS1 and ITS4 primers using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) and the fragment analyses were conducted using ABI Prism 3130 Genetic Analyzer. Both the agarose gel electrophoresis and the Sanger sequencing were conducted as described elsewhere (Chen et al., 2014).

Molecular phylogeny study: For the phylogenetic analysis, the raw sequence data were assembled using Sequencher version 5.4.6 sequence assembly software (Gene Codes Corporation) and later BLASTn search was performed with the assembled sequence for determining the best hits. Based on this BLAST search, the in-group and the out-group members were selected for the phylogenetic tree construction. The assembled sequence was aligned with the nucleotide sequences of the predetermined in-group and out-group members obtained from the NCBI GenBank database using the ClustalW algorithm of MEGAX software (Kumar et al., 2018). The phylogenetic tree that reveals the evolutionary relationship of ANK Akata & Altuntaş 690 was constructed using the Maximum Likelihood method and GTR nucleotide substitution model with invariant + gamma distribution (Nei and Kumar, 2000). The phylogenies of the specimens were predicted using the bootstrap method with applying 1000 bootstrap replicates (Felsenstein, 1985).

Results

Agaricaceae Chevall.

Battarrea phalloides (Dicks.) Pers. (1801), (Figure1-3).

Syn.: *Lycoperdon phalloides* Dicks. (1785), *Dendromyces stevenii* Libosch. (1814), *Phallus campanulatus* Berk. (1842), *Ithyphallus campanulatus* (Berk.) Sacc. (1888), *Sphaericeps lignipes* Welw. & Curr. (1868), *Sphaerocybis lignipes* (Welw. & Curr.) Clem. (1909), *Battarrea stevenii* (Libosch.) Fr. (1829), *B. gaudichaudii* Mont. (1834), *B. guicciardiniana* Ces. (1875), *B. muelleri* Kalchbr. (1880), *B. tepperiana* F. Ludw. (1889), *B. guachiparum* Speg. (1898), *B. patagonica* Speg. (1898), *B. laciniata* Underw. ex V.S. White (1901), *B. levispora* Massee (1901), *B. franciscana* Copel. (1904), *B. phalloides* var. *stevenii* (Libosch.) Cleland & Cheel (1916), *B. katzlerae* Ulbr. (1936), *B. phalloides* f. *stevenii* (Libosch.) Calonge (2004).

Macroscopic and microscopic features: Basidioma initially developing underground is ovoid,

covered by a two-layered peridium. **Mature basidioma** consisting of a long stipe with an apical spore sac. **Spore sac** 30-90 mm across, convex to hemispherical covered by a smooth, whitish, grayish endoperidium exposing a sticky, rust-brown spore mass at times. **Gleba** cinnamon to rust-brown, powdery at maturity. **Stipe** 120-350 × 5-15 mm, cylindrical and hollow. **Stipe surface** longitudinally striate, fibrous to scaly in circles, grayish to pale brown, often covered by rust-brown spore mass. **Volva** at the base of stipe, sac shaped, whitish covered by rust-brown spore mass, often disappears. **Basidia** not seen. **Basidiospores** 4-6 µm diam, globose to broadly elliptical, yellowish to yellow-brown and warted with short and smooth pedicel, densely verruculose, sometimes coalescing to form anastomosing ridges. **Elaters** 4-7 µm broad, thin-walled, cylindrical to narrowly clavate, consist of spiral threads, pale yellow to honey-colored. **Pseudocapillitium** 4-5 µm broad, mostly thin-walled, smooth, hyaline to pale yellow or honey-colored and septate.

Ecology: Widely distributed but rare, summer to late autumn, solitary to scattered, in warm temperate, Mediterranean to tropical climate, frequently distributed in several kind of xerophytic vegetation, arid and semiarid regions and dry savannas steppes, coastal dunes, and woodlands; on dry, usually sandy, more rarely chalky or calcareous soils (Calonge, 1998; Howladar et al., 2013; Ivančević et al., 2016; Shepherd and Cooper, 2017; Abdel-Azeem and Nafady, 2019).

Distribution: Africa (Tunisia, Algeria, Libya, Egypt, Morocco, Equatorial Guinea, São Tomé and Príncipe, Cape Verde, Congo, Somalia, Namibia, Mauretania, Ethiopia, Angola, Kenya, Burundi Mozambique and South Africa), Asia (Azerbaidjan, Georgia, Armenia, Israel, Iraq, Pakistan, Iran, Saudi Arabia, China, India, Yemen and Mongolia), Europe (Greece, Bulgaria, Romania, Ukraine, Macedonia, Hungary, Serbia, Crotia, Cyprus, Slovakia, Austria, Poland, Spain, Czech Republic, Germany, Belgium, England, France, Russia, Italy, Malta, Switzerland and Turkey), North America (USA, Canada, Puerto Rico, Jamaica and Mexico), South America (Peru, Argentina, Chile, Ecuador, Brazil and Uruguay), Australia (Commonwealth of Australia and New Zealand) (Pegler et al. 1995, Watling et al. 1995, Calonge 1998, Nieves-Rivera 1998, Jacobson et al. 1999, Denchev and Assyov, 2010, Kreisel 2001, Esqueda et al. 2002, Gates and Ratkowsky, 2004, Yılmaz Ersel and Solak 2004, Sobestiansky, 2005; Hong and Li, 2006; Allı et al. 2007, Madrid 2007, Lacheva, 2012, Seyidova and Hüseyin, 2012, Howladar et al. 2013, Martín et al. 2013, Yousaf et al. 2013, Ivančević et al. 2016, Karadelev and Rusevska 2016, Shepherd and Cooper, 2017, Abdel-Azeem and Nafady 2019).

Material examined: TURKEY—Muğla: Bodrum, Turgutreis, in meadow, sea level, 37° 01' N, 27°15' E, 12.12.2019, ANK Akata & Altuntaş 690.

Molecular phylogenetic characterisation: The ITS rDNA sequence of ANK Akata & Altuntaş 690 determined by conventional PCR and subsequent sequencing was submitted to NCBI GenBank with the accession number MT823465. By considering the BLASTn results of the ITS sequence of ANK Akata & Altuntaş 690, the ITS sequences of the genera *Mycenastrum*, *Tulostoma*, *Bovista*, and *Lycoperdon*, some of the well-supported genera of the gasteroid fungi, were choosen as ingroup members and the ITS sequence of *Pluteus squarrosus* Iqbal Hosen & T.H. Li was selected as the outgroup member for revealing the evolutionary relationship of ANK Akata & Altuntaş 690. As a result of the phylogenetic analysis, five distinct clades appeared along with an outgroup (Figure 4). While the clade 5 contained *Battarrea* species and the specimen Ank Akata & Altuntaş 690, the Clades 1, 2, 3, and 4 included species from the genera *Bovista*, *Lycoperdon*, *Mycenastrum*, and *Tulostoma* respectively. On the other hand, *Pluteus squarrosus* was divaricated separately from the rest of the fungal taxa and constituted an outgroup as predicted. The BLASTn analysis conducted with the ITS sequence of Ank Akata & Altuntaş 690 revealed evidence for more than 99.80 % similarities with *B. phalloides* species. The phylogenetic analysis conducted with the ITS sequence of the specimen, further verified the significant evolutionary relationship of the specimen with *B. phalloides* with a bootstrap value of 100%.

Discussions

B. phalloides is a terricolous and distinctive saprobic species which can be easily recognized by its unique appearance such as umbrella-shaped basidiome, up to 400 mm long fibrous to scaly stipe covered by brown spore mass at maturity. The species appears at summer to late autumn, especially growing on dry, sandy soils of arid and semiarid regions from sea level up to over 2.500 m high and distributed in sixty-five countries within the five continents. Despite its wide distribution, *B. phalloides* is a rare species included in the Red List of Armenia, Bulgaria, Czech Republic, England, Macedonia, Poland, Romania, Russia and Slovakia (Denchev and Assyov, 2010; Rimóczi et al. 2011; Fraiture and Otto, 2013; Karadelev and Rusevska, 2016; Ivančević et al., 2016; Smith et al., 2016; Shepherd and Cooper, 2017).

Regarding the identification of fungal taxa which exhibit enormous genetic diversity, the morphological data may not always be conclusive for the accurate identification of fungal species. Therefore, the sequence data from the preserved genomic DNA regions such as ITS, nrSSU and nrLSU are taken into consideration as a hallmark in

molecular taxonomic studies for over decades (Raja et al., 2017). Apart from this, ITS is one of the most useful and widely used DNA barcoding marker and therefore confer crucial information for molecular phylogenetic studies. Hence, in this study, we benefited from the ITS region for the molecular identification of Ank Akata & Altuntaş 690. The phylogenetic analysis carried out with the ITS region pointed out as much as 100% genetic similarity between the *B. phalloides* and the specimen (GenBank ID: MT823465.1) (Figure 4).

With the current study, *B. phalloides* was reported from Muğla province for the first time and it was the fifth record from Turkey. The distribution of Turkish *Battarrea phalloides* was given in Figure 5 and Table 1.

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Figure 1. Basidiomata of *Battarrea phalloides*.

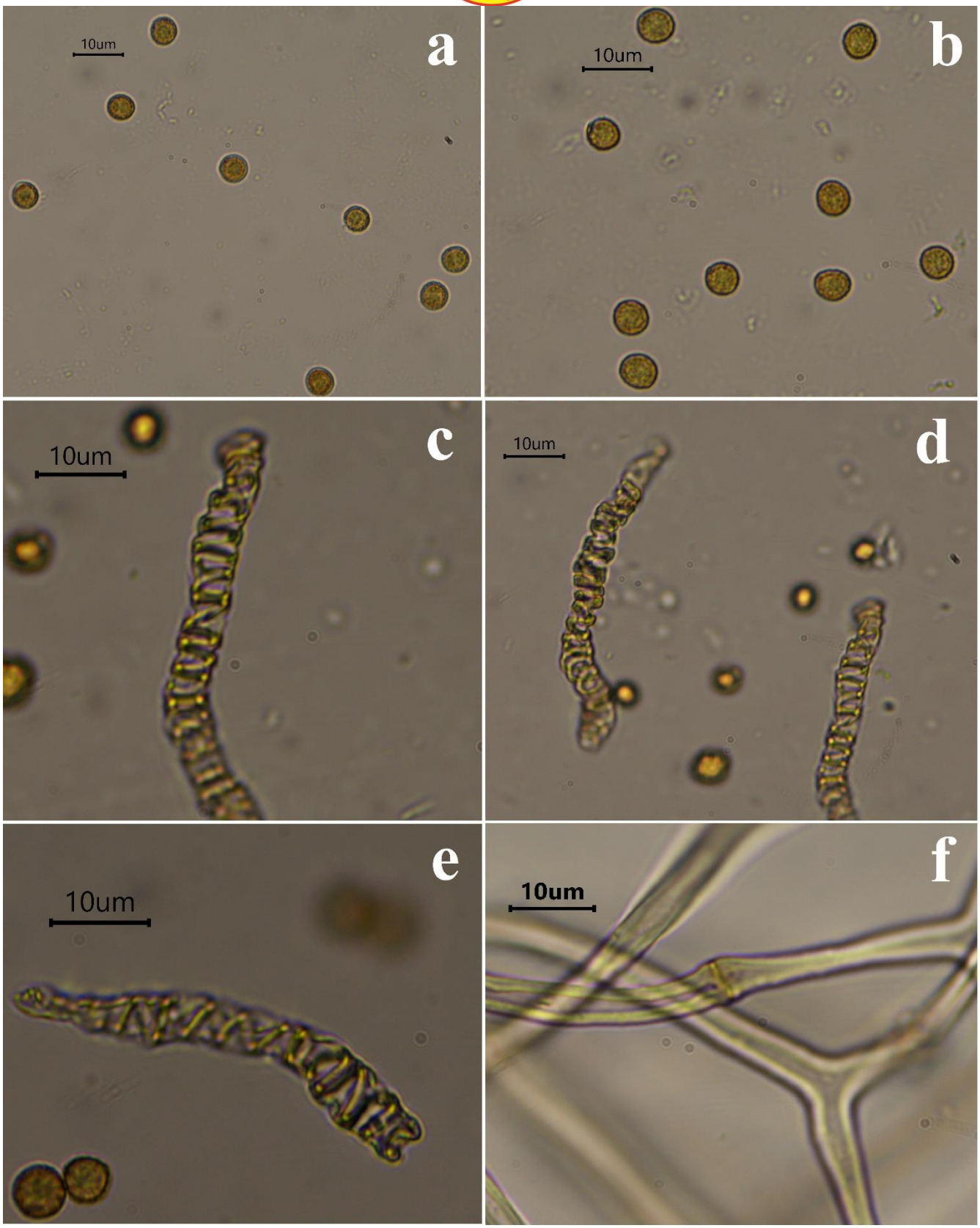

Figure 2. *Battarrea phalloides*: **a-b.** spores, **c-e.** elaters, **e.** pseudocapillitial threads.

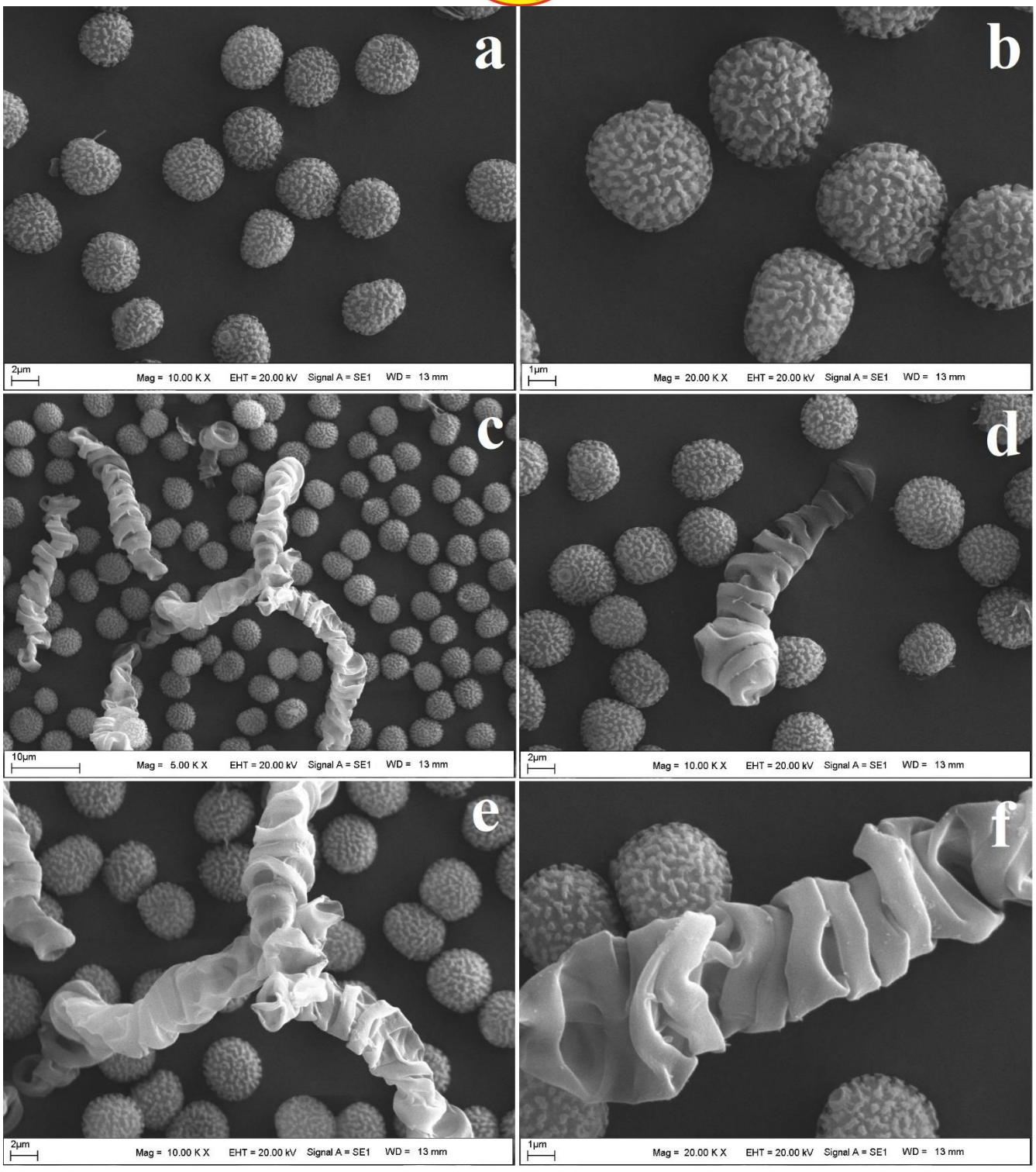

Figure 3. *Battarrea phalloides*: as viewed by a scanning electron microscope (SEM): **a-b.** spores, **c-f.** spores and elaters.

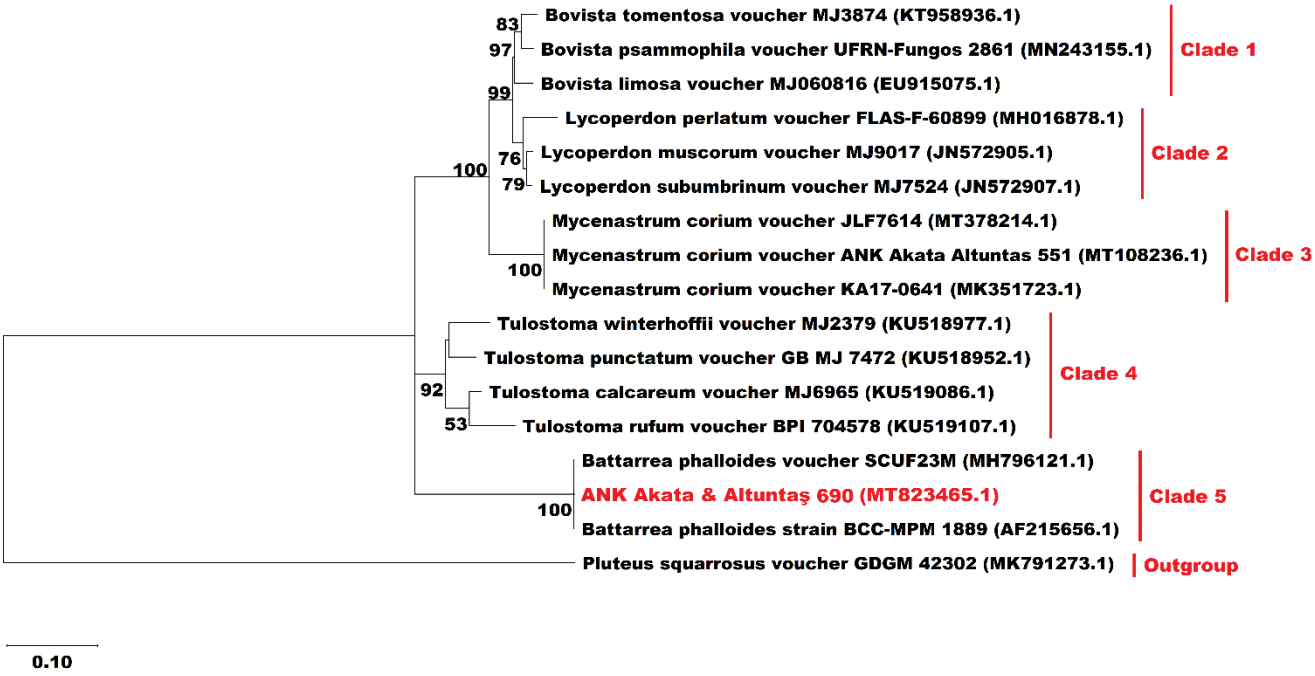

Figure 4. The Maximum Likelihood phylogenetic tree showing the evolutionary relationships of 17 fungal taxa deduced from their ITS region. Percentage bootstrap values (>50%) were stated next to the branches. All the reference sequences utilized in the phylogenetic analysis were retreived from GenBank and their accession numbers were indicated in parantheses. *Pluteus squarrosus* was used as the outgroup member. The scale bar (lower left) exhibits a genetic distance of 0.1.

Figure 5. Distribution of *Battarrea phalloides* in Turkey.

Table 1. Studies on *Battarrea phalloides* in Turkey.

STUDIES	LOCATIONS
Watling et al., 1995	Yalova
Ersel and Solak, 2004	İzmir
Allı et al. 2007	Aydın
Adanacıoğlu et al., 2016	İzmir
Current study	**Muğla**

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Investigation of Bacterial and Fungal Load of Five Printing House in Kahramanmaraş City

Ufuk YILMAZ[1]*, Ferudun KOÇER[2]
Ahmet TUTUŞ[3], Sinan SÖNMEZ[4]

**Sorumlu yazar:kufu27@hotmail.com*

[1]Kahramanmaras Sutcu Imam University, Forest Industry Engineering
Orcid ID: 0000-0001-8240-1294/kufu27@hotmail.com
[2]Kahramanmaras Sutcu Imam University, Bioengineering Sciences
Orcid ID: 000-0002-8749-7106/kocerferudun@gmail.com
[3]Kahramanmaras Sutcu Imam University, Forest Industry Engineering
Orcid ID: 0000-0003-2922-4916/atutus@ksu.edu.tr
[4] Marmara University - School of Applied Sciences / Department of Printing Technologies
Orcid ID: 0000-0003-3126-9590/ ssonmez78@gmail.com

Abstract: Employee health is one of the most important issues in the printing sector as well as in all sectors. In addition to the chemical substances used in printing, some of the pathogenic bacteria and fungi that are formed in the environment may threaten the working health if factors such as the printing materials used, humidity and temperature in the environment can not be controlled. In our study, it was aimed to determine the bacterial and fungal load in the internal environment of the printing house operating in different regions in Kahramanmaraş Province. In the scope of the study, petri plate method was used for indoor sampling. Rose Bengal Streptomycin Agar (RSBA) was used for sampling. After sampling, incubation was performed at 27^0C for 7 days. After incubation, morphological characteristics of fungal colonies were examined and pure cultures were transfer to selective media. Fungus colonies were identified as genus and species level with morphological characteristics. According to the results of the study, it was determined that the printing house staff were exposed to indoor pathogens intensely. The most fungi are *Penicillium* and *Aspergillus* species were found to be followed by *Cladosporium* and *Alternaria* species. Among these species, species known as allergy sources have been identified. In such environments, it is necessary to take preventive measures for these microorganisms.

Keywords: Printing house, fungus, bacteria, allergy

Kahramanmaraştaki Beş Matbaanın Bakteri ve Mantar Yükünün İncelenmesi

Öz: Çalışan sağlığı tüm sektörlerde olduğu gibi matbaacılık sektöründe de en önemli konulardan birisidir. Matbaacılıkta kullanılan kimyasal maddelerin yanı sıra kullanılan baskı altı malzemeleri, ortamdaki nem, sıcaklık gibi etkenlerin kontrol altında tutulamaması durumunda ortamda oluşan bazı patojen bakteriler ve mantarlar çalışan sağlığını tehdit edebilir. Bu çalışmada Kahramanmaraş ilinde bulunan farklı mahallelerde faaliyet gösteren matbaaların iç ortamında bulunan bakteri ve mantar yükünün belirlenmesi amaçlanmıştır. Çalışma kapsamında iç ortamdan petri plak yöntemi ile örnekleme yapılmıştır. Örnekleme için Rose Bengal Streptomisin Agar (RSBA) kullanılmıştır. Örnekleme sonrası laboratuarda 7 gün süre ile bırakılmıştır. İnkübasyon sonrası fungal koloniler morfolojik özellikleri incelenerek seçici besiyerlerine saf kültürleri elde edilmiştir. Fungus kolonileri morfolojik kriterler doğrultusunda cins ve tür düzeyinde tanımlamaları yapılmıştır. Çalışma sonuçlarına göre matbaalarda birçok fungus türüne maruziyet olduğu belirlenmiştir. Bu fungus türlerinin başında *Penicillium* ve *Aspergillus* türlerinin yer aldığı, bunları *Cladosporium* ve *Alternaria* türlerinin takip ettiği belirlenmiştir. Bu türler içerisinde alerji kaynağı olarak bilinen türler tespit edilmiştir. Bu gibi ortamlarda bu mikroorganizmaları önleyici tedbirler alınması gerekliliği ortaya konulmuştur.

Anahtar kelimeler: Matbaa, mantar, bakteri, alerji

Introduction

With the developing industrialization in the world, the number of diseases caused by industrial working environments has increased. For this reason, studies are carried out to determine occupational diseases originating from the working environment. With the development of technology, the printing industry has become connected with almost all sectors (Yavuz, 2016). According to social security Institution statistics, Turkey in the printing industry in 2012 395 cases of occupational diseases were observed. 395 occupational disease cases were seen in the printing sector in 2012. 173 employees have become permanently incapacitated as a result of occupational disease. The causes of these diseases are the pathogen and allergen bacteria and fungi that are constantly growing in the printing house as well as the hazardous chemicals used in the facility environment. There are many studies examining the bacterial and fungal concentrations for the determination of indoor air quality (Adams et al 2014; Jafari et al, 2015; Adams et al,2015; Hanson et al, 2016; Nevalainen et al, 2015; Güneş Et al, 2016; Weikl et al, 2016; Ogbu et al, 2016; Benammar et al, 2017; Pokhum et al, 2018).Bacterial infections and fungal allergens constitute a significant expense in countries' health expenditures.

The purpose of this study determine the bacterial and fungal load in different printing houses. Determination of indoor air fungal and bacterial load is important in determining the microorganisms that may be the source of disease and taking necessary precautions.

Material and metod

Sampling was made during the working hours of the staff in 5 different printing house operating intensively in Kahramanmaraş city center.

Preparation of media: Rose-bengal-Streptomycin Agar (RBA) was used for first medium for the isolation and diagnosis of fungi (Imali et al, 2011a). The isolates obtained after incubation were inoculated into flat agar tubes for long-term storage on special media (Malt Extract Agar, Czapek Dox Agar) for diagnosis (Biyik et al. 2005). Plate Count Agar (PCA) was used to determine bacterial load.

Sampling from the station: In the samples, Gravity Based Petri Plate Method was used. Simultaneously at all stations, it is taken from a height of 1.5 meters above the ground. Five (5) petri plates containing the appropriate medium were provided to contact with air by leaving the lid open for 15 minutes. The closed plates were wrapped with stretch film and brought to the laboratory for incubation. Incubation was performed for 48 hours at 37 ^{o}C for growth of bacteria and 7-10 days at 27 °C for growth of fungi (Sarıca et al, 2002).

Identification: The total number of microfungi was determined according to the macroscopic appearance obtained at the end of incubation. The following sources were used for identification of microfungi; Hasenekoglu (1991), Samson and Pitt (2000), Campbell and Johnson (2013) and Walsh et al. (2018). Based on these sources, identification of genus and species level were examined under light microscope and macroscopic and microscopic structures.

Results

The number of bacteria in the indoor air of the printing house and the genera and species level of microfungi definitions were made. The following Table 1 shows the percentages of bacteria and microfungi.

Table 1: Percentage of bacteria and microfungi determined in the study

Station	Bacteria (%)	Microfungi (%)
1	35,90	7,14
2	14,53	39,29
3	5,98	0,00
4	39,32	53,57
5	4,27	0,00

When Table 1 was examined, it was seen that the highest bacterial load was in station number 4 and the least bacterial load was in station number 5. At the same time, microfungi were not found in stations 3. and 5. while fungal diversity was determined to be the highest in station 4. When the table was examined, it was determined that the bacteria rate was the highest (39.32%) and the microfungus percentage was the highest (53.57%) in the 4th station.It was determined that the stations with the lowest bacterial density are stations 3 and 5. The majority of bacteria and microfungus organisms were found to be in the paper stack area in the printing house. It should be noted that the higher the bacterial flora in the indoor air of the studied facility, the higher the number of microfungi. Therefore, ventilation systems should be developed and widespread use in working environments.

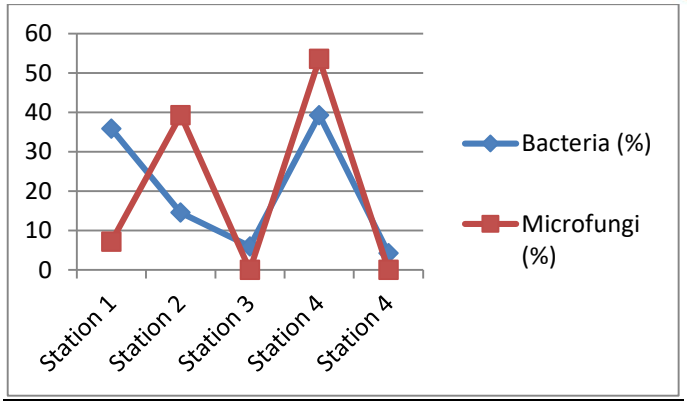

Figure 1. Graphical representation of bacteria and fungi

Table 2 below shows the microfungus species identified in the study.

Table 2. Microfungus species identified in the study

Mikrofungi genus and species	
***Aspergillus* P. Micheli ex Haller**	Samson and Pitt, (2000) Campbell and Johnson, (2013)
Aspergillus niger Tiegh.	
Aspergillus flavus Link	
Aspergillus fumigatus Fresen.	
***Alternaria* Nees**	Hasenekoğlu, (1991)
Alternaria alternata (Fr.) Keissl.	
Alternaria brassicicola (Schwein.) Wiltshire	
***Penicillium* Link**	Samson and Pitt, 2000 Pitt, (2000)
Penicillium sp.	
Penicillium chrysogenum Thom	
Penicillium brevicompactum Dierckx	
Penicillium commune Thom	
Penicillium thomi Maire	
***Mucor* Fresen.**	Hasenekoğlu, (1991)
Mucor hiemalis Wehmer	
***Cladosporium* Link**	Hasenekoğlu, (1991)
Cladosporium cladosporiodes (Fresen.) G.A. de Vries	
Cladosporium oxysporum Berk. & M.A. Curtis	
Cladosporium oxysporum Berk. & M.A. Curtis	

In our country, many studies have been conducted to determine the indoor air fungal and bacterial load (Imali et al, 2011a; Sarıca et al, 2002; Aydogdu et al, 2005;). The determination of indoor air fungal and bacterial concentration is important for the prevention of parameters affecting human health in different working environments. Demirel et al. (2017) reported that *Aspergillus* flavus fungus is a thermotelorant and may be an infectious agent. In our study, *Aspergillus* species were identified in the sampling area. *Aspergillus* sp. spores, which cause allergen sources and other diseases, were first identified (Sugeçti et al. 2018) [Table 2]. *Aspergillus* species, which is a dominant species were studied intensively by many scientist Pitt and Taylor (2014), Samson and Pitt (2000), İmalı et al.(2011b),,, Yang et al.(2016), Do Nascimento et al. (2019).

Alabbasy (2019), stated that *Penicilium* spp.,*Aspergillus* spp.,*Alternaria* spp*. and Cladosporium* spp. were found in his study on paper moneys. *Penicilium* species were found to be the most common species in the printing facilities where sampling is taken within the scope of the study. This was followed by *Aspergillus*, *Alternaria, Cladosporium* and *Mucor* respectively. Some species belonging to these genera are found to be common in the air and spores have allergen properties (Imalı et al, 2011b)[Fig. 1]. It can also be considered that the paper origin used also contributes to the reproductive environment of the microfungi. The following Figure 1, shows the images of the microfungi obtained in the study.

Routine health screening of working people is important as an indicator of immune system parameters, early detection and prevention of bacteria and fungal infections. In addition, improvement of working conditions (air filtration (Burrell, 1991) removal of biological resources (Nevalainen et al, 2015) etc.) is necessary to protect employee health. It is possible to develop immunoprotective antifungal strategies (prophylaxis, empirical and preventive).

Conclusion

Identifying and identifying microfungi in the workplace is important for eliminating employee exposure. *Penicilium*,*Aspergillus*, *Alternaria*, *Cladosporium* and *Mucor* species which are commonly found in airborne microfungi have been reported to be allergen. It has been demonstrated that the necessary controls of the personel working in closed environments should be made and that they should be included in the diseases list such as COPD, asthma and allergy..

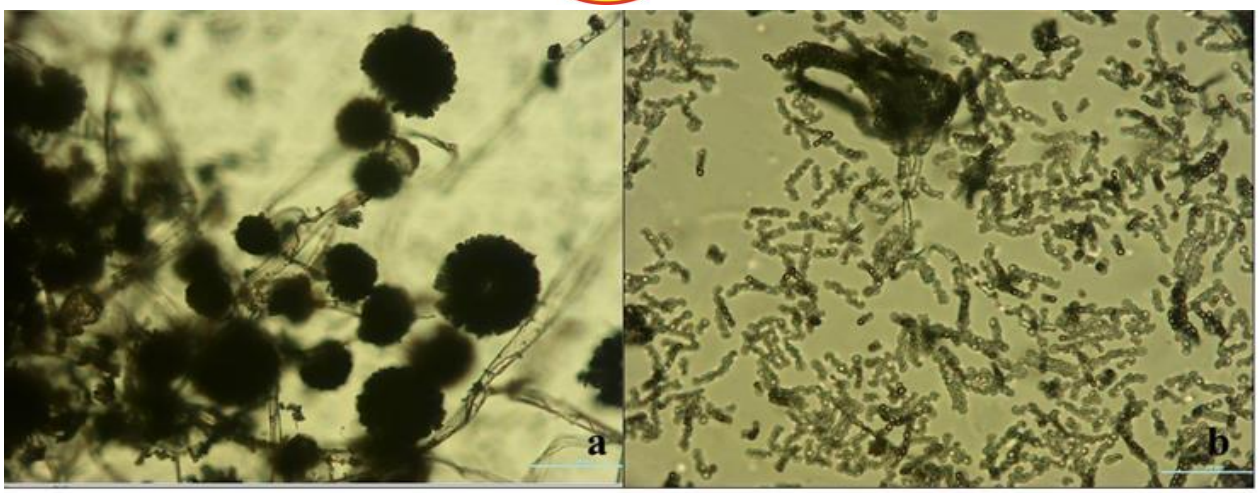

Figure 2. Images of microfungi obtained in the study [a: *Aspergillus*, b: *Penicillium*] (40x)

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Research Article
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First Records of *Cortinarius leucoluteolus* and *C. roseocastaneus* from Turkey

Ertuğrul SESLİ

Corresponding author: ertugrulsesli@trabzon.edu.tr

Trabzon University, Fatih Faculty of Education, Mathematics and Science Education, Department of Biology, Trabzon, Turkey.
Orcid ID: //orcid.org/0000-0002-3779-9704

Abstract: *Cortinarius leucoluteolus* and *C. roseocastaneus* are reported for the first time from Turkey and provided here with definitions, photos, and a short discussion. *Cortinarius leucoluteolus* has a yellowish white or ochre pileus; ochre or pale flesh colour wavy lamellae; generally curved, whitish or creamy and hard stipe; ovoid, elliptical or amygdaliformis basidiospores and clavate basidia. *Cortinarius roseocastaneus* has a broad umbonate, dark brown, chestnut honey colored or slightly reddish brown pileus; sparse, pale brown, dark yellowish to brown lamellae; a cylindrical, slightly enlarged, mostly curved, whitish fibrillose or pale brown stipe; ovoid or ellipsoid basidiospores and clavate basidia.

Key words: Basidiomycota, *Cortinarius,* new record, taxonomy

Cortinarius leucoluteolus ve *C. roseocastaneus*'un Türkiye'den İlk Kayıtları

Öz: *Cortinarius leucoluteolus* ve *C. roseocastaneus* Türkiye'den ilk kez rapor edilmiş ve burada tanımlar, resimler ve kısa bir tartışma ile birlikte verilmiştir. *Cortinarius leucoluteolus* sarımsı beyaz veya koyu sarı bir şapkaya; koyu sarı veya soluk et rengi dalgalı lamellere; genellikle eğri, beyaz veya krem rengi ve sert bir sapa; yuvarlak, eliptik veya badem biçiminde bazidiyosporlara ve çomak şeklinde bazidiyumlara sahiptir. *Cortinarius roseocastaneus* tepe çıkıntılı, koyu kahverengi veya kestane balı renginde veya hafif kırmızımsı kahverengi bir şapkaya; seyrek, soluk kahverengi, koyu sarıdan kahveye doğru değişen renkte lamellere; silindirik, hafifçe genişlemiş, çoğunlukla eğri, beyazımsı lifli veya soluk kahverengi bir sapa; yuvarlağımsı veya eliptik bazidiyosporlara ve çomak şeklinde bazidiyumlara sahiptir.

Anahtar kelimeler: Basidiomycota, *Cortinarius*, taksonomi, yeni kayıt

Introduction

Cortinarius leucoluteolus Rob. Henry first-time was described from France by Henry (1983). It is easily recognised with dry, yellowish buff or opaque yellowish pileus. *Cortinarius roseocastaneus* Niskanen, Liimat. & Kytöv. was initially described by Liimatainen et al. (2014) from Finland, Varsinais-Suomi, Turku, Ruissalo, Honkapirtti in deciduous forest. It is characterized by dark brown to blackish brown, hygrophanous pileus, brown to dark brown lamellae, obovoid to weakly lacrymoid basidiospores.

The largest type study of *Cortinarius* was made by Liimatainen et al. (2014). Before the present study some studies have been carried out in Turkey including *Cortinarius* species from different part of Turkey (Türkekul, 2003; Sesli, 2006; Kaya et al., 2009; Kaşık et al., 2011; Uzun et al., 2013; Akata et al., 2015; Sesli, 2018; Sesli and Liimatainen, 2018; Sesli, 2020).

The aim of the present study is to contribute to Turkish Mycota by introducing two *Cortinarius* species collected from Trabzon.

Material and method

Basidiomata were collected and photographed from Sevinç neighborhood, Maçka, Trabzon, Turkey. Investigation of basidiospores, basidia, pileipellis and other microscopic structures important for diagnosis were performed according to Clémençon (2009) in the Mycology Laboratory of Trabzon University. In order to view and examine the basidiospores, a piece of basidioma is placed in water. After waiting for a while, basidiospores were obtained by tightening with the

forceps. To view the structures of the other microscopic elements, superficial and transverse sections were taken by hand with a new razor blade under a Zeiss Stemi 2000-C microscope, and mounted in 3% NH_3 solution, stained with aqueous 4% Congo red and examined under a Zeiss Axio Imager A2 trinocular microscope. The remaining material was dried on an electrical heater, packed and placed into fungarium cupboard after some days of processing in the deep freezer.

Bulgular / Results

Cortinariaceae R. Heim ex Pouzar

Cortinarius leucoluteolus Rob. Henry, Bull. Trimest. Soc. Mycol. Fr. 99(1): 75 (1983) (Figure 1)

Pileus 40–60(–70) mm convex to plane, irregular, pale yellowish, yellowish white or ochre; margin generally broken, sometimes lobed. Umbo absent. Lamellae emarginate, smooth, ochre, pale flesh colour, darker when injured, wavy, L= 50–60 × I= 1–2. Content whitish, smell indistinct. Stipe cylindrical, generally curved, 80–100(–120) × 8–10(–12) mm, whitish to creamy, nudus, sometimes covered with reddish brown remnant of cortina, hard, tapering towards base. Basidiospores [n= 40] subovoid to ellipsoid or amygdaliformis, smooth, slightly granulated, (5.6–)6.5–7.4(–7.6) × (3.2–)3.5–4.5(–4.8) µm; on average 6.8 × 4.1 µm. Basidia clavate, 38–42 × 7–9 µm; on average 38.8 × 7.9 µm and 4-spored. Pileipellis consists of cylindrical hyphae. Clamp connections present at all tissues. Marginal cells 22–30 × 2.9–3.4 µm.

Specimen examined: TURKEY, Trabzon, Maçka, Sevinç neighborhood, Göller area, gregarious with hornbeam and spruce (*Carpinus betulus* L. and *Picea orientalis* L.), 40°51'34.14" N and 39°37'40.26" E, 1000 m alt., 18 Sept. 2019, E. Sesli, KATO Fungi 4101.

Cortinarius roseocastaneus Niskanen, Liimat. & Kytöv., in Liimatainen, Index Fungorum 196bis: 1 (2014) (Figure 2).

Pileus 20–30 mm, convex to plane, irregular, with a broad umbo, dark brown, chestnut honey colored or slightly reddish brown, lighter and striped towards the edge, darker at the centre, hygrophanous. Margin often slit. Content very thin, dark brown in pileus, pale brown in stipe. Lamellae subdecurrent to decurrent, sparse, pale brown, dark yellowish to brown, L= 25–35, I= 1–3. Stipe cylindrical, slightly enlarged towards base and sometimes tapering, mostly curved, whitish silky-fibrillose, pale brown, darkening at the base, 40–60 × 3–5 mm. Universal veil whitish, pinkish or vinaceous reddish. Odor indistinct. Basidiospores [n= 62] sub-obovoid, ellipsoid to weakly lacrymoid, (6.7–)7–9(–9.5) × (3.9–)4–5(–5.5) µm; on average 7.8 × 4.5 µm, Q= 1.56–1.75, Qm= 1.65, with a low suprahilar depression, moderately verrucose. Basidia clavate, (20–)21–27(–31) × 7–9(–9.3) µm; on average 24.9 × 8.1 µm. Pileipellis made up of cylindrical, regular, parallel hyphae. Clamp connections present at all tissues. Specimen examined: TURKEY, Trabzon, Maçka, Sevinç neighborhood, Kıran area, gregarious and caespitose, with *Quercus petraea* (Mattuschka) Liebl., 40°50'50.25" N and 39°37'41.44" E, 741 m alt., 03 Nov. 2016, E. Sesli, KATO Fungi 3838.

Discussions

In this study, two *Cortinarius* species (*C. leucoluteolus* and *C. roseocastaneus*) are recorded for the first time from Turkey and provided here with descriptions, field and microscopic photos. Our results about the new records generally matched very well with the original descriptions. The findings connected with KATO F. 4101 are in accordance with the original description by Henry (1983) (*C. leucoluteolus*). *Cortinarius roseocastaneus* described by Liimatainen (2014) has 15–30 mm, very dark brown to blackish brown, hygrophanous pileus, brown or dark brown lamellae, 30–60 × 2–4 mm, cylindrical stipe, 7.0–8.4 × 4.5–5.2 um, obovoid to weakly lacrymoid basidiospores. Our findings are nearly same with those results (pileus dark brown, or slightly reddish brown, 20–30 mm, hygrophanous. Lamellae pale brown, dark yellowish to brown. Stipe cylindrical, 40–60 × 3–5 mm. Basidiospores sub-obovoid, ellipsoid to weakly lacrymoid, 7–9 × 4–5 µm). We think that some small morphologic differences are due to the different ecological conditions.

Acknowledgements

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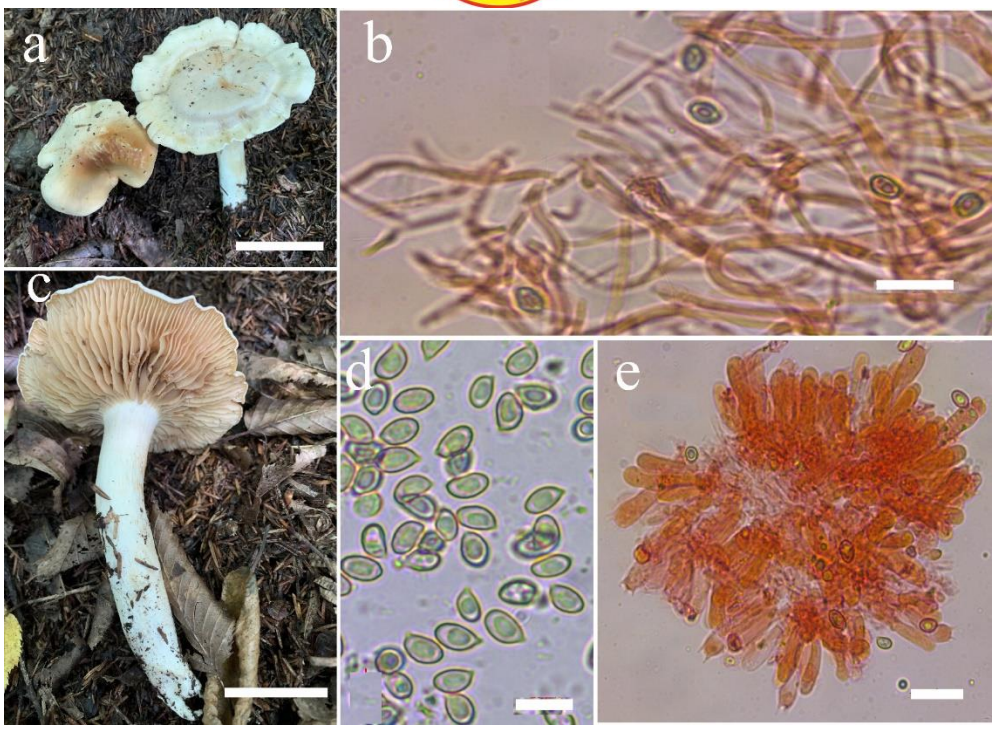

Figure 1. *Cortinarius leucoluteolus*: a and c- basidiomata, b- pileipellis, d- basidiospores, e-basidia (bars: a and c= 35 mm, b= 20 µm, d= 10 µm, e= 20 µm).

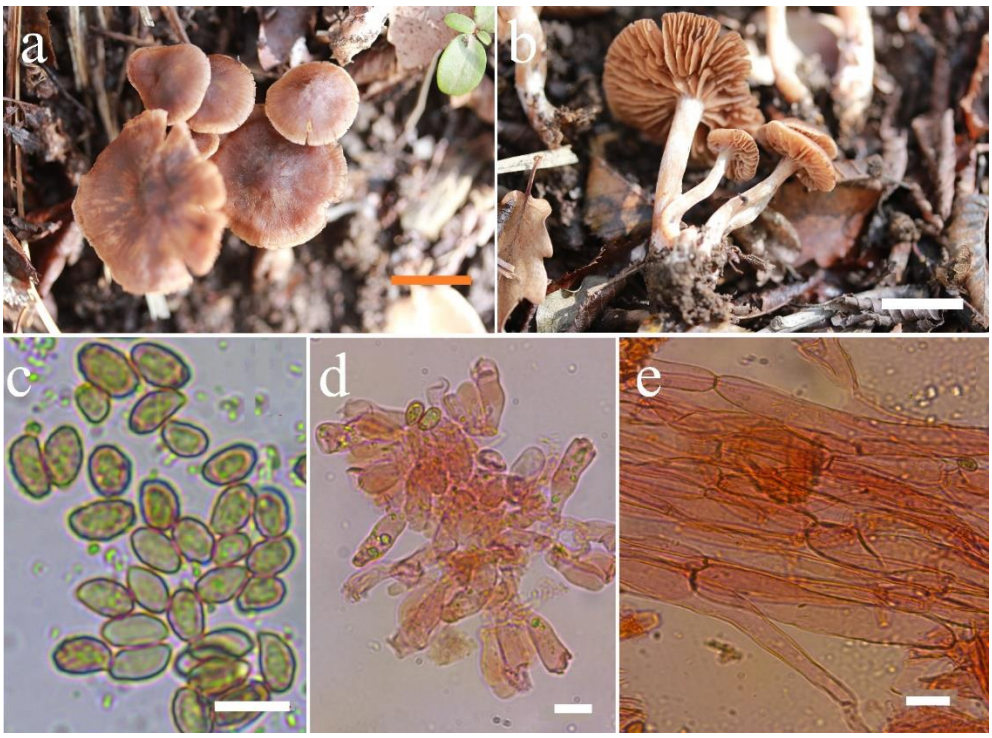

Figure 2. *Cortinarius roseocastaneus*: a and b- basidiomata, c- basidiospores, d-basidia, e-pileipellis (bars: a and b= 15 mm, c and d= 10 µm, e= 20 µm).

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Üç *Lactarius* Türünün Antioksidan Ve Enzim İnhibitör Aktiviteleri Üzerine Karşılaştırmalı Bir Çalışma

Ramazan CEYLAN[1*], Güneş AK[1]
Ilgaz AKATA[2], Gökhan ZENGİN[1]

**Sorumlu yazar: biyoram7@gmail.com*

[1] Selcuk Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Konya.
Orcid No: 0000-0002-7795-8482/biyoram7@gmail.com
Orcid No: 0000-0002-9539-0763/akguneselcuk@gmail.com
Orcid No: 0000-0001-6548-7823 /gokhanzengin@selcuk.edu.tr
[2]Ankara Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Ankara.
Orcid No:0000-0002-1731-1302/akata@ankara.edu.tr

Öz: Mantarlar tarih öncesi çağlardan beri mucize gıda ve tıp malzemeleri olarak kabul edilir. Günümüzde, insanoğlu sentetik bileşiklere karşı alternatif doğal hammadde arayışı içindedir ve bu yüzden mantarlar bu yolda büyük bir hazinedir. Bu bağlamda, sunulan çalışmada üç *Lactarius* (*L. salmonicolor* Heim et Leclair*, L. deliciosus* (L. ex Fr.) S.F. Gray ve *L. aurantiacus* (Pers.) Gray) türünün antioksidan ve enzim inhibisyon özelliklerini belirlemeyi amaçladık. Ek olarak, her bir ekstraktın toplam fenolik içeriklerini belirledik. Antioksidan özellikler serbest radikal süpürme (2,2-diphenil-1-pikrilhidrazil (DPPH) ve 2,2'-azino-bis(3-etilbenzothiazolin-6-sulfonik asit (ABTS)), indirgeme gücü (bakır (II) iyonu indirgeme antioksidan kapasite (CUPRAC) ve demir (III) iyonu indirgeme gücü (FRAP)), fosfomolibdat ve metal şelatlama testlerin içeren farklı kimyasal testler ile değerlendirildi. Enzim inhibitör özellikler ise kolinesterazlar (asetilkolinesteraz (AChE) ve butirilkolinesteraz (BChE)), α-amilaz ve α-glukozidaza karşı araştırıldı. Genel olarak, *L. delicious* ve *L salmonicolor* en güçlü serbest radikal süpürme ve indirgeme gücü yeteneklerini sergilediler. Bununla birlikte, *L. aurantiacus* fosfomolibdat testinde en etkili olandı. En güçlü AChE inhibisyonu *L. salmonicolor*'da elde edilirken en yüksek α-glukozidaz inhibisyon etkileri *L. delicious* ve *L. aurantiacus* ile sağlandı. Tüm eksraklar benzer α-amilaz inhibisyonu sergilediler. Mevcut çalışma test edilen *Lactarius* türlerinin, farmasötikler veya nutrasötikler gibi fonksiyonel ürünleri tasarlamak için doğal hammaddeler olarak kabul edilebileceğini öne sürdü.

Anahtar kelimeler: *Lactarius*, antioksidan, enzim inhibisyonu, temel bileşen analizi

A Comparative Study on Antioxidant and Enzyme Inhibitory Activities of Three *Lactarius* species

Abstract: Mushrooms are considered a miracle food and medical items since prehistoric ages. Nowadays, humanity is searching for alternative natural raw materials against synthetic ones and thus mushrooms are a big treasure in this way. In this context, we aimed to determine the antioxidant and enzyme inhibitory properties of three Lactarius species (*L. salmonicolor* Heim et Leclair*, L. deliciosus* (L. ex Fr.) S.F. Gray, and *L. aurantiacus* (Pers.) Gray) in the presented study. In addition, total phenolic content was determined for each extract. Antioxidant properties of the tested extracts were evalauted by different chemical methods including free radical scavenging (2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)), reducing power (cupric reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP)), phosphomolybdenum and metal chelating. Enzyme inhibitory properties were investigated against cholinesterases (acetylcholinesterase (AChE) and butyrlcholinesterase (BChE)), α-amylase and α-glucosidase. Generally, *L. delicious* and *L salmonicolor* exhibited the strongest free radical scavenging and reducing power abilities. However, *L. aurantiacus* exhibited the most activity in phosphomolybdenum assays. The highest AChE inhibitory effect was obtained by *L. salmonicolor* while the best α-glucosidase inhibitory effects were provided by *L. delicious* and *L. aurantiacus*. All extracts exhibited similar α-amylase inhibition abilities. The presented study suggested that the tested *Lactarius* species might be

considered as natural raw materials for designing functional products such as pharmaceuticals or nutraceuticals.

Key words: *Lactarius*, antioxidant; enzyme inhibition, principal component analysis

Giriş

Doğal ürünler ve bunların biyolojik etkinlikleri son yıllarda bilim dünyasının en popüler konuları arasındadır. Doğal ürünler ve bunların eczane raflarında yer alan uygulamaları gün geçtike sentetik ürünlerin yerini almakta ve bu durum yeşil devrim olarak ifade edilmektedir. 2015 yılında Nobel tıp ödülünün doğal ürünlere layık görülmesi ile yeni bir dönüm noktası yaşanmıştır. Bu durum başta gelişmekte olan ülkeler olmak üzere dünya üzerinde doğal kaynakların kullanımında yeni bir boyut kazanmıştır (Koparde, Doijad, & Magdum, 2019; Mathur & Hoskins, 2017).

Doğal kaynaklar arasında mantarların son yıllarda insanlarla olan ilişkileri oldukça dikkat çekicidir. Sanayi devrimini takiben mantarlar insan diyetinin önemli bir parçası haline gelmiştir. Bu durumun temelinde düşük lipid ve karbohidrat içeriği, yüksek protein, vitamin ve mineral içeriğine sahip olmaları yatmaktadır. Besleyici özellikleri dışında, mantarlar farmasötik ve kozmesötik endüstriler içinde değerli ham madde kaynakları olarak değerlendirilmektedir (Sevindik, 2018; Sharifi-Rad et al., 2020).Tahminlere göre, dünya çapında mantar sayısının 12.000 ile 22.000 arasında olduğu rapor edilmiş olup ve bunların 2000 tanesinin yenilebilir nitelikte oldukları bildirilmektedir (Rathore, Prasad, & Sharma, 2017; Sharifi-Rad et al., 2020; Wasser 2002). Yaklaşık 35 yenilebilir mantar türü ticari olarak yetiştirilirken, tıbbi amaçlar için yaklaşık 200 yabani tür kullanılmaktadır. Dünya üzerinde en çok kültürü yapılan tür olarak *Agaricus bisporus* (L.) Sing. birinci sırada yer alırken, bunu sırasıyla *Lentinus edodes* (Berk.) Pegler ve *Pleuroteus* (Fr.) P. Kumm. takip etmektedir (Rathore et al., 2017). Son zamanlarda mantarlar üzerine yapılan kimyasal ve biyolojik çalışmalar farmasötik anlamda değerli bileşiklerin varlığını ortaya koymuştur (Li et al., 2020; Milovanovic, Zengin, Maksimovic, & Tadic, 2020; Wang et al., 2020) ve sentetik bileşiklerin yerine geçebilecek çok sayıda yeni biyoaktif bileşik farmasötik endüstrisinin kullanımına sunulmuştur. Bu noktadan hareketle, mantarlar ve bunlar üzerine yapılacak yeni çalışmalar yeni hammadde kaynaklarının tespit edilmesine olanak sağlaması bakımından önem arz etmektedir.

Lactarius cinsi (Russulaceae) gıda ve tıbbi olarak kullanılan gelecek vaad eden türleri bünyesinde bulunduran oldukça önemli bir cinstir. Bu cinsin en iyi bilinen türü *L. deliciosus* olup birçok ülkede besin ve gıda kaynağı olarak kullanılmaktadır (Adanacioglu et al., 2017). Bu noktadan hareketle, *L. deliciosus* (L. ex Fr.) S.F. Gray üzerine çok sayıda kimyasal karakterizasyon ve biyolojik aktivite çalışmaları gerçekleştirilmiş (Alkan et al., 2020; Hasar, Dogan, & Demirel, 2020; Rasalanavho, Moodley, & Jonnalagadda, 2020; Rosa et al., 2020; Su, Ding, Fu, & Hou, 2019; Volcao et al., 2020) ve ilgili mantar gıda ve farmasötik önemi ortaya konulmuştur. Bununla birlikte diğer *Lactarius* taksonları üzerine yapılan çalışmalara halen sınırlı seviyededir. Bu bilgiler ışığında mevcut çalışmada, *Lactarius* cinsine ait üç türün (*L. salmonicolor* Heim et Leclair, *L. delicious* ve *L. aurantiacus (*Pers.) Gray,) antioksidan ve enzim inhibisyon özelliklerinin karşılaştırmalı olarak incelenmesi amaçlanmıştır. Antioksidan kapasitenin belirlenmesinde serbest radikal süpürme (ABTS ve DPPH), indirgeme gücü (FRAP ve CUPRAC), metal şelatlama ve fosformolibdat testleri uygunlamıştır. Enzim inhibisyon testleri olarak ise küresel sağlık problemleri ile ilişkili kolinesteraz, α-amilaz ve α-glukozidaz enzimleri kullanılmıştır. Elde edilecek sonuçlar literatüre yeni bilgiler sağlamasının yanı sıra bu cins üzerine yapılacak olan yeni çalışmalara ufuk açacaktır.

Materyal ve Metot

Mantar örnekleri ve ekstrakların hazırlanması

L. salmonicolor (Bolu), *L. deliciosus* (Muğla) ve *L. aurantiacus* (Kastamonu) 2019 yılında gerçekleştirilen arazi çalışmalarında toplanmıştır ve mantar örneklerin taksonomik olarak tanımlanmaları Dr. Ilgaz Akata (Ankara Üniversitesi, Fen Fakültesi) tarafından gerçekleştirilmiştir. Mantar örnekleri kurutma fırınında 40-45 °C kurutulduktan sonra laboratuvar tipi öğütücü ile öğütülerek toz haline getirilmiştir. Toz haline getirilen mantar örneklerinin aynı hafta ekstraksiyonları yapılmıştır. Ekstraksiyon metodu olarak maserasyon metodu seçilmiş olup her bir mantar örneğinin 5 g'ı 100 mL metanol ile oda sıcaklığında 24 saat süreyle masere edilmiştir. Elde edilen ekstraktlar süzülüp rotary-evaporator yardımıyla çözücünün uzaklaştırılması

sağlanmıştır. Ele geçen ham ekstraklar analiz edilinceye kadar + 4 °C'de saklandı.

Toplam fenolik içerik ve antioksidan kapasite tayin testleri

Mantar ekstraklarının toplam fenolik içerikleri Folin-Ciocalteu metodu kullanılarak belirlendi. Metoda ait deneysel prosedür daha önce yapılan çalışmalarımızda verilmiş olup (Zengin & Aktumsek, 2014; Zengin, Sarikurkcu, Aktumsek, & Ceylan, 2014), testin sonuçları gallik asit eşdeğeri olarak verilmiştir.

Antioksidan kapasite testleri olarak DPPH, ABTS, FRAP, CUPRAC, metal şelatlama ve fosfomolibdat testleri seçilmişitr. Bu test sistemlerine ait deneysel prosedürler önceki çalışmalarımızda belirtilmiş olup (Zengin & Aktumsek, 2014; Zengin et al., 2014), testlerin sonuçları troloks (TE) ve EDTA (EDTAE) eşdeğerleri olarak verilmiştir.

Enzim inhibisyonuna yönelik testler

Mantar ekstraktlarının α-amilaz, α-glukozidaz, AChE ve BChE enzimlerine karşı enzim inhibitör etkisi test edildi. Bu deneylerin prosedürü bizim önceki çalışmamıza göre gerçekleştirilmiş olup (Zengin, 2016) kolinesteraz inhibitör aktiviteleri galantamine eşdeğer olarak hesaplandı (mgGALAE/g). α- amilaz ve α-glukozidaz inhibitör aktiviteleri akarboza eşdeğer olarak hesaplandı (mmolAKAE/g).

İstatiksel değerlendirme

Sonuçlar üç paralel ölçümün ortalaması ve standard sapmaları şeklinde verildi. Ekstraklar arasında her hangi bir fark olup olmadıkları tek yönlü varyans analizi (ANOVA, Tukey testi) ile belirlenmiştir. Ayrıca ekstraklara uygulanan testler arasında Pearson korelasyon, temel bileşen (PCA) ve kümeleme (cluster) analizleri yapılmıştır. İstatistiksel değerlendirmeler Statistica 8.0 programı kullanılarak gerçekleştirilmiştir.

Bulgular

Çalışılan *Lactarius* ekstraklarının toplam fenolik içerikleri Folin-Ciocalteu metodu ile belirlenmiş ve toplam fenolik içerik bakımından en zengin tür *L. salmonicolor* (9.70 mg GAE/g) olrak bulunmuştur. Bu türü sırasıyla *L. deliciosus* (9.64 mg GAE/g) ve *L. aurantiacus* (6.55 mg GAE/g) takip etmektedir (Tablo 1). Bununla birlikte, *L. salmonicolor* ve *L. deliciosus*'un istatistik olarak toplam fenolik içerikleri bakımından aralarında herhangi bir fark yoktur (p>0.05). Toplam fenolik içerik sonuçlarına benzer şekilde serbest radikal süpürme etkinliklerinde hem DPPH hem de ABTS testlerinde çalışılan türler arasında en etkin olanı olarak *L . deliciosus* (11.71 mg TE/g ve 65.09 mg TE/g) belirlenmiş ve bunu sırasıyla *L. salmonicolor* (10.42 mg TE/g ve 54.53 mg TE/g) ve *L. aurantiacus* (4.04 mg TE/g ve 30.82 mg TE/g) izlemektedir (Tablo 1). Serbest radikal süpürme etkinliğinin tersine fosfomolibdat testinde çalışılan türler *L. aurantiacus*>*L salmonicolor*> *L. deliciosus* şeklinde sıralanmaktadır. Çalışmamızda *Lactarius* taksonlarının indirgeme güçleri CUPRAC ve FRAP testleri kullanılarak belirlenmiştir ve her iki test sisteminde de en yüksek etkinlik *L . deliciosus*'da tespit edilmiştir ve en düşük aktivite ise *L. aurantiacus*'da gözlemmiştir (Tablo 2). Metal şelatlama aktivitesi bakımından üç türde benzer aktiviteler sergilemiştir (7.54-7.81 mg EDTAE/g) ve istatistik açıdan aralarında herhangi bir fark gözlenmemiştir (p<0.05).r

Tablo 1. Çalışılan *Lactarius* türlerinin toplam fenolik içerikleri, radikal süpürme etkinlikleri ve fostomolibdat testindeki aktiviteleri*

Örnekler	Toplam fenolik içerik (mg GAE/g özüt)	Fosfomolibdat (mg TE/g özüt)	DPPH süpürme aktivitesi (mg TE/g özüt)	ABTS süpürme aktivitesi (mg TE/g özüt)
L. salmonicolor	9.70±0.17^{a}	152.11±4.97^{b}	10.42±0.59^{b}	54.53±0.78^{b}
L. deliciosus	9.64±0.21^{a}	144.58±2.84^{b}	11.71±0.34^{a}	65.09±0.23^{a}
L. aurantiacus	6.55±0.15^{b}	232.33±4.86^{a}	4.04±0.16^{c}	30.82±0.71^{c}

*Değerler üç parallel ölçümün ortalaması ± standard sapmasıdır. GAE: Gallik asit eşdeğeri; TE: Troloks eşdeğeri. Aynı sütundaki farklı harfler (a, b ve c) örnekler arasındaki farkı göstermektedir (p<0.05).

Tablo 2. Çalışılan *Lactarius* türlerinin indirgeme gücü ve metal şelatlama yetenekleri*

Örnekler	FRAP (mg TE/g özütt)	CUPRAC (mg TE/g özüt)	Metal şelatlama yeteneği (mg EDTAE/g özüt)
L. salmonicolor	10.42±0.07^{b}	23.48±0.40^{b}	7.68±0.14^{a}
L. deliciosus	14.75±0.24^{a}	28.39±0.26^{a}	7.54±0.13^{a}
L. aurantiacus	7.74±0.13^{c}	20.26±0.36^{c}	7.81±0.71^{a}

*Değerler üç parallel ölçümün ortalaması ± standard sapmasıdır. TE: Troloks eşdeğeri; EDTAE: EDTA eşdeğeri. Aynı sütundaki farklı harfler (a, b ve c) örnekler arasındaki farkı göstermektedir (p<0.05).

Lactarius türlerinin enzim inhibisyon özellikleri AChE, BChE, α-amilaz ve α-glukozidaz enzimleri kullanılarak araştırılmış ve sonuçlar Tablo 3'de verilmiştir. En güçlü AChE inbihitor özellik *L. salmonicolor*'da gözlenmiş (0.90 mg GALAE/g) ve diğer iki *Lactarius* türü benzer inhibisyon yetenekleri sergilemiştir (0.84 mg GALAE/g, p>0.05). BChE inhibisyonu açısından ise *L. delicious* (1.17 mg GALAE/g) ve *L. aurantiacus*'un (1.16 mg GALAE/g) sergiledikleri aktiviteler birbirlerine yakın olup, *L. salmonicolor*'dan (0.89 mg GALAE/g) daha yüksektir. α-amilaz enzimini inhibe etme yetenekleri bakımından çalışılan *Lactarius* türleri benzer aktiviteler sergilemiştir ve aralarında istatisksel olarak herhangi bir fark gözlenmemiştir (p> 0.05*).L. delicious* (12.89 mmol ACAE/g) ve *L. aurantiacus (*12.92 mmol ACAE/g) α-glukozidaz inhibisyonu bakımından oldukça etkili olup, *L. salmonicolor* ise en düşük inhibisyonu sergilemiştir.

Tablo 3. Çalışılan *Lactarius* türlerinin enzim inhibitör özellikleri*

Örnekler	AChE inhibisyonu (mg GALAE/g özüt)	BChE inhibisyonu (mg GALAE/g özüt)	α- Amilaz inhibisyonu (mmol ACAE/g özüt)	α- Glukozidaz inhibisyonu (mmol ACAE/g özüt)
L. salmonicolor	0.90±0.03^{a}	0.89±0.02^{b}	0.22±0.01^{a}	2.62±.0.04^{b}
L. deliciosus	0.84±0.04^{b}	1.17±0.09^{a}	0.21±0.01^{a}	12.89±0.41^{a}
L. aurantiacus	0.84±0.01^{b}	1.16±0.02^{a}	0.21±0.01^{ab}	12.92±1.15^{a}

*Değerler üç parallel ölçümün ortalaması ± standard sapmasıdır. GALAE: galantamin eşdeğeri; ACAE: akarboz eşdeğeri. Aynı sütundaki farklı harfler (a ve b) örnekler arasındaki farkı göstermektedir (p<0.05).

Tartışma

Fenolik bileşikler sergiledikleri biyolojik aktiviteler ile son yıllarda bilim dünyasının en ilgi çekici moleküllerini oluşturmaktadır. Öyle ki, bu bileşikler antioksidan, antimikrobiyal, antikanser ve anti-inflamatuvar aktiviteler başta olmak üzere oldukça geniş yelpazede biyolojik etkinliklere sahiptir (Cory, Passarelli, Szeto, Tamez, & Mattei, 2018; Tanase, Coșarcă, & Muntean, 2019). Yapısal olarak bünyelerindeki hidroksil grubunun varlığı bunları kimyasal olarak güçlü elektron dönorları yapmakta ve bu yolla serbest radikallerin ortadan kaldırılmasında etkili görev alırlar. Yapılan çalışmalara diyetteki fenolik bileşik miktarının artırılmasıyla oksidatif stres kaynaklı hastalıklara yakalanma ve bunlardan ölüm oranları arasında ters bir ilişki rapor edilmiştir (Gao et al., 2020; Pandey & Rizvi, 2009). Bu noktadan hareketle doğal ekstrakların fenolik içeriklerinin belirlenmesi, ekstrakların potansiyelleri hakkında ilk bakış açısını oluşturmaktadır. Çalışmamızda *Lactarius* türlerinin toplam fenolik içerikleri 6.55-9.64 mg GAE/g arasında bulunmuştur. Literatür taraması yapıldığında *Lactarius* türlerinin toplam fenolik içerikleri bakımından farklı sonuçlar gözlemlenmiştir (Alkan et al., 2020; Ayvaz, Aksu, & Kır, 2019; Bozdogan et al., 2018; Kosanic, Rankovic, Rancic, & Stanojkovic, 2016; Ozen, Kizil, Yenigun, Cesur, & Turkekul, 2019; Radzki, Slawinska, Jablonska-Rys, & Gustaw, 2014; Rosa et al., 2020; Su et al., 2019; Volcao et al., 2020). Bu sonuçlar mantarın toplanma yeri, zamanı veya ekstraksiyon prosedürlerinde kullanılan metotların farklı olmasıyla açıklanabilir (Boonsong et al., 2016; Petrovic et al., 2014; Zeng et al., 2013) . Ayrıca, son yıllarda spektrofotometrik metotların dezavantajlarının rapor edilmesi (Sánchez-Rangel, Benavides, Heredia, Cisneros-Zevallos, & Jacobo-Velázquez, 2013) elde edilen bu sonuçları şüpheli hale getirmiştir. Bu nedenle ileri kromatografik teknikler kullanılarak elde edilen toplan fenolik sonuçların doğrulanması gerekmektedir.

Oksidatif stres terimi son yıllarda bilim dünyasında en sık karşılaşılan terimlerden olup, birçok hastalığın ilerleyişinin doğrudan veya dolaylı olarak oksidatif stresin rol aldığı yapılan çalışmalarla ortaya konulmuştur. Oksidatif stres durumu serbest radikal miktarının artması ve bunu önleyen iç savunma sisteminin yetersiz kalması ile ortaya çıkan bir durumdur (Chatterjee, 2016; Liguori et al., 2018; Sevindik, 2019). Bu noktada antioksidan bileşikler olarak bilinen bir grup bileşik oksidatif stres durumunun iyileştirilmesinde önemli görevler üstlenirler. Bu açıdan, antioksidatif özelllikler sergileyen kimyasal sentez yoluyla elde edilmiş BHA, BHT veya PG bir grup bileşik gıda katkı maddeleri olarak kullanılmaktadır. Bununla birlikte, son yıllarda bu sentetik antioksidanların sergiledikleri yan etkileri bunların kullanımlarını kaygılı hale getirmiştir (Augustyniak et al., 2010; Silva & Lidon, 2016). Bu nedenle, bu sentetik antioksidanların daha güvenli ve doğal kaynaklardan elde edilen antioksidanlar ile yer değiştirilmesi gerekmektedir. Mevcut çalışmada *Lactarius* türlerinin antioksidan özellikleri farklı test sistemleri kullanılarak belirlenmiş ve bu şekilde çalışılan ekstrakların antioksidan özellikleri hakkında daha kesin bir sonuca varılması amaçlanmıştır. DPPH ve ABTS radikalleri antioksidan kapasite çalışmalarında en sık kullanılan radikaller olup ekstrakların radikal süpürme özelliklerinin değerlendirilmesini sağlar. Bitkisel antioksidanlar elektron veya hidrojen vererek radikalleri etkisiz hale getirmektedir.

İndirgeme gücü antioksidan parametreler arasında oldukça önemli bir yere sahip olup antioksidan bileşiklerin elektron verme yeteneğini ortaya koymaktadır. Bir molekül ne kadar kolay elektron verebiliyorsa o derece güçlü antioksidan etkinlikler sergiler. Bu açıdan çalışmamızda *Lactarius* ekstraklarının indirgeme güçleri CUPRAC ve FRAP testleri ile belirlenmiştir. Hem serbest radikal süpürme hemde indirgeme gücü testlerinde *L. delicious* en yüksek etkinliğe sahiptir. Ayrıca *L. deliciosus* fenolik içerik bakımından da *L. salmonicolor* ile birlikte en

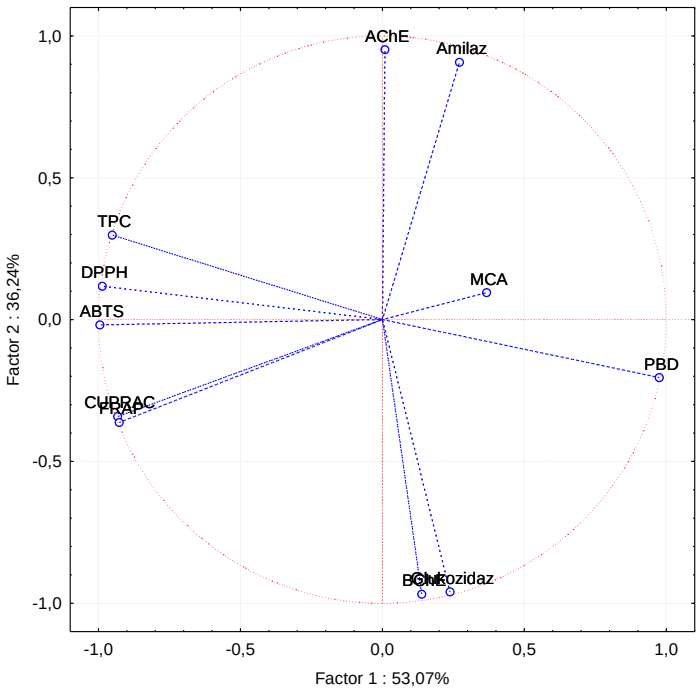
yüksek içeriğe sahip olan tür olarak belirlenmiştir. Dolayısıyla, toplam fenolik içerik ile radikal süpürme ve indirgeme güçleri arasında pozitif bir korelasyonun varlığından bahsedilebilir ve bu olgu korelasyon analizi ile ortaya konulmuştur. Bu korelasyon sonuçlarını doğrular nitelikte bir çok çalışmada toplam fenolik içerik ile radikal süpürme ve indirgeme güçü arasında pozitif bir ilişkinin varlığı belirtilmiştir (Lim, Pang, Yusoff, Abdul Mudalip, & Gimbun, 2019; Mwamatope, Tembo, Chikowe, Kampira, & Nyirenda, 2020; Sarikurkcu et al., 2020; Vidal-Gutiérrez et al., 2020; Wu, Wu, Cai, Li, & Wang, 2020). Bununla birlikte fosfomolibdat ve metal şelatlama testleri ile toplam fenolik içerik arasında herhangi bir korelasyon gözlenmemiştir. Bu testlerden fosfomolibdat testi toplam antioksidan kapasite testlerinden bir olarak değerlendirilmekte ve fenolikler dışında diğer antioksidan bileşiklerde bu test sisteminde etkili olabilirler (Llorent-Martínez et al., 2017; Zengin et al., 2020). Metal şelatlama testinde ise, çeşitli araştırmacılar fenolik bileşiklerin metal şelatlama özelliklerini antioksidan özellikleri arasında oldukça küçük bir etkinlik olarak rapor etmişlerdir (Rice-Evans, Miller, & Paganga, 1997; Wang, Jonsdottir, & Ólafsdóttir, 2009). Bu açıdan *Lactarius* türlerinin metal şelatlama özellikleri fenolik olmayan örneğin peptidler, sülfidler gibi şelatlayıcı ajanların varlığı ile açıklanabilir. Literatür taramasında *Lactarius* türlerinin antioksidan özellikleri ile çeşitli çalışmalar mevcuttur (Alkan et al., 2020; Bozdogan et al., 2018; Hasar et al., 2020; Rosa et al., 2020). Her ne kadar genel olarak *Lactarius* türleri orta seviyede antioksidan etkinlik sergileselerde, sentetiklerin kullanımındaki kaygılar, bu türlerin doğal antioksidanları bir kaynağı olarak kullanılabilieceğini göstermektedir.

Son on yılda bazı hastalıkların küresel ölçekte görülme oranları giderek artmakta ve bu noktada acil eylem planlarına ihtiyaç vardır. Bu hastalıkların başında şeker hastalığı (diyabet), Alzheimer hastalığı ve obezite gelmektedir (Rauf & Jehan, 2017). Farmasötik endüstri bu hastalıkların kontrolünü sağlamak ve görülme sıklığını azaltmak için yoğun bir şekilde çalışmakta ve bu çalışmalarda enzim önemli hedefler olarak değerlendirilmektedir. Enzim inhibisyon teorisi olarak bilinen bu teori hastalıkların patolojilerinde etkin rol oynayan enzimlerin inhibe edilmesiyle hastalık semptomlarının hafifletilmesini içerir. Bu teoriye göre hastalıkların patolojilerindeki enzimler örneğin Alzheimer hastalığı için asetilkolinesteraz, şeker hastalığı için amilaz ve glukozidaz ve obezite için lipaz enzimleri hedeflerimizdir. Örneğin kolinesteraz enziminin inhibe edilmesi Alzheimer hastalarında sinaptik boşluklarda azalan asetilkolin seviyelerinin yükseltilmesine ve böylece bilinçsel faaliyetlerin artırılmasına yardımcı olur (Mishra, Kumar, & Panda, 2019). Yine şeker hastalarında kan şeker seviyesinin yükselmesini geçici süre önlemek için α-amilaz ve α-glukozidaz enzimleri inhibe edilir ve bu durum ortaya çıkacak komplikasyonların kontrol altında tutulmasını sağlayabilir (Chinsembu, 2019). Bu amaçla sentetik olarak çok sayıda enzim inhibitörü farmasötik endüstride üretilmiştir . Örneğin takrin ve galatamin Alzheimer hastalığı için; akarboz ise şeker hastalığı için yaygın olarak kullanılan inhibitörlerdir. Bununla birlikte, bu bileşiklerin başta gastrointestinal problemler ve toksik özellikleri olmak üzere çeşitli yan etkilerinin bulunması bunların doğal inhibitörler ile yer değiştirmesi gerekliliğini ortaya koymuştur (Papoutsis et al., 2021; Pope & Brimijoin, 2018). Bu bağlamda, mevcut çalışmada üç *Lactarius* türünün enzim inhibitör özellikleri araştırılmıştır ve türlerin tamamı çalışılan enzimler üzerine inhibitör etkilere sahiptir. Korelasyon analiz sonuçları temelinde elde edilen sonuçlar değerlendirildiğinde enzim inhibitör özellikler ile toplam fenolik içerik arasında herhangi bir korelasyonun olmadığı ve fenolikler dışındaki bileşiklerin enzim inhibisyon testlerinde etkin rol oynadığı sonucuna varılabilir. Bu yaklaşım literatürdeki mevcut olan çalışmalarda da gözlemlenmiş ve fenolikler dışında birçok bileşiğin alkaloidler, terpenoidler gibi bileşiklerin inhibitör olarak rol oynayabileceğini göstermiştir (Ma, Li, Hou, & Wei, 2019; Spínola, Llorent-Martínez, & Castilho, 2020). Literatür taraması yapıldığında *Lactarius* üyelerinin enzim inhibitör özellikleri üzerine sınırlı sayıda çalışmanın varlığı gözlemlendi (Alkan et al., 2020; Ayvaz et al., 2019; Ozturk, Tel, Ozturk, & Duru, 2014). Bu yüzden, elde edilen sonuçlar literatüre değerli katkılar sağlayacaktır.

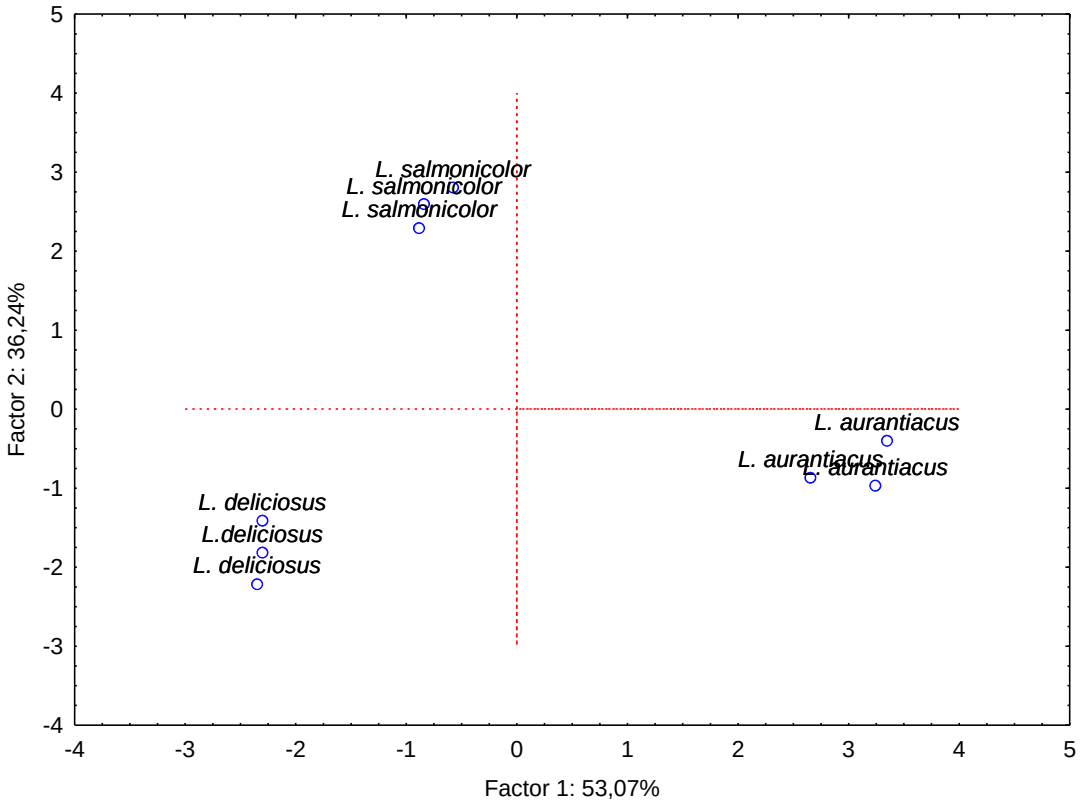
Tablo 4. Uygulanan biyolojik aktivite testleri arasındaki Pearson korelasyon değerleri (p<0.05).

	TPC	DPPH	ABTS	CUPRAC	FRAP	PBD	MCA	AChE	BChE	Amilaz	Glukozidaz
TPC	**1.00**	0.98	0.95	0.79	0.78	-0.99	-0.26	0.28	-0.41	0.01	-0.51
DPPH	0.98	**1.00**	0.98	0.88	0.87	-0.99	-0.31	0.09	-0.25	-0.16	-0.36
ABTS	0.95	0.98	**1.00**	0.94	0.94	-0.97	-0.29	-0.03	-0.11	-0.28	-0.21
CUPRAC	0.79	0.88	0.94	**1.00**	1.00	-0.84	-0.33	-0.32	0.22	-0.55	0.11
FRAP	0.78	0.87	0.94	1.00	**1.00**	-0.83	-0.31	-0.36	0.23	-0.56	0.13
PBD	-0.99	-0.99	-0.97	-0.84	-0.83	**1.00**	0.27	-0.19	0.33	0.09	0.42
MCA	-0.26	-0.31	-0.29	-0.33	-0.31	0.27	**1.00**	0.11	0.06	0.19	0.00
AChE	0.28	0.09	-0.03	-0.32	-0.36	-0.19	0.11	**1.00**	-0.89	0.82	-0.90
BChE	-0.41	-0.25	-0.11	0.22	0.23	0.33	0.06	-0.89	**1.00**	-0.83	0.97
Amilaz	0.01	-0.16	-0.28	-0.55	-0.56	0.09	0.19	0.82	-0.83	**1.00**	-0.78
Glukozidaz	-0.51	-0.36	-0.21	0.11	0.13	0.42	0.00	-0.90	0.97	-0.78	**1.00**

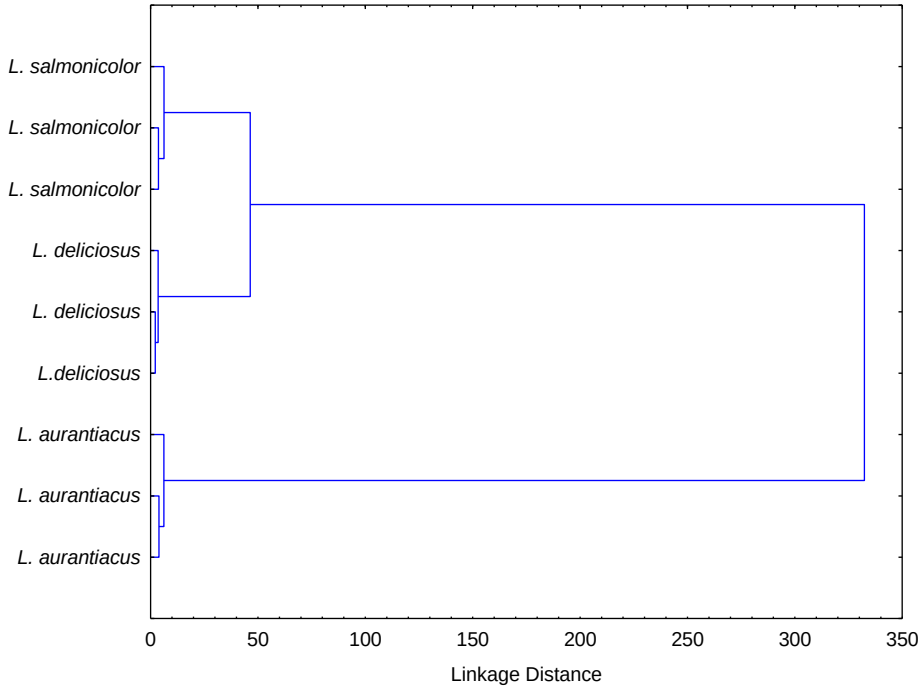
TPC: Total fenolik içerik; PBD: Fosfomolibdat; MCA: Metal şelatlama



Şekil 1. Uygulanan biyolojik aktivite testleri kullanılarak yapılan temel bileşen analizi (PCA)



Şekil 2. *Lactarius* türlerinin biyolojik aktivite sonuçları kullanılarak yapılan temel bileşen analizi (PCA)



Şekil 3. *Lactarius* türlerinin biyolojik aktivite sonuçları kullanılarak yapılan kümeleme analizi (cluster)

Biyolojik aktivite testlerinden elde edilen sonuçlar temel bileşen (PCA) ve kümeleme (cluster) analizleri ile incelendi ve sonuçlar Şekil 1-3'de gösterilmiştir. Temel bileşen analizi açık bir şekilde korelasyon analizi ile uyumlu olup TPC (total fenolik içerik), DPPH, ABTS, FRAP ve CUPRAC testlerinin aynı düzlemde yer aldığını göstermekte ve sonuçlar yüksek korelasyonun varlığını bize vurgulamaktadır. Enzim inhibisyon testleri ile MCA (metal şelatlama aktivitesi) ve PBD (fosfomolibdat) ise farklı düzlemlerde dağılmakta ve bu durum fenolik bileşikler dışındaki bileşiklerin bu testlerde etkili olabileceğini doğrulamaktadır. Şekil 2 ve 3'de ise çalışılan *Lactarius* türlerinin dağılımı görünmekte ve açık bir şekilde çalışılan türler iki gruptan toplanmaktadır. Küme 1'i *L. salmonicolor* ve *L. deliciosus* oluştururken, Küme 2'de ise *L. aurantiacus* yer almaktadır. Bu noktada ele geçen bu kümeleme analizli *Lactarius* türlerinin kemotaksonomik açıdan gruplandırılmasında da faydalı olabilir.

Sonuç olarak mevcut çalışmada üç *Lactarius* türünün antioksidan ve enzim inhibitör özellikleri karşılaştırmalı olarak değerlendirildi ve genel olarak ekstrakların orta seviyede biyolojik etkinlikler sergiledikleri gözlendi. Özellikle serbest radikal ve indirgeme gücü testlerin ile fenolik içerik arasında güçlü korelasyonun bulunması türlerdeki fenoliklerin temel antioksidan moleküller olabileceğini varsayımını çıkarmamızı sağlamaktadır. Yine özellikle *Lactarius* türlerinin sergiledikleri enzim inhibitör özellikler bu taksonların önemli potansiyellere sahip olduklarını göstermektedir. Çalışmamızın sonuçları çalışılan *Lactarius* türlerinin biyoaktif ajanların doğal bir kaynağı olabilieceğini ortaya koymaktadır. Bununla birlikte, ileri araştırmalar ile bu türlerin toksik potansiyellerinin belirlenmesi ve kimyasal parmak izlerinin çıkarılması önerilmektedir.

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Antioxidant and Oxidant Potantials and Element Contents of *Chroogomphus rutilus* (Agaricomycetes)

Mustafa SEVİNDİK[1*]

**Sorumlu yazar: sevindik27@gmail.com*

[1] University of Osmaniye, Bahçe Vocational School, Department of Food Processing, Osmaniye, Turkey
Orcid ID: 0000-0001-7223-2220/ sevindik27@gmail.com

Abstract: In this study, antioxidant and oxidant status of ethanol extract of *Chroogomphus rutilus* (Schaeff.) O.K. Mill. were determined. In addition, the element contents of the cap and stipe parts were measured. Antioxidant (TAS) and oxidant states (TOS) were determined using Rel Assay Diagnostics kits. Element contents (Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn) were determined using atomic absorption spectrophotometer. As a result of the study, the TAS value of *C. rutilus* was determined 2.769±0.100, the TOS value was 9.437±0.238 and the OSI value was 0.341±0.007. In addition, it was determined that Cu, Mn, Fe, Cd and Zn elements were accumulated more in the cap part of the mushroom, and Cr, Ni and Pb elements were more accumulated in the stipe part. As a result, it was determined that *C. rutilus* has antioxidant potential. It can be used as a natural material in pharmacological designs.

Key words: Antioxidant, *Chroogomphus rutilus*, Element, Medicinal mushroom, Oxidant

Chroogomphus rutilus'un (Agaricomycetes) Antioksidan ve Oksidan Potansiyelleri ve Element İçerikleri

Öz: Bu çalışmada *Chroogomphus rutilus* (Schaeff.) O.K. Mill. antioksidan ve oksidan durumları belirlenmiştir. Ayrıca şapka ve sap kısımlarının element içerikleri ölçülmüştür. Antioksidan (TAS) ve oksidan durumları (TOS) Rel Assay Diagnostics kitleri kullanılarak belirlenmiştir. Element içerikleri (Cr, Cu, Mn, Fe, Ni, Cd, Pb ve Zn) atomik absorbsiyon spektrofotometresi kullanılarak belirlenmiştir. Çalışma sonucunda *C. rutilus*'un TAS değeri 2.769±0.100, TOS değeri 9.437±0.238 ve OSİ değeri 0.341±0.007 olarak belirlenmiştir. Ayrıca mantarın şapka kısmında Cu, Mn, Fe, Cd ve Zn elementlerinin daha fazla, sap kısmında ise Cr, Ni ve Pb elementlerinin daha fazla biriktiği belirlenmiştir. Sonuç olarak *C. rutilus*'un antioksidan potansiyelinin olduğu ve farmakolojik dizaynlarda doğal materyal olarak kullanılabileceği belirlenmiştir.

Anahtar kelimeler: Antioksidan, *Chroogomphus rutilus*, Element, Tıbbi mantar, Oksidan

Introduction

Mushrooms are organisms that appear in different ecosystems, especially after rainfall. They have been used by people for different purposes since ancient times. Mushrooms are often used as a food source. However, looking at the ancient sources, it is found that it was used in the treatment of diseases and in religious ceremonies. (Özparlak et al., 2016; İnci et al., 2019). There are species with different characteristics among mushrooms. Some of these species stand out with their nutritious properties, while others stand out with their medicinal properties. Nutritious mushrooms are rich in protein, including most essential amino acids (Baba et al., 2012; Gedik et al., 2019). They are also rich in most water-soluble vitamins and pro-vitamin D (Kurtzman, 1997). Medicinal mushrooms contain biologically active compounds. (İnci and Kırbağ, 2018). In many studies, wild mushrooms have been reported to have anti-aromatase, anti-angiogenic, hypoglycemic, immunomodulatory, anti-inflammatory, insecticidal, antiallergic, anticancer, antioxidant, antimicrobial, antiproliferative and DNA protective effects (Wang et al., 2002; Song et al., 2004; Han et al., 2006; Chen et al., 2008; Jedinak et al., 2011; Alkan et al., 2017; Bal et al., 2017; Wang et al., 2018; Canlı et al., 2019).

C. rutilus, an edible species, is a cosmopolitan species that is brown color. It has ectomycorrhizal association especially with *Pinus* members (Ji et al., 2011). In our study, antioxidant and oxidant potentials and levels of some elements of *C. rutilus* were determined.

Material and metod

C. rutilus samples were collected from Bahçe/Osmaniye (Turkey) region. 30 g of collected mushroom samples were extracted with ethanol (EtOH) for approximately 6 hours at 50 °C using the soxhlet device (Gerhardt EV 14). The extracts obtained were concentrated with a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

Total Antioxidant and Oxidant status: Total antioxidant and oxidant states of EtOH extract of *C. rutilus* were determined using Rel Assay TAS and TOS kits. Trolox (TAS) and hydrogen peroxide (TOS) were used as calibrators (Erel, 2004, 2005). The oxidative stress index (OSI (Arbitrary Unit = AU)) of the mushroom was applied as follows (Erel, 2005).

$$OSI\ (AU): \frac{TOS\ (\mu mol\ H2O2\ equiv./L)}{TAS\ (mmol\ Trolox\ equiv./L\ )X\ 10}$$

Element Analyses: In order to determine the elemental analysis (Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn) of *C. rutilus*, the samples were dried at 80 °C up to constant weighing. 0.5 g of the mushroom samples were mineralized using a microwave solubilizer (Milestone Ethos Easy) in a mixture of 9 mL HNO_3 + 1 mL H_2O_2. After the mineralization process, the element contents were determined using an atomic absorption spectrophotometer device (Agilent 240FS AA) (Sevindik et al., 2017).

Results and Discussion

Total Antioxidant and Oxidant Status: Living organisms of the ecosystem produce oxidant compounds as a result of metabolic activities in their bodies (Monaghan et al., 2009). While these oxidant compounds act as catalysts in some metabolic activities in low amounts, high levels can cause serious damage to the living organism. Endogenous antioxidants come into play to prevent this harmful process from occurring (Willcox et al., 2004). However, in cases where endogenous antioxidants are insufficient, supplemental antioxidants are required. In this context, it is important to identify natural products with antioxidant potential to be taken through diet (Sevindik, 2020). TAS, TOS and OSI values of EtOH extract of *C. rutilus* were determined in our study. The findings obtained are shown in Table 1.

Table 1. TAS, TOS and OSI values of *C. rutilus*

Sample	TAS (mmol/L)	TOS (µmol/L)	OSI
Chroogomphus rutilus	2.769±0.100	9.437±0.238	0.341±0.007

* Values are presented as mean±SD; Experiments were made in 5 parallels

It has been emphasized in previous studies that *C. rutilus* has antioxidant potential using different methods (DPPH Free Radical Scavenging Assay, β-Carotene/Linoleic Acid Assay, ABTS Cation Radical Decolorisation Assay, Cupric Reducing Antioxidant Capacity (CUPRAC), Ferrous Ions Chelating Activity) (Ji et al., 2011; Çayan et al., 2014; Zhang et al., 2017; Zhang et al., 2020). In this study, the antioxidant potential of *C. rutilus* was determined for the first time using TAS kits. There are studies on different wild mushrooms using TAS kits. TAS value of *Laetiporus sulphureus* (Bull.) Murrill was reported as 2.195 mmol/L, TOS value was 1.303 µmol/L and OSI value was 0.059 (Sevindik et al., 2018). TAS value of *Suillus granulatus* (L.) Roussel was reported as 3.143 mmol/L, TOS value was 18.933 µmol/L and OSI value was 0.603 (Mushtaq et al., 2020). TAS value of *Tricholoma virgatum* (Fr.) P. Kumm. was reported as 3.754 mmol/L, TOS value was 8.362 µmol/L and OSI value was 0.223 (Selamoglu et al., 2020). TAS value of *Clavariadelphus truncatus* Donk was reported as 2.415 mmol/L, TOS value was 3.362 µmol/L and OSI value was 0.140 (Sevindik, 2018). TAS value of *Lycoperdon molle* Pers. was reported as 7.52 mmol/L (Emsen et al., 2019). TAS value shows the whole of the antioxidant compounds in the mushroom (Bal, 2018). Compared to these studies, it was determined that the TAS value of *C. rutilus* was higher than *L. sulphureus* and *C. truncatus* and lower than *L. molle, S. granulatus* and *T. virgatum*. The reason for the difference in TAS value among mushroom species is thought to be due to the mushroom's potential to produce antioxidant compounds. It has also been determined that *C. rutilus* has an antioxidant potential.

In addition, the TOS value shows the whole of the oxidant compounds produced by the fungus (Bal, 2018). The variability in TOS values is due to the species' potential to produce oxidant compounds and their hatitat. The OSI value shows how much oxidant compounds produced in the mushroom are suppressed by endogenous antioxidants. As the OSI value increases, it is seen that the antioxidant defense system in the mushroom is insufficient (Bal, 2018). It is seen that the TOS and OSI values of *C. rutilus* were higher than *L. sulphureus, C.*

truncatus, T. virgatum and *C. truncatus*, but lower than *S. granulatus*. These results show that *C. rutilus* is weak in suppressing the oxidant compounds it produces. As a result, it is recommended not to consume excessively in case of consumption of mushrooms.

Element Contents: Mushrooms play an important role in ensuring the ecological cycle. In this context, they serve in the breakdown of organic cover. As a result of this process, they accumulate elements at different levels in their bodies (Dursun et al., 2006; Sarıkürkçü et al., 2020). In our study, the Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn levels accumulated in the cap and stipe parts of *C. rutilus* were measured. The findings obtained are shown in Table 2.

Table 2. Element Contents of cap and stipe of *C. rutilus* (mg.kg^{-1})

Parts	Cr	Cu	Mn	Fe	Ni	Cd	Pb	Zn
Cap	1.40±0.27	57.13±1.73	26.13±1.89	108.78±3.76	0.77±0.13	3.76±0.63	4.18±0.44	168.34±4.04
Stipe	2.54±0.11	49.11±0.80	15.82±0.53	36.99±2.31	1.14±0.09	1.08±0.16	8.96±0.61	129.18±2.52

*Values are presented as mean±S.D.; n=3 (Experiments were made as 3 parallel)

As a result of the elemental analysis of *C. rutilus*, it is seen that Cr, Ni and Pb elements accumulate more in the stipe part, while Cu, Mn, Fe, Cd and Zn contents are accumulated more in the cap part. The lowest and highest element levels in element analysis on wild mushrooms were reported as 9.63-42.7 for Cr, 60.33-95 for Cu, 18.1-103 for Mn, 14.6-835 for Fe, 0.67-5.14 for Ni, 2.71-7.5 for Cd, 2.86-16.54 for Pb and 29.8-158 for Zn mg.kg^{-1}. (Svoboda and Chrastny, 2008; Zhu et al., 2010; Gebrelibanos et al., 2016). In our study, it is seen that the Cr, Cu and Ni contents of *C. rutilus* are lower than the literature ranges of the cap and stipe parts. It has been determined that the cap and stipe parts of Mn, Fe and Pb contents are within the literature ranges. Less accumulation of Cd content in the stipe parts compared to literature ranges was observed. It was determined that the Cd accumulated in the cap part is within the range of the literature. It was observed that the Zn content of the mushroom was higher than the literature ranges in the cap part, and it was within the literature ranges in the stipe part. In this context, it is seen that *C. rutilus* accumulates more elements in the cap part.

Conclusion

In this study, the antioxidant and oxidant potential of *C. rutilus* was determined. In addition, the element contents of the cap and stipe parts were determined. As a result of the studies, it was seen that the mushroom has an antioxidant potential. It was also determined that it accumulated more elements in the cap part. As a result, it is thought that mushrooms can be a natural source of antioxidants in pharmacological designs.

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Haloalkalitolerant and Haloalkaliphilic Fungal Diversity of Acıgöl/Turkey

Fatma AYVA[1], Rasime DEMİREL[2]*, Semra ILHAN[3], Lira USAKBEK KYZY[4], Uğur ÇİĞDEM[5], Niyazi Can ZORLUER[6], Emine IRDEM[5], Ercan ÖZBİÇEN[4], Esma OCAK[5], Gamze TUNCA[4]

** Corresponding author e-mail: rasime.demirel@gmail.com*

[1] Eskisehir Technical University, Faculty of Science, Department of Biology, TR-26470 Eskisehir, Turkey
Orcid ID: 0000-0002-7072-2928/ ayvafatma@gmail.com
[2] Eskisehir Technical University, Faculty of Science, Department of Biology, TR-26470 Eskisehir, Turkey
Orcid ID: 0000-0001-8512-1597/ rasime.demirel@gmail.com
[3] Eskişehir Osmangazi University, Faculty of Science and Letters, Department of Biology, TR-26040 Eskisehir, Turkey
Orcid ID: 0000-0002-3787-2449/ silhan@ogu.edu.tr
[4] Eskişehir Osmangazi University, Graduate School of Natural and Applied Sciences, Department of Biology, TR-26040, Eskisehir, Turkey
Orcid ID: 0000-0002-9424-4473/ lira199322@gmail.com
Orcid ID: 0000-0001-5646-6115/ ercanozbıcen@hotmail.com
Orcid ID: 0000-0002-4126-8340/ gamzetuncaa@gmail.com
[5] Eskişehir Osmangazi University, Graduate School of Natural and Applied Sciences, Department of Biotechnology and Biosafety, TR-26040, Eskisehir, Turkey
Orcid ID: 0000-0003-4790-494X/ ugur.cigdem@hotmail.com
Orcid ID: 0000-0002-2955-298X/ emineirdem@gmail.com
Orcid ID: 0000-0002-9085-4151/ eocak@ogu.edu.tr
[6] Eskişehir Osmangazi University, Faculty of Science and Letters, Programme of Biology, TR-26040 Eskisehir, Turkey
Orcid ID: 0000-0002-2394-2194/ nicazor@gmail.com

Abstract: Microfungi are the most common microorganisms found in range from environment. They are well known as producer of some product in industrial and food fields, decomposer of organic matter, of important mycotoxins, reason major economic and health effects on plant, animal and human life. Because of these reasons, studies on the determination of biodiversity of microfungi are vitally important. Aim of this study is investigation of biodiversity of microfungi in Acıgöl Lake that is the second largest alkaline lake in the world.
For this purpose, the water sample was compositely taken from a saltern of Acıgöl Lake in November 2019. The samples have been analysed in terms of pH, and salinity. To isolate and enumerate the fungal species from water, filtration method and different DRBC medium types were used. Salt tolerance range of isolates were determinated.
A total of 260 CFUs/L and 65 CFUs/L were counted from DRBC and DRBC28 media, respectively. After purification steps, totally 52 isolates were obtained and identified by using conventional methods and multi locus genes sequencing.
The results indicated that the Acıgöl Lake Region has rich for *Aspergillus* (25%) and *Penicillium* (27%) genera, respectively. Although other members of the genus were determined in the region, other members were found to be 48% in total. In addition, *Cladosporium acalypha* and *Penicillium sizovae* was determined as a new recorded for Turkey. The fact that the microfungus biodiversity determined by this study has the ability to produce toxins (such as *Aspergillus flavus*), contains pathogenic (such as plant pathogen *Fusarium* genus members) and saprophyte species, has been identified as an issue to be considered for public health.

Key words: Acıgöl, Lake, Microfungus, Biodiversity

Acıgöl/Türkiye'deki Haloalkalitolerant ve Haloalkalifilik Fungus Çeşitliliği

Öz: Mikrofunguslar, çevrede en yayın olarak bulunan mikroorganizmalardır. Bu organizmalar, organik maddelerin ayrıştırıcısı, endüstriyel ve gıda alanlarında bazı ürünlerin üreticisi, önemli mikotoksinlerin, bitki, hayvan ve insan yaşamı üzerindeki önemli ekonomik ve sağlık etkilerinin nedeni olarak bilinirler. Bu nedenlerden dolayı mikrofungusların biyolojik çeşitliliğinin belirlenmesine yönelik çalışmalar hayati önem taşımaktadır. Bu çalışmanın amacı, dünyanın ikinci büyük alkali gölü olan Acıgöl Gölü'ndeki mikrofungusların biyolojik çeşitliliğinin araştırılmasıdır.

Bu amaçla, su örneği Kasım 2019'da Acıgöl Gölü'ndeki tuzladan kompozit olarak alınmıştır. Su örneği pH ve tuzluluk açısından analiz edilmiştir. Mantar türlerinin sudan izole edilmesi ve sayılması için filtrasyon yöntemi ve farklı DRCB besi ortamları kullanılmıştır. İzolatların tuz tolerans aralığı belirlenmiştir.

DRBC ve DRBC28 ortamlarından sırasıyla toplam 260 KOB/L ve 65 KOB/L sayılmıştır. Saflaştırma adımlarından sonra, toplam 52 izolat elde edilmiştir ve geleneksel yöntemler ve çoklu lokus genleri dizilimi kullanılarak izolatlar tanımlanmıştır.

Sonuçlar, Acıgöl Gölü bölgesinin sırasıyla *Aspergillus* (% 25) ve *Penicillium* (% 27) cinsleri bakımından zengin olduğunu göstermiştir. Bölgede cinsin diğer üyeleri belirlenmekle birlikte toplam diğer üyeler % 48 oranında bulunmuştur. Ayrıca *Cladosporium acalypha* ve *Penicillium sizovae* Türkiye için yeni kayıt olarak belirlenmiştir. Bu çalışma ile belirlenen mikrofungus biyoçeşitliliğinin toksin üretme kabiliyetine sahip olması, patojenik ve saprofit türleri içermesi, halk sağlığı için dikkate alınması gereken bir konu olarak belirlenmiştir.

Anahtar kelimeler: Acıgöl, Göl, Mikrofungus, Biyoçeşitlilik

Introduction

Microfungi are important eukaryotic microorganisms for plants, animals and humans with their beneficial and harmful activities (Amaeze et al., 2010). Microfungi are commonly found in soil, air, in various food products, from fresh waters to seas and in extreme environments (Kayış et al., 2018). Some microfungi have been noted in many extreme environments such as salt water, rock surface, ocean bottoms, hot ecological regions. Many species developed, especially *Acremonium, Alternaria, Aspergillus, Cladosporium, Fusarium* and *Penicillium* genera members have been observed in different level saline environments (Orwa et al., 2020).

Saline lakes are alkaline with pH values often ranging between 9 and 12. This condition is characterized by particularly high concentrations of carbonate salts. The presence of sodium chloride and other dissolved salts at high concentration creates salty waters (Salano et al., 2017). Fungi in saline environments have been found to produce extremoliths and extremozymes to cope with osmotic stress. They can also prevent water loss by accumulating K^+ ions (Orwa et al., 2020).

Many abilities have been discovered in haloalkaliphilic microfungi such as absorbing salt ions, producing organic acids, providing macromolecules beneficial for health (Wei and Hong-Zhang, 2019). As a result of many studies, haloalkaliphilic microfungi are rare, while haloalkalitelorans are more intense (Eliades et al., 2011). Microfungi growing in extreme environments are of great importance for humans, animals and plants as microbial biodiversity. These microfungi are important pathogens of humans, animals and plants, and they have many properties, such as producing certain enzymes and compounds, producing low-molecular-weight low metabolites, mycotoxins for this reason, it is very important to reveal the biotechnological features (Schuster and Kahmann, 2019).

Haloalkalitolerant microfungi are important for some enzymes in biotechnological field as well as molecular characterization in adaptation (Eliades et al., 2011). It supports both ecological roles and industrial applications with microfungi isolated from extreme environments (Sharma et al., 2016). Some halotolerant fungi are able to produce different hydrolytic enzymes such as cellulase (CMCase), amylase, protease, lipase, and laccase with tolerance to salt (Li et al., 2018).

More studies are needed to discover more features of these microorganisms. For these reason, the main idea of this study investigation of biodiversity of microfungi in

Acıgöl Lake that is the second largest alkaline lake in the world and located between Afyonkarahisar-Denizli-Isparta city boundaries, in the southwest Anatolia, Turkey.

Material and Metod

Research Area, Features of Acıgöl Lake and Sampling

The research area is Acıgöl Lake, which is one of most important sodium sulphate production fields in Turkey, located between Afyonkarahisar-Denizli-Isparta provinces boundaries (Map 1). It is a tectonic lake and has center coordinates 37º 49' N 29º 48' E, and is 836 m altitude above the sea level. The average depth of the lake is 150 and 210 cm with an average area of 41.34 km^2. Acıgöl Lake, which is fed by precipitation, ground-water and the springs that occur along tectonic faults, has no water output other than evaporation and industrial activities. The modern Acıgöl Lake is the second largest alkaline lake in the world, with active precipitation of sodium, calcium and magnesium salts and its surface varies greatly due to seasonal drought (Kuşçu et al., 2017).

Sampling, Isolation and Enumeration of Microfungi

The water sample was compositely taken from a saltern of North site (37°51′14.8″N 29°52′32.2″E) of Acıgöl Lake in November 2019 (Map 1). Variables such as sample pH and salinity (%) are analyzed in Eskişehir Osmangazi University Biology Department Laboratories.

Map 1. Acıgöl Lake and sampling point

To isolate and enumerate the fungal species from water, 20 ml of water sample has been filtered through the sterile Cellulose Nitrate Membrane Filters (pore size 0.45µm, Ø 47 mm Sartorius) and placed onto the Petri plates containing DRBC and DRBC with water sample (DRBC+28% salty) media with chloramphenicol (100 mg/L). For DRBC28 medium, untreated saltern water from the Acıgöl Lake has been used. The plates have been incubated for 5 weeks at 25°C. Fungal colony forming units (CFUs) were counted on 3rd, 5th, 7th, 14th and 30th days of incubation, and subcultures were made of all of the morphologically distinct colonies from each Petri dish on Malt Extract Agar (Merck) slants and kept at 4°C. Individual pure strains have been deposited in the culture collection of the Department of Biology, Eskisehir Osmangazi University (Turkey).

Water samples of ten aliquots have been filtered in parallel and the average number of colonies has been calculated as CFUs/1000 ml. Water activities of the media have been determined using the water activity meter (Aqualab, Decagon Devices, USA).

Halotolerans and Haloalkalitolerant Tests

In the first step, the isolated fungi were inoculated with three point technique in Petri plates containing PDA supplemented with 0%, 5%, 10%, 15% and 20% NaCl. After 7 days incubation at 27 ºC, colony diameters were measured in mm.

In the second step, the isolates with halotolerant properties were inoculated in plates containing PDA adjusted to high pH values (8 and 10) in addition to salt. After 7 days incubation at 27 ºC, colony diameters were measured in mm. The buffer solutions used in the pH settings of the media were used from Grum et al (2016).

Morphological and Multi Locus Gene

Taxonomic identification of fungal isolates was based on their cultural characteristics and morphological structures. Briefly, for identification, isolates were streaked onto potato dextrose agar (PDA) (MERCK) and incubated at 25°C for 7 days. Subsequently, colony diameters were measured and fungal cultures were examined under stereomicroscope (Prior James Swift, England) and a high-resolution light microscope (OLYMPUS CH20BIMF200, OLYMPUS Optical Co, Ltd. Japan) to determine colonial and morphological features. For morphological examinations fungal material was mounted in a modified mounting medium, Lacto-Cotton Blue, as proposed by Sime et al. (2002). Microfungi obtained from Acıgöl Lake were identified to genus level according to Barnett and Hunter (1999).

The isolates which are accepted as *Aspergillus* and *Penicillium* genus members of haloalkalitolerant and haloalkaliphilic microfungi were initially detected at the

genus level according to their microscopic and colonial characteristics.

For traditional identification of *Penicillium* species, Czapek Yeast Extract Agar (CYA; incubation at 25°C and 37°C), Malt Extract Agar (MEA; at 25°C), Yeast Extract Agar (YES; at 25°C) and Creatine Sucrose Agar (CREA; at 25°C) were used. For traditional identification of *Aspergillus* species, CYA (at 25°C and 37°C), MEA (at 25°C) and CREA (at 25°C) were used. The isolates were incubated for 7 days. At the end of incubation, the isolates were distinguished at the species level according to their morphological and microscopic characteristics (Klich, 2002; Samson et al. 2010; 2011).

All isolates were grown on Potato Dextrose Agar (PDA) at 25 °C 7 days for DNA extraction. Fungal genomic DNA was extracted by using ''Mobio Ultraclean Microbial DNA Isolation Kit'' according to the manufacturer's instructions. Obtained DNA used as template for PCR amplification of internal transcribed spacer (ITS) regions of the rDNA genes, part of the β-tubulin (BenA) and calmodulin (CaM), genes. The ITS regions and BenA genes were amplified using described methods (Visagie et al. 2014). For CaM, the CL1 and CL2 primer sets were used (Serra et al. 2006). PCRs were performed by using a Veriti® 96-Well Thermal Cycler (Applied Biosystems®) using described methods (Visagie et al. 2014).PCR products were confirmed by agarose gel electrophoresis (1% w/v in 1xTAE) and visualized by GelRed staining and examined via the Gel Documentation System (Uvitec M02 4611). Sequencing reactions were performed with the Applied Biosystems (3130 XL Genetic Analyser) by the RefGen Biotechnology (http://www.refgen.com).

Data Analysis

The data obtained as a result of the sequence were compared with the NCBI GenBank Database type (Altschul et al., 1990; https://blast.ncbi.nlm.nih.gov/Blast.cgi). The closest Blast result to each taxon was reported.

Alignment of all data obtained as a result of comparisons was carried out using Muscle in the MEGA X software program and together with other sequences of type species obtained from NCBI GenBank (Kumar et al., 2018). Aligned datasets were analyzed in MEGA X 1000 repeats (1000 bootstrap) using the Maximum Likelihood (ML) analysis based on the Tamura-Nei model (Tamura and Nei, 1993). All positions with less than 50% site coverage, containing gaps, or missing data were eliminated.

The author and current species names of the isolates have been standardized according to the Index Fungorum website (http://www.indexfungorum.org/names/names.asp)

Results and Discussions

Halotolerans and Haloalkalitolerant Tests

Microfungi are common microorganisms found in range habitats from soil, air, water and extreme environments (Chavez et al., 2015). As a matter of fact, we were faced with a remarkable fungal biomass values in our study of isolation from Acıgöl that is an important in sodium sulphate production area in Turkey. A total of 52 and 13 isolates were obtained from water sample by using DRBC and DRBC28 media, respectively. Similarly isolates numbers, the highest colony count were recorded from DRBC as 260 CFUs/L. A total of 65 CFUs/L colonies were counted from DRBC28 medium. It is seen that the number of isolates and colonies obtained from 20% (percentage of colonies) and 19,12% (percentage of isolates) are from DRBC28 medium containing 28% salt. A total of 58 isolates from obtained isolates were detailed in this study.

Many studies have demonstrated that fungal biodiversity is high in hypersaline and salty soil environments (Plemenitaš et al., 2014).

Fungi living in hipersaline environments need a minimum salt concentration to adapt to different salt concentrations. Concentrations of Na^+ ions are much greater than K^+ ions. Therefore, the mechanisms that maintain a stable and high intracellular K^+ / Na^+ ratio are crucial to survival in such environments (Plemenitaš et al., 2014). Studies investigating the effects of different salt concentrations on fungal growth; They determined that at low salt concentrations fungal colonies had large diameters and that the colony diameters decreased with the increase in salt concentration, and that alkaline pH values limited the growth of fungal colonies. Another remarkable record is that the pH values of the culture media increase to the alkaline level; It is also the decrease in the level of sport creation (Samson et al., 2010; Kayış et al., 2018).

Isolates were found to have a certain amount of salt tolerance. When the growth performance of the isolates at different salt concentrations was evaluated, it was determined that colonies with the largest diameter were formed in the medium containing 5% salt concentration compared to the medium with 0% salt concentration (Figure 1). While isolates were exhibited moderately

smaller colonies than 0% on 10% and 15% salinity media, micro colonies or not colony formation were on 20% salinity medium (Figure 2).

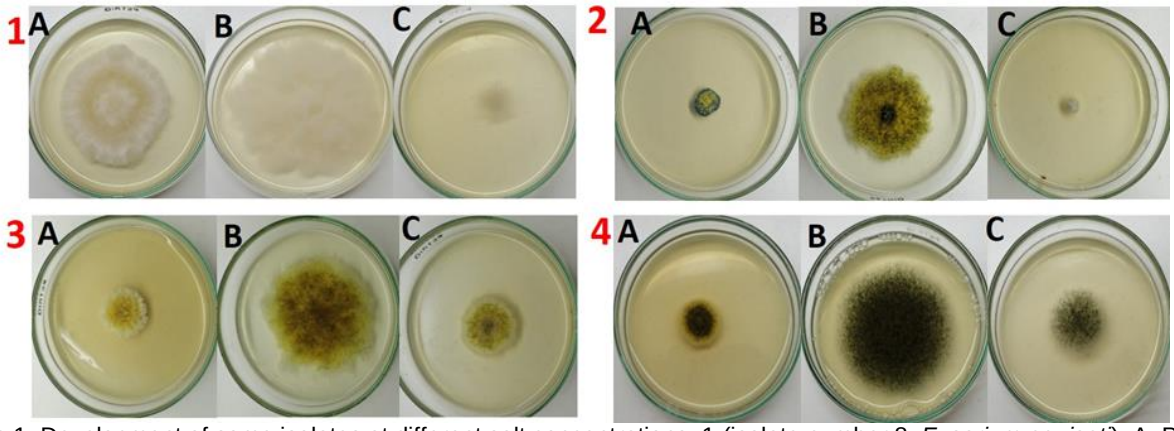

Figure 1. Development of some isolates at different salt concentrations. 1 (isolate number 8, *Fusarium equiseti*); A: PDA 0% salt, B: PDA 5% salt, C: PDA 10% salt; **2** (isolate number 48, *Aspergillus cristatus*) A: PDA 0% salt, B: PDA 5% salt, C: PDA 20% salt; **3** (isolate number 50, *Aspergillus pseudoglaucus*) A: PDA 0% salt, B: PDA 5% salt, C: PDA 10% salt; **4**; (isolate number 54, *Aspergillus amstelodami*) A: PDA 0% salt, B: PDA 5% salt, C: PDA 15% salt.

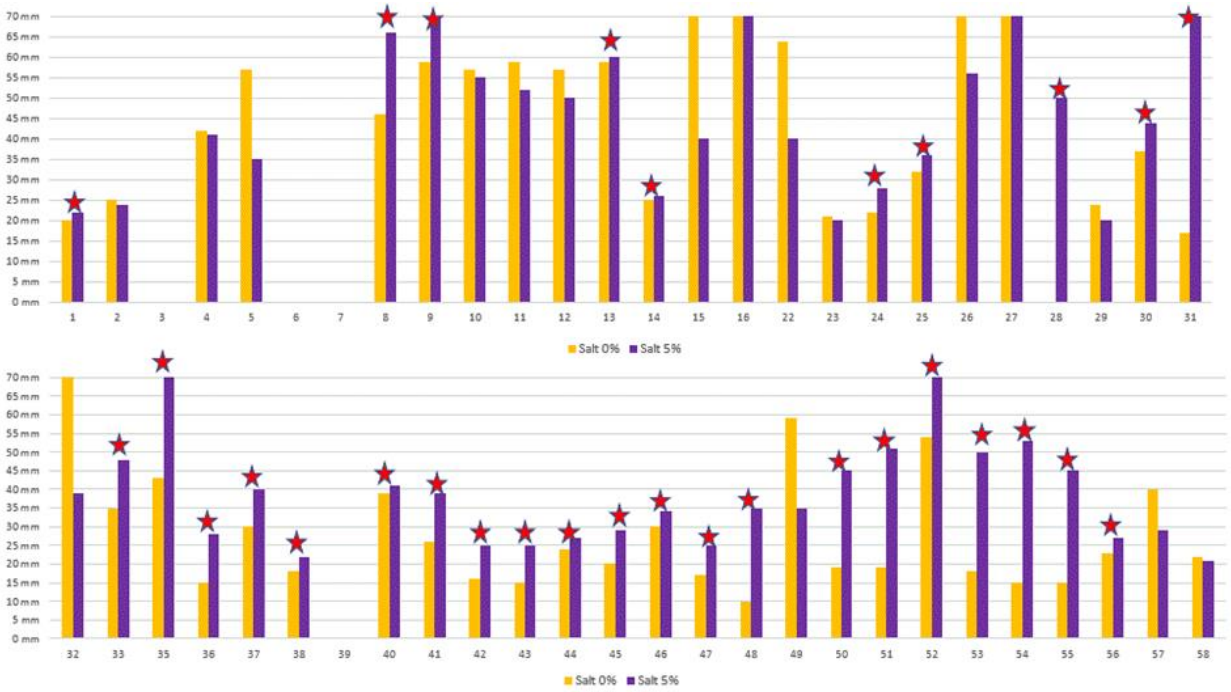

Figure 2. The development of organisms at different salt concentrations (Labeled isolates formed larger colonies on 5% salt medium than of 0%.

Morphological and Multi Locus Gene Identification

The dominant fungal group in the hypersaline and alkaline environments known as *Aspergillus* sp. and *Penicillium* sp. genera in addition to *Alternaria* sp., *Fusarium* sp.and melanized fungi (Plemenitaš et al., 2014; Orwa et al., 2020). According to morphologic and multi-locus genes sequencing results, the isolates were found to be members of *Acremonium* (2%), *Alternaria* (13 %), *Aspergillus* (25%), *Cladosporium* (6%), *Fusarium* (19%), *Penicillium* (27%), *Rhizopus* (2%), and *Trichoderma* (2%). The *Penicillium* genus was recorded as a common genus in Acıgöl Lake with 27% (Figure 3). When we focused on biodiversity and distribution of members of the *Penicillium*, *P. brevicompactum* Dierckx 1901 was determined as the most common with 36%.

This is followed by *P. dipodomyicola* (Frisvad, Filt. & Wicklow) Frisvad 2000 (21.5%), *P. sizovae* Baghd. 1968 (21.5%) and *P. bilaiae* Chalab. 1950, *P. chrysogenum* Thom 1910, *P. solitum* Westling 1911 (each 7%). Up to now, many species of *Penicillium* have been reported from saline habitats such as saline soil, hypersaline regions, hypersaline lakes (Chung et al., 2019). In addition to the widespread determination of *P. brevicompactum* especially in the salterns, *P. chrysogenum, P. citrinum, P. digitatum* has also been recorded with a high frequency (Yadav et all, 2018).

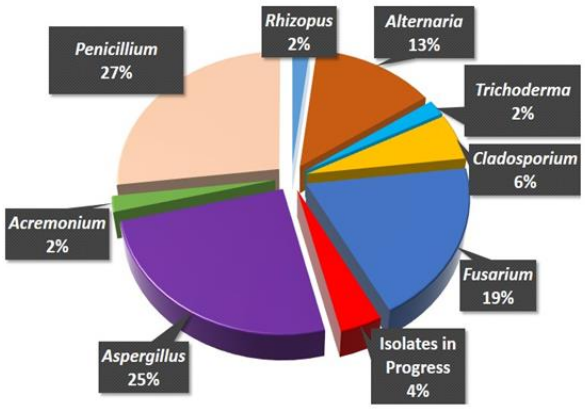

Figure 3. Distribution of isolates in level of genus

About distribution of *Aspergillus* genus from investigated area, although this genus is less in number than *Penicillium* in the research area, it is remarkable that it is higher in terms of diversity. Another remarkable issue is the prevalence of members with strong body structures such as sexual reproduction and sclerotium. *Aspergillus amstelodami* (L. Mangin) Thom & Church 1926, *A. intermedius* Blaser 1976, *A. ochraceus G.* Wilh. 1877 and *A. terreus* Thom 1918 species were recorded as common species (each 15%). This is followed by A. *alliaceus* Thom & Church 1945, *A. cristatus* Raper & Fennell 1965, *A. flavus* Link, Mag. Gesell. naturf. Freunde 1809, *A. pseudoglaucus* Blochwitz 1929 and *A. tubingensis* Mosseray 1934 (each 8%). Already, Eurotiales order within Ascomycota is most important group of both xerotolerant and halotolerant species (Gunde-Cimerman et al., 2009).

Fusarium genus follows *Aspergillus* and *Penicillium* genera at a rate of 19%. *F. solani* (*Neocosmospora solani) (Mart.) L. Lombard & Crous 2015* 40%, *F. keratoplasticum* (*Neocosmospora keratoplasticum*) (Geiser, O'Donnell, D.P.G. Short & Ning Zhang) Sand.-Den. & Crous 2017 20% were obtained. *F. equiseti* (Corda) Sacc. 1886, *F. fujikuroi* Nirenberg 1976, and *Fusarium sp*. species were obtained at a rate of 10%.

All *Alternaria* genus were identified as *Alternaria alternata* (Fr.) Keissl. 1912. *Cladosporium* breed members were obtained in 6% (*C. acalyphae* Bensch, H.D. Shin, Crous & U. Braun 2010, *C. cladosporioides* (Fresen.) G.A. de Vries 1952 and *C. pseudocladosporioides* Bensch, Crous & U. Braun 2010 33.3%). In addition to their ability to survive in low water activity, these black fungi can protect themselves and survive against ultraviolet rays that occur directly due to their pigments and indirectly due to habitat components. Therefore, the frequency of being encountered in hypersalin environments worldwide is high (Chung et al., 2019). Other species obtained; *Acremonium sclerotigenum* (Moreau & R. Moreau ex Valenta) W. Gams 1971, *Rhizopus oryzae* A. Fisch. 1892 and *Trichoderma harzianum* Rifai 1969 (Table 1).

Saline waters are chemically rich by Cl^+ and Na^+ ions. It also contains ions such as Mg^{2+}, SO_4^{2-}. Chemical structure of water together with other environmental factors such as temperature, humidity, wind, sun can affect microbial diversity (Chung et al., 2019). Intense water evaporation and leaching of surrounding rocks with Ca^{2+} and Mg^{2+} deficiency cause the formation of water with high pH values (Bondarenko et al., 2018). In our study, it has been determined that this kind of environmental characteristics may be suitable for the development of certain breed members.

Microfungi that develop only in saline environments are called halophilic (Wei and Hong-Zhang, 2018). The species identified as a result of the study are also seen in different environments such as air, soil, food, and we can say that these microfungi are halotolerant because they have also developed in different salt ratios. Some of the *Acremonium, Aspergillus, Fusarium* and *Penicillium* species are known to be secondary metabolite producers. Secondary metabolites cause anticancer, antioxidant, antiviral and antibacterial effects (Orwa et al., 2020). In this study, it is seen that the microfungi isolated from Acıgöl Lake are rich in these species. Since lake water is rich in potassium, sodium and sulfate, it is used by some businesses (denizli.gov.tr). Therefore, determination of the microfungal diversity of the lake and the characteristics of the species obtained are of great importance. Identified species are known to cause beneficial and harmful effects in economic, biotechnological, food industry, agricultural activities and many other areas.

According to the checklists (Asan (2004), Asan et al. (2016) and Asan (2017)); *Cladosporium acalypha* (23) and *Penicillium sizovae* (37, 46, 56) was determined as a new recorded for Turkey. Contribution to future studies can be made by determining the fungal variety of Acıgöl Lake in terms of Halotolerant and Haloalkalitolerant and the development of these species in different concentrations of salt.

Table 1. Distribution of species isolated from Acıgöl Lake

Species Name	GenBank accession numbers	Number of the isolate (Percentage)
Acremonium sclerotigenum	MT472511	58 (1.92)
Alternaria alternata	MT472471, MT472472, MT472473, MT472474, MT472475, MT472476, MT472482	10, 11, 12, 13, 14, 15, 26 (13.46)
Aspergillus flavus	MT472489	35 (1.92)
Aspergillus alliaceus	MT472488	33 (1.92)
Aspergillus intermedius	MT472504, MT472506	**51, 53** (3.85)
Aspergillus amstelodami	MT472507, MT472508	**54, 55** (3.85)
Aspergillus cristatus	MT472502	**48** (1.92)
Aspergillus ochraceus	MT472486, MT472505	**31, 52** (3.85)
Aspergillus pseudoglaucus	MT472503	50 (1.92)
Aspergillus terreus	MT472484, MT472485	**29**, **30**(3.85)
Aspergillus tubingensis	MT472487	**32** (1.92)
Cladosporium acalyphae	MT472479	**23** (1.92)
Cladosporium cladosporioides	MT472481	**25** (1.92)
Cladosporium pseudocladosporioides	MT472480	**24** (1.92)
Fusarium equiseti	MT472469, MT472470	**8, 9** (3.85)
Fusarium fujikuroi Nirenberg 1976	MT472465	**4** (1.92)
Neocosmospora keratoplastica	MT472463, MT472478	**2, 22** (3.85)
Neocosmospora solani	MT472462, MT472464, MT472466, MT472467	**1, 3, 5, 6** (7.69)
Fusarium sp.	MT472468	**7** (1.92)
Penicillium bilaiae	MT472498	**44** (1.92)
Penicillium brevicompactum	MT472490, MT472493, MT472496, MT472497, MT472501	**36, 39, 42, 43, 47** (9.62)
Penicillium chrysogenum	MT472510	**57** (1.92)
Penicillium solitum	MT472494	**40** (1.92)
Penicillium dipodomyicola	MT472492, MT472495, MT472499	**38, 41, 45** (5.77)
Penicillium sizovae	MT472491, MT472500, MT472509	**37, 46, 56** (5.77)
Rhizopus arrhizus	MT472477	**16** (1.92)
Trichoderma harzianum	MT472483	**28** (1.92)
Isolates in Progress	-	**27, 49** (3.85)

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Macrofungi Determined in Ayrancı and Yeşildere (Karaman) Districts

Ahmet ÇETİNKAYA[1], Yasin UZUN[2],
Abdullah KAYA[3*]

**Sorumlu yazar: kayaabd@hotmail.com*

[1]Ayrancı Social Assistance and Solidarity Foundation, 70100 Karaman, Turkey
Orcid ID: 0000-0001-9794-4363/ ahmet_cetinkayaaa@hotmail.com
[2]Karamanoğlu Mehmetbey University, Ermenek Uysal & Hasan Kalan Health Services Vocational School, 70400, Karaman, Turkey
Orcid ID:0000-0002-6423-6085 / yuclathrus@gmail.com
[3]Gazi University, Science Faculty, Department of Biology, 06500 Ankara, Turkey
Orcid ID: 0000-0002-4654-1406 / kayaabd@hotmail.com

Abstract: This study was carried out on macrofungi samples collected from Ayrancı and Yeşildere districts of Karaman between 2014 and 2018. Seventy four species, belonging to 58 genera, 42 families, 13 orders and 7 classes within Ascomycota and Basidiomycota were determined. The list of the taxa is presented together with their habitats and localities.

Key words: Biodiversity, macrofungi, taxonomy, Turkey

Ayrancı ve Yeşildere (Karaman) Yörelerinde Belirlenen Makromantarlar

Öz: Bu çalışma Ayrancı ve Yeşildere (Karaman) yörelerinden 2014 ve 2018 yılları arasında toplanan örnekler üzerinde gerçekleştirilmiştir. Ascomycota ve Basidiomycota bölümleri içinde yer alan 7 sınıf, 13 takım, 42 familya ve 58 cinse ait 74 tür belirlenmiştir. Türlerin listesi habitat ve lokaliteleri ile birlikte verilmiştir.

Anahtar kelimeler: Biyoçeşitlilik, makromantarlar, taksonomi, Türkiye

Introduction

Karaman is one of the subsequently established provinces and officially located in Central Anatolian Region of Turkey. Though some regions of the province take place at the transition zone of Mediterranean and Central Anatolian Regions, majority of the landscape is located within the latter region. Ayrancı and Yeşildere are also the districts, where the research was conducted, that take place at the north of Taurus Mountains and within the Central Anatolian Region (Figure 1). The research area is situated between 37°05′-37°25′ north latitudes and 33°22′-33°51′ east longitudes and takes place in C4 according to Davis' grid square system. According to Emberger's formula (Akman, 1999), the area has a Mediterranean climate. The annual precipitation is 331.7 mm, and the average temperature is 12 °C.

Though steppe vegetation is the dominant vegetation in the region, some naturally growing and planted *Pinus nigra* J.F.Arnold, *Juniperus excelsa* M.Bieb. and some *Quercus* L. populations are localized at higher portions of the region and around Ayrancı and Yeşildere dam lakes. *Salix* L and *Populus* L. trees are dominant along the stream sides together with some *Platanus* L. trees.

Kaşık et al. (2000), Öztürk et al. (2001), Doğan and Öztürk (2006) and İleri et al. (2020) presented some lists and some new records (Doğan et al., 2003; İleri et al., 2019; Çetinkaya et al., 2020; Çetinkaya and Uzun, 2021) were reported about the macrofungi of Karaman. But a research related to macrofungal biodiversity of Ayrancı and Yeşildere districts haven't been conducted.

The work aims to determine the macrofungal composition of the region and make a contribution to the mycobiota of Karaman and Turkey.

Material and method

The macrofungi samples were collected between 2014 and 2018 from the region within the boundaries of

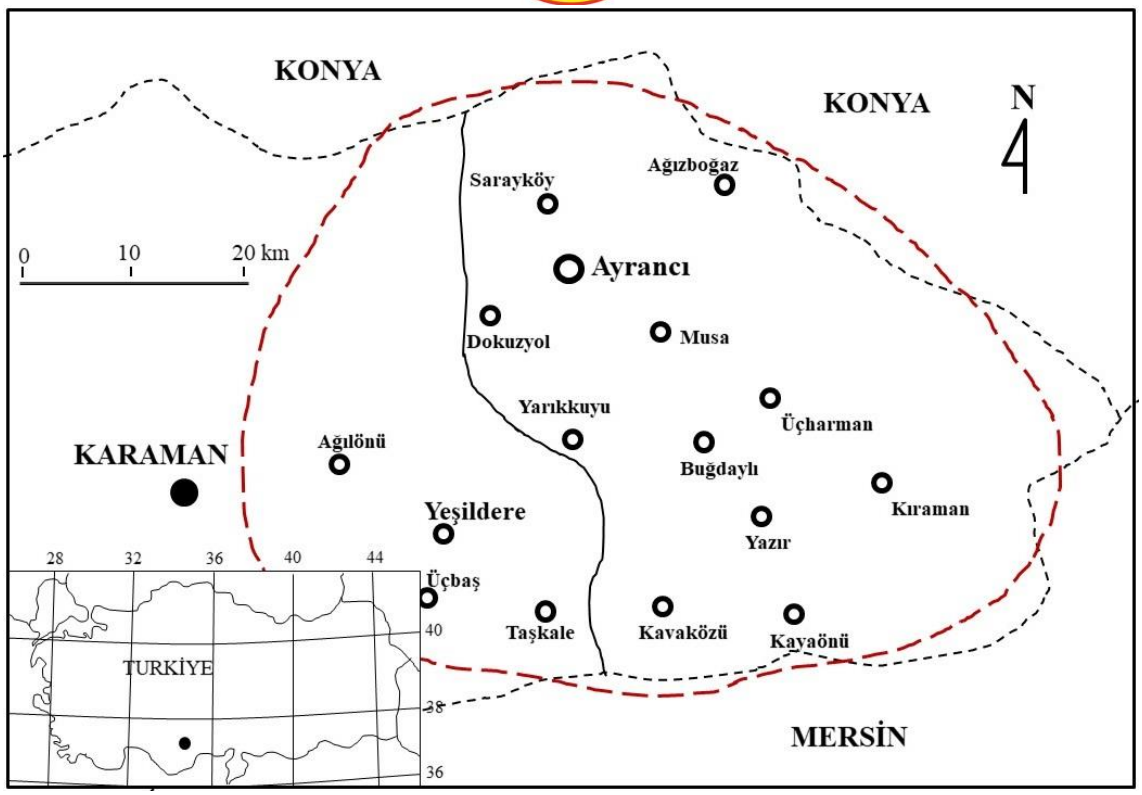

Figure 1. Map of the research area

Ayrancı and Yeşildere districts of Karaman (Figure 1, Table1). The fruit bodies were photographed at their natural habitats and ecologic characters were recorded. Detailed investigations related to their macroscopy and microscopy were carried out in the fungarium. Microscopic investigations were carried out under a Nikon Eclipse Ci-S trinocular microscope. The samples were identified by comparing the obtained data with Watling (1973), Phillips (1981), Moser (1983), Cappelli (1984), Breitenbach and Kränzlin (1984, 1986, 1991, 1995, 2000), Miller and Miller (1988), Ellis and Ellis (1990), Buczacki (1992), Hansen and Knudsen (1992, 1997), Jordan (1995), Pegler et al. (1995), Bessette et al. (1997, 2007), Medardi (2006), Hausknecht (2009), Antonin and Noordeloos (2010), Thompson (2013) and Beug et al. (2014).

The specimens are kept at Karamanoğlu Mehmetbey University, Kamil Özdağ Science Faculty, Department of Biology.

Results

Seventy four macromycete taxa were determined from the research area. The taxa are listed in alphabetical order, considering the taxonomic categories from division to species. Kirk et al., (2008) and Index Fungorum (accessed on 15 September 2020) was followed for the systematics of the taxa.

Ascomycota Caval-Sm

Dothideomycetes O.E. Erikss. & Winka

Patellariales D. Hawksw. & O.E. Erikss.

Patellariaceae Corda

1. ***Patellaria atrata*** (Hedw.) Fr: On dead *Salix* sp. stump, locality 12, 1.06.2014, AÇK. 19; locality 4, 25.10.2014, AÇK. 90; locality 5, 25.10.2014, AÇK. 92; locality 22, 27.09.2014, AÇK. 80.

Leotiomycetes O.E. Erikss. & Winka

Helotiales Nannf. ex Korf & Lizoň

Helotiaceae Rehm

2. ***Hymenoscyphus caudatus*** (P. Karst.) Dennis: (Çetinkaya and Uzun, 2021).

Lachnaceae Raitv.

3. ***Belonidium sulphureum*** (Fuckel) Raitv.: On dead *Phragmites* sp. stems, locality 23, 14.06.2014, AÇK. 58.

Mollisiaceae Rehm

4. ***Mollisia hydrophila*** (P. Karst.) Sacc.: On dead *Phragmites* sp. stems, locality 23, 14.06.2014, AÇK. 62.

Orbiliomycetes O.E. Erikss. & Baral

Orbiliaceae Nannf.

5. ***Orbilia auricolor*** (A. Bloxam) Sacc.: On dead *Salix* sp. stump, locality 21, 17.05.2014, AÇK. 14.

Table 1. Collection localities of the macromycete samples

Loc. No	Locality name	Coordinates	Altitude (m)
1	Ağılönü village	37°13′N-33°22′E	1050
2	Ağızboğaz village	37°25′N-33°51′E	1100
3	Around Ayrancı Dam Lake	37°18´N-33°44´E	1190
4	Around Ayrancı Dam Lake	37°18′N-33°45′E	1200
5	Around Ayrancı Dam Lake	37°20′N-33°43′E	1150
6	Buğdaylı village	37°15′N-33°45′E	1260
7	Dokuzyol village	37°18′N-33°36′E	1130
8	From Musa village to Ağızboğaz village	37°19′N-33°45′E	1220
9	Kavaközü village	37°10′N-33°44′E	1560
10	Kayaönü village	37°07′N-33°49′E	1590
11	Kıraman village	37°16′N-33°50′E	1310
12	Musa village	37°19′N-33°44′E	1185
13	Sarayköy village	37°23′N-33°40′E	1150
14	Taşkale village	37°08´N-33°33´E	1225
15	Taşkale village	37°08′N-33°34′E	1330
16	Üçbaş village	37°07′N-33°28′E	1300
17	Üçharman village	37°17′N-33°48′E	1260
18	Yarıkkuyu village	37°15′N-33°42′E	1310
19	Yazır village	37°12′N-33°43′E	1460
20	Yeşildere village	37°09´N-33°25´E	1150
21	Yeşildere village	37°09′N-33°27′E	1130
22	Yeşildere village	37°09'N-33°28'E	1140
23	Yeşildere village	37°09′N-33°29′E	1160
24	Yeşildere village	37°09′N-33°30′E	1170
25	Yeşildere village	37°09′N-33°31′E	1220
26	Yeşildere village	37°09'N-33°32'E	1200

Pezizomycetes O.E. Erikss. & Winka

Pezizales J. Schröt.

Ascobolaceae Boud. ex Sacc.

6. ***Thecotheus lundqvistii*** Aas.: (Çetinkaya et al., 2020).

Helvellaceae Fr.

7. ***Dissingia leucomelaena*** (Pers.) K. Hansen & X.H. Wang: Among needle litter under *Pinus* sp., locality 23, 25.04.2015, AÇK. 179; Among grass in mixed forest, locality 5, 26.04.2015, AÇK. 207.

8. ***Helvella acetabulum*** (L.) Quél.: On soil among grass in mixed forest, locality 16, 15.05.2015, AÇK. 243.

9. ***Helvella fusca*** Gillet: On soil in mixed forest, locality 4, 26.04.2015, AÇK. 200.

10.***Helvella lacunosa*** Afzel.: On soil in mixed forest, locality 5, 26.04.2015, AÇK. 191.

11.***Helvella solitaria*** P. Karst.: On soil in mixed forest, locality 21, 17.05.2014, AÇK. 02.

Morchellaceae Rchb.

12.***Morchella deliciosa*** Fr.: On soil among needle litter under *Pinus* sp., locality 16, 15.05.2015, AÇK. 244.

Pezizaceae Dumort.

13.***Peziza succosa*** Berk.: On sandy soil at streamside, locality 24, 14.06.2014, AÇK. 043.

14.***Terfezia albida*** Ant. Rodr., Mohedano & Bordallo: In soil among *Helianthemum* sp., locality 8, 26.04.2015, AÇK.203; locality 1, 07.05.2015, AÇK. 210.

15.***Terfezia boudieri*** Chatin: In soil among *Helianthemum* sp., locality 5, 16.05.2015, AÇK. 267; locality 13, 09.04.2018, K. 14398; locality 2, 12.05.2018, K. 14495.

16.***Terfezia claveryi*** Chatin: In soil among *Helianthemum* sp., locality 5, 26.04.2015, AÇK. 197; locality 1, 15.05.2015, AÇK. 238.

Pyronemataceae Corda

17.***Geopora arenicola*** (Lév.) Kers: In sandy soil among grass, locality 5, 26.04.2015, AÇK. 190; locality 24, 10.11.2015, AÇK. 335.

18.***Geopora sumneriana*** (Cooke) M. Torre: In soil among needle litter in mixed forest, locality 5, 09.05.2015, AÇK. 227.

19.***Parascutellinia violacea*** (Velen.) Svrček: On sandy soil at streamside, locality 23, 29.10.2015, AÇK. 289.

20.***Picoa juniperi*** Vittad.: In soil among *Helianthemum* sp., locality 1, 07.05.2015, AÇK. 209; locality 5, 16.05.2015, AÇK. 258.

21.***Picoa lefebvrei*** (Pat.) Maire: In soil among *Helianthemum* sp., locality 8, 26.04.2015, AÇK. 204; locality 1, 07.05.2015, AÇK. 211.

22.***Pyronema domesticum*** (Sowerby) Sacc.: On ash, locality 22, 27.09.2014, AÇK. 070.

23.***Pyronema omphalodes*** (Bull.) Fuckel: On ash, locality 12, 25.10.2014, AÇK. 099.

24.***Trichophaeopsis bicuspis*** (Boud.) Korf & Erb: On decaying *Populus* sp. twigs, locality 22, 29.10.2014, AÇK. 149.

Sordariomycetes O.E. Erikss. & Winka

Diaporthales Nannf.

Valsaceae Tul. Ve C. Tul.

25.***Valsa sordida*** Nitschke: On *Populus* sp. stump, locality 24, 25.04.2015, AÇK. 166; locality 23, 15.05.2015, AÇK. 257.

Hypocreales Lindau

Nectriaceae Tul. & C. Tul.

26.***Nectria peziza*** (Tode) Fr.: On *Populus* sp. stump, locality 24, 14.06.2014, AÇK. 55; 29.10.2014, AÇK. 135; locality 23, 10.11.2015, AÇK. 331; locality 5, 26.04.2015, AÇK. 187; 09.05.2015, AÇK. 231.

Diatrypaceae Nitschke

27.***Diatrype stigma*** (Hoffm.) Fr.: On dead *Salix* sp. branches, locality 25, 20.04.2014, AÇK. 101.

Xylariaceae Tul. & C. Tul.

28.***Kretzschmaria deusta*** (Hoffm.) P.M.D. Martin: On *Populus* sp. stump, locality 23, 27.09.2014, AÇK. 84.

29.***Nemania serpens*** (Pers.) Gray: On *Populus* sp. stump, locality 25, 14.06.2014, AÇK. 40.

Basidiomycota R.T. Moore

Agaricomycetes Doweld

Agaricales Underw.

Agaricaceae Chevall.

30.***Agaricus campestris*** L.: On soil among grass, locality 7, 25.10.2014, AÇK. 97; locality 5, 26.04.2015, AÇK. 206; locality 11, 09.04.2018, K. 14395.

31.***Coprinus comatus*** (O.F. Müll.) Pers.: On soil among grass, locality 5, 30.10.2015, AÇK. 308.

32.***Cyathus olla*** (Batsch) Pers.: On decaying *Populus* sp. stump, locality 25, 25.04.2015, AÇK. 171; locality 5, 31.10.2015, AÇK. 311.

33.***Leucoagaricus leucothites*** (Vittad.) Wasser: On soil among grass in mixed forest, locality 23, 27.09.2014, AÇK. 85.

34.***Macrolepiota excoriata*** (Schaeff.) Wasser: Among grass in poplar grove, locality 26, 29.10.2014, AÇK. 115.

Bolbitiaceae Singer

35.***Conocybe apala*** (Fr.) Arnolds: On manured soil among grass, locality 17, 09.04.2018, K. 14396.

36.***Conocybe deliquescens*** Hauskn. & Krisai: On soil among grass, locality 26, 29.10.2015, AÇK. 277.

Crepidotaceae Singer

37.***Crepidotus mollis*** (Schaeff.) Staude: On decaying *Populus* sp. twigs, locality 21, 17.05.2014, AÇK. 06; locality 23, 27.09.2014, AÇK. 83.

38.***Crepidotus variabilis*** (Pers.) P. Kumm.: On decaying *Populus* sp. twigs, locality 12, 25.10.2014, AÇK. 94.

Cyphellaceae Lotsy

39.***Chondrostereum purpureum*** (Pers.) Pouzar: On decaying *Populus* sp. stump, locality 23, 29.10.2014, AÇK. 138; locality 24, 29.10.2015, AÇK. 287.

Hymenogastraceae Vittad.

40.***Hymenogaster bulliardii*** Vittad.: In soil under mixed wood, locality 12, 01.06.2014, AÇK. 23.

41.***Hymenogaster olivaceus*** Vittad.: In soil under mixed wood, locality 12, 01.06.2014, AÇK. 24.

Inocybaceae Jülich

42.***Inocybe rimosa*** (Bull.) P. Kumm.: On soil among grass, locality 12, 01.06.2014, AÇK. 18; locality 15, 14.06.2014, AÇK.030.

Marasmiaceae Roze ex Kühner

43.***Calyptella capula*** (Holmsk.) Quél.: On dead *Helianthus* sp. stem, locality 23, 29.10.2014, AÇK. 128.

Mycenaceae Overeem

44.***Mycena acicula*** (Schaeff.) P. Kumm.: On soil under *Populus* sp., locality 21, 17.05.2014, AÇK. 13.

Niaceae Jülich

45.***Merismodes anomala*** (Pers.) Singer.: On decaying *Populus* sp. twigs, locality 23, 14.06.2014, AÇK. 54.

Physalacriaceae Corner

46.***Armillaria mellea*** (Vahl) P. Kumm.: On soil under *Populus* sp., locality 22, 29.10.2014, AÇK. 143.

Pleurotaceae Kühner

47.***Pleurotus ostreatus*** (Jacq.) P. Kumm.: On *Populus* sp. stump, locality 22, 29.10.2014, AÇK. 144; locality 5, 26.04.2015, AÇK. 194; 07.11.2015, AÇK. 328; locality 10, 27.10.2018, K. 14723.

Pluteaceae Kotl. & Pouzar

48.***Pluteus romellii*** (Britzelm.) Sacc.: On *Populus* sp. stump, locality 23, 29.10.2014, AÇK. 133; locality 22, 15.05.2015, AÇK. 245.

Psathyrellaceae Vilgalys, Moncalvo & Redhead

49.***Coprinellus disseminatus*** (Pers.) J.E.Lange: On damp soil, locality 21, 17.05.2014, AÇK. 03; locality 26, 14.06.2014, AÇK. 38; locality 22, 27.09.2014, AÇK. 71; locality 23, 27.09.2014, AÇK. 81; locality CC, 07.05.2015, AÇK. 214; locality 5, 16.05.2015, AÇK. 259.

50.***Coprinellus micaceus*** (Bull.) Vilgalys, Hopple & Jacq. Johnson: Around decaying *Populus* sp. stump, locality 12, 01.06.2014 AÇK. 16; locality 14, 14.06.2014, AÇK. 39; locality 22, 27.09.2014, AÇK. 73; locality 23, 25.04.2015, AÇK. 173; locality CC, 07.05.2015, AÇK. 218; locality 1, 15.05.2015, AÇK. 239.

51.***Coprinopsis atramentaria*** (Bull.) Redhead, Vilgalys & Moncalvo: On soil among grasses around *Populus* sp. stump, locality 23, 29.10.2015, AÇK. 290.

52.***Coprinopsis nivea*** (Pers.) Redhead, Vilgalys & Moncalvo: On decaying cow dung, locality 1, 07.05.2015, AÇK. 212; locality 23, 15.05.2015, AÇK. 253; locality 17, 09.04.2018, K. 14397.

53.***Psathyrella candolleana*** (Fr.) Maire: On damp soil among grasses in poplar grove, locality 1, 17.05.2014, AÇK. 05; locality 23, 14.06.2014, AÇK. 48; locality 26, 29.10.2014, AÇK. 116; locality 9, 27.10.2018, K. 14724.

Schizophyllaceae Quél.

54.***Schizophyllum amplum*** (Lév.) Nakasone: On decaying *Populus* sp. twigs, locality 22, 27.09.2014, AÇK. 66; locality 26, 29.10.2014, AÇK. 114; locality 24, 25.04.2015, AÇK. 165; loclity V, 07.05.2015, AÇK. 220; localit R, 29.10.2015, AÇK. 280; locality 12, 09.11.2014, AÇK. 161; locality 5, 09.05.2015, AÇK. 232; 31.10.2015, AÇK. 313.

55.***Schizophyllum commune*** Fr.: On decaying *Populus* sp. stump, locality 26, 29.10.2014, AÇK. 104; locality 19, 27.10.2018, K. 14725.

Strophariaceae Singer & A.H. Sm.

56.***Cyclocybe cylindracea*** (DC.) Vizzini & Angelini: On soil around *Populus* sp. stump, locality 21, 17.05.2014, AÇK. 08; locality 6, 27.10.2018, K. 14726.

57.***Deconica coprophila*** (Bull.) P. Karst.: On decaying cow dung, locality 20, 25.04.2015, AÇK. 183.

58.***Pholiota limonella*** (Peck) Sacc.: On *Salix* sp. trunk, locality 5, 30.10.2015, AÇK. 309; locality 18, 27.10.2018, K. 14727.

Boletales E.-J. Gilbert

Diplocystidiaceae Kreisel

59.***Astraeus hygrometricus*** (Pers.) Morgan: On soil in *Quercus* sp. forest, locality 20, 25.04.2015, AÇK. 182.

Gomphidiaceae Maire ex Jülich

60.***Chroogomphus rutilus*** (Schaeff.) O.K. Mill.: Among needle litter in mixed forest, locality 5, 16.05.2015, AÇK. 266.

Rhizopogonaceae Gäum. & C.W. Dodge

61.***Rhizopogon luteolus*** Fr.: In soil among needle litter, locality 5, 24.05.2014, AÇK. 15.

62.***Rhizopogon roseolus*** (Corda) Th. Fr.: In soil among needle litter, locality 5, 16.05.2015, AÇK. 265; 30.10.2015, AÇK. 304.

Sclerodermataceae Corda

63.***Scleroderma areolatum*** Ehrenb.: On soil under mixed forest, locality 23, 27.09.2014, AÇK. 86.

Suillaceae Besl & Bresinsky

64.***Suillus collinitus*** (Fr.) Kuntze: On soil among grass under *Pinus* sp., locality 5, 30.10.2015, AÇK. 305.

Hymenochaetales Oberw.

Hymenochaetaceae Donk

65.***Phellinus igniarius*** (L.) Quél.: On *Salix* sp. trunk, locality 23, 25.04.2015, AÇK. 178.

Polyporales Gäum.

Fomitopsidaceae Jülich

66.***Fomes fomentarius*** (L.) Fr.: On *Salix* sp. stump, locality 24, 25.04.2015, AÇK. 170; locality 16, 15.05.2015, AÇK. 248.

67.***Laetiporus sulphureus*** (Bull.) Murrill: On *Salix* sp. trunk, locality 15, 14.06.2014, AÇK. 33; locality 22, 27.09.2014, AÇK. 69; locality 26, 29.10.2014, AÇK.110.

Meruliaceae Rea

68.***Bjerkandera adusta*** (Willd.) P. Karst.: On *Salix* sp. stump, locality 12, 25.10.2014, AÇK. 100.

Polyporaceae Fr. ex Corda

69.***Lentinus tigrinus*** (Bull.) Fr.: On *Populus* sp. stump, locality 22, 27.09.2014, AÇK. 76; locality 23, 25.04.2015, AÇK. 174; locality 1, 15.05.2015, AÇK. 242; locality 24, 29.10.2015, AÇK. 286; locality 3, 30.10.2015, AÇK. 298; locality 5, 31.10.2015, AÇK.0314; locality 12, 07.11.2015, AÇK. 319.

70.***Trametes hirsuta*** (Wulfen) Lloyd: On *Populus* sp. stump, locality 5, 16.05.2015, AÇK. 264.

71.***Trametes trogii*** Berk.: On *Populus* sp. stump, locality 12, 01.06.2014, AÇK. 27; locality 22, 29.10.2014, AÇK. 147.

72.***Trametes versicolor*** (L.) Lloyd: On *Populus* sp. stump, locality 22, 27.09.2014, AÇK. 79.

Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David

Stereaceae Pilát

73.***Stereum hirsute*** (Willd.) Pers.: On dead *Quercus* sp. stump, locality 23, 27.09.2014, AÇK. 87; locality 24, 10.11.2015, AÇK. 332; On *Populus* sp. stump, locality 16, 26.04.2015, AÇK. 202.

Tremellales Fr.

Tremellaceae Fr.

74.***Tremella mesenterica*** Retz.: On dacaying *Populus* sp. stump, locality 23, 29.10.2015, AÇK. 288.

Discussions

A list of 74 macromycete taxa were presented from Ayrancı and Yeşildere districts of Karaman. Twenty nine of them (19 *Pezizales,* 3 *Helotiales,* 3 *Xylariales,* 1 *Diaporthales,* 1 *Hypocreales,* 1 *Orbiliales,* 1 *Patellariales*) belong to *Ascomycota* and 45 (29 *Agaricales,* 7 *Polyporales,* 6 *Boletales,* 1 *Hymenochaetales,* 1 *Russulales,* 1 *Tremellales*) to *Basidiomycota.*

Pyronemataceae was found to be the most crowded family in the region. Two of the families (*Agaricaceae, Psathyrellaceae*) are resembled with 5 taxa, 3 of them (*Helvellaceae, Pezizaceae, Polyporaceae*) are resembled with 4 taxa, one (*Strophariaceae*) is resembled with 3 taxa, 7 (*Bolbitiaceae, Crepidotaceae, Fomitopsidaceae, Hymenogastraceae, Rhizopogonaceae, Schizophyllaceae, Xylariaceae*) are resembled with 2 taxa, while the rest of the families are resembled with only one taxon in the region.

Helvella L. was found to be the most crowded genus in the research area with 4 taxa. Two genera (*Terfezia* (Tul. & C. Tul.) Tul. & C. Tul., *Trametes* Fr.) are resembled with 3 taxa and 9 (*Conocybe* Fayod, *Coprinellus* P. Karst., *Coprinopsis* P. Karst., *Crepidotus* (Fr.) Staude, *Geopora* Harkn., *Picoa* Vittad., *Pyronema* Carus, *Rhizopogon* Fr., *Schizophyllum* Fr.) with two taxa while the rest of the 38 genera are resembled with only one taxon in the research area.

Thirty four of the determined taxa are lignicolous, 20 are terricolous, 3 are coprophilous, 3 are herbicolous and 2 are pyrophilous. Twelve of them were also determined to be hypogeous or semihypogeous.

Literatural data indicates that 24 of the determined taxa are edible, 47 are inedible and 3 are more or less poisonous. *Agaricus campestris, Morchella deliciosa, Picoa juniperi, Picoa lefebvrei, Pleurotus ostreatus, Terfezia albida, T. boudieri* and *T. claveryi* are collected and consumed by local public. Two of them have regional commercial value. *Terfezia boudieri* and *T. claveryi* are heavily collected and sold during especially May and June.

The determined taxa were also compared with the studies carried out in close environs and some similarities were observed. These studies and the similarity percentages are given in Table 2. The reason for this similarity may be the common climate and vegetation.

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Table 2. Similarity percentages of neighbouring studies with Ayrancı & Yeşildere and their close environs

	# of Identical taxa	Total taxa	Similarity (%)
Aktaş et al. (2003)	15	74	20.27
Alkan et al. (2010)	19	134	14.17
Çelik et al. (2020)	29	89	32.58
Doğan and Öztürk (2006)	27	202	13.37
Doğan et al. (2007)	21	95	22.11
İleri et al. (2020)	30	84	35.71
Kaşık and Öztürk (2000)	10	47	21.28

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Pleurotus eryngii' nin Misel Biyokütlesinin ve Farklı Olgunlaşma Seviyesindeki Gövdelerinin Ekstraksiyonunun Optimizasyonu ve Antidiyabetik Özelliklerinin Belirlenmesi

Nurcan DOĞAN[1*], Cemhan DOĞAN[1]

**Corresponding author: nurcan.dogan@bozok.edu.tr*

[1]Department of Food Technology, Yozgat Boğazlıyan Vocational High School, Yozgat Bozok University 66400, Yozgat, Turkey
Orcid ID: 0000-0001-5414-1819/nurcan.dogan@bozok.edu.tr
Orcid ID: 0000-0002-9043-0949 /cemhan.dogan@bozok.edu.tr

Öz: Yenilebilir mantarlar, tarih öncesi zamanlardan beri küresel olarak tüketilen bir gıda maddesidir. Mantarların bu nama sahip olmalarında duyusal özellikleri başta olmak üzere, besleyici değeri ve tıbbi özellikleri etkili olmuştur. Ancak ülkemiz bazında bakıldığında yenilebilir mantarlar üretim, tüketim ve bilimsel çalışmalar açısından yeterli düzeye ulaşamamıştır. Bu çalışmada, *Pleurotus eryngii* mantarının yaşam döngüsünden farklı parçalarının ekstraksiyon koşullarının optimizasyonu, Yanıt Yüzey Yöntemi (YYY) esas alınarak araştırılmıştır. Optimize edilmiş ekstraktların toplam fenolik madde miktarı (TPC), antioksidan ve antidiyabetik etkileri incelenmiştir. Ektrasksiyon parametleri olan; sıcaklık, sıvı / katı oranı ve solvent konsantrasyonu, örneklerin TPC ve antioksidan kapasitelerini önemli ölçüde etkilemiştir. Mantarların genç meyve gövdesi miselyum biyokütlesine göre daha yüksek antioksidan ve antidiyabetik aktivite göstermiştir. Ancak meyve gövdesi yaşlandıkça bu etkiler kısmen azalmıştır. Sonuç olarak mantarların hem meyve gövdesi hem de miselyum biyokütlesi birçok biyo-fonksiyonel özelliğe sahiptir. Miselyum biyokütlesinin üretim süresinin kısa oluşu ancak aktivitesinin meyve gövdesine göre azlığı değerlendirilmesi gereken konulardan biri olup, hasat zamanının gecikmesi ile de bu özellikleri önemli ölçüde azalması yadsınamaz bir gerçektir. Bu çalışma ile *P.eryngii* mantarının doğal antioksidan ve anti-diyabetik gıda olma potansiyeli ortaya konulmuştur.

Anahtar kelimeler: *Pleurotus eryngii*, Miselyum biyokütle, Antioksidian, antidiyabetik, YYY

Optimization of Extraction of Mycelial Biomass and Parts at Different Maturation Levels of *Pleurotus eryngii* and, Determination of İts Antidiabetic Properties

Abstract: Edible mushrooms have been consumed as a global food since prehistoric times. Its sensory properties, its nutritional value, and medicinal properties have been affected in having this famous. However, edible mushrooms could not reach a sufficient level in production, consumption, and scientific studies in our country. In this study, optimization of extraction conditions of parts of the life-cycle of *Pleurotus eryngii* mushroom was investigated based on Response Surface Method (RSM). Total phenolic content (TPC), antioxidant and antidiabetic effects of optimized extracts were investigated. Extract parameters; temperature, liquid/solid ratio, and solvent concentration significantly affected the TPC and antioxidant capacities of the samples. The young fruit body of mushrooms showed higher antioxidant and antidiabetic activity than mycelium biomass. However, these effects partially decreased as the fruit body ages. As a result, both the fruit body and mycelium biomass have many bio-functional properties. The short production time of mycelium biomass, but the low bioactivity than the fruit body, is one of the issues that needs to be evaluated. This research has shown that *P.eryngii* mushrooms have potential as natural antioxidants and anti-diabetic food.

Key words: *Pleurotus eryngii*, Mycelium biomass, Antioxidant, anti-diabetic, RSM

Giriş

Makromantarlar, insanoğlu tarafından yüzyıllar boyunca çeşitli amaçlarla kullanılagelmiştir. Yenilebilir mantarlar, uygun ekolojik şartlarda, ormanlarda, organik madde yönünden zengin topraklarda, çürümekte olan dal ve kökler üzerinde saprofit olarak kendiliğinden yetişmektedir.

Tarih öncesi zamanlardan beri doğadan toplanan mantarlar kutsal görülmüş, Romalılar tarafından ‘‘Tanrıların yiyeceği’’ olarak anılmış, Yunanlılar tarafından da savaşlarda savaşçılara güç verdiğine inanılmıştır (Arora, 2008). Firavunlar mantarları lezzetli gıdalar olarak addetmişlerdir (Daba ve ark., 2008). Yüzyıllardır yenilebilir mantarlar, halk ilacı olarak, uzun ömürlülüğü arttırmak hatta halüsinojen (magic mushroom) olarak tüketilmiştir (Manzi ve ark., 1999).

Ancak, doğadan toplanan mantarlar hala önemli bir besin ve geçim kaynağı olmakla birlikte, miktarının az olması, mevsime bağımlı olması ve zehirli olanlarının kolay ayırt edilememesi gibi sebeplerden ötürü kültüre alınması çalışmaları başlamıştır (Arora ve Shepard, 2008).

Tarihsel kayıtlarda ilk kültüre alınıp yetiştirilen mantar MS 600 yıllarda yetiştirilen *Auricularia auricula* (wood ear, judae, jelly ear, kulak mantarı) olup, onu sırasıyla MS 800-900 yıllarında kültüre alınan *Flammulina velutipes* (Enokitake, Enoki, kış mantarı) ve MS 1000-1100 yıllarında *Lentinula edodes* (shiitake, meşe mantarı) takip etmiştir. Dünyada en fazla üretim ve tüketimi olan *Agaricus bisporus* (beyaz şapkalı mantar)’un yetiştirilmesi ise MS 1600’lü yıllara, *Pleurotus* cinsi mantarların üretimi ise 1900’lü yıllara dayanır.

Tarihsel süreçte her ne kadar ilk kültüre alınan mantarlar farklı olsa da günümüzde ticari üretim kapasitesine bakıldığında; *Agaricus bisporus* (beyaz şapkalı mantar) dünyada üretimi yapılan mantar türleri arasında birinci sırada, *Pleurotus* cinsi mantarlar ise ikinci sırada yer almaktadır (Öztürk ve Çopur, 2009). Dünya üzerinde binden fazla *Pleurotus* türü tanımlanmıştır. Ancak bununla birlikte *Pleurotus* cinsinde sadece 50 kadar tür kabul edilmektedir (Miles ve Chang, 2004). *Pleurotus* cinsi mantarlar arasında da lezzeti ve gastronomik üstünlüklerinden dolayı *P. eryngii* (King oyster mushroom, kral mantarı, çaşır, çakşır)’ye olan talebin gün geçtikçe arttığı bilinmektedir (Ohga ve Royse, 2004).

Mantarların sahip olduğu besin bileşikleri, miktarı, cins, tür, alttür, hasat zamanı, depolama ve kompost üretiminde kullanılan substratlara göre değişebilmekle birlikte (Sturion ve Oetterer, 1995) yüksek protein ve vitamin içeriğinin yanı sıra; diyet lifi, mineraller ve β-glukan içeriği bakımından zengin, düşük yağ oranına sahip olup, kalorisi son derece düşük olan değerli bir gıdadır (Jablonsky ve ark., 2005; Sanmee ve ark., 2003; Vetter, 2003).

Mantarlar yüksek besin değerinin yanı sıra tıbbi özellikleri ile de son derece dikkat çekicidir. Mantarlar çeşitli ülkelerde farklı hastalıklarda tedavi amaçlı olarak kullanılmakta olup, genel olarak, antiviral, antibakteriyel, antikolesterol, antikarsinojenik, antihipertansif ve antioksidan özellikleri ile dikkat çekmektedir (Cohen ve ark., 2002; Daba ve Ezeronye, 2003; Gunde-Cimerman, 1999; Sarangi ve ark., 2006; Singh ve Mishra, 2008; Kris-Etherton ve ark., 2002). Ayrıca antidiyabetik aktivitesi ile Tip 2 diyabetin tedavisinde ince barsaklarda bulunan ve disakkaritlerle kompleks karbonhidratları absorbe olabilir forma dönüştüren özellikle α-glukozidaz ve α-amilaz enzimlerini inhibe ederek monosakkaritlertin oluşumunu ve emilimini kısıtlaması Su ve ark. (2013) gibi tıbbi özellikleri de son dönemin çalışma konuları arasındadır.

Hastalıkların birçoğunun çevresel faktörlerle ve özellikle tüketilen gıdalarla ilişkilendirilmesi, tüketicilerin satın aldıkları gıdaları sorgulamaya başlamalarına sebep olmuştur. Bu nedenle, gıdaların insan sağlığı üzerine etkileri her geçen gün daha da önem kazanmaktadır. Bilinçli tüketiciler artık, gıdalardan beslenmenin yanı sıra ilave faydalar getirmesini beklemektedirler. Böylelikle günümüz insanı bir nevi “öze dönüş” içerisindedir. Tıbbın babası olarak nitelenen Hipokrat (MÖ 400) “Gıdalarınızın ilaç, ilaçlarınızın da gıda olmasını sağlayın” (Let food be thy medicine and medicine be thy food) derken gıdaların sağlık için önemini vurgularken özünde gıda-ilaç ayrımını net olarak yapamamaktadır (Totelin, 2015).

Sahip oldukları besin ve tıbbi özelliklerinden dolayı mantarlar hem direkt olarak tüketilerek hem de bünyelerinde bulunan etken maddelerin ekstrakte edilmesiyle, birçok hastalığın tedavisinde veya önlenmesinde destekleyici gıda ajanı olarak kullanılması, ayrıca yeterli ve dengeli beslenmede de etkin olarak kullanıldığı ve gelecekte de artan oranda kullanılacağı düşünülmektedir. Dolayısıyla doğadan toplanan yenilebilir mantarlar ile bu miktarlara ulaşılamayacağından kültür mantarı üretimi önem kazanacaktır.

Ülkemizde kültür mantar üretimi, yeni bir alan olmakla birlikte hızlı bir değişim ve gelişme içerisindedir. 1983 yılında yıllık mantar üretimi 1400 ton civarındayken, 2018 yılında 65000 tona ulaşmıştır (Erkan ve Pekşen, 2019). Günümüzde, dünyanın birçok ülkesinde kültür mantarı yetiştiriciliği bir endüstri halindeyken, ülkemizde yetiştiriciliği istenen düzeyde yapılamamaktadır. Bu sebeple mantar çeşitliliğini arttırmak ve pazarı çeşitlendirmek için, ülkemizde farklı cins ve türde mantar üretimlerine de ivme kazandırılması gerekmektedir.

Bu çalışmada, ülkemizde üretim ve tüketim potansiyeli yüksek olan egzotik mantarlardan, *Pleurotus eryngii* mantarının yaşam döngüsünden farklı parçalarının ekstraksiyon koşullarının optimizasyonu, YYY esas alınarak araştırılmıştır. Optimize edilmiş ekstraktların toplam fenolik madde miktarı, antioksidan ve antidiyabetik etkileri incelenmiştir.

Materyal ve metot

Materyal

Sıvı kültür üretiminde kullanılan; açık renkli malt özütü, kavak talaşı, maya, misel taşıyıcısı olarak kullanılan; yulaf, K_2PO_4, $MgSO_4$ ve kompost yapımında kullanılmış olan kepek, kavak talaşı Yozgat ilindeki lokal tedarikçilerden temin edilmiştir. Polietilen microsac ambalajlar Type 14A, Unicorn Bags, TX, USA'den, diğer tüm kimyasallar aksi belirtilmedikçe Merck KGaA'dan temin edilmiştir.

Mantarlar

Çalışma kapsamında kullanılmış *Pleurotus eryngii* stok kültürden elde edilen hiflerin uygun substrat karışımına inoküle edilip uygun şartlarda inkübasyona tabi tutulması ile elde edilmiştir. Farklı işlem ve gelişim evrelerine göre 3 farklı kısım incelenmiştir. Birincisi Miselyum biyokütle (MB) ikincisi genç meyve (GM) ve üçüncüsü de genç meyvenin 5 gün sonra toplanmasıyla elde edilen olgun meyve (OM) olarak adlandırılmıştır. Genç meyve hasat zamanı, sap ve şapka dahil olmak üzere fruit body (meyve gövdesi) 9 cm olarak belirlenmiştir.

Metot

Sıvı kültür üretimi

Sıvı kültür üretimi Stamets (2011) metotu modifiye edilerek uygulanmıştır. Öncelikle 1. aşamada 100 ml distile su 121 °C de 2 saat sterilize edilmiş ve 25 °C'ye soğutulmuştur. Ardından 9 mm cork borer ile 2 parça kesit alınarak waring blender içine konulmuş, 2 sefer olarak 3 sn çalıştırılır ve 5 s beklenilerek homojenize edilmiştir. 2. aşamada da 90 ml broth için; 20 g/L açık renkli malt özütü, 5 g/L maya, 1 g/L K_2PO_4 and 0.5 g/L $MgSO_4$ karışımı 250 ml'lik erlenmayerde hazırlanarak, 121 ^oC'de 2 saat sterilize edilmiştir. Homojenizasyonun ardından 10 ml miselyum bakımından zenginleştirilmiş sıvı sterilize edilmiş broth içine aktarılmıştır. Hazırlanan karışım Lee ve ark. (2009) metodu modifiye edilerek, mantar hiflerinin oksijen ihtiyacını karşılamak için çalkalamalı su banyosunda 25 ℃ de 14 gün boyunca inkübe edilmiştir. 10-14 gün içerisinde transparan görüntüsü kaybolup bulanık bir görüntü oluştuğunda süreç tamamlanmıştır.

Miselyum biyokütle üretimi

Elde edilen sıvı kültür 4000 rpm de 5 dak santrifüj (Nüve, Turkey) edilmiştir. Santrifüj sonunda oluşan çökelti MB olarak tanımlanmıştır.

Tohumluk misel üretimi

%1 $CaSO_4$ ve %0,5 $CaCO_3$ ile harmanlanmış yulaf, 0,5 µ delik çapına sahip filtreli microsac torbalarda (Type 14A, Unicorn Bags, TX, USA) 121 °C' de 2 saat sterilize edilmiştir.İnkübe edilen sıvı kültürden steril şırınga ile 10 ml alınmış ve %55 nem içeriğine sahip steril yulaf tanesi üzerine inoküle edilmiştir. İnokülasyonu tamamlanan microsac torbalarda görsel olarak tam bir kolonizasyon oluşana kadar (10-15 gün) 25 ^oC 'de inkübe edilmiştir (Stamets, 2011).

Mantarların üretimi

Üretilen tohumluk misel %65 nem içeriğine sahip steril besi ortamına %5 oranında inoküle edilmiştir. Besi ortamında bazal substrat olarak kullanılan ince kıyılmış kavak talaşına, katkı mateyali olarak ¼ oranında buğday kepeği ilave edilmiştir. Ayrıca ortamın asitliğini ayarlamak için komposta, %1 $CaCO_3$ ilave edilmiştir. Hazırlanan kompost 0,5 µ delik çapına sahip filtreli polietilen microsac ambalajlara 1 kg olarak doldurularak (Type 14A, Unicorn Bags, TX, USA), 121 °C de 4 saat sterilize edilmiştir. Yaklaşık 3 haftalık 25 °C ve %85 nem ve karanlık ortamda inkübasyona bırakılmıştır. Sürecin akabinde torbaya çapraz şekilli 3 cm uzunluğunda kesikler atılarak sıcaklık 17 °C ye düşürülmüş, nem %95 çıkarılmış, ortamdaki CO_2 miktarının 800 ppm altında olması sağlanmış ve günde 12 saat ışık verilmiştir. Mantarların çalışmaya uygun toplama zamanları dikkate alınarak flaşlar (hasat) yapılmıştır.

Örneklerin kurutulması

Toplanan tüm örnekler flaş zamanlarını müteakip 0,5 cm kalınlığında kesilmiş ve etüvde 40 °C de 12 saat boyunca kurutulmuştur.

Örneklerin ekstraksiyonu

Kurutulmuş örnekler daha sonra bir bıçaklı değirmen (Bosch MKM6000, Almanya) kullanılarak toz haline getirilmiştir. Ön denemelerde en iyi solvent olarak metanol ve su seçildiği için çalışmada solvent olarak metanol ve su kullanılmıştır. Toz örnekler (1 g), vidalı kapaklı tüplerde çeşitli konsantrasyonlarda hazırlanan sulu metanol karışımına ilave edilmiştir. Ekstraksiyon, deney tasarımında belirtilen sıcaklık, solvent/ katı oranı ve % metanol konsantrasyonunda 60 dakika süreyle çalkalamalı bir su banyosunda (Wisd, Kore) ekstrakte edilmiştir.

Deneme dizaynı ve istatistiksel analiz

Maksimize edilmiş Toplam fenolik madde miktarı (TPC) ve antioksidan kapasitesine göre optimum ekstraksiyon koşullarını belirlemek amacıyla (sıcaklık, solvent/ katı oranı ve % metanol konsantrasyonu) Yanıt Yüzey Yönteminin (YYY) Box-Behnken Tasarımı (BBD) kullanılmıştır. Örneklerin TPC ve antioksidan (DPPH) kapasitesi yanıt olarak seçilmiş, bu yanıtlar üzerine sıcaklık, solvent/ katı oranı ve metanol konsantrasyonu gibi ekstraksiyon koşulları ise bağımsız değişkenler olarak belirlenmiştir (Tablo 1).

Tablo 1. Bağımsız değişkenlerin seviyeleri

Bağımsız Değişkenler	Birim	Sembol	Kodlanmış değerler		
			-1	0	1
Sıcaklık	°C	X_1	30	45	60
Sıvı/katı oranı	mL g^{-1}	X_2	10	20	30
Metanol konsantrasyonu	%	X_3	30	55	80

Tablo 2 de verilen deneme dizaynında da gösterildiği üzere 15 deneme noktasında çalışılmıştır.

Tablo 2. Box Behnken deneme dizaynına göre deneysel ve tahminlenen veriler

Den. nok.	Kodlanmış değişkenler			Miselyum Biyokütle (MB)				Genç Meyve (GM)				Olgun Meyve (OM)			
	X_1 (°C)	X_2 (%)	X_3 (mL g^{-1})	TPC		DPPH		TPC		DPPH		TPC		DPPH	
				Den.	Tah.	Den.	Tah.	Den.	Tah.	Den.	Tah.	Den.	Tah.	Den.	Tah.
1	-1	-1	0	2.33	2.36	18.97	18.86	3.69	3.77	24.04	24.52	2.99	2.96	21.31	21.62
2	1	-1	0	2.67	2.71	17.66	17.76	3.99	4.13	25.52	25.64	3.50	3.55	25.56	25.25
3	-1	1	0	2.02	1.98	17.05	16.95	3.00	2.86	20.36	20.24	2.87	2.82	20.78	20.07
4	1	1	0	2.14	2.11	16.49	16.60	3.54	3.46	24.23	23.75	3.12	3.15	22.99	23.70
5	-1	0	-1	1.21	1.19	17.05	16.99	3.10	3.15	22.87	22.79	2.87	2.93	20.14	21.38
6	1	0	-1	1.59	1.56	16.64	16.37	3.32	3.31	23.14	23.41	3.55	3.53	25.41	25.01
7	-1	0	1	2.10	2.14	18.13	18.40	3.22	3.24	22.90	22.63	3.05	3.07	22.23	21.38
8	1	0	1	2.23	2.25	17.52	17.58	4.08	4.04	26.55	26.64	3.47	3.41	25.00	25.01
9	0	-1	-1	1.91	1.91	18.12	18.29	3.87	3.75	25.77	25.38	3.33	3.30	24.10	24.06
10	0	1	-1	1.35	1.41	17.36	17.52	3.07	3.16	22.14	22.34	3.14	3.13	23.34	22.51
11	0	-1	1	2.78	2.72	20.51	20.35	4.45	4.36	27.16	26.96	3.40	3.41	24.01	24.06
12	0	1	1	2.24	2.24	18.23	18.06	3.25	3.37	23.44	23.83	3.00	3.03	21.68	22.51
13	0	0	0	2.89	2.91	21.60	21.33	4.85	4.99	27.55	27.87	4.10	3.89	26.98	26.59
14	0	0	0	2.91	2.91	21.40	21.33	5.12	4.99	27.99	27.87	3.75	3.89	26.22	26.59
15	0	0	0	2.94	2.91	21.00	21.33	5.00	4.99	28.07	27.87	3.81	3.89	26.57	26.59

Den. nok.; Deneme noktası, Den. Deneysel değer,. Tah. Tahmini değer

Yanıtlar ve bağımsız değişkenler arasındaki ilişki, ikinci dereceden polinom regresyon eşitliği kullanılarak ifade edilmiştir.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

Y: öngörülen yanıt, β0: kesişme, β_1, β_2, β_3: doğrusal katsayılar, β_{11}, β_{22}, β_{33}: etkileşim katsayıları, β_{12}, β_{13}, β_{23}: ikinci dereceden katsayılar, X_1, X_2, X_3: bağımsız değişkenler RSM, Design Expert 11.0.0 yazılımı (Stat-Ease Inc., Minneapolis, MN) kullanılarak gerçekleştirildi. Bağımsız değişkenlerin yanıt üzerindeki etkisini gösteren 3D yüzey grafikleri, Mathematica yazılımında (sürüm 7; Wolfram Research, Champaign, IL) Design Expert 11.0.0 yazılımının çıktılarının işlenmesiyle oluşturulmuştur. İki bağımsız değişken ve bir yanıttan oluşan 3 boyutlu yüzey grafiklerinde diğer bağımsız değişkenin değeri orta noktasında sabitlenmiştir. Veriler arasındaki farkların önemini belirlemek için SPSS 22.0 istatistik paket programı (SPSS Inc., Chicago, IL) kullanılmış olup, duncan çoklu karşılaştırma testi ile grup ortalamaları karşılaştırılmıştır.

Toplam fenolik madde miktarı (TPC)

Ekstraktların TPC'si Singleton ve ark. (1999)'nın yöntemine göre belirlenmiştir. 0.4 ml seyreltilmiş özüt, 2 ml 10 kat seyreltilmiş Folin & Ciocalteu fenol reaktifi ve 1.6 mL % 7.5 Na_2CO_3 bir test tüpünde karıştırılmıştır. Karanlık bir yerde 60 dakika inkübasyondan sonra, karışımın absorbansı 765 nm'de okunmuştur (Shimadzu UV-1700 spektrofotometre, Shimadzu Corp, Kyoto, Japonya). TPC değeri, standart bir gallik asit eğrisi (20-100 mg /L) ile kalibre edilerek hesaplanmış ve gallik asit eşdeğeri (GAE) olarak ifade edilmiştir.

Antioksidan kapasite

Antioksidan kapasite tayini 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing ability of plasma (FRAP) yöntemi ile belirlenmiştir. Analizin sonuçları Trolox eşdeğeri (µM TE/g örnek) olarak ifade edilmiştir. DPPH analizi için 0,1 mL numune 3.9 mL 25 mg / L konsantrasyonda metanolik DPPH solüsyonu içeren test tüpüne yerleştirdikten sonra karanlık bir yerde oda sıcaklığında 30 dakika inkübasyondan sonra, 515 nm de okunmuştur (Brand-Williams ve ark., 1995). ABTS analizinde, 30 mg ABTS ve

7,8 mL 2,46 mM potasyum peroxodisülfattan oluşan ABTS çözeltisi 16 saat inkübe edilmiştir. Bu çözelti daha sonra fosfat tamponlu salin (PBS) ile seyreltilerek 0.70 ± 0.05'lik bir absorbansa ayarlanmıştır. Bundan sonra 1950 µL seyreltilmiş ABTS solüsyonu ve 50 µL örnek 30 saniye vortekslenip 6 dakika inkübe edildikten sonra 734 nm'de okunmuştur (Çam ve ark., 2009). FRAP analizi Benzie ve Strain (1996)'ya göre yapıldı. 10 µL örnek ve 20 mM ferric chloride, 30 mM sodium acetate ve 10 mM TPTZ'den oluşan FRAP reaktifi bir test tüpünde karıştırıldı. 30 dakika inkübasyondan sonra, absorbans 593 nm'de okundu.

Antidiyabetik aktivite

Örneklerin antidiyabetik aktiviteleri, α-amilaz ve α-glukozidaz inhibisyon aktiviteleri kullanılarak belirlenmiştir. Ekstraktlar uygun konsantrasyon aralığına seyreltilmiştir. Hazırlanan bu ekstraktların α-glukozidaz ve α-amilaz enziminin çalışmasını inhibe edici özellikleri enzimatik-spektrofotometrik olarak belirlenmiştir. Bu amaçla denemeler fizyolojik ortamları temsil etmesi açısından 37 °C de gerçekleştirilmiştir. α-glukozidaz inhibisyon aktivitesini belirlemek için, 50 µL örnek, 1250 µL 67 mM monopotasyum fosfat ve 50 µL α-glukozidaz bir test tüpünde karıştırılıp 37 °C'de 5 dakika inkübe edilmiştir. Enzimatik reaksiyonu başlatmak için tüpe 125 µL 10 mM 4-nitrophenyl-D-glucopyranoside solüsyonu eklenmiştir. 20 dakika sonra, reaksiyonu durdurmak için tüpe 2 mL 0.1 M sodyum karbonat ilave edildikten sonra 400 nm'de okunmuştur. α -amilaz inhibisyon aktivitesini belirlemek için, 1 mL örnek, 1 mL patates nişastası solüsyonu ve 1 mL 20 mM monosodyum fosfat bir test tüpünde karıştırılarak 37 °C'de 5 dakika inkübe edilmiştir. Enzimatik reaksiyonu başlatmak için tüpe 1 mL α -amilaz solüsyonu ilave edildikten 30 dakika sonra, 0.5 mL 5.31M sodyum potasyum tartarat (2 M sodyum hidroksit ile hazırlanmış) ve 0.5 mL 96 mM 3,5-dinitrosalisilik asit solüsyonu tüpe eklenmiştir. Reaksiyonu durdurmak için karışım 5 dakika kaynar suda tutulduktan sonra absorbans 540 nm'de okunmuştur (Cam ve ark., 2020; McDougall ve ark., 2005). Yukarıda açıklanan her iki yöntem için antidiyabetik aktivite, aşağıdaki denklem kullanılarak hesaplanılmıştır.

$$Antidiyabetik\ aktivite(\%) = \frac{ABS_{kontrol} - ABS_{örnek}}{ABS_{örnek}} x100$$

$ABS_{kontrol}$ kontrolün absorbasını ve $ABS_{örnek}$ ise numunenin absorbansını ifade eder. α -amilaz ve α-glukozidazın % 50'sini inhibe eden konsantrasyon IC_{50} olarak ifade edilmiştir.

Enzimin çalışmasını %50 oranında inhibe eden konsantrasyon (IC_{50}) ise örnek miktarına karşılık inhibisyon yüzdesinin bulunduğu grafikten elde edilmiştir. Pozitif kontrol olarak akarboz standartı kullanılmıştır.

Bulgular ve Tartışma

Solvent seçimi için ön çalışmalar

Solvent seçimi ön çalışmalar neticesinde belirlenmiştir. Genç meyve etanol, methanol, aseton ve su (hidrosol) ile ekstrakte edilmiş ve Antioksidan (DPPH) ve TPC miktarı belirlenmiştir. Bu amaçla 1 gram kuru örnek, % 80 konsantrasyona sahip 20 mL çözücü ile karıştırılarak 45 °C'de 60 dakika inkübe edilmiştir. Süpernatant, çökeltiden ayrıldıktan sonra analiz edilmiştir ve sonuçlar TPC 4.79±0.48 mg GAE/g ve antioksidan kapasitesi 26.32±1.76 µmol TE/g ile diğer çözücü ekstraktlarına kıyasla en yüksek metanolik ekstrata ait olduğunu göstermiştir. Çözücü ve bileşenin polaritesine ek olarak, bileşenlerin suda çözünürlüğü, biyoaktif bileşenlerin ektraksiyonunda kritik bir rol oynar (Barwick, 1997). Etanol, metanol, aseton ve damıtılmış suyun çözücü polarite indeksleri sırasıyla 5.2, 6.6, 5.4 ve 9'dur (Snyder, 1974). Mantar örnekleri için damıtılmış suyun yüksek polarite indeksine rağmen düşük ekstraksiyon performansının nedeni, bu mantarlardaki bazı biyoaktif bileşenlerinin suda çözünmemesiyle açıklanabilir. Öte yandan metanol, en iyi iyonizasyon potansiyeline sahip alkoldür. Bu durum mantar anyonlarının, kolaylaştırılmış elektron transferi yoluyla diğer çözücülere göre metanolde daha fazla çözünmesine neden olmuş olabilir (Litwinienko ve Ingold, 2003).

Model Doğrulama

Modelden üretilen 2. dereceden polinom denklemleri ve regresyon (p-değeri), belirleme katsayısı (R^2), ayarlanmış R^2 (R^2_{adj}), tahmini R^2 (R^2_{pred}) ve uyumsuzluk gibi istatistiksel parametreler Tablo 3. de verilmiştir. Tüm yanıtların varyans analizinin (ANOVA) p değerinin 0,05'ten küçük olduğu görülmüştür, bu da seçilen modelin anlamlı olduğunu göstermektedir. 15 deney noktasında verilen yanıtların deneysel ve öngörülen değerlerinin birbirine yakın olması güvenilir bir modelin göstergesidir (Tablo 4). Genel olarak, modelin uygunluğunu değerlendirmek için, uyum değeri eksikliğine ‘‘Lack of fit’’ (model uyumsuzluğu) hipotezi ile F testi uygulanır. Örneklerin TPC ve DPPH için belirlenen uyum eksikliği önemsiz bulunmuştur. Yanıtların R^2 değerleri, ikinci dereceden modelin yeni değerleri (tahmini değer) tahmin etme yeteneği için mantarın 3 farklı kısmı (MB, GM, OM) için sırasıyla 0.922-0.990 değişmiştir (Table 3). Dahası, R^2pred ile R^2_{adj} arasındaki fark 0.2'den küçüktür. Adj-R^2 ile pre-R^2 değerleri arasındaki farkın 0.2 değerinden küçük olması seçilen modelin uygunluğunu göstermektedir (Myers ve ark., 1995). Tüm istatistiksel

değerlendirmeler, yazılımdan elde edilen 2. dereceden kuadratik modellerin, yanıtlar üzerindeki etkisini belirlemek ve süreç optimizasyonunda kullanılabileceğini göstermektedir. Modelin belirlenmesinde, önemsiz olan faktörler ($p>0.05$) 2. derece polinomiyal denklemden çıkarılarak modifiye edilmiştir. Önemsiz faktörlerin elemine edilmesi, genel tahminin doğruluğunu artırmak ve daha az önemlileri denklemden çıkartarak faktörleri yorumlarken daha efektif değerlendirilmesi ile açıklanabilir (Friedman ve ark., 2001).

Tablo 3. Polinomiyal denklemler ve model uyumluluğunu belirlemek için istatistiksel parametreler

Örnek	Yanıtlar	2.derece polinomiyal denklemler	Regression (p-value)	R^2	R^2_{adj}	R^2_{pred}	Model uyumsuzluğu
MB	TPC	$2.91+0.121X_1-0.243X_2+0.411X_3-0.455X_1^2-0.168X_2^2-0.675X_3^2$	<0.0001	0.990	0.982	0.959	0.083
	DPPH	$21.33-0.361X_1-0.766X_2+0.653X_3-2.21X_1^2-1.29X_2^2-1.49X_3^2$	<0.0001	0.972	0.951	0.896	0.393
GM	TPC	$4.99+0.240X_1-0.393X_2+0.205X_3-0.833X_1^2-0.603X_2^2-0.728X_3^2$	<0.0001	0.960	0.930	0.850	0.328
	DPPH	$27.87+1.16X_1-1.54X_2+0.766X_3+0.845X_1X_3-2.55X_1^2-1.79X_2^2-1.46X_3^2$	0.0001	0.967	0.933	0.844	0.143
OM	TPC	$3.89+0.233X_1-0.136X_2+0.004X_3-0.375X_1^2-0.392X_2^2-0.277X_3^2$	0.0003	0.930	0.877	0.783	0.892
	DPPH	$26.59+1.81X_1-0.774X_2-2.01X_1^2-1.92X_2^2-1.39X_3^2$	<0.0001	0.922	0.878	0.775	0.179

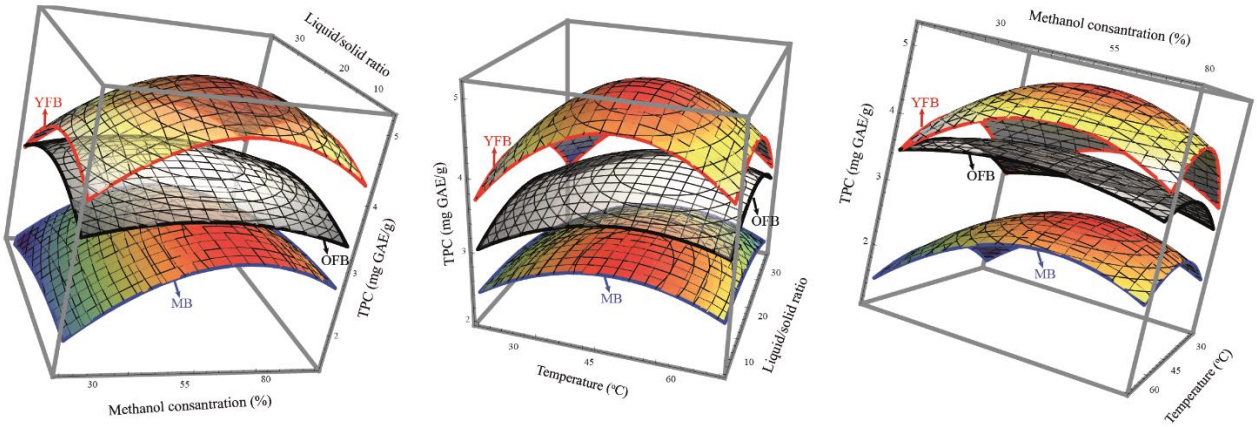
Ektraksiyon şartlarının TPC üzerine etkisi

MB, GM ve OM kısımlarında, ekstraksiyon işlem koşullarının TPC değeri üzerindeki etkisi Şekil 1.'de verilmiştir.

Bağımsız değişkenlerden sıcaklık ve sıvı/katı oranı önemli ölçüde etkilerken ($p<0.05$), metanol konsantrasyonu bazı kısımlarında önemsiz çıkmıştır ($p>0.05$). Genel olarak, sıvı/katı oranı 10'dan 20'ye çıktıkça TPC, kütle transferi prensibine uygun olarak önemli ölçüde artmıştır. Ancak daha sonra azalmıştır. Bu, sıvı/katı oranı belli bir seviyedeyken maksimum miktarda fenolik ekstrakte edilerek ve bu seviyeden sonra artan sıvı/katı oranının fenolik konsantrasyonunun azaltılmasıyla açıklanabilir.

Metanol konsatrasyonunun değişimi, OM kısmı hariç diğer kısımlarında hem TPC hem de antioksidan kapasitesi üzerine etkili olmuştur. Fenolikler gibi biyoaktif bileşenlerin biyokütleden çözücüye transferi büyük ölçüde çözücü ile çözünen madde arasındaki polarite farkına bağlıdır. Bu nedenle, alkol ve su kombinasyonundan oluşan çözücüler, yalnızca bir tür alkol kullanan çözücülerden daha verimli özütleme performansı sağlayabilir (Markom ve ark., 2007).

Ekstraksiyon işlemi sırasında fenoliklerin geri kazanılmasına sıcaklığın etkisi ile ilgili iki farklı durumdan bahsedilebilir. Birincisi, sıcaklığın dokuları yumuşatması, fenoliklerin çözünürlüğünü artırması ve buna bağlı olarak fenolik madde miktarının difüzyon kabiliyetini belirli bir seviyeye çıkarmasıdır. Ayrıca yüksek sıcaklık, solvent viskozitesini ve yüzey gerilimini düşürerek fenolik transferin verimini artırır. İkincisi, aşırı sıcaklığın kimyasal ve termal bozunma sonucu ekstrakte edilen fenolik miktarının azalmasına neden olmasıdır (Dent ve ark., 2013). Bu nedenle sıcaklık, ekstraksiyon prosesleri için en önemli parametrelerden biridir. Burada sadece sıcaklık göz önüne alındığında MB için 44.95 °C , GM için 47.71 °C ve OM için ise 50.31 °C TPC için kırılma noktası olarak göze çarpmaktadır.



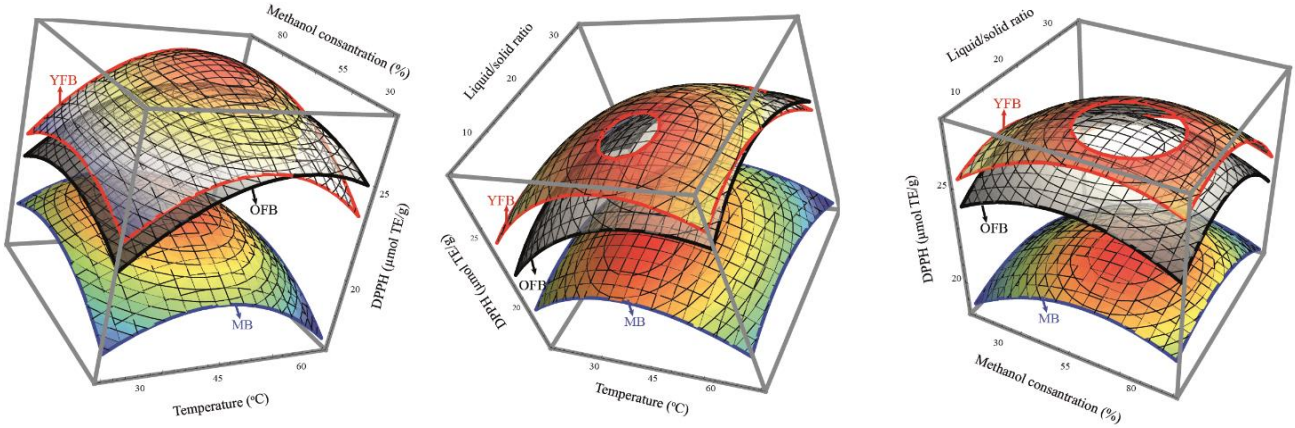
Şekil 1. MB, GM ve OM kısımlarında ekstraksiyon koşullarının TPC üzerindeki etkisi üzerine üst üste bindirilmiş 3-D yüzey grafikleri

Ektrasksiyon şartlarının DPPH üzerine etkisi

İkinci dereceden denklemlerin DPPH üzerindeki etkisi istatistiksel olarak önemli olarak tespit edilmiştir ($p<0.05$). Sıcaklık ve sıvı/katı oranı orta düzeyde sabit tutulduğunda, metanol konsantrasyonun antioksidan kapasitesi üzerine etkisi TPC'de olduğu gibi quadratic eğri etkisi göstermiştir (Şekil 2). Bu tür bir etki, bazı fenolik bileşenlerin antioksidan etkiye sahip olması nedeniyle TPC ile antioksidan kapasite arasında pozitif bir korelasyon olduğunun da bir işaretidir. Çalışmamıza uygun olarak Liyana-Pathirana (2005), su-alkol kompozit (%50) çözücülerin antioksidan bileşenlerin ekstraksiyonunda en yüksek performansı gösterdiğini belirtmişlerdir.

Sıcaklık, antioksidan kapasiteyi etkileyen bir başka önemli faktördür ve MB için 44.95 °C , GM için 47.71 °C ve OM için ise 50.31 °C antioksidan kapasitesi için tıpkı TPC de olduğu gibi kırılma noktası olarak göze çarpmaktadır. Genel olarak, mantar türlerinin MB ve GM kısımlarının maksimum antioksidan ve TPC miktarlarına daha düşük sıcaklıklarda ulaşılırken, OM kısımlarında daha yüksek sıcaklık derecelerinde ulaşılmıştır. Mantarlar, kitin, glukan ve glikoproteinlerin çapraz bağlanmasıyla oluşan hücre duvarlarına sahiptir (Bowman ve Free, 2006).

OM'nin maksimum antioksidan kapasitesi ve TPC miktarına diğer kısımlardan daha yüksek sıcaklıklarda ulaşılmasının nedeni, OM'nin kalınlaşmış hücre duvarının TPC ve antioksidan bileşikleri serbest bırakmak için daha yüksek sıcaklığa ihtiyaç duyması ve ayrıca hücre duvarının aşırı sıcaklık nedeniyle bozulan bileşiklere bir miktar koruma sağlaması olabilir. Sıvı/katı oranının antioksidan kapasitesi üzerinde TPC'ye benzer şekilde ~20 mL g^{-1} pik ile eğri etkisi belirlenmiştir. Bu durum, sıvı/katı oranı 20 mL g^{-1}'ye çıktıkça çözücü ile temas edebilen antioksidan bileşenlerin artmasıyla açıklanabilir. Bununla birlikte, bu çözücü miktarı daha da arttıkça, çözeltideki bileşenler seyreleceğinden, ölçülen antioksidan kapasitesi azalmaktadır.



Şekil 2. MB, GM ve OM kısımlarında ekstraksiyon koşullarının DPPH üzerindeki etkisi üzerine üst üste bindirilmiş 3-D yüzey grafikleri

Optimizasyon ve optimum noktadaki deneysel verilerin doğrulanması

Mantar örneklerinin farklı parçalarının için en iyi ekstraksiyon koşullarını belirlemek için sayısal optimizasyon ayrı ayrı gerçekleştirilmiştir. İstenen yanıtlar için kriterler TPC ve DPPH için maksimum olarak belirlenmiş olup, en yüksek istenirlik değerine sahip faktör kombinasyonları optimum nokta olarak seçilmiştir. Arzu edilirlik yaklaşımı, bir dizi yanıta "puan" atayan ve bu puanı maksimize eden faktör ayarlarını seçen bir yöntemdir (Natrella, 2010). Optimal bağımsız değişken seviyeleri ve numuneler için arzu edilirlik değerleri Tablo 4'de gösterilmiştir.

Modelin doğruluğunu ölçmek için, modelin öngördüğü tahmin değerler ile deneysel değerler karşılaştırılmıştır. Deneysel veriler ile tahmin edilen veriler arasında %5'ten daha az fark bulunduğundan modelin uyumlu olduğu söylenebilir (Tablo 4). Dolayısıyla ikinci derece modelin öngördüğü değerlerin güvenilir olduğu sonucuna varılmıştır.

Tablo 4. Ekstraksiyon koşulları ve optimum noktadaki deneysel ve tahmin edilen değerler

Örnekler	Optimum şartlar				Yanıtlar	Tahmini değerler	Deneysel değerler	Fark (%)
	Sıcaklık (°C)	Sıvı/katı oranı(mL g^{-1})	Metanol konsantrasyonu (%)	Arzu edilirlik				
MB	43.7	16.6	61.6	0.99	TPC	3.02	3.10±1.03	2.6
					DPPH	21.57	21.74±0.11	0.78
GM	47.2	16.7	59.5	0.99	TPC	5.09	5.15±0.25	1.16
					DPPH	28.39	28.10±1.09	1.02
OM	49.9	18.0	54.7	0.93	TPC	3.94	3.88±0.32	1.52
					DPPH	27.05	27.18±0.55	0.48

Optimum ektraksiyon koşullarındaki TPC, antioksidan ve antidiyabetik aktivite

Optimum noktalarda alınan ekstraktların TPC, antioksidan ve antidiyabetik etkileri Tablo 5. de verilmiştir. Antioksidan ve antidiyabetik etkileri arasındaki hiyerarşisi şu şekildedir: GM> OM> MB. Oksijen radikalleri, hücre içinde DNA, RNA ve protein hasarına neden olabilen kanserle ilgili moleküllerdir. Antioksidanlar, bu radikalleri engelledikleri için kansere karşı koruyucu olabilirler (Lambert ve Elias, 2010). Bu çalışmada antioksidan kapasiteyi değerlendirmek için DPPH, FRAP ve ABTS yöntemleri kullanılmıştır çünkü antioksidanların radikalleri inhibe etme mekanizmaları birbirinden farklıdır.

GM'nin DPPH, FRAP ve ABTS değerleri sırasıyla MB'den % 29.25, % 44.28 ve % 40.10 daha yüksek bulunmuştur. Ancak meyve gövdesi yaşlandığında (OM) antioksidan etkisini sırasıyla % 3.38, % 24.69 ve % 22.27 oranında kaybetmiştir. Muhtemelen yaşlanma süreci oksidatif reaksiyonları artırarak antioksidan bileşenlerin parçalanmasını hızlandırdığı düşünülmektedir. Sonuçlar, FRAP değerinin 1,76 ila 4,92 µmol TE / g arasında değiştiği önceki çalışmadan daha yüksektir (Atila ve ark., 2018).

Tablo 5. Optimum ekstraksiyon koşullarında TPC, antioksidan ve antidiyabetik aktiviteleri

Örnek	Antioksidan Kapasitesi				Antidiayabetik aktivite		
	TPC (mg GAE g^{-1})	DPPH (µmol TE g^{-1})	FRAP (µmol TE g^{-1})	ABTS (µmol TE g^{-1})	α-glucosidase IC_{50} (µg mL^{-1})	α-amylase IC_{50} (µg mL^{-1})	UD
MB	3.10±1.03^{c}	21.74±0.11^{b}	31.01±0.10^{c}	40.99±1.05^{c}	227.42±0.58^{a}	285.50±1.66^{a}	3x161.34 g
GM	5.15±0.25^{a}	28.10±1.09^{a}	44.74±1.55^{a}	57.43±0.33^{a}	164.51±0.70^{c}	210.72±0.13^{c}	3x116.71 g
OM	3.88±0.32^{b}	27.18±0.55^{a}	35.88±0.46^{b}	46.97±1.47^{b}	203.11±1.24^{b}	232.51±0.57^{b}	3x144.09 g
Akarboz	-	-	-	-	35.24±1.73^{d}	72.96±3.18^{d}	3x25 mg

UD: Uygulama Dozu; günlük olarak diyabetik bir hastaya verilen ve α-glukozidaz inhibisyonu temelinde hesaplanan 3 x 25 mg tabletlerle akarboza eşdeğer mg *P. eryngii* tozunu ifade eder. Aynı sütunda farklı harfler örneklerin istatistiksel olarak farklı olduğunu göstermektedir ($p < 0.05$)

Tip 2 diyabet için birincil tedavi yaklaşımlarından biri, karbonhidratları parçalayan a-glukozidaz ve a-amilaz gibi enzimlerin inhibisyonuna dayanır. Bu çalışmada, *P.* eryngii'nin farklı parçalarının optimize edilmiş özütlerinin bu enzimleri engellemedeki etkinliği test edilmiştir. β-Glukan, polisakkaritler, D-treitol, D-arabinitol, palmitik asit ve α-D-glukan, terpenoidler ve streoridler vb. bileşikler mantarlarda antidiyabetik etkiler gösteren önemli bileşenlerdir (De Silva ve ark., 2012). GM'nin antidiyabetik etkisi MB'den ve OM'den daha düşük olduğu

tespit edilmiştir. Muhtemelen antidiyabetik etkinin MB da daha düşük çıkması meyveye dönüştükçe antidiyabetik etki gösteren bileşiklerin artmasından kaynaklanmaktadır. OM de antidiyabetik etkinin düşük çıkmasının sebebinin ise mantarın sahip olduğu antidiyabetik bileşikler meyvenin yaşlanması ile bozunduğundan kaynaklandığı düşünülmektedir. Mantar çeşitlerinin antidiyabetik etkisi araştırıldığı önceki çalışmalarda, genel olarak, GM kısmı dikkate alındığında daha düşük α-amilaz inhibisyonu gösterirken, daha yüksek alfa α-glukozidaz inhibisyonu etkisi göstermiştir (Stojkovic ve ark., 2019). Diyabetli kişiler, antidiyabetik bir ilaç olan akarbozu günlük 3x25 mg'lık bir başlangıç dozunda kullanması önerilmektedir (Lebovitz, 1995). Çalışmamızda, α-glukozidazın akarboz tarafından inhibisyonuna göre yapılan hesaplamayla tüm mantar çeşitleri arasında GM kısmında en etkili olan mantar tozunun 3x116.71 g/gün dozunda kullanılması gerekmektedir (Tablo 5).

Literatür çalışmalarına bakıldığında mantarların antioksidan ve antidiyabetik özelliklerinin ortaya konulduğu birçok çalışma mevcuttur (Suleria ve ark., 2019). Ancak *P.eryngii*'nin yaşamsal döngülerindeki farklı parçaların ektraksiyonunun optimizasyonu ve antidiyabetik potansiyelinin belirlendiği bir çalışmaya rastlanılmamıştır. Söz konusu çalışmanın literature katkı sağlayacağı düşünülmektedir.

Sonuç olarak; son yıllarda hastalıkların çoğalması ve bu hastalıkların yediklerimiz ile ilişkilendirilmesi, İnsanların sağlık konusunda gıdalarına daha fazla önem vermelerini sağlamıştır. Bilinçli tüketiciler hastalıktan önce, sağlık önlemlerinin alınmasında kimyasal içerikli takviyeler yerine, doğal ürünlere rağbet göstermektedir. Yenilebilir mantarlar besinsel ihtiyaçları karşılaması yanında sağlık üzerinede de ilave faydalar sağlaması ile son derece popüler konumda olup, içeriğindeki farklı bileşenlerin tespiti ve farklı yenilebilir mantarların literatüre kazandırılması ile de popülaritesi gün geçtikçe artmaktadır. Ülkemiz kültür mantarı üretim ve tüketim konusunda geride kalmış görünse de istatistiksel veriler bu durumun pozitif yönde iyileştiğini göstermektedir. YYY, bu çalışmada antioksidan kapasitesini ve fenolik içeriği en üst düzeye çıkaran ekstraksiyon koşullarının optimizasyonunda başarıyla uygulanmıştır. Sonuçlar, optimize edilmiş özütlerin yüksek düzeyde TPC, antioksidan ve antidiyabetik etkilerinin sahip olduğunu göstermiştir. Çalışmadan da anlaşıldığı üzere hasat zamanının biyoaktif bileşenler üzerinde etkili olduğu önem kazanmıştır. Ancak biyoaktivitesi miktarca az olmasına rağmen MB'nin kısa üretim periyodu ve nispeten kolay üretilebilir oluşunun değerlendirilmesi gerektiği düşünülmektedir.

Teşekkür

Bu çalışmaya 6602b/BMYO/17-125 numaralı Bilimsel Araştırma Projeleri ile destek sağlayan Yozgat Bozok Üniversitesi Proje Koordinasyon Uygulama ve Araştırma Merkezi'ne teşekkürlerimi sunarım.

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First Report of *Octospora neerlandica* from Asian Continent

Osman BERBER[1], Yasin UZUN[2]*
Abdullah KAYA[3]

**Sorumlu yazar: yuclathrus@gmail.com*

[1]Karaman Provincial Directorate of Agriculture and Forestry, 70100, Karaman, Turkey
Orcid ID: 0000-0002-0265-4441 / ober70@hotmail.com
[2]Karamanoğlu Mehmetbey University, Ermenek Uysal & Hasan Kalan Health Services Vocational School, 70400, Karaman, Turkey
Orcid ID:0000-0002-6423-6085 / yuclathrus@gmail.com
[3]Gazi University, Science Faculty, Department of Biology, 06560 Ankara, Turkey
Orcid ID: 0000-0002-4654-1406 / kayaabd@hotmail.com

Abstract: The bryophillic ascomycete species, *Octospora neerlandica* Benkert & Brouwer, is reported as a new record from Turkey, based on the identification of the samples collected from Niğde province. A brief description and photographs, related to the macroscopy and microscopy of the species, are provided.

Key words: Biodiversity, bryophillic ascomycete, new record, *Pyronemataceae,* Turkey

Octospora neerlandica'nın Asya Kıtasından İlk Kaydı

Öz: Briyofilik askomiset türü olan, *Octospora neerlandica* Benkert & Brouwer, Niğde'den toplanan örneklerin teşhis edilmesiyle, Türkiye'den yeni kayıt olarak rapor edilmiştir. Türün kısa bir betimlemesi ve makroskobi ve mikroskobisine ilişkin fotoğrafları verilmiştir.

Anahtar kelimeler: Biyoçeşitlilik, briyofilik askomiset, yeni kayıt, *Pyronemataceae,* Türkiye

Introduction

Octospora Hedw is an ascomycete genus within the family Pyronemataceae Corda. Moss associated apothecia, typical marginal hyphae, and ellipsoid to globose or rounded, sometimes ornamented, guttulate spores generally characterize the genus (Yao and Spooner, 1996). Kirk et al. (2008) reports the presence of 84 species of the genus, eighty one of which also exist in Europe (Benkert, 2007).

Octospora itzerottii Benkert and *O. leucoloma* Hedw. were the first two *Octospora* species reported in Turkey (Çolak and Kaygusuz, 2017; Uzun et al., 2017). In the following two years, eleven members of the genus, *O. areolata* (Seaver) Caillet & Moyne, *O. axillaris* (Nees) M.M. Moser, *O. coccinea* (P. Crouan & H. Crouan) Brumm., *O. excipulata* (Clem.) Benkert, *O. gemmicola* Benkert, *O. grimmiae* Dennis & Itzerott, *O. lilacina* (Seaver) Svrček & Kubička, *O. musci-muralis* Graddon, *O. orthotrichi* (Cooke & Ellis) K.B. Khare & V.P. Tewari, *O. polytrichi* (Schumach.) Caillet & Moyne and *O. rustica* (Velen.) J.Moravec were also presented as new records for the mycobiota of Turkey by Uzun et al. (2018) and Uzun and Kaya (2019). But the current checklists (Sesli and Denchev, 2014; Solak et al., 2015) on Turkish macromycota and the later contributions (Alkan et al., 2018; Doğan et al., 2018; Işık and Türkekul, 2018; Acar et al., 2019; Allı et al., 2019; Berber et al., 2019; Keleş, 2019; Şelem et al., 2019; Türkekul and Işık, 2019; Akçay, 2020; Çağli and Öztürk, 2020; Çelik et al., 2020; İleri et al., 2020; Sadullahoğlu and Uzun, 2020; Sesli, 2020; Uzun et al., 2020), indicate that, *O. neerlandica* hasn't been reported from Turkey before.

The study aims to make a contribution to the mycobiota of Turkey.

Material and method

The fruit body of *O. neerlandica* was collected from Ulukışla district of Niğde province, in 2018, during a routine field trip performed to determine the macrofungal biodiversity of the district. The sample was photographed at its natural habitat, and ecological characteristics and geographic position were noted. Then it was put in a

paper box and transferred to the fungarium where it was dried and prepared as fungarium material. Microscopic investigations were carried out on dry samples. A Nikon Eclipse Ci-S trinocular microscope was used for microscopic investigation and a DS-Fi2 digital camera was used to get microstructural photographs. The sample was identified with the help of Benkert and Brouwer (2004). The specimen is kept at Karamanoğlu Mehmetbey University, Kâmil Özdağ Science Faculty, Department of Biology.

Results

Ascomycota Caval-Sm

Pezizomycetes O.E.Erikss. & Winka

Pezizales J.Schröt.

Pyronemataceae Corda

Octospora neerlandica Benkert & Brouwer, Persoonia 18(3): 381 (2004)

Macroscopic and microscopic features: Apothecium 1.8 mm, margin fimbriate, membranaceous, hymenial surface pinkish-orange to pale orange (Figure 1a), outside portion somewhat paler. Asci 210-290 × 13-18 µm, cylindrical, 8-spored, spores uniseriate Paraphyses slender, generally straight, some slightly curved, enlarged up to 3-7 µm, at the apex (Figure 1b). Ascopores 16-18 × 11-12.8 µm, ellipsoid, one oil drop generally accompained by smaller droplets, spore ornamentation constists of an irregular reticulum with variably formed meshes (Figure 1c).

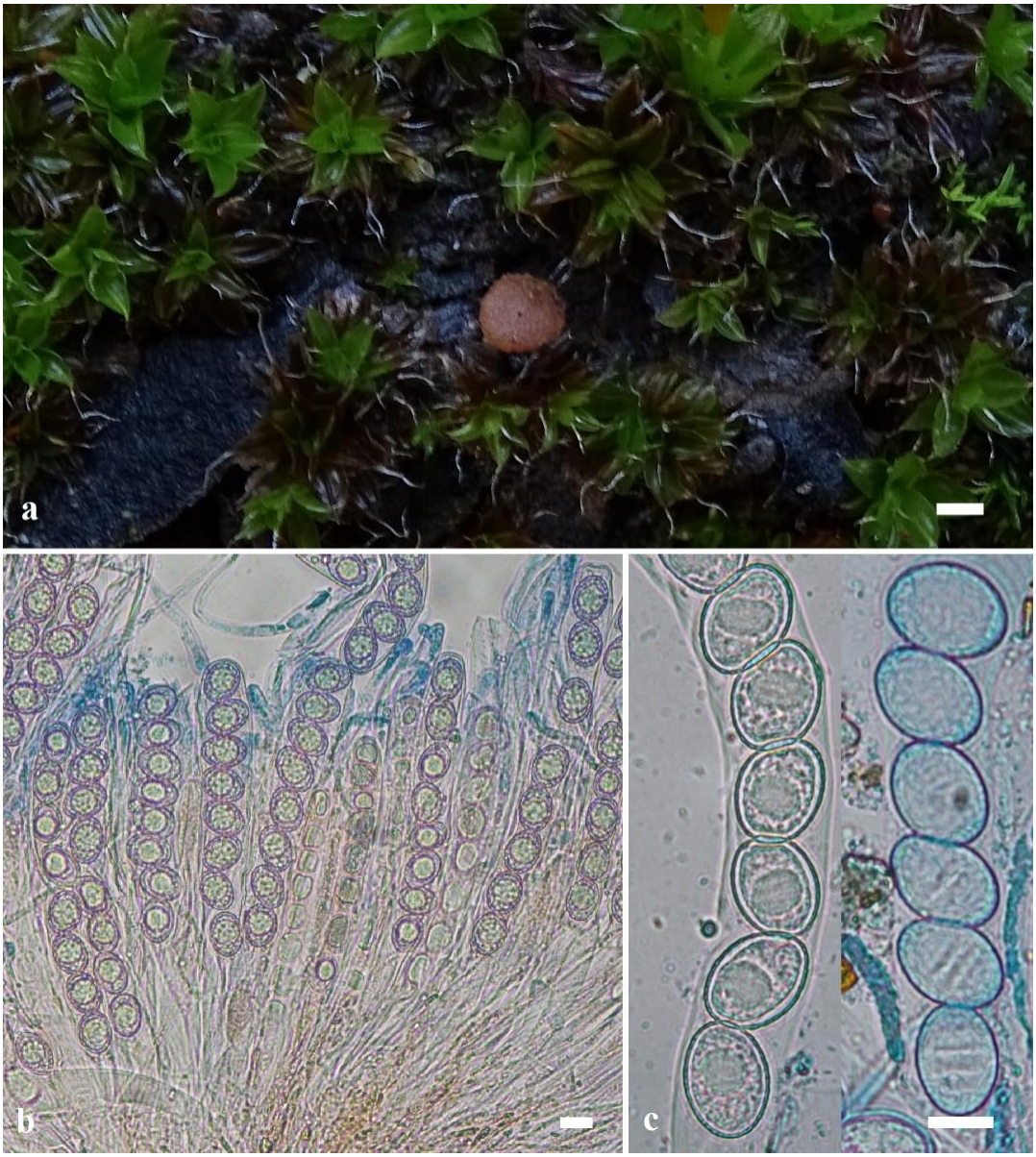

Figure 1. Ascocarp (a), asci and paraphyses (b) and ascospores (c) of *Octospora neerlandica* (bars: a- 1 mm, b,c- 10 µm)

Octospora neerlandica was reported to grow on sandy soil among or directly on the the stems of the mosses *Tortula ruralis* (Hedw.) Gaertn., Meyer, & Scherb., *T. ruraliformis* (Besch.) Ingham or *T. virescens* (De Not.) De Not. (Benkert and Brouwer, 2004).

Specimen examined: Turkey, Niğde, Ulukışla, Çiftehan village, on soil among mosses under *Pinus* sp., 37°30′N, 34°47′E, 930 m, 07.01.2018, O.Ber-108.

Discussion

Octospora neerlandica is reported for the first time for the mycobiota of Turkey. It is the 14th member of the genus *Octospora* in Turkey (Çolak and Kaygusuz, 2017; Uzun et al., 2017, 2018; Uzun and Kaya, 2019). Macroscopic, microscopic and habitat characteristics of Turkish collection are in agreement with Benkert and Brouwer (2004).

Due to their morphological similarities and very small size, *Octospora* species can easily be overlooked and it is very hard to separate their species from each other morphologically (Itzerott, 1977). But the spore characteristics and substrate preferation of *O. neerlandica* makes it a very distinctive species. The spore ornamentation of this species constists of an irregular reticulum with variably formed meshes. Though *Lamprospora seaveri* Benkert has a remarkable similar spore ornamentation, it has globose spores and is associated with other moss species. Likewise very little is known about the infection of the memsers of the moss genus *Tortula* Hedw. by *Octospora* species. Although *O. crosslandii* (Dennis & Itzerott) Benkert, has been observed in association with *Tortula* species together with many other moss species, the globose spores of this species easily differentiates it from *O. neerlandica* (Benkert and Brouwer, 2004).

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Research Article
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A New Record for Turkish Mycobiota from Selim (Kars) District

İsmail ACAR[1]*, Yusuf UZUN[2], Ayşenur KALMER[3],
Ayten Tekpınar DİZKIRICI[3], Yaşar ÖĞÜN[4]

**Corresponding author: iacar2011@gmail.com*

[1*]Department of Organic Agriculture, Başkale Vocational High School, Van Yuzuncu Yil University, Van, Turkey
Orcid ID: 0000-0002-6049-4896/ iacar2011@gmail.com
[2]Van Yüzüncü Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Sciences, 65080, Van, Turkey
Orcid ID: 0000-0002-0537-4517/ yusufuzun2004@yahoo.com
[3]Department of Molecular Biology and Genetics, Van Yuzuncu Yil University, Van, Turkey
Orcid ID: 0000-0001-6176-8812/ aysenurkalmer@gmail.com
[3]Department of Molecular Biology and Genetics, Van Yuzuncu Yil University, Van, Turkey
Orcid ID: 0000-0002-0578-5092/ aytendizkirici@gmail.com
[4]Milli Piyango Anadolu High School, Van, Turkey
Orcid ID: 0000-0002- 3462- 8088/ begumbugra@windowslive.com

Abstract: *Hebeloma cylindrosporum* (*Hymenogastraceae*, *Basidiomycota*) from Selim (Kars) district is described as a new record species for Turkish mycota. The species is assigned to the genus *Hebeloma*, section *Scabrispora*. A comprehensive description, photographs, and comparisons with related species based on morphological and phylogenetical features are provided. The phylogenetic position within the genus is provided based on the DNA sequence of nuclear ribosomal internal transcribed spacer (nrITS) region. Phylogenetic analyses show that the species is located within a well-supported section *Scabrispora*.

Key words: *Basidiomycota*, *Hebeloma*, fungal phylogeny, new record.

Selim (Kars) yöresinden Türkiye Mikobiotası için Yeni Bir Kayıt

Öz: *Hebeloma cylindrosporum* (*Hymenogastraceae*, *Basidiomycota*), Türkiye'nin Selim (Kars) ilçesinden Türk mikotası için yeni kayıt tür olarak tanımlanmıştır. Tanımlanan tür, *Hebeloma* cinsine ait *Scabrispora* seksiyonunda yer almaktadır. Detaylı deskripsiyon, fotoğraflar ve morfolojik ve filogenetik karakterlere dayalı olarak yapilan cins icindeki filogenetik pozisyonu verilmiştir. Cins içindeki filogenetik ilişkilerinin belirlenmesi transkribe edici iç aralayıcı (ITS) bölgenin dizisine dayanılarak sağlanmıştır. Yapılan filogenetik analizler türün *Scabrispora* seksiyonunda yer aldığını göstermiştir.

Anahtar Kelimeler: *Basidiomycota*, *Hebeloma*, fungal filogeni, yeni kayıt

Introduction

Hebeloma (Fr.) P. Kumm. *(Hymenogastraceae, Agaricales)* is a genus of ectomycorrhizal fungi that contains 31 species in Turkey (Sesli and et al., 2020) Beker and his colleagues (2016) divided the genus into thirteen sections (*Denudata*, *Hebeloma*, *Sinapizantia*, *Sacchariolentia*, *Velutipes*, *Theobrominum*, *Naviculospora*, *Scabrispora*, *Myxocybe*, *Pseudoamarescens*, *Duracinus*, *Porphyrospora*, *Syrjense*). Among these sections, the section *Scabrispora* is characterized by rooting basidiomes, cylindrical spores, and mostly cylindrical cheilocystidia.

In 2007, some samples of *Hebeloma cylindrosporum* Romagn. were collected from Kars province of Turkey. However, they have not been characterized until 2020. Fortunately, the collected specimens have been preserved in suitable Fungarium conditions. As a result, they were well-preserved to perform scientific analyses on them. For identification of the samples, not only microscopic and macroscopic characters but also molecular data were used. As macroscopic characters;

(pileus, lamellae, and stipe) as microscopic characters; (basidia, spores, pileipellis, hyphae, and cheilocystidia) were utilized. DNA sequences of nuclear ribosomal internal transcribed spacers (nr ITS) region including ITS1, 5.8S, ITS2 sub-regions were also used as molecular characters to determine the phylogenetic relationships and positions of *H. cylindrosporum* within *Hebeloma* genus.

The purpose of the present study is to describe *Hebeloma cylindrosporum* as a new record in Turkey using both morphological and molecular data.

Material and Metod

Taxon sampling and morphological studies

Fungal samples were collected from Selim (Kars) region of Turkey in 2007. During the fieldwork, specimens were photographed using a Canon (EOS 60D) camera equipped with Tokina 100 mm macro lens. Macroscopic characters were recorded from the fresh materials. At least 40 spores, 20 basidia, and cheilocystidia were measured under a Leica DM500 research microscope by using distilled water and Melzer's reagent solution. Measurements were made with Leica Application Suite (version 3.4.0) program and described based on the terminology of Beker et al. (2016). Dried samples were deposited in the Fungarium of Van Yüzüncü Yıl University (VANF).

Molecular studies

Genomic DNA was extracted from the dried basidiomata using the CTAB method (Doyle and Doyle 1987). The purity and quantity of the extracted DNA were determined using NanoDrop2000c UV–Vis Spectrophotometer (Thermo Scientific) and 0.8% agarose gel electrophoresis. DNA amplification was performed in a 25 µl volume mixture containing genomic DNA (10 ng/µl), 10× PCR Buffer, MgCl2 (25 mM), dNTP mixture (10 mM), selected primer pair (10 µM), Taq polymerase (5u/µl) and sterile water. Amplification of ITS region was performed using primer pair N- nc18S10 5'AGGAGAAGTCGTAACAAG3' / C26A 5'GTTTCTTTTCCTCCGCT3' (Wen and Zimmer 1996). After amplification, PCR products were run in a 1 % agarose gel and visualized by staining with Gelred dye. Positive reactions were sequenced with forward and reverse PCR primers using ABI 3730XL automated sequencer (Applied Biosystems, Foster City, CA, USA).

The sequences that were taken from forward and reverse primer were assembled and edited using Alibee Multiple Alignment 3.0 software from the GeneBee website (www.genebee.msu.su/genebee.html). Ambiguous sites were checked manually and corrected. Sequence data of ITS region were deposited in GenBank and accession numbers were added to the manuscript.

Sequence alignment and phylogenetic analysis

Two sequences of *Hebeloma cylindrosporum* generated from the current study and additional sequences retrieved from NCBI software (Appendix 1) were combined and analyzed together to see phylogenetic relationship and position of the studied species within *Hebeloma* genus. *Galerina paludosa* (Fr.) Kühner was chosen as outgroup. All sequences were aligned with ClustalW program (Thompson et al., 1994) and adjusted manually where it was necessary.

Phylogenetic tree was constructed using two different methods; Maximum Likelihood (ML) and Maximum Parsimony (MP). The appropriate model of nucleotide evolution for phylogenetic analyses was determined using MEGA 6.0 and the model with the lowest BIC (Bayesian Information Criterion) score was used to describe the substitution model the best (Tamura *et al*. 2013). Tamura-Nei model (Tamura and Nei, 1993) was used for two analyses. To test branch support, bootstrap analysis was used with 1000 replicates (Felsenstein, 1985). In the ML method, initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then the topology with superior log likelihood value was selected. The Tree-Bisection-Reconnection (TBR) search method was employed with 100 random addition replications to construct the MP trees and the consensus tree inferred from 10 most parsimonious trees was used. All positions containing gaps and missing data were eliminated.

Results

Taxonomy

Basidiomycota R.T. Moore
Agaricomycetes Doweld
Agaricales Underw.
Hymenogastraceae Vittad.
Hebeloma (Fr.) P. Kumm. (TURPKOKAN)
Hebeloma cylindrosporum Romagn. **Figure 1.**

Pileus 10-55 mm; convex to planoconvex, the margin curved inward, sometimes crenulate, eroded, and wavy with age; color at center yellowish-brown to ochraceous to dark brick and color at margin cream to buff or yellowish; when young with a cortina. **Lamellae** adnexed to emarginate; the presence of tears absent or visible; cream color when young, then brown. **Stipe**: 25-80 × 3-8 mm and 4-11 mm at the base; cylindrical, usually clavate

towards the base; floccose fibrillose, pruinose, sometimes with rooting. **Spores**: 7-10.6 × 4-5.5 µm, cylindrical, occasionally ellipsoid; yellow-brown; usually guttulate; dextrinoid, with or without perispore. **Basidia**: 19-28 × 5.5-7.5 µm; cylindrical to clavate; four spored, rarely two spored. **Cheilocystidia**: 18-38 × 3-6.35(apex) × 2.5-5.6(median) × 3-6.8(basal) µm, cylindrical, occasionally clavate-lageniform. **Pileipellis:** ixocutis maximum hyphae width 5.5 µm, cylindrical, ellipsoid, and sausage-shaped (Figure 1).

Specimens examined

Turkey, Kars, Selim, N 40°27'15.76" and E 42°33'13.94", 2137 m, 10.06.2007, gregarious, under *Pinus* and *Picea* sp., Y. Uzun (Selim) VANF7881.

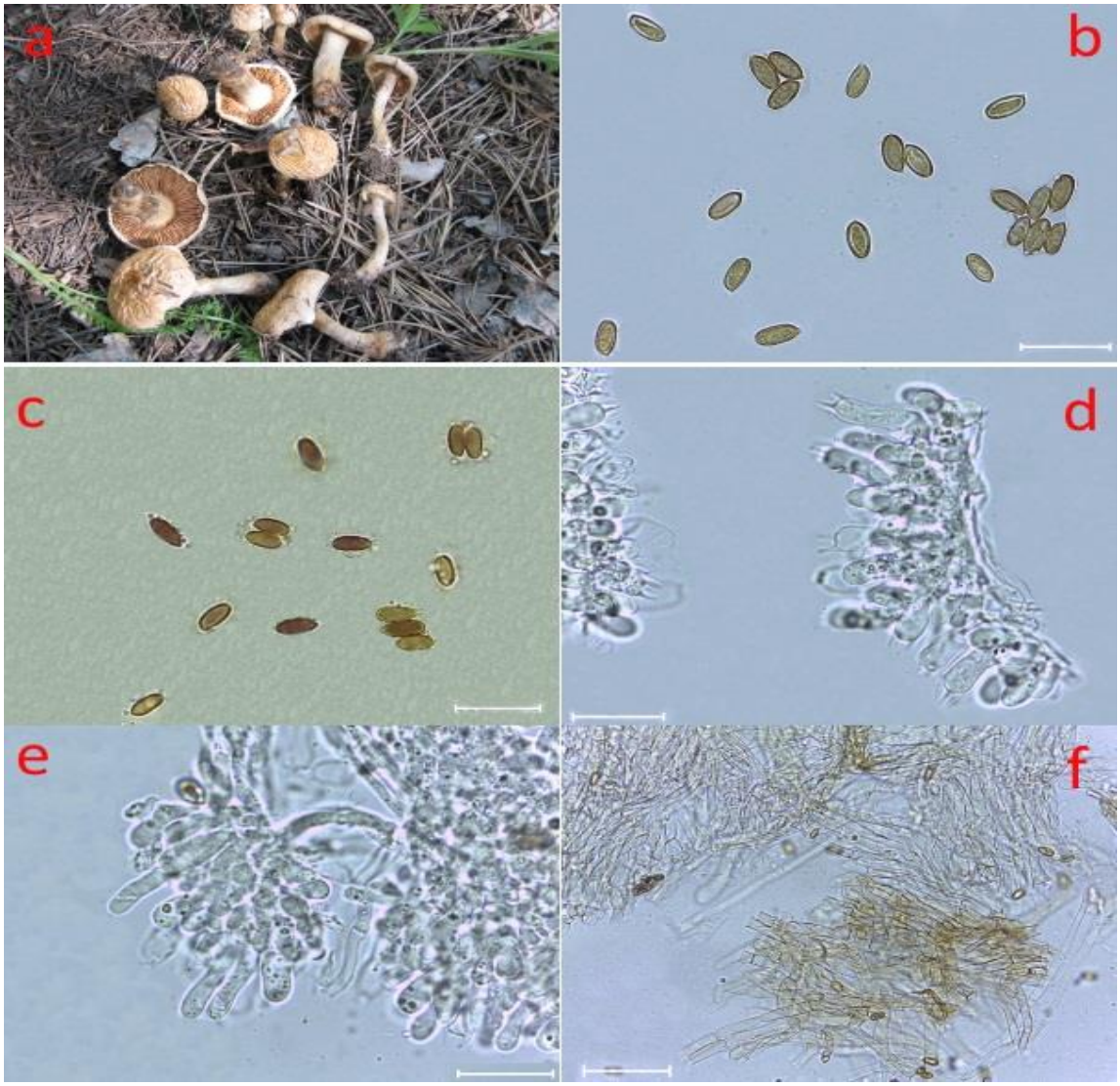

Figure 1. *Hebeloma cylindrosporum* a. Basidiomata b. Spores in distilled water c. Spores in Melzer's reagent d. Basidia e. Cheilocystidia f. Pileipellis.

Molecular analysis

The amplified DNA fragment of the ITS region was approximately 650 bp in length encompassing complete ITS1, 5.8S, and ITS2 subregions. Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1997) analysis was performed using the National Center for Biotechnology Information (NCBI) database. The sequence of *Hebeloma cylindrosporum* matched their representatives with 99% identity values.

ITS data matrix comprised a total of 66 sequences including studied two samples and one outgroup sample. The aligned data included a total of 683 positions, of which 520 were conserved, and 147 were variable (77 variable sites in ITS1, 1 in 5.8S, and 70 in ITS2 subregion) nucleotides. Accession numbers of ITS region for studied

two samples were assigned as MW131677 and MW131678, respectively.

The topologies of the MP and ML phylogenetic trees had no considerable differences, so only one tree (ML) was given to indicate phylogenetic relationships and taxonomic position of studied species. Phylogenetic trees constructed based on ITS separated the species at section levels (Figure 2). The phylogenetic relations of ten sections can be observed in the constructed tree. The Scabrispora clade consists of two clusters and all *Hebeloma cylindropsorum* samples (studied and downloaded from NCBI) grouped with a 100% bootstrap value (Figure 2). Even close relationships among *H. cylindropsorum*, *H. birrus* and *H. circinans were seen in the tree, where they can be separated phylogenetically.* Similar separation among mentioned species were also detected when spore morphology was taken into account. *Hebeloma cylindropsorum* has mostly cylindrical and less ornamented spores while the others have amygdaloid spore shape.

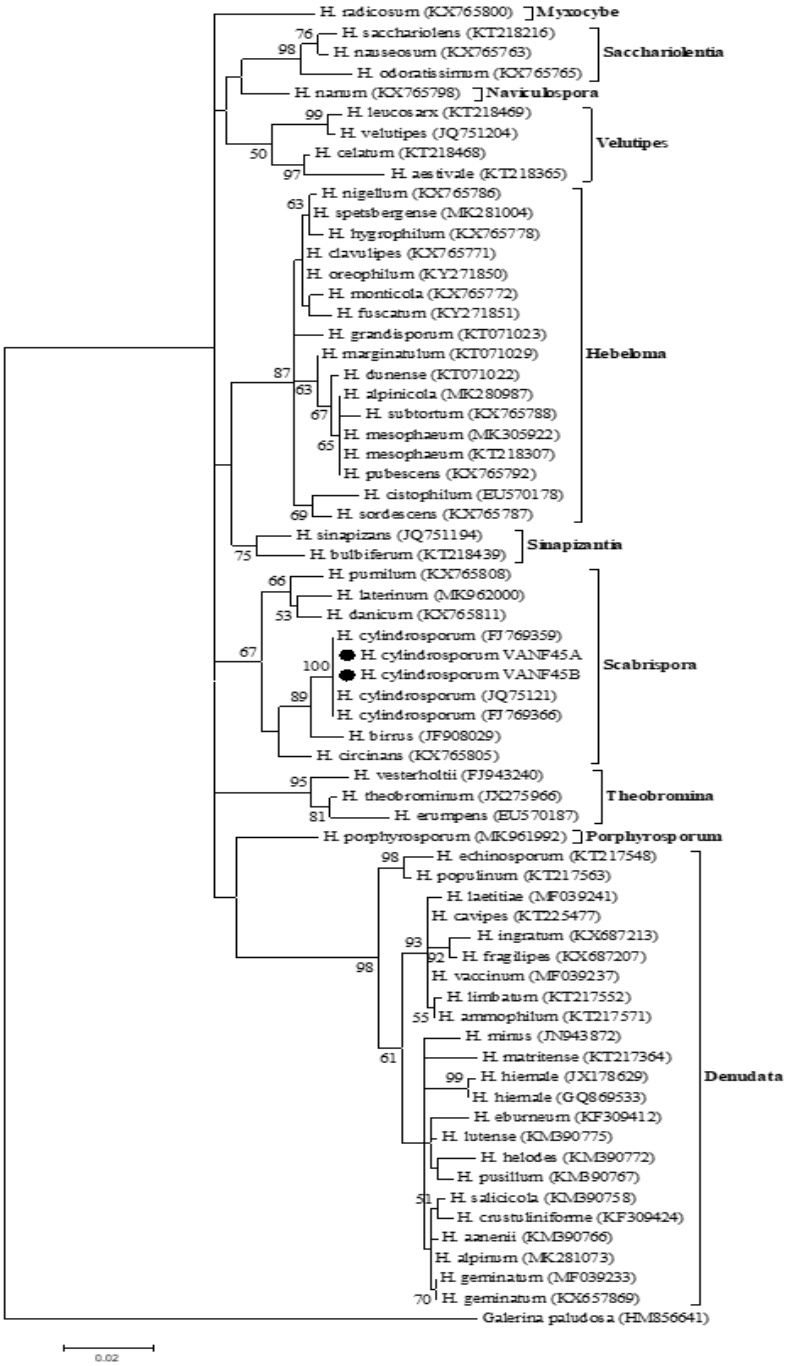

Figure 2. Phylogenetic tree of *Hebeloma* species based on ML analysis of the ITS region. The black circle indicates studied specimens. *Galerina paludosa* was used as an outgroup. Bootstrap values higher than 50% were indicated on the branches.

Discussions

Hebeloma cylindrosporum which belongs to the sect. *Scabrispora* can be distinguished from relative species by its yellow-brown, sticky pileus, thick and buff and tendency rooting stipe, emarginate to adnate lamellae, cylindrically cheilocystidia, dextrinoid mostly cylindrical spores.

The species of *Scabrispora* section are characterized by usually rooting basidiomas, cylindrically shaped spores, and cylindrically most cheilocystidia (Vesterholt 2005; Beker et al. 2016). The studied taxon that carries distinctive characters of section *Scabrispora* was introduced with the current study.

Hebeloma cylindrosporum is a mostly studied fungus in worldwide due to its superior ectomycorrhizal symbiosis feature (Laurans et al. 2001; Marmeisse et al. 2004; Aquino and Plassard, 2004; Doré et al. 2014; Becquer et al. 2018; Khullar and Reddy, 2020). The species is specifically associated with *Pinus*, even though *Quercus*. Reliable description is necessity when economic importance is considered. As observed from the results of the study, both morphological and molecular characters are very useful to describe *Hebeloma* species. The Turkish name of the genus is taken from the book "The Checklist of Fungi of Turkey" (Sesli and et al., 2020).

The current study is providing the morphological and molecular identification of the new record taxon for Turkey and the total number of *Hebeloma* species is increased from 31 to 32 by addition of *H. cylindrosporum*.

Acknowledgement

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Appendix 1

ITS sequences downloaded from NCBI database

H. aanenii (KM390766), *Hebeloma aestivale* (KT218365), *H. alpinicola* (MK280987), *H. alpinum* (MK281073), *H. ammophilum* (KT217571), *H. birrus* (JF908029), *H. bulbiferum* (KT218439), *H. cavipes* (KT225477), *H. celatum* (KT218468), *H. circinans* (KX765805), *H. cistophilum* (EU570178), *H. clavulipes* (KX765771), *H. crustuliniforme* (KF309424), *H. cylindrosporum* (FJ769359, JQ75121, FJ769366), *H. danicum* (KX765811), *H. dunense* (KT071022), *H. eburneum* (KF309412), *H. echinosporum* (KT217548), *H. erumpens* (EU570187), *H. fragilipes* (KX687207), *H. fuscatum* (KY271851), *H. geminatum* (KX657869, MF039233), *H. grandisporum* (KT071023), *H. helodes* (KM390772), *H. hiemale* (JX178629), *H. hygrophilum* (KX765778), *H. ingratum* (KX687213), *H. laetitiae* (MF039241), *H. laterinum* (MK962000), *H. leucosarx* (KT218469), *H. limbatum* (KT217552), *H. lutense* (KM390775), *H. marginatulum* (KT071029), *H. matritense* (KT217364), *H. mesophaeum* (KT218307, MK305922), *H. minus* (JN943872), *H. monticola* (KX765772), *H. nanum* (KX765798), *H. nauseosum* (KX765763), *H. nigellum* (KX765786), *H. odoratissimum* (KX765765), *H. oreophilum* (KY271850), *H. populinum* (KT217563), *H. porphyrosporum* (MK961992), *H. pubescens* (KX765792), *H. pumilum* (KX765808), *H. pusillum* (KM390767), *H. radicosum* (KX765800), *H. sacchariolens* (KT218216), *H. salicicola* (KM390758), *H. sinapizans* (JQ751194), *H. sordescens* (KX765787), *H. spetsbergense* (MK281004), *H. subtortum* (KX765788), *H. theobrominum* (JX275966), *H. vaccinum* (MF039237), *H. velutipes* (JQ751204), *H. vesterholtii* (FJ943240), *Galerina paludosa* (HM856641).

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Macromycetes Determined in Çamburnu Nature Park and Close Environs (Trabzon)

Yılmaz ORUÇ[1], Ali KELEŞ[2],
Yasin UZUN[3], Abdullah KAYA[4*]

**Sorumlu yazar: kayaabd@hotmail.com*

[1]Yüzüncü Yıl University, Department of Strategy Development, 65080 Van, Turkey
Orcid ID: 0000-0002-1238-481X / oruc_2747@hotmail.com
[2]Yüzüncü Yıl University, Education Faculty, Department of Mathematics and Science Education, 65080 Van, Turkey
Orcid ID: 0000-0002-9087-0805 / alikeles61@hotmail.com
[3]Karamanoğlu Mehmetbey University, Ermenek Uysal & Hasan Kalan Health Services Vocational School, Department of Pharmacy Services, 70400, Karaman, Turkey
Orcid ID:0000-0002-6423-6085 / yuclathrus@gmail.com
[4]Gazi University, Science Faculty, Department of Biology, 06500 Ankara, Turkey
Orcid ID: 0000-0002-4654-1406 / kayaabd@hotmail.com

Abstract: This study was carried out the macrofungi samples collected from Çamburnu Nature Park (Sürmene/Trabzon). As a result of field and laboratory studies, 109 macromycete species belonging to four classes, 12 orders, 41 families and 64 genera within Ascomycota and Basidiomycota were determined. The species are presented in alphabetical order together with their habitats and localities.

Key words: Biodiversity, macrofungi, Black Sea Region, Turkey

Çamburnu Tabiat Parkı ve Yakın Çevresinde (Trabzon) Belirlenen Makromantarlar

Öz: Bu çalışma Çamburnu Tabiat Parkı (Sürmene/Trabzon)'ndan toplanan makromantar örnekleri üzerinde gerçekleştirilmiştir. Arazi ve laboratuvar çalışmaları sonucunda Askomikota ve Bazidiyomikota bölümleri içinde yer alan dört sınıf, 12 takım, 41 familya ve 64 cinse ait 109 makromantar türü belirlenmiştir. Türler habitat ve lokaliteleri ile birlikte alfabetik sırada verilmiştir.

Anahtar kelimeler: Biyoçeşitlilik, makromantarlar, Karadeniz Bölgesi, Türkiye

Introduction

The checklists presented on the macromycetes of Turkey indicate that more than 2.500 species are currently known (Doğan et al., 2005; Sesli and Denchev, 2014; Solak et al., 2015; Sesli et al., 2020; Uzun and Kaya, 2020). Though a remarkable portion of these species were presented from the districts situated along Black Sea coasts (Sesli, 1993; Pekşen and Karaca, 2003; Demirel et al., 2010; Akata et al., 2014, 2016; Keleş et al., 2014; Akata and Uzun, 2017; Sesli et al., 2018; Keleş, 2019a,b; Uzun and Kaya, 2019; Yakar et al., 2019; Sesli, 2020), many unstudied or less studied areas still exist in the region. Çamburnu Nature Park area is also among these regions. Though some new records (Keleş and Oruç, 2017) were presented from the region, a research on the macrofungi growing within the boundaries of Çamburnu Nature Park has not been conducted.

Çamburnu Nature Park (Fig. 1) is located in Sürmene district of Trabzon province within Eastern Black Sea Region of Turkey. The Nature Park covers an area of 51 decares and situated at distance of about 8, 7 and 1 km to Sürmene, Of district centers and Çamburnu county center respectively. Phytogeographically the area falls in Colchis sector of the Euro-Siberian floristic area within Holarctic flora kingdom (Davis, 1965; İnandık, 1969; Anşin, 1983).

The area comprises 153 plant taxa within 123 genera and 62 families (Yetmen and Aytaç, 2017). Çamburnu Nature Park is also an important region as being one of the two localities in Turkey where the scotch pine (*Pinus*

sylvestris L. ssp. *kochiana* (Klotzsch ex K. Koch) Eliçin) can reach the coast. Though scotch pine is the dominant tree population in the region, chestnut, spruce, hornbeam and bearded alder are also common tree populations.
The study aims to determine the macrofungal composition of the region, and to make a contribution to the mycobiota of Turkey.

Material and method

Field studies were performed within the boundaries of Çamburnu Nature Park and its close environs (Table 1) between 2014-2016, and 439 macrofungi samples were collected. Macro photos of the samples were taken, and ecological characteristics and geographical coordinates were noted. By asking to the locals, edibility of the samples by local public were also investigated. Then they were transferred to the fungarium and dried in an air-conditioned room. The dried samples are kept in polyethylene bags in VANF. Macroscopic and microscopic investigations were performed in the fungarium. A Leica trinocular light microscope were used for microscopic investigations. The data were compared with relevant literature (Breitenbach and Krânzlin, 1984-2000; Buczacki, 2012; Bresinsky and Besl, 1990; Ellis and Ellis, 1990; Phillips et al., 1991; Jordan, 1995; Krânzlin, 2005; Phillips, 2006; Bessette et al., 2007; Antonin and Noordeloos, 2010; Kuo and Methven, 2014) and the samples were identified. The samples are kept at Yüzüncü Yıl University Fungarium (VANF).

Figure 1. Map of the research area

Results

One hundred and nine macrofungi species were determined from the research area. The taxa are listed in alphabetical order in accordance with Index Fungorum (accessed on 01 January 2021) was followed for the systematics of the species.

Table 1. Localities of the collected macrofungi samples

L. No	Locality name	Coordinates	Altitude (m)
1	Entrance of Çamburnu	N 40°55.534'; E 40°13.390'	18
2	Eastern part of Çamburnu	N 40°55.437'; E 40°12.856'	45
3	Western part Çamburnu	N 40°55.422'; E 40°12.749'	56
4	Southern part of Çamburnu	N 40°55.400'; E 40°12.790'	61 m
5	Northern part of Çamburnu	N 40°55.436'; E 40°12.771'	31 m
6	Çamburnu district centre	N 40°54.435'; E 40°13.151'	350 m
7	Çamburnu district centre	N 40°53.685'; E 40°12.707'	440 m
8	Çamburnu district centre	N 40°54.887'; E 40°12.097'	18 m
9	Kemerli neighborhood	N 40°54.874'; E 40°12.101'	77 m
10	Çamburnu picnic area	N 40°55.462'; E 40°12.974'	25 m
11	Çamburnu picnic area	N 40°55.450'; E 40°12.980'	29 m
12	Çamburnu picnic area	N 40°55.470'; E 40°12.955'	32 m
13	Çamburnu picnic area	N 40°55.483'; E 40°12.952'	19 m
14	Çamburnu picnic area	N 40°55.387'; E 40°12.749'	19 m
15	Çamburnu picnic area	N 40°55.533'; E 40°12.997'	15 m
16	Çamburnu picnic area	N 40°55.230'; E 40°12.631'	11 m
17	Çamburnu picnic area	N 40°55.580'; E 40°12.711'	40 m
18	Çamburnu picnic area	N 40°55.287'; E 40°12.843'	25 m
19	Çamburnu picnic area	N 40°55.331'; E 40°12.874'	30 m
20	Çamburnu picnic area	N 40°55.491'; E 40°12.985'	23 m
21	Around Çamburnu waste facilities	N 40°54.128'; E 40°12.604 '	334 m
22	Around Nemerli mosque	N 40°54.755'; E 40°12.595'	156 m
23	Around Çamburnu recreation facilities	N 40°55.436'; E 40°12.776'	21 m
24	Around Çamburnu waste facilities	N 40°53.692'; E 40°12.661'	433 m
25	Around Çamburnu old hotel	N 40°55.354'; E 40°12.746'	107 m
26	Southern part of Maritime Faculty campus area	N 40°55.390'; E 40°12.599'	16 m
27	Southern part of Maritime Faculty campus area	N 40°55.287'; E 40°12.714'	18 m
28	Southern part of Maritime Faculty campus area	N 40°55.640'; E 40°12.887'	21 m
29	Southern part of Maritime Faculty campus area	N 40°55.492'; E 40°12.611'	19 m
30	Southern part of Maritime Faculty campus area	N 40°55.163'; E 40°12.443'	13 m
31	Southern part of Maritime Faculty campus area	N 40°55.311'; E 40°12.477'	14 m
32	Nothern part of Maritime Faculty campus area	N 40°55.378'; E 40°12.613'	13 m
33	Nothern part of Maritime Faculty campus area	N 40°55.099'; E 40°12.237'	17 m
34	Nothern part of Maritime Faculty campus area	N 40°55.127'; E 40°12.803'	12 m
35	Nothern part of Maritime Faculty campus area	N 40°55.431'; E 40°12.722'	10 m
36	Nothern part of Maritime Faculty campus area	N 40°55.127'; E 40°12.301'	13 m
37	Nothern part of Maritime Faculty campus area	N 40°55.444'; E 40°12.555'	11 m
38	Nothern part of Maritime Faculty campus area	N 40°55.608'; E 40°12.757'	9 m
39	Nothern part of Maritime Faculty campus area	N 40°55.699'; E 40°12.903'	13 m
40°	Nothern part of Maritime Faculty campus area	N 40°55.271'; E 40°12.638'	14 m
41	Around forestry management directorate	N 40°55.379'; E 40°12.790'	93 m
42	Around forestry management directorate	N 40°55.121'; E 40°12.895'	127 m
43	Around forestry management directorate	N 40°55.263'; E 40°12.711'	79 m
44	Around forestry management directorate	N 40°55.541'; E 40°12.921'	133 m

Ascomycota Whittaker
Pezizomycetes O.E. Erikss. & Winka
Pezizales J. Schröt.
Helvellaceae Fr.

1. ***Helvella crispa*** (Scop.) Fr.: On soil under mixed wood, locality 44, 18.11.2015, YO & AK 269.

2. ***Helvella elastica*** Bull.: On soil under mixed wood, locality 41, 18.11.2015, YO & AK 264.

3. ***Helvella lacunosa*** Afzel.: On soil among leaf litter under mixed wood, locality 18, 18.11.2015, YO & AK 270.

4. ***Helvella latispora*** Boud.: Among grass at mixed forest edge, locality 6, 18.11.2015, YO & AK 295.

Pyronemataceae Corda

5. ***Aleuria aurantia*** (Pers.) Fuckel: On soil, locality 7, 18.11.2015, YO & AK 311.

Sordariomycetes O.E. Erikss. & Winka
Xylariales Nannf.
Xylariaceae Tul. & C. Tul.

6. ***Xylaria polymorpha*** (Pers.) Grev.: On decaying stump, locality 34, 14.09.2014, YO & AK 22.

Basidiomycota R.T. Moore
Agaricomycetes Doweld
Agaricales Underw.
Agaricaceae Chevall.

7. ***Agaricus campestris*** L.: Meadow, locality 35, 14.09.2014, YO & AK 30.

8. ***Agaricus moelleri*** Wasser: Meadow, locality 14, 13.10.2014, YO & AK 69; under pine forest, locality 36, 27.10.2016, YO & AK 365.

9. ***Coprinus comatus*** (O.F. Müll.) Pers.: Meadow, locality 22, 17.09.2016, YO & AK 438.

10. ***Leucocoprinus brebissonii*** (Godey) Locq.: (Keleş and Oruç, 2017).

11. ***Macrolepiota mastoidea*** (Fr.) Singer: Among grass in forest clearing, locality 42, 21.10.2015, YO & AK 215.

12. ***Macrolepiota procera*** (Scop.) Singer: Among needle litter under pine forest, locality 20, 29.10.2014, YO & AK 112; 18.05.2016, YO & AK 317; under mixed wood, locality 4, 29.10.2014, YO & AK 147.

Amanitaceae R. Heim ex Pouzar

13. ***Amanita caesarea*** (Scop.) Pers.: On soil under pine forest, locality 18, 14.09.2014, YO & AK 7; locality 17, 21.10.2015, YO & AK 181; under chesnot trees, locality 41, 21.10.2015, YO & AK 213; under mixed wood, locality 16, 17.09.2016, YO & AK 325; locality 3, 17.09.2016, YO & AK 336.

14. ***Amanita citrina*** Pers.: On soil under pine forest, locality 15, 29.10.2014, YO & AK 116; locality 25, 29.10.2014, YO & AK 148.

15. ***Amanita gemmata*** (Fr.) Bertill.: On soil under pine forest, locality 20, 05.2016, YO & AK 315; under mixed wood, locality 19, 18.05.2016, YO & AK 321.

16. ***Amanita mairei*** Foley: Among grass, locality 23, 14.09.2014, YO & AK 18.

17. ***Amanita muscaria*** (L.) Lam.: Among grass in pine forest clearing, locality 18, 14.09.2014, YO & AK 10.

18. ***Amanita phalloides*** (Vaill. ex Fr.) Link: On soil under pine forest, locality 17, 18.05.2016, YO & AK 313.

19. ***Amanita rubescens*** Pers.: On soil under pine forest, locality 16, 14.09.2014, YO & AK 12; under mixed wood, locality 41, 21.10.2015, YO & AK 254.

20. ***Amanita vaginata*** (Bull.) Lam.: On soil under pine forest, locality 15, 29.10.2014, YO & AK 121.

Bolbitiaceae Singer

21. ***Bolbitius titubans*** (Bull.) Fr.: On soil under mixed wood, locality 24, 17.09.2016, YO & AK 436.

22. ***Conocybe semiglobata*** Kühner & Watling: Among grass under burned pine trees, locality 1, 29.10.2014, YO & AK 153.

Clavariaceae Chevall.

23. ***Ramariopsis subtilis*** (Pers.) R.H. Petersen: On soil under pine forest, locality 39, 27.10.2016, YO & AK 362.

Entolomataceae Kotl. & Pouzar

24. ***Entoloma rhodopolium*** (Fr.) P. Kumm.: Among needle litter under pine forest, locality 10, 21.10.2015, YO & AK 194.

Hydnangiaceae Gäum. & C.W. Dodge

25. ***Laccaria amethystina*** Cooke: Among needle litter, under pine forest, locality 20, 18.11.2015, YO & AK 308.

26. ***Laccaria laccata*** (Scop.) Cooke: On soil under pine forest, locality 40, 27.10.2016, YO & AK 359.

Hygrophoraceae Lotsy

27. ***Hygrocybe conica*** (Schaeff.) P. Kumm.: On soil under mixed wood, locality 14, 27.10.2016, YO & AK 418.

Hymenogastraceae Vittad.

28.***Gymnopilus picreus*** (Pers.) P. Karst.: Among needle litter under pine forest, locality 17, 14.09.2014, YO & AK 39.

29.***Hypholoma fasciculare*** (Huds.) P. Kumm.: On decaying stump, locality 26, 29.10.2014, YO & AK 143; locality 42, 18.11.2015, YO & AK 256.

Inocybaceae Jülich

30.***Inocybe dulcamara*** (Pers.) P. Kumm: Among leaf litter, roadside, locality 25, 29.10.2014, YO & AK 151.

31.***Inocybe obsoleta*** (Quadr. & Lunghini) Valade: Under pine forest, locality 13, 14.09.2014, YO & AK 9; locality 43, 14.09.2014, YO & AK 15.

32.***Inocybe queletii*** Konrad: On soil under mixed wood, locality 20, 21.10.2015, YO & AK 240.

*33.****Inocybe tenebrosa*** Quél.: Under mixed wood, locality 44, 18.11.2015, YO & AK 266.

Incertae sedis

34.***Clitocybe rivulosa*** (Pers.) P. Kumm.: On soil under mixed wood, locality 44, 18.11.2015, YO & AK 255.

35.***Cyathus striatus*** (Huds.) Willd.: On decaying twigs under pine forest, locality 37, 14.09.2014, YO & AK 36.

36.***Lepista sordida*** (Schumach.) Singer: Among grass, locality 20, 18.11.2015, YO & AK 306.

Lycoperdaceae Chevall.

37.***Lycoperdon marginatum*** Vittad.: Among needle litter under pine forest, locality 17, 14.10.2015, YO & AK 64.

38.***Lycoperdon perlatum*** Pers.: Among grass, locality 38, 14.09.2014, YO & AK 27.

Lyophyllaceae Jülich

39.***Lyophyllum decastes*** (Fr.) Singer: On soil under pine forest, locality 40, 18.11.2015, YO & AK 291.

Mycenaceae Roze

40.***Hemimycena delectabilis*** (Peck) Singer: On decaying twigs, locality 39, 14.09.2014, YO & AK 24.

41.***Mycena flavescens*** Velen.: On soil under pine forest, locality 38, 14.09.2014, YO & AK 38.

42.***Mycena leptocephala*** (Pers.) Gillet: On soil under mixed wood, locality 27, 27.10.2016, YO & AK 368.

Omphalotaceae Bresinsky

43.***Gymnopus erythropus*** (Pers.) Antonín, Halling & Noordel.: Under pine forest, locality 37, 14.09.2014, YO & AK 35.

Physalacriaceae Corner

44.***Armillaria borealis*** Marxm. & Korhonen: On decaying stump, locality 36, 13.10.2014, YO & AK 71.

45.***Armillaria cepistipes*** Velen.: On decaying stump, locality 12, 29.10.2014, YO & AK 126.

46.***Armillaria mellea*** (Vahl) P. Kumm.: Under *Alnus* sp., locality 23, 29.10.2014, YO & AK 133.

47.***Hymenopellis radicata*** (Relhan) R.H. Petersen: Under mixed wood, locality 28, 21.10.2015, YO & AK 196.

Pleurotaceae Kühner

48.***Pleurotus ostreatus*** (Jacq.) P. Kumm.: On soil under mixed wood, locality 21, 17.09.2016, YO & AK 437.

Psathyrellaceae Vilgalys, Moncalvo & Redhead

49.***Coprinellus disseminatus*** (Pers.) J.E. Lange: Around decaying stump under mixed wood, locality 9, 17.09.2016, YO & AK 439.

50.***Coprinellus micaceus*** (Bull.) Vilgalys, Hopple & Jacq. Johnson: On decaying stump, locality 11, 21.10.2015, YO & AK 184.

51.***Coprinopsis gonophylla*** (Quél.) Redhead, Vilgalys & Moncalvo: Among grass under burned pine trees, locality 1, 29.10.2014, YO & AK 155.

52.***Coprinopsis urticicola*** (Berk. & Broome) Redhead, Vilgalys & Moncalvo: On decaying Urtica sp. remains, locality 35, 14.09.2014, YO & AK 34.

53.***Parasola leiocephala*** (P.D. Orton) Redhead, Vilgalys & Hopple: Around *Alnus* sp. stump, locality 44, 25.07.2016, YO & AK 434.

54.***Psathyrella candolleana*** (Fr.) Maire: Among grass, locality 34, 14.09.2014, YO & AK 28; under mixed wood, locality 10, 18.05.2016, YO & AK 314; among leaf litter, locality 2, 25.07.2016, YO & AK 432.

55.***Psathyrella multipedata*** (Peck) A.H. Sm.: On soil among grass under *Alnus* sp., locality 17, 14.09.2014, YO & AK 16.

56.***Psathyrella piluliformis*** (Bull.) P.D. Orton: Under tangerine trees, locality 8, 27.10.2016, YO & AK 385.

Schizophyllaceae Quél.

57.***Schizophyllum commune*** Fr.: On decaying pine stump, locality 1, 29.10.2014, YO & AK 157; on decaying tree branches, locality 7, 18.05.2016, YO & AK 320.

Tricholomataceae R. Heim ex Pouzar

58.***Tricholoma sciodes*** (Pers.) C. Martín: Among needle litter under pine forest, locality 10, 29.10.2014, YO & AK 111.

59.***Tricholomopsis rutilans*** (Schaeff.) Singer: On decaying stump under pine forest, locality 33, 29.10.2014, YO & AK 144; 21.10.2015, YO & AK 206; 18.11.2015, YO & AK 292; 27.10.2016, YO & AK 361.

Boletales E.-J. Gilbert

Boletaceae Chevall.

60.***Cyanoboletus pulverulentus*** (Opat.) Gelardi, Vizzini & Simonini: Among grass at forest edge, locality 1, 21.10.2015, YO & AK 229.

61.***Imleria badia*** (Fr.) Vizzini: On soil under pine trees, locality 32, 14.09.2014, YO & AK 51; under mixed wood, locality 7, 21.10.2015, YO & AK 251.

62.***Leccinellum pseudoscabrum*** (Kallenb.) Mikšík: On soil under pine forest, locality 10, 21.10.2015, YO & AK 189.

63.***Leccinum versipelle*** (Fr. & Hök) Snell: On soil under mixed wood, locality 20, 21.10.2015, YO & AK 193.

64.***Porphyrellus porphyrosporus*** (Fr. & Hök) E.-J. Gil: Among needle litter under pine forest, locality 17, 14.09.2014, YO & AK 37.

Diplocystidiaceae

65.***Astraeus hygrometricus*** (Pers.) Morgan: On soil under mixed wood, locality 7, 21.10.2015, YO & AK 235.

Gomphidiaceae Maire ex Jülich

66.***Chroogomphus confusus*** Y.C. Li & Zhu L. Yang: (Keleş, 2019a).

Paxillaceae Lotsy

67.***Paxillus involutus*** (Batsch) Fr.: On soil under pine trees, locality 33, 14.09.2014, YO & AK 54.

68.***Paxillus rubicundulus*** P.D. Orton: Among grass at pine forest edge, locality 29, 13.10.2014, YO & AK 80; [40], 21.10.2015, YO & AK 222.

Sclerodermataceae Corda

69.***Scleroderma areolatum*** Ehrenb.: Among grass, under mixed wood, locality 10, 14.09.2014, YO & AK 4.

70.***Scleroderma citrinum*** Pers.: On soil under pine trees, locality 19, 21.10.2015, YO & AK 185.

Suillaceae Besl & Bresinsky

71.***Suillus bovinus*** (L.) Roussel: Among grass under mixed wood, locality 18, 21.10.2015, YO & AK 211.

72.***Suillus granulatus*** (L.) Roussel: Among needle litter under pine forest, locality 17, 21.10.2015, YO & AK 208.

73.***Suillus luteus*** (L.) Roussel: On soil under pine trees, locality 34, 14.09.2014, YO & AK 32; locality 41, 21.10.2015, YO & AK 223.

74.***Suillus tomentosus*** Singer: (Keleş, 2019b).

Tapinellaceae C. Hahn

75.***Tapinella atrotomentosa*** (Batsch) Šutara: On decaying stump, locality 30, 13.10.2014, YO & AK 96.

Cantharellales Gäum.

Hydnaceae Chevall.

76.***Cantharellus cibarius*** Fr.: On soil under mixed wood, locality 35, 13.10.2014, YO & AK 70.

77.***Cantharellus cinereus*** (Pers.) Fr.: On soil under mixed wood, locality 7, 21.10.2015, YO & AK 249.

78.***Clavulina cinerea*** (Bull.) J. Schröt.: Among leaf litter under *Castanea* sp., locality 20, 21.10.2015, YO & AK 243.

79.***Clavulina coralloides*** (L.) J. Schröt.: On decaying stump, locality 43, 18.11.2015, YO & AK 257; under mixed wood, locality 6, 18.11.2015, YO & AK 298.

80.***Craterellus tubaeformis*** (Fr.) Quél.: On soil under mixed wood, locality 42, 18.11.2015, YO & AK 261.

81.***Hydnum repandum*** L.: On soil under pine trees, locality 18, 29.10.2014, YO & AK 115.

82.***Hydnum rufescens*** Pers.: Among grass under mixed wood, locality 6, 27.10.2016, YO & AK 391.

Gomphales Jülich

Gomphaceae Donk

*83.***Ramaria flavescens*** (Schaeff.) R.H. Petersen: On soil under pine trees, locality 40, 14.09.2014, YO & AK 47.

Hymenochaetales Oberw.

Hymenochaetaceae Donk

84.***Phellinus igniarius*** (L.) Quél.: On decaying stump, locality 17, 14.09.2014, YO & AK 29.

Phallales E. Fisch.
Phallaceae Corda
85.***Clathrus ruber*** P. Micheli ex Pers.: On soil under pine trees, locality 31, 13.10.2014, YO & AK 79; under *Alnus* sp., locality 23, 29.10.2014, YO & AK 134; under mixed wood, locality 5, 27.10.2016, YO & AK 417.

86.***Pseudocolus fusiformis*** (E. Fisch.) Lloyd: On soil under fruit trees, locality 26, 13.10.2014, YO & AK 82; meadow, locality 8, 27.10.2016, YO & AK 379.

Polyporales Gäum.
Fomitopsidaceae Jülich
87.***Fomitopsis pinicola*** (Sw.) P. Karst.: On decaying stump, locality 10, 21.10.2015, YO & AK 182.

Polyporaceae Fr. ex Corda
88.***Daedaleopsis confragosa*** (Bolton) J. Schröt.: On decaying *Corylus* sp. twigs, locality 10, 21.10.2015, YO & AK 192.

89.***Trametes hirsuta*** (Wulfen) Lloyd: On decaying *Alnus* sp. stump, locality 27, 29.10.2014, YO & AK 141; on dead branches, locality 17, 18.05.2016, YO & AK 318.

90.***Trametes ochracea*** (Pers.) Gilb. & Ryvarden: On decaying *Alnus* sp. stump, locality 28, 29.10.2014, YO & AK 142; locality 16, 27.10.2016, YO & AK 423.

91.***Trametes versicolor*** (L.) Lloyd: On decaying *Alnus* sp. stump, locality 8, 27.10.2016, YO & AK 384.

Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David
Auriscalpiaceae Maas Geest.
92.***Auriscalpium vulgare*** Gray: On decaying *Abies* sp. cones, locality 29, 27.10.2016, YO & AK 372.

93.***Lentinellus cochleatus*** (Pers.) P. Karst.: On decaying *Fagus* sp. stump, locality 39, 14.09.2014, YO & AK 50.

Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David
Russulaceae Lotsy
94.***Lactarius deliciosus*** (L.) Gray: Among needle litter under pine trees, locality 30, 13.10.2014, YO & AK 75.

95.***Lactarius fulvissimus*** Romagn.: On soil under mixed wood, locality 15, 14.09.2014, YO & AK 11.

96.***Lactarius semisanguifluus*** R. Heim & Leclair: Under pine forest, locality 38, 27.10.2016, YO & AK 354.

97.***Lactarius tabidus*** Fr.: On soil under pine forest, locality 14, 29.10.2014, YO & AK 131.

98.***Russula amoenolens*** Romagn.: On soil under pine forest, locality 13, 14.09.2014, YO & AK 5.

99.***Russula cyanoxantha*** (Schaeff.) Fr.: On soil under pine forest, locality 12, 14.09.2014, YO & AK 8.

100. ***Russula delica*** Fr.: On soil under mixed forest, locality 31, 13.10.2014, YO & AK 74.

101. ***Russula emetica*** (Schaeff.) Pers.: On soil under pine forest, locality 11, 14.09.2014, YO & AK 6.

102. ***Russula olivacea*** (Schaeff.) Fr.: On soil under pine forest, locality 10, 14.09.2014, YO & AK 13.

103. ***Russula parazurea*** Jul. Schäff.: On soil under *Corylus* sp., locality 11, 13.10.2014, YO & AK 67.

104. ***Russula queletii*** Fr.: On soil under pine forest, locality 36, 17.09.2016, YO & AK 355.

105. ***Russula rhodopus*** Zvára: On soil under pine forest, locality 12, 21.10.2015, YO & AK 179.

106. ***Russula xerampelina*** (Schaeff.) Fr.: On soil under pine forest, locality 28, 13.10.2014, YO & AK 73.

Stereaceae Pilát
107. ***Stereum hirsutum*** (Willd.) Pers.: On decaying stump, locality 13, 18.05.2016, YO & AK 316.

Thelephorales Corner ex Oberw.
Bankeraceae Donk
108. ***Sarcodon squamosus*** (Schaeff.) Quél.: On soil under pine forest, locality 32, 14.09.2014, YO & AK 48.

Dacrymycetes Doweld
Dacrymycetales Henn.
Dacrymycetaceae J. Schröt.
109. ***Calocera viscosa*** (Pers.) Fr.: On soil under pine forest, locality 33, 14.09.2014, YO & AK 49.

Discussions

The study in Çamburnu Nature Park revealed 109 macromycete species belonging to four classes, 12 orders, 41 families and 64 genera.

Six of them belong to *Ascomycota* (*Pezizomycetes* 5, *Sordariomycetes* 1) and 103 to *Basidiomycota* (*Agaricomycetes* 102, *Dacrymycetes* 1). *Agaricales* were found to be the most diverse order in the region, comprising almost half of the (%49) determined species.

It is followed by *Boletales, Russulales, Cantharellales* and *Pezizales* with 16, 16, 7 and 5 species respectively. The results indicate that the most diverse family is *Russulaceae* in the region. It comprises 13 species. It is followed by *Amanitaceae, Psathyrellaceae, Agaricaceae,* and *Cantharellaceae* with 8, 8, 6 and 6 species respectively. Similarly the first five most crowded genera in the region were found to be *Russula, Amanita, Helvella, Inocybe* and *Lactarius* with 9, 8, 4, 4, 4 species respectively. The abundance of decidious and coniferous trees, and grassy areas in the region seems to favor the diversity of the members of some families such as *Russulaceae* and *Agaricaceae*.

Among the 109 determined macromycete species, 44 (%40.37) are edible, 51 (%46.79) are inedible and 14 (%12.84) are poisonous. *Lactarius delicious* is heavily collected and consumed by locals with the name "kanlıca". *Agaricus campestris* is also collected and consumed in the region but no specific name was assigned for it.

Macromycetes determined in research area were also compared with the studies carried out in close environs and some similarities were observed. These studies and the similarity percentages are given in Table 2. The reason for this similarity may be the common climate and vegetation.

The study presents the macrofungal biodiversity of Çamburnu nature park and its close environs and makes a regional contribution to the mycobiota of Turkey.

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Table 2. Similarity percentages of neighbouring studies with Çamburnu and its close environs

	# of Identical taxa	Total taxa	Similarity (%)
Sesli (1993)	16	62	25.81
Pekşen and Karaca (2003)	38	169	22.49
Demirel et al. (2010)	31	126	24.60
Keleş et al. (2014)	28	127	22.05
Akata et al. (2014)	46	236	19.49
Akata et al. (2016)	43	182	19.49
Akata and Uzun (2017)	44	205	21.46

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Review Article
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Potential Cardiogenic Effects of Poisonous Mushrooms

Mustafa SEVİNDİK[1*], Betül ÖZDEMİR[2], Nady BRAIDY[3], Hasan AKGÜL[4], Ilgaz AKATA[5], Zeliha SELAMOGLU[6]

**Sorumlu yazar: sevindik27@gmail.com*

[1] Department of Food Processing, Bahçe Vocational School, Osmaniye Korkut Ata University, Osmaniye, Turkey
Orcid ID: 0000-0001-7223-2220/ sevindik27@gmail.com
[2] Nigde Omer Halisdemir University, Faculty of Medicine, Depart. of Cardiology, Nigde, Turkey
Orcid ID: 0000-0003-4725-9522/ betulozaltun@ohu.edu.tr
[3] New South Wales Uni., Sch. of Psychiatry, Centre for Healthy Brain Ageing, Sydney, Australia
Orcid ID: 0000-0002-0497-5572/ n.braidy@unsw.edu.au
[4] Akdeniz University, Faculty of Science, Department of Biology, Antalya, Turkey
Orcid ID: 0000-0001-8514-9776/ hakgul@akdeniz.edu.tr
[5] Ankara University, Faculty of Science, Department of Biology, Ankara, Turkey
Orcid ID: 0000-0002-1731-1302/ akatailgaz@gmail.com
[6] Nigde Omer Halisdemir University, Faculty of Medicine, Depart. of Cardiology, Nigde, Turkey
Orcid ID: 0000-0001-9056-6435/ zselamoglu@ohu.edu.tr

Abstract: Natural resources have been the savior of people at every stage of human history. Mushrooms are very valuable food sources, especially in the rainy seasons. They can be grouped as poisonous, edible or inedible, depending on their nutritional status. These groups have positive or negative effects. Some types of mushrooms have different medicinal properties thanks to their bioactive compounds. It is necessary to characterize their toxicological profiles, especially before using mushroom species for human consumption, as toxic substances are identified even in some edible species. Mycetism, known as mushroom poisoning, is an international health problem. There are those that cause deadly poisoning and limited symptoms of poisoning in mushrooms. Poisonous mushrooms are divided into many classes as cytotoxic, neurotoxic, myotoxic, gastroirritan mushrooms according to their types or symptoms they cause. It is known that mushroom poisoning can cause serious toxicity on the liver, kidneys and central nervous system. However, its effect on heart function has not been determined yet. This study focused on poisonous mushrooms and their cardiological effects.

Key words: Cardiogenic effects, poisonous mushrooms, syndromes, wild mushroom

Zehirli Mantarların Potansiyel Kardiyojenik Etkileri

Öz: İnsanlık tarihinin her aşamasında doğal kaynaklar insaların kurtarıcısı olmuştur. Mantarlar özellikle yagmur mevsimlerinde yoğun olarak bulunan oldukça değerli gıda kaynaklarıdır. Mantarlar beslenme durumlarına göre zehirli, yenir veya yenmez olarak gruplandırılabilir. Bu grupların olumlu veya olumsuz etkileri vardır. Bazı mantar türleri yapılarındaki biyoaktif bileşikler sayesinde farklı tıbbi özelliklere sahiptir. Özellikle insan tüketimi için mantar türlerini kullanmadan önce, bazı yenilebilir türlerde bile toksik maddeler tanımlandığı için toksikolojik profillerini karakterize etmek gerekir. Mantar zehirlenmesi (MP) olarak bilinen mycetism, uluslararası bir sağlık sorunudur. Mantarlar içerisnde ölümcül zehirlenmelere neden olanlar ve zehirlenme semptomları sınırlı olanlar vardır. Zehirli mantarlar türlerine veya neden oldukları semptomlara göre sitotoksik, nörotoksik, miyotoksik, gastroirritan mantarlar olarak birçok sınıfa ayrılır. Mantar zehirlenmesinin karaciğer, böbrekler ve merkezi sinir sistemi üzerinde ciddi toksisiteye neden olabileceği bilinmektedir. Ancak bunun kalp fonksiyonları üzerindeki etkisi henüz belirlenmemiştir. Yapılan bu araştırmada zehirli mantarlar ve görülen kardiyolojik etkileri üzerinde durulmuştur.

Anahtar kelimeler: Kardiyojenik etkiler, zehirli mantarlar, sendromlar, yabani mantar

Introduction

Mushrooms are living organisms that reproduce by spores and do not contain chlorophyll (Akgül et al., 2017). Spores in humid environments germinate and form fruiting bodies, especially after rains. Although there are 140.000 mushroom species identified from past to present, approximately 2.000 of these are safe for human health (Sevindik, 2018). Mushrooms were originally known as a source of food. Many studies in later processes have revealed that mushrooms have medicinal properties (Rathee et al., 2012; Sevindik et al., 2018; Gürgen et al., 2020; Selamoglu et al., 2020). Medicinal effects of some mushroom species such as antioxidant, antilipidemic, antimicrobial, anticancer, anti-inflammatory, anti-proliferative, DNA protective, hepatoprotective have been known (Lull et al., 2005; Canli et al., 2016; Liu et al., 2017; Sevindik et al., 2017; Wasser, 2017; Bozdogan et al., 2018; Muszyńska et al., 2018; Mushtaq et al., 2020; Sevindik and Akata, 2019; Sevindik, 2020). In addition, mushrooms rich in glucans can stimulate the immune system and provide people with health benefits. In addition, mushrooms contain high protein content, vitamins, fibers and minerals (Mhanda et al., 2015; Kim et al., 2016; Süfer et al., 2016; Sevindik et al., 2020). Since toxic compounds are identified even in some edible species, it is necessary to characterize their toxicological profiles before being consumed by humans.

Results

Mushroom Poisoning

Mushroom poisonings known as mycetism or mycetismus is an international health problem. Especially in the spring and autumn, high mortality is observed with the appearance of mushrooms after heavy rains (Tegzes et al., 2002; Eren et al., 2010). Some mushrooms are known to be poisonous in the mushroom kingdom, and cases of mycetism are reported annually in many countries around the world. Many of cases of these mycetism are due to the mixing of species from their empirical and traditional knowledge with their toxic counterparts (White et al., 2003; Flesch and Saviuc, 2004; Jo et al., 2014; Ozaltun and Sevindik, 2020). Among the known mushroom species today, about 100 species are poisonous to humans, but new toxic compounds are constantly identified within the fungi (Eren et al., 2010; Yardan et al., 2010). Toxicity differences can be observed even among the species of the same genus in the mushroom kingdom. Even one type of the same breed can be poisonous, while the other type can be eaten. Different toxins and different clinical symptoms are observed even among poisonous species (Kirchmair et al., 2012; Méndez-Navarro et al., 2016). Some of the poisonous mushroom species can be fatal, while some species have limited symptoms. People who consume poisonous mushroom species often have gastrointestinal symptoms like vomiting, nausea, diarrhea. But depending on the type of poisonous mushroom, signs of poisoning can reach serious levels. Although the duration of symptoms and symptoms varies depending on the amount of mushroom consumption and the type of toxic compound, it appears between 30 minutes and 2 hours on average. Mushroom poisonings are classified as early diagnosis and late diagnosis. Early diagnosis is made in the first 6 hours after consumption, and late detection is done after 6 hours (Nordt and Manoguerra, 2000; Eren et al., 2010). The onset of early or late symptoms is a marker for prognosis (Levine et al., 2011). Since the compound that provides toxicity in cases of mycetism is different, the symptoms change. Symptoms of cases of mycetism have a very wide range. Therefore, early diagnosis and interventions are very important for patients. Mycetism is usually diagnosed based on clinical findings and the presence of toxin in plasma, serum and urine (Durukan et al., 2007; De Oliveira, 2009). However, in most countries, toxin determination is not performed in daily practice. Generally, mushroom consumption history and clinical findings are very important for diagnosis. A detailed history should be obtained from patients in case of cases of mycetism. In addition to the importance of early diagnosis for effective treatment methods, it is the prevention of absorption of toxins by applying activated charcoal after gastric lavage. In addition to this treatment, treatment is supported with different drugs such as beta-lactam antibiotic, silymarin complex and antioxidant drugs (eg ascorbic acid, cimetidine and NAC). Hemodialysis is not recommended unless renal failure develops as a result of excessive thirst. Liver transplantation is considered a life-saving procedure in cases of acute massive hepatic necrosis (Barriot et al., 2000; Enjalbert et al., 2002).

Syndromes

Mycetism can generally be examined under two groups. Group 1 syndromes are poisonings that show symptoms between 30 minutes and 6 hours after consumption. Healing is seen in a short time with stomach wash or symptomatic treatments. Group 2 syndromes are poisonings that show symptoms between 6-24 hours.

These types of poisoning are serious poisonings because their symptoms are delayed and cause liver and kidney diseases. Poisoning treatments should be started in a hospital setting without wasting time (Diaz, 2005; Kaufmann, 2007; White et al., 2019).

Group 1 syndromes

Muscarinic Syndrome

The mushrooms that cause this syndrome are muscarine-containing species, such as *Clitocybe* (Fr.) Staude and *Inocybe* (Fr.) Fr. members. Clinical symptoms appear on average between 30 minutes and 2 hours following consumption (Beuhler and Graeme, 2005). Clinical symptoms seen include bradycardia, miosis, salivary secretion, lacrimation, diarrhea, nausea, vomiting and bronchospasm. Depending on the amount of consumption, symptoms manifest themselves on average for 6-24 hours. Symptoms are usually eliminated by applying activated charcoal and fluid therapy (Azzolina et al., 2011; Lima et al., 2012; Karimi and Razavi, 2014).

Gastrointestinal Syndrome

The mushroom species that cause this syndrome are Omphalotus olearius (DC.) Singer, *Chlorophyllum molybdites* (G. Mey.) Massee, *Entoloma lividum* Quél., *Hypholoma fasciculare* (Huds.) P. Kumm, *Boletus satanas* (Current name: *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang), *Agaricus xanthodermus* Genev. and *Russula emetica* (Schaeff.) Pers. species (Azzolina et al., 2011; Karimi and Razavi, 2014). Symptoms usually appear between 1-6 hours. The symptoms seen are vomiting, nausea, diarrhea, abdominal pain, drowsiness and blurred vision, similar to muscarin syndrome. The treatment method applied in muscarinic syndrome is applied (Beuhler and Graeme, 2005; Karimi and Razavi, 2014).

Coprinus Syndrome

The mushroom species that causes this syndrome is *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys & Moncalvo. The syndrome gets its name from this species. Generally, symptoms appear within 30 minutes of consumption. Alcohol consumption with this fungus causes symptoms to be more severe (Azzolina et al., 2011; Karimi and Razavi, 2014). Symptoms include flushing, nausea, vomiting, weakness, agitation, palpitations, and tingling in the legs. Symptoms usually disappear spontaneously about 2 hours after stopping alcohol consumption. However, it can present serious problems in people with heart conditions (Danel et al., 2001; Karimi and Razavi, 2014).

Psilocybin Syndrome

The mushrooms that cause this syndrome are species belonging to the genus *Psilocybe* (Fr.) P. Kumm, *Stropharia* (Fr.) Quél., *Conocybe* Fayod and *Panaeolus* (Fr.) Quél. Generally, *Gymnopilus spectabilis* (Current name: *Phaeolepiota aurea* (Matt.) Maire), *Panaeolus foenisecii* (Current name: *Panaeolina foenisecii* (Pers.) Maire), *Conocybe cyanopus* (G.F. Atk.) Kühner, *Psilocybe caerulescens* Murrill, and *P. cubensis* (Earle) Singer are mushroom species that contain psilocybin. Symptoms usually manifest between 30 minutes and 2 hours. Visual hallucinations are common among symptoms (Peden et al., 1982; Passie et al., 2002; Karimi and Razavi, 2014). Other symptoms include increased heart rate, mydriasis, chills, and sweating. Effects can be seen for up to 8 hours. However, visual hallucinations usually lose their effect after 1 hour. In addition, there may be difficulties in visual and auditory senses due to continuous use. Healing usually happens without treatment. Symptoms disappear after an average of 8 hours (Hasler et al., 2002; Beuhler and Graeme, 2005; Karimi and Razavi, 2014).

Pantherina-Muscaria Syndrome

The mushrooms that cause this syndrome are *Amanita muscaria* (L.) Lam. and *A. pantherina* (DC.) Krombh species. The compound that causes poisoning is ibotenic acid. Symptoms appear between 30 minutes and 2 hours on average following consumption (Azzolina et al., 2011). Symptoms include visual impairment, difficulty speaking, visual hallucinations, delirium, salivation, dizziness, fatigue, vivid dreams and deep sleep. Symptoms usually disappear completely after 24 hours. Atropine can be used for long-term symptoms or clinical situations (Tsujikawa et al., 2006; Lima et al., 2012; Karimi and Razavi, 2014).

Group 2 syndromes

Orellanus syndrome

The mushrooms that cause this syndrome are *Cortinarius orellanus* Fr. and *C. orellanoides* (Current name: *Cortinarius rubellus* Cooke). The effects of orellanine on the digestive system are seen on average 36-48 hours. Its effect on the kidneys is seen after 7-17 days (Beuhler and Graeme, 2005; Wessely et al., 2007). Symptoms include anorexia, headache, dry mouth,

burning in the tongue and lips, vomiting, diarrhea, chills, joint and muscle pain. It is not understood that the symptoms are due to mushroom poisoning, since the duration of symptoms is long. If kidney impairment has occurred, kidney transplantation is required. Complete recovery may take a long time (Eigler et al., 1997; Tegzes and Puschner, 2002; Karimi and Razavi, 2014).

Gyromitra syndrome

Syndrome-causing species are mushrooms containing Gyromitrin, such as *Gyromitra esculenta* (Pers.) Fr. and *G. californica* (W. Phillips) Raitv.. Symptoms appear between 6-48 hours after consumption (Michelot and Toth, 1991; Azzolina et al., 2011). The first symptoms are gastrointestinal symptoms such as vomiting, abdominal pain or diarrhea. Clinical symptoms include vertigo, sweating, dysarthria, coordination disorder, seizures, hemolysis, methemoglobinemia, rhabdomyolysis, myalgia and hypoglycemia abnormalities. If it is used for a long time, it may cause liver damage. Treatment is symptomatic (Brooks and Graeme, 2005; Goldfrank, 2009; Karimi and Razavi, 2014).

Phalloides syndrome

Amanita phalloides (Vaill. ex Fr.) Link, *A. verna* (Bull.) Lam., *A. virosa* Bertill. and *Galerina* Earle species are fungi that cause this syndrome. Symptoms appear on average between 6-24 hours following consumption. The first symptoms are nausea, vomiting, severe abdominal pain and bloody diarrhea. These symptoms persist for an average of 1 day. It then enters the 1-2-day process called pseudo-healing. During this period, the symptoms disappear (Vogel et al., 1984; Alves et al., 2001; Vanooteghem et al., 2014). Although the patient looks clinically well, enzymes rise rapidly in the blood test. In the next process, necrosis begins in the liver and kidneys. As a result, jaundice, stomach and intestinal bleeding and hepatic coma are seen. If early diagnosis and treatment is not made, the patient die within an average of 6-16 days. (α, β, γ,) amanitins and phallotoxins are compounds responsible for poisoning (Mas, 2005; Unverir et al., 2007). Activated charcoal use may be useful in adsorbing toxic compounds in the gastrointestinal tract. Hemoperfusion is required within 48 hours of consumption to remove toxin from the blood. For this reason, with the onset of symptoms, the treatment should be started immediately. However, continuous monitoring, fluid supplementation, and penicillin therapy are essential parts of the treatment of Phalloides syndrome. In addition, liver transplantation can be successfully applied in cases of severe poisoning and inadequate treatments (Donnelly and Wax, 2005; Karimi et al., 2011; Karimi and Razavi, 2014).

Rhabdomyolysis syndrome

The mushrooms responsible for the syndrome are *Tricholoma equestre* (L.) P. Kumm. and *Russula subnigricans* Hongo. Symptoms begin to appear within an average of 24-72 hours after consumption (Azzolina et al., 2011; Karimi and Razavi, 2014). Symptoms appear as diarrhea, nausea and vomiting. In severe cases of poisoning, muscle weakness, fatigue, muscle pain and rhabdomyolysis, kidney failure and metabolic acidosis are observed. Treatment is symptomatic (Karlson-Stiber and Persson, 2003; Beuhler and Graeme, 2005; Karimi and Razavi, 2014).

Cardiogenic effects of poisonous mushrooms

Mycetism cases are generally known to have serious toxic effects on the liver, kidneys and central nervous system. However, the effects of mushroom poisoning on heart functions have not been determined much. In previous studies, several cases of left ventricular systolic functions have been reported with increased cardiac biomarkers with increased cardiogenic shock in the absence of mycardism-related myocardial infarction (Unverir et al., 2007; Nieminen et al., 2008; Faulstich and Wieland, 2019). ECG abnormalities can often be seen in mycetism cases. ECG abnormality frequently seen in mycetism cases is sinus tachycardia. In addition, other ECG abnormalities are sinus arrhythmia, ST / T inversion, 1st degree AV block and QT prolongation. Inflammatory damage in the pericardial space may be the cause of tachycardia in mycetism cases. The cardiotoxic effects of mycetism are still not fully disclosed. The data in the literature are mainly based on several case reports and series. In a study on mice under consumption of *Tricholoma flavovirens* (Current name: *Tricholoma equestre* (L.) P. Kumm), plasma CK and CKMB activities and plasma bilirubin concentrations were higher than those in the control group. In addition, pericardial inflammation was observed from histological samples of long-term mushroom supplemented mice (Nieminen et al., 2008; Azzolina et al., 2011).

In a different study, it was reported that a mycetism case increased the level of troponin I (Avcı et al., 2014).

In another mycetism case, the patient's liver and kidney function tests found high amylase and cardiac levels. Cardiac markers may be elevated due to the fact that amatoxins bind actin filaments in myocardiocytes or kidney cells, or their effects as circulating antitroponin antibodies. Myocardiocytes or amatoxins that bind actin filaments in kidney cells may cause elevated heart markers. In addition, a temporary decrease in systolic function was observed in this reported mycetism case (Altıntepe et al., 2014). In another study, after 5 hours after mushroom consumption, the patient observed an increase in ST in low leads and high cardiac markers. As a result of angiogram, the coronary arteries were found at normal levels. It has been suggested that ECG changes and elevation of biomarkers in mycetism case may be related to temporary vasospasm (Kalcik et al., 2015). In cases of mycetism, hypertension or hypotension may occur. Hypotension can be seen due to its mechanism of action on the renin-angiotensin system. Hypertension may occur as a result of endothelial damage of toxins of vasoconstrictive agents (Kalcik et al., 2015).

C. atramentarius is a type of fungus that causes Coprinus syndrome. When this mushroom is consumed before or during alcohol consumption, erythema of the face and hands, swelling and rash on the hands, tachycardia, hypotension, dyspnea, nausea, vomiting and shock can be seen. The symptoms seen are similar to the symptoms seen when a patient with disulfiram, which is used to treat alcohol, is drinking alcohol. Disulfiram has been reported to be isolated from *C. atramentarius* (Stolman, 2013). In a study, reversible ECG changes were observed in most patients during the controls of patients receiving alcohol therapy. As a result of toxic interactions between disulfiram and alcohol, cardiac arrhythmias and myocardial infarction have been described (Jr Tyler, 1963; Stolman, 2013). Cardiac arrhythmias and myocardial infarction have been described by toxic interactions between disulfiram and alcohol, while in most patients observe reversible ECG changes during controlled reactions (Markham and Hoff, 1953; Wessely et al., 2007). Due to the similarities between these two reactions, it can be expected that disorders of heart function may occur after alcohol consumption of *C. atramentarius*.

Although edible mushrooms constitute a healthy food group for livings, cardiogenic harmful effects of poisonous mushrooms that are have been observed. Studies and observations on this subject are very limited. The number of cases in the case series with the subject is not sufficient. Despite few studies, the harmful effects of some mushrooms are known. In this study, the cardiological effects of poisonous mushrooms are emphasized.

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YAYIN İLKELERİ

SELÇUK ÜNİVERSİTESİ MANTARCILIK UYGULAMA Ve ARAŞTIRMA MERKEZİ'nin yayınladığı **MANTAR DERGİSİ (e-ISSN 2147 6845);** Ulusal veya Uluslararası Mikoloji alanıyla ilgili araştırma sonuçlarını içeren orijinal araştırma ve derleme makalelerin yayınlandığı elektronik HAKEMLİ bir dergidir.

Dergiye yayınlanmak üzere sunulan makaleler, Baş Editör tarafından konusu açısından dergide yayınlanmasının uygunluğuna karar verildikten sonra, derginin yazım kurallarına göre ön kontrolden geçirilir. Sonra Editör Kurulu aracılığı ile ilgili uzmanlık alanındaki hakemlere bilimsel yönden değerlendirilmek üzere gönderilir. Baş Editör, Bilimsel Hakemlerin eleştiri ve önerileri ile yazarın bunlara verdiği cevaplar doğrultusunda eserin yayınlanıp, yayınlanamayacağına karar verir. Yayınlanması uygun görülmeyen eserler hakkında yazarlara bilgi verilir. Yayınlanması uygun görülen eserlerin matbaa provası yazarlara gönderilir ve son kontrol okuması yapılır. Son okumada imla ve şekilsel hatalar dışında düzeltme veya ekleme yapılmaz. Derginin yayın dili Türkçedir. İngilizce dilinde de yayın kabul edilebilir.

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Sisteme yüklenen makalelerin bir an önce işleme alınabilmesi için; "Hakem Öneri Formu" ve "Son Kontrol Formu" renkli olarak taranmış şekilde sisteme ek dosya olarak yüklenmelidir.

MAKALE YAZIM KURALLARI

Makale, **A4 boyutunda,** kenarlarda **2 cm** boşluk bırakılacak şekilde, 1.5 **aralıklı**, **arial** yazı karakterinde, **10** punto kullanılarak Word 2003 veya daha üst sürümdeki programla yazılmalıdır. Makalenin word dosyasında otomatik numaralandırma kullanılmamalıdır. Makalenin bölümleri sırayla şöyle olmalıdır;

(Türkçe Makaleler için);

Türkçe Başlık, Yazarlar(sorumlu yazar belirtilmelidir) ve adresleri, E-postaları, Orcid numaraları, Öz, Anahtar kelimeler, İngilizce Başlık, Abstract, Key words, Giriş, Materyal ve Metot, Bulgular, Tartışma, Kaynaklar.

(İngilizce makaleler için);

İngilizce Başlık, yazarlar(sorumlu yazar belirtilmelidir) ve adresleri, E-postaları, Orcid numaraları, Abstract ve Key words, Türkçe Başlık, Öz ve Anahtar kelimeler, Introduction, Material and Method, Results, Discussion, References.

Derleme çalışmalarda da mevcut başlıkların (materyal ve metot hariç) kullanılması gerekir. Bulgular ve Tartışma başlıkları tek başlık altında verilebilir.

Yazar gerekli görürse alt başlıklar kullanabilir. Her bölüme ait başlıklar kalın ve ilk harfleri büyük yazılmalıdır. Metin içinde geçen tüm bilimsel isimler italik olmalı, eğer başlık içerisinde yer alıyorsa hem italik hem de kalın olmalıdır. Tür isimleri ilk geçtikleri yerde yazarlarıyla birlikte verilmelidir. Daha sonraki yerlerde sadece takson isimleri yazılmalıdır. Bölümler arasında bir satır boşluk bırakılmalıdır.

Başlık: Türkçe makalelerde; makalenin Türkçe başlığı 14 punto, İngilizce başlığı 12 punto, İngilizce makalelerde ise İngilizce başlık 14 punto, Türkçe başlık 12 punto, sadece baş harfleri büyük ve kalın olmalıdır. Yazar isimlerinin baş harfi ve soyadı büyük olmalı, adresler ismin altına yazılmalı, sorumlu yazarın e-mail adresi mutlaka belirtilmelidir. Akademik unvanlar makalede yer almamalıdır.

Anahtar kelimeler: 4-10 kelimeden oluşmalıdır.

Giriş: Araştırma konusu mümkün olduğu kadar güncel olarak kısa ve özlü değerlendirilir. Çalışmanın amacı da belirtilmelidir.

Kaynaklar: Kaynaklar metinde (soyadı, tarih) parantez içinde belirtilerek yazılmalıdır. Kaynaklar bölümü alfabetik sıraya göre 10 punto olarak yazılmalı ve yararlanılan eserlerin Yazar (yazarlarının) Adı ve soyadının ilk harfleri büyük olmalıdır. Kitap, makale ve tam metin yayımlanmış bildiri isimlerinin ilk harfleri büyük yazılmalıdır. **Lisansüstü tezler kaynak olarak gösterilemez.**

Kaynak künyeleri **APA stilinde** aşağıdaki sıraya uygun olarak yazılmalıdır (2019'dan itibaren).

Periyodik ise aşağıdaki örneğe uygun olarak:

Aktaş, S., Kaşık, G., Doğan, H. H. ve Öztürk, C. (2006). Two New Taxa Records for the Macrofungi of Turkey. *Tr.J.of Bot., 30* (4) 209-212.

Kitap ise aşağıdaki örneğe uygun olarak:

Kaşık, G., Öztürk, C., Doğan, H. H., Aktaş, S. ve Demirel, G. (2005). *Mikoloji Laboratuvarı*. Konya: Marifet Ofset Matbaa ve Kağıtçılık.

Bilimsel Toplantı kitabı ise aşağıdaki örneğe uygun olarak:

Önay, A. O., Kaşık, G., Alkan, S. ve Öztürk, C. (2018). *Pleurotus ostreatus*'un Misel Gelişmesine Humik Maddelerin Etkisinin Araştırılması. Ö. Türkmen ve M. Paksoy (Ed.), *II. International Eurasian Agriculture and Natural Sciences Congress Book of Full Text*, (ss.22-29). Bakü-Azerbaijan.

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S.Ü. Mantarcılık Uygulama ve Araştırma Merkezi Müdürlüğü

Mantar Dergisi Editörlüğü

Fen Fakültesi Binası B Blok Zemin Kat42079 Kampüs/KONYA E-posta: **mantarcilik@gmail.com**

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S.Ü. Mantarcılık Uygulama ve Araştırma Merkezi Müdürlüğü
Mantar Dergisi Editörlüğü
Fen Fakültesi Binası B Blok Zemin Kat42079 Kampüs/KONYA/TÜRKİYE E-posta: **mantarcilik@gmail.com**

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İÇİNDEKİLER(*CONTENTS*)

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Nisan 2021 *Cilt:12* *Sayı:1*
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