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Mantarcılık
Uygulama ve Araştırma
Merkezi
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Effects of Olive Mill Wastewater on Mycelial Growth of Some Macrofungi

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Abstract: Olive oil mills produce a liquid waste called olive black water in the olive oil production process. Olive mill waste water from factories specifically in Turkey and surrounding Mediterranean countries where olive oil is produced the most in the world is being disposed of into the environment such as seas and rivers without any precautions being taken. It was focused on determination of effect of olive mill waste water which containing high organic matter on mycelial growth of several macrofungi in this study. It was found that the mycelia of *Rhodonia placenta*, *Trametes versicolor* and *Pleurotus ostreatus* showed the best development in 5%, concentration of olive mill wastewater. On the other hand, *Schizophyllum commune* mycelia grew best in 10% concentration. The lowest mycelial growth rate for all of the fungal species were observed in 70% concentration. Our aim is to cultivate various mushroom species using blackwater with high organic matter content.

Keywords: Environment, Fungi, Mycelial, Olive, Olive Blackwater

Zeytin Karasuyunun Bazı Makromantarların Misel Gelişimi Üzerine Etkileri

Öz: Zeytin üretimininin dünyada en fazla olduğu ülkemiz ve diğer Akdeniz ülkelerinde bulunan zeytinyağı fabrikaları, üretim sürecinde zeytin kara suyu adı verilen bir sıvı atık üretmektedir. Zeytin karasuyunun deniz ve nehir gibi alıcı su kaynaklarına hiçbir önlem alınmadan atılması ciddi çevre sorunlarına neden olmaktadır. Bu çalışmanın amacı yüksek organik madde içeriğine sahip zeytin karasuyunun bazı makrofungusların misel gelişimine etkisinin belirlenmesidir. *Rhodonia placenta*, *Trametes versicolor* ve *Pleurotus ostreatus* misellerinin gelişimi %5 zeytin karasuyu içeren ortamlarda, *Schizophyllum commune* misellerinin gelişiminin ise %10 zeytin karasuyu içeren ortamlarda yüksek olduğu belirlenmiştir. Tüm mantarlarda en düşük misel gelişimin ise %70 zeytin karasuyu içeren ortamlarda olduğu gözlemlenmiştir.

Anahtar kelimeler: Çevre, Mantarlar, Misel, Zeytin, Zeytin Karasuyu

Introduction

Olive and olive oil are two important agricultural products in Mediterranean countries, and almost 95% of global olive oil is produced and consumed within these countries. Türkiye is also among the most important olive producing countries after Spain, Italy and Greece. With about one million hectares of olive land (approximately 123 million fruit bearing and 43 million non-fruiting olive trees), Türkiye is the second producer of table olives and the fourth in olive oil production in the world (Anonynus, 2018, 2019; Özaltaş et al., 2016). Olives and olive oil are produced primarily in the Aegean and Marmara regions, especially within the boundaries of Aydın, Balıkesir, Bursa, Çanakkale, İzmir, Manisa and Muğla provinces (Şengül et al., 2000).

During olive oil production process, olive mill wastewater (OMW) is also produced as a by-product.

Dispose of OMW to the environment without any precautions, serious environmental problems are encountered. Due to its high organic matter content, discharged black water decreases the dissolved oxygen concentration of natural water resources such as rivers, lakes and seas, very quickly. Therefore OMW becomes very difficult for all the macro and microorganisms to survive in their own habitats (Oruç, 1995). The dark color of the OMW also spoils the bright appearance of the water, and reproduction of photosynthetic aquatic plants and alg populations were decreased, or completely stopped by preventing the penetration of sunlight into the water.

The film layer formed the oil contained in OMW also prevents the penetration of atmospheric oxygen into the water (Şengül et al., 2000). Over time, only anaerobic microorganisms develop in the water and putrefaction begins. At the same time, it also causes soil pollution due to its acidic nature and high salt and phenolic content (Oruç, 2012).

The black water produced during the olive processing process is a highly complex mixture and contains organic and inorganic components as well as polyphenols, tannins, amino acids and other biochemical compounds. It is also known that the resulting water exhibits acidic properties and contains high amounts of organic acids and phenols. The release of these compounds into the environment can lead to serious problems with water resources and soil fertility (Diamantis et al., 2022). OMW is also rich in suspended solids (AKM), pectins, sugar, phenol compounds and vegetable oils. However, its environmental impacts have become more pronounced in recent years due to the significant increase in production in the last 35 years, the small sized and very scattered production facilities, and the direct discharge of OMW into the soil or groundwater. For this reason, the attention paid to the treatment of OMW has increased gradually (Rozzi & Malpei, 1997; Chowdhury et al., 2013; Galanakis, 2017). On the other hand, OMW has a high energy source potential due to the simple and complex sugars and aromatic compounds it contains (Oruç, 2012).

Many studies have been carried out in Türkiye to both reduce the phenol content of OMW and to use it as a waste (Zervakis et al., 1996; Yesilada et al., 1995; Yürekli et al.,1999; Yeşilada et al., 1999; Kahraman &Yeşilada 2001; Aypar et al., 2011; Özcan & Topçuoğlu 2001; Apohan & Yesilada 2017; Cibelli et al., 2017). On the other hand there is not any published report to investigate the effect of OMW on mycelial growth of macrofungi in Türkiye.

This study was conducted to investigate the effect of OMW mycelial growth mycelium oroduction by some fungal species, such as *Trametes versicolor* (L.) Lloyd (Turkish name: Hindikuyruğu), *Pleurotus ostreatus* (Jacq.) P. Kumm. (Turkish name: İstiridye mantarı), *Schizophyllum commune* Fr. (Turkish name: Kımuk), *Rhodonia placenta* (Fr.) Niemelä, K.H. Larss. & Schigel. (Turkish name: Ağaçpeteği) (Sesli ve ark., 2020).

Material and Metod

Mycelia of some macrofungi (Table 1), obtained from Muğla Sıtkı Koçman University Mushroom Research and Application Center (MUMMER), were inoculated to the media containing the OMW at different proportions (Table 1).

Table 1. The species and codes of macrofungi

Fungus code	Type name	Collection
1	*Rhodonia placenta*	MAD-698-R
2	*Trametes versicolor*	MAD-697
3	*Pleurotus ostreatus*	MUMMER-3
4	*Schizophyllum commune*	MUMMER-8

Five liters of OMW were obtained from an olive oil factory from Muğla. The OMW is composed of olive washing waters, olive pulp water, water added to olive paste in the centrifugation step, and water coming from washing extraction plants. The OMW stored at 4°C until used. Test solutions of different concentrations were prepared by adding distilled water to the OMW with the ratios given (Table 2).

Table 2. Experiment codes for the tested concentrations

Solution code	Waste fluid (%)	Distilled water (%)
Control	0	100
A	5	95
B	10	90
C	20	80
D	30	70
E	50	50
F	70	30

Malt extract agar (Merck, Germany) was prepared with each solution (X, A, B, C, D, E, F) as experiment medium. Media pH was adjusted to 6.0. After sterilization of the solutions for 15 minutes in an autoclave, set to 121°C and 1.5psi, they were poured to 10 cm petri plates and left to cool at room temperature. The petri dishes were put in an incubator at 35°C for 24 hours to control the presence of any contamination.

Fungal mycelial discs (6.0 mm) were placed in the centre of petri dishes contain prepared media. Then, the inoculated media were incubated at 28°C. The horizontal growth diameter of each fungus colony were measured with a caliper every 24 hours over a period of 7 days.

Results

The growth rate of fungal isolates were presented in figures 1-4.

The mycelium of *Rhodonia placenta* showed the best growth in A1 (5%) which was followed by B1 (10%), C1 (20%), D1 (30%), E1 (50%), X1 (0%) and F1 (70%) respectively (Fig. 1).

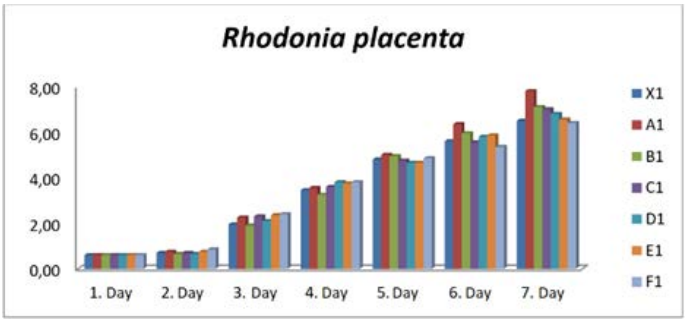

Figure 1. Growth of *Rhodonia placenta* during 7 days

The colony diameter of *Trametes versicolor* was the largest in A2 (5%) which was followed by the others within the order X2 (0%), B2 (10%), C2 (20%), D2 (30%), E2 (50%) and F2 (70%) (Fig. 2).

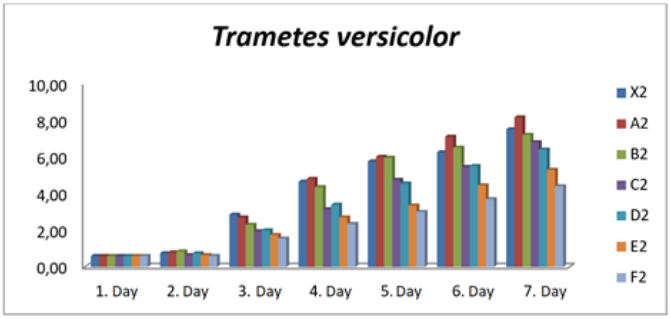

Figure 2. Growth of the fungus of the Turkey Tail (*Trametes versicolor*) over 7 days

Pleurotus ostreatus mycelium showed the most growth in A3 (5%) which was followed by B3 (10%), C3 (20%), X3 (0%), D3 (30%), E3 (50%), and F3 (70%) respectively (Fig. 3).

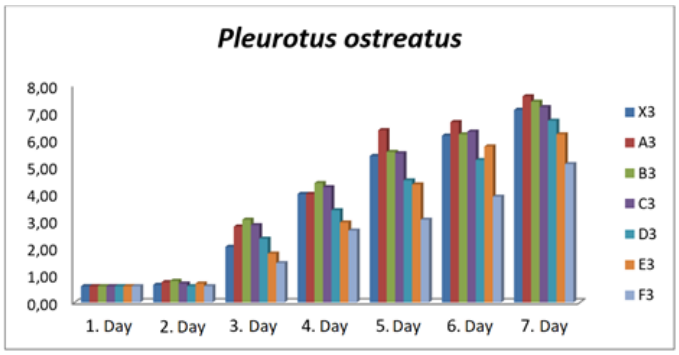

Figure 3. Growth of the fungus of the Oyster Mushroom (*Pleurotus ostreatus*) over 7 days.

The mycelium of *Schizophyllum commune* reached the largest growth diameter in B4 (10%) which were followed by C4 (20%), D4 (30%), E4 (50%), A4 (5%), X4 (0%) and F4 (70%) (Fig. 4).

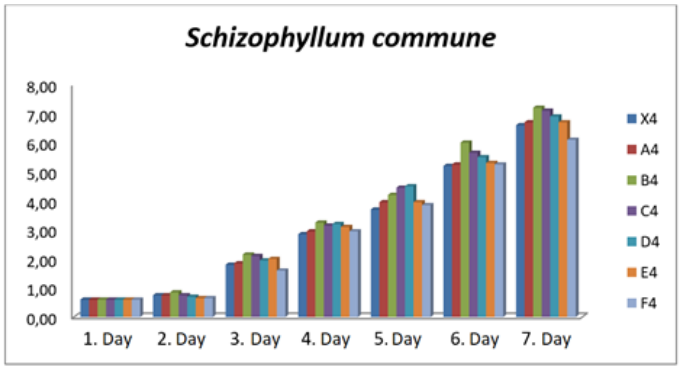

Figure 4. Growth of the fungus of Splitgill (*Schizophyllum commune*) over 7 days.

Discussions

Olive mill wastewater, mostly discharged from the olive oil factories in Türkiye and other Mediterranean countries, pose an important threat to aquatic environments by damaging living things and their ecosystems. In this study, the effect of olive mill wastewater on fungal mycelium growth was investigated.

It was found that, the mycelia of *Rhodonia placenta*, *Trametes versicolor* and *Pleurotus ostreatus* showed the best development in 5% (A) concentration of olive mill wastewater. On the other hand, *Schizophyllum commune* grew best in 10% (B) concentration. The lowest growth rate for all of the fungal mycelia were observed in 70% (F) concentration.

The data obtained for *Trametes versicolor* and *Pleurotus ostreatus* are in agreement with those reported by Galli et al., (1988) and Saiz-Jimmenez and Gomez-Alarcon (1986).

As a result of our study, the positive effect of OMW on fungal mycelial growth was seen as determined as a result of the research.

Since it has a possitive effect on mycelial growth of *Pleurotus ostreatus*, which is popularly known as oyster, beech or poplar mushroom, addition of olive mill wastewater to the compost of *P. ostreatus* as a nitrogen source will allow more efficiency to be obtained. In some countries, *T. versicolor*, *P. ostreatus* and *S. commune* have also been grown for medical purposes, and have been used in capsule form, especially in far east countries (Rogers, R. 2011; Hobbs, C.1986). An increase in the yield of the mushroom produced will also contribute to the economies of the countries in mediterranean basin.

Author Contributions

All authors have equal contribution.

Conflict of Interest

There is no conflict of interest with any institution or person

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Hakan ALLI).

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Contribution to the Macrofungal Biodiversity of Yahyalı District

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Abstract: This contributory study was carried out on the macrofungi samples collected from Yahyalı district of Kayseri province. As a result, 69 macromycete species belonging to 56 genera, 35 families and 7 orders within *Agaricomycetes* and *Pezizomycetes* were determined. Including the previously reported species, a total of 147 macromycete species were compiled from the region. The list of the determined species was provided together with their habitats, collection dates, voucher numbers, or the citations for those reported before.

Keywords: Mycobiota, taxonomy, Kayseri, Türkiye

Yahyalı Yöresi Makromantar Biyoçeşitliliğine Katkılar

Öz: Katkı niteliğindeki bu çalışma Kayseri ilinin Yahyalı ilçesinden toplanan makromantar örnekleri üzerinde gerçekleştirilmiştir. Sonuç olarak, *Agaricomycetes* ve *Pezizomycetes* sınıfları içinde yer alan 7 takım, 35 familya ve 56 cinse ait 69 makromantar türü tespit edilmiştir. Önceden rapor edilmiş türler de dahil edilerek bölgeden toplamda 147 makromantar türü derlenmiştir. Derlenen türler, habitatları, toplanma tarihleri, toplayıcı numaraları veya önceden rapor edilenlerin atıflarıyla birlikte verilmiştir.

Anahtar kelimeler: Mikobiyota, taksonomi, Kayseri, Türkiye

Introduction

Yahyalı is a district of Kayseri province, and situated between 37°40′-38°12′ northern latitudes and 35°06′-35°37′ eastern longitudes. The terrain generally exhibits a mountainous structure except for the southern parts of the Develi Plain, which also includes the Sultan Marshes National Park. Though step vegetation dominates the region, naturally growing forest areas exist in the southern parts of the district. Some local plantations also exist in the northern parts. *Salix* and *Populus* species are abundant along river and streamsides.

More than 2600 naturally growing macromycete taxa of Türkiye were presented as checklists (Sesli et al., 2020; Uzun, 2023). New contributions are being made to this number either as local lists (Çelik and Alma, 2023; Polat and Keleş, 2022; Kuru and Allı, 2023) or as new records (Çetinkaya and Uzun, 2021; Akçay et al., 2022, 2023; Şengül Demirak et al., 2022; Kaygusuz et al., 2023; Sesli, 2023a,b; Yeşilyurt et al., 2023).

Two lists (Kaşık et al., 2003; Türkoğlu and Gezer, 2006) and some new records (Türkoğlu et al., 2007) were presented related with the macromycetes of Yahyalı. But almost all of the reported taxa within both lists were addressed to the southern regions of Yahyalı. Except for five species which were reported from near Ağcaşar Dam, no collection were made from the northern regions of the district.

The study aims to determine the macromycetes growing in the northern regions of Yahyalı and make a contribution to the macrofungal biodiversity of Türkiye.

Material and method

Macrofungi samples were collected from different localities (Table 1) in northern regions of Yahyalı district, during routine visits between 2006 and 2021. Descriptive data related to macromorphology and ecology was based on the photographs and notes obtained during field works. Micromorphological data was obtained from the dry samples upon the investigations carried out under a light microscope. Identification was performed with the help of Breitenbach and Kränzlin (1984, 1986, 1991, 1995, 2000), Hansen and Knudsen (1997), Hausknecht (2009), Bessette et al. (1997, 2007), Bessette and Bessette (2006), Siegel and Schwarz (2016). The specimens are kept at Gazi University, Science Faculty, Department of Biology.

During compilation of the overall list, previously reported taxa were rechecked from IndexFungorum (2023), and the current names were included for those synonymized.

Table 1. Collection localities of the macrofungi samples

Loc. No	Locality name	Coordinates	Altitude (m)
1	Ağcaşar village	38°09′N-35°22′E	1100
2	Aladağlar, Ağsu	37°53′N-35°21′E	2000
3	Aladağlar, Düşmüş	37°57′N-35°20′E	1820
4	Aladağlar, Göğoluk	37°55′N-35°18′E	2250
5	Aladağlar, Kayaardı	37°59′N-35°24E	1700
6	Aladağlar, Köşkderesi	37°58′N-35°19′E	1810
7	Aladağlar, Kürsiyen	37°59′N-35°22′E	1780
8	Sekidağı	38°06′N-35°16′E	1450
9	Sekidağı	38°08′N-35°17′E	1320
10	Sekidağı	38°08′N-35°18′E	1340
11	Boyacılı quarter	38°06′N-35°22′E	1250
12	Boyacılı quarter	38°06′N-35°21′E	1160
13	Çadırkaya village	38°10′N-35°13′E	1150
14	Çağlayan village	38°04′N-35°18′E	1305
15	Çağlayan village	38°03N-35°18′E	1320
16	Çağlayan village	38°03N-35°17′E	1450
17	Çiğilli quarter	38°06′N-35°21′E	1160
18	Çubuklu village	38°09′N-35°16′E	1220
19	Derebağ village	38°04′N-35°17′E	1360
20	Derebağ village	38°04′N-35°18′E	1330
21	Göğnük	38°06′N-35°24′E	1340
22	İlyaslı village	38°10′N-35°18′E	1080
23	İsmet quarter	38°07′N-35°21′E	1150
24	Kirazlı village	38°04′N-35°19′E	1270
25	Kirazlı village	38°04′N-35°20′E	1240
26	Kirazlı village	38°04′N-35°21′E	1230
27	Kocahacılı village	38°11′N-35°23′E	1090
28	Kocahacılı village	38°11′N-35°24′E	1100
29	Mustafabeyli village	38°10′N-35°21′E	1110
30	Senirköy village	38°12′N-35°15′E	1078
31	Yenice quarter	38°04′N-35°25′E	1220
32	Yerköy village	38°11′N-35°21′E	1090
33	Yuları village	38°09′N-35°19′E	1100

Results

Ascomycota

Pezizomycetes

Pezizales

Caloscyphaceae

1. ***Caloscypha fulgens*** (Pers.) Boud.: Among needle litter in coniferous forest, locality 11, 27.04.2019, K. 15304.

Helvellaceae

2. ***Dissingia leucomelaena*** (Pers.) K. Hansen & X.H. Wang: Among needle litter in coniferous forest, locality 11, 27.04.2019, K. 15306.
3. ***Helvella acetabulum*** (L.) Quél.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Morchellaceae

4. ***Morchella conifericola*** Taşkın, Büyükalaca & H.H. Doğan: (Taşkın et al., 2016).
5. ***Morchella deliciosa*** Fr.: Among needle litter, locality 11, 27.04.2019, K. 15303.
6. ***Morchella elata*** Fr.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
7. ***Morchella esculenta*** (L.) Pers.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
8. ***Morchella mediterraneensis*** Taşkın, Büyükalaca & H.H. Doğan: (Taşkın et al., 2016).
9. ***Morchella semilibera*** DC.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
10. ***Morchella vulgaris*** (Pers.) Gray: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Pezizaceae

11. ***Sarcosphaera coronaria*** (Jacq.) J. Schröt.: Among needle litter in coniferous forest, locality 11, 23.05.2021, K. 15384.

Pyronemataceae

12. ***Geopora arenicola*** (Lév.) Kers: (Kaşık et al., 2003).
13. ***Geopora sumneriana*** (Cooke ex W. Phillips) M. Torre: Among needle litter in coniferous forest, locality 11, 23.05.2021, K. 15385.

Basidiomycota

Agaricales

Agaricaceae

14. ***Agaricus bresadolanus*** Bohus: (Türkoğlu and Gezer, 2006).
15. ***Agaricus campestris*** L.: On soil among grass, locality 9, 27.10.2019, A.Şahin 42.
16. ***Agaricus sylvicola*** (Vittad.) Peck: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
17. ***Agaricus xanthodermus*** Genev.: Among grass, locality 30, 27.04.2019, K. 15313.
18. ***Coprinus comatus*** (O.F. Müll.) Pers.: Among grass, locality 7, 14.05.2014, A.Şahin 26.
19. ***Lepiota clypeolaria*** (Bull.) P. Kumm.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
20. ***Lepiota cristata*** (Bolton) P. Kumm.: Meadow, locality 33, 27.04.2019, K. 15311.
21. ***Leucoagaricus leucothites*** (Vittad.) Wasser: Among grass, locality 1, 03.11.2018, A.Şahin 38.
22. ***Macrolepiota excoriata*** (Schaeff.) Wasser: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
23. ***Macrolepiota procera*** (Scop.) Singer: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Amanitaceae

24. ***Amanita vaginata*** (Bull.) Lam.: Among grass around *Quercus* sp., locality 16, 22.05.2021, K. 15367.

Bolbitiaceae

25. ***Conocybe apala*** (Fr.) Arnolds: Among grass, locality 19, 22.05.2021, K. 15375.
26. ***Conocybe aporos*** Kits van Wav.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
27. ***Conocybe deliquescens*** Hauskn. & Krisai: Meadow, locality 27, 27.04.2019, K. 15310.
28. ***Conocybe velata*** (Velen.) Watling: (Türkoğlu et al., 2007).

Cortinariaceae

29. ***Cortinarius obtusus*** (Fr.) Fr.: (Türkoğlu and Gezer, 2006).
30. ***Phlegmacium saginum*** (Fr.) Niskanen & Liimat.: (Kaşık et al., 2003).

Cyphellaceae

31. ***Chondrostereum purpureum*** (Pers.) Pouzar: On decaying *Populus* sp. trunk, locality 25, 26.10.2012, K. 7464.

Entolomataceae

32. ***Entoloma sinuatum*** (Bull.) P. Kumm.: (Kaşık et al., 2003).

Hygrophoraceae

33. ***Hygrocybe nigrescens*** (Quél.) Kühner: (Kaşık et al., 2003).
34. ***Hygrophorus agathosmus*** (Fr.) Fr.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
35. ***Hygrophorus chrysodon*** (Batsch) Fr.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Hymenogastraceae

36. ***Gymnopilus penetrans*** (Fr.) Murrill: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
37. ***Hebeloma eburneum*** Malençon: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
38. ***Hebeloma mesophaeum*** (Pers.) Quél.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
39. ***Psilocybe coronilla*** (Bull.) Noordel.: Among grass, locality 13, 27.04.2019, K. 15315.

Incertae sedis

40. ***Clitocybe odora*** (Bull.) P. Kumm.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
41. ***Clitocybe phyllophila*** (Pers.) P. Kumm.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
42. ***Clitocybe rivulosa*** (Pers.) P. Kumm.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
43. ***Cystodermella granulosa*** (Batsch) Harmaja: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
44. ***Crucibulum laeve*** (Huds.) Kambly: On decaying Populus sp. twigs, locality 14, 22.05.2021, K. 15374.
45. ***Cyathus olla*** (Batsch) Pers.: On decaying *Populus* sp. trunk, locality 20, 22.05.2021, K. 15377.
46. ***Lepista nuda*** (Bull.) Cooke: On soil among needle litter, locality 11, 23.05.2021, K. 15381.
47. ***Lepista personata*** (Fr.) Cooke: Among grass, locality 6, 05.05.2019, A.Şahin 39.
48. ***Leucocybe candicans*** (Pers.) Vizzini, P. Alvarado, G. Moreno & Consiglio: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
49. ***Leucocybe connata*** (Schumach.) Vizzini, P. Alvarado, G. Moreno & Consiglio: (Kaşık et al., 2003).
50. ***Lichenomphalia umbellifera*** (L.) Redhead, Lutzoni, Moncalvo & Vilgalys: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
51. ***Melanoleuca cognata*** (Fr.) Konrad & Maubl.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
52. ***Melanoleuca exscissa*** (Fr.) Singer: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
53. ***Melanoleuca graminicola*** Kühner & Maire: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
54. ***Melanoleuca stridula*** (Fr.) Singer: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
55. ***Melanoleuca substrictipes*** Kühner: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
56. ***Panaeolina foenisecii*** (Pers.) Maire: Meadow, locality, 27, 27.04.2019, K. 15309.
57. ***Panaeolus fimicola*** (Pers.) Gillet: (Kaşık et al., 2003).
58. ***Panaeolus olivaceus*** F.H. Møller: (Kaşık et al., 2003).
59. ***Panaeolus papilionaceus*** (Bull.) Quél.: On manured soil among grass, locality 18, 27.04.2019, K. 15314.

Inocybaceae

60. ***Inocybe cincinnata*** (Fr.) Quél.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
61. ***Inocybe curvipes*** P. Karst.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
62. ***Inocybe geophylla*** P. Kumm.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
63. ***Pseudosperma rimosum*** (Bull.) Matheny & Esteve-Rav.: (Kaşık et al., 2003).

Lycoperdaceae

64. ***Bovista plumbea*** Pers.: Among grass, locality 18, 22.05.2021, K. 15376.
65. ***Calvatia gigantea*** (Batsch) Lloyd: (Kaşık et al., 2003).
66. ***Lycoperdon molle*** Pers.: Among leaf litter under *Quercus* sp., locality 15, 22.05.2021, K. 15369.
67. ***Lycoperdon perlatum*** Pers.: Among leaf litter under *Quercus* sp., locality 16, 22.05.2021, K. 15370.

Lyophyllaceae

68. ***Lyophyllum transforme*** (Lapl.) Singer: (Kaşık et al., 2003).

Marasmiaceae

69. ***Crinipellis scabella*** (Alb. & Schwein.) Murrill: Among grass, locality 11, 23.05.2021, K. 15379.
70. ***Marasmius oreades*** (Bolton) Fr.: Meadow, locality 28, 25.05.2014, K. 8949.
71. ***Marasmius wynneae*** Berk. & Broome: Among leaf litter under *Quercus* sp., locality 15, 22.05.2021, K. 15373.

Mycenaceae

72. ***Mycena metata*** (Fr.) P. Kumm.: (Türkoğlu and Gezer, 2006).
73. ***Mycena polygramma*** (Bull.) Gray: (Kaşık et al., 2003).
74. ***Panellus stipticus*** (Bull.) P. Karst.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
75. ***Xeromphalina campanella*** (Batsch) Kühner & Maire: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
76. ***Xeromphalina junipericola*** G. Moreno & Heykoop: (Doğan and Karadelev, 2009).

Omphalotaceae

77. ***Gymnopus dryophilus*** (Bull.) Murrill: Among leaf litter under Quercus sp., locality 28, 24.05.2014, K. 8948; locality 16, 22.05.2021, K. 15368.

Paxillaceae

78. ***Paxillus involutus*** (Batsch) Fr.: Among grass, locality 5, 05.10.2014, A.Şahin 12.

Physalacriaceae

79. ***Armillaria mellea*** (Vahl) P. Kumm.: Around *Populus* sp. stump, locality 12, 27.10.2012, K. 7480; locality 16, 02.12.2019, A.Şahin 44.
80. ***Oudemansiella melanotricha*** (Dörfelt) M.M. Moser: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
81. ***Strobilurus tenacellus*** (Pers.) Singer: On decaying *Pinus* sp. cones, locality 1, 24.05.2014, K. 8951.

Pleurotaceae

82. ***Pleurotus eryngii*** (DC.) Quél.: On *Ferula* sp. remains, locality 21, 30.04.2016, A.Şahin 29.
83. ***Pleurotus ostreatus*** (Jacq.) P. Kumm.: On *Salix* sp. stump, locality 24, 26.10.2012, K. 7458.

Pluteaceae

84. ***Pluteus romellii*** (Britzelm.) Lapl.: On decaying twigs, locality 29, 27.04.2019, K. 15312.
85. ***Volvariella bombycina*** (Schaeff.) Singer: On *Populus* sp. stump, locality 27, 29.10.2014, A.Şahin 21.
86. ***Volvopluteus gloiocephalus*** (DC.) Vizzini, Contu & Justo: On soil among grass, locality 30, 28.05.2017, A.Şahin 31.

Psathyrellaceae

87. ***Candolleomyces candolleanus*** (Fr.) D. Wächt. & A. Melzer: Around *Salix* sp. stump, locality 2, 19.05.2012, A.Şahin 3; locality 12, 27.10.2012, K. 7481.
88. ***Coprinellus disseminatus*** (Pers.) J.E. Lange: Around decaying *Populus* sp, trunk, locality 12, 27.10.2012, K. 7478.
89. ***Coprinellus micaceus*** (Bull.) Vilgalys, Hopple & Jacq. Johnson: Around *Salix* sp., locality 22, 08.10.2006, K. 3820; around *Populus* sp. trunk, locality 25, 26.10.2012, K. 7457.
90. ***Coprinellus xanthothrix*** (Romagn.) Vilgalys, Hopple & Jacq. Johnson: On manured soil among grass, locality 17, 27.10.2012, K. 7476.
91. ***Coprinopsis atramentaria*** (Bull.) Redhead, Vilgalys & Moncalvo: Around decaying Salix sp. stump, locality 4, 18.06.2011, A.Şahin 1.
92. ***Coprinopsis nivea*** (Pers.) Redhead, Vilgalys & Moncalvo: On manured soil, locality 10, 21.06.2019, A.Şahin 40.

Schizophyllaceae

93. ***Schizophyllum commune*** Fr.: On decaying *Populus* sp. trunk, locality 27, 27.04.2019, K. 15308.

Strophariaceae

94. ***Agrocybe molesta*** (Lasch) Singer: (Kaşık et al., 2003).
95. ***Agrocybe paludosa*** (J.E. Lange) Kühner & Romagn. ex Bon: (Kaşık et al., 2003).
96. ***Agrocybe pediades*** (Fr.) Fayod: Among grass, locality 14, 27.04.2019, K. 15316.
97. ***Deconica coprophila*** (Bull.) P. Karst.: On cow manure, locality 3, 07.06.2014, A.Şahin 7.
98. ***Hypholoma fasciculare*** (Huds.) P. Kumm.: Around *Populus* sp. stump, locality 27, 16.11.2019, K. 15355.
99. ***Pholiota aurivella*** (Batsch) P. Kumm.: On *Salix* sp. stump, locality 26, 26.10.2012, K. 7467.
100. ***Pholiota limonella*** (Peck) Sacc.: (Türkoğlu and Gezer, 2006).
101. ***Pholiota populnea*** (Pers.) Kuyper & Tjall.-Beuk.: On *Populus* sp. stump, locality 28, 08.11.2014, A.Şahin 22.

Tricholomataceae

102. ***Tricholoma imbricatum*** (Fr.) P. Kumm.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
103. ***Tricholoma populinum*** J.E. Lange: Around *Populus* sp. stump, locality 22, 08.10. 2006, K. 3821.
104. ***Tricholoma terreum*** (Schaeff.) P. Kumm.: On soil among needle litter, locality 1, 27.04.2019, K. 15305.
105. ***Tricholoma virgatum*** (Fr.) P. Kumm.: (Kaşık et al., 2003).

Tubariaceae

106. ***Cyclocybe cylindracea*** (DC.) Vizzini & Angelini: Around *Salix* sp. stump, locality 29, 18.10.2014, A.Şahin 15.
107. ***Tubaria furfuracea*** (Pers.) Gillet: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Auriculariales

Incertae sedis

108. ***Guepinia helvelloides*** (DC.) Fr.: (Türkoğlu et al., 2007).

Boletales

Boletaceae

109. ***Neoboletus erythropus*** (Pers.) C. Hahn: (Kaşık et al., 2003).
110. ***Suillellus luridus*** (Schaeff.) Murrill: Around *Quercus* sp., locality 8, 01.07.2019, A.Şahin 41.

Diplocystidiaceae

111. ***Astraeus hygrometricus*** (Pers.) Morgan: Among leaf litter under *Quercus* sp., locality 16, 22.05.2021, K. 15371.

Gomphidiaceae

112. ***Chroogomphus rutilus*** (Schaeff.) O.K. Mill.: Among needle litter, locality 11, 23.05.2021, K. 15383.

Omphalotaceae

113. ***Omphalotus olearius*** (DC.) Singer: (Kaşık et al., 2003).

Rhizopogonaceae

114. ***Rhizopogon luteolus*** Fr.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Sclerodermataceae

115. ***Pisolithus arhizus*** (Scop.) Rauschert: On soil among grass, locality 28, 24.05.2014, K. 8950.
116. ***Scleroderma bovista*** Fr.: In soil among grass, locality 9, 27.06.2014, A.Şahin 10.

Suillaceae

117. ***Suillus grevillei*** (Klotzsch) Singer: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

118. ***Suillus luteus*** (L.) Roussel: Among grass under *Pinus* sp., locality 1, 27.04.2019, K. 15307.

Geastrales

Geastraceae

119. ***Geastrum fimbriatum*** Fr.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Gomphales

Clavariadelphaceae

120. ***Clavariadelphus truncatus*** Donk: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Gomphaceae

121. ***Ramaria flava*** (Schaeff.) Quél.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Hymenochaetales

Hymenochaetaceae

122. ***Inocutis rheades*** (Pers.) Fiasson & Niemelä: (Kaşık et al., 2003).
123. ***Inonotus hispidus*** (Bull.) P. Karst.: On *Malus* sp. stump, locality 26, 26.10.2012, K. 7466; locality 31, 18.10.2014, A.Şahin 16; locality 32, 17.11.2019, K. 15358.
124. ***Phellinus igniarius*** (L.) Quél.: On *Salix* sp. stump, locality 24, 26.10.2012, K. 7459.

Hyphodontiaceae

125. ***Kneiffiella floccosa*** (Bourdot & Galzin) Jülich & Stalpers: (Doğan et al., 2011).

Incertae sedis

126. ***Trichaptum abietinum*** (Pers. ex J.F. Gmel.) Ryvarden: (Kaşık et al., 2003).

Polyporales

Fomitopsidaceae

127. ***Fomitopsis pinicola*** (Sw.) P. Karst: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).

Laetiporaceae

128. ***Laetiporus sulphureus*** (Bull.) Murrill: On *Salix* sp. stump, locality 27, 08.11.2014, A.Şahin 24.

Phanerochaetaceae

129. ***Bjerkandera adusta*** (Willd.) P. Karst.: On decaying *Populus* sp. trunk, locality 12, 27.10.2012, K. 7479.

Polyporaceae

130. ***Cerioporus squamosus*** (Huds.) Quél.: (Kaşık et al., 2003).
131. ***Ganoderma adspersum*** (Schulzer) Donk: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
132. ***Lentinus arcularius*** (Batsch) Zmitr.: On decaying *Quercus* sp. twigs, locality 15, 22.05.2021, K. 15372.
133. ***Lentinus brumalis*** (Pers.) Zmitr.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
134. ***Lentinus tigrinus*** (Bull.) Fr.: Around decaying *Populus* sp. trunk, locality 12, 16.11.2019, K. 15354.
135. ***Neolentinus cyathiformis*** (Schaeff.) Della Magg. & Trassin.: Around *Quercus* sp. stump, locality 29, 19.05.2015, A.Şahin 27.
136. ***Neolentinus lepideus*** (Fr.) Redhead & Ginns: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
137. ***Trametes gibbosa*** (Pers.) Fr.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
138. ***Trametes pubescens*** (Schumach.) Pilát: On decaying *Prunus* sp. twigs, locality 25, 26.10.2012, K. 7463.
139. ***Trametes*** trogii Berk.: On decaying *Populus* sp. trunk, locality 22, 08.10.2005, K. 3819.

Russulales

Russulaceae

140. ***Lactarius deliciosus*** (L.) Gray: On soil, among needle litter, locality 11, 16.11.2019, K. 15352.
141. ***Russula delica*** Fr.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
142. ***Russula grisea*** Fr.: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
143. ***Russula olivaceoviolascens*** Gillet: (Türkoğlu et al., 2007).

Stereaceae

144. ***Stereum hirsutum*** (Willd.) Pers.: On decaying *Populus* sp. trunk, locality 25, 26.10.2012, K. 7471.

Thelephorales

Bankeraceae

145. ***Boletopsis leucomelaena*** (Pers.) Fayod: (Kaşık et al., 2003; Türkoğlu and Gezer, 2006).
146. ***Hydnellum glaucopus*** (Maas Geest. & Nannf.) E. Larss., K.H. Larss. & Kõljalg: (Kaşık et al., 2003).

Thelephoraceae

147. ***Thelephora terrestris*** Ehrh. ex Fr.: Among needle litter, locality 11, 23.05.2021, K. 15380.

Discussion

Sixty nine macromycete species belonging to 56 genera, 35 families and 7 orders were determined. Fourty three of them are new for the district. New localities were presented for the remaining 26 species. The compilation of the determined and previously reported taxa revealed a list of 147 macrofungi species growing within the boundaries of Yahyalı district.

Thirteen of them (%8.84) belong to *Ascomycota* and 134 (%91.16) to *Basidiomycota.* The determined taxa are distributed in 10 orders (*Agaricales* 94, *Pezizales* 13, Polyporales 13, Boletales 10, Hymenochaetales 5, Russulales 5, *Thelephorales* 3, *Gomphales* 2, *Auriculariales* 1, *Geastrales* 1) (Fig. 1).

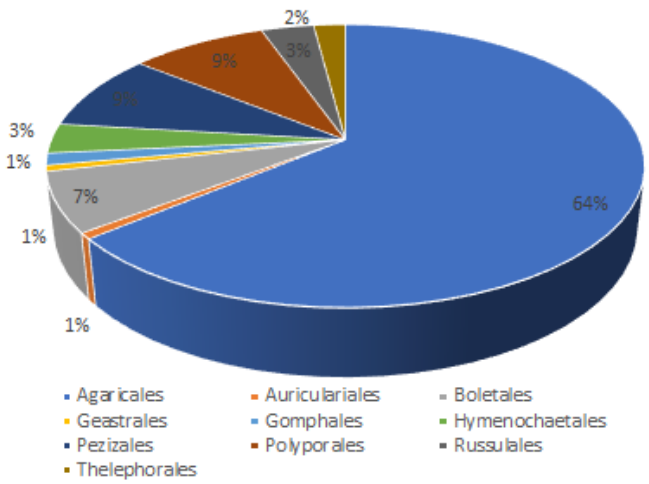

Figure 1. Order-wise distribution of the compiled species

Twenty two of the compiled 147 species are currently in Incertae sedis position. The remaining 125 taxa are distributed in 47 families. *Agaricaceae* and *Polyporaceae* are the two most crowded families in the region each with 10 species. They were followed by *Strophariaceae, Morchellaceae, Psathyrellaceae* and *Mycenaceae* with 8, 7, 6 and 5 taxa respectively. *Bolbitiaceae, Hymenogastraceae, Inocybaceae, Lycoperdaceae, Russulaceae* and *Tricholomataceae* were found to comprise 4 species in the region. *Hygrophoraceae, Hymenochaetaceae, Marasmiaceae, Physalacriaceae* and *Pluteaceae* followed them each with 3 taxa. Ten families (*Bankeraceae, Boletaceae, Cortinariaceae, Helvellaceae, Omphalotaceae, Pleurotaceae, Pyronemataceae, Sclerodermataceae, Suillaceae, Tubariaceae*) are resembled in the region with 2 taxa, while the remaining 20 families are resembled with only one taxon.

According to the compiled list, *Morchella* is the most crowded genus in the region with 7 species. It is followed by *Melanoleuca* with 5 species. In the region, four of the genera (*Agaricus, Clitocybe, Conocybe, Tricholoma*) are resembled with 4 taxa, eight of them (*Agrocybe, Coprinellus, Inocybe, Lentinus, Panaeolus, Pholiota, Russula, Trametes*) are represented with 3 taxa and 15 of them (*Coprinopsis, Geopora, Hebeloma, Hygrophorus, Lepiota, Lepista, Leucocybe, Lycoperdon, Macrolepiota, Marasmius, Mycena, Neolentinus, Pleurotus, Suillus, Xeromphalina*) are represented with 2 taxa while the remaining 65 genera are resembled with only one taxon.

The compiled list bare similarities to some extend with those studies (Kaşık et al., 2001, 2002a,b; Atila and Kaya, 2013; Çevik et al., 2021; Berber et al., 2022) carried out in neighboring regions. This similarity would be due to the similarities of flora and vegetations of the regions.

The study contributes to the macrofungal biodiversity of Yahyalı district by adding 43 taxa, new for the district, and new localities for 26 previously reported taxa. The compilation of overall list of macromycetes growing in Yahyalı district is also another contribution.

Author Contributions

All authors have equal contribution.

Conflict of Interest

There is no conflict of interest with any institution or person.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Abdülkadir ŞAHİN, Yasin UZUN, Abdullah KAYA).

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Assessment of Some Nutrient Contents and Heavy Metal Accumulation in Some Wild Edible Mushrooms in Türkiye

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Abstract: In this study, it was aimed to identify mushrooms gathered from two different regions and localities of Türkiye and to determine their heavy metal and nutrient contents. Four of the mushrooms (*Coprinus comatus* (O.F. Müll.) Pers, *Cantharellus cibarius* Fr., *Pleurotus ostreatus* (Jacq.) P. Kumm. and *Lactarius glyciosmus* (Fr.) Fr.) from nearby settlements, while the others (*Hydnum repandum* L., *Pleurotus eryngii* (DC.) Quél and *Lactarius delicious* (L.) Gray)) were collected from rural areas. All species have known and consume by local peoples. All identified species were given along with their trophic status, habitats, locations, Turkish names and edibility. Concentrations of elements were determined based on dry weight. The analysis of samples indicated that different result obtained from mushrooms. As (nd, 1.5-17.43), Ba (1.48-10.81), Cd (nd, 1.4-43.46), Co (nd, 12.0- 42.79), Cr (nd, 5.0-14.92), Cu (12.95-143.45), K (nd, 30085- 52680), Mg (nd, 5056-5955.9), Mn (52.45- 187.25), Mo (nd, 1.22-57.53), Ni (43.46-565), Pb (318.9-1483.5), Sb (nd, 0.14-4.12), Si (nd, 3.18-87.83), Ti (20.32-302.2), V (67.66-102.3), Zn (1026.8-2422.0), Ca (411.5 -2077), Na (752.5-2105.5) and Fe (470.5-1093.5) were determined and the elements studied were given in mg/kg. As a result, it was determined that *C. comatus*, *H. repandum*, *C. cibarius* and *P. eryngii* had the lowest content of heavy metals and *P. ostreus* had the highest value in terms of calcium and magnesium contents. Therefore, it has been determined that these mushroom species may have important beneficial effects to human health. It is thought that it can be used as a source in future studies.

Keywords: Atomic absorption, Food analysis, Heavy metals, Minerals, Mushrooms

Türkiye'de Yenilen Bazı Yabani Mantarlarda Bazı Besin İçerikleri ve Ağır Metal Birikiminin Değerlendirilmesi

Öz: Bu çalışmada, Türkiye'de iki farklı bölge ve lokalitelerinden toplanan yenilebilir yabani bazı mantar örneklerinin teşhis edilmesi ve ardından ağır metal ve besin içeriklerinin belirlenmesi amaçlanmıştır. Bu mantarların dördü (*Coprinus comatus* (O.F. Müll.) Pers*, Cantharellus cibarius*

Fr*, Pleurotus ostreatus* (Jacq.) P. Kumm. ve *Lactarius glyciosmus* (Fr.) Fr.) yakın yerleşim yerlerinden, diğerleri ise (*Hydnum repandum* L.*, Pleurotus eryngii* (DC.) Quél *ve Lactarius deliciosus* (L.) Gray) kırsal alanlardan toplanmıştır. Tüm türler yerel halk tarafından tanınmakta ve besin amaçlı tüketilmektedir. Teşhis edilen tüm türlerin, trofik durumları, habitatları Türkçe adları ve yenilebilirlikleri ile birlikte verilmiştir. Element konsantrasyonları kuru ağırlığa göre belirlenmiştir. Analiz edilen mantar türlerinden elde edilen sonuçların farklılık gösterdiği belirlenmiştir. Sonuçlara göre; As (nd, 1,5-17,43), Ba (1,48-10,81), Cd (nd, 1.4-43.46), Co (Nd, 12.0- 42.79), Cr (nd, 5.0-14.92), Cu (12.95-143.45), K (nd, 30085- 52680), Mg (nd, 5056-5955.9), Mn (52.45- 187.25), Mo (nd, 1.22-57.53), Ni (43.46-565), Pb (318.9-1483.5), Sb (nd, 0.14-4.12), Si (nd, 3.18-87.83), Ti (20.32-302.2), V (67.66-102.3), Zn (1026.8-2422.0), Ca (411.5 -2077), Na (752.5-2105.5) ve Fe (470.5-1093.5) belirlenmiş ve incelenen elementler mg/kg olarak verilmiştir. Sonuç olarak, ağır metaller bakımından en düşük değere sahip mantarlar: *C. comatus*, *H. repandum*, *C. cibarius* ve *P. eryngii,* kalsiyum ve magnezyum içeriği bakımından ise en yüksek değere ise *P. ostreus*'un sahip olduğu belirlenmiştir. Dolayısıyla bu mantar türlerinin insan sağlığı açısından önemli potansiyele sahip olduğu görülmektedir. Daha sonra yapılacak ilgili çalışmalarda kaynak olarak kullanılabileceği düşünülmektedir.

Anahtar Kelimeler: Atomic absorption, Besin İçeriği, Ağır Metaller, Mineraller, Mantarlar

Introduction

Fresh or dried macrofungi cooked in various ways are consumed as a nutrient. Mushrooms give flavor to the food thanks to their unique flavors and tissues. Although natural fungi are thought to have lower nutritional properties than vegetables, they have a high nutritional value in many foods. Even some species that are able to breed, such as meat, eggs, and milk show comparable significant nutritional properties (Boa, 2004). Natural mushrooms are very rich in terms of protein, amino acids, vitamins, minerals, and carbohydrates content. Button mushrooms are healthy food sources because they are a good source of bioactive compounds such as protein, vitamins, polyphenolics and minerals (Cavusoglu et al., 2021; Şaran et al., 2022).They also have low-calorie values due to containing almost no fat (Agahar-Murugkar, Subbulakshmi, 2005). *P. eryngii* may contain chemicals that strengthen into the connective tissue (Nozaki et al., 2008) *P*. *eryngii* can work as a cholesterol-lowering agent of the intake of nutrients in the diet (Jumpup Alam et al., 2011). It has been shown in a preliminary study that consumption of *P. ostreatus* (oyster mushroom) reduces cholesterol levels by an effect associated with beta-glucan content (Rop et al., 2009). *P*. *ostreatus* and *P*. *eryngii* are sold in local markets in Van province. *P*. *eryngii* is known as a heliz or mantis mushroom in Van. *P*. *ostreatus* is known as poplar mushroom or oyster mushroom in this region. *H. repandum* is a mycorrhizal fungi (Jumpupto et al., 2002) whose fructifications are grown alone or forming groups under coniferous trees or non-evergreen (Arora, 1986; Sterry and Hughes, 2009). It is known as a anchusa mushroom in Ordu territory.

The nutrient and chemical composition of *H. repandum* as a common edible species has been the subject of various scientific studies. *H. repandum* fructifications include 10.7% moisture and 9.2% cinders. The organic acid composition (54%) (100 grams of mushroom, 0.31 grams per dry weight) contains citric acid and malic acid (Ayaz et al., 2011). In another study, percentages of essential amino acids were evaluated as follows: valine, 3.9 %; leucine, 14.5%; carbohydrate, 3.2 %; threonin, 4.4 %; lysine, 4.2 %; trytophane, 1.4 %. The content of lipid (expressed as percentage of dry matter) was found 4.7 %. As big fatty acids, oleat was (20.3 %); lineoleate (47.5 %); linolenate (23.9 %); three dimensional (0.9 %), stearate (4 %). Mycosterol content is 628 grams of ergosterol and was recorded as 85 mg of fungisterol per 100 grams of dry substance. It has been reported that Chanterellus species contain antioxidants, amino acids, beta carotene, and canthaxanthin, and also contain significant amounts of vitamin D (Pilz et al., 2003). It is known as chicken tirmiti mushroom in Ordu area.

L. deliciosus in Türkiye is very popular mushroom. Pine forests in the Eastern Black Sea region constitute a suitable habitat for these species. It is known as the name of Kanlıca or Çıntar throughout the Black Sea region and in the Ordu area. *L. glyciosmus* is known as hazelnut tirmitii. *H. repandum*, chanterelle, *Lactarius delicious*, and *L. glycosomes* are sold in local markets in the Ordu province of Türkiye. *P. ostreatus* and *P. eryngii* are sold in local markets in Van province of Türkiye. *C. comatus* is known as ink mushroom or horse tail mushroom. Not only do mushrooms all over the world gain merely nutritious properties and taste but also have a medical value in terms of their chemical structure and functional functions (Kalač, 2009).

Mushrooms produce secondary metabolites with a variety of interesting biological activities, and are seen as an important potential for the discovery of new drugs. Many types of macrofungi are used as therapeutic agents

in diseases such as gastric cancer, cardiovascular diseases, tuberculosis, liver, heart diseases, inflammation, back pain, gonorrhea, bleeding, abdominal pain, and diabetes (Chang and Miles, 1989). These therapeutic biological activities are mediated by polysaccharides found in fungi such as especially beta glucans. There are many publications in the literature about the isolation and biological activity of polysaccharides produced by medicinal fungi. It is well known that the pharmacological effects and therapeutic potentials of these compounds are important for human health (Barros et al., 2007). Certain macrofungi are found to harbour copper, cadmium, mercury, lead, arsenic, cobalt, iron and nickel in high concentrations (Tyler, 1982; Kalač and Svoboda, 2000). Lalotra P. and his colleagues found that *Amanita augusta* and *Boletus subvelutipes* mushrooms carried heavy metal in extreme quantities. Many researchers have been carried out on metal contents of mushrooms especially for edible fungi (Demirbas, 2000; Lepsova and Majestrı'k, 1988). The heavy metal concentration in the fungi is a reflection of the pH and organic matter content of the soil (Gast et al., 1988).

It is thought that present study has supported previous studies and will contribute to future studies because the studies investigating the heavy metal and nutrient contents of the mushrooms are important and some species in the present study have different results each other.

Material and Metod

Macrofungi samples (*H. repandum*, *C. comatus*, *L. delicious*, *C. cibarius*, and *L. glycosomes*, *P. ostreatus* and *P. eryngii*) were collected in Türkiye's Ordu and Van provinces between 2015 and 2016 years. Diagnosis of fungal specimens was made using (Phillips, 1981; Moser, 1983; Breitenbach and Karnzlın, 1984-2000; Buczacki, 1989; Bresinsky and Besl, 1990; Jordan, 1995; Kibby, 1997) reference sources for macroscopic and microscopic data obtained using mycology techniques. The types of fungi used in this study, trophic status, habitats, locations, Turkish names and their edibility are given in Table 1.

Experimental

Samples of dried mushrooms were crushed in porcelain mortar. Grinded samples were sifted out on a 75 mesh sieve. After weighing to 1 gram (2 repeats), they were taken into the proselen crucibles. 2 ml ethanol/H_2SO_4 (95,5% by volume) were added on each of them. Muffule furnace heated to 550 °C was burned until burning to ashes (3 hours). 4 mL of 3 N HCl were added to the samples removed from the oven. Pure distilled water was added until the final volume of the supernatant was 25 mL. The blue band was filtered on the filter paper. The blue band was filtered through the filter paper. The prepared solution was read by AAS (Atomic Absorption spectrophotometer). Heavy metal and nutrient concentrations of mushroom samples were determined.

Tablo 1. Code, Family and Species, Trophic status, location, habitat, Turkish name and edibility of mushroom species (Sesli ve ark.,2020) i

Code	Family and species	Tropic Status	Location	Habitat	Turkish Name	Edibility
1	Cantharellaceae J.Schröt. ***Cantharellus cibarius*** Fr.	Mycorrhizal	Ordu, Kabadüz	Under *Corylus maxima*	Sarıkız Mantarı	Edible
2	Russulaceae Lotsy ***Lactarius deliciosus*** (L.) Gray	Mycorrhizal	Ordu, Kabadüz	In pine forest	Kanlıca Mantarı	Edible
3	***Lactarius glyciosmus*** (Fr.) Fr.	Mycorrhizal	Ordu, Kabadüz	Under *Corylus maxima*	Tatlı Sütlüce Mantarı	Edible
4	Hydrangea Chevall. ***Hydnum repandum*** L.	Mycorrhizal	Ordu, Kabadüz, Çambaşı	Deciduous and coniferous wood	Sığır Dili Mantarı	Edible
5	Pleurotaceae Kühner. ***Pleurotus eryngii*** (DC.) Quél.	Saprotrophic	Van, Gürpınar	It grows in association with the roots, *Ferula* sp.	Çakşır Mantarı	Edible
6	***Pleurotus ostreus*** (Jacq.) P. Kumm.	Saprotrophic	Van, Edremit	On stumps of *Populus* sp.	İstiridye Mantarı	Edible
7	Agaricaceae Chevall. ***Coprinus comatus*** (O.F. Müll) Pers.	Saprotrophic	Van, Merkez	In meadows	Söbelen Mantarı	Edible

Results and Discussions

All metal concentrations were determined as dry weight. Arsenic value was the highest in *P. ostreatus* (17.43±0.002 mg/kg) and the lowest value of as was found in *H. repandum* (1.5±0.002 mg/kg). Nothing was found in *C. cibarius*. Cd value was the highest in *P. ostreatus* (17.43±0.002 mg/kg) and the lowest for *P. eryngii* (1.4±0.0001 mg/kg). Cd value was not found in *L. glyciosmus*. Co value was the highest in *P. ostreatus* (42.79±0.0004 mg/kg) and the lowest for *C. cibarius* (12±0.0002 mg/kg). It was not found in *H. repandum*. *C. cibarius* (143.45±0.003 mg/kg) collected from the settlement areas was found the highest concentration of

Cu, and also *P. eryngii* (12.95±0.0027 mg/kg) collected from rural area had the lowest concentration. The highest of Fe concentration was found to be 1093.5 ± 0.0027 mg/kg in *P. ostreatus* collected from settlement area and was found to be 258.5 ± 0.0027 in *H. repandum* collected from the most rural area. Mn concentration of *L. glyciosus* collected from settlement area was found to be 187.25 ± 0.002 mg/kg and the lowest concentration of *C. cibarius* was found to be 52.45 ± 0.00035 mg/kg. The highest concentration of Ni was found in the settlement areas of *L. glycosomes* (565.8±0.0008 mg/kg) and the *H. repandum* (43.46±0.0002 mg/kg) collected from the rural areas was the lowest. The highest Pb concentration was found in the *L. glycosomes* (1483.5±0.005 mg/kg) collected from the settlement areas and the *P. eryngii* (318.9±0.0015 mg/kg) collected from rural areas was found the lowest value. The highest concentration of Zn was found in *C. cibarius* collected from the settlement areas and the *H. repandum* collected from the rural areas was the lowest concentration.

In this study, the accumulation of 16 heavy metals (As, Ba, Cd, Co, Cu, Cr, Fe, Mn, Mo, Ni, Pb, Sb, Si, Ti, V and Zn) and nutrients (Ca, K, Mg and Na) contents were investigated in 7 wild edible mushrooms (*C. cibarius*, *L. glyciosmes*, *H. repandum*, *C. comatus*, *P. eryngii*, *P. ostreatus* and *L. delicious*). The average heavy metal concentration and nutrients in the sporocarp of the wild-grown edible mushrooms is given in Table 2.

In addition to their nutritional values, mushrooms change their element content depending on the substrate content they use, as they play a role in organic matter breakdown in nature (Sevindik et al., 2015; Sevindik et al, 2018). For this reason, heavy metal concentrations of mushrooms are much higher than agricultural crops, fruits and vegetables (Liu et al., 2015). Although heavy metals such as iron (Fe), cobalt (Co), copper (Cu), manganese (Mn), chromium (Cr) and zinc (Zn) are required for living things, arsenic (As), cadmium (Cd) and Heavy metals such as lead (Pb) are considered harmful (Liu et al., 2015).

Arsenic has a carcinogenic effect when taken in excessive amounts to the human body, while dermatitis problems and allergic effects may occur if nickel is taken too much (Okut, 2019). The amount of arsenic was found in the range of 1.5 - 17.43 mg/kg (Koch et al., 2000). When Ni content was examined, it varied between 43.46 - 408 mg/kg. It was found to be relatively higher than the results of 44.6-127 (Demirbaş, 2001).

Barium is an element that is directly effective in human nutrition and Ba and Sb amounts in our study support the results of Koyyalamudi et al. (2013), which is a similar study.

High levels of Cd can lead to cancer, diarrhea, stomach problems, and death-affecting effects on the central nervous system. The amount of Cd was similar to the study of Tüzen et al. (2007) in *P. eryngii* cultivar and it was observed that the value in *Hydnum repandum*, *C. comatus*, *C. cibarius*, *L. delicious*, and *P. ostreatus* varieties were higher.

Co is one of the essential elements for the human body in small amounts and skin problems are encountered especially in its deficiency. Co element was found to be between 12 and 42.79 mg/kg in our study and it was found to be higher than the values determined in similar studies (Sarıkürkçü et al., 2011; Sevindik et al., 2015; ilker et al., 2019).

Because of its ability to increase glucose tolerance in type-2 diabetes mellitus patients (Anderson, 2000), chromium is considered essential to man. The recommended dietary intake for chromium is 0.035 mg / day for male and 0.025 mg/day for the female (Anonymous, 2001). Mushrooms could be thought as a potential source of this element. When the intervals determined in terms of the concentration of Cr ions are compared with previous studies, it was found to be similar. (Sarıkürkçü et al., 2011; Sevindik et al., 2015; ilker et al., 2019).

Copper plays a role with iron in the activity of the cytochrome oxidase enzyme. This activity is transformed into Cu + and Cu ++ and transports electrons to oxygen. It is present in the active group of the lysyl oxidase enzyme. This enzyme assists in cross-linking between collagen, elastin, and polypeptides. Besides catalase, phenyloxidase, and ascorbic acid oxidase, it is also necessary for iron to be used regularly in the body. Iron does not bind hemoglobin without copper (Çavuşoğlu, 2018). The difference concentration value of copper was seen as a significant result both in rural areas and in rural areas and in residential areas. It has been reported that the copper concentration in fungus does not constitute a risk for human health incase of 100-300 mg/kg in dry material (Kalač and Svoboda, 2000). The difference concentration value of copper was seen as a significant result both in rural areas and in residential areas. It has been reported that the copper concentration in fungus does not constitute a risk for human health incase of 100-300 mg/kg in dry material (Kalač and Svoboda, 2000). The amount of Cu varied between 12.95 - 143.45 mg/kg in our study and the results found in similar studies were 10-70 mg/kg (Kalač, 2009), 18.9-64.8 mg/kg (Tüzen et al., 2009), 10.3-145 mg/kg (Sesli and Tüzen, 1999; Işıloğlu et al., 2001), 10.60- 144.20 mg/kg (Yamaç et al., 2007), 11.6-41.9 mg/kg (Demirbaş, 2001), 3.80-32.6 mg/kg (Ouzouni et al., 2007) and 8.2-19.3 mg/kg (Colak et al., 2007).

Potassium is the main component of fluids in the cells. It provides acid-base balance, regulates blood pressure, acts in the transmission of nerve stimuli, and is effective in muscle contraction. When the K value is examined, *H. repandum*, *C. comatus*, *C.* can not be detected in the varieties, as a matter of fact, similar studies conducted by the researchers support this (Akın et al., 2019; Mendil et al., 2005; Sesli, 2007; Sesli and Dalman, 2006; Tuzen et al., 2007; Turkmen and Budur, 2018). Values determined in *L. delicious*, *L. glycosomes*, *P. ostreatus* and *P. eryngii* varieties are in the range of 30085-52680 mg/kg and are similar to the studies of other researchers (Demirbaş, 2001; Sesli, 2006; Sesli and

Tuzen, 2006; Pekşen et al., 2007; Pekşen et al., 2008; Ayaz et al., 2011a; Ayaz et al., 2011b; Turfan et al., 2018).

Magnesium regulates energy metabolism in the body and the working of muscle and nervous systems, and helps in the forming of bones and teeth and in the regulation of blood pressure (Samur, 2008). In the *C. comatus*, *P. ostreatus* mushroom species, the Mg value is found to be 5056 - 5955 mg/kg, respectively (Pekşen et al., 2007; Ayaz et al., 2011a; Akın et al., 2019), showing the results of the researchers. In *H. repandum*, *C. cibarius*, *P. eryngii*, *L. delicious*, *L. glycosome*s mushroom species, Mg value is both in our study and in similar studies conducted by researchers (Mendil et al., 2005; Sesli and Dalman, 2006; Sesli, 2007; Tuzen et al., 2007; Turkmen and Budur, 2018)

Manganese has an important role in growth and reproductive functions, carbohydrate and lipid metabolism, protein synthesis, mucopolysaccharide production, phosphorylation, and bone formation. Mn ion concentrations were 12.9-93.3 mg/kg (Kalac and Svaboda, 2000), 5.5-135 mg/kg (Gençcelep et al., 2009), 18.1-103 mg/kg (Mendil et al., 2005) and it supports our results.

Molybdenum is generally an essential element for nitrogen fixation in enzyme activations and legumes for plants. It is found in the structure of nitrogenase and nitrate reductase enzymes. It is necessary for biological nitrogen binding and the formation of amines by reducing nitrate in plants (Kacar and Katkat, 2010). Plants also need to make protein to molybdenum (Plaster, 1992).

When the amount of Mo is examined, the results in general were similar to the results in the study conducted by (Kiremedijian-Schumacher et al., 1994; Ekiz et al., 1995; Shankar and Prasad, 1998; Koyyalamudi et al., 2013). However, this ratio was higher in *C. comatus* species.

With lead accumulating in the body, acute and chronic poisoning occurs, leading to negative effects on the kidneys and causing death (Heyes, 1997). Pb amount value (Kalač, 2009; Yamaç et al., 2007; Ouzouni et al., 2007) was determined at a higher rate compared to similar studies. Silicon is among the 25 elements necessary for the normal development and nutrition of the human body and is the third most abundant element (Sripanyakorn et al., 2005). When the Si and V values were examined together, the results we found were found to be relatively higher than those of Koyyalamudi et al. (2013). However, the amount of Si was similar to the results of Koyyalamudi et al. (2013) in *C. comatus* species.

Titanium is an element that does not cause toxic effects and does not harm the human body. The amount of Ti was determined in the range of 20.32 - 302.2 mg/kg (Vetter, 1994; Györfi et al., 2010), and it was found to be higher than the results they found in their studies.

Zinc is involved in the structure of enzymes that have metabolic functions in the body. In our study, the amount of Zn was found in the range of 1026 - 2422 mg/kg. It was found higher compared to previous studies (Mendil et al., 2005; Dalman, 2006; Sesli and Tuzen, 2006; Sesli, 2007; Akın et al., 2019).

Calcium is an essential element for the construction of bones and teeth, for muscle contraction, for the work of nerves, for the supply of normal blood pressure, for blood clotting, and for keeping cells together (Samur, 2008).When the Ca concentration is examined, the results we find are in line with the values found in similar studies (Sesli, 2006; Sesli and Tuzen, 2006; Ayaz et al., 2011b; Akın et al., 2019; Bulam et al., 2019).

Sodium is very important for the continuation of nerve and muscle functions. Its main task is to provide liquid pumping and to allow food to pass through cell membranes. Excessive amounts of sodium contribute to high blood pressure. The values determined in terms of Na were found in the range of 752.5 - 2105.5 mg/kg (Ayaz et al., 2011), which is higher than the results.

Adequate iron level in a diet was reported to be very important in order to decrease the incidence of anemia (Uzun et al., 2011).It has been reported that there is no reported toxic effect of Fe element, especially in children, Fe element, which is taken too much, has a toxic effect and 60 mg/kg Fe intake may have a fatal effect (Kulhari et al., 2013). In studies with mushroom Fe concentration, 4.15-51.42 mg/kg (Sarıkürkçü et al., 2011), 211 - 628 mg/kg (Mendil et al., 2005), 319.2 - 379.1 mg/kg (Sevindik et al., 2015), 102 - 1580 mg/kg (Soylak et al., 2005), 173.1 - 5044 mg/kg (İlker et al., 2019) have been reported to vary with our findings.

Conclusion

Naturally grown mushroom species have different characteristics and nutritional content. Additionally, they have great importance for researchers, producers and consumers. The differences among the macrofungi species found in nature from the point of both visually and nutritional value have been proved their proportion in biodiversity. As a result, in this study, macro and micronutrient contents of seven natural fungal species were vary from each other. Additionally, *C. comatus*, *H repandum*, *C cibarius*, *P. eryngii* and *P. ostreus* species come to the fore in terms of heavy metals and nutrient content among the mushroom species included in the study. In conclusion, in this study, it was attempted to determine the changes of mineral content in seven wild mushroom species Ordu and Van/Türkiye. Therefore, in the light of the data obtained from this study, it was answered which fungus species are more valuable for human health It was concluded that *P. ostreatus* mushroom species was the richest in terms of nutrient content and *H. repandum* and *P. ostreatus* in terms of heavy metals. It is thought that it can be used as a resource in future studies.

Tablo 2 Heavy metal and mineral concentration

Heavy metal and mineral concentration (Avg. mean + std) mg/kg Dry weight							
	Cantharellus cibarius	***Lactarius glycosomes***	***Coprinus comatus***	***Hydnum repandum***	***Pleurotus eryngii***	***Lactarius delicious***	***Pleurotus ostreatus***
As	Nd	10.38±0.0015	4.86±0.003	1.5±0.002	10.21±0.0005	11.47±0.0025	17.43±0.002
Ba	2.97±0.0030	7.6±0.00031	3.76±0.00025	2.78±0.00015	3.98±0.0002	1.48±0.0004	10.81±0.0002
Cd	16.35±0.00013	Nd	4.52±0.00015	3.87±0.00005	1.4±0.0001	6.03±0.003	43.46±0.0003
Co	12±0.0002	32.25±0.0003	13.24±0.0002	Nd	19.11±0.0002	18.64±0.0002	42.79±0.0004
Cr	Nd	14.92±0.003	Nd	Nd	Nd	Nd	5±0.0003
Cu	143.45±0.003	104.65±0.00015	46.89±0.0025	49.49±0.0035	12.95±0.0027	26.4±0.0025	29.25±0.004
K	Nd	52680±0.85	Nd	Nd	30085±0.35	41665±0.3	46840±1.15
Mg	Nd	Nd	5056±0.045	Nd	Nd	Nd	5955.5±0.085
Mn	52.45±0.00035	187.25±0.002	57.53±0.00055	58.95±0.0007	88.15±0.002	104.05±0.001	177.9±0.004
Mo	1.22±0.00025	1.34±0.0003	57.53±0.00018	Nd	Nd	Nd	2.89±0.00015
Ni	119.67±0.0006	565.8±0.0008	131.45±0.00085	43.46±0.0002	62.93±0.0003	312.9±0.001	408.6±0.0015
Pb	1018.3±0.0135	1483.5±0.005	470.9±0.0015	334.4±0.003	318.9±0.0015	347.45±0.0035	488.05±0.0015
Sb	0.14±0.002	Nd	Nd	4.12±0.0015	1.2±0.001	1.56±0.001	Nd
Si	87.83±0.0165	55±0.01	3.18±0.015	Nd	Nd	Nd	36.98±0.01
Ti	37.15±0.00125	52.42±0.002	29.51±0.0001	20.32±0.0014	302.2±0.00085	30.2±0.001	183.35±0.0015
V	98.72±0.0045	80.58±0.003	101.58±0.0065	102.3±0.0045	86.02±0.002	100.54±0.005	67.66±0.0055
Zn	2422±0.008	1370.5±0.004	1451±0.003	1026.8±0.0025	1334±0.003	1679.5±0.0075	1382.5±0.003
Ca	580.5±0.0075	916.5±0.0395	1552.5±0.0215	411.5±0.0155	519.5±0.0025	851±0.0.0190	2077±0.0320
Na	1137.5±0.0215	1324±0.0230	2105.5±0.0105	1038±0.0530	906±0.0230	752.5±0.0625	1479.5±0.0105
Fe	470.5±0.0355	1009±0.0040	559±0.0030	258.5±0.0095	960±0.0140	478±0.0050	1093.5±0.0075

Author Contributions
All authors have equal contribution.

Conflicts of interest
The authors declare no competing interests.

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A New Locality Record from the Order of Helotiales; *Cistella grevillei*

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Abstract: In this article, new findings regarding the presence of a saprophytic fungus, *Cistella grevillei* (Berk.) Raitv., in Bingöl province are presented. The fungus has been identified using morphological data and represents one of the two species of this genus in Türkiye. The article provides a comprehensive description of the macroscopic and microscopic characteristics of the fungus, along with information on where and when it was collected, accompanied by photographs.

Keywords: Ascomycota, Morphology, New record, Türkiye

Helotiales Takımından Yeni Bir Lokalite Kaydı; *Cistella grevillei*

Öz: Bu makalede, Bingöl ilinde saprofit bir mantar olan *Cistella grevillei* (Berk.) Raitv.'nin varlığına dair yeni bulgular sunulmaktadır. Mantar morfolojik veriler kullanılarak tanımlanmış olup, bu cinsin Türkiye'deki iki türünden birini temsil etmektedir. Makalede, mantarın makroskobik ve mikroskobik özelliklerini içeren kapsamlı bir tanım sunmakta, ayrıca fotoğrafları ile birlikte nerede ve ne zaman toplandığına dair bilgileri içermektedir.

Anahtar Kelimeler: Ascomycota, Morfoloji, Yeni kayıt, Türkiye

Introduction

Helotiales is a diverse group of apothecial ascomycetes that possess inoperculate asci. With approximately 3000-4000 known taxa, it is one of the largest groups in this category (Kirk et al., 2008; Baral, 2016). These fungi typically have small apothecia, typically less than 2 mm in diameter, that can be sessile or stipitate, brightly colored or dark, and either superficial or erumpent on the plant host. The shape of the apothecia can be cup-shaped, discoid, turbinate funnel-shaped, or clavate (Korf, 1973). Most members of this group are saprophytic and live on decaying wood and fallen leaves, but some are symbiotic, parasitic, or pathogenic to other organisms. While many members are relatively easy to isolate, inducing the formation of apothecia in vitro is challenging (Müller and Loeffler, 1976). Although some members produce an asexual state, many do not. Despite the diversity of these fungi, taxonomic and ecological studies are still lacking (Hosoya, 2021). Quijada et al., in their study in 2015 the generic type species of *Cistella* and *Hyphodiscus*, have not been included in any phylogenetic analyses.

In recent years, there has been a significant increase in the number of studies conducted on Ascomycetes in Türkiye (Acar et al. 2020; Uzun and Kaya 2020; Sadullahoğlu and Uzun 2020; Acar 2021; Çetinkaya and Uzun 2021; Kaplan and al. 2021; Kesici and Uzun 2021; Acar and Quijada 2022; Akçay et al. 2022; Tekpınar Dizkırıcı and Acar 2022; Uzun and Kaya 2022, Kaşık et al. 2022; Acar and Dizkırıcı, 2023; Akçay et al., 2023). Despite the increasing activity in studies on Ascomycetes in the country, the current checklist of Turkish fungi only reports one species belonging to the Cistella genus (Sesli et al., 2020) and Solak and Türkoğlu (2022). Previously, Uzun et al. (2017) provided a genus record as Discocistella grevillei (Berk.) Svrček. Our report on Cistella grevillei represents both the second record of the genus in Türkiye and the second locality record for this species. Considering that the current checklist of Turkish fungi does not yet include a significant number of

species from this genus, our report makes a significant contribution to the mycological knowledge of the country.

Material and Metod

Macrofungi specimens were collected from Bingöl province on branches of on leaf petiole Populus sp. in 2021. Bingöl is situated in the Eastern Anatolian region of Türkiye and is bordered by Erzurum and Erzincan to the north, Tunceli and Elâzığ to the west, Diyarbakır to the south, and Muş to the east. In the course of conducting field studies, we documented the geographical coordinates and characteristics of samples based on their morphology and ecology. We captured images of fresh specimens using a Canon (EOS 60D) camera fitted with a Tokina 100 mm macro lens, while images of dried specimens were taken with a Leica EZ4 stereo microscope.

We undertook microscopic examinations at Yüzüncü Yıl University, as per the Clémençon (2009) method. Using a Leica DM500 research microscope with oil immersion, we observed the microscopic characteristics in water. The Leica Application Suite (version 3.4.0) program was used to measure at least 30 asci, ascospores, paraphyses and hairs.

To identify and compare our collection with other species in the genus, we referred to several sources, including Hansen and Knudsen (2000); Raitviir (2004); Quijada et al., (2015). The studied specimen has been deposited at the Fungarium of the Van Flora Application and Research Center, Van Yüzüncü Yıl University (VANF).

Results

The identified species is described below, along with details of its location, collection date, fungarium number, and accompanying figures.

Cistella grevillei (Berk.) Raitv.

Apothecia 0.2–0.7 mm, broadly sessile, dispersed to gregarious, whitish, white to pink-white, margin shortly hairy (Figure 1). **Asci** 32–50 × 3.5–6.1 µm, spores 2-seriate, cylindrical, 8-spored, arising from croziers. **Ascospores** 6–9.5 × 1.5–2 µm, hyaline, multiguttulate at poles, straight to slightly curved, cylindric to clavate. **Paraphyses** terminal cell 9–16 × 1.8–2.5 µm, cell below 10–17 × 1.6–2.8 µm, lanceolate, cylindric to sublanceolate, 3–4 septate, not branched, without guttules. **Hairs** 14–45 × 3–5 µm, 1–4 septate cylindric, clavate to subclavate, densely spiny, straight to sinuous (Figure 2).

Specimen Examined: **TÜRKİYE, Bingöl,** Sarıçiçek village, 38° 54′01"N, 40° 36′55"E, 1063 m, on petiole of *Populus* sp., 10.04.2021, VANF Acar. 1201.

Figure 1. Ascomata of *Cistella grevillei*

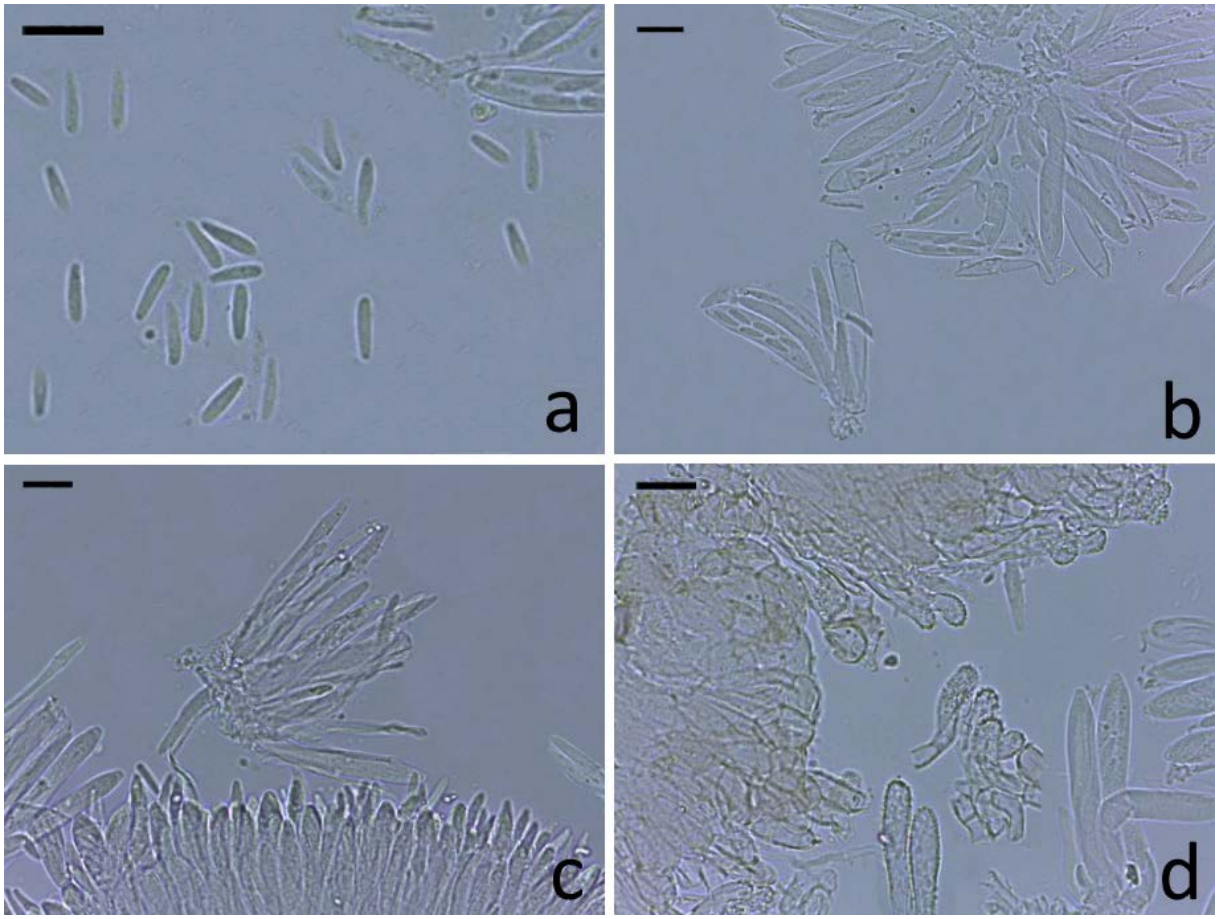

Figure 1. Microscopic characters of *Cistella grevillei* **a**. ascospores, **b**. asci, **c**. paraphyses and asci, **d**. hairs
Scale bar: 10 µm

Discussions

The example presented in the manuscript aligns quite well with the existing definitions (Hansen and Knudsen 2000; Raitviir 2004; Quijada et al., 2015). Raschle (1978) documented significantly longer spores for his samples (6–15 µm). The most similar species is *Cistella hungarica* (Rehm ex Kuntze) Raitv., but there are notable differences compared to the Canarian specimen: (1) ascospores in *C. grevillei* are longer (8–9.8 µm vs *6.3–7.5 µm); (2) the terminal cell in paraphyses in *C. grevillei* is shorter (*8.7–15.2 µm vs *12.5–21.3 µm); and (3) the color of apothecia is white in *C. grevillei* and yellowish in *C. hungarica*. Raitviir (2004) utilized ascus and ascospore size to differentiate between the two taxa, which is also adopted here, although Raschle (1978) reported exceptionally large variability in ascus length (Quijada et al., 2015).

Dicocistella grevillei (Berk.) Svrček was recorded at the genus level by Uzun et al. (2017). In addition, Acar (2021) also provided the first genus-level record for *Cistella dentata* (Pers.) Quél. in Türkiye. When considering Mycobank and Index Fungorum, it can be observed that *Dicocistella grevillei* is synonymous with *Cistella grevillei*. One of the most characteristic features of the *Cistella* genus is the presence of a hair structure within the microscopic characters, which is of significance for genus identification. However, Uzun et al. (2017) did not provide any information about the hair structure in their study. With this research, the species description of *Cistella grevillei* has been supported, and a new locality has been added.

Author Contributions

All authors have equal contribution.

Conflict of Interest

There is no conflict of interest with any institution or person

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (İsmail ACAR).

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Investigation of Aroma Components of *Tuber aestivum* and *Tuber borchii* Collected From Different Location in Türkiye

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Abstract: Truffle is an important food source for wild animals and a significant part of the ecosystem, indicating a healthy forest. With their unique aromas, truffles are also a crucial food source for humans. The volatile substances released by *Tuber* not only appeal to our sense of smell but also contribute to various biological activities. Different aromas result in different tastes; therefore, aroma is essential in defining the organoleptic properties and quality of underground fungi. This research aims to investigate according to the percentage the chemical components considering the habitat and mycorrhizal host tree species of naturally occurring summer truffle *Tuber aestivum* Vittad. and white truffle *Tuber borchii* Vittad. in Southwest Anatolia and Marmara regions. For this purpose, the aroma components of *T. aestivum* and *T. borchii* collected from two different regions were analyzed using the HS-SPME-GC/MS system. Accordingly, a total of 59 different compounds (volatile organic compounds) were identified, including 20 compounds in *T. aestivum* from Muğla, 13 compounds in *T. aestivum* from Kırklareli, 44 compounds in *T. borchii* from Muğla, and 33 compounds in *T. borchii* from Kırklareli, belonging to different classes of compounds. While terpenes such as limonene (37.62%), *p*-cymene (4.79%), and *β*-pinene (4.12%) were major compounds in *T. aestivum* collected from Muğla, 2-octen-1-ol (46.78%), 1-octen-3-ol (40.44%), and 3-octanol (2.62%) predominantly constituted the aroma in *T. aestivum* from Kırklareli. The characteristic sulfur compounds commonly found in *Tuber* species were present in 42.19% of *T. borchii* collected from Kırklareli and 12.15% from Muğla. When comparing *T. borchii* grown in Kırklareli and Muğla, 3-methyl-4,5-dihydrothiophene (29.53% and 6.73%) *p*-methyl thiobenzaldehyde (2.75% and 5.42%), and methionine (9.91% in Kırklareli, not detected in Muğla) were found in different percentage rates. Based on the data obtained in this study, the classification of both *Tuber* species, with respect to their geographical origin, was determined using hierarchical cluster analysis (HCA). Accordingly, it is show that of the aroma of *Tuber* species of the chemical components and the aroma components to the formation should be cultivated after optimizing the ecological conditions that contribute to the symbiotic life of the plant species they provide.

Keywords: *Tuber aestivum, Tuber borchii,* Volatile organic compound (VOC), Aroma, Truffle.

Türkiye'nin Farklı Lokasyonlarından Toplanan *Tuber aestivum* ve *Tuber borchii*'nin Aroma Bileşenlerinin Araştırılması

Öz: Trüf, sağlıklı bir ormanın belirtisi, yabani hayvanların besin kaynağı olmaları yönüyle, ekosistemin önemli bir parçası ve eşsiz aromalarıyla insanlar için önemli bir besin kaynağıdır. *Tuber*'in saldığı uçucu maddeler, sadece koku alma duyumuza hitap etmenin ötesinde çeşitli biyolojik aktivitelere de katkı sağlamaktadır. Farklı aromalar farklı tada sahiptir, dolayısıyla aroma, yer mantarlarının organoleptik özelliklerini ve kalitesini tanımlamada esastır. Bu araştırmada,

Güney Batı Anadolu'da ve Marmara'da doğal olarak yetişen yaz trüfü *Tuber aestivum* Vittad. *ve* beyaz trüf *Tuber borchii* Vittad.'nin yayılış gösterdiği habitat, mikorizal olarak yetiştiği ağaç türü dikkate alınarak aromanın yüzdesine göre kimyasal bileşenlerin araştırılması amaçlanmıştır. Bu amaçla, iki farklı bölgeden toplanılan *T. aestivum* ve *T. borchii*'nin aroma bileşenleri HS-SPME-GC/MS sisteminde analiz edildi. Buna göre, farklı sınıflarda *T. aestivum*'da; Muğla'da 20, Kırklareli'de 13, *T. borchii*'de; Muğla'da 44 ve Kırklareli'de 33 aroma bileşeni (uçucu organik bileşik) olmak üzere her iki trüfte birbirinden farklı toplam 59 bileşik belirlendi. Muğla'dan toplanılan *T. aestivum*'da limonen (%37.62), *p*-simen (% 4.79) ve *β*-pinen (%4.12) gibi terpenlerin majör bileşikler olmasına karşılık Kırklareli'ndeki *T. aestivum*'da 2- okten-1-ol (%46.78), 1-okten-3-ol (%40.44) ve 3-oktanol (%2.62) aromanın çok büyük bir kısmını oluşturmaktadır. *Tuber* türlerinde yaygın olarak bulunan karakteristik kükürt bileşikleri; Kırklareli'den toplanılan *T. borchii*'de %42.19 oranında bulunurken, Muğla'da %12.15 oranında bulunmaktadır. Kırklareli ve Muğla'da yetişen *T. borchii* karşılaştırıldığında; 3-metil-4,5-dihidrotiyofen (%29.53 ve %6.73), *p*-metiltiyobenzaldehid (%2.75 ve %5.42) ve metiyonin Kırklareli'de %9.91 oranında iken Muğla'da tespit edilmemiştir. Bu veriler dikkate alınarak her iki *Tuber* türünün coğrafi kökene dayalı sınıflandırılması hiyerarşik küme analizi (HCA) kullanılarak belirlenmiştir. Buna göre *Tuber* türlerinin aromasının kimyasal bileşenlerin oluşumuna katkı sağlayan ekolojik şartların ve simbiyotik yaşam sağladıkları bitkilerin türlerine göre aroma bileşenleri bakımından optimize edildikten sonra kültüre alınması gerektiğini göstermektedir.

Anahtar kelimeler: *Tuber aestivum*, *Tuber borchii*, Uçucu organik bileşik (VOC), Aroma, Trüf

Introduction

Mushrooms are consumed as food because of their nutritional values, aromas and their own smell. Many types of fungi used as a nutritious food are specially collected and used in food in many parts of the world. Edible fungi are important natural sources for healthy eating, considering their low calories, as well as essential fatty acids. Therefore, it is continued to be seen as a popular diet throughout the world (Taş et al., 2021). The "truffle" also known as "hypogeous mushroom" is a complex family, which includes mainly genus *Picoa, Tirmania, Tuber* and *Terfezia*. Truffle is beyond functional food and, it creates the opportunity to develop new products in gastronomy cuisine because of its particular aromas. Truffle grows with mansion plant (pine, linden, oak, nuts) as ectomicorizal mushrooms. They release a very intense aroma and chemical smell signals in their habitat where truffle fungi mature (Trappe & Maser, 1977; Taş et al., 2021). The studies of truffle species to cultivate is more about *Tuber* species because of their commercial interest. Many of the edible *Tuber* spp. (*Tuber magnatum* Pico, *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad., *Tuber borchii* Vittad.) found in the The Mediterranean area have a privileged place in various cuisines in many parts of Europe, America and East Asia.

Aroma is one of the important factors to assess truffle quality (Feng et al., 2019). Truffle species are mushrooms, especially in communities with high socioeconomic levels, which are attractive and provide high added value depending on their aroma. Beyond the functional food aspect of truffle species, they differentiate from other mushrooms and nutrients because of their aroma. Therefore, they create the opportunity to develop new products in terms of gastronomy. With this approach, truffle restaurants serve in developed countries. *Tuber* species' aromas in truffles are standing out in this group because they generate more interest than others in terms of their flavors. The studies of truffle species to cultivate are more about Tuber species due to the interest they receive in the market. The genus *Tuber* breed is located in the Pezizales order of the Ascomycota branch in the *Tuberaceae* (Lee et al., 2020). It contains ectomycorrhizal species of fungi that are economically valuable (Trappe, 1979). In the Northern Hemisphere, approximately 180 species are identified in the group (Zambonelli et al., 2016). The species in *Tuber* are grouped according to their season and morphology, based on where they grow. For example, there are summer, winter, black, and white truffles. Among the major winter truffle species, the winter white truffle (*Tuber magnatum* Pico) is found in Piedmont, Italy, while the winter black truffle (*Tuber melanosporum* Vittad) is found in Perigord. The summer black truffle is known as the most sought-after *Tuber* species in world cuisine, and it grows naturally in various regions in Europe. *Tuber borchii* Vittad. is widely grown in the Bianchetto region of France, and *Tuber aestivum* known as summer truffle is found in the *T. borchii* known as white truffle region of Italy (Zambonelli et al., 2016; Sesli et al., 2020).

Tuber species detected in studies on hypogeous mushrooms in Türkiye are listed as follows: *Tuber brumale* Vittad. (Öztürk et al., 1997), *Tuber borchii* Vittad. (Kaya, 2009; Elliot et al., 2016), *Tuber aestivum* Vittad. (Gezer et al., 2014), *Tuber excavatum* Vittad. (Castellano

& Türkoğlu, 2012), *Tuber mesentericum* Vittad. (Castellano & Türkoğlu, 2012), *Tuber nitidum* Vittad. (Castellano & Türkoğlu, 2012), *Tuber rufum* Picco (Türkoğlu & Castellano, 2014), *Tuber puberulum* Berk. & Broome (Elliot et al., 2016), *Tuber ferrugineum* Vittad. (Elliot et al., 2016), *Tuber fulgens* Quél (Akata et al., 2020), *Tuber macrosporum* Vittad. (Doğan, 2021).

It is suggested that the aroma components of truffles can be detected with plants in a symbiotic relationship, but the ecological role of essential substances they have in truffle-plant interactions remains speculative (Splivallo et al., 2011). Several literature studies are focused on the origin of the odorants and the identity of the predecessors emitted by truffles (Vahdatzadeh et al., 2015; Vahdatzadeh & Splivallo, 2018).

The aim of this study is to investigate according to the percentage the chemical components of the aroma of naturally occurring summer truffle *Tuber aestivum* Vittad. and white truffle *Tuber borchii* Vittad. in the Southwest Anatolia and Marmara regions of Turkey, taking into consideration the habitat where they are distributed and the tree species they are mycorrhizally associated with.

Material and Methods

Truffle Material

Summer truffle (*Tuber aestivum*) and white truffle (*Tuber borchii*) were collected in Southwest Anatolia and Marmara in Türkiye. Both species are naturally grow in the region (Figure 1). The specimens collected from the field were processed into fungarium material at Muğla Sıtkı Koçman University, Natural Products Research Laboratory. Details about the locations of truffles, the species of trees where they are found mycorrhizally, the Fungarium number, and the time of collection are given in Table 1.

Extraction of the volatile compounds

The volatile compounds of truffle samples collected from different locations were examined using solid-phase microextraction (SPME) and SHIMADZU gas chromatography (GC) and VARIAN 2100 gas chromatography-mass spectrometry (GC-MS) systems. Before analysis, the fiber was preconditioned and thermally cleaned in the injection port of a gas chromatograph according to the instructions provided by the supplier. Analyses were carried out by weighing 5 g of truffle sample in 20 mL vials and adding 5 mL of a 20% sodium chloride solution. The samples were maintained and magnetically stirred for 30 minutes at 50 °C to allow equilibration. Subsequently, the fiber was introduced into the vial and exposed to the headspace above the sample for 30 minutes. Once the extraction step was completed, the SPME fiber was removed from the vial and inserted into the injection port of the GC–MS for thermal desorption of the volatile compounds (Duru et al., 2021).

GC–FID and GC–MS analyses

For GC–FID analyses, a flame ionization detector (FID) and Rxi-5Sil MS (Restek) fused silica non-polar capillary column (30 m × 0.25 mm I.D., film thickness 0.25 µm) were used. The detector and injector temperatures were set to 270°C and 250°C, respectively. Helium was used as a carrier gas at a flow rate of 1.4 mL/min. The initial oven temperature was kept at 60°C for 5 minutes, then increased to 280°C, and maintained at this temperature for 5 minutes. The Class GC10 GC computer program (SHIMADZU) was used to identify the percentage of volatile compounds. The analyses were performed in triplicate, and the data were averaged to obtain the mean values. GC–MS analyses were performed using an ion trap MS spectrometer (VARIAN) and an Rxi-5Sil MS (Restek) fused silica non-polar capillary column (30 m × 0.25 mm I.D., film thickness 0.25 µm). Samples were injected in splitless mode, and helium was used as a carrier gas at a flow rate of 1.4 mL/min. The injector port and MS transfer line temperatures were set to 220°C and 290°C, respectively. The ion source temperature was 200°C, and EI – MS measurements were taken at 70 eV ionization energy. The mass range was m/z 28 to 650 amu. The scan time was 0.5 s with delays between scans of 0.1 s. The oven temperature was initially kept at 60°C for 5 minutes, then increased to 280°C at a rate of 4 °C/min and maintained at this temperature for 5 minutes. The essential compounds were identified based on GC retention indices and computer matching with Wiley, ADAMS, and NIST 08 MS databases. Additionally, models of mass spectra reported in the literature were compared, and injection with reference compounds was done when possible (Adams, 2007).

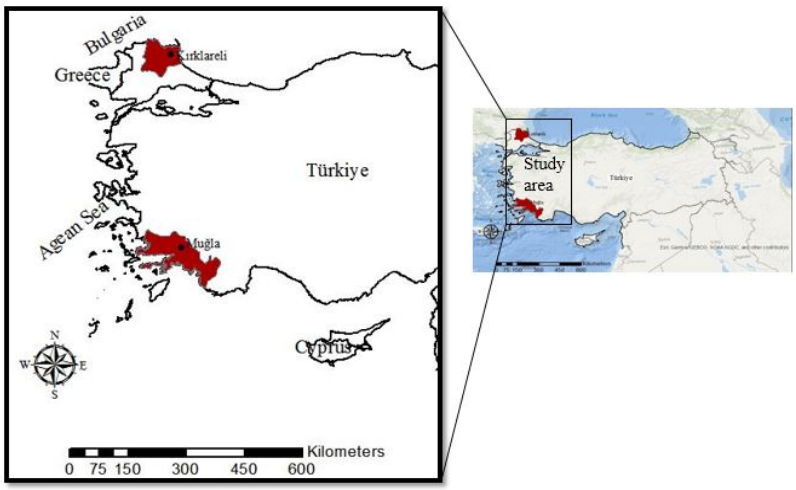

Figure 1. Location map of the *Tuber aestivum* and *Tuber borchii* collected from the field

Table 1. Location map of the *Tuber aestivum (summer truffle)* (a) and *Tuber borchii* (white truffle)

***Tuber* species name**	**Mycorrhizal tree species and altitude**	**Fungarium number**	**Collected season**
 ***a) T. aestivum (summer truffle)***	Muğla/Menteşe, in *Pinus brutia* Ten. *forest,* 660 m.	CK 1014	March 2021
	Kırklareli/Vize, *oak* and *hornbeam mix forest,*165m.	CK 1016	July 2021
 ***b) T. borchii (white truffle)***	Muğla/Menteşe, in *Pinus brutia* Ten. *forest,* 640 m,	CK 1022	April 2021
	Kırklareli/Vize, *oak* and *hornbeam mix forest,*175m.	CK 1029	March 2021

Statistical analysis

MINITAB 16.0 software was used to perform all statistical computations for chemometric investigations of the volatile chemicals in *Tubers* samples using hierarchical cluster analysis (HCA). With the Ward Linkage approach and Euclidean distance, hierarchical relations were obtained using cluster analysis.

Results

Volatile compounds

The aroma components of *T. aestivum* and *T. borchii,* which were gathered at different locations in Türkiye (fluencer organic components), were analyzed using SPME–GC–MS. The chemical analysis results of truffle samples for aroma components are presented in Table 2 as percentages (%). Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$). A total of 59 compounds were identified from the two different *Tuber* species, including alkanes and alkenes, sulfur compounds, alcohols, aldehydes, ketones, esters, aromatic compounds, terpenes, and others. These compounds differ both according to the percentage the chemical components of aromas according to the *Tuber* species and the region where they grew up. In addition to the sulfur-containing compounds characteristic of truffles, compounds such as octanal, [E]-2-octenal and 3-octanone, which are commonly found in the aroma of edible mushrooms, are found in all samples analyzed.

Table 2. Volatile organic compounds of *Tuber aestivum* and *Tuber borchii* from different regions (%) *

Class of compounds	Compounds	RI	*T.aestivum* (Muğla region) (%)	*T.aestivum* (Kırklareli region) (%)	*T.borchii* (Muğla region) (%)	*T.borchii* (Kırklareli region) (%)
Sulfur compounds	Methional	872	-	-	-	9.91 ^{c} ± 0.45
	3-Methylthiopropanal	885	5.9 ^{b} ± 0.15	-	-	-
	3-Methyl-4.5-dihydrothiophene	1125	-	-	6.73 ^{c} ± 0.20	29.53 ^{d} ±1.02
	p-(Methylthio) benzaldehyde	1346	-	-	5.42 ^{c} ± 0.13	2.75 ^{a} ± 0.08
Alkanes and Alkenes	3-Octene	799	-	-	1.10 ^{a} ± 0.03	1.88 ^{a} ± 0.07
	Hexadecane	1612	-	-	8.94 ^{d} ± 0.38	-
Alcohols	1-Octen-3-ol	961	-	40.44 ^{d} ± 1.08	1.21 ^{a} ± 0.04	11.71 ^{c} ± 0.35
	3-Octanol	992	0.52 ^{a} ± 0.02	2.62 ^{b} ± 0.06	-	-
	2-Octen-1-ol	1024	7.05 ^{b} ± 0.25	46.48 ^{d} ± 1.15	-	-
	3-Octen-1-ol	1062	0.43 ^{a} ± 0.01	1.50 ^{b} ± 0.05	-	-
	Octanol	1068	-	0.59 ^{a} ± 0.02	0.37 ^{a} ± 0.01	-
	Nonanol	1089	-	-	4.22 ^{b} ± 0.12	6.37 ^{b} ± 0.18
	(E)-3-Decen-1-ol	1232	-	-	2.06 ^{b} ± 0.09	0.58 ^{a} ± 0.02
	Hexadecanol	1825	-	-	1.18 ^{a} ± 0.02	-
Esters	Ethyl. (E)-3-hexenoate	1012	-	-	1.16 ^{a} ± 0.02	-
	Ethyl phenylacetate	1212	5.04 ^{b} ± 0.12	-	-	-
	Linalyacetate	1242	-	-	0.41 ^{a} ± 0.01	0.25 ^{a} ± 0.01
	Bornylacetate	1275	-	-	1.30 ^{a} ± 0.03	0.49 ^{a} ± 0.01
	Methyl caproate	1313	-	-	0.43 ^{a} ± 0.01	0.32 ^{a} ± 0.01
	Lauric acid methyl ester	1514	-	-	1.34 ^{a} ± 0.03	-
	Isopropyllaurate	1621	-	-	4.60 ^{b} ± 0.15	-
	Methylmyristate	1708	-	-	6.18 ^{c} ± 0.18	-
	Methylpalmitate	1907	-	-	1.77 ^{a} ± 0.07	-
Aldehydes	Heptanal	880	-	-	-	2.41 ^{a} ± 0.10
	2-Methylene-hekzanal	892	1.48 ^{a} ± 0.05	-	-	-
	cis-2-Heptenal	935	1.04 ^{a} ± 0.04	-	0.50 ^{a} ± 0.02	0.52 ^{a} ± 0.02

	Octanal	983	2.14 ^{a} ± 0.08	0.22 ^{a} ± 0.01	0.45 ^{a} ± 0.01	2.99 ^{a} ± 0.10
	[E]-2-Octenal	1039	5.83 ^{b} ± 0.15	2.43 ^{b} ± 0.05	0.70 ^{a} ± 0.03	6.95 ^{b} ± 0.15
	cis-2-Decenal	1250	-	-	0.44 ^{a} ± 0.01	0.21 ^{a} ±0.01
	5-methyl-2-phenyl-2-hexenal	1493	-	-	-	0.27 ^{a} ± 0.01
	Myristaldehyde	1591	-	-	2.11 ^{b} ± 0.04	-
Ketones	1-Octen-3-one	959	-	-	2.90 ^{b} ± 0.05	-
	3-Octanon	988	1.95 ^{a} ± 0.05	4.78 ^{c} ± 0.18	10.06 ^{d} ± 0.45	3.26 ^{b} ± 0.10
	1-(2.8.8-Trimethyl-5.6.7.8-tetrahydro-4H-cycloheptafuran-5-yle) ethanone	1632	-	-	2.28 ^{b} ± 0.05	0.17 ^{a} ±0.01
Aromatic compounds	Styrene	862	-	-	0.50 ^{a} ± 0.02	0.79 ^{a} ± 0.03
	Benzaldehyde	956	10.36 ^{c} ± 0.45	-	5.02 ^{c} ± 0.12	0.93 ^{a} ± 0.02
	Phenylacetaldehyde	1043	0.41 ^{a} ± 0.01	0.2 ^{b} ± 0.00	-	5.07 ^{b} ± 0.15
	Acetophenone	1058	-	-	1.68 ^{a} ± 0.06	0.45 ^{a} ± 0.01
	Phenylethanol	1081	-	0.12 ^{a} ± 0.00	1.12 ^{a} ± 0.05	-
	4-methyl acetophenon	1152	-	0.21 ^{a} ± 0.01	-	-
	Azulene	1156	-	-	5.25 ^{c} ± 0.15	-
	Cinnamaldehyde	1262	-	-	0.66 ^{a} ± 0.02	-
Terpenes	*α*-Pinene	928	0.87 ^{a} ± 0.02	-	2.61 ^{b} ± 0.11	0.38 ^{a} ± 0.01
	β-Pinene	963	4.12 ^{b} ± 0.15	-	-	-
	p-Cymene	1027	4.79 ^{b} ± 0.17	-	0.49 ^{a} ± 0.01	0.4 ^{a} ± 0.01
	Limonene	1032	37.62 ^{d} ± 1.40	0.21 ^{a} ± 0.01	-	0.53 ^{a} ± 0.02
	Eucalyptol	1035	-	-	1.39 ^{a} ± 0.05	5.22 ^{b} ± 0.15
	β-trans-Ocimene	1047	-	-	1.38 ^{a} ± 0.05	3.01 ^{b} ± 0.12
	β-Linalool	1083	-	-	3.95 ^{b} ± 0.12	1.78 ^{a} ± 0.06
	Borneol	1134	-	-	1.27 ^{a} ± 0.05	0.23 ^{a} ± 0.00
	α-Terpineol	1175	-	-	0.90 ^{a} ± 0.03	-
	Thymol	1278	0.93 ^{a} ± 0.03^{a}	-	1.45 ^{a} ± 0.05	0.15 ^{a} ± 0.00
	Carvacrol	1290	7.24 ^{b} ± 0.30	-	1.44 ^{a} ± 0.05	0.19 ^{a} ± 0.01
	α-Terpinylacetate	1334	-	-	0.64 ^{a} ± 0.02	0.16 ^{a} ± 0.00
	α-Himachalen	1414	0.55 ^{a} ± 0.02	0.20 ^{a} ± 0.01	-	-

	Cedreneoxide	1426	-	-	0.82 [a] ± 0.03	-
	Geranylacetone	1429	-	-	1.17 [a] ± 0.05	0.14 [a] ± 0.00
Others	2-İndanone	1128	1.72 [a] ± 0.07	-	-	-
	γ-Nonalacton	1342	-	-	0.40 [a] ± 0.01	-
Total			100.00	100.00	100.00	100.00

* Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).

The characteristic compounds of truffle species, sulfur compounds, were found to be more abundant in *T. borchii* than in *T. aestivum*. In *T. borchii*, which grows in the Kırklareli region, sulfur compounds account for 42.19% of the aroma, while in the samples collected from Muğla, they represent only 12.15%.

In this study, the aroma of *T. aestivum* was analyzed in Muğla, and 20 volatile organic compounds were identified. In Kırklareli, 13 components were identified. Additionally, the aroma of *T. borchii* was analyzed in Muğla, and 44 volatile organic compounds were identified. In T. *borchii* from Kırklareli, 33 volatile organic compounds were identified. Overall, a total of 59 different compounds were identified between the two truffles studied. The structures of all these compounds were elucidated.In summer truffle *T. aestivum* collected from Muğla and Kırklareli, the major and common compounds are as follows: 2-octen-1-ol (7.05% and 46.48%, respectively), limonene (37.62% and 0.21%), [E]-2-octenal (5.83% and 2.43%), 3-octanone (1.95% and 4.78%), octanal (2.14% and 0.22%), 3-octanol (0.52% and 2.62%), 3-octen-1-ol (0.43% and 1.50%), phenylacetaldehyde (0.41% and 0.20%), and α-himachalen (0.55% and 0.20%).Similarly, in the aroma of white truffle *T. borchii*, which grows in Muğla and Kırklareli, the major and common compounds are as follows: 1-octen-3-ol (1.21% and 11.71%, respectively), 3-octanone (10.06% and 3.26%), benzaldehyde (5.02% and 0.93%), nonanol (4.22% and 6.37%), [E]-2-octenal (0.70% and 6.95%), 3-octene (1.10% and 1.88%), (E)-3-decen-1-ol (2.06% and 0.58%), octanal (0.45% and 2.99%), acetophenone (1.68% and 0.45%), bornyl acetate (1.30% and 0.49%), and linalyl acetate (0.41% and 0.25%), which are the main compounds commonly found in both regions.

In white truffle *T. borchii*, which grows in both regions, the following terpenoids were found: *α*-pinene, *p*-cymene, eucalyptol, *β*-trans-ocimene, *β*-linalool, borneol, thymol, carvacrol, *α*-terpinyl acetate, and geranyl acetone. Methional was detected at 9.91% in *T. borchii* collected from the Kırklareli region. Additionally, among other sulfur compounds, 3-methyl-4,5-dihydrothiophene was detected at 6.73% in Muğla and 29.53% in Kırklareli, while p-(methylthio) benzaldehyde was found at 5.42% in Muğla and 2.75% in Kırklareli, and these compounds were common major compounds in both regions. The aromas of both species commonly resemble garlic, spices, and musk smells.

Chemometric analysis

A chemometric analysis was conducted to determine the variability of volatile organic compounds in Tuber species concerning their types and geographical variations. Hierarchical cluster analysis (HCA) was employed using the suggested dendrogram in Table 2, representing data related to sulfur and alcohol compounds, the major compound classes for both truffle species. The data are interconnected and complementary (Figure 2).

When evaluated in terms of sulfur and alcohol compounds, which are the characteristic and main components of aroma in both truffle species, it was observed that the examined species clustered separately in the Hierarchical Clustering Analysis (HCA) *T. aestivum* grown in Kırklareli formed a distinct cluster compared to other specimens, while *T. borchii* and *T. aestivum* grown in Muğla clustered together. In addition, the *T. borchii* sample taken from Kırklareli showed some similarities with other *Tuber* species in Muğla, but still formed a separate cluster.

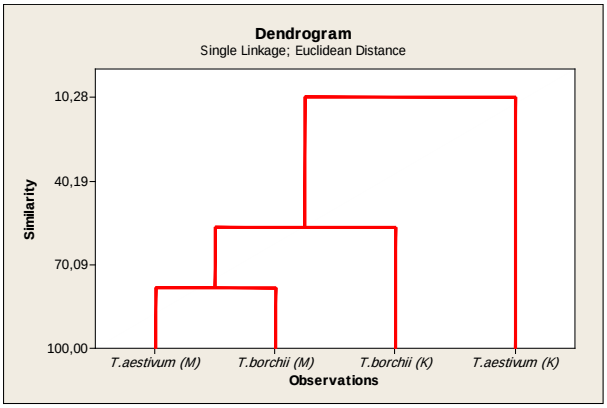

Figure 2. Dendrogram results obtained by Euclidean distance and Ward Linkage method

Discussion

The aromas of *Tuber* species are characterized by sulfur compounds, and they are found at the highest rate

(42.19%) in *T. borchii* collected from Kırklareli, while in *T. borchii* collected from Muğla, they are found at 12.15%. The high content of 3-methyl-4,5-dihydrothiophene and 1-octen-3-ol, identified as predominant in *T. borchii*, align with the results of previous studies reported in the literature (Bellesia et al., 2001; Splivallo & Ebeler, 2015; Lee et al., 2020). In *T. borchii* collected from a forest mixed with oak and hornbeam trees in Kırklareli, the major compounds were 3-methyl-4,5-dihydrothiophene (29.53%), methional (9.91%), and *p-(*methylthio) benzaldehyde (2.75%). It was observed that the sulfur-content aroma has a richer content and strongly varied when viewed sensorially. Among the sulfur compounds, 3-methyl-4,5-dihydrothiophene and *p*-(methylthio) benzaldehyde were detected, and methional was found in both Muğla and Kırklareli samples. Literature studies reported that methional is determined in both *T. borchii* and *T. magnatum* aromas (Vita et al., 2015; Niimi et al., 2021). Some compounds, such as octanal, [E]-2-octenal, and 3-octanone, which are widely found in the aroma of mushrooms, were common in all the analyzed samples (Splivallo et al., 2012; Molinier et al., 2015; Zambonelli et al., 2016; Schmidberger & Schieberle, 2017; Strojnik et al., 2020; Šiškovič et al., 2021).

In the aroma components of *Tuber* species, dimethyl sulfide, dimethyl disulfide, 3-methyl-1-butanol, 1-octen-3-ol, 3-methyl butanal, bis(methylthio)methane are reported to be the most frequently found components (Spanier et al., 2000; Zeppa et al., 2004; Piloni et al., 2005; March et al., 2006; Gioacchini et al., 2008; Cullere et al., 2010; Splivallo et al., 2011; Wang & Marcone, 2011; Beara et al., 2014; Vita et al., 2015; Zhang et al., 2016; Vahdatzadeh & Splivallo, 2018; Feng et al., 2019). As the quantities of sulfur compounds increase, the more distinctive garlic aroma, which is a characteristic of truffles, becomes more pronounced (Costa et al., 2015; Schmidberger & Schieberle, 2017; Mustafa et al., 2020).

The alcohol content is higher in the aroma of *T. aestivum* collected from Kırklareli, while terpenes are more abundant in the samples collected from Muğla. This observation in these two chemical components may be related to the species of the plant with which the truffles grow symbiotically, rather than regional differences in truffle growth. This result supports earlier research reports that truffles differentiate according to the plant species, soil microbiota, and other elements of their habitat (Mello et al., 2006; Vita et al., 2015; Vahdatzadeh et al., 2015; Büntgen et al., 2017; Mustafa et al., 2020; Šiškovič et al., 2021).However, the sample of *T. aestivum* collected from a forest of *Pinus brutia* Ten. in Muğla was richer in this content compared to the samples collected from the Kırklareli province. The major components in this sample included 38.36% limonene, 5.68% *p*-cymene, and 4.12% *β*-pinene terpene compounds.The major compounds in the Muğla sample were 3-methyl thiopropanal and benzaldehyde, in line with results from the literature (Mustafa et al., 2020; Šiškovič et al., 2021).

Hierarchical Clustering Analysis (HCA) was performed in terms of sulfur-containing compounds characteristic of truffles and alcohol compounds, which are the major compound class in the flavor of both truffles. In the dendogram obtained in this way, *T. borchii* specimen grown in Kırklareli clustered differently compared to other specimens, while *T. borchii* and *T. aestivum* in Muğla clustered together, partially similar to other *Tuber* species in Muğla. Appears to be clustered together. These data show that the type of truffle and the region where it grows are the factors that directly affect both the chemical component and the amount of their aroma.

According to the results obtained in this study, it can be concluded that regional differences cause quantities of the aroma changes in the major compound content, and the species of trees in which the ecological environment and/or ectomycorrhizal association can also cause differences in aroma. The aromas of truffle types are factors that directly affect their value in the market. Therefore, scientific studies have been conducted in recent years on truffle cultivation. Factors such as the soil structure of the truffle to be cultured, the ecological conditions, climate, and the tree species where truffles grow can all affect their aroma. These results indicate that after optimizing the aroma components to form preferred compounds in truffles, the ecological conditions and the types of symbiotic life-providing plants need to be considered for the cultivation of summer truffle *T. aestivum* and white truffle *T. borchii.* Recently, there has been promising interest in truffle cultivation in our country. However, we believe that in the future, marketing problems need to be addressed to optimize these mushrooms in terms of aroma components.

Author contributions

Cansu Korkmaz collection of truffle samples, preparation of fungarium, identifications, preparation of samples for analysis, realization of HS-SPME-GC/MS analysis of Mehmet Emin Duru truffles, mass analysis and interpretation of results.

Conflicts of interest

The authors declare no competing interests.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Cansu KORKMAZ, Mehmet Emin DURU).

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Leucoglossum leucosporum, A New Record for Turkish Mycobiota

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Abstract: *Leucoglossum leucosporum* (Benkert & Hardtke) Arauzo is reported as a new record from Türkiye, based on the identification of the samples collected from Pazar district of Rize province. It is the first member of the genus *Leucoglossum* S. Imai determined in Türkiye. A brief description of the species is provided together with the photographs, related to the macroscopy and microscopy.

Keywords: Biodiversity, New record, *Geoglossaceae*, Türkiye

Leucoglossum leucosporum, Türkiye Mikobiyotası İçin Yeni Bir Kayıt

Öz: *Leucoglossum leucosporum* (Benkert & Hardtke) Arauzo Rize'nin Pazar ilçesinden toplanan örneklerin teşhis edilmesiyle, Türkiye'den yeni kayıt olarak rapor edilmiştir. Bu *Leucoglossum* S. Imai cinsinin Türkiye'de belirlenen ilk üyesidir. Türün kısa bir betimlemesi, makroskobi ve mikroskobisine ilişkin fotoğrafları ile birlikte verilmiştir.

Anahtar kelimeler: Biyoçeşitlilik, Yeni kayıt, *Geoglossaceae*, Türkiye

Introduction

Leucoglossum S. Imai is a small genus of Geoglossaceae family. It was first erected by Imai (1942). The members of the genus are characterized by dark coloured setose ascomata and the ascospores which remain hyaline till the late maturity (Imai, 1942; Fedosova and Kovalenko, 2015). Members of the genus resemble some *Trichoglossum* Boud. species. Due to this similarity they have been regarded as the members of *Trichoglossum* for a long time, till the reuse of the generic name, *Leucoglossum,* by Arauzo and Iglesias (2014), depending on the results of morphological and phylogenetic studies. The genus comprises only two species, *L. durandii* (Teng) S. Imai and *L. leucosporum* (Benkert & Hardtke) Arauzo (IndexFungorum, 2023).
Though some geoglossoid, *Geoglossum lineare* Hakelier (Güngör et al., 2013), *G. umbratile* Sacc. (Güngör et al., 2015a), *Trichoglossum hirsutum* (Pers.) Boud (Akata and Kaya, 2013), *T. variabile* (E.J. Durand) Nannf. (Güngör et al., 2015b), *T. walteri* (Berk.) E.J. Durand (Uzun, 2021), cudonioid, *Cudonia circinans* (Pers.) Fr. (Pilát, 1937), and spathularioid, *Spathularia flavida* Pers. (Sesli, 1998), *S. nigripes* (Quél.) Sacc. (Uzun, 2021) and *Spathulariopsis velutipes* (Cooke & Farl.) Maas Geest. (Akata and Kaya, 2013), fungi have so far been reported from Türkiye, any member of the genus *Leucoglossum* were presented before (Sesli et al., 2020; Akçay et al., 2023).

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

Material and Method

Ascomata of the *Leucoglossum leucosporum* were collected from Pazar district of Rize province, in 2022,

during a field survey. Photographs of the ascomata were taken at their natural habitats, and notes were taken related to their morphology, ecology and geopraphy. Then the samples were put in a paper bag and transferred to the fungarium. Macromorphological characteristics of the collection were observed in fresh material while micromorphological structures were observed in dried material. A Nikon Eclipse Ci-S trinocular light microscope was used for microscopic investigation, and a DS-Fi2 digital camera and a Nikon DS-L3 displaying apparatus were used to obtain images related to micromorphology. The sample was identified with the help of Benkert and Hardtke (1988), Arauzo and Iglesias (2014), Fedosova and Kovalenko (2015) and Kučera et al. (2021).

The specimen is kept at Karamanoğlu Mehmetbey University, Science Faculty, Department of Biology.

Results

Ascomycota
Geoglossomycetes
Geoglossales
Geoglossaceae

Leucoglossum leucosporum (Benkert & Hardtke) Arauzo, in Arauzo & Iglesias, Errotari 11: 186 (2014)

Syn: [*Trichoglossum leucosporum* Benkert & Hardtke]

Macroscopic and microscopic features: Ascomata 17-35 mm tall, geoglossoid in appearance, clavate, stipitate, without a distinct transition zone between fertile part and stipe, black. Hymenial part forms 1/3 to 1/2 of the total length of the fruit body, lanceolate to clavate, laterally compressed, with a grove, setose. Stipe cylindrical to somewhat compressed and enlarged towards the fertile part, somewhat darker, setose, seta much more noticeable.

Asci 140–165 × 14–16,5 µm, clavate, with somewhat fusiform apex, with euamyloid apical apparatus, 8-spored. Paraphyses filiform, pigmented, slgihtly enlarged towards the apex (up to 7.5 µm), apical cells sometimes slightly swollen, straight, declinate or hooked. Ascospores (39)42–52.3 (56) × 5–6 (7) µm, cylindrical, slightly curved, rarely straight, some slightly tapering towards one end, aseptate when young, hyaline, become 1-2 septate (rarely 3-7), with one or several lipid drops. Setae up to 360 x 12 µm, simple, straight, thick walled, dark brow to blackish.

Leucoglossum leucosporum was reported to grow on soil among grass and mosses, in meadow, pasture, forest road border and cave (Benkert & Hardke, 1988; Arauzo & Igleasis, 2014; Fedosova ond Kovalenko, 2015; Kučera et al., 2021).

Figure 1. Ascocarps of *Leucoglossum leucosporum*

Specimen examined: Rize, Pazar, Suçatı village, 41°06′N-40°52′E, 475 m, 11.12.2022. Yuzun 7332.

Discussions

Leucoglossum leucosporum is easily recognizable by the presence of setae and the ascospores remaining aseptate or few septate and hyaline for a long period up to maturation. Macro and microscopic characteristics of Turkish collection generally coincide with previous descriptions (Benkert & Hardke, 1988; Arauzo & Igleasis, 2014; Fedosova and Kovalenko, 2015; Kucera et al., 2021), except the spore lenght of Russian collection. Fedosova ond Kovalenko (2015) reported the length of ascospores up to 77.9 µm, but the measured spore length of our collection reached up to 56 µm. The brownish colour and 9-15 septation of over-mature spores were also not observed.

Leucoglossum leucosporum has so far been reported from Austria, Czech Republic, Germany, Netherlands, Russia, Slovakia, Spain and Switzerland (Benkert and Hardtke 1988; Arauzo and Iglesias 2014; Fedosova and Kovalenko 2015; Kucera et al., 2020).

With this study *Leucoglossum leucosporum* was added to the mycobiota of Türkiye as the first member of the genus *Leucoglossum*.

Author Contributions

All authors have equal contribution.

Conflict of Interest

There is no conflict of interest with any institution or person.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Yasin UZUN, Abdullah KAYA).

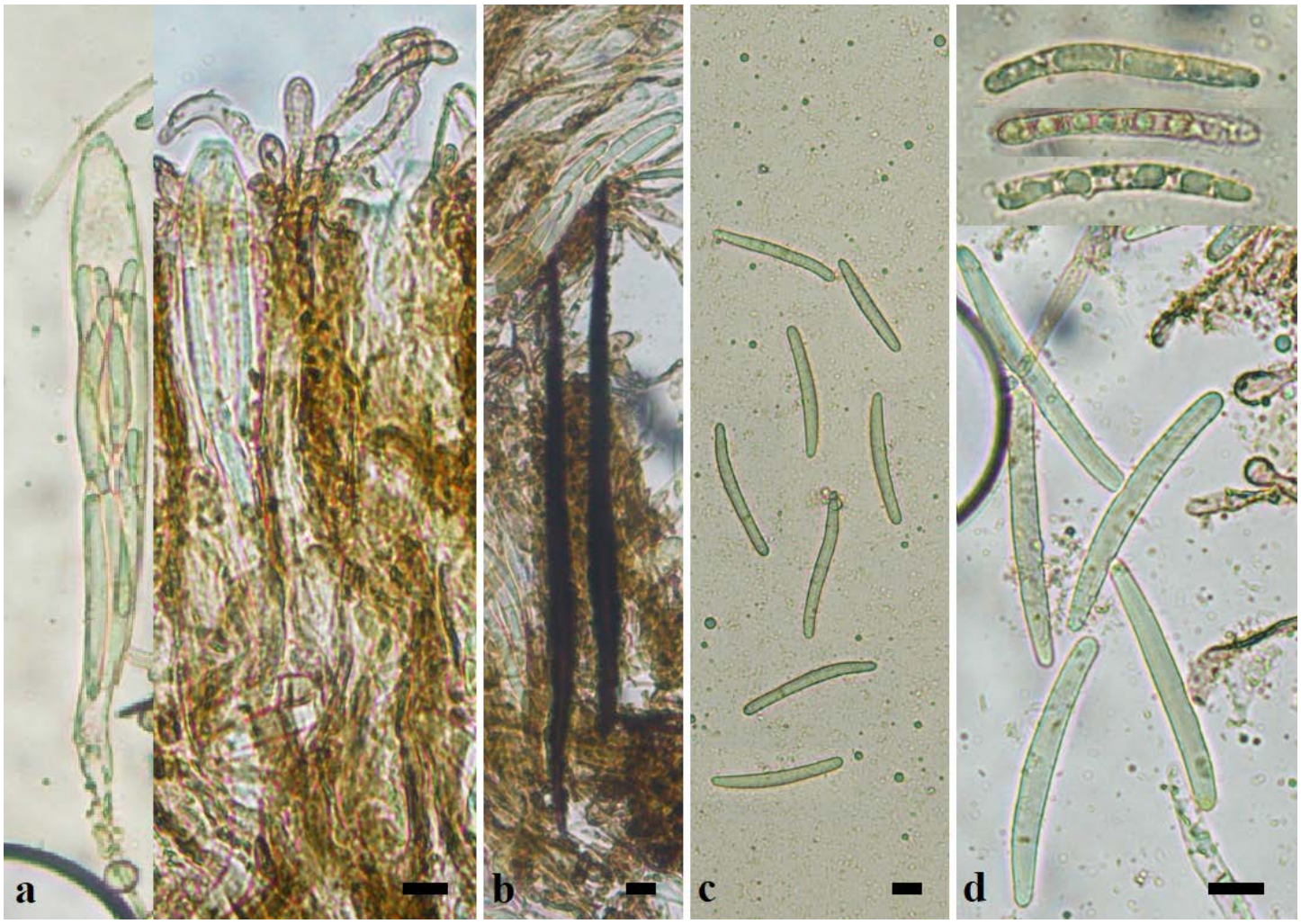

Figure 2. Asci and paraphyses (a), setae (b), ascospores (c-d) of *Leucoglossum leucosporum* (bars: 10 µm) (a-d: Melzer)

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In Vitro Antagonistic Activity of Entomopathogenic Fungi Against *Phytophthora infestans*

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Abstract: Potato downy mildew is a disease caused by a fungus called *Phytophthora infestans* (Mont.) de Bary, which is mainly seen in potatoes and tomatoes, but also in other culture and wild plants belonging to the Solanaceae family. This fungus is quite important both from an economic and historical point of view. In case of an epidemic, it can cause full crop deterioration or serious losses in potatoes. Although cultural and chemical control methods are generally used in the control of the disease, there is no specific biological control method in this regard. Entomopathogenic fungi (EPFs) are generally used as biological control agents in the control of insect pests. Recently, there are many studies showed that these fungi can live endophytically on various cultivated plants and provide beneficial properties to the plants they live with. In this study, it was aimed to determine the *in vitro* antagonistic activities of different entomopathogenic fungi previously isolated from potato fields and identified by molecular methods against *P. infestans*. Earlier work determined that these fungi had a lethal effect on *Leptinotarsa decemlineata* (Say, 1824). Antagonistic activity tests were performed according to the "direct opposition method" and percentage (%) activity values were calculated. As a result of the tests, the most effective isolates were found to be *Beauveria* sp. SK-14 (75.23%) and *Metarhizium* sp. SK-24 (76.23%). It is thought that the results obtained will contribute to the biocontrol of diseases and pests in potatoes.

Keywords: Entomopathogenic fungi, Potato downy mildew, Antagonistic activity, Biological control

Phytophthora infestans'a Karşı Entomopatojenik Fungusların *In Vitro* Antagonistik Aktivitesi

Öz: Patates mildiyösü *Phytophthora infestans* (Mont.) de Bary adı verilen bir fungus tarafından oluşturulan başta patates ve domates olmak üzere Solanaceae familyasına ait diğer kültür ve yabani bitkileri de görülen bir hastalıktır. Bu fungus hem ekonomik hem de tarihsel açıdan oldukça önemlidir. Salgın durumunda patateste tam mahsul bozulmasına veya ciddi kayıplara neden olabilmektedir. Hastalığın mücadelesinde genellikle kültürel ve kimyasal mücadele yöntemleri kullanılmakla beraber bu konuda spesifik bir biyolojik mücadele yöntemi bulunmamaktadır. Entomopatojenik funguslar ise zararlı böceklerle mücadelede biyolojik mücadele etmeni olarak yaygın bir şekilde kullanılmaktadır. Son zamanlarda ise bu fungusların çeşitli kültür bitkileri üzerinde endofitik olarak yaşayabildiğini ve birlikte yaşadığı bitkilere faydalı özellikler sağladığına dair birçok çalışma bulunmaktadır. Bu çalışmada ise daha önceden patates tarlalarından izole edilen ve moleküler yöntemlerle tanımlanan çeşitli entomopatojenik fungusların *P. infestans*'a karşı *in vitro* antagonistik etkilerinin belirlenmesi amaçlanmıştır. Daha önce yapılan

çalışmada bu fungusların *Leptinotarsa decemlineata* (Say, 1824) üzerinde öldürücü etkiye sahip olduğu belirlenmiştir. Antagonistik aktivite testleri "direct opposition method" yöntemine göre yapılmış ve yüzde (%) aktivite değerleri hesaplanmıştır. Testler sonucunda en etkili izolatların *Beauveria* sp. SK-14 (%75.23) ve *Metarhizium* sp. SK-24 (%76.23) olduğu tespit edilmiştir. Elde edilen sonuçların patatesteki hastalık ve zararlılarının biyolojik mücadelesine katkı sağlayacağı düşünülmektedir.

Anahtar kelimeler: Entomopatojenik fungus, Patates mildiyösü, Antagonistik aktivite, Biyolojik mücadele

Introduction

Potato downy mildew caused by *Phytophthora infestans* (Mont) de Bary (Mildiyö) is one of the best-known, the most studied and still one of the most important crop loss factors of all potato diseases in potato production all over the world (Kassa & Hiskias, 1996; Jones, 1998). Worldwide losses in potato production due to this disease are estimated to exceed $5 billion per year, and therefore this pathogen is considered a threat to global food security (Latijnhouwers et al., 2004; Sesli et al., 2020). In our country, this disease is widely seen in all regions of Turkey, including the microclimate areas of the Central Anatolia region. Cultural and chemical control methods are widely used against this disease. In the cultural control, various methods such as removing diseased piles, removing them from the fields and preferring various storage practices are used (T.C. Ministry of Agriculture and Forestry, 2017). In the chemical control, the use of various fungicides such as metalaxly is recommended. However, it has been recently reported that some fungicide-resistant strains have been developed due to the use of fungicides (Matson et al., 2015). In addition, the side effects of various chemicals used in agriculture and forestry on the environment, human and animal health have been known issue for a long time. For this, it is preferred to use safer methods such as biological control will bring solutions in the long term.

Insect pathogenic fungi are a considerable agent in the natural control of insect pests and some arthropods, and these fungi often cause epizootics covering large areas in insect pest populations. These fungi have been used as microbial control agents for over 200 years. In general, many insect orders are susceptible to fungal diseases, and entomopathogenic fungi have good potential as microbial control agents against insect pests (Roberts, 1989). Many entomopathogenic fungi infect their hosts directly from the cuticle and therefore do not need to be eaten by their hosts. This feature makes entomopathogenic fungi a leading candidate in the control of insect pests that feed on plant sap or animal blood. Today, there are many commercial preparations from entomopathogenic fungi around the world, and they are used against various pests in both agriculture and forestry (Goettel et al., 2005). So far, 700 species of entomopathogenic fungi belonging to at least 90 genera have been described, and some species, such as *Beauveria bassiana* (Bals.) Vull. (Böcekküfü), *Metarhizium anisopliae* (Metch) Sorok (Böceksaran), *Isaria fumosorosea* (Wize) Brown (Hoşnut izarya) (=*Paecilomyces fumosoroseus*) and *Verticillium lecanii* (Zimm.) (Şamdan küfü), are commercially produced and used in many countries in the control of many pests (Rath, 2000; Sesli et al., 2020). For example, *B. bassiana* is used in Brazil against the banana root borer *(Cosmopolites sordidus* (Germar)), in China against the pine caterpillar *(Dendrolimus* spp.), and in Europe against aphids and European corn borer (*Ostrinia nubilalis* Hub.) (Goettel et al., 2005; Sevim et al., 2015).

In addition to the use of entomopathogenic fungi as microbial control agents against harmful insect species, recent studies have also shown that these fungi can live endophytically and epiphytically with plants (Vega et al., 2008). The term endophyte was first introduced by the German scientist Heinrich Anton De Bary (1884) and is now defined as bacteria or fungi that live within plant tissues and do not cause any visible symptoms in the plant (Wilson, 1995). Fungal endophytes of many plants have been identified in studies so far, and most of these plants include agriculturally important crops such as wheat, bananas, and tomatoes (Watts et al., 2023). These fungal endophytes are also known to protect plants against insects, pathogens, and nematodes (Vega, 2018). Apart from this, today, fungal endophytes have become a point of interest in biotechnology due to their potential to be used as genetic vectors, secondary sources of metabolites and use as biological control agents (Ting et al., 2021; Larran et al., 2007; Hubbard et al., 2013).

So far, some studies have determined that various entomopathogenic fungi showed antifungal activity against fungal disease agents. Of these, it has been determined that various entomopathogenic fungi, especially *B. bassiana*, showed anti-fungal activity

against *Rhizoctonia* sp. (Dalindiren) and *Botrytis cinerea* (Pers.) & F (Kurşuni küf), which cause disease in potatoes (Barra-Bucarei et al., 2019; Tomilova et al., 2020; Sesli et al., 2020). In this study, the *in vitro* antagonistic effects of various entomopathogenic fungi previously isolated from potato fields against *P. infestans* was investigated. It is thought that the results obtained are useful in the biological control of potato downy mildew disease.

Material and Metod

Antagonistic Activity of Entomopathogenic Fungal İsolates

The *in vitro* antagonistic activity of entomopathogenic fungal isolates (24) obtained from potato fields against *P. infestans* was determined. The entomopathogenic fungal isolates were both morphologically and molecularly identified in our previous study (Keçili et al., 2022). The antagonistic avtivities of the entomopathogenic fungal isolates were determined according to the "direct opposition method" defined by Dennis and Webster (1971). Briefly, a disc of P. *infestans* mycelium actively growing on PDA medium with a diameter of 5 mm was taken and placed at a distance of 1 cm away from the approximate edge of the 120 mm PDA medium. For entomopathogenic fungal isolates, an actively growing mycelium disc of the same size (5 mm) was taken and placed 1 cm away from the opposite side of the petri dish. These petri dishes were then be left to incubate at 28°C and in the dark. Only *P. infestans* mycelium disc was present in the control group. To calculate the percentage of inhibition, the radial growth of fungi in both the control group and the inhibition tests were measured by caliper on day 10. All testes were repeated three times on different occasion. The percentage of inhibition was calculated using the following formula: (Royse & Ries, 1977; Landum et al., 2016).

$$I\ (\text{Inhibition percentage}) = \left(\frac{\text{R1 (colony radius in control)} - \text{R2 (colony radius in test)}}{\text{R1}}\right) \times 100$$

As a result of the experiments, the interaction between the two fungi was expressed on a scale from 1 to 4 (Dharmaputra, 2003). 1; growth inhibition of *P. infestans* after contact with entomopathogenic fungi, 2; *P. infestans* interbreeding with entomopathogenic fungi, but growth is slow and at varying rates, 3; Mutual inhibition in the range of less than 0.2 cm and 4; mutual inhibition greater than 0,2 cm.

Statistical Analysis

The percentage data calculated from the antagonistic activity tests was analyzed using Minitab 17 software. The difference among the entomopathogenic fungal isolates in terms of percentage (%) inhibition against *P. infestants* was determined ANOV followed by Tukey test to make multiple comparision. All data was tested using Levene statistics with respect to variance homogeneity.

Results

Based on ANOVA analysis, all isolates caused different antagonistic activities against *P. infestans* ($F=3,47$, $df=23$, $p<0,05$). The highest activities were obtained from *Beauveria* sp. SK-14 (75,23%) and *Metarhizium* sp. SK-24 (76,23%) (Figure 1). The other antagonistic activitiy values were ranged from 48,4% to 70,23%. All activity values, isolates and scale values were given on table 1.

Discussions

The use of microbial populations as biological control agents is not only effective against insect pests. but also safe for the environment. human health. and non-target organisms. In this sense. the controlling of pest populations with entomopathogenic fungi (such as *Beauveria* and *Metarhizium)* is an alternative method to chemical insecticides and one of the most desirable agricultural practices (Sharma & Sharma. 2021). Recently. it has been shown that these fungi can live endophytically with various agriculturally important plants (Quesada Moraga, 2020). These entomopathogenic fungal endophytes have also been shown to protect plants against both insect pests and various plant pathogens (Mantzoukas & Eliopoulos. 2020; Bamisile et al. 2021). In a previous study. various entomopathogenic fungi were obtained from potato fields and found to have good lethal effects on *L. decemlineata* (Keçili et al., 2022). In this study. the antagonistic effects of 24 previously isolated entomopathogenic fungal isolates against *P. infestans*, one of the most important disease agents of potatoes, were examined.

Table 1. The *in vitro* antagonistic activity of the entomopathogenic fungal isolates against *P. infestans* and their scale values.

No	Species	Isolate	The percentage inhibition ± standard error	Scale value
1	*Beauveria* sp. (Böcekküfü)	SK-1	62.30 ± 3.72	1
2		SK-5	67.07 ± 2.06	1
3		SK-8	69.45 ± 3.94	1
4		SK-12	56.25 ± 2.49	3
5		SK-14	75.23 ± 3.80	4
6		SK-16	66.93 ± 3.17	1
7		SK-17	54.35 ± 1.89	1
8		SK-28	62.49 ± 9.44	4
9		SK-40	60.72 ± 11.79	3
10		SK-45	63.33 ± 2.72	4
11	*Metarhizium* sp. (Böceksaran)	SK-3	64.20 ± 0.43	4
12		SK-9	61.33 ± 3.94	4
13		SK-10	48.40 ± 18.9	4
14		SK-15	60.89 ± 1.27	4
15		SK-21	61.71 ± 1.09	1
16		SK-22	66.49 ± 3.92	4
17		SK-24	76.23 ± 3.16	4
18		SK-27	59.81 ± 7.53	4
19		SK-29	56.44 ± 1.83	4
20		SK-37	64.63 ± 3.08	4
21		SK-42	62.14 ± 1.55	4
22		SK-47	62.93 ± 2.25	4
23		SK-49	63.56 ±0.11	4
24		SK-50	70.23 ± 0.69	4

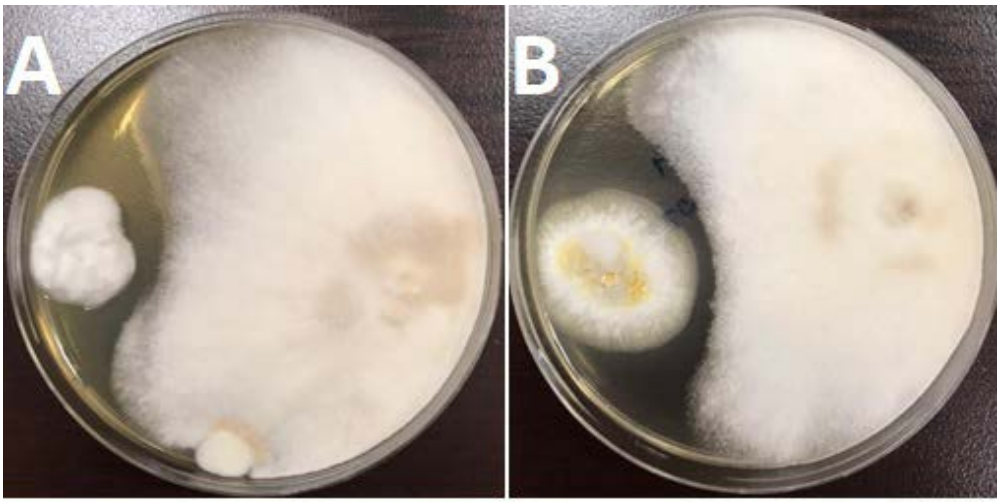

Figure 1. The antagonistic activity pictures of the most effective isolates against *P. infestans*. A; *Beauveria* sp. SK-14 (75.23%). B; *Metarhizium* sp. SK-24 (76.23%).

Many previous studies have shown that entomopathogenic fungal endophytes suppress various plant pathogens and reduce disease symptoms in plants. For example. *B. bassiana and M. brunneum* Petch. (Böcek Saran) can live endophytically in wheat and increase plant growth by suppressing *Fusarium culmorum* (WG) Smith Sacc (Başak küfü). In the same study, it has been also shown that they reduced the disease symptoms in wheat (Jaber, 2018; Sesli et al., 2020). Jaber & Alananbeh (2018) investigated the entophytic colonization of *B. bassiana* and. *M. brunneum* in sweet peper (*Capsicum annuum* L.) and showed that both fungal entomopathogens significantly inhibited all three *Fusarium* species in this pepper. Lozano-Tovar et al. (2017) investigated the antifungal activity of two entomopathogenic fungal isolates (*B. bassiana* and *M. brunneum*) against the olive pathogens *Verticillium dahliae* Kleb. (Avcı şamdan küfü) and *Phytophthora megasperma* Drechs. (Mildiyö) (Sesli et al., 2020). They found that the

entomopathogenic fungus *M. brunneum* produces antifungal compounds that reduce the number of the pathogen propagules in the soil and the severity of *Verticillium* wilt. Kang et al. (2018) also investigated the biocontrol potential of *Isaria javanica* (Bally) Samson & Hywel-Jones (Gürbüz izarya) against aphids and plant fungal pathogens (*Fusarium oxysporum* (Fo) (Sebze küfü) and *Colletotrichum gloeosporioides* Penz. & Sacc. (Ağuantraknozu), *Phytophthora capsica,* Leonian (Biber mildiyösü) and *Rhizoctonia solani* Kühn. (Hırçın dalindiren) (Sesli et al., 2020). They found that *I. javanica* was pathogenic to aphids and had the antifungal activity against these four plant pathogens. Therefore. they suggested that this entomopathogenic fungus can be considered as a dual biocontrol agent against aphids and fungal diseases in red pepper. There are many more similar studies in the literature.

Our study is the first to include antifungal activity of entomopathogenic fungi against *P. infestans*. Our results suggest that the two most effective isolates identified in this study (*Beauveria* sp. SK-14 and *Metarhizium* sp. SK-24*)* can be used simultaneously for controlling both the potato beetle and *P. infestans.* However. to fully prove this. *in vivo* plant trials need to be conducted.

Author contributions

All authors have equal contribution

Conflict of interest

The authors declare that there is no competing interest

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Işılay AKÇA, Şerife ACAR, Zeliha Çağla TARAKÇI, Ali SEVİM).

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Determination of the Connection Between the Asthma Patients and Mycobiota in the Environment They Live in

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Abstract: In the 12-month period between February 2014 and January 2015, this study was carried out in the homes of 55 asthma patients living in 14 different regions of Istanbul (Ataşehir, Bulgurlu, Fikirtepe, Hasanpaşa, İçerenköy, Moda, Göztepe, Çekmeköy, Ümraniye, Altayçeşme Neighborhood, Esenkent Neighborhood, Feyzullah Neighborhood, Gülsuyu Neighborhood, Yalı Neighborhood). Air ideal (Biomerieux, France) air vacuuming device was used to determine the fungal flora in the domestic ambient air of the relevant patients. In this context, in order to prevent bacterial growth, Streptomycin antibiotic was added and Rose Bengali potato dextrose agar was placed in the slot of the device and the air filter of the device was installed. The device, which was placed at a height of 75-85 cm from the ground, was operated for 3-5 minutes and 200 liters of domestic ambient air was vacuumed. A total of 1071 microfungi colonies isolated in the study were found to belong to a total of 10 genuses and 23 species. The obtained genera are *Alternaria* (Arıküfü), *Aspergillus* (Asper), *Aureobasidium* (Karamaya), *Chaetomium* (Günoku), *Cladosporium* (Havaküfü), *Fusarium* (Solduran), *Mucor* (Ekmekküfü), *Paecilomyces* (Günküfü), *Penicillium* (Penisilyum) and *Rhizopus* (Karaküf). Among them, the most isolated genera were *Aspergillus, Penicillium, Cladosporium, Alternaria* and *Fusarium*. The most isolated species in this study were *Aspergillus niger* (Kara asper), *Fusarium poae* (Buğday solduran), *Alternaria alternata* (Astımküfü), *Cladosporium cladosporioides* (Karakökküfü), *Penicillium brevicompactum* (Sağlam penisilyum), *Cladosporium macrocarpum* (İrikurutan), *Cladosporium sphaerospermum* (Güllekurutan) and *Penicillium glabrum* (Bol penisilyum). In the 12-month period, the lowest microfungi concentration was observed in January and the highest microfungi concentration was observed in May. During the study, the temperature of the sample areas were measured with a thermometer and the humidity rates were measured with a hygrometer. In this study, the types of allergen microfungus that cause the onset of asthma disease or the progression of the degree of disease are stated. These were determined as *Alternaria alternariae* (Fıstık küfü), *Alternaria alternata*, *Aspergillus fumigatus* (Kıran asper), *Aspergillus niger*, *Aureobasidium pullulans* (Karamaya), *Chaetomium globosum* (Top günoku), *Cladosporium cladosporioides*, *Cladosporium herbarum* (Yaygınkurutan), *Cladosporium sphaerospermum*, *Penicillium chrysogenum* (Penisilyum), *Penicillium glabrum*.

Keywords: İstanbul, Asthma, Indoor air, Microfungi

Astım Hastalarının Yaşadıkları Ortamlardaki Mikobiyotanın Astım Hastalığıyla İlişkisinin Belirlenmesi

Öz: Şubat 2014-Ocak 2015 tarihleri arasındaki 12 aylık zaman periyodunda, İstanbul'un 14 farklı bölgesinde (Ataşehir, Bulgurlu, Fikirtepe, Hasanpaşa, İçerenköy, Moda, Göztepe, Çekmeköy, Ümraniye, Altayçeşme Mahallesi, Esenkent Mahallesi, Feyzullah Mahallesi, Gülsuyu Mahallesi, Yalı Mahallesi) yaşayan 55 astım hastasının ev ortamlarında bu çalışma gerçekleştirilmiştir. İlgili hastaların ev içi hava ortamında bulunan fungal floranın belirlenmesi amacıyla Air Ideal (Biomerieux, France) hava vakumlama cihazı kullanılmıştır. Bu bağlamda bakteriyel üremeyi önlemek amacıyla Streptomisin antibiyotiği eklenmiş Rose Bengalli patates dekstroz agarlar cihazın yuvasına yerleştirilmiş ve cihazın hava filtresi takılmıştır. Yerden 75-85 cm yüksekliğe konulan cihaz 3-5 dakika çalıştırılarak 200 litre ev içi ortam havası vakumlanmıştır. Araştırmada izole edilen toplam 1071 mikrofungus kolonisinin toplam 10 genus ve 23 türe ait olduğu saptanmıştır. Elde edilen cinsler *Alternaria*, *Aspergillus, Aureobasidium*, *Chaetomium, Cladosporium, Fusarium, Mucor*, *Paecilomyces*, *Penicillium* ve *Rhizopus*'tur. Bunların içerisinde en fazla izole edilen cinsler; *Aspergillus, Penicillium, Cladosporium, Alternaria* ve *Fusarium* olmuştur. Bu çalışmada en fazla izole edilen türler; *Aspergillus niger, Fusarium poae, Alternaria alternata, Cladosporium cladosporioides, Penicillium brevicompactum, Cladosporium macrocarpum, Cladosporium sphaerospermum* ve *Penicillium glabrum* olmuştur. 12 aylık zaman periyodunda en az mikrofungus konsantrasyonu Ocak ayında, en fazla mikrofungus konsantrasyonu ise Mayıs ayında görülmüştür. Çalışma süresince örneklem alanlarının termometre ile sıcaklığı, higrometre ile nem oranlarının ölçümleri gerçekleştirilmiştir. Bu çalışmada astım hastalığının başlamasına ya da hastalık derecesinin ilerlemesine neden olan allerjen mikrofungus türleri belirtilmiştir. Bunlar; *Alternaria alternariae, Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aureobasidium pullulans*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum*, *Penicillium glabrum* olarak tespit edilmiştir.

Anahtar kelimeler: İstanbul, Astım, İç ortam havası, Mikrofungus

Introduction

The discovery of the existence of microfungi began after the invention of the simple microscope by Anton van Leeuwenhoek and Robert Hooke in the seventeenth century, which primarily relied on the system of lenses. Especially due to the fact that it causes various diseases, the importance given to microfungi has increased, and studies have been started on microfungi in order to diagnose the microfungi infections that cause these diseases and to find a treatment for these infections (Tümbay, 1983). Like all groups of microorganisms, microfungi are present in excess amounts in the air. Microorganisms in the air continue their vital activities on water, soil, plants, animals and humans that provide optimum conditions for their lives. Microfungi are present everywhere on our planet. The increase in the density and number of microfungi depends on basic factors such as high-water activity, high temperature, high carbon dioxide rate (Çolakoğlu, 1996).

Since there are fungal elements that cause various health problems, it is important to detect and diagnose them correctly. Since fungi have very different characteristics, they have morphological and microscopic differences. While the basic criteria in this distinction are counted as macroscopic features such as colony shape and size of fungi, colony colors, observation of exudation on the colony surface, full adhesion of colonies to the agar surface and easy separation from the agar surface in powder consistency, the sports shapes, sizes, surface shapes of fungi being different, the shape and size of phialides and conids being different, and the morphologies of hyphae being different can be counted as microscopic features. All these different macroscopic and microscopic characteristics are due to the difference in the genetic heritage of the fungi. The reasons for the difference can be the environmental conditions, especially the humidity and nutrient status of the environment, and the age status of the culture, apart from the genetic characteristics. So much so that differences are observed between young and old colonies of the same fungus species. Since the characteristics of phialide, conidi and hyphae in different regions of the same colonies may vary, all characteristics should be examined in detail in the differentiation of fungi (Arda, 2000).

It has been observed that the molds in the indoor air of the houses and their fungal spores cause the onset and progression of respiratory system diseases,

especially asthma and rhinitis (Miller, 1992). Fungal pathogens in the air that cause respiratory system diseases are also known to cause allergic reactions (Verhoeff and Burge, 1997). *Alternaria, Cladosporium* and *Penicillium* mold were the most isolated pathogen types in the ambient air of the houses where asthma patients live (Şen and Asan, 2001). Species belonging to important genera such as *Aspergillus, Aureobasidium, Paecilomyces, Rhizopus* and *Ulocladium* were also detected in the hospital ambient air (Çolakoğlu and Karaltı, 2011).

This study aims to determine the species of microfungi that make up the fungal flora in the homes of asthma patients living in certain regions of Istanbul and to determine the types of mold that cause or affect the course of the disease and to reveal the relationship between the species isolated in this manner and the disease. Therefore, between February 2014 and January 2015, samples were taken via air sampler from the habitats of 55 asthma patients in 14 different regions of Istanbul for 1 year and isolated species were identified and asthma-related species were specified.

Material and Metod

Samples were taken from the houses of asthma patients living in the regions specified in Table 1 within the borders of Istanbul province between February 2014 and January 2015 and in the number of asthma patients specified in these regions.

Samples were taken from specific areas of the homes of asthma patients in specified areas each month for one year. Sampling was performed with the Air Ideal (Biomerieux, France) device. The specified device was operated at a height of 75-85 cm from the ground for 3-5 minutes and samples were taken by vacuuming 200 liters of air into the medium. This procedure was applied for a year, in 14 different regions of Istanbul, where 55 asthma patients lived. Rose Bengali Peptone Dextrose Agar was used as the main culture medium in which the samples were taken. 30 mg/l streptomycin was added while preparing cultures to prevent bacterial growth (Salo et al., 2008).

Petri plates, containing Peptone Dextrose Agar with Rose Bengal and streptomycin, used for isolation were kept in the laboratory at room temperature (20-26 °C) for 7 days for incubation. Fungus growths were examined during this period. Each fungus colony where reproduction occurred were passaged to Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Czapek's Agar (CZ) culture media. These Petri plates were also incubated at room temperature (20-26 °C) for 7-10 days. Pure cultures of microfungi were obtained as a result of incubation. In this step, all colony macromorphologies such as colony shapes, sizes, structures, diameters and sizes, exudation and pigmentation states were examined and noted from the surface and reverse region of the colonies (Yoltaş et al., 2010).

Preparates were prepared for each fungus colony obtained purely for the purpose of genus and species diagnosis of microfungi. For the microscopic examination of the preparations, a cotton-blue lactophenol solution, which is functional in genus diagnoses and stains the fungus cell walls, and a yellow-colored picric acid-dyed lactophenol solution, which is functional in fungus species diagnoses, were used. A drop of these solutions was dripped on the microscope slide and the micelle and fructification organs of the microfungus were transferred from the previously prepared pure fungus culture to the area where the solution drops were located on the slide with the help of an extract sterilized by passing through the flame and closed with a lamella. Then, the preparate was covered with a colorless-transparent nail polish to prevent contact with air and causing spoilage.

Each of the preparates prepared from the pure cultures of the microfungi was examined separately with an optical microscope. The organs of the microfungi such as hyph, conidiophore, conidi, phialid, etc. were measured 50 times and averaged. The diagnosis of microfungi was carried out using domestic and foreign sources. In order to perform the measurements in the study, an ocular micrometer was placed in the microscope eyepieces.

In order to compare the isolated fungus concentration with the temperature and humidity values criteria, temperature and humidity values were measured with a thermometer and hygrometer in the homes of asthma patients in the sampled regions during the study. These values are given in Table 2.

Within the scope of micro and macromorphological genus identifications Barnett and Hunter (1999) was used as the diagnostic key and as the diagnostic key for species identifications, the relevant literature sources (Ellis, 1965; Klich, 2002; Pitt, 1979; Pitt and Hocking, 2009; Samson et al., 2004) were used. Molecular diagnostic studies of microfungi for gene regions such as its, Beta-Tubulin and Actin also support our study results according to traditional culture and morphological characteristics (Asan et al., 2018).

Table 1. Names of regions sampled and number of patients

	NAME OF THE DISTRICT	NAME OF THE NEIGHBORHOOD	NUMBER OF PATIENTS
1	Ataşehir	Atatürk Neighborhood	1
2	Çekmeköy	Merkez Neighborhood	4
3	Kadıköy	Fikirtepe Neighborhood	3
4	Kadıköy	Göztepe Neighborhood	5
5	Kadıköy	Hasanpaşa Neighborhood	4
6	Kadıköy	İçerenköy Neighborhood	1
7	Kadıköy	Moda Neighborhood	6
8	Maltepe	Altayçeşme Neighborhood	5
9	Maltepe	Esenkent Neighborhood	1
10	Maltepe	Feyzullah Neighborhood	5
11	Maltepe	Gülsuyu Neighborhood	9
12	Maltepe	Yalı Neighborhood	6
13	Ümraniye	Atakent Neighborhood	4
14	Üsküdar	Bulgurlu Neighborhood	1
	Total		**55**

Table 2. Temperature and humidity values measured in the homes of asthma patients (February 2014-January 2015)

	Months											
Name of the District-Region	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Ataşehir-Atatürk Neighborhood	14°C 53%	17°C 51%	17°C 45%	19°C 79%	22°C 70%	26°C 78%	27°C 69%	22°C 74%	15°C 91%	16°C 89%	14°C 57%	15°C 48%
Ataşehir-İçerenköy Neighborhood	17°C 42%	20°C 54%	19°C 45%	20°C 78%	20°C 70%	26°C 73%	25°C 64%	24°C 69%	22°C 80%	19°C 74%	18°C 61%	16°C 40%
Çekmeköy-Merkez Neighborhood	16°C 51%	14°C 53%	15°C 46%	22°C 78%	21°C 68%	28°C 73%	26°C 69%	24°C 74%	16°C 88%	17°C 78%	17°C 61%	12°C 44%
Kadıköy-Fikirtepe Neighborhood	15°C 54%	18°C 55%	18°C 46%	20°C 79%	23°C 72%	25°C 76%	26°C 69%	24°C 75%	16°C 90%	17°C 89%	15°C 58%	13°C 40%
Kadıköy-Göztepe Neighborhood	14°C 53%	17°C 51%	17°C 44%	19°C 78%	22°C 70%	26°C 77%	27°C 68%	23°C 74%	15°C 92%	16°C 90%	14°C 56%	15°C 47%
Kadıköy-Hasanpaşa Neighborhood	16°C 50%	18°C 51%	19°C 46%	20°C 80%	23°C 68%	24°C 78%	27°C 70%	24°C 72%	18°C 93%	18°C 90%	14°C 55%	11 °C 40%
Kadıköy-Moda District	16°C 53%	17°C 55%	17°C 44%	19°C 80%	22°C 67%	26°C 73%	27°C 65%	24°C 75%	15°C 92%	16°C 90%	14°C 57%	14°C 42%
Maltepe-Altayçeşme Neighborhood	17°C 42%	20°C 53%	19°C 44%	20°C 79%	20°C 71%	25°C 73%	24°C 64%	24°C 69%	22°C 80%	19°C 73%	18°C 60%	18°C 40%
Maltepe-Esenkent Neighborhood	16°C 51%	14°C 53%	15°C 44%	22°C 77%	21°C 69%	28°C 72%	26°C 68%	24°C 74%	16°C 87%	17°C 79%	17°C 60%	14°C 44%
Maltepe-Feyzullah Neighborhood	15°C 49%	15°C 53%	16°C 47%	20°C 80%	21°C 72%	27°C 70%	26°C 68%	25°C 72%	17°C 86%	17°C 80%	19°C 63%	17°C 40%
Maltepe-Gülsuyu Neighborhood	9°C 52%	11 °C 58%	13°C 40%	20°C 82%	20°C 73%	26°C 78%	24°C 66%	21°C 73%	14°C 85%	15°C 74%	9°C 59%	4°C 43%
Maltepe-Yalı Neighborhood	17°C 49%	17°C 54%	19°C 43%	19°C 82%	22°C 73%	24°C 77%	25°C 66%	24°C 73%	17°C 91%	16°C 90%	15°C 56%	15°C 40%
Ümraniye-Atakent Neighborhood	16°C 49%	18°C 51%	19°C 45%	20°C 80%	23°C 69%	25°C 77%	27°C 69%	24°C 75%	17°C 90%	18°C 88%	16°C 56%	13°C 41%
Üsküdar-Bulgurlu Neighborhood	15°C 55%	18°C 57%	18°C 48%	21°C 80%	24°C 72%	26°C 77%	28°C 70%	24°C 75%	17°C 88%	18°C 85%	15°C 58%	12°C 39%

Results

From the aerial samples taken from the houses of 55 asthma patients living in 14 different regions of Istanbul, 23 species of 10 genera were isolated and a total of 1071 colonies were examined. The most isolated microfungus genus was *Aspergillus* with 23.34%, followed by Penicillium with 22.53%, Cladosporium with 21.48%, Alternaria with 11.57%, Fusarium with 8.87%, Aureobasidium with 4.38%, Mucor with 4.11%, Rhizopus with 2.42%, Paecilomyces with 1.21%, and Chaetomium with 0.09% (Table 3).

The Turkish nomenclature of the genus and species of microfungi isolated within the scope of thestudy was made according to the Turkish Fungus List (Sesli et al., 2020) (Table 3-4).

Table 3. Colony count and percentage ratios of isolated microfungus genuses

Name of the Genus	Number of Colonies	Percentage of the Colonies
Alternaria (Arıküfü)	124	11,57
Aspergillus (Asper)	250	23,34
Aureobasidium (Karamaya)	47	4,38
Chaetomium (Günoku)	1	0,09
Cladosporium (Havaküfü)	230	21,48
Fusarium (Solduran)	95	8,87
Mucor (Ekmekküfü)	44	4,11
Paecilomyces (Günküfü)	13	1,21
Penicillium (Penisilyum)	241	22,53
Rhizopus (Karaküf)	26	2,42
Total	**1071**	**100**

The percentages of species isolated throughout the study were *Aspergillus niger* with 15.96%, *Fusarium poae* with 8.87%, *Alternaria alternata* with 7.93%, *Penicillium brevicompactum* with 7.47%, *Cladosporium cladosporioides* with 7.47%, *Penicillium glabrum* with 6.54%, *Cladosporium sphaerospermum* with 6.54%, *Cladosporium macrocarpum* with 6.54%, *Aspergillus fumigatus* with 4.58%, *Aureobasidum pullulans* with 4.38%, *Penicillium citrinum* (Limon penisilyum) with 2.80%, *Aspergillus acidus* (Ekşi asper) with 2.80%, *Mucor circinelloides* (Halkaküf) with 2.71%, *Rhizopus microsporus* (Küçükküf) with 2.42%, *Penicillium chrysogenum* with 2.36%, *Alternaria alternariae* with 2.24%, *Penicillium commune* (Zonlu penisilyum) with 2.24%, *Mucor racemosus* (Salkımküf) with 1.40%, *Alternaria tenuissima* (Narinküf) with 1.40%, *Paecilomyces variotii* (El günküfü) with 1.21% , *Penicillium digitatum* (Yeşil penisilyum) with 1.12%, *Cladosporium herbarum* with 0.93%, *Chaetomium globosum* with 0.09% (Table 4).

Table 4. Colony number and percentage rates of isolated microfungus species

Name of the Species	Number of Colonies	Percentage of the Colony
Alternaria alternariae (Fıstık küfü)	24	2,24
Alternaria alternata (Astımküfü)-Pathogen	85	7,93
Alternaria tenuissima (Narinküf)	15	1,40
Aspergillus acidus (Ekşi asper)	30	2,80
Aspergillus fumigatus (Kıran asper)-Pathogen	49	4,58
Aspergillus niger (Kara asper)-Pathogen	171	15,96
Aureobasidium pullulans (Karamaya)-Pathogen	47	4,38
Chaetomium globosum (Top günoku)-Pathogen	1	0,09
Cladosporium cladosporioides (Karakökküfü)-Pathogen	80	7,47
Cladosporium herbarum (Yaygınkurutan)-Pathogen	10	0,93
Cladosporium macrocarpum (İrikurutan)	70	6,54
Cladosporium sphaerospermum (Güllekurutan)-Pathogen	70	6,54
Fusarium poae (Buğday solduran)	95	8,87
Mucor circinelloides (Halkaküf)	29	2,71
Mucor racemosus (Salkımküf)	15	1,40
Paecilomyces variotii (El günküfü)	13	1,21
Penicillium brevicompactum (Sağlam penisilyum)	80	7,47
Penicillium chrysogenum (Penisilyum)-Pathogen	25	2,36
Penicillium citrinum (Limon penisilyum)	30	2,80
Penicillium commune (Zonlu penisilyum)	24	2,24
Penicillium digitatum (Yeşil penisilyum)	12	1,12
Penicillium glabrum (Bol penisilyum)-Pathogen	70	6,54
Rhizopus microsporus (Küçükküf)	26	2,42
Total	**1071**	**100**

Within the scope of the study, the most microfungus isolation by months was realized in May with a maximum rate of 19.61%. This was followed by June with a rate of 14.00%, November with a rate of 10.74%, October with a rate of 9.15%, July with a rate of 7.47%, April with a rate of 7.00%, February with a rate of 6.54%, March with a rate of 6.07%, August with a rate of 5.60%, September with a rate of 4.95%, and December with a rate of 4.67%. The least microfungal isolation occurred in January with a rate of 4.20% (Table 5).

Table 5. Distribution of isolated microfungi colonies by months and percentage ratios

Months	Number of Colonies	Percentage of the Colonies
February	70	6,54
March	65	6,07
April	75	7,00
May	210	19,61
June	150	14,00
July	80	7,47
August	60	5,60
September	53	4,95
October	98	9,15
November	115	10,74
December	50	4,67
January	45	4,20
Total	**1071**	**100**

Within the scope our study, if we specify the relationship between the measurements of temperature and humidity values that vary according to the seasons and isolated allergen-pathogen microfungus species according to the spring, summer, autumn and winter seasons, respectively; *Alternaria alternata* species at 27,06%, 29,41%, 34,12% and 9,41% ratios, *Aspergillus fumigatus* species at 26,53%, 30,61%, 28,57% and 14,29% ratios, *Aspergillus niger* species at 19,88%, 41,52%, 13,45% and 25,15% ratios, *Aureobasidium pullulans* species at 2,13%, 29,79%, 53,19% and 14,89% ratios, *Chaetomium globosum* species at 100%, 0,00%, 0,00% and 0,00% ratios, *Cladosporium cladosporioides* species at 42,50%, 12,50%, 40,00% and 5,00% ratios, *Cladosporium herbarum* species at 66,67%, 0,00%, 22,22% and 11,11% ratios, *Cladosporium sphaerospermum* species at 45,72%, 35,71%, 2,86% and 15,71% ratios, *Penicillium chrysogenum* species at 48,00%, 36,00%, 16,00% and 0,00% ratios and *Penicillium glabrum* species were isolated at 41,43%, 42,86%, 14,29% and 1,42% ratios

Discussions

Microfungi, whose existence was detected after the discovery of the microscope, continue their vital activities all over the world. Microfungi are known to spread the most through the air. Due to the increasing number of diseases and infections caused by microfungi, there is a lot of scientific research and studies on microfungi that spread with air.

In studies on the subject in the literature, it has been stated that the microfungus concentration in the environment is directly related to the temperature and humidity values, so that the mould density is high in seasons with high temperature and humidity values, on the contrary, the mould density is low in seasons with low air temperature and humidity values (Çolakoğlu and Karaltı, 2011). The relevant situation was revealed within the scope of the study (Table 2,5).

Depending on the temperature and humidity values, microfunguses belonging to the *Alternaria* genus were isolated mostly in the spring and autumn period in March, April, May, September, October and November, while microfunguses belonging to the *Cladosporium* genus were isolated in February, March, April, May and November, and microfunguses belonging to the *Aspergillus* and *Penicillium* genuses were isolated much more in the spring and autumn seasons throughout the year (Karaltı and Çolakoğlu, 2012). The results obtained in our study are in line with this condition.

Alternaria, *Aspergillus*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* have been reported to be the main microfungi that cause respiratory system infections in humans, especially asthma (Zock et al., 2002). The increase or decrease in mold flora in the air environment in the house directly affects the course of lower respiratory tract diseases, especially asthma (Ünlü et al., 2003). Even in immunocompetent patients, too much exposure to an allergen-pathogenic microfungus species such as *Aspergillus fumigatus* can reveal the clinical picture of pulmonary aspergillosis (Huseynov et al., 2020). Within the scope of our study, the genus and species of microfungus isolated from the air throughout the year were specified, and pathogenic mold life forms that cause respiratory diseases such as allergies and asthma; *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aureobasidium pullulans*, Chaetomium *globosum*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum* and *Penicillium glabrum* were reported in the light of current scientific data (Table 4).

Author Contributions

All authors contributed equally to this work.

Conflict of Interests

There is no conflict of interest with any person, institution or organization.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Aras Fahrettin KORKMAZ, Günay Tülay ÇOLAKOĞLU, İskender KARALTI).

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Comparison of Epiphytic Lichen Diversity on the Base and Trunk of *Quercus robur* Population in Görükle Campus Area of Bursa Uludag University (Bursa, Türkiye)

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Abstract: This study was carried out in a pedunculate oak grove located next to the Faculty of Agriculture in the Görükle campus area of Bursa Uludag University. Differences in epiphytic lichen diversity on the base and trunk of *Quercus robur* L. were analyzed. A significant difference in epiphytic lichen diversity between the base and trunk of the tree was found. The community structure of epiphytic lichens on *Q. robur* is characterized by the Physcietum adscendens association within the alliance of Xanthorion parietinae. The diversity of epiphytic lichens and the number of species are higher on the trunk rather than at the base of the trees.

Keywords: Physcietum, Species diversity, Species richness, Xanthorion

Bursa Uludağ Üniversitesi (Bursa, Türkiye) Görükle Kampüs Alanındaki *Quercus robur* Populasyonunun Taban ve Gövdesi Üzerindeki Epifitik Liken Çeşitliliğinin Karşılaştırılması

Öz: Bu çalışma, Bursa Uludağ Üniversitesi Görükle yerleşkesi alanında Ziraat Fakültesi yanında bulunan saplı meşe korusunda gerçekleştirilmiştir. *Quercus robur* L.'un taban ve gövdesindeki epifitik liken çeşitliliğindeki farklılıklar analiz edilmiştir. Ağacın tabanında ve gövdesinde epifitik liken çeşitliliğinde önemli bir fark bulunmuştur. *Q. robur* üzerindeki epifitik likenlerin topluluk yapısı, Xanthorion parietinae alyansı içindeki Physcietum adscendens birliği ile karakterize edilmektedir. Epifitik likenlerin çeşitliliği ve tür sayısı gövdede ağacın tabanında olduğundan daha fazladır.

Anahtar kelimeler: Physcietum, Tür çeşitliliği, Tür zenginliği, Xanthorion

Introduction

Lichens are poikilohydric organisms and highly sensible to an increase of light intensity. They are not very efficient at controlling their water content. Therefore, they are very sensitive to changes in the microclimate (Rheault et al., 2003). The main site factors controlling the diversity and distribution of epiphytic lichens are light intensity and moisture. Because of the lower trunks receive much less light than upper trunk of trees, epiphytic lichen diversity and biomass are generally higher in the sun-exposed upper canopy than in trunk bases. Epiphytic lichen cover increases with increasing humidity (Hauck, 2011). Small

changes in the microclimate affect the distribution and species composition of epiphytic lichens (Öztürk et al., 2019).

The epiphytic lichen species richness, density and composition varies between different fractions of a tree (Caruso and Thor, 2007; Hauck and Meifiner, 2002). The species richness and community structure of epiphytic lichens are changing from the base up the trunk (Marmor et al., 2013; Muchnik and Blagoveschenskaya, 2022). According to Castillo-Campos et al. (2019), species richness increases from the lowest part to the highest part of a tree.

Additionally, the impact of anthropogenic and agricultural activities in and around the settlements greatly alters epiphytic lichen variety and community structure (Wolseley et al., 2006; Shukla and Upreti, 2011; Garrido-Benavent et al., 2015). The importance of host tree species, size, bark, and habitat characteristics for epiphytic species were also investigated (Mitchell et al., 2021; Fazan et al., 2022).

The lichen biota of Türkiye is very rich with a total of 2000 lichenicolous and lichenized fungi taxa (Güvenç et al., 2020). Recently, studies on epiphytic lichen vegetation have also been carried out in Türkiye (Çobanoğlu and Sevgi, 2009; Sevgi et al., 2010; Öztürk and Güvenç, 2010).

The aim of this study is to determine the differences in epiphytic lichen diversity on the base and trunk of *Quercus robur*, located next to Faculty of Agriculture in Bursa Uludağ University campus.

Material and Metod

Study area

This study was carried out in the Görükle campus area of Bursa Uludag University. Görükle campus area is located 20 km from Bursa city center in the Marmara Region. The campus has a total area of 1600 hectares, of which 691.65 hectares are forest area, 374.8 hectares are agricultural land, and 168.87 hectares are landscaped garden-woodland. The area is located between 40º23'81"-40º21'76" north latitudes and 28º88'57"-28º85'83" east longitudes. Görükle campus area is under the influence of Mediterranean climate (Akman 1999). The mean annual temperature is 14.4°C, and the mean annual rainfall is 691.9 mm in the Görükle campus area. The campus area has a wide variety of different plants, natural and planted and a total of 252 species, 71 subspecies and 33 varieties were recorded from here. Most of these taxa are Mediterranean element, followed by Euro-Siberian and Irano-Turanian elements, respectively (Tarımcılar and Kaynak, 1994; 1995). A total of 78 lichen species have been recorded in the studies conducted in the Görükle campus area so far (Güvenç and Aslan, 1994; Oran and Öztürk, 2011; Oran, 2019).

Collection of lichen samples

This study was conducted in the base and trunk of the trees in pedunculate oak grove (Alt. 120 m, 40°13′29″N-28°51′39″E), located next to the Faculty of Agriculture in the Görükle campus area of Bursa Uludağ University on 27 December 2022. Five pedunculate oak trees (*Quercus robur* L.) were randomly selected in this area. The collection of lichen samples was carried out on both the base and trunk of the same tree.

Lichen samples were collected using the methods suggested by Asta et al. (2002). The sampling grid templates, each having five 10x10 cm contiguous quadrats were placed on the north (N), east (E), south (S) and west (W) sides both 10-15 cm above the ground of the base, and 150 cm above the ground on the tree trunk. As result, lichen samples were collected for a total of 40 subunits from each oak tree. All lichen species found in each subunit of the sample grid were recorded. The frequency of each species was calculated as the number of subunits at either base or trunk where it was present. The cover value of each species was calculated as the surface area covered by the subunits at the base or trunk. The circumference of the tree was measured 30 cm above the ground at the base and 170-180 cm above the ground at the trunk, corresponding to the middle of the sampling grid templates.

Statistical Analyses

Frequency and cover are the most commonly used parameters as a measure of the importance of taxa in epiphytic communities and habitats (Lara and Mazimpaka, 1998). Importance values of lichens were used for statistical evaluation. The importance value is as the sum of the % relative cover and % relative frequency values of each species in the sampling plots (Pirintsos et al., 1993).The cover and frequency of lichen species were calculated according to the north, south, east, west side on the base and trunk for each trees. The statistical analyses were conducted using the IBM SPSS Statistics 23 sotware. The Mann-Whitney U test was used to analyze whether the diversity of epiphytic lichens on the base and trunk of a tree was different. If the p-value is $\geq$ 0.05, there is no statistical difference between the base and trunk of a tree, or if the p-value is $<$ 0.05, the epiphytic lichen diversity is suggested to be significant different between the base and trunk of a tree. The Kruskal-Wallis H test was used to analyze whether epiphytic lichen diversity differs in different directions (north, south, east, west) on the body of a tree. If the p-value is $\geq$ 0.05, there is no statistical difference between the diversity of epiphytic lichens in different directions on the body of a

tree, or if the p-value is < 0.05, a significant difference is suggested. PCA ordination diagram of the species and trees according to the base and trunk parts of the tree body was obtained using the indirect linear model with Principal Component Analysis (PCA) in CANOCO 4.5 (Ter Braak and Smilauer, 2002).

Results

In this study, a total of 22 epiphytic lichen species were determined on *Quercus robur*. While five species (*Catillaria nigroclavata* (Nyl.) J. Steiner, *Physcia aipolia* (Ehrh. ex Humb.) Fürnr., *Physconia enteroxantha* (Nyl.) Poelt, *Ramalina pollinaria* (Westr.) Ach. and *Scoliciosporum chlorococcum* (Graewe ex Stenh.) Vězda) were found only on the base of the tree, three species (*Evernia prunastri* (L.) Ach., *Parmelina tiliacea* (Hoffm.) Hale and *Physconia perisidiosa* (Erichsen) Moberg) were found only on the trunk of the tree (Table 1). *Xanthoria parietina* (L.) Th. Fr. has a highest frequency and cover value on both the base and the trunk of the tree, followed by *Physcia adscendens* H. Olivier and *Phaeophyscia orbicularis* (Neck.) Moberg, respectively. Other common species found in all sampling frames at both the trunk and base of the tree were *Athallia cerinella* (Nyl.) Arup, Frödén & Søchting, *Caloplaca cerina* (Hedw.) Th. Fr, *Lecania cyrtella* (Ach.) Th. Fr., *Lecanora chlarotera* Nyl., *Lecidella elaeochroma* (Ach.) M. Choisy and *Rinodina pyrina* (Ach.) Arnold.

When the means importance values of epiphytic lichens on the base and trunk parts of the tree are compared, there is a significant difference (Z: -2.402, $p<0.05$) between the base and trunk. The biggest difference between the base and trunk parts of the tree is in the south direction (Z: -2.611, $p<0.01$).

Table 1. Mean percent of relative frequency (RF%) and relative cover (RC%) values of epiphytic lichens on the base and trunk of *Quercus robur*

		BASE								TRUNK							
		North		South		East		West		North		South		East		West	
Species	Abbrev.	RF%	RC%	RF%	RC%	RF%	RC%	RF%	RC%	RF%	RC%	RF%	RC%	RF%	RC%	RF%	RC%
Athallia cerinella	Atha cer	3.33	0.15	3.23	1.07	4.44	0.44	2.22	0.40	1.18	0.13	14.51	0.56	4.00	0.71	4.89	0.41
Caloplaca cerina	Calo cer	0.67	0.06	10.67	2.09	4.67	0.82	5.33	0.50	3.53	0.26	11.76	1.00	2.35	0.19	4.71	0.45
Catillaria nigroclavata	Cati nig	-	-	-	-	-	-	1.33	0.47	-	-	-	-	-	-	-	-
Evernia prunastri	Ever pru	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.18	0.06
Lecania cyrtella	Leca cyr	3.33	0.35	4.67	0.35	0.67	0.12	6.00	1.15	1.18	0.03	3.53	0.10	-	-	8.24	0.84
Lecanora chlarotera	Leca chl	4.00	0.94	0.67	0.06	1.33	0.12	6.67	1.82	4.71	0.64	1.18	0.06	2.35	0.13	3.53	0.26
Lecidella elaeochroma	Leci ela	4.67	1.00	0.67	0.06	-	-	4.00	0.94	4.71	0.51	1.18	0.06	2.35	0.13	2.35	0.19
Melanelixia subaurifera	Mela sub	0.67	0.18	-	-	-	-	0.67	0.06	-	-	-	-	1.18	0.13	-	-
Parmelina tiliacea	Parm til	-	-	-	-	-	-	-	-	1.18	0.39	-	-	-	-	-	-
Phaeophyscia orbicularis	Phae orb	9.33	1.94	8.00	4.05	13.33	9.22	8.00	1.00	22.35	12.93	28.24	18.39	29.41	17.56	25.88	20.71
Physcia adscendens	Phys ads	13.33	6.81	12.00	4.46	9.33	7.58	12.67	6.11	18.82	8.23	21.18	3.15	15.29	3.67	24.71	3.02
Physcia aipolia	Phys aip	2.00	1.00	1.33	0.59	-	-	-	-	-	-	-	-	-	-	-	-
Physcia stellaris	Phys ste	2.67	0.59	8.00	2.06	4.00	1.23	5.33	2.35	14.12	3.99	14.12	2.57	15.29	3.15	16.47	2.89
Physconia enteroxantha	Psco ent	0.67	0.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Physconia perisidiosa	Psco per	-	-	-	-	-	-	-	-	-	-	2.35	0.39	-	-	-	-
Pleurosticta acetabulum	Pleua ce	0.67	0.06	-	-	0.67	0.18	-	-	-	-	1.18	0.58	-	-	-	-
Poeltonia grisea	Poel gri	-	-	0.67	0.23	1.33	0.35	-	-	-	-	-	-	1.18	0.13	-	-
Polyozosia hagenii	Polyh ag	0.67	0.06	0.67	0.03	-	-	2.00	0.15	-	-	2.35	0.10	1.18	0.03	2.35	0.06
Ramalina pollinaria	Rama pol	-	-	-	-	-	-	1.33	0.41	-	-	-	-	-	-	-	-
Rinodina pyrina	Rino pyr	2.67	0.18	8.67	1.06	4.67	0.47	6.00	0.79	4.71	0.29	7.06	0.35	2.35	0.06	7.06	0.45
Scoliciosporum chlorococcum	Scol chl	-	-	-	-	-	-	2.00	0.21	-	-	-	-	-	-	-	-
Xanthoria parietina	Xant par	16.67	83.52	16.67	45.52	16.67	51.51	16.67	76.65	29.41	87.07	28.24	61.35	28.24	62.06	29.41	89.39

Abbrev.: Abbreviation of species names

When the means importance values of epiphytic lichens in the north, south, east and west directions of the trees were compared, no significant difference was found (H: 3.209, $p>0.05$) (Table 2).

In the comparison of the differences between the base and trunk of the epiphytic lichen species, *Phaeophyscia orbicularis* (Z: -2.309, $p<0.05$), *Physcia adscendens* (Z: -2.021, $p<0.05$) and *Physcia stellaris* (Z: -2.309, $p<0.05$) were significant for the tree trunk (Table 3).

A PCA ordination diagram is provided in Figure 1. The first axis was associated with the change in epiphytic lichen diversity on trees from base to trunk of trees. The first and second axes of the PCA explained 60.2 % and

32.5 %, respectively, of the total variance in the species data. The upper left side of the first axis represents the trunk of the trees, and the lower central side represents the base of the trees. On the upper and lower left side of the first axis, there are trees with high epiphytic lichen diversity on the trunk, and there are trees with low lichen diversity in the center. On the contrary, trees with high diversity at the base are located in the lower right, and those with low diversity are located in the central right. The diversity of epiphytic lichens on the base (1) and trunk (6) of a tree is similar. Lichen diversity differs only on the southern side of the tree. Therefore, numbers 1 and 6 are side by side in the ordination diagram of PCA. Similarly, the diversity of epiphytic lichens found at the base (5) and trunk (10) of tree is quite similar. The importance values of epiphytic lichen species on the trunk of the tree are higher than those at the base (Table 2). For this reason, samples 5 and 10 are distributed to each other in PCA ordination. The species with the highest importance are *Xanthoria parietina, Phaeophyscia orbicularis, Physcia adscendens* and *Physcia stellaris,* respectively. These are followed by *Caloplaca cerina, Rinodina pyrina, Athallia cerinella, Lecania cyrtella, Lecanora chlarotera* and *Lecidella elaeochroma*, respectively. *Phaeophyscia orbicularis, Physcia adscendens* and *Physcia stellaris* show significant differences between the base and trunk of the trees. These species have significant differences for the trunk of the tree. Species with high importance values are located in the upper and lower left parts of the second axis of the PCA orientation, and those with lower values are located in the right central parts of the second axis (Table 3, Figure 1).

In our study, the most common species collected from all sample squares on *Quercus robur* are *Athallia cerinella, Caloplaca cerina, Lecanora chlarotera, Phaeophyscia orbicularis, Physcia adscendens, Physcia stellaris, Rinodina pyrina* and *Xanthoria parietina*. Eight of the 22 epiphytic lichen species in this study belong to the Physciaceae family.

Due to intensive fertilization in the agricultural areas of the Faculty of Agriculture, nitrophilous species such as *Lecanora chlarotera, Parmelia sulcata, Parmelina tiliacea, Phaeophyscia orbicularis, Physcia adscendens, Physcia stellaris, Physcia tenella, Pleurosticta acetabulum*, *Polyozosia hagenii* and *Xanthoria parietina* were frequently encountered on trees (Oran, 2019).

Table 2. Comparison of means ± standart deviation (SD) of importance value of epiphytic lichen diversity according to the base and trunk parts of the trees and the direction (North, South, East and West) of the body of the tree.

	Trees	Circumference of tree (cm)	Richness		North	South	East	West	Means	Kruskal-Wallis H	df	p-value
			Number	Means								
BASE	1	100	15	9.75	9.09±20.04	6.45±10.49	6.21±14.75	10.21±13.36	7.99±1.98	3.503	3	0.320
	2	108	9	5.25	6.73±21.74	6.43±13.90	3.94±10.80	7.19±28.99	6.07±1.46			
	3	132	9	4.75	5.76±19.19	3.88±7.98	4.02±10.07	6.58±19.40	5.06±1.32			
	4	98	10	6.25	7.41±23.65	6.95±21.16	7.61±20.65	7.00±22.81	7.25±0.32			
	5	93	12	8.25	7.97±23.84	7.54±19.32	8.48±28.65	8.38±17.39	8.09±0.43			
	Means±SD	N=5	**11±2.55**	**6.85±2.10**	**7.39±1.26**	**6.25±1.40**	**6.05±2.06**	**7.87±1.47**	**6.89±1.64**			
TRUNK	6	84	12	8.50	9.09±22.86	14.33±20.16	8.98±17.50	11.57±22.95	10.99±0.88	3.206	3	0.361
	7	92	8	4.75	7.70±24.53	9.41±19.11	8.49±19.34	11.91±35.53	9.38±0.35			
	8	118	7	4.75	10.46±27.54	7.87±18.47	4.70±13.80	9.33±22.48	8.09±2.53			
	9	88	5	4.25	10.22±30.61	9.39±25.85	10.35±32.18	10.35±26.35	10.08±1.83			
	10	80	12	7.75	12.61±28.56	10.26±30.50	11.37±31.52	13.55±31.28	11.95±2.50			
	Means±SD	N=5	**8.8±3.11**	**6±1.97**	**10.02±1.82**	**10.25±2.44**	**8.78±2.55**	**11.34±1.60**	**10.10±2.18**			
Means±SD		N=10	**9.9±2.92**	**6.43±1.97**	**8.70±2.02**	**8.25±2.82**	**7.42±2.61**	**9.61±2.33**	**8.49±2.50**	3.209	3	0.360
Mann-Whitney U					2.500	.000	3.000	1.000	1.000			
Z					-2.095	-2.611	-1.984	-2.402	-2.402			
p-value					**0.036**	**0.009**	**0.047**	**0.016**	**0.016**			

Table 3. Comparison of means ± standard deviation of importance value of epiphytic lichen species according to the base and trunk parts of the trees (Mann-Whitney U test) and the direction (North, South, East and West) of the body of the tree (Kruskal-Wallis H test).

Species	BASE				TRUNK				Mann-Whitney U	Z	p-value	Kruskal-Wallis H	df	p-value
	North	South	East	West	North	South	East	West						
Athallia cerinella	3.48	4.30	4.88	2.63	1.31	15.07	4.71	5.30	5.000	-0.866	0.386	3.167	3	0.367
Caloplaca cerina	0.73	12.75	5.49	5.83	3.79	12.76	2.55	5.16	7.000	-0.289	0.773	5.500	3	0.139
Catillaria nigroclavata	-	-	-	1.80	-	-	-	-	6.000	-1.000	0.317	3.000	3	0.392
Evernia prunastri	-	-	-	-	-	-	-	1.24	6.000	-1.000	0.317	3.000	3	0.392
Lecania cyrtella	3.69	5.02	0.78	7.15	1.21	3.63	-	9.07	6.000	-0.577	0.564	6.167	3	0.104
Lecanora chlarotera	4.94	0.73	1.45	8.49	5.35	1.24	2.48	3.79	8.000	0.000	1.000	6.000	3	0.112
Lecidella elaeochroma	5.67	0.73	-	4.94	5.22	1.24	2.48	2.55	7.000	-0.289	0.773	6.000	3	0.112
Melanelixia subaurifera	0.84	-	-	0.73	-	-	1.31	-	7.000	-0.331	0.741	1.531	3	0.675
Parmelina tiliacea	-	-	-	-	1.56	-	-	-	6.000	-1.000	0.317	3.000	3	0.392
Phaeophyscia orbicularis	11.27	12.05	22.56	9.00	35.28	46.63	46.97	46.59	**0.000**	**-2.309**	**0.021**	1.500	3	0.682
Physcia adscendens	20.15	16.46	16.91	18.78	27.06	24.33	18.96	27.73	**1.000**	**-2.021**	**0.043**	2.167	3	0.539
Physcia aipolia	3.00	1.92	-	-	-	-	-	-	4.000	-1.512	0.131	2.357	3	0.502
Physcia stellaris	3.25	10.06	5.23	7.68	18.10	16.69	18.45	19.36	**0.000**	**-2.309**	**0.021**	3.000	3	0.392
Physconia enteroxantha	1.14	-	-	-	-	-	-	-	6.000	-1.000	0.317	0.667	3	0.881
Physconia perisidiosa	-	-	-	-	-	2.74	-	-	6.000	-1.000	0.317	3.000	3	0.392
Pleurosticta acetabulum	0.73	-	0.84	-	-	1.76	-	-	7.000	-0.331	0.741	1.531	3	0.675
Poeltonia grisea	-	0.90	1.69	-	-	-	1.31	-	6.000	-0.661	0.508	5.906	3	0.116
Polyozosia hagenii	0.73	0.70	-	2.15	-	2.45	1.21	2.42	4.500	-1.016	0.309	3.247	3	0.355
Ramalina pollinaria	-	-	-	1.74	-	-	-	-	6.000	-1.000	0.317	3.000	3	0.392
Rinodina pyrina	2.84	9.72	5.14	6.79	5.00	7.41	2.42	7.51	7.000	-0.289	0.773	5.500	3	0.139
Scoliciosporum chlorococcum	-	-	-	2.21	-	-	-	-	6.000	-1.000	0.317	3.000	3	0.392
Xanthoria parietina	100.19	62.19	68.18	93.32	116.49	89.59	90.29	118.80	4.000	-1.155	0.248	5.500	3	0.139

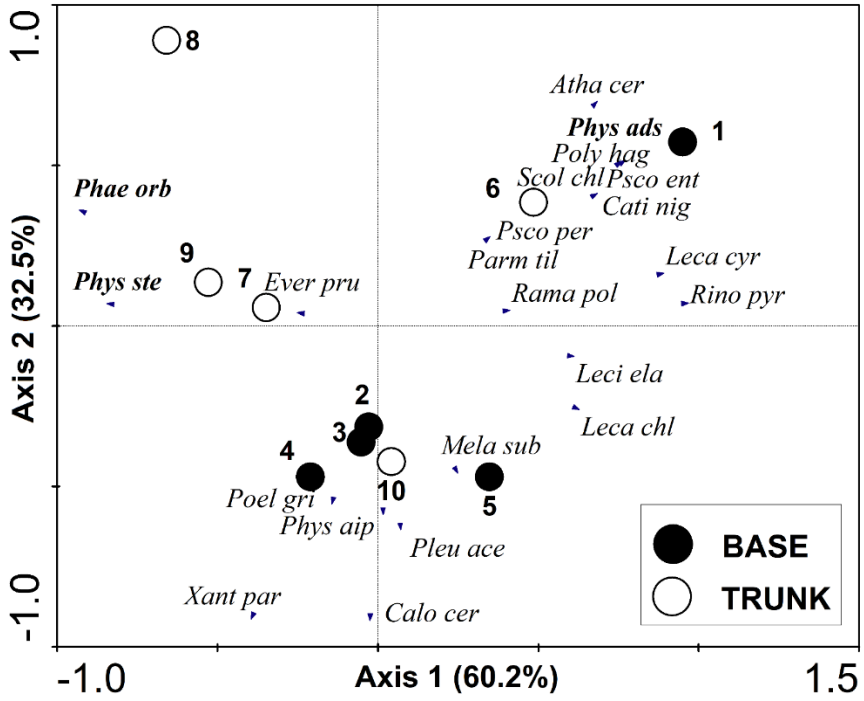

Figure 1. PCA Ordination diagram of the species and trees according to the base and trunk parts of the tree body.

Discussions

The area chosen as the study area in this study is under the influence of anthropogenic and agricultural activities in and around the settlement. We found that the richness (number of species) (R^2=0.7835) were significantly increased from the base to trunk of *Quercus robur* (Figure 2). The circumference of the tree was measured 30 cm above the ground at the base and 150 cm above the ground at the trunk, corresponding to the middle of the sampling grid templates.

The average number of species was found to be less in the lower part of the tree than in the upper parts (Muchnik and Blagoveschenskaya, 2022). It was determined that species richness increased from the lowest parts to the highest parts of the tree (Castillo-Campos et al. 2019). These results are similar to our results.

Epiphytic lichen diversity varies depending on the tree species (Öztürk and Güvenç, 2010; Sevgi et al., 2010), age (Fazan et al., 2022), bark and site characteristics (Mitchell et al., 2021). It has been determined that the community structure of epiphytic lichen vegetation varies according to the characteristics of the sampling areas (elevation, aspect, slope, tree diameter classes and stand type) (Çobanoğlu and Sevgi, 2009).

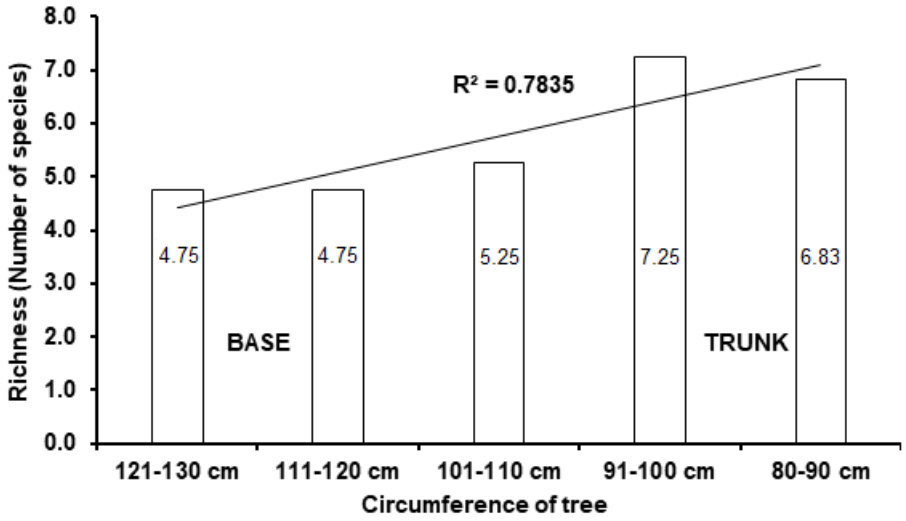

Figure 2. The relationship between species richness and trunk circumference thickness from the base to the trunk of the tree.

Epiphytic lichen diversity in host *Quercus* trees in London city parks has been shown to decrease significantly with increasing tree size. The community structure of epiphytic lichens on these oak trees was characterized by the *Physcietum adscendens* association within the alliance of *Xanthorion parietinae*. *Physcietum adscendens* consists of species adapted to nutrient-enriched substrates and high light intensities (Llewellyn et al., 2020). *Lecidella elaeochroma, Physcia adscendens* and *Xanthoria parietina* were the most abundant on oak trees in the vicinity of agricultural areas and settlements (Garrido-Benavent et al., 2015; Wolseley et al., 2006). According to Filippini et al. (2020), the frequency of the species belonging to the Physciaceae family on the trees increase with the increase in cultivated areas. *Phaeophyscia orbicularis, Physcia adscendens, P. aipolia, P. stellaris, Physconia enteroxantha, P. perisidiosa, Poeltonia grisea* and *Rinodina pyrina* species belonging to the Physciacea family were detected on *Quercus robur*. Of those, *Phaeophyscia orbicularis, P. adscendens, P. stellaris* and *Rinodina pyrina* were found in high frequency at the base and trunk of all sampling trees. This result is consistent with the results of Filippini et al. (2020). As result, our findings shows to be compatible with many source information in terms of characteristic species and association characteristic for *Querus*.

Author Contributions

All authors have equal contribution.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Şule ÖZTÜRK, Şaban GÜVENÇ, Seyhan ORAN, Abdoulaye YENDE).

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