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Pulmonary Aspergillosis: A Case Report of Invasive Aspergillosis Caused by *Aspergillus fumigatus*

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Abstract: *Aspergillus* – a genus consisting of mold species widely distributed in the environment. The spectrum of pulmonary diseases includes invasive aspergillosis (IA), chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA).

We report a case of invasive aspergillosis caused by *Aspergillus fumigatus* in a 65-year-old patient admitted with complaints of shortness of breath, general weakness, and malaise. Diagnosis of "probable" aspergillosis was established based on microbiological and radiological investigations. During microbiological analysis *A.fumigatus* was isolated an identified based on cultural and morphological characteristics. Despite the medical treatment, the patient's complaints worsened. He refused artificial lung ventilation apparatus.

Probably, chronic obstructive pulmonary disease (COPD) together with late diagnosis and absence of appropriate treatment resulted in the development of IA and lethal outcome. CPA develops in immunocompetent patients, suffering from tuberculosis or other diseases accompanied by the formation of cavities in lungs. This case demonstrates the importance of reliable and timely diagnosis of aspergillosis in order to provide patients with adequate treatment.

Taking into account difficulty of obtaining of punctate for histopathological examination and absence of radiological signs physicians should give more attention to the probability of aspergillosis in patients with chronic disease in order to apply adequate therapy and reduce the number of lethal outcomes.

Key words. *Aspergillus fumigatus*, Invasive aspergillosis, CPA, COPD

Pulmoner Aspergilloz: *Aspergillus fumigatus* 'un Etken Olduğu Invaziv Aspergilloz Vakası

Öz: Aspergillus, küf türlerinden oluşan ve çevrede yaygın olarak bulunan bir mantar türüdür. Akciğer hastalıkları spektrumuna invaziv aspergilloz (İA), kronik pulmoner aspergilloz (KPA) ve alerjik bronkopulmoner aspergilloz (ABPA) gibi hastalıklar dahildir.

Nefes darlığı, genel halsizlik ve rahatsızlık yakınmaları ile başvuran 65 yaşındaki bir hastada Aspergillus fumigatus'un neden olduğu invaziv aspergilloz vakasını sunuyoruz. Mikrobiyolojik ve radyolojik araştırmalara dayanarak "olası" aspergilloz tanısı konuldu. Mikrobiyolojik incelemede A.fumigatus kültürel ve morfolojik özelliklerine göre tanımlandı. Tıbbi tedaviye rağmen hastanın durumu daha da kötüleşti. Hasta mekanik ventilasyon cihazını reddetti.

Muhtemelen, kronik obstrüktif akciğer hastalığında (KOAH) geç tanı ve uygun tedavinin olmaması İA gelişimine ve letal sonluğa neden olmuştur. KPA, akciğerlerde kavite oluşumu ile sonuçlanan tüberküloz veya diğer hastalıklara maruz kalan immünokompetan hastalarda gelişir. Bu olgu, hastalara yeterli tedaviyi sağlamak için aspergilloz tanısının güvenilir ve zamanında konulmasının önemini göstermektedir.

Histopatolojik inceleme için biyopsi alınmasının zorluğu ve radyolojik bulguların bulunmaması göz önünde bulundurularak, doktorlar yeterli tedaviyi uygulamak ve ölümcül sonuçların sayısını azaltmak için kronik hastalığı olan hastalarda aspergilloz olasılığına daha fazla dikkat etmelidir.

Anahtar kelimeler. *Aspergillus fumigatus*, İnvaziv aspergilloz, KPA, KOAH

Introduction

Aspergillus – a genus consisting of mold species widely distributed in the environment. Despite the fact that inhalation of mold spores is a frequent phenomenon only in a few cases it causes pulmonary infections. Clinical features, course, and prognosis mainly depend on organism's immune status. The role of genetic factors should be taken into account as well. The spectrum of pulmonary diseases includes invasive aspergillosis (IA), chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA). IA occurs in solid organ and hematopoietic stem cell transplant (HSCT) recipients. Insufficiency of the immune system in these patients leads to the development of acute invasive diseases. CPA develops in immunocompetent patients with chronic pulmonary diseases. Tuberculosis is among the most frequent risk factors of CPA. Other risk factors are an atypic mycobacterial infection, sarcoidosis, chronic obstructive pulmonary diseases (COPD), bronchiectasis, lung cancer, ABPA, pneumothorax. The proportion of patients with previously treated tuberculosis varies between 15.3 and 93 %. In addition, during ABPA allergic response to conidia entered the organism causes fungal sensibilization (Kosmidis & Denning, 2015).

Case report

A 65-year-old man was hospitalized in the Department of Pulmonology and Allergology of Educational-Therapeutic Clinic of Azerbaijan Medical University with complaints of shortness of breath, general weakness, and malaise. According to the information given by relatives, he had been considering himself unhealthy for a long time and underwent stationary and ambulatory treatment in Scientific-Research Institute of Lung Diseases (Baku, Azerbaijan). The general condition of the patient was severe, conscience was clear. Objective examinations revealed pallor of the skin and mucous membranes, fever (38,8 ˚C). There were no visible deformations of joints and bone tissue. Lymphatic nodes were not enlarged. Heart rate– 146 bpm, blood pressure - 90/60 mmHg. Palpation of the heart area did not reveal any pathological pulsations and protuberances. The boundaries of relative cardiac dullness were widened. During auscultation cardiac sounds were normal, the accent of II tone was heard above aorta. Respiratory system: RR – 28 breaths per minute. On percussion dullness of lower lobes was observed. Blood oxygen saturation value vas low (SPO_2=52%). Gastrointestinal tract examination: the tongue was moist, swallowing action - painless. Stomach during palpation was soft, liver and spleen were palpable. Defecation was normal. Urogenital system: negative tapping symptom, painful urination. Computed tomography of thoracic organs showed diffuse emphysematous alterations caused bronchiectasis accompanied by cysts of different sizes, pleural thickening, and calcifications. Secondary reduction of the left lung upper lobe volume and hearth boundaries, calcified lymphatic nodes and atherosclerotic plaques of the aorta were observed. Bone structure examination revealed thoracal kyphosis, osteodegenerative alterations in cervical and thoracal vertebrae. Conclusion: massive destructive processes in left upper lobe accompanying diffuse emphysematous alterations in both lungs, characteristic for previous lung tuberculosis.

Microbiological investigation

Sputum sample sent to Clinic Microbiological Laboratory (The department of Microbiology and Immunology) of Azerbaijan Medical University was inoculated onto 5%-sheep blood, Eosin methylene blue (EMB) and Sabouraud Dextrose (supplemented with chloramphenicol) agars and incubated at 37ºC and 45ºC. After 2 days of incubation growth of white velvety colonies was observed. The colonies color changed to blue-green with age (Fig. 1). In order to avoid contamination

collection of sputum sample from the patient was repeated. For morphological identification, lactophenol cotton blue mount (LCBM) was used. Microscopic examination revealed septate hyphae, smooth walled conidiophores, and chains of conidia arising from a single row of phialides located on club-shaped vesicles. Echinulate conidia (2.5 to 3 micrometer) formed on the upper 2/3 of the vesicle is characteristic for *Aspergillus fumigatus* (Fig. 1).

The diagnosis of "probable" aspergillosis was established based on microbiological and radiological investigations (De Pauw et al., 2008) and voriconazole was administered. Despite the medical treatment, the patient's complaints of breath shortness, fatigue, weakness, malaise worsened. He refused artificial lung ventilation apparatus and died a day later.

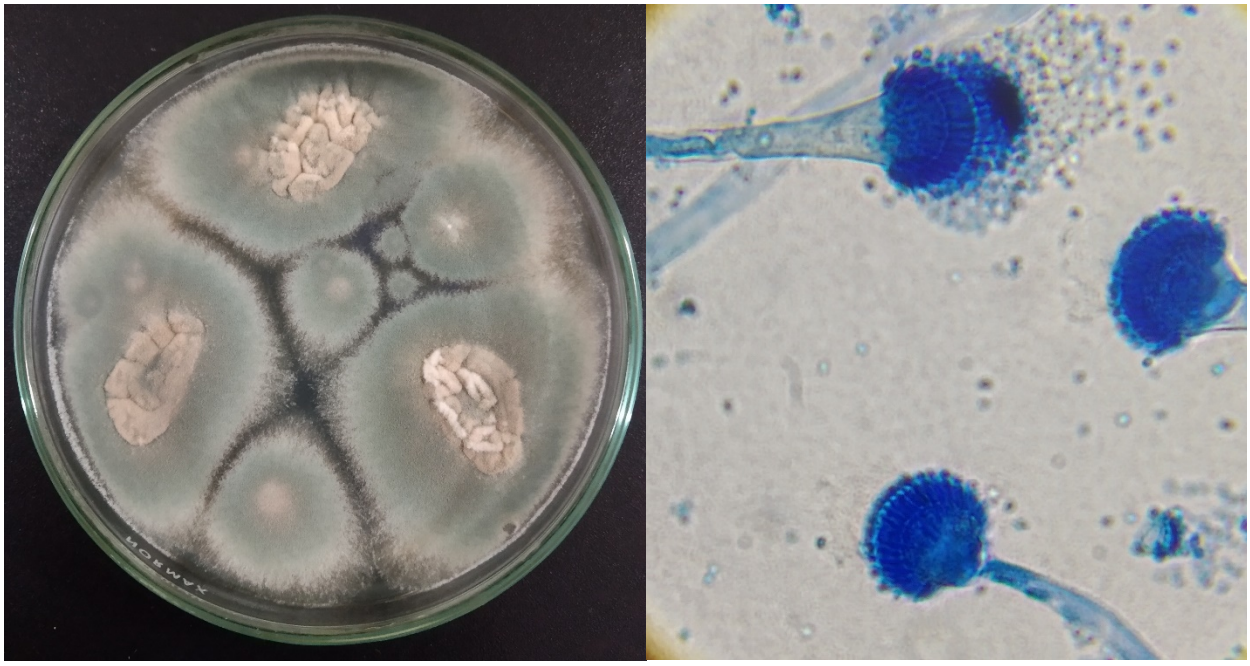

Figure 1. *A.fumigatus* colonies on SDA medium (left) and microscopy with lactophenol cotton blue stain (right).

Discussion

Pulmonary aspergillosis has many clinic variations including IA, chronic necrotizing aspergillosis (CNA), CPA and ABPA (Walsh et al., 2008). The most frequent causing agents are 4 species: *A. fumigatus*, *A. flavus*, *A. niger* and *A. terreus (Steinbach et al., 2012).* The main manifestations are shortness of breath, chronic sputum discharge, chest discomfort, fatigue, loss of weight. The complexity of diagnosis of aspergillosis is due to nonspecific symptoms and absence of classic risk factors. 3 levels of certainty exist regarding the diagnosis: "proven", "probable" and "possible" aspergillosis. The diagnosis of "proven" IA is based on histopathological examinations and positive culture from normally sterile organism site. "Probable" diagnosis is based on the presence of risk factors, clinical manifestations, radiological signs and microbiological examination (De Pauw et al., 2008).Clinical manifestation of aspergillosis depends on 2 factors: immune status of organism and presence of concomitant disease. IA develops in immunosupressive patients, ABPA – in atopic patients and patients with hyperreactive immune system, CPA – in immunocompetent patients (Maghrabi & Denning, 2017).This case demonstrates the importance of reliable and timely diagnosis of aspergillosis in order to provide patients with adequate treatment. According to Betancourt et al. (2015) the diagnosis of aspergillosis is accompanied with difficulties related to the absence of classic radiological signs and negative results of the microbiological analysis. Furthermore, the presence of tuberculosis can complicate the task. The comprehensive diagnosis was made by a histopathological diagnosis of material obtained after surgical manipulations (Betancourt, Garofoli, Sandhu, Boma, & Sy, 2015). In case of IA described by Naaraayan et al. (2015) the diagnosis of aspergillosis was made after

histopathological examination as well. The positive result of sputum cultivation was obtained only after a 13-rd day of patient's admission (Naaraayan, Kavian, Lederman, Basak, & Jesmajian, 2015). In the presented case differential diagnosis was made with tuberculosis. Probably, COPD together with late diagnosis and absence of appropriate treatment resulted in the development of IA and lethal outcome. CPA develops in immunocompetent patients, suffering from tuberculosis or other diseases accompanied by the formation of cavities in lungs (Thompson & Patterson, 2011). Radiologically it can be represented by aspergilloma, nodules or various thin-/thickwalled cavities. In the presented case, CT results confirmed the presence of diffuse emphysematous alterations in lungs favoring the development of CPA. Around 1.2 million people in the World have CPA as a consequence of tuberculosis (Denning, Pleuvry, & Cole, 2011). Isolation of pathogen from sputum can represent colonization, in particular in immunocompetent patients. Controversially, positive culture in immunosuppressed patients and recipient of HSCT and solid organs indicates a high probability of aspergillosis.

Conclusion

In the represented case based on anamnesis, clinical manifestations and radiological signs of aspergillosis was suspected and a microbiological investigation was performed. Taking into account difficulty of obtaining of punctate for histopathological examination and absence of radiological signs physicians should give more attention to the probability of aspergillosis in patients with chronic lung disease in order to apply adequate therapy and reduce the number of lethal outcomes.

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Fungal Biodiversity on Slippers and Carpets Dusts in Three Mosques of Edirne City, Turkey

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Abstract: This study was conducted for the purpose of identifying the microfungi types and numbers in carpets, carpet dusts and slippers in three mosques in the Edirne City and surveying of microfungi during perform an ablution. It was isolated 78.937 CFU/g microfungi in total during 12 months from the samples taken between the dates of October 2008 and September 2009 from the stations. Of the microfungi 83 CFU/g were dermatophyte. It was identified only one dermatophyte in the slippers. It was identified 24 fungal species in carpet samples. The genus *Penicilium* was on the first rank with 18.553 CFU/g and 49.03 % in carpets, followed by *Trichoderma* with 13.666 CFU/g and 25 %, and followed *Cladosporium* ranked three with 96.666 CFU/g and 12.34 %. It was found the dermatophyte *Trichophyton rubrum* in the mosques only for once (July 2009). Statistical analysis for identifying whether the fungal types and the total microfungi concentrations are related with various meteorological factors. The highest value in indoor carpets was isolated as 6.084 CFU/g on the October. As a result, it was identified that the carpet dust fungus concentrations in three mosques are within the range of healthy limit values.

Key words: Carpet dust, mosque, microfungus, dermatophyte, fungal biodiversity, Edirne

Türkiye'de Edirne Şehrinin Üç Camisinde Halı Tozları ve Terliklerde Mantar Biyoçeşitliliği

Öz: Bu çalışma, Edirne ilindeki 3 farklı caminin halı, halı tozu ve terliklerde mikrofungus tipleri ve sayılarının belirlenmesi, iç ortam halısında ve abdest alma esnasında kullanılan terliklerde dermatofit varlığının araştırılması amacıyla yapılmıştır. İstasyonlardan Ekim 2008 – Eylül 2009 tarihleri arasında alınan örneklerden, 12 ay süresince, toplam olarak 78937 KOB/g mikrofungus izole edilmiştir. Mikrofunguslardan 83 KOB/g'ı dermatofit fungustur. Terliklerde dermatofit tespit edilmemiştir. Halı örneklerinde 24 fungal cins tespit edilmiştir. Halıda tespit edilen mikrofunguslar arasında ilk sırayı 18533 koloni ve % 49,03 ile *Penicillium* cinsi almıştır. Bunu 13666 koloni ve % 25 ile *Trichoderma* cinsi, 9667 koloni ve % 12,34 ile *Cladosporium* cinsi üçüncü olarak takip etmiştir. Sadece bir camide bir kez (Temmuz 2009) *Trichophyton rubrum* bulunmuştur. Fungal cinslerin ve toplam mikrofungus konsantrasyonlarının çeşitli meteorolojik faktörlerle ilişkili olup olmadığını tespit etmek için istatistik analiz yapılmıştır. İç ortam halısındaki en yüksek değer 2. istasyonda Ekim ayında 6084 KOB/g izole edilmiştir. Sonuç olarak, üç camideki halı tozu fungus konsantrasyonlarının sağlıklı sınır değerleri arasında olduğu tespit edilmiştir.

Anahtar kelimeler: Halı tozu, cami, mikrofungus, dermatofit, fungal biyoçeşitlilik, Edirne.

Introduction

All kinds of decorative purposed covering materials in homes, offices or schools, especially wallpapers and carpet undersides create environments in which the fungi can grow (Ozyaral et al., 1988). It is known that it cumulates too much dust in the carpets in comparison with the flat surfaces such as wooden floors or nylon floors. Thus, the studies conducted have shown that some carpets involve too much allergens and create much more fungus compared to flat surfaces and air (Beguin ve Nolard, 1999).

Environmental fungi are related with many diseases in the humans. These diseases can be listed as atopic allergic dermatitis, allergic rhinitis, asthma, extrinsic allergic alveolitis, hypersensitivity pneumonitis, sick building syndrome (SBS) and liver cancer with its toxins such ad aflatoxin (Abdu-Wahab, 2006; El-Nagerabi et al., 2012).

There are convenient conditions for growing of fungi in historical buildings, museums and libraries (Kasprzyk, 2008). The buildings which involve high fungus concentration are the oldest buildings and their connection with the fungi has not been explained completely yet (Macher, 2001). The age of the buildings is an important factor that affect the indoor fungal spore concentration (Sivasubramani et al., 2004).

Mosques are historical buildings which are visited for praying and for touristic purposes. Researching the fungal concentration in mosques' indoor carpets can play an important role in identifying the possible risk which can be created by fungi and can reveal the necessity to take precautions. Specifying the possible microbiota in the carpets is important for protecting the health of the visitors who come to the mosque. It is also important to research especially the air quality of a place as well as the carpet dust by considering the infecting effects of microfungi. It is expected that the indoor air quality have the most acceptable level in collective areas of usage because of that it plays an important role in triggering allergic reactions especially in atopic persons. The studies on the indoor carpet-sourced microbiota in the mosques are limited.

Muslims go mosques for praying collectively five times in a day. They perform a practice named kotow (touching their forehead and nose, hands, knees and foot fingers to the ground) in a part of the salaat worship. In the study, it was tried to specify the fungal concentration, fungal types, seasonal distribution of fungi which can possibly be faced by the people when they get kotow position, and the dermatophyte factors in the slippers used during performing ablution.

Material and Method

Sampling, fungal isolation and identification

Research materials were collected from 5 m^2 area in total as 1 m^2 areas from the four corners and the exact middle area with a vacuum sweeper between the dates of October 2008-September 2009 from three different mosques in the Edirne City.

It was used a vacuum sweeper (BEKO TT-635, Turkey) in the practice of sampling from carpet dusts. The vacuum sweeper dust bags was transferred to 250 ml sterile containers after sampling from the carpets. Dust samples was weighed as 0.1 gr. and was dissolved in a sterile peptoned water involving Tween 80. The samples were kept in this liquid for 10 minutes, and then vortexed and it was waited for 15 minutes for the subsidence of dust (Macher, 2001).

It was transferred 1 ml for each liquid to 3 petri plates for microfungi by taking with pipette among the material and subsided dust flowing in the tube. Then, it was added potato dextrose agar (PDA) to these three petri plates, it was mixed by shaking 1 ml liquid and PDA petri slightly together, and all petri plates were observed daily. The petri plates was kept waiting in 37^{o}C incubator between 7 and 14 days. They transferred to 5 petri plates by taking 1 ml liquid samples by pipette from the tube in the same manner for dermatophytes. It was cultivated cycloheximide added sabouraud dextrose agar to two of these petri plates and sabouraud dextrose agar to three of them. One petri involving cycloheximide added sabouraud dextrose agar was incubated at 25^{0}C and another petri was incubated at 35^{0}C. Two petri plates involving sabouraud dextrose agar were incubated at 25^{0}C and one petri was incubated at 35^{0}C. All petri plates was kept waiting between 7 and 14 days.

The petri plates with colony forming unit (CFU) were calculated and it was calculated the average of the colonies counted from numerous petri plates in the same dilution. The microfungi concentration in the dust sample was expressed as CFU/g by using the formula below:

[Number of colonies in the petri (CFU) x Overall volume (ml)] **/** [dilution factor (10^{-X}) x cultivated volume (ml) x dust mass (g)] = CFU/g (Macher, 2001).

Also dematiaceous fungi isolated and did not create spores was cultivated as spot-on from the stock cultures in the tubes to PDA and malt extract agar (MEA) media. These plates were left for incubation for

seven days at 25^{0}C by performing spot-on cultivations to czapek agar (CA), czapek yeast autolysate agar (CYA), czapek yeast 20 % sükroz agar ($CY_{20}S$) and malt ekstrat agar (MEA) media of the types belong to *Aspergillus*. It was performed cultivation also to another petri plate which involves CYA and it was left for fertility for seven days (Klich, 2002).

It was used three CYA, 25 % glycerol nitrate agar ($G_{25}N$) and MEA media for identifying the types belong to *Penicillium* (Pitt et al., 2000). The samples cultivated in CYA media was left for incubation at 5°C, 25°C and 37°C, and the samples cultivated in $G_{25}N$ and MEA media was left for incubation at 25°C for seven days. At the end of the incubation process, it was examined the characteristics such as colony diameter, texture, shape, color from above and below, sporulation, zonation, exudation, pigmentation and the existence of various macroscopic structures of the fungal colonies in the petri plates which involve media intrinsic to types microscopically. It was examined the stereo microscope and colony texture, the incidence way and the measurements of various parts with light microscope and the features such as peripheral characteristics and colors. It was made naming by using algorithms defined previously according to the specified characteristics (Klich, 2002; Pitt et al., 2000; Pitt, 1979; Samson et al., 2002; Booth, 1971; Nelson et al., 1983; Gerlach ve Nirenberg, 1982; Barnett ve Hunter, 1999; Ellis, 1971; Ellis ve Ellis, 1997; Hasenekoglu, 1991).

The isolated types belong to dermatophyte was cultivated spot-on and they left for incubation for seven days at 25°C. The samples which are estimated as dermatopyhte fungus macroscopically and microscopically were cultivated in test media for identifying various vitamin requirements, and they were named by using algorithms defined previously (Sutton et al., 1998).

Characteristics of research stations

All stations are situated in the Edirne City Center. The stations have been using for worship and visit purposes between the hours 5:00 AM and 23:00 PM approximately. The first and the second station are historical buildings which have been visiting also for touristic purposes as well as worship, while the third station is a historical building using only for worship.

Moisture and temperature measurement

The temperature and moisture values of all stations were measured by the help of thermometer and hygrometer device (TFA-Dostmann GmbH, Germany) during sampling.

Statistical analyses

In the statistical evaluation, the relations between the microfungi isolated as for seasons and months and fungal concentrations and the various meteorological factors were examined with Spearman Correlation Analysis. It was used Mann Whitney U test in identifying whether there is a significant difference between the indoors environments and the outdoor environment of the mosque. It was used Kruskal Wallis test in identifying whether there is a significant difference between the stations and the overall fungal numbers. $P<0.05$ was accepted as the statistical significance limit value.

Results

It was isolated 78.937 microfungi CFU/g in total in 108 petri plates for fungi in carpet environment samples for the purpose of specifying the fungal intensity in the indoor carpets of the mosques. The distributions of the isolated fungal colony numbers according to months and stations were given in Table 1.

When the distributions of the isolated fungal colony quantities according to months and stations were examined, it was found the maximum fungal colony in the 3rd station with 40.89 % and the minimum fungal colony in the 1st station with 25.52 %. When the distributions of the colony numbers according to months were examined, it was found the maximum fungal colony in the month of October with 19.73 %. It was observed the minimum fungal colony on the month of May with 3.92 % (Table 1).

In the presented study, it was specified 24 fungal genera and 58 species in the samplings from the dusts over the carpets. In the general distribution between microfungi belong to the indoor carpets, the *Penicillium* (23.60 %) was on the first rank. The *Trichoderma* (17.40 %) was identified as second, the *Cladosporium* (12.31 %) as third, and the *Alternaria* (10.08 %) as fourth frequent microfungi. The microfungi in the first four ranks consisted 63.39 % of the overall colony quantity.

When the distributions of the isolated fungal colony quantities according to months were examined, it was found the maximum fungal colony in the month of October all the year around with 19.73 %. The month of September was on the second rank with 10.61 % and the month of June was on the third rank with 9.32 %. It was observed the minimum microfungus colony in the month of May with 3.92 % (Table 2).

Table 1. Distribution of fungal colony numbers isolated from carpet dust of the mosques between the dates of October 2008 and September 2009 according to the months and stations (CFU/g).

Months	1st station	2nd station	3rd station	Total
October	4499	4916	6084	15499
November	1083	1333	2366	4782
December	1665	3416	2333	7414
January	999	2667	750	4416
February	917	1083	2416	4416
March	1666	1500	3666	6832
April	1667	2084	1918	5669
May	416	833	1831	3080
June	2417	2083	2833	7333
July	1667	1500	3166	6333
August	1582	1750	1500	4832
September	2416	2583	3332	8331
Total	20994	25748	32195	
Total		78937		78937

Table 2. Fungal genera list the months in which they were isolated

From curpet dust (CFU/gr)	*Months (CFU/gr)
Acremonium spp. (1833)	9(167), 6(333),10(167), 12(1166)
Alternaria spp. (7914)	2(333), 3(333), 5(83), 6(167), 7(583), 8(1083), 9(1333), 10(3166), 11(83), 12(750)
Apiospora sp. (83)	5 (83)
Arthrinium sp. (83)	5 (83)
Aspergillus spp. (7082)	1(583), 2(584), 3(666), 4(2084), 5(333), 6(583), 7(750), 9(417), 10(333), 11(666), 12(83)
Chaetomium spp. (1416)	1(250), 2(250), 3(250), 5(250), 6(83), 9(167), 11(83), 12(83)
Cladosporium spp. (9667)	2(500), 3(1000), 5(250) 6(1834), 8(333), 9(1000), 10(2667), 11(750), 12(1333)
Drechslera sp. (83)	11 (83)
Fusarium spp. (2083)	6 (250), 7(1000), 9(83), 10 (500), 11(250),
Gliocladium spp. (166)	4(83), 8(83)
Gliomastix spp. (166)	12(83), 5(83)
Hirsutella sp. (83)	6(83)
Mucor spp. (2000)	12(83), 2(167), 4(167), 6(833), 9(750)
Penicillium spp.(18532)	1(2333), 2(2166), 3(3000), 4(1501), 5(1250), 6(500), 8(1000), 9(333), 10(3249), 11(1033), 12(2167)
Pithomyces spp. (333)	9 (333)
Rhizopus spp. (2917)	10(167), 11(250), 12(250), 2(83), 3(1167), 4(167), 5(83), 6(167), 7(167), 9(416)
Scopulariopsis spp. (2168)	1(417), 4(167), 5(83), 6(834), 10(167), 11(417), 12(83)
Sordaria sp. (83)	1(83)
Staphylotrichum sp. (333)	1(250), 2(83)
Trichoderma sp. (13666)	1(417), 2(83), 3(167), 4(750), 5(333), 6(583),

	7(3750), 8(1583), 9(1833), 10(3333), 11(667), 12(167)
Trichothecium sp. (167)	11(167)
Ulocladium spp.(2333)	5(83), 6(250), 8(250), 9(583), 10(167), 12(1000)
Verticillium sp. (83)	11(83)
Sterile (5246)	1(83), 2(167), 3(249), 4(750), 5(83), 6(833), 8(333), 9(916), 10(1583), 11(83), 12(166)
Trichophyton (83)	7(83)
Not identified (334)	8(167), 11(167)

*Months: January (1) is accepted as the beginning.

Trichophyton sp. was isolated from the carpet in the third station in the month of July once as a dermatophyte factor (Table 2). It was not isolated any dermatophyte type in any ablution slippers.

Discussion

The fungal concentrations accepted for carpet environment in literature sources varies between $2x10^4$ and 10^5 CFU/g (Esis, 2004). In its declaration published on the year of 2004, Global Risk Control Service (GRCS) emphasized that there are not any acceptable standards about building and workplace indoor surface contaminations, and that air and dust samples should be evaluated together for evaluating the potential contamination correctly (Esis, 2004). GRCS remarked the limit value in building and workplace indoor dust samples as 100.000 CFU/g. In the report of European Collaborative Action (ECA), <200-500 CFU/m^3 or <20.000 CFU/g concentration in dust samples was classified as low level (Celtik et al., 2011). As it was stated in the study previously published, it was identified that the fungal concentrations in the air in the same environments do not exceed the limit values (Tikvesli et al., 2018). In this study, it was considered that the mosque carpet environments are in accordance with the limit values identified by the Global Risk Control Service.

Niemeier et al. (2006) remarked that the concentration of fungal spores can be measured from the dust covering the floor, that between 20 and 40% of the houses in North Europe and Canada have fungal contamination, and that this value are much higher than the tropical and subtropical countries (Niemeier et al., 2006). They pointed the most widespread spores in the dust samples as *Aspergillus, Penicillium* and *Cladosporium* spores. In their study, Niemeier et al. (2006) identified much more type fungus in dust samples in comparions with the air samples when they compare the dust samples and the air samples belong to carpets or floors, and that the floor dust can be a source of indoor air fungi (Niemeier et al., 2006). When the findings of the study conducted previously in the air of the same environment (Tikvesli et al., 2018) were compared with the findings of this study, it was identified types and kinds in the carpet environment similar with the air environment. Identification of common types and kinds in carpet and air environment can be the evidence of that the microfungi hanged in air environment fall on the floor or they get mixed in the air environment from the floor.

When the distributions of the fungal spore numbers accumulated on the carpet surface in this study, it was seen that while the maximum fungus spore is in autumn season in which almost one third of overall fungal load [with 28614,2 CFU/g (36,44 %)] was identified, they isolated in close values in the following three seasons. Chao et al. (2002) identified the *Cladosporium* type as the most widespread type, and they also identified its maximum frequency in the winter, and then respectively in the seasons of autumn, spring and summer. They remarked that it reaches 12.000 CFU/g the highest concentration in the month of July. It was not seen any significant seasonal influence in *Aspergillus* and *Penicillium* types. They identified a level under 100 CFU/g in *Fusarium* all over the year (Chao et al., 2002). In our study, it was identified the most intense type as *Trichoderma* type in summer and autumn seasons, and *Penicillium* type in spring and winter seasons. *Penicillium* type which had been identified most widespread all over the year was identified as 3249.9 CFU/g the highest in the month of October.

In the study conducted by Ramachandran et al. on the year of 2005, they alleged that heat is the one and only variable for carpet fungal concentrations. They reached the conclusion of that heat causes decrease in carpet fungal levels (Ramachandran et al., 2005). Chao et al. (2002) specified in their study on carpet fungal

concentrations that heat has a positive effect on the fungal concentration (Chao et al., 2002). Buildings with good isolations and covering the floor with carpets provide conditions such as heat and moisture, and that causes a gradual increase of fungi (Ceylan et al., 2006). In the present study, we identified as a result of the correlation analyses made between moisture and heat values that there is not any relation between the fungal numbers on indoor carpet floor and the monthly average indoor moisture and heat.

As a result of the correlation analyses of the types identified as widespread in carpet environment, it was not identified any significant relation of it with moisture and heat in the correlation analyses of *Penicillium*, *Aspergillus* and *Alternaria* genera on the carpet surface. In conclusion, it is thought in this study that only *Trichoderma* and *Cladosporium* genera are affected from indoor conditions. *Trichoderma* genera had displayed an antagonistic situation with *Penicillium* and a synergistic situation with *Cladosporium*. So, it is thought in this study that *Trichoderma* and *Cladosporium* genera display similar seasonal characteristics. In this study, it could be observed the reproduction of *Trichoderma* genera by help of PDA used during the fungal isolation from carpet environment. *Trichoderma* genera was specified on the second rank and in all months following *Penicillium*. As a result, because of that it could be succeeded to isolate *Trichoderma* with the method we used in the study, the role of *Trichoderma* could be presented.

The most important problem of indoor spaces is the increased moisture (Wong et al., 2008). The moisture ratios of indoors over 70 % increase the risk of fungus formation (www.jivs.net/jivs/dosya/2003.pdf). Because of that the fungal concentration is higher in smaller spaces under favorable conditions, in case of inadequate ventilation and due to human activities, the fact that the 3rd station have all of these characteristics can explain the excess of fungal diversity and concentration. The section that has the minimum microfungus concentration is the worship space of the 1st station. The reason of this can be that the indoor of this station was good ventilated in contrast to the 3rd station, and that the 1st station is far from contamination affects because of the mosque floor and other sections have been cleaning and ventilating regularly in spite of that the station accepts many touristic visitor as well as there are individuals who have regularly been coming for worship.

Aspergillus is one of the most frequently isolated types among the dust samples (Stark et al., 2005). It is known that these fungi have allergenic features. In our study, *Penicillium* is on the first rank among the fungi identified in carpet environment, and it was specified in all months except the month of July. Although *Aspergillus* type is on the forth rank, it was not identified in all months except the month of August.

It has been conducted many studies in the indoor and outdoor environments of various buildings hospitals, schools, habitable houses, textile factories, farm houses, piggeries, slaughterhouses and caves. It was remarked that dusts involve too much air-sourced fungal spores which cause hypersensitivity in humans (Awad, 2002). However, people gather together in mosques as crowded groups and in this study, it was tried to specify whether the moisture of the mosques specified have a ratio that support fungal reproduction by conducting moisture control in those mosques. It was concluded that moisture does not have an effect in increasing the fungal concentration because of that it was not identified any relation between the overall indoor fungus quantity on carpet floor and the indoor moisture.

Carpet is an important allergenic reservoir (Tranter et al., 2009). It was showed in the studies that the carpets involve more allergenic (Beguin and Nolard, 1999). Old and damaged carpets may be a large reservoir for microfungi (Roberts et al., 1999). It is quite important to examine the air quality and the fungal concentration in carpet dust in such places in which the people gather together in crowded groups by considering the infecting effects of fungi. There are studies which show that there is strong connections between dust and health symptoms (Niemeier et al., 2006). Stark et al. (2005) alleged that the development of diseases of the 5 years old children with allergenic rhinitis and the fungal concentration (Stark et al., 2005). They especially pointed that they have isolated *Aspergillus* and then *Cladosporium* most widespread from the dust samples. They identified that the fungi with highest concentration are *Aureobasidium*, *Aspergillus*, *Alternaria*, yeast and the fungi which do not form spores. In this study, it is thought that fungi can create a potential risk about allergenic rhinitis due to that *Penicillium*, and then *Trichoderma*, *Cladosporium* ve *Alternaria* genera were isolated respectively of their frequency.

Celtik et al. (2011) have researched the fungal concentration in floor dusts in 10 elementary schools. In

mentioned study, they have isolated most frequently the types of *Cladosporium* (30.8 %), *Penicillium* (25.8 %), *Alternaria* (8.8 %) and *Aspergillus* (6.6 %). Celtik et al. (2011) interpreted their own study as that the fungal contamination is in low level in comparison with the ECA report. In our study, the first four fungal genera which are frequently isolated are *Penicillium* with 23.60 %, *Trichoderma* with 17.40 %, *Cladosporium* with 12.31 % and *Alternaria* with 10.08 %, and they constitute 63.39 % of the overall colony numbers. In this study, the highest value in indoor carpet environment was specified in the 3rd station in the month of October as 6.083 CFU/g. When the limit values of both ECA and GRCS are taken as a basis, it was evaluated that the fungus concentration in the three mosques in this study are between the healthy limit values (Hasenekoglu, 1991).

When the distributions of fungus spore accumulated on the carpet floor according to the stations, the maximum fungi were isolated in the third station. The reason why there are more indoor carpet environment fungal concentration in the third station can be about that it has the maximum fungal concentration in its indoor air, that it is smaller than the others, that the ventilation is not adequate with limited number of windows, and that all the facades of the building is surrounded with a garden area in which there are various plants and trees.

Dermatophytes are pathogenic fungi which cause dermatophytosis which is also defined as tinea infections by infecting the keratinized tissues (skin, hair and nails) of humans and animals (Hryncewicz-Gwozdz et al., 2011). *T. rubrum* is a filamentous fungus which cause 90 % of dermatophytosis in humans by affecting the epidermis (Garcia-Madrid et al., 2011). In our study, it was isolated only *Trichophyton rubrum* (83,3 CFU/g) in the third station once only in the month of July as a dermatophytosis factor. It could be found two literature which research the dermatophytosis factors from the carpet environment in the mosques which are one of the collective life spaces. In these researches, it was applied the single sampling method. In the study presented here, it is thought that the follow-up by months characteristic is also important.

Yenişehirli et al. (2012) researched dermatophytosis factor fungi from the carpet floor environments of 30 mosques in Tokat in worshippers. They took 160 samples from the carpet floor and 40 samples from the people with a cotton swab. As a result of the study, they identified 144 culture positive samples in total 200 samples including 113 from carpet samples and 31 from humans. They have isolated *Epidermophyton floccosum, Trichophyton mentagrophytes, T. rubrum, T. tonsurans* and *T. verricosum* dermatophytes in carpets and humans together. As a result, they remarked that fungus and contaminated carpet environment and humans can be reservoirs in terms of the contamination of these fungi (Yenişehirli et al., 2012).

Raboobee et al. (1998) researched the dermatophytosis factor fungi from the floor of the ablution section and the indoor carpet environment of eight mosques in Durban region. They also took nail and foot skin samples from 77 people who came regularly for worship to those mosques at least one time in a week. As a result of the study, they identified the tinea pedis and unguium prevalence in the people who have infection symptoms through culture positivity or microscopic evaluation as 85 %. They isolated the dermatophytes in all mosque floors. They specified *T. rubrum* and *Candida spp.* from the ablution section and *T. rubrum*, *T. verricosum* and *T. violaceum* from the carpet. In conclusion, they allege that the infected individuals can transfer fungi to the mosque floor and that other people can be infected from here (Raboobee et al, 1998).

In contrast to the studies of Raboobee et al. (1998) and Yenişehirli et al. (2012), the existence of dermatophytosis factors were found as scarcely any when it was followed up for 12 months in the mosque carpets in Edirne. It was also thought that this study is the third study in the literature with its characteristic of conducting on mosque carpets. When it is considered that it could be specified different findings from the first study, it was concluded that it should be conducted more studies on this subject (Raboobee et al. 1998; Yenişehirli et al., 2012).

It was thought in our study that it should be researched the dermatophyte load in the areas which can involve other risk factors such as ablution sections and slippers and even maybe it should be identified the dermatophytosis ratios in individuals who use the mosque regularly as for the society because of it was identified that the mosque carpets do not pose an important risk.

In our study presented here, it was identified only one kind dermatophyte factor (*Trichophyton rubrum*) for only once in the carpet dust of only one mosque station. Identifying dermatophyte factors in the mosque carpets

for only once makes we think that the mosque carpets are not very important in the contamination of infections between the people in the study field.

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The Powdery Mildew Fungi of Aladağlar and Bolkar Mountains in Turkey

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Abstract: In this study, sixty-one species of Powdery mildew (Erysiphales) in Aladağlar and Bolkar mountains were determined. The research was carried out between 2013 and 2016. The sixty-one species of Powdery mildew were observed from 37 family, 83 genera and 94 host species and make a contribution to Turkish mycobiota.

Key words: *Erysiphales*, New records, Parasite, Turkey

Aladağlar ve Bolkar Dağları (Türkiye) Külleme Mantarları

Öz: Bu çalışmada Aladağlar ve Bolkar dağlarında bulunan 61 külleme mantarı ve konakçıları tanımlanmıştır. Çalışma 2013 ve 2016 yılları arasında yapılmıştır. 61 külleme türü 37 familya, 83 cins ve 94 tür konakçı üzerinde belirlenmiştir ve Türkiye mycobiatasına katkılar sağlanmıştır.

Anahtar kelimeler: *Erysiphales*, Yeni kayıtlar, Parazit, Türkiye

Introduction

The powdery mildews (Erysiphales) represent a large group of common, obligate plant pathogens of cosmopolitan distribution, usually easily recognizable by their obvious symptoms, i.e. superficial white powdery patches or films of the anamorphic, composed of mycelium, conidiophores and conidia, on leaves, stems and other plant organs. Powdery mildews are assigned to an order and to a family of its own. The economic importance of these fungi in phytopathology and plant protection is enormous. Some species cause serious damages on a number of cultivated plants, such as cereals, vegetables, flowers, fruit trees, ornamental plants, etc. (Braun and Cook 2012).

According to the checklist on Turkish powdery mildews and recent literature (Akata et. al., 2019; Akdeniz and Sert, 2019; Kabaktepe et al., 2015, Churakov et al., 2018), 143 species belong to 14 teleomorphic and anamorphic genera and they were observed on 674 host plants species within the 322 genera and 72 families.

On the other hand, Turkey is one of the most comprehensively explored regions of powdery mildews in the world.

The purpose of the current study is to determine the powdery mildews (Erysiphales) of Aladağlar and Bolkar mountains and make a contribution to mycobiota of Turkey.

Material and Method

Host plants were collected from Aladağlar and Bolkar mountains (Kayseri, Niğde, Konya, Karaman, Mersin, Adana) in Turkey between 2013 and 2016. The Flora of Turkey (Davis, 1965-1985; Davis et al., 1988) was the main source used for the identification of the host specimens. Morphological observations of herbarium specimens were conducted according to the procedure outlined in Kabaktepe and Akata. (2019).

Herbarium samples used in this study were deposited into Inönü University herbaria (INU).

While systematic of the fungal taxa were in accordance with Braun (2017), and Index fungorum (www.indexfungorum.org: accessed 1 March 2020),

current names of the host plant taxa were confirmed according to the plant list (www. theplantlist.org). Identification of the fungal samples was performed according to Braun and Cook (2012).

Results

Fungi

Ascomycota

Leotiomycetes

Erysiphales

Erysiphaceae

Blumeria graminis (DC.) Speer: On *Avena barbata* Pottex ex Link (*Poaceae*), Adana: Akçatekir plateau, 920-1000 m, 22.05.2014, Ş. Kabaktepe 7510; On *Bromus sterilis* L. (*Poaceae*), Niğde: Çamardı, Emlivalley, 1800-1900 m, 25.06.2015, Ş. Kabaktepe 8122; Kayseri: Yahyalı, Kirazlıbağ village, 1250-1400 m, 25.09.2013, Ş. Kabaktepe 7152; On *Elymus hispidus* (Opiz) Melderis (*Poaceae*), Konya: Halkapınar, 5 km from Kayasaray to Çakıllar, 1350-1400 m, 14.07.2014, Ş. Kabaktepe 7565; On *Festuca cappadocica* (Hack.) Markgr.-Dann. (*Poaceae*), Mersin: Between Gülek to Çamlıyayla, Kurt çukuru area, 550-600 m, 26.06.2015, Ş. Kabaktepe 8161; On *Hordeum bulbosum* L. (*Poaceae*), Adana: Aladağ, 600-650 m., 23.04.2014, Ş. Kabaktepe 7375; On *Milium effusum* L. (*Poaceae*), Kayseri: Yahyalı, Hacer forest, Yedigöller, 2000-2100 m, 24.06.2015, Ş. Kabaktepe 8101; On *Phleum pratense* L. (*Poaceae*), Niğde: Çamardı, Emli valley, 1800-1900 m, 25.06.2015, Ş. Kabaktepe 8120; On *Secale cereale* L. (*Poaceae*), Kayseri; Yahyalı, 3-5 km from Ulupınar to Aksu valley, 1100 m, 21.05.2014, Ş. Kabaktepe 7454; On *Triticum aestivum* L. (*Poaceae*), Kayseri; Yahyalı, 3-5 km from Ulupınar to Aksu valley, 1100 m, 21.05.2014, Ş. Kabaktepe 7463; Kayseri, Yahyalı, 2 km from Çamlıca to Ulupınar, 1050 m, 21.05.2014, Ş. Kabaktepe 7471; Mersin: Toroslar, 5 km from Değnek to Tırtar, 1400 m, 23.05.2014, Ş. Kabaktepe 7512.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Erysiphe alphitoides (Griffon & Maubl.) U. Braun& S. Takam.: On *Quercus cerris* L. (*Fagaceae*), Adana: Aladağ, 22. km from Gerdibi to Kamışlı, 980 m, 18.09.2014, Ş. Kabaktepe 7815; Niğde, Ulukışla, 10. km from Kılan to Darboğaz, 1500-1550 m, 29.08.2015, Ş. Kabaktepe 8284; Kayseri: Yahyalı, Derebağ waterfall, 1330 m, 17.09.2014, Ş. Kabaktepe 7778; On *Quercus coccifera* L. (*Fagaceae*), Konya: Halkapınar, Yassıkaya village, 1460 m, 31.10.2014, Ş. Kabaktepe 7939; Kayseri: Yahyalı, 18. km from Kapuzbaşı to Aladağ, Pos jungle, 920 m, 18.09.2014, Ş. Kabaktepe 7795; On *Quercus infectoria* Olivier (*Fagaceae*), Kayseri: Yahyalı, Derebağ waterfall, 1350-1450 m, 29.10.2014, Ş. Kabaktepe 7896; Mersin: Çamlıyayla, İnköy village, 850-900 m, 31.10.2014, Ş. Kabaktepe 7948; Mersin: Çamlıyayla, 2. km from Sebil to Cehennem River, 1100-1150 m, 01.11.2014, Ş. Kabaktepe 7963; Mersin: Çamlıyayla, Sebil, Cehennem river, Bağdut area, 900-960 m, 01.11.2014, Ş. Kabaktepe 7971; Mersin: Çamlıyayla, Sinap Castle, 1100-1200 m, 26.08.2015, Ş. Kabaktepe 8202; On *Quercus pubescens* Willd. (*Fagaceae*), Kayseri, Yahyalı, Derebağ waterfall, 1350-1450 m, 14.10.2015, Ş. Kabaktepe 8338.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Erysiphe aquilegiae DC.: On *Ranunculus* sp. (*Ranunculaceae*), Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7823.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Erysiphe astragali DC.: On *Astragalus* sp. (*Fabaceae*), Kayseri: Yahyalı, Derebağ Derebağ waterfall, 1350-1450 m, 14.10.2015, Ş. Kabaktepe 8333.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Erysiphe atraphaxis (Golovin) U. Braun & S. Takam.: On *Atraphaxis billardieri* Jaub. & Spach (*Polygonaceae*), Mersin: Çamlıyayla, Sebil, Cehennem River, Bağdut area, 900-960 m, 01.11.2014, Ş. Kabaktepe 7974.
Distribution: China, Mongolia (Braun and Cook, 2012).

Erysiphe berberidis DC.: *Berberis crataegina* DC. (*Berberidaceae*), Niğde: Çamardı, Demirkazık, Cımbar throat, 1600-1750 m, 30.10.2014, Ş. Kabaktepe 7909; Kayseri: Yahyalı, 5. km from Ulupınar to Hacer foest, 1450 m, 24.06.2015, Ş. Kabaktepe 8189.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Erysiphe buhrii U. Braun: On *Arenaria rotundifolia* M. Bieb. (*Caryophyllaceae*), Niğde, Çamardı, Emli valley, 1900-2200 m, 16.10.2015, Ş. Kabaktepe 8434; On *Minuartia juniperina* (L.) Maire & Petitm. (*Caryophyllaceae*), Mersin: Çamlıyayla, Saydibi plateau, 1860 m, 09.10.2013, Ş. Kabaktepe 7355.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Erysiphe caulicola (Petr.) U. Braun: On *Astragalus* sp. (*Fabaceae*), Niğde: Ulukışla, 10 km from Kılan to Darboğaz, 1500-1550 m, 29.08.2015, Ş. Kabaktepe 8283.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Erysiphe coluteae (Kom.) U. Braun & S. Takam.: On *Colutea cilicica* Boiss. & Balansa (*Fabaceae*), Konya: Halkapınar, İvriz dam lake, 1080-1130 m, 28.08.2015, Ş. Kabaktepe 8263.
Distribution: Cosmopolitan in Asia (Braun and Cook, 2012).

Erysiphe convolvuli DC.: On *Convolvulus arvensis* L. (*Convolvulaceae*), Kayseri: 30. km from Develi to Yahyalı, 1030 m, 05.10.2013, Ş. Kabaktepe 7258; Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7829; Konya, Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8380.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Erysiphe cruciferarum Opiz ex L. Junell: On *Alyssum strigosum* Banks & Sol. (*Brassicaceae*), Adana: Aladağ, Gerdibi village, 27.09.2013, Ş. Kabaktepe 7238; Kayseri, Yahyalı, 3-5 km from Ulupınar to Aksu valley, 1100 m, 21.05.2014, Ş. Kabaktepe 7451; Kayseri: Yahyalı, Derebağ waterfall, 1400-1600 m, 23.06.2015, Ş. Kabaktepe 8072; Kayseri, Yahyalı, Derebağ Derebağ waterfall, 1350-1450 m, 14.10.2015, Ş. Kabaktepe 8336.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Erysiphe flexuosa (Peck) U. Braun & S. Takam.: On *Aesculus hippocastanum* L. (*Sapindaceae*), Kayseri: Yahyalı, Derebağ waterfall, 1330 m, 17.09.2014, Ş. Kabaktepe 7775.
Distribution: Cosmopolitan in Europe, Canada, USA (Braun and Cook, 2012).
Erysiphe geraniacearum U. Braun & Simonyan: On *Geranium tuberosum* L. (*Geraniaceae*), Adana: Pozantı, Horoz village, 1200-1250 m, 15.07.2014, Ş. Kabaktepe 7616.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Erysiphe guarinonii (Briosi & Cavara) U. Braun & S. Takam.: On *Laburnum anagyroides* Medik. (*Fabaceae*), Konya, Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8324.
Distribution: Australia, Germany, Denmark, United Kingdom, Switzerland, Italy, Poland (Braun and Cook, 2012).
Erysiphe heraclei DC.: On *Seseli libanotis* Koch (*Apiaceae*), Mersin: Toroslar, 3 km from Aslanköy to Fındıkpınarı, 20.09.2014, Ş. Kabaktepe 7873; Adana: 8. km from Mansurlu to Yahyalı, 1450-1500 m, 25.08.2015, Ş. Kabaktepe 8180; Kayseri, Yahyalı, Derebağ waterfall, 1350-1450 m, 14.10.2015, Ş. Kabaktepe 8337; Konya, Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8372; On *Torilis arvensis* (Huds.) Link (*Apiaceae*), Kayseri: Yahyalı, Ulupınar, 1800-2000 m, 16.07.2014, Ş. Kabaktepe 7637.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Erysiphe knautiae Duby: On *Morina persica* L. (*Morinaceae*), Niğde: Çamardı, Demirkazık village, Cımbar throat, 1650-1800 m, 15.07.2014, Ş. Kabaktepe 7587; Niğde: Çamardı, Demirkazık, Cımbar throat, 1600-1750 m, 30.10.2014, Ş. Kabaktepe 7911.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Erysiphe lycopsidis R.Y. Zheng & G.Q. Chen: On *Anchusa leptophylla* Roem. & Schult. (*Boraginaceae*), Kayseri, Yahyalı, 3-5 km from Ulupınar to Aksu valley, 1100 m, 21.05.2014, Ş. Kabaktepe 7457; Kayseri: Yahyalı, Derebağ waterfall, 1350-1450 m, 29.10.2014, Ş. Kabaktepe 7893; Kayseri: Yahyalı, Ulupınar, Hacer forest, 1450 m, 24.06.2015, Ş. Kabaktepe 8109; Kayseri, 18. km from Develi to Yahyalı, 1000-1050 m, 14.10.2015, Ş. Kabaktepe 8315; On *Cynoglossum montanum* L. (*Boraginaceae*) Mersin: Toroslar, Atlılar village, 1250 m, 27.06.2015, Ş. Kabaktepe 8170.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Erysiphe mayorii S. Blumer: On *Cirsium vulgare* (Savi) Ten. (*Asteraceae*), Konya, Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8369.
Distribution: Cosmopolitan in Europe, Iran (Braun and Cook, 2012).
Erysiphe necator Schwein.: On *Vitis vinifera* L. (*Vitaceae*), Kayseri: Yahyalı, Derebağ waterfall, 1250 m, 05.10.2013, Ş. Kabaktepe 7276; Mersin: Çamlıyayla, Sebil, Cehennem River, 1100-1150 m, 01.11.2014, Ş. Kabaktepe 7956; Kayseri, 18 km from Develi to Yahyalı, 1000-1050 m, 14.10.2015, Ş. Kabaktepe 8321; Konya, 3 km from Ereğli to Halkapınar, 950 m, 15.10.2015, Ş. Kabaktepe 8398.
Distribution: Cosmopolitan in Europe, Iran (Braun and Cook, 2012).
Erysiphe pisi DC: On *Consolida orientalis* (J. Gay.) Schrödinger (*Fabaceae*), Kayseri, Yahyalı, 3-5 km from Ulupınar to Aksu valley, 1100 m, 21.05.2014, Ş. Kabaktepe 7459; Adana: Pozantı, Dağdibi village, 27.09.2013, Ş. Kabaktepe 7230; On *Medicago* x *varia* Martyn (*Fabaceae*), Kayseri: Yahyalı, Derebağ waterfall, 1350-1450 m, 29.10.2014, Ş. Kabaktepe 7886; Kayseri: Yahyalı, Kirazlıbağ village, 1250-1400 m, 25.09.2013, Ş. Kabaktepe 7154; Konya, 3 km from Ereğli to Halkapınar, 950 m, 15.10.2015, Ş. Kabaktepe 8400; On *Vicia cracca* L. (*Fabaceae*) Mersin, Çamlıyayla, Kadıncık valley, Kuzbağı area, 1350-1450 m, 17.10.2015, Ş. Kabaktepe 8479.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Erysiphe platani (Howe) U. Braun & S. Takam.: On *Platanus orientalis* L. (*Platanaceae*), Kayseri, Yahyalı, Derebağ waterfall, 1270 m, 17.09.2014, Ş. Kabaktepe 7768; On *Platanus orientalis* L. (*Platanaceae*), Kayseri: Yahyalı, Kapuzbaşı, 650-700 m, 18.09.2014, Ş. Kabaktepe 7791.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Erysiphe polygoni DC.: On *Polygonum bistorta* L. (*Polygonaceae*), Kayseri: Yahyalı, Kirazlıbağ village, 1150-1200 m, 14.10.2015, Ş. Kabaktepe 8357; Adana: Pozantı, Horoz village,1300-1350 m, 15.07.2014, Ş. Kabaktepe 7591; On *Rumex patientia* L. (*Polygonaceae*), Niğde, Ulukışla, Kılan, Eskiköy area, 1680-1750 m, 29.08.2015, Ş. Kabaktepe 8295.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Erysiphe thesii L. Junell: On *Thesium arvense* Horv. (*Santalaceae*), Niğde, Ulukışla,14 km from Kılan to Darboğaz, 1600-1650 m, 29.08.2015, Ş. Kabaktepe 8289.
Distribution: Cosmopolitan in Europe (Braun and Cook, 2012).
Erysiphe tortilis (Wallr.) Link: On *Cornus sanguinea* L. (*Cornaceae*), Konya: Halkapınar, İvriz dam lake, 1080-1130 m, 28.08.2015, Ş. Kabaktepe 8262.
Distribution: Armenia, Georgia, Iran, Cosmopolitan in Europe (Braun and Cook, 2012).
Erysiphe urticae (Wallr.) S. Blumer: On *Urtica dioica* L. (*Urticaceae*), Mersin: Çamlıyayla, Saydibi plateau, 1800-1880 m, 09.10.2013, Ş. Kabaktepe 7356.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Golovinomyces biocellatus (Ehrenb.) V.P. Heluta: On *Ajuga salicifolia* (L.) Schreb. (*Lamiaceae*), Niğde: Çamardı, Demirkazık village, Cımbar throat, 1650-1800 m, 15.07.2014, Ş. Kabaktepe 7580; On *Nepeta nuda* L. (*Lamiaceae*), Konya, Halkapınar, Yassıkaya village, 1850 m, 14.07.2014, Ş. Kabaktepe 7557.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Golovinomyces cichoracearum (DC.) V.P. Heluta: On *Achillea arabica* Kotschy (*Asteraceae*), Mersin: Çamlıyayla, Sebil – Cehennem River, 1400 m,

27.06.2015, Ş. Kabaktepe 8162; On *Crepis foetida* L. (*Asteraceae*), Konya: Halkapınar, 5 km from Kayasaray to Çakıllar, 1350-1400 m, 14.07.2014, Ş. Kabaktepe 7558b; On *Inula aucheriana* DC. (*Asteraceae*), Mersin: Tarsus, Söğütlü village, 575 m, 08.10.2013, Ş. Kabaktepe 7332b; Niğde: Çamardı, Demirkazık village, Cımbar, 1650-1800 m, 15.07.2014, Ş. Kabaktepe 7588; On *Lactuca tuberosa* Jacq. (*Asteraceae*), Konya: Halkapınar, Kayasaray village, 1650-1750 m, 13.07.2014, Ş. Kabaktepe 7542; On *Taraxacum* sp. (*Asteraceae*), Konya: Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8397.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Golovinomyces cucurbitacearum (R.Y. Zheng & G.Q. Chen) Vakal. & Kliron.: On *Cucurbita pepo* L. (*Cucurbitaceae*), Adana: 8 km from Mansurlu to Yahyalı, 1450-1500 m, 25.08.2015, Ş. Kabaktepe 8179; Kayseri: 6 km from Yahyalı to Kirazlıbağ, 1250-1400 m, 14.10.2015, Ş. Kabaktepe 8348.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Golovinomyces cynoglossi (Wallr.) V.P. Heluta: On *Anchusa leptophylla* Roem. & Schult. (*Boraginaceae*), Konya, 18 km from Ereğli to İvriz, 1100 m, 15.10.2015, Ş. Kabaktepe 8364; On *Cerinthe minor* L. (*Boraginaceae*) Konya: Halkapınar, Kayasaray village, 1650-1750 m, 13.07.2014, Ş. Kabaktepe 7542.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Golovinomyces depressus (Wallr.) V.P. Heluta: On *Arctium minus* (Hill) Bernh. (*Asteraceae*), Adana: Pozantı, Horoz Throat, 950-1000 m, 30.10.2014, Ş. Kabaktepe 7917.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Golovinomyces orontii (Castagne) V.P. Heluta: On *Acanthus dioscoridis* L. (*Acanthaceae*), Konya, Halkapınar, Kayasaray village, 1650-1750 m, 13.07.2014, Ş. Kabaktepe 7548; On *Helianthus annuus* L. (*Asteraceae*), Adana: 8 km from Mansurlu to Yahyalı, 1450-1500 m, 25.08.2015, Ş. Kabaktepe 8177.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Golovinomyces sordidus (L. Junell) V.P. Heluta: On *Plantago major* L. (*Plantaginaceae*), Niğde: Çamardı, Demirkazık, Cımbar Throat, 1600-1750 m, 30.10.2014, Ş. Kabaktepe 7906.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Golovinomyces valerianae (Jacz.) V.P. Heluta: On *Centranthus longiflorus* Steven (*Caprifoliaceae*), Niğde: Çamardı, Demirkazık, Cımbar Throat, 1600-1750 m, 30.10.2014, Ş. Kabaktepe 7910; Kayseri, Yahyalı, Derebağ waterfall, 1350-1450 m, 14.10.2015, Ş. Kabaktepe 8328; On *Valeriana dioscoridis* Sm. (*Caprifoliaceae*) Adana:5 km from Pozantı to Ömerli, 850 m, 20.05.2014, Ş. Kabaktepe 7444.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Leveillula chrozophorae U. Braun: On *Chrozophora tinctoria* (L.) A. Juss. (*Euphorbiaceae*), Kayseri, Yahyalı, Çamlıca village, 1050-1100m, 16.07.2014, Ş. Kabaktepe 7654.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Leveillula duriaei (Lév.) U. Braun: On *Phlomis pungens* Willd. (*Lamiaceae*), Niğde: Ulukışla, 3-4 km from Darboğaz to Yazıgülü plateau, 1550 m, 19.09.2014, Ş. Kabaktepe 7839; Konya, Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8375; On *Stachys sparsipilosa* R.Bhattacharjee & Hub.-Mor. (*Lamiaceae*), Mersin: Çamlıyayla, Olukkaya village, 990 m, 22.05.2014, Ş. Kabaktepe 7487; Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7822; On *Teucrium chamaedrys* L. (*Lamiaceae*), Konya, Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8388.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Leveillula geraniacearum Eliade ex U. Braun: On *Geranium tuberosum* L. (*Geraniaceae*), Kayseri: Yahyalı, Derebağ waterfall, 1350-1450 m, 29.10.2014, Ş. Kabaktepe 7890.

Distribution: Bulgaria, France, Georgia, Romania, Serbia (Braun and Cook, 2012).

Leveillula lactucarum Durrieu & Rostam: On *Chondrilla juncea* L. (*Asteraceae*), Kayseri: 30 km from Develi to Yahyalı, 1030 m, 05.10.2013, Ş. Kabaktepe 7255; Mersin: Tarsus, Gülek village, 1240 m, 08.10.2013, Ş. Kabaktepe 7320; Kayseri, 18 km from Develi to Yahyalı, 1000-1050 m, 14.10.2015, Ş. Kabaktepe 8313; Konya: Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8386; On *Lactuca sativa* L. (*Asteraceae*), Adana, Pozantı, Karanfil mountain, 1150-1200 m, 16.10.2015, Ş. Kabaktepe 8453; Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7822.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Leveillula lappae (Castagne) U. Braun: On *Centaurea virgata* Lam. (*Asteraceae*), Kayseri, Yahyalı, Derebağ waterfall, 1270 m, 17.09.2014, Ş. Kabaktepe 7770; Çamardı, Demirkazık, Yarpuz II area, 2300-2400 m, 25.06.2015, Ş. Kabaktepe 8116; Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7825; Mersin: Çamlıyayla, Sebil, Cehennem River, Suçatı area, 1800-2000 m, 27.06.2015, Ş. Kabaktepe 8165; On *Cirsium arvense* (L.) Scop. (*Asteraceae*), Niğde, Ulukışla, 15-20 km from Darboğaz to Yassıkaya, 1550-1600 m, 15.10.2015, Ş. Kabaktepe 8422; On *Crepis foetida* L. (*Asteraceae*), Konya, Halkapınar, İvriz village, 1200-1300 m,) 15.10.2015, Ş. Kabaktepe 8381.

Distribution: Cosmopolitan (Braun and Cook, 2012).

Leveillula papilionacearum (Kom.) U. Braun: On *Astragalus* sp. (*Fabaceae*), Konya: Halkapınar, İvriz village 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8392; On *Coronilla varia* L. (*Fabaceae*), Adana: 8 km from Aladağ to Mansurlu, 960 m, 20.05.2015, Ş. Kabaktepe 8046; On *Lathyrus pratensis* L. (*Fabaceae*), Kayseri: Yahyalı, Kapuzbaşı, 650-700 m, 18.09.2014, Ş. Kabaktepe 7790; On *Medicago sativa* L. (*Fabaceae*), Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7821; Konya, Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8387; On *Ononis spinosa* L. (*Fabaceae*), Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7824; On *Vicia villosa* Roth

(*Fabaceae*), Adana. Aladağ, 600-650 m. 23.04.2014, Ş. Kabaktepe 7374; Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7818; Konya, 18km from Ereğli to İvriz, 1100 m, 15.10.2015, Ş. Kabaktepe 8360; Konya, Halkapınar, Çakıllar village, Aydos mountain, 1350-1400 m, 15.10.2015, Ş. Kabaktepe 8407; Niğde, Ulukışla, Darboğaz village, 1550-1600 m, 15.10.2015, Ş. Kabaktepe 8424.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Leveillula picridis (Castagne) Durrieu & Rostam: On *Centaurea kotschyi* (Boiss. & Heldr.) Hayek (*Asteraceae*), Niğde, Ulukışla, Darboğaz village, 1550-1600 m, 15.10.2015, Ş. Kabaktepe 8415; On *Echinops ritro* L. (*Asteraceae*), Konya: Halkapınar, Yassıkaya villge, 1460 m, 31.10.2014, Ş. Kabaktepe 7942; On *Picris hieracioides* L. (*Asteraceae*), Niğde, Çamardı, Elek village, 27.09.2013, Ş. Kabaktepe 7222.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Leveillula taurica (Lév.) G. Arnaud: On *Glaucium grandiflorum* Boiss. & A. Huet (*Papaveraceae*), Konya: Halkapınar, Kayasaray village, 1650-1750 m, 13.07.2014, Ş. Kabaktepe 7543; Kayseri, Yahyalı, Derebağ waterfall, 14.10.2015, Ş. Kabaktepe 8332; On *Papaver rhoeas* L. (*Papaveraceae*), Adana: Pozantı, Ömerli village, 1100-1150 m, 15.07.2014, Ş. Kabaktepe 7601; On *Peganum harmala* L. (*Zygophyllaceae*), Kayseri: Yahyalı, Çubuklu village, 26.09.2013, Ş. Kabaktepe 7179; On *Scabiosa argentea* L. (*Dipsecaceae*), Niğde, Çamardı, Emli valley, 1900-2200 m, 16.10.2015, Ş. Kabaktepe 8433.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Leveillula verbasci (Jacz.) Golovin: On *Verbascum* sp. (*Scrophulariaceae*), Niğde, Ulukışla, 10 km from Kılan to Darboğaz, 1500-1550 m, 29.08.2015, Ş. Kabaktepe 8286.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Neoerysiphe galeopsidis (DC.) U. Braun: On *Sideritis libanotica* Labill. (*Lamiaceae*), Mersin: Çamlıyayla, Kadıncık valley, 1850-1900 m, 18.07.2014, Ş. Kabaktepe 7691; *Marrubium vulgare* L. (*Lamiaceae*), Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7826; Konya, Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8384; On *Stachys sparsipilosa* R. Bhattacharjee & Hub.-Mor. (*Lamiaceae*), Kayseri: Yahyalı, Ulupınar, Yedigöller area, 1800-2000 m, 16.07.2014, Ş. Kabaktepe 7633.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Neoerysiphe galii (S. Blumer) U. Braun: On *Galium verum* L. (*Rubiaceae*), Niğde, Çamardı, Demirkazık village, 1850-2000 m, 26.09.2013, Ş. Kabaktepe 7213; Kayseri: Yahyalı, Kayapınar plateau, 1600-1650 m, 23.06.2015, Ş. Kabaktepe 8081.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Phyllactinia corni H.D. Shin & M.J. Park: On *Cornus mas* L. (*Cornaceae*), Mersin: Çamlıyayla, Sebil, Cehennem River, 1100-1150 m, 01.11.2014, Ş. Kabaktepe 7962.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Phyllactinia fraxini (DC.) Fuss: On *Fraxinus angustifolia* Vahl (*Oleaceae*), Mersin: Mezitli, Tepeköy village, 1140 m, 20.09.2014, Ş. Kabaktepe 7883; Mersin: Çamlıyayla, Sebil, Cehennem River, 1100-1150 m, 01.11.2014, Ş. Kabaktepe 7961.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Phyllactinia guttata (Wallr.) Lév.: On *Corylus avellana* L. (*Betulaceae*), Konya, 18 km from Ereğli to İvriz, 1100 m, 15.10.2015, Ş. Kabaktepe 8365.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Phyllactinia mali (Duby) U. Braun: On *Crataegus monogyna* Jacq. (*Rosaceae*), Kayseri: 30 km from Develi to Yahyalı, 1030 m, 05.10.2013, Ş. Kabaktepe 7263; Mersin, Tarsus, Gülek throat, 1020-1100 m, 08.10.2013, Ş. Kabaktepe 7322; Mersin: Tarsus, Söğütlü village, 575 m, 08.10.2013, Ş. Kabaktepe 7333; Adana: Aladağ, 7 km from Kökez to Gerdibi, 1020 m, 18.09.2014, Ş. Kabaktepe 7799.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Phyllactinia nivea (Castagne) U. Braun: On *Ulmus glabra* Huds. (*Ulmaceae*), Kayseri: Aladaglar National Park, Ulupınar village, 1080 m, 07.10.2013, Ş. Kabaktepe 7291; On *Crataegus monogyna* Jacq. (*Rosaceae*), Kayseri: Yahyalı, Derebağ waterfall, 1350-1450 m, 29.10.2014, Ş. Kabaktepe 7888; Mersin: Çamlıyayla, Sebil, Cehennem River, 1100-1150 m, 01.11.2014, Ş. Kabaktepe 7960.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Phyllactinia paliuri U. Braun: On *Paliurus spina-christi* P. Mill. (*Rhamnaceae*), Kayseri: Yahyalı, Aladaglar National Park, Ulupınar village, 900-950 m, 07.10.2013, Ş. Kabaktepe 7281; Mersin: Toroslar, 4 km from Tırtar to Aslanköy, 1350 m, 20.09.2014, Ş. Kabaktepe 7864; Adana: Pozantı, Horoz throat, 950-1000 m, 30.10.2014, Ş. Kabaktepe 7919.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Phyllactinia pistaciae H.D. Shin & Y.J. Choi: On *Pistacia terebinthus* L. (*Anacardiaceae*), Adana: Pozantı, Horoz throat, 950-1000 m, 30.10.2014, Ş. Kabaktepe 7923.
Distribution: Iran, Kazakhstan, Kyrgyzstan, Uzbekistan, Turkmenistan, Greece (Braun and Cook, 2012).

Phyllactinia pyri-serotinae Sawada: On *Cotoneaster nummularia* Fisch. & C.A. Mey. (*Rosaceae*), Kayseri: Yahyalı, Derebağ waterfall, 1350-1450 m, 29.10.2014, Ş. Kabaktepe 7889; Adana: Aladağ, Gerdibi village, 975 m, 18.09.2014, Ş. Kabaktepe 7803; Kayseri: Yahyalı, Ulupınar village, Aksu valley, 1050-1100 m, 25.08.2015, Ş. Kabaktepe 8187; On *Crataegus monogyna* Jacq. (*Rosaceae*), Konya: Halkapınar, Yassıkaya village, 1800-1850 m, 29.08.2015, Ş. Kabaktepe 8379; Kayseri: Yahyalı, Derebağ waterfall, 1350-1450 m, 29.10.2014, Ş. Kabaktepe 7891; On *Crataegus szovitsii* Pojark., Kayseri: Yahyalı, Derebağ waterfall, 1350-1450 m, 14.10.2015, Ş. Kabaktepe 8341; Mersin: Tarsus, Gülek, Karboğazı, 1750-1850 m, 16.10.2015, Ş. Kabaktepe 8462.
Distribution: Cosmopolitan (Braun and Cook, 2012).

Podosphaera aphanis (Wallr.) U. Braun & S. Takam.: On *Alchemilla holocycla* Rothm. (*Rosaceae*), Niğde:

Dündarlı, 1600 m, 30.10.2014, Ş. Kabaktepe 7895. On *Potentilla recta* L. (*Rosaceae*), Kayseri: Yahyalı, Aladaglar National Park, Hacer forest, 1550-1600 m, 07.10.2013, Ş. Kabaktepe 7300.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Podosphaera dipsacacearum (Tul. & C. Tul.) U. Braun& S. Takam.: On *Scabiosa argentea* L. (*Dipsecaceae*), Mersin: Toroslar, 4 km from Tırtar to Aslanköy, 1350 m, 20.09.2014, Ş. Kabaktepe 7864.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Podosphaera ferruginea (Schltdl.) U. Braun & S. Takam.: On *Sanguisorba minor* L. (*Rosaceae*), Mersin: Tarsus, Söğütlü village, 575 m, 08.10.2013, Ş. Kabaktepe 7332; Konya, Halkapınar, İvriz village, 1200-1300 m, 15.10.2015, Ş. Kabaktepe 8371.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Podosphaera fusca (Fr.) U. Braun & Shishkoff: On *Xanthium strumarium* L. (*Asteraceae*), Mersin: Tarsus, Söğütlü village, 575 m, 08.10.2013, Ş. Kabaktepe 7336.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Podosphaera pannosa (Wallr.) de Bary: On *Prunus domestica* L. (*Rosaceae*), Mersin: Çamlıyayla, 7 km from Fakılar to Kadıncık valley, 1200-1300 m, 18.07.2014, Ş. Kabaktepe 7732; On *Rosa canina* L. (*Rosaceae*), Niğde: Ulukışla, 3-4 km from Darboğaz to Yazıgülü plateau, 1550 m, 19.09.2014, Ş. Kabaktepe 7843.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Podosphaera plantaginis (Castagne) U. Braun & S. Takam.: On *Plantago major* L. (*Plantaginaceae*), Niğde: Ulukışla, Emirler village, 1440-1460 m, 19.09.2014, Ş. Kabaktepe 7835.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Podosphaera tridactyla (Wallr.) de Bary: On *Ceracus incana* var. *incana* (Pall.) Spach (*Rosaceae*), Kayseri: Yahyalı, Aladaglar National Park, Hacer forest, 950-1000 m, 24.06.2015, Ş. Kabaktepe 8085.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Sawadaea bicornis (Wallr.) Homma: On *Acer monspessulanum* L. (*Sapindaceae*), Karaman: 2-3 km from Ayrancı to Çat, 1050-1100 m, 28.08.2015, Ş. Kabaktepe 8254.
Distribution: Cosmopolitan (Braun and Cook, 2012).
Sawadaea tulasnei (Fuckel) Homma. On *Acer monspessulanum* L. (*Sapindaceae*), Niğde: Çamardı, Demirkazık village 1850-2000 m, 26.09.2013, Ş. Kabaktepe & I. Akata 7218.
Distribution: Cosmopolitan in Europe, China, Iran, Japan, Korea, Russia (Braun and Cook, 2012).

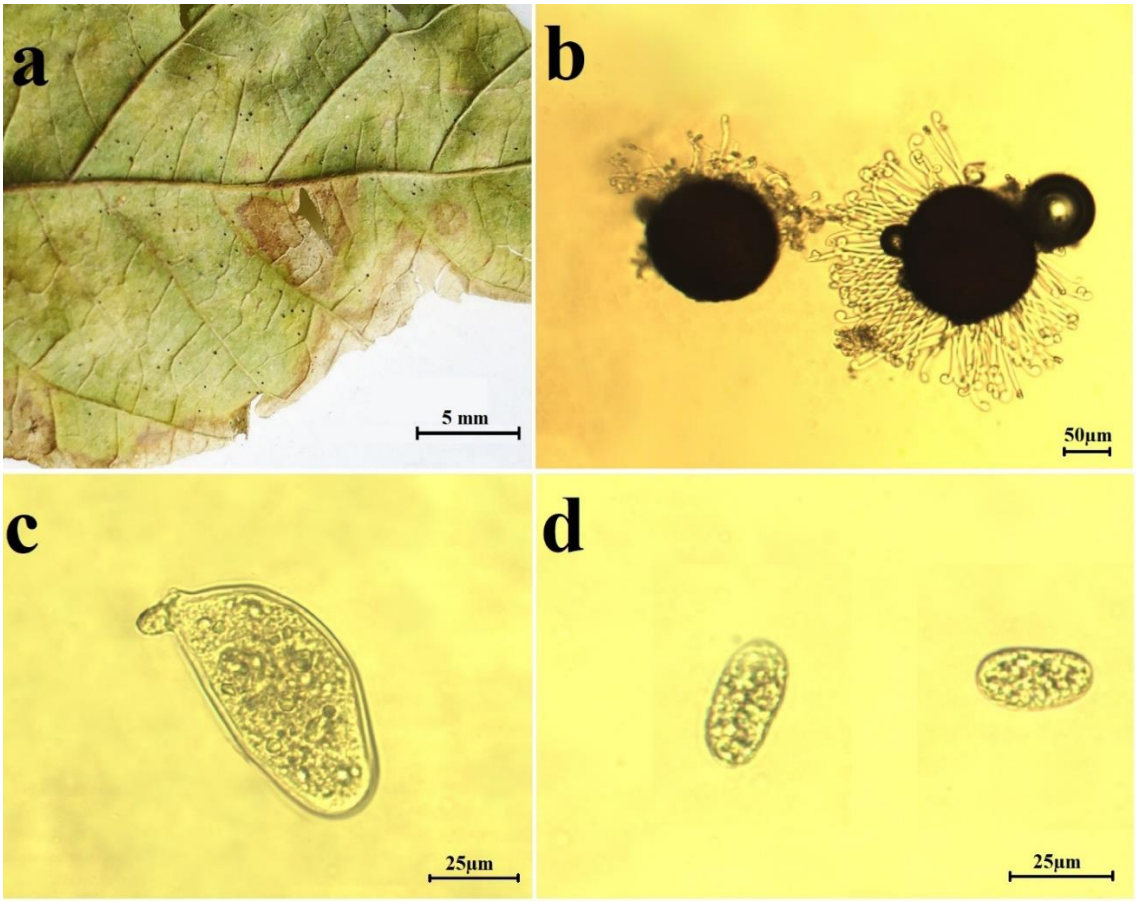

Figure 1. *Sawadaea tulasnei* on *Acer monspessulanum***: a.**, the host plant affected by the powdery mildews **b.**, .chasmothecia as viewed under the light microscope **c. a** single ascus **-d.** ascospores.

Discussions

As a result, Sixty-one powdery mildews species of eight genera and one family were listed. The samples belonging to Sixty-one powdery mildews were observed on 94 host species within the 83 genera and 37 families.

The species numbers of genera are as follows; *Erysiphe* 24, *Leveillula* 9, *Golovinomyces* 8, *Phyllactinia* 8 and *Podosphaera* 7, Neoerysiphe 2, Sawadaea 2, and Blumeria 1.

With the current study, we make a contrubion of Turkey mycobiota and the number of Turkish powdery mildew species are 144.

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Antioxidant and Antimicrobial Activities of *Armillaria mellea* and *Macrolepiota procera* Extracts

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Abstract: Mushrooms have been used extensively, owing to their nutritional and medicinal value, for thousands of years. This study designed for the determine of antioxidant and antimicrobial potential of two edible mushrooms *Armillaria mellea* (Vahl) P.Kumm. and *Macrolepiota procera* (Scop.) Singer. Antioxidant activity was detected method by DPHH free radical scavenging. *M.procera* extract had more potent free radical scavenging activity than *A.mellea* extract (IC_{50}: 0.191, 1.19 mg/mL). The concent of the components with antioxidant properties, such as total phenols,β-caratone and lycopene were determined by spectrophotometric methods. Finally, the antimicrobial potential was determined with a agar well diffusion method on 14 microorganisms. *A. mellea* methanol extract formed against to *Klebsiella pneumaniae* ATCC 13883, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923,10±1 mm inhibition zone diameter. *M.procera* methanol extract formed against to *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumaniae* ATCC 13883, 9±1 mm inhibition zone diameter. This research has shown that these two edible mushrooms have potential as natural antioxidants.

Key words: Antimicrobial activity, Antioxidant activity, *Armillaria mellea, Macrolepiota procera,* Mushroom

Armillaria mellea and *Macrolepiota procera* Ekstrelerinin Antioksidan ve Antimikrobiyal Etkileri

Öz: Mantarlar, binlerce yıldır besin ve tıbbi değerleri nedeniyle yaygın olarak kullanılmaktadır. Bu çalışma iki yenilebilir mantar olan *Armillaria mellea* (Vahl) P.Kumm ve *Macrolepiota procera* (Scop.) antioksidan ve antimikrobiyal potansiyelinin belirlenmesi için tasarlanmıştır Antioksidan aktivitesi DPHH serbest radikal süpürme yöntemi ile tespit edildi. *M.procera* methanol ekstresi *A.mellea* methanol ekstresinden daha güçlü serbest radikal süpürücü aktivitesine sahiptir (IC_{50}: 0.191, 1.19 mg/mL). Toplam fenol, β-karoten ve likopen gibi antioksidan özelliklere sahip bileşenlerin konsantrasyonu spektrofotometrik yöntemlerle belirlendi. Son olarak, antimikrobiyal aktivitesi 14 mikroorganizma üzerinde denenmiş olup agar difüzyon yöntemi ile kullanılmıştır. *A. mellea* metanol ekstraktı *Klebsiella pneumaniae* ATCC 13883, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 mikroorganizmlarına karşı 10 ± 1 mm inhibisyon zon çapı oluşturdu. *M.procera* metanol ekstresi ise *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumaniae* ATCC 13883 mikroorganizmlarına karşı 9 ± 1 mm inhibisyon zon çapı oluşturdu Bu araştırma, iki yenilebilir mantarın doğal antioksidanlar olarak potansiyele sahip olduğunu göstermiştir.

Anahtar Kelimeler: Antimikrobiyal aktivite, Antioksidan aktivite, *Armillaria mellea, Macrolepiota procera*, Mantar

Introduction

Free radicals are atoms or molecules that have one or more unpaired electrons. Free radicals can form homolytic and heterolytic products or redox reactions as well as reactive oxygen species and reactive nitrogens. Reactive oxygen species include oxygen-bearing free radicals (Turpaev, 2002). Due to their unbalanced nature, reactive oxygen and nitrogen species (including free radicals) may trigger many biochemical reactions in cell. There must be a balance between the production and accumulation of these reagents in cells and tissues and the inactivation of these forms in living organisms (Son 2012; Pizzino et al. 2017). This unbalanced situation causes oxidative stress that affects the structure of cells membranes, lipids, proteins, lipoproteins and DNA (Young and Woodside 2001; Droge 2002). In addition, overproduction of free radicals can cause oxidative damage to biomolecules, (lipids, proteins, DNA), eventually leading to many chronic diseases such as cancer, cardio-vascular diseases and inflammation in humans. Oxidative stress in cells can result from either an increase in the levels of reactive oxygen species, or a reduction of the natural cell antioxidant capacities (Racchi et al. 2002).

Antioxidants can be defined as molecules that can delay or prevent oxidation of the substrate when they encounter a low amount of oxidizable substrate (Becker et al. 2004). In some cases, the amount of antioxidant in cell may be insufficient for intracellular protection. In such cases, external antioxidant supplementation will contribute to the renovation of this balance again (Valko et al. 2007).

In recent decades, the presence of antimicrobial resistance in pathogenic bacteria has been one of the most worrying human health issues. It is estimated that antibiotic resistance causes 25,000 deaths per year in the European Union and 700,000 deaths worldwide. In addition, antimicrobial resistance in bacteria can cause serious economic damage, with an estimated cost of 1.5 trillion dollars worldwide in health costs and lost productivity (Arteaga et al. 2019).

Mushrooms are distinctive organisms which constitute their own kingdom. Some characteristics relate them more closely to animals than to plants. In many ways fungi are important supporters of life on earth, as decomposers of dead matter and nutrient recyclers. On the other hand, benefits from edible (wild and grown) mushrooms and truffles are widely recognized (Willis 2018). Mushrooms have been used extensively for medical properties for centuries (Muszyńska et al. 2017). Mushrooms contain many non-toxic and bioactive compounds that can be used as a source in the pharmaceutical and nutrition (Dong et al. 2006; Ji et al. 2007; Sevındık et al. 2017). They can produce a large variety of secondary metabolities, such as steroids, alkoloids, terpenoids, organic acids and phenolic compounds (Kosanic et al. 2016; Bal et al.2019). Nowadays, especially in Japan, Korea and China, various mushroom extracts are used as potential additives in chemotherapy and radiation treatments (Sullivan et al. 2006; Borchers et al. 2008). The mushroom's compounds possess anticancer and antiviral effects, immunosuppressive, and lipid-lowering effects in the blood (Bobek and Galbavy 2001; Nowacka et al. 2015; Aytar and Ozmen 2020). Therefore, antimicrobial compounds are a rich source of natural antibiotics since they can be isolated from many mushroom species (Yamaç and Bilgili 2006; Barros et al. 2007;Sevindik et al.2018). For example, glucans found in cell walls are known for their immunomodulatory properties and their secondary metabolites have been shown to be effective against bacteria and viruses (Suzuki et al. 1990).

A. mellea is used in traditional medicine. These species that grow naturally in our country are known as honey mushrooms. Traditional medicine is used as a treatment for dizziness, headache, nerve weakness, insomnia, numbness in the arms and legs, and remittance in elderly patients with stroke (Yang et al. 1989). In modern pharmacology, the effects of polysaccharides isolated from *A. mellea* against vertigo, aging and allergy have also been demonstrated in many studies (Yang et al. 2007; Lai and Ng 2013). *M. procera* is a kind of saprobic fungi. It is popularly known as the parasol mushroom (Arora et al. 2003). *M. procera* is a rich source for carbohydrates (glycerol, mannitol, glucose, trehalose, lepiotan) 15.9%, saturated acids 81.95%, unsaturated fatty acid 19.51%, monosaturated acids 62.44%, polysaturated acids 10.95%, palmitic acid 62.44%, dien 17.40%, oleic acid 62.44%, linoleic acid, chitin, proteins, fiber, vitamins, minerals.(Kumari and Atri 2004;Ouzouni and Riganakos 2007; Falandysz et al. 2008; Yılmaz et al. 2013).

The main objectives of the current study were to evaluate the phenolic, β-Carotene and lycopene profıle, antioxidant and antimicrobial properties of *A. mellea* and *M. procera* in Turkey (popular edible mushroom species).

Material and Method

Mushrooms material

A. mellea and *M. procera* samples were collected from Bozkır district of Konya in 2012. The mushroom samples used in this study was identified by Dr. Ilgaz AKATA from Ankara University.

Preparation of mushroom extracts

The dried mushroom samples were extracted by maceration in 1:4 (w/v) biomass /solvent ratio with methanol for 2 weeks at room temperature in a dark environment. The obtained methanolic extracts were filtered through filter paper. After filtration the solvent was evaporated in a rotary evaporator (Heidolph, Germany) at 50 °C under reduced pressure and the solid extracts were stored at +4°C until further use.

Determination of total phenolic content

Total phenolic of each mushroom extract was quantified according to the method of Folin-Ciocalteu (Siddhuraju and Becker, 2003) using gallic acid as standard. Briefly, 0.1 mL of extracts (1 mg/mL) were mixed with 0.2 mL of diluted Folin-Ciocalteu reagent (1:1 with water). After incubation at room temperature for 3 min,1 mL 2% sodium carbonate was added to the reaction mixture. The absorbance was read at 760 nm by spectrophotometer after 1 h of incubation at room temperature in the dark. The total phenolic concent values are expressed as gallic equivalent (GAE) in milligrams per gram of dried extract (mg GAE/g). All measurements were performed in triplicate.

Determination of β-carotene and lycopene content

β-Carotene and lycopene content of the extracts were determined according to the method described by (Nagata and Yamashita,1992) with slight modification. Briefly, dried samples (100 mg) were mixed with acetone/hexane (4:6, v/v). After incubation for 1 min. The absorbance of the supernatants was read at 453, 505, 645 and 663 nm by spectrophotometer. Contents of β-carotene and lycopene were calculated according to the following equation:

Lycopene (mg/100 ml) = −0.0458 A_{663} + 0.372 A_{505} − 0.0806 A_{453}; β-carotene (mg/100 ml) = 0.216 A_{663} − 0.304 A_{505} + 0.452 A_{453}.

1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging capacity of the extracts were analyzed according to the method described by (Braca et al. 2001) with slight modifications. Briefly,0.5 mL extracts with different concentrations were mixed with a methanolic solution of DPPH radical (0.1mM) freshly prepared. After incubation for 30 min at room temperature in the dark, absorbance was read at was added to extracts solutions at 517 nm by spectrophotometer (Shimadzu UV-1800,Japan) against a blank (extract only). Same procedure with a solution without the extract was applied as a control group. Butylated hydroxytoluene (BHT) was used as a reference standard. The percentage of DPPH radical scavenging effect was calculated according to the following equation:

DPHH scavenging activity (%inhibation)= [($A_{control}$-A_{sample})/$A_{conrtol}$]×100, where $A_{conrtol}$ is the absorbance of the control and A_{sample} is the absorbance of the reaction mixture or standard. A curve of extract concentration versus %inhibation was created to determine the concentration of the extract needed to cause a %50 decrease of the beginning DPHH concentration. This value calculated by linear regression analysis is known as a IC_{50}.

Antimicrobial activity

The antimicrobial activities of mushroom extracts were determined by agar well method and evaluated against bacterial strains on *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Enterococcus hirae* ATCC 9790, *Klebsiella pneumaniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* RSKK 96029, *Salmonella typhimurium* ATCC 14028 and fungal strains *Candida tropicalis* Y-12968, *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258. For comparison ampicillin and chloramphenicol were used a standard antibiotics.

In the agar well method, bacterial strains were allowed to incubate at 37 ° C for 24 hours in Nutrient Agar medium and yeast strains were incubated for 48 hours at 30 ° C in Malt Extract Agar medium. The post-incubation microorganisms were adjusted to 0.5 McFarland blur. Muller – Hinton Agar (for bacterial strains) and Malt Extract Agar (for yeast strains) were spread on a petri with a 1% suspension of microorganism suspension. With the punch, 6 mm in diameter wells are opened at specific points of the medium. The opened wells were placed in a volume of 50 µL from mushroom extracts at a concentration of 150 mg/mL and left to incubate. The diameter of the inhibition zones formed after incubation is measured in mm. Chloramphenicol, ampicillin were used for antimicrobial activity.

Results

Determination of total phenolic content

The total phenolic contents of the methanolic extracts of mushrooms, evaluated by Folin-Ciocalteu method, are shown in Table 1. As a shown in table, the mushrooms *A. mellea* and *M. procera* presented phenolic contents with 20.87 ± 0.88 and 36.25±0.35 mg GAE/g extract, respectively.

Determination of β-carotene and lycopene content

The β-carotene and lycopene content of *A. mellea* and *M. procera* methanol extracts are shown in Table 1. As a shown in table, the mushrooms *A. mellea* and *M. procera* demonstrated β-carotene contents with 0.032 ± 0.04 and 0.091 ± 0.09 µg/mL, respectively. The content of lycopene was lower than the concentration of β-carotene in the mushrooms studied. The mushrooms *A. mellea* and *M. procera* content of lycopene were found 0.011 ± 0.02 and 0.059 ± 0.02 µg/mL, respectively.

Determination of DPPH radical scavenging activity

The scavenging DPPH radicals of the studied methanol extracts are indicated in Table 2. As a shown in table, the free radical scavenging activity of the mushroom extracts was evaluated by DPPH assay comparing the IC_{50} value of synthetic chemical BHT, which was 0.096 ± 0.005 mg/mL. *M. procera* methanol extract revealed the best antioxidant properties (IC_{50} 0.191 ± 0.07 mg/mL).*A.mellea* methanol extract exhibited the lowest IC_{50} 1.19 ± 0.03 mg/mL.

Antimicrobial activity

Antimicrobial activities of the mushrooms extract against the test microorganisms is shown in Table 3. *A. mellea* methanol extract formed against to *K.pneumaniae* ATCC 13883, *B.subtilis* ATCC 6633, *S. aureus* ATCC 25923,10±1 mm inhibition zone diameter. *M.procera* methanol extract formed against to *E. faecalis* ATCC 29212, *K.pneumaniae* ATCC 13883, 9±1 mm inhibition zone diameter.

The antimicrobial activity was compared with the standard antibiotics, ampicillin and chloramphenicol. The results showed that standard antibiotics had stronger activity than tested samples as shown in Table 3. In a negative control, DMSO had no inhibitory effect on the tested organisms.

Table1. Total phenolic contents of the MeOH extracts as mg GAE $g^{-1}$and β-Carotene and lycopene content.

Sample	Total phenolic content (mg GAE/g extract)	β -Carotene (µg/mL)	Lycopene (µg/mL)
A.mellea	20.87±0.88	0.032±0.04	0.011±0.02
M.procera	36.25±0.35	0.091±0.09	0.059±0.02

Values epresent the mean with the error bars representing standard deviation, n = 3.

Table 2. DPPH radical scavenging activities of the extracts. Scavenging activity is expressed as IC_{50} (µg/mL) ± SD (n=3)

Sample	IC_{50} (mg/mL)
A.mellea	1.19±0.03
M.procera	0.191±0.07
BHT	0.09647±0.005

Table 3. Antimicrobial activity results (zone diameter / mm)

Test microorganisms	*A.mellea*	*M.procera*	Amphicillin	Chloramphenicol
E. faecalis ATCC 29212	-	9 ± 0	27±0	20±0
K. pneumaniae ATCC 13883	10± 1	9 ± 0	-	31±1
B. subtilis ATCC 6633	10± 1	-	23±1	21±0
S. aureus ATCC 25923	10± 1	-	44±1	24±0

Discussions

Metabolic syndromes affect people of all age groups. Natural compounds are noteworthy because chemical compounds are perceived to be incompatible with the human body. Therefore, the search for new and natural bioactive compounds has been become of great interest (Asatiani et al. 2018). In this study, the antioxidant and antimicrobial activity of methanolic extracts of mushrooms *A. mellea* and *M. procera* have been evaluated.

The antioxidant activity of mushrooms increased with the increased in the concentration of samples, higher the antioxidant property lower the IC_{50} values. A lower IC_{50} values means better radical scavenging activity (Abdelaaty et al. 2015). The scavenging DPPH radicals of the studied methanol extracts are indicated in Table 2. *M.procera* extract had more potent free radical scavenging activity (IC_{50}: 0.191 mg/mL) than *A.mellea* extract (IC_{50}:1.19 mg/mL). In previous studies, the antioxidant activities of *A.mellea* and *M.procera* extracts have been reported. In the study of Akata et al. 2012, found that the DPPH radical scavenging effect methanolic extract of *A. mellea* IC_{50}: 4.51 mg/mL. In another study Strapac et al. 2016 reported that the DPPH radical scavenging effect methanolic extract of *A. mellea* IC_{50}: 6.44 mg/mL. Lung and Chang 2011, Popescu et al. 2016 demonstrated the DPPH radical scavenging effect methanolic extract of *A. mellea* IC_{50} 7.83, 4.03 mg/mL, respectively. In addition, Lung and Chang 2011, investigated that dried mycelia and mycelia-free broths obtained by *A. mellea* submerged cultures are extracted with methanol and hot water and investigated for antioxidant properties. Methanolic extracts from dried mycelia and mycelia-free broth and hot water extracts from dried mycelia by *A. mellea* submerged cultures show good antioxidant properties as evidenced by low IC_{50} values (<10 mg/mL). In a anathor study of Kalyonu et al. 2010, found that the DPHH radical inhibition value ethanolic extract mycelia of *A.mellea* %2.85 (extract 1mg/mL). On the other hand, similar studies of *M. procera* conducted previously. In the study of Akata and Zengin 2019, found that the DPPH radical scavenging effect methanolic extract of *M. procera* IC_{50} 14.73 ± 0.55 mg/mL. In another study Kosanic et al. 2016, raported that methanolic extract *M. procera* IC_{50}: 0.311 ± 0.3 mg/mL. It was also Fernandes et al. 2013, reported that different extraction methods in the dry, fresh, freeze and irradiated methanol extracts of *M. procera* were 2.7, 4.9, 3.7, 7.9 mg/mL, respectively. The differences observed in this studys could be due to the growing conditions and the extraction method. This current study also indicated that *A.mellea* and *M.procera* extract possesses DPPH free radical scavenging activity which has demonstrated the antioxidant potential of the studied mushrooms.

Phenolic compounds are aromatic hydroxylated compounds with one or more aromatic rings and one or more hydroxyl groups. They include phenolic acids, flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, lignans, tannins, stilbenes and oxidized polyphenols. Furthermore, some of them stimulate synthesis of endogenous antioxidant molecules in the cell (Shanchez 2017). The total phenolic contents of the methanolic extracts of mushrooms, evaluated by Folin-Ciocalteu method, are shown in Table 1. The mushrooms *A. mellea* and *M. procera* presented phenolic contents with 20.87 ± 0.88 and 36.25±0.35 mg GAE/g extract, respectively. The results suggest that most of the phenolic compounds in *M. procera.* Phenolic content in extracs of *A.mellea* was reported before as having 21.68 mg GAE/g (Zavastin et al. 2015), and this value is very similar to what we obtained in this study (20.87 mg GAE/g). On the other hand, Sarıkurkcu et al. 2015 reported that total phenolic content of 2.56, 4.24 mg GAE/g for methanol and water extract of *M.procera* from Turkey. Hussein et al. 2015 found that a phenolic content of 136 mg GAE/g for *M.procera* from Tanzania. The differences observed in this studys could be due to the growing regions.

β-carotene is a light yellow or orange pigment and the precursor of vitamin A. Antioxidant β-carotene prevents oxidation of unsaturated fats and the formation of free radicals. Carotenoids have been reported to act as radical scavengers due to the extensive system of conjugated double bonds in their molecule, and β-carotene is an excellent scavenger of singlet oxygen (Heinonen et al. 1994). Lycopene, which is an important derivative of carotenoids, is the most powerful antioxidant and has more radical aggregation activity (Kelkel et al. 2011). In this study, the amount of β-carotene and lycopene were considerably different among two studied samples (Table 1). Similar results also reported by Lung and Chang 2011;Strapac et al. 2016; Vishwakarma et al. 2016.

The antimicrobial activities of mushroom extracts were determined by agar well method. Antimicrobial activities of the mushrooms extract against the test microorganisms are shown in Table 3. *A. mellea* methanol extract formed against to *K.pneumaniae* ATCC 13883, *B.subtilis* ATCC 6633, *S. aureus* ATCC 25923,10±1 mm inhibition zone diameter. *M.procera* methanol extract formed against to *E. faecalis* ATCC 29212, *K.pneumaniae* ATCC 13883, 9±1 mm inhibition zone diameter. Similar to our results, numerous researchers found antimicrobial activity for *A. mellea* and *M. procera.* (Yamac and Bilgili 2006; Barranco et al.2010; Kalyoncu et al.2010; Kosanic et al. 2016). In this study, methanol extract of tested mushrooms exhibited a same antimicrobial effect than previously reported for other studies.

As a result, *A. mellea* and *M. procera* have high antioxidant activity at low concentrations of methanol extracts. However, both mushroom have low

antimicrobial activity. Wild mushroom samples have high free radical scavenging activity, they can be used health beneficial antioxidant supplement.

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Determination of Elemental Composition of Some Wild Growing Edible Mushrooms

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Abstract: The aim of this study was to determine and elaborate the mineral contents and the some highly toxic elements of wild grown-edible mushrooms. The potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd) and lead (Pb) contents of twenty edible mushrooms, collected from Gümüşhane province, Turkey, were analyzed. The studied mushrooms varied widely in their content of both essential and toxic deleterious elements. The minimum and maximum mineral contents of mushrooms were determined as mg/kg dw for K (4170-15747), Mg (295-2095), Ca (100-2778), Mn (3.82-170.25), Fe (50.25-1121.53), Zn (22.99-91.76), Cu (5.89-135.35), Ni (1.05-6.07), Cd (0.06-7.29) and Pb (0.02-32.31) were determined. The potassium content was found to be higher than those of the other minerals in all the mushrooms. Lead and cadmium were present but at concentrations that are not hazardous to human health except for *Armillaria ostoyae.* The K, Mg, Ca, Mn, Fe, and Ni concentrations were determined to be high in *Agrocybe dura.*

Key words: Minerals, Toxic element, Wild edible mushrooms

Yabani Büyüyen Bazı Yenilebilir Mantarların Element Bileşiminin Belirlenmesi

Öz: Bu çalışmanın amacı, yabani yetişmiş mantarların mineral içeriğini ve bazı yüksek derecede toksik elementlerini belirlemek ve detaylandırmaktır. Gümüşhane ilinden toplanan yirmi yenilebilir mantarın Potasyum (K), magnezyum (Mg), kalsiyum (Ca), manganez (Mn), demir (Fe), çinko (Zn), bakır (Cu), nikel (Ni), kadmiyum (Cd) ve kurşun (Pb) içeriği analiz edilmiştir. İncelenen mantarlarda, hem yararlı hem de toksik elementler çok geniş aralıkta değişiyordu. Mantarların minimum ve maksimum mineral içerikleri, K (4170-15747), Mg (295-2095), Ca (100-2778), Mn (3.82-170.25), Fe (50.25-1121.53) için mg/ g kurumadde olarak belirlendi. Örneklerde Zn (22.99-91.76), Cu (5.89-135.35), Ni (1.05-6.07), Cd (0.06-7.29) ve Pb (0.02-30.46 mg/kg kurumadde) aralığında belirlendi. Potasyum içeriğinin, tüm mantarlarda diğer minerallerden daha yüksek olduğu bulunmuştur. Mantarlarda kurşun ve kadmiyum *Armillaria ostoyae* dışındaki mantarlarda insan sağlığı için tehlikeli olmayan konsantrasyonlarda belirlenmiştir. Ayrıca *Agrocybe dura'da* K, Mg, Ca, Mn, Fe ve Ni konsantrasyonlarının yüksek olduğu belirlendi.

Anahtar kelimeler: Mineral, Toksik element, Yabani yenebilir mantarlar

Introduction

Wild edible mushrooms are valued for their unique taste, aroma, nutritional value, and medicinal potentials (Falandysz and Gucia, 2008). In the last few decades, the interest in services of urban ecosystems has greatly increased. Mushrooms have been a popular delicacy in many countries, particularly in central and east Europe. Wild mushrooms are also a popular food source in Turkey. Mushrooms have a long history of use in traditional Chinese medicine. Mushrooms have also been reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia and cancer. These functional characteristics are mainly due to their chemical composition (Kalaĉ, 2016; Zsigmond *et al.,* 2018). The consumption of wild edible mushrooms is increasing, even in the developed world, due to their good contents of proteins and trace minerals (Kalaĉ, 2016). Nevertheless, mushrooms are also

recognized as efficient trace metal accumulators (Rzymski *et al.,* 2017).

Studies have shown that some mushroom species contain high concentrations of nutritional trace elements such that the fruiting bodies of higher mushrooms are generally considered rich sources of mineral constituents, including the heavy metals (Falandysz *et al*., 2001, 2003). Mushrooms are an important part of diet in many world regions. Wild edible species collected for culinary reasons have already been found to uptake several toxic elements easily (including Hg, Ni, Cd and Pb) from the ambient environment and accumulate them in aboveground parts (Falandysz *et al*., 2015). Therefore, the place from which they are collected is crucial if the specimen is to be safe for human health. Unfortunately, some mushroom species are able to accumulate relatively high levels of these elements which can pose a threat to human health. However, other elements can also be harmful for mushroom consumers (e.g. As, Cd, Cr (III), Hg or Pb). Trace metal contents in mushrooms are usually higher than those in agricultural crop plants, vegetables, fruits or even animal tissue (Falandysz *et al*., 2015; Kalaĉ, 2016).

Exposure to toxic elements in humans occurs through a variety of routes, including the inhalation of air pollutants or contaminated soil particles, and the consumption of contaminated foods. For non-occupationally exposed individuals, the most likely source of trace elements intake is the diet. Consumers of delicacies such as mushrooms, which may not have been extensively investigated for toxic metal contents, could be exposed via the diet. Information about heavy metal concentrations in foods and their consumers' dietary intake is very important for assessing their health risks to humans. Exposure to elevated levels of mineral elements, such as Cd, Hg, Pb, Cr, and Ni could lead to both acute and chronic health hazards (Falandysz *et al*., 2015).

The macrofungi are collected to make a substantial contribution to food intake. Therefore, it is necessary to know the levels of essential elements in edible mushrooms (Isıloğlu *et al*., 2001). The bioavailability of iron in mushrooms is therefore high and up to 90% of the iron present can be absorbed (Kalaĉ and Svoboda, 2000). The contents of trace metals are related to species of mushroom, collecting area of the sample, age of fruiting bodies and mycelium, and distance from any source of pollution. Metals, such as iron, copper, zinc and manganese are essential metals, since they play an important role in biological systems. Lead and cadmium are non-essential metals as they are toxic, even in traces. The essential metals can also produce toxic effects when the metal intake is excessively elevated (Tuzen *et al.,* 2007; Kalaĉ, 2016). Accurate and adequate food composition data are invaluable for estimating the adequacy of intakes of essential nutrients and assessing exposure risks from intake of toxic non-essential heavy metals (Soylak *et al.,* 2003; Mleczek *et al.,* 2018).

Mushrooms have become increasingly attractive as functional foods for their potential beneficial effects on human health. Due to the toxic minerals they carry, mushrooms should be taken into consideration during their consumption as human food. Also, mushrooms are important in the ecosystem because they are able to biodegrade the substrate, to collect heavy metal (Kalaĉ, 2016).

Turkey has a large edible mushroom potential and is becoming an important exporter of wild mushrooms. Trace metal levels in wild mushroom samples in Gümüşhane province have not yet been determined. The purpose of this study is to determine toxic and essential elements (K, Mg, Ca, Mn, Fe, Zn, Cu, Ni, Cd and Pb in fruit bodies of several mushroom species from Gümüşhane, Turkey.

Material and Method

Samples

The thirty macrofungi samples were collected during field trips in Gümüşhane province, Turkey. Colour slides of the macrofungal specimens were taken in their natural habitats during fields studies. After relevant notes were taken of their morphological and ecological features, they were put in private prepared boxes and brought to the laboratory. Their spore prints were taken and spore dimensions were measured using an ocular micrometer. Then, dried specimens were placed in locked polyethylene bags and kept in a deep freezer at -20 ^{0}C to protect against parasites.

Identification of the specimens was performed according to Breitenbach and Kranzlin (1984–2000), Bresinsky and Besl (1990), Buczacki (1989) and Dahncke and Dahncke (1989). All specimens are kept in the Herbarium of Yuzuncu Yıl University, Department of Biology (VANF). Table 1 shows the taxa of wild edible macrofungi.

Method

Atomic absorption spectrophotometer (Varian Techtron Model AAS 1000, Varian Associates, Palo Alto, CA) was used for the determination of the minerals (Ca, Mg, K, Fe, Zn, Cu, Mn, Pb, Ni and Cd) in dried fruit bodies of macrofungi. Each dried mushroom sample was weighed as 4-5 g and placed in a porcelain crucible and ashed at 550°C for 18-20 h.; then the ash was dissolved in 1ml concentrated HNO3, evaporated to dryness, heated again at 550°C for 4 h, treated successively with 1 ml HNO_3 and 1 ml H_2O_2 and then diluted with double deionized water up to a volume of 25 ml. Three blank samples were treated in the same way. The species, which were digested in an acid solution of HNO_3, were passed through the AAS system using different lamps, and calibrated with related minerals in different concentrations for different micronutrients (AOAC, 1990).

Table 1. Taxa of wild edible macrofungi collected from Gümüşhane region of Turkey

No	Class, Family and taxa of macrofungi	Habitat	KHN no:
1.	*Agaricus bisporus* (J.E. Lange) Imbach	Gümüşhane, Torul, Köprübaşı district, meadow area (Agaric)	842
2.	*A. langei (F.H. Møller) F.H. Møller*	Gümüşhane, Torul, Zigana Mountein, Zigana tunnel around, conifer forest	865
3.	*Coprinus comatus* (O.F. Müll.) Pers.	Gümüşhane, Torul, Günay Wİllage, meadow area	1318
4.	*Hydnum repandum* L.	Gümüşhane, Torul, Zigana Mountein, Zigana tunnel around, conifer forest	879
5.	*Marasmius oreades* (Bolton) Fr.	Gümüşhane, Torul, Köprübaşı district meadow area	845
6	*Armillaria ostoyae* (Romagn.) Herink	Gümüşhane, Kürtün, Örümcek Mountein, mixed forest, on stumps of poplar tree	1221
7.	*Agrocybe dura* (Bolton) Singer	Gümüşhane, Torul, Köprübaşı district	1434
8.	*A. praecox* (Pers.) Fayod	Gümüşhane, Geçitkaya Willage, on stumps of poplar tree	1102
9.	*Pleurotus eryngii* (DC.) Quél.	Gümüğhane, Bozkır, On Heliz residues	874
10.	*P. ostreatus* (Jacq.) P. Kumm.	Gümüşhane, Near Akçahısar Castle, on stumps of poplar tree (Poplar mushroom)	3425
11.	*Cyclocybe cylindracea* (DC.) Vizzini & Angelini	Gümüşhane, Güvercinlik Willage, on stumps of poplar tree	1093
12.	*Cantharellus cibarius Fr.*	Gümüşhane, Torul, Zigana Mountein, Zigana tunnel around, conifer forest	889
13	*Clavulina cinerea* (Bull.) J. Schröt.	Gümüşhane, Kürtün, Örümcek Mountein, mixed forest	889
14.	*Leccinum scabrum* (Bull.) Gray	Gümüşhane, Günay Willage, conifer forest	1320
15.	*Suillus granulatus* (L.) Roussel	Gümüşhane, Günay Willage, conifer forest	1311
16.	*S. luteus* (L.) Roussel	Gümüşhane, Köse, conifer forest	1443
17.	*Cerioporus squamosus* (Huds.) Quél.	Gümüşhane, Torul, Köprübaşı district, on stumps of poplar tree	854
18.	*Lactarius deliciosus* (L.) Gray	Gümüşhane, Torul, Günay willage, meadow area	1321
19.	*L. salmonicolor* R. Heim and Leclair	Gümüşhane, Torul, Zigana Mountein, Zigana tunnel around, conifer forest	885
20.	*Russula delica* Fr.	Gümüşhane, Torul, Zigana Mountein, Zigana tunnel around, conifer forest	882

To check for possible contamination by reagents or glassware, blanks containing 4ml of ultrapure concentrated HNO_3 and 4 ml H_2O_2 were run together with analytical samples and every batch of analytical samples was run together with the standard matrix. The values of Ca, Mg, K, Fe, Zn, Cu, Mn, Pb, Ni and cadmium Cd were calculated as mg/kg dw. Detection limit is defined as the concentration corresponding to three times the Standard deviation of ten blanks. Detection limit values of elements as mg/kg in AAS were found to be 0.012 for K, 0.003 for Mg, 0.015 for Ca, 0.029 for Mn, 0.060 for Fe, 0.013 for Zn, 0.041 for Cu, 0.063 for Ni, 0.032 for Cd and 0.10 for Pb. The results were within limits of quantification for above minerals (calculated as 10-fold of standard deviation from ten replicates of the instrumental blank solution) 0, 2, 4, 8 and 16 mg/g or mg/kg, respectively. Correlation coefficients of the mineral result were determined between r=0.9932 and 0.9999. Mushrooms were selected normally harvested for consumption (pileus+stipe). For all the mushroom species, at least three samples were analysed.

Results and Discussion

Data on potassium and nine metals most frequently determined in mushrooms from Gümüşhane, Turkey are given for 20 species in Table 2. All the metal concentrations were determined on a dry weight basis.

The most abundant element was found to be potassium, (ranging from 4170 to 15747 mg/kg dw), followed by magnesium, calcium and iron while the most variable mineral was calcium. Mushrooms contained a wide range of minerals, particularly manganese and magnesium. The lowest Mg, Zn and Ni contents were observed in *Russula delica* (Table 2). Wild-grown mushrooms are able to accumulate in their fruiting bodies large amounts of both macro- and micro-elements that are essential to fungi and its consumers. Mushrooms can also be specifically enriched with toxic elements such as As, Hg, Cd and Pb. Potassium (K) and phosphorus (P) are two prevailing elements in fruiting bodies and are usually followed by Ca, Mg, Na and Fe (Falandysz and Borovicka, 2013; Okoro and Achuba, 2012).

The mean contents of elements detected in the mushrooms of all the investigated species (Table 2) generally decreased in the following order: K > Mg > Ca > Fe > Mn > Zn > Cu > Ni > Cd > Pb. These elements can be divided according to their level in dry matter into five groups: i) those exceeding 1000 mg/kg (K, Mg and Ca); (ii) ranging from 100 to 1000 mg/kg (Mn, Fe and Zn); (iii) ranging from 10 to 100 mg/kg (Cu); (iv) ranging from 1 to 10 mg/kg (Ni); and (v) below 1 mg/kg (Cd and Pb).

Potassium content was higher than other minerals in all mushrooms in this study, varying between 4170 (*Lactarius salmonicolor*) and 15747 mg/kg dw (*Cantharellus cibarius*). In general, most of the mushrooms studied contained considerably high amounts of potassium. The levels of essential elements in mushroom species were higher than those of toxic elements. Gençcelep *et al.* (2009) reported the potassium contents of wild edible mushrooms as being between 12600 and 29100 mg/kg dw. Wang *et al*. (2014) found that potassium content was between 16000 (*S. rugoso-annulata*) and 37000 (*C. cornucopioides*) mg/kg in dry matter. Sanmeea *et al.* (2003) reported that potassium accumulation in mushrooms could rise up to 45200 mg/kg. Liu *et al.* (2012) reported that the lowest potassium value (1300 mg/kg dw) was measured in *Melanoleuca gigantea* and *Melanoleuca arcuata*, the highest potassium value (4600 mg/kg dw) was found in *Boletus griseus*. The greatest concentrations of K were obtained in C. *cibarius* (41823 mg/kg), whereas the lowest was in *Boletus edulis* (11269 mg/kg) (Cvetkovic, 2015). Usually potassium content in mushrooms varies between 20000 and 40000 mg/kg dw. The overall data indicates that mushrooms may contain elevated levels of potassium. In this study, potassium levels were lower than reported values in the literature. Showing that mushrooms are an excellent source of potassium in the human diet.

Magnesium content was 295 mg/kg dw in *Russula delica* and 2095 mg/kg dw in *Agaricus bisporus*. The level of magnesium reported in this study was relatively low compared to earlier published reports (Demirbaş, 2000) which was magnesium content ranged from 330 mg/kg dw in *Tricholoma anatolicum* to 6560 mg/kg dw in *Morchella deliciosa*. In our previous study, the concentration levels of Mg in *Morchella vulgaris* 1920 mg/kg, *Helvella lacunosa* 1190 mg/kg, *Lepista nuda* 3410 mg/kg were found (Gençcelep *et al.,* 2009). Liu *et al.* (2002) reported that the magnesium contents of the mushrooms ranged from 84 mg/kg dw in *Leucopaxillus giganteus* to 550 mg/kg dw in Macrocybe *gigantea*. Previously reported magnesium contents in mushrooms varied between 800 and 1800 mg/kg dw (Kalaĉ, 2010). The lowest magnesium value, 248 mg/kg dw *Bolereus tomentipes*, was found by Li *et al.* (2011). Sanmeea et al. (2003) reported that mature *Astraeus hygrometricus* had 1600 mg/kg of Mg concentrations. In this study, magnesium concentrations of the same fungus species were found to be lower than the other studies. As a result, environmental factors are very important to amount of metal concentrations in mushrooms.

Magnesium levels in this study are in agreement with the value reported in the literature.

In the present study, the calcium contents of the mushrooms ranged from 100 mg/kg dw in *Armillaria ostoyae* to 2778 mg/kg dw in *Coprinus comatus*. In our previous study, the concentration levels of Ca in *Morchella vulgaris* 870 mg/kg, *Helvella lacunosa* 470 mg/kg, *Lepista nuda* 8800 mg/kg were found (Gençcelep

et al., 2009). Previously reported calcium contents of mushrooms varied from 100 to 500 mg/kg dw (Kalaĉ, 2010). The calcium contents in our mushroom samples are higher than the values reported in the literature. The accumulation of metals in mushrooms has been found to be affected by environmental and fungal factors.

Table 2. Taxa of wild edible macrofungi collected from Gümüşhane region of Turkey (mg/kg dw)

No	*Taxa*	K	Mg	Ca	Mn	Fe	Zn	Cu	Ni	Cd	Pb
1	*Agaricus bisporus*	7624	2095	936	33.07	217.99	61.06	69.19	4.89	0.42	4.97
2	*Agaricus langei*	5386	1273	238	13.98	104.18	47.19	46.85	3.30	0.54	1.94
3	*Coprinus comatus*	9548	1553	2778	20.18	277.66	48.34	47.26	6.07	1.18	1.39
4	*Hydnum repandum*	6334	481	103	16.82	1121.53	34.68	11.55	2.09	0.28	4.56
5	*Marasmius oreades*	12124	888	761	30.78	262.92	91.76	58.48	1.91	0.15	0.02
6	*Armillaria ostoyae*	8636	732	100	32.89	242.11	57.13	31.11	2.27	7.29	32.31
7	*Agrocybe dura*	12066	1507	1542	170.25	395.66	40.06	17.60	3.67	1.98	1.76
8	*Agrocybe praecox*	7786	979	573	14.69	133.54	38.34	24.00	1.87	0.52	0.56
9	*Pleurotus eryngii*	7839	1838	205	8.15	103.86	56.69	9.39	2.68	0.17	1.13
10	*Pleurotus ostreatus*	9975	1514	465	10.57	242.09	55.29	5.89	2.03	0.57	1.18
11	*Cyclocybe cylindracea*	9264	544	160	21.31	259.91	30.10	19.09	2.11	0.23	0.91
12	*Cantharellus cibarius*	15747	686	439	18.73	174.42	82.22	46.91	2.91	1.65	6.51
13	*Clavulina cinerea*	15737	412	184	35.84	355.20	49.75	135.35	3.88	3.92	30.46
14	*Leccinum scabrum*	8040	752	178	3.82	98.14	48.57	34.59	2.91	0.06	0.92
15	*Suillus granulatus*	5618	593	277	5.18	166.02	40.31	16.72	1.71	0.13	0.75
16	*Suillus luteus*	6449	736	184	7.53	113.96	52.30	14.84	1.67	0.12	1.10
17	*Cerioporus squamosus*	7384	1574	102	4.70	50.25	44.47	15.16	2.61	1.99	1.05
18	*Lactarius deliciosus*	7121	579	222	12.45	144.01	57.12	8.85	1.61	0.88	1.39
19	*Lactarius salmonicolor*	4170	529	237	17.45	73.93	34.50	13.70	1.38	1.22	3.19
20	*Russula delica*	5198	295	119	8.13	106.39	22.99	26.90	1.05	2.55	4.17
	Mean	8602	978	490	24.32	232.18	49.64	32.67	2.63	1.29	5.01
	±SD	±3212	±529	±646	±35.75	±229.52	±16.30	±30.08	±1.23	±1.79	±9.18
	Minimum	4170	295	100	3.82	50.25	22.99	5.89	1.05	0.06	0.02
	Maximum	15747	2095	2778	170.25	1121.53	91.76	135.35	6.07	7.29	32.31

But, it seems to be higher when compared to the concentrations obtained by Sanmeea *et al.* (2003) (100-2400 mg/kg dw). Andreea *et al.* (2018) suggest that the Ca has a considerable role in the integrity of the cell and the vacuolar membranes, and it slows enzymatic browning reactions.

Manganese was also determined in all mushrooms. The manganese content of the mushrooms studied in the present work ranged from 3.82 mg/kg dw in *Leccinum scabrum* to 170.25 mg/kg dw in *Agrocybe dura*. The reported manganese values in the literature for mushrooms were 14.2-69.7 mg/kg, 21.7-74.3 mg/kg and 7.1-81.3 mg/kg, 5.54-135 mg/kg dw (Gençcelep *et al.,* 2009; Soylak *et al.* (2005), respectively. The manganese values in this study are found lower than in the literature.

The iron content of the mushrooms ranged from 50.25 mg/kg dw in *Cerioporus squamosus* to 1121.53 mg/kg dw in *Hydnum repandum*. Iron values in mushroom

samples (as reported) ranged from 31.3-1190 mg/kg (Sesli and Tüzen, 1999), 56.1-7162 mg/kg (Işıloğlu *et al.,* 2001), 50.1-842.0 mg/kg (Gençcelep *et al.,* 2009), respectively. Wang *et al.* (2014) found that iron (Fe) content in *Thelehhora ganhajun* was 1500 mg/kg dw, which is particularly higher than in other mushrooms. The iron values in the present study are in lower than with reported values in the literature. It is known that adequate iron in a diet is very important in order to decrease the incidence of anemia.

Mushrooms are known as zinc accumulators and the sporophore: substrate ratio for Zn ranges from 1 to 10 mg/kg (Işıloğlu *et al.,* 2001). The zinc content was the lowest (22.99 mg/kg dw) in *Russula delica*, whereas it was highest (91.76 mg/kg dw) in *Marasmius oreades* (Table 2). The reported literature zinc content ranged between 22.10 and 185 mg/kg dw (Gençcelep *et al.,* 2009; Kalaĉ, 2001; Kaya and Bağ, 2001). Sarıkürkçü *et al.* (2012) found the highest Zn values in *Helvella leucopus* and *Tricholoma auratum* (354 and 356 mg/kg, respectively). In this study, some mushroom species have higher zinc content more than 50 mg/kg (*Agaricus bisporus, Pleurotus eryngii, Lactarius deliciosus, Suillus luteus, Pleurotus ostreatus, Cantharellus cibarius* and *Armillaria ostoyae*). These mushrooms species were collected from locations near the downtown of Gümüşhane. Therefore, metal accumulation may be more owing to soil pollution. Zn is an essential nutrient that has an important role in biological systems. Zinc is necessary for the functioning of various enzymes and plays an essential role in DNA, RNA, and protein synthesis. The major symptoms of zinc deficiency are delayed growth and slow maturation (WHO, 1996).

Minimum and maximum values of copper were 5.89 and 135.35 mg/kg dw in *Pleurotus ostreatus* and *Clavulina cinerea*. Copper contents of mushroom samples in the literature have been reported to be in the range of 4.71-51.0 mg/kg (Tüzen *et al.,* 1998) and 10.3-145 mg/kg (Sesli and Tüzen, 1999). Copper contents found in this study parallel those reported in the literature.

In the present study, the Cu concentrations detected in mushrooms were lower than previously reported in the literature but only one mushroom (*Clavulina cinerea*, 135.35 mg/kg) higher than 100mg/kg in Table 2. Keleş *et al.* (2017*)* found that copper values in *Leccinum versipelle* (102.40 mg/kg) and *Russula delica* (128.94 mg/kg) were collected near the downtown of Erzincan, Turkey, therefore copper contents of these samples were found higher than the others. In our previous study, the concentration levels of Cu in *Pleurotus ostreatus* 47.1 mg/kg and *Lepista nuda* 26.6 mg/kg were found (Gençcelep *et al.,* 2009). Cu is an essential element. Enzymes containing copper are important for the body to transport and use iron. In previous studies, Cu concentrations in edible mushrooms were found to be between 100 and 300 mg/kg, which was not considered a health risk (Kalaĉ and Svoboda, 2000). In 1996, a joint FAO/International Atomic Energy Agency/ WHO official report set an upper limit for the safe range of population mean exposures for adults of 0.2 mg/kg body weight per day (WHO, 1996).

Nickel was determined all mushrooms. *Coprinus comatus* contained high nickel content with an amount of 6.07 mg/kg dry matter. The reported Ni values for wild-growing mushrooms were 0.4-15.9, 0.4-2, 1.72-24.1 mg/kg (Işıloglu, 2001; Kalaĉ and Svoboda, 2000, Soylak *et al.,* 2005), respectively. The Ni levels are generally in agreement with previous studies. The obtained Ni levels in some mushrooms (*Coprinus comatus*, *Agaricus bisporus, Clavulina cinerea, Agaricus langei* and *Agrocybe dura)* are higher than the allowed amount (0.05-5 mg/kg) of National Academy of Sciences (1975) for plants and foods (NAS, 1975) (Table 2). Nickel has been linked to lung cancer and the tolerable upper intake level for this toxic element is reported as 1 mg/day (FNB, 2001).

Cadmium is known as a principal toxic element, since it inhibits many life processes. Cadmium has been associated with renal damage; cancer and childhood aggression (JECFA, 2011). Mushroom, in particular, can be very rich in cadmium. Cadmium was measured as the lowest detected in *Leccinum scabrum* (0.06 mg/kg dw) and it was the highest in *Armillaria ostoyae* (7.29 mg/kg dw) which is relatively high compared to reported literature data (Mendil et al., 2005) Cd levels were found generally lower than 2.0 mg/kg for the other mushrooms species, in this study. Long-term exposure to high levels of Cd may lead to considerable accumulation in the liver and kidneys, particularly the renal cortex, resulting in kidney damage (WHO,1989). Thus, cadmium seems to be the most deleterious one among heavy metals in mushrooms. It is acceptable daily or weekly intake may be easily reached by consumption of an accumulating mushroom species (Kalaĉ *et al.,* 2004). In the case of this element the JECFA authority has established a provisional tolerable monthly intake (PTMI) at a level of 0.025 mg/kg bodyweight (JECFA, 2011), which accounts for 1.5 mg per month for a 60-kg adult.

Pb concentrations of mushroom samples were generally low, except *Armillaria ostoyae* and *Clavulina cinerea* with an amount of 32.31 and 30.46 mg/kg dw. The Pb levels of all other samples were not higher compared to the reported Pb values for mushrooms by Tüzen *et al.,* (1998) (2.35 mg/kg), Kalaĉ and Svoboda (2000) (0.5-20 mg/kg) and Kaya and Bag (2010) (2.166 mg/kg). Sarikürkcü *et al.,* (2012) found the lowest Pb value in *Lyophyllum decastes* (0.5 ± 0.19 mg/kg). This is followed by *Morchella esculenta* (0.9 ± 0.29 mg/kg). In *Rhizopogon roseolus, Volvoriella gloiocephala* and *Cyclocybe cylindracea*, Pb contents were found equal or above 4.0 mg level (6.2 ± 0.44, 5.9 ± 0.11 and 4.0 ± 0.50 mg/kg), respectively. Pb is used for a number of industrial,

domestic, and rural purposes for example, in lead batteries and in leaded petrol. A significant source of exposure to lead is via the diet. Lead is a cumulative toxin that can primarily affect the blood, nervous system, and kidneys. In the blood at high concentrations, lead inhibits red blood cell formation and eventually results in anemia (Çayır *et al.,* 2010). The provisional tolerable weekly intake (PTWI) of Pb was set at 0.025 mg/kg bodyweight (the equivalent of 0.0036 mg/kg body weight per day) (JECFA, i.e., 1.5 mg weekly (and 0.21 mg daily) for an adult weighing 60 kg.

Aiming to assess the health risks associated to the consumption of the mushrooms with regard to the toxic metal content, we used the PTWI (Provisional Tolerable Weekly Intake) values given by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA) (FAO/WHO, 2011). The PTWI is the maximum amount of a contaminant to which a person can be exposed per week over a lifetime without an unacceptable risk of health effects. The level is provisional, because it is subject to review when new information becomes available. According to the FAO/WHO the PTWI values given in mg/ kg body weight (bw) are the followings: 0.015 for As, 0.007 for Cd and 0.025 for Pb. In 2011 the values for As and Pb were withdrawn by the Committee because of the need for further research, the level of Cd was changed to 0.025 PTMI (Provisional Tolerable Monthly Intake). In our evaluation, we used the values given in 2009 for As and Pb, and the values given in 2011 for Cd. We have also calculated the recommended weekly amount (RWA) as the maximum safe intake in kg of fresh mushroom considering the PTWI value as one.

Present human health risk assessments of elements like toxic metals or metalloids are traditionally based on the total content in foods and food consumption, although, the amount of an ingested element in the diet does not always reflect the amount that is accessible to the consumer. The terms bioaccessibility and bioavailability are often used indiscriminately although their meaning is slightly different. Bioaccessibility defines the fraction of a contaminant ingested with food that is released from its matrix into the digestive juice and has the potential to be absorbed by the intestines during the digestion. Bioavailability, however, refers to the proportion of a contaminant ingested with food that is absorbed by the intestine with the subsequent potential to reach the systemic circulation and exert toxic effects (Versantvoort *et al.,* 2005; Zsigmond *et al.,* 2018).

The results of nutritionally valuable minerals show that twenty mushroom species contained high amounts of potassium, calcium, magnesium and iron. Most of them contain little lead, nickel, cadmium or copper. Minerals in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance (Kalaĉ and Svoboda*,* 2000).

Mushrooms collected from urban and industrial areas polluted with toxic compounds (Ni, As, Hg, Cd and Pb) usually show highly elevated content of such constituents in flesh (Falandysz and Borovicka, 2013). The content of toxic elements in the studied mushrooms was generally low and safe, although all investigated species contained a relatively high content of rare-earth elements (REEs), particularly of Cd ve Pb.

Finally, Principal Component Analysis (PCA) was used to display the differences among all the observations (all mushroom species characterized by all elements) (Figure 1). The results of PCA of the mean values of minerals of wild mushroom samples are depicted in a 3-dimensional plot (Figure 1). Results from the PCA showed that principal components (PC) 1, 2 and 3 described about 68.71% of the total variation of sample: 31.12% PC1, 23.80% PC2 and 13.79% PC3. Principal component 1 was heavily loaded on Ca, Ni and Mg, component 2 was loaded on Cd, Pb and Cu whereas PC3 was loaded Zn, K, Fe and Mn. The PCA analysis showed that Mn, Ni, Ca and Mg were positively correlated to each other, and the correlation was very high. Figure 1 also presents the positive low correlation between Fe and Zn. Results also showed that there is negative significant relationship among Zn and K attributes (Figure 1).

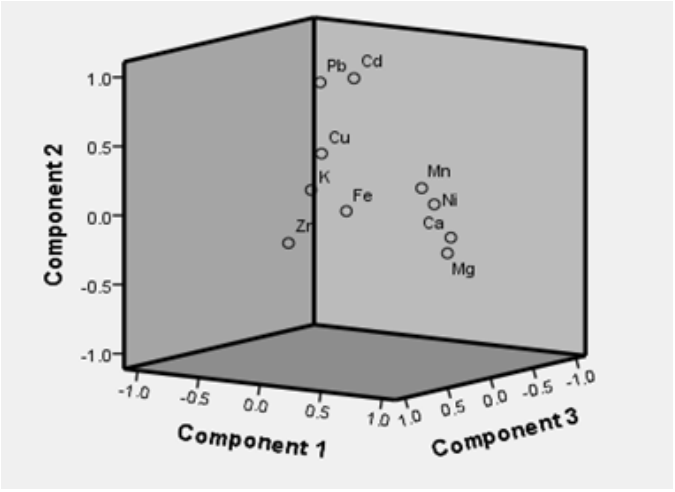

Figure 1. Principal component analysis threeplot of minerals contents of wild mushrooms.

Ideally, mushrooms used for human consumption should be characterized by the lowest possible level of toxic elements, and contain relevant mineral content in their fruiting bodies. In summary, the present research investigated the elemental composition of mushrooms from the wild edible mushrooms for dietary purposes in Turkey over the last few years. The studied mushrooms were found to vary widely in their elemental composition. Some mushrooms demonstrated a lower variability in element content and the lowest content of toxic or potentially toxic elements when compared with other

tested species. This would seem to indicate that wild edible mushrooms (as well as other mushrooms investigated previously) may constitute an important dietary source of these elements for humans. The studied mushrooms were found to be generally safe with respect to the content of potentially toxic elements, although increased contents of Ni, Cd and Pb in some of the studied species requires further attention as regards the bioavailability of these elements from mushrooms. Further studies are, however, necessary to elucidate any potential risks arising from the presence of rare-earth elements (REEs) in these foodstuffs.

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Thecotheus lundqvistii, A New Coprophilous Ascomycete Record for Turkey

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Abstract: The fimicolous *Thecotheus* Boud. species, *Thecotheus lundqvistii* Aas., is reported in Turkey for the first time. A brief description of the species and the photographs related to its macro and micromorphologies are provided.

Key words: Biodiversity, Macrofungi, Taxonomy, Karaman

Thecotheus lundqvistii, Türkiye İçin Yeni Bir Koprofil Askomiset Kaydı

Öz: Gübre üzerinde gelişim gösteren bir *Thecotheus* Boud. türü, *Thecotheus lundqvistii* Aas., Türkiye'de ilk kez rapor edilmiştir. Türün kısa betimlemesi ve makro ve mikromorfolojilerine ilişkin fotoğrafları verilmiştir.

Anahtar kelimeler: Biyoçeşitlilik, Makromantarlar, Taksonomi, Karaman

Introduction

Thecotheus Boud. is an ascomycete genus within the family *Ascobolaceae.* Members of the genus are characterized by membranous to fleshy rough or papillate disc shaped ascomata, operculate, diffusely amyloid, 8-32-spored asci, ellipsoidal, symmetrical to slightly or greatly inequilateral, smooth to verrucose, biapiculate or non-apiculate ascospores (Doveri, 2007). Most members of the genus are coprophilous, often with a specific dung preference and widespread, especially in temperate regions (Doveri and Coué, 2008; Kirk et al., 2008).

Though Kirk et al. (2008) give the species number of *Thecotheus* as 17, Index Fungorum (accessed on 15 April 2020) lists 26 conformed *Thecotheus* species. But the current checklists (Sesli and Denchev, 2014; Solak et al., 2015) on Turkish macromycota and the later contributions (Kaşık et al., 2017; Akçay et al., 2018; Işık and Türkekul, 2018; Sadullahoğlu and Demirel, 2018; Acar et al., 2019; Keleş, 2019; Sesli, 2019; Yıldız et al., 2019; İleri et al., 2020; Uzun et al., 2020) indicate that only two of them, *Thecotheus pelletieri* (Crouan) Boud. and *Thecotheus holmskioldii* (E.C.Hansen) Eckblad, of the genus have so far been recorded from Turkey (Kaya and Uzun, 2015; Uzun et al., 2018).

Here we report *Thecotheus lundqvistii* Aas as a new member of the Turkish *Thecotheus*. The study aims to make a contribution to the mycobiota of Turkey.

Material and Method

The *Thecotheus* samples were collected from Yeşildere village of Karaman province in 2015. The fructification organs were photographed at their natural habitats and ecologic characters were recorded. Detailed investigations related to their macroscopy and microscopy were carried out in the fungarium. A Nikon Eclipse Ci-S trinocular light microscope was used microscopic investigations, and a Nikon DS-Fi2 camera was used for photographing micromorphologic structures. The samples were identified by comparing the obtained

data with relevant literature (Doveri et al., 2000; Nagao et al., 2003; Doveri, 2007; Richardson, 2007, 2008; Doveri and Coué, 2008; Bronckers, 2011). The specimens are kept at Karamanoğlu Mehmetbey University, Kâmil Özdağ Science Faculty, Department of Biology.

Results

Ascomycota Caval-Sm

Pezizomycetes O.E.Erikss. & Winka

Pezizales J.Schröt.

Ascobolaceae Boud. ex Sacc.

Thecotheus lundqvistii Aas

Macroscopic and microscopic features: Apothecia 0.8-3 mm in diam., rounded at first, then cup shaped to cylindrical with a subiculum-like base, grey brownish to whitish. Hymenial surface smooth to finely rough or pruinose. Outer surface covered with small grayish granulations which are more visible near the margin. Asci 260-300 x 21-28 µm, cylindrical, operculate, tapering towards the base, uniseriate, 8-spored. Paraphyses filiform, hyaline, septate, enlarged at the apices. Ascospores 24-29 x 12-14 µm, ellipsoid with an apiculus of 2-3 µm at each pole, ornamented with minute granules or subreticulate structures, surrounded by gelatinous envelope. *Thecotheus lundqvistii* was reported to grow on cow dung as solitary, gregarious or in large groups (Garcia and Ormad, 2010).

Specimen examined: Karaman, Yeşildere village, on cow dung as solitary or in large groups, 37°09′N-33°25′E, 1150 m, 25.04.2015, AÇK. 181.

Figure 1. Ascocarps of *Thecotheus lundqvistii* on cow dung

Discussions

Thecotheus lundqvistii is reported as new record for the mycobiota of Turkey. After the reports of *T. pelletieri* and *T. holmskioldii* (Kaya and Uzun, 2015; Uzun et al., 2018), it seems to be the third member of the genus *Thecotheus* in Turkey. General characteristics of Turkish specimens are in agreement with those given by Richardson (2007, 2008) and Garcia and Ormad (2010).

Thecotheus lundqvistii is similar to *T. harasisus* and *T. holmskjoldii* in terms of ecological and some macro and micromorphological characters. All the three species grow on corprophilous substrata. Larger ascospores (29-38 x 14-18 µm) of *T. holmskjoldii* easily differentiates it from *T. lundqvistii* (24-29 (30) x 12-14 µm) (Nagao et al., 2003; Uzun et al., 2018). The subiculum-like base of apothecia and the verruculose ascospore ornamentation differs *T. lundqvistii* both from *T. harasisus* and *T. holmskjoldii* (Bronckers, 2011).

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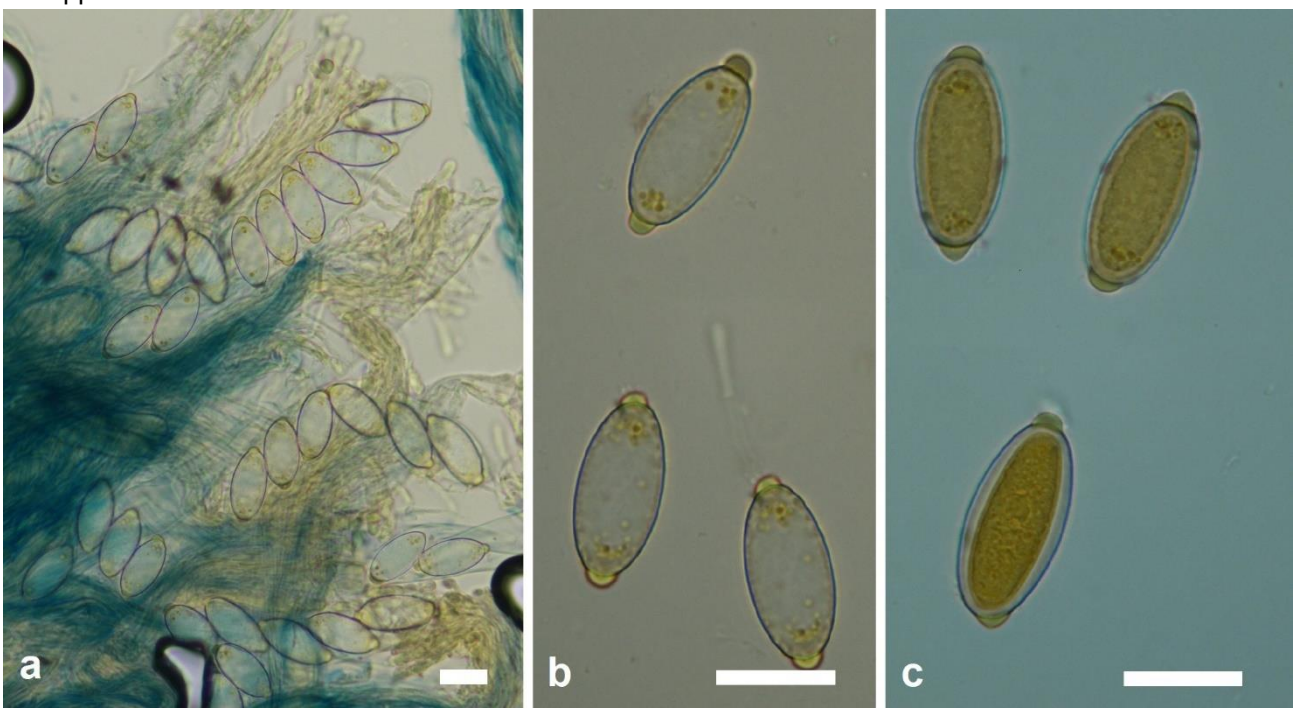

Figure 2. Asci, paraphyses and ascospores (a), and ascospores (b,c) of *Thecotheus lundqvistii*

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Moleküler Yöntemlerin Kullanımı İle Türkiye *Morchella* (Kuzugöbeği) Genetik Çeşitliliğine Katkılar

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Öz: Türkiye'de "kuzugöbeği" olarak bilinen *Morchella* cinsi, Türkiye'nin de içinde bulunduğu birçok ülkede yoğun olarak tüketilmekte ve bilimsel olarak da üzerindeki ilgiyi her zaman korumaktadır. Bu mantar cinsinde yapılan sistematik çalışmalar popülerliğini sürdürmekte, yeni türler tanımlanmakta ve sinonimler belirlenmektedir. Sunulan bu çalışmada da Türkiye'nin farklı lokasyonlarından toplanmış ve 50 örnekten oluşmuş bir koleksiyonun morfolojik ve filogenetik değerlendirilmesi yapılmıştır. Filogenetik analizlerde, transkripsiyonu yapılamayan (ITS rDNA) ve 28S rDNA (LSU) gen bölgeleri kullanılmıştır. Çalışma sonunda, bu gen bölgelerine göre, *Morchella dunensis*, *M. tridentina*, *M. importuna*, *M. eximia*, *M. dunalii*, *M. mediterraneensis* ve *M. deliciosa* türleri belirlenmiştir.

Anahtar kelimeler: *Morchella*, Kuzugöbeği, ITS rDNA, 28S rDNA, Türkiye

Conributions to The Genetic Diversity of Turkish *Morchella* (Kuzugöbeği) with Using Molecular Techniques

Abstract: *Morchella* genus called as "kuzugöbeği" in Turkey, is consumed extensively in many countries including Turkey and it always maintains its scientific interest. Systematic studies on the genus *Morchella* have continued, new species have also been identified and synonyms have been determined. In this study, phylogenetic and morphological assessment of a collection consisting 50 samples collected from different locations of Turkey were performed. In phylogenetic analysis, internal transcribed spacer (ITS rDNA) and 28S rDNA (LSU) gene regions were used. At the end of the study, *Morchella dunensis*, *M. tridentina*, *M. importuna*, *M. eximia*, *M. dunalii*, *M. mediterraneensis* and *M. deliciosa* have been identified based on these gene regions.

Key words: *Morchella*, Morel, ITS rDNA, 28S rDNA, Turkey

Giriş

Türkiye'de kuzugöbeği mantarı olarak bilinen *Morchella* cinsi üyeleri, ticari önemi nedeniyle üzerinde en fazla çalışılan yenir özellikte olan doğa mantarlarındandır. Dünyada geniş bir ticaret hacmine sahip olan bu cins üyelerinin, satış fiyatı oldukça yüksektir. Türkiye *Morchella* cinsi yönünden oldukça zengin bir genetik çeşitliliğe sahip olup, her sene ülkenin farklı yörelerinde

yetişen bu mantarlar yerel halk tarafından toplanarak yurt dışına ihraç edilmektedir. Günümüze kadar Türkiye'de bulunan *Morchella* cinsi türlerinin toplanarak morfolojik, mikroskobik ve moleküler yöntemlerle tanımlanması ile ilgili çalışmalar Taşkın ve ark. (2010, 2012) tarafından başlatılmış, bu çalışmalar sonucunda ülkemizde 20 adet kuzugöbeği mantarı türünün varlığı kayıt altına alınmıştır. Yine, *M. anatolica* olarak isimlendirilen 1 tür de Işiloğlu ve ark. (2010) tarafından belirlenmiş ve böylece Türkiye'de 21 türün varlığı moleküler verilerle desteklenerek belgelenmiştir. Ancak Türkiye'de hala arazi çalışması yapılmamış, *Morchella* cinsi üylerinin yetiştiği birçok alan olduğu tahmin edilmekte ve bu bağlamda Türkiye'nin kuzugöbeği mantarı çeşitliliğinin 21 adet türle sınırlı olmadığı düşünülmektedir. Türkiye (Taşkın ve ark., 2010, 2012), Kuzey Amerika (Kuo ve ark., 2012; O'Donnell ve ark., 2011), Çin (Du ve ark., 2012a, 2012b), Kıbrıs (Loizides ve ark., 2015, 2016) ve Avrupa (Clowez, 2012; Richard ve ark., 2015) tarafından ilk çok genli analizler, *Morchella*'nın gerçekten de tür açısından zengin bir cins olduğunu doğrulamıştır: atasal *Morchella rufobrunnea* grubu (beyaz kuzugöbekleri), *Morchella esculenta* grubu (sarı kuzugöbekleri) ve geç farklılaşan *Morchella elata* grubu (siyah kuzugöbekleri). Sonrasında yeni türlerin tanımlanmasına devam edilmiştir (Baroni ve ark., 2018; Clowez ve ark., 2014, 2015, 2020; Du ve ark., 2019; Elliott ve ark., 2014; Loizides ve ark., 2016; Taşkın ve ark., 2016; Voitk ve ark., 2014, 2016). Bu filogenetik ve taksonomik atılımlarla sağlanan stabiliteye rağmen, *Morchella* türlerinin morfolojik olarak tanımlanması hala zorlayıcılığını korumaktadır (Loizides, 2017).

Yukarıda sunulan bilgilerin ışığında, tamamlanan bu çalışmanın amacı; Türkiye'den toplanan yeni *Morchella* örneklerinin, morfolojik-mikroskobik ve moleküler yöntemlerle tanımlanarak ülkemiz coğrafyasının kuzugöbeği mantarı zenginliğinin ortaya çıkarılmasına katkıda bulunulmasıdır. Sadece morfolojik ve mikroskobik yöntemlerle yapılan tanımlamalar, mantarlar üzerinde çevresel faktörlerin etkisi nedeniyle, çok belirgin olan bazı türler dışında yanıltıcı olabilmektedir.

Materyal ve Metot

Morfolojik analizler

Arazi çalışması

2016-2018 yılı ilkbahar sezonunda, Türkiye'nin farklı illerinden toplanmış olan 50 *Morchella* (kuzugöbeği) cinsi örnekleri, çalışmanın materyalini oluşturmuştur (Tablo 1). Arazi çalışmaları sırasında toplanan taze mantar örneklerinin öncelikle renkli fotoğrafları çekilmiş, morfolojik ve ekolojik özellikleri, yetişme yerinin özellikleri, toplandığı yükselti, coğrafi koordinatları, tarih ve numaraları kaydedilmiştir. Toplanan her bir mantar örneği için gelişmenin bütün evrelerine ait bireylerin bulunmasına özen gösterilmiş, daha sonra bu örnekler taşınabilir mantar kurutma makinesinde 40 ^{0}C'de 8 saat boyunca kurutularak, kilitli plastik torbalar içerisinde muhafaza edilmiştir. Kurutulan örnekler Osmaniye Korkut Ata Üniversitesi Fungaryum'unda saklanmaktadır.

Laboratuvar çalışması

Laboratuvar çalışması esnasında *Morchella* örneklerinin çeşitli mikroskobik yapıları stereo ve binoküler mikroskop altında incelenmiştir. Mikroskobik yapılar incelenirken mantar örneklerinin himenyum tabakasından ince kesitler alınarak, distile su, %5 lik KOH, melzer ayıracı ve kongo kırmızısı çözeltisinden oluşan preparatlar hazırlanmıştır. Askuslar, parafizler ve askosporlar vb. mikroskobik yapıların şekli, rengi, çeper kalınlığı, yüzey görünümü, askus içinde yer alan spor sayısı ve dizilimi teşhiste veri olarak kullanılmak üzere not alınmış ve bu yapıların enleri ve boyları, mikrometrik oküler yardımıyla ölçülerek, 10-20 ölçümün en küçük ve en yüksek değeri arasındaki aralık saptanmış ve boyutları belirlenmiştir. Örneklerin teşhisinde mevcut literatür kullanılmıştır (Baroni ve ark., 2018; Clowez, 1997; Clowez ve ark., 2014; 2015; Du ve ark., 2019; Kuo ve ark., 2012; Loizides ve ark., 2015, 2016; Richard ve ark., 2015; Taşkın ve ark., 2010, 2012, 2016; Voitk ve ark., 2014, 2016).

Tablo 1. Çalışmada kullanılan koleksiyona ait bilgiler (*****): "Bilinmiyor" olarak belirtilen örnekler toplayıcılardan veya firmalardan temin edilmiştir.)

HT No	MN	Lokasyon-Yıl	Koordinat	Habitat
HT554	44	Çanakkale 2016	Bilinmiyor*	Bilinmiyor*
HT559	10	Sinop 2016	Bilinmiyor*	Bilinmiyor*
HT560	15	Adana 2016	Bilinmiyor*	Yanık alan
HT562	52	Çanakkale 2016	Bilinmiyor*	*Pinus brutia*
HT567	104	Çanakkale 2016	Bilinmiyor*	Bilinmiyor*
HT587	14	Çanakkale 2016	K39°56.463' D026°32.318'-455 m	*Pinus brutia*
HT593	66	Çanakkale 2016	K39°56.372' D026°31.485'-386 m	*Pinus brutia*
HT601	65	Çanakkale 2016	K39°40.713' D026°35.511'-521 m	*Pinus brutia*

HT605	30	Erzincan 2016	K39°52.288’ D038°57.495’-5 m	*Pinus sylvestris* *Populus tremula*
HT628	84	Edirne 2017	K40°39.195’ D026°39.671’-84 m	Yanık saha
HT629	110	Edirne 2017	K40°39.195’ D026°39.671’-84 m	Yanık saha
HT630	94	Edirne 2017	K40°39.147’ D026°39.637’-82 m	Yanık saha
HT631	35	Edirne 2017	K40°39.633’ D026°39.686’-90 m	Yanık saha
HT633	81	Edirne 2017	K40°39.029’ D026°39.599’-48 m	Yanık saha
HT636	75	Edirne 2017	K40°39.029’ D026°39.599’-48 m	Yanık saha
HT637	71	Edirne 2017	K40°39.694’ D026°39.677’-77 m	Yanık saha
HT640	76	Edirne 2017	K40°39.704’ D026°39.688’-93 m	Yanık saha
HT641	41	Edirne 2017	K40°39.197’ D026°39.613’-102 m	Yanık saha
HT643	39	Edirne 2017	K40°39.679’ D026°39.677’-85 m	Yanık saha
HT644	107	Edirne 2017	K40°39.213’ D026°39.683’-58 m	Yanık saha
HT647	96	Edirne 2017	K40°39.199’ D026°39.671’-89 m	Yanık saha
HT648	73	Edirne 2017	K40°39.713’ D026°39.717’-86 m	Yanık saha
HT650	49	Edirne 2017	K40°39.210’ D026°39.633’-88 m	Yanık saha
HT651	106	Edirne 2017	K40°39.198’ D026°39.680’-80 m	Yanık saha
HT653	95	Edirne 2017	K40°39.676’ D026°39.678’-75 m	Yanık saha
HT654	72	Edirne 2017	K40°39.179’ D026°39.638’-86 m	Yanık saha
HT655	47	Edirne 2017	K40°39.685’ D026°39.719’-86 m	Yanık saha
HT668	37	Adana-Feke 2017	K37°42.163’ D035°48.500’-639 m	*Pinus brutia*, *Arbutus* spp.
HT669	98	Adana-Feke 2017	K37°42.484’ D035°46.513’-732 m	*Pinus brutia*, *Arbutus* spp.
HT670	25	Adana-Feke 2017	K37°42.484’ D035°46.513’-732 m	*Pinus brutia*, *Arbutus* spp.
HT671	55	Adana-Feke 2017	K37°42.475’ D035°46.512’-741 m	*Pinus brutia*, *Arbutus* spp.

HT672	51	Adana-Feke 2017	K37°42.819' D035°48.464'-625 m	*Pinus brutia*, *Arbutus* spp.
HT677	80	Balıkesir-Kazdağları 2018	K39°36.414' D026°50.556'-407 m	*Pinus brutia*, *Cistus* spp.
HT678	74	Balıkesir-Kazdağları 2018	K39°35.462' D026°51.178'-194 m	Yanık saha, *Pinus brutia*
HT679	93	Balıkesir-Kazdağları 2018	K39°35.198' D027°00.367'-1 m	Sebze bahçesi, bir tane *Pinus brutia* var
HT680	40	Balıkesir-Kazdağları 2018	K39°35.462' D026°51.178'-208 m	Yanık saha, *Pinus brutia*
HT682	17	Isparta 2018	K37°47.365' D031°19.549'-1695 m	*Juniperus* spp., *Abies* spp.
HT683	42	Isparta 2018	K37°47.361' D031°19.549'-1722 m	*Juniperus* spp., *Abies* spp.
HT685	22	Isparta 2018	K37°47.363' D031°19.550'-1721 m	*Juniperus* spp., *Abies* spp.
HT687	105	Isparta 2018	K37°47.362' D031°19.550'-1734 m	*Juniperus* spp., *Abies* spp.
HT688	24	Isparta 2018	K37°47.364' D031°19.549'-1695 m	*Juniperus* spp., *Abies* spp.
HT689	5	Isparta 2018	K37°47.471' D031°19.553'-1734 m	*Juniperus* spp., *Abies* spp.
HT690	90	Isparta 2018	K37°46.584' D031°20.213'-1629 m	*Juniperus* spp., *Abies* spp.
HT691	63	Isparta 2018	K37°47.363' D031°19.548'-1694 m	*Juniperus* spp., *Abies* spp.
HT693	4	Isparta 2018	K37°47.361' D031°19.550'-1719 m	*Juniperus* spp., *Abies* spp.
HT694	3	Isparta 2018	K37°47.360' D031°19.552'-1717 m	*Juniperus* spp., *Abies* spp.
HT697	86	Isparta 2018	K37°47.365' D031°19.550'-1698 m	*Juniperus* spp., *Abies* spp.
HT698	83	Adana-Aladağ 2017	K37°27.519' D035°15.226'-1186 m	*Pinus brutia*, *Pinus nigra*, *Abies* spp.
HT707	56	Isparta-Keçiborlu 2017	K37°54.539' D030°17.517'-986 m	Bilinmiyor*
HT715	103	Kars 2018	Bilinmiyor*	Bilinmiyor*

Moleküler analizler

DNA izolasyonu, kurutulmuş mantarlardan hazır kit (Eurx GeneMATRIX marka Bitki ve Mantar DNA Saflaştırma Kiti) kullanılarak yapılmıştır. İzolasyona başlamadan önce kit protokolüne göre, spin kolonlar tampon P (40 µl) ile aktive edilmiştir. Kurutulan mantar örneklerinden, yaklaşık 50 mg alınarak bir homojenizatör (IKA T-10B, Almanya) yardımıyla eppendorf tüplerinin içerisinde toz haline gelinceye kadar öğütülmüştür. Toz haline getirilen örneklerin üzerine sırasıyla Lyse-F tamponu (400 µl), RNaz A (10 µl, 20 mg/ml), Proteinaz K (10 µl, 10 mg/ml) ilave edilip vortekslendikten sonra, su banyosunda 65°C'de 30 dk boyunca inkübe edilmiştir. Su banyosundan çıkarılan örneklerin üzerine tampon AC (130 µl) ilave edilerek, 12.000 devir/dk'da 10 dk boyunca santrifüj edilmiştir. Süpernatant kısım (yaklaşık 400 µl) yeni bir eppendorf tüpe aktarılıp, üzerine sırasıyla Sol P tamponu (350 µl) ve etanol (250 µl, %96'lık) ilave edilerek tüpler hafif bir şekilde alt üst edilmiştir. Daha sonra yine süpernatant kısım spin kolonlarına alınarak, 1 dk boyunca

11.500 devir/dk'da santrifüj edilmiştir. Toplama tüpleri içerisindeki sıvı kısım döküldükten sonra, spin kolonların üzerine Wash PX tamponu ilave edilerek, tekrar 1 dk boyunca 11.500 devir/dk'da santrifüj edilmiştir (bu işlem en az iki kez tekrarlanmıştır). Spin kolonlar yeni bir eppendorf tüp içerisine konularak üzerine 80°C'ye kadar ısıtılmış elution tamponu (200 µl) konulup 3 dk bekletildikten sonra, 1 dk boyunca 11.500 devir/dk'da santrifüj edilmiştir. İçerisinde 200 µl genomik DNA solüsyonu bulunan ependorf tüpleri kullanılıncaya kadar, 20°C'de buzdolabında bekletilmiştir.

Herbir örnek için PCR karışımı, toplamda 50 µl olarak, şu şekilde hazırlanmıştır: 35.8 µl steril ddH_2O, 5 µl 10X PCR buffer, 5 µl $MgSO_4$ (50 mM), 1.0 µl dNTP (10 mM), 0.8 µl ileri primer (10 pM), 0.8 µl geri primer (10 pM), 1.0 µl Taq polimeraz enzimi ve 0.6 µl genomik DNA. PCR reaksiyon şartları; 94°C'de 5 dk süreyle bir döngü; 94°C'de 30 s, ITS için 50°C'de, LSU için 53°C'de ve 72°C'de 90 s süreyle 30 döngü; 72°C'de 10 dakikalık bir döngü ve +4°C'de bekletme şeklinde ayarlanmıştır. ITS ve 28S rDNA gen bölgeleri için sırasıyla ITS4 (TCC TCC GCT TAT TGA TAT GC) - ITS5 (GGA AGT AAA AGT CGT AAC AAG G) ve NL1 (GCA TAT CAA TAA GCG GAG G) – NL4 (GGT CCG TGT TTC AAG ACG G) primer çiftleri kullanılmıştır (O'Donnell ve ark., 1997; White ve ark., 1990). Elde edilen PCR ürünleri, 1xTAE buffer (Sambrook ve Russell, 2001) içerisindeki agoroz jel (%1.5-UltraPure TM Agarose)'de elektroforez yardımıyla koşturulduktan sonra, etidyum bromit ile boyanarak UV görüntüleme sistemi yardımıyla görüntülenmiştir.

PCR ürünlerinin temizlenmesi ve DNA dizi analizleri, hizmet alımı şeklinde gerçekleştirilmiştir. Çalışmada, transkripsiyonu yapılamayan bölge (ITS rDNA, yaklaşık 700 baz çifti; White ve ark., 1990) ile 28S rDNA bölgesi (LSU, yaklaşık 600 baz çifti; O'Donnell ve ark., 1997) kullanılmıştır. Öncelikle tüm örneklerin, ITS rDNA gen bölgesinin dizi analizi yapılmıştır. Elde edilen DNA dizileri Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI) programı kullanılarak hizalanmış ve aynı programda %100 benzerlik gösteren tür grupları belirlenmiştir. Daha sonra oluşan bu tür gruplarından birer örnek seçilerek, LSU gen bölgesi ile DNA dizi analizi yapılmıştır. Satırlardaki dizin verilerinin düzenlemesinde (ileri ve geri primerlerin karşılaştırılarak hataların düzeltilmesi) Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI) programı kullanılmış ve sonrasında ITS ve 28S rDNA gen dizileri ile birleştirilerek (yaklaşık 1300 baz çifti uzunluğunda) her iki genin kombinasyonu ile bir filogenetik ağaç oluşturulmuştur. Filogenetik ağacın oluşturulması, MEGA 7.0 programı kullanımıyla Maximum Likelihood (ML) analizi ile yapılmıştır. Türlerin belirlenmesi, elde edilen DNA dizilerinin Genbankası verileri ile karşılaştırılarak gerçekleştirilmiştir.

Bulgular

Morchellaceae Rchb. (1828).

Morchella Dill. ex Pers. (1794).

1. *Morchella deliciosa* Fr. (1822) (Şekil 2).

Syn.: *Morilla deliciosa* (Fr.) Quél. (1892), *Morchella deliciosa* var. *incarnata* Quél. (1892), *M. deliciosa* var. *elegans* Boud. (1897), *M. deliciosa* var. *purpurascens* Boud.(1897), *M. conica* var. *deliciosa* (Fr.) Cetto (1988).

Makroskobik ve mikroskobik özellikler

Askokarp 50-70 mm boyunda, şapka ve saptan meydana gelmiştir. **Şapka** 30-40 × 20-25 mm, koni şeklinde, bal peteği görünümünde, üzerinde çukurlar ve kaburga benzeri çıkıntılar mevcut, griden kahverengiye değişen tonlardadır. **Sap** 20-30 × 10-20 mm, silindir şeklinde ve taban kısmı şişkinleşmiş, içi boş, beyazdan sarıya değişen tonlardadır. **Etli kısım** sert, elastik ve kalındır. **Askuslar** 300-320 × 20-22 µm, silindir veya çomak şeklinde, sekiz sporlu, renksiz ve inamiloittir. **Parafizler** 13-14 µm geniştiğinde, silindir veya çomak şeklinde, bölmeli ve renksizdir. **Askosporlar** 23-26 × 13.5-15 µm, eliptik, pürüzsüz, kalın duvarlı, renksiz ve inamiloittir. GenBank numaları: MT435013 ve MT430970.

2. *Morchella dunalii* Boud. (1887) (Şekil 3).

Syn.: *Morchella fallax* Clowez & Luc Martin in Clowez (2012).

Makroskobik ve mikroskobik özellikler

Askokarp 80-90 mm boyunda, şapka ve saptan meydana gelmiştir. **Şapka** 50-60 × 20-30 mm, silindir, yumurta veya koni şeklinde, bal peteği görünümünde, kaburga benzeri boyuna çıkıntılara sahip, açık kahverengi veya grimsi kahverengi, bazen daha koyu kahverengi tonlardadır. **Sap** 20-30 × 15-20 mm, silindir şeklinde ve taban kısmı şişkinleşmiş, içi boş, başlangıçta beyaz, zamanla beyaz zemin üzerinde sarıdan açık kahverengiye değişen tonlarda renklenmeler görülür. **Etli kısım** sert, elastik ve kalındır. **Askuslar** 290-340 × 19-23 µm, silindir veya çomak şeklinde, sekiz sporlu, renksiz ve inamiloittir. **Parafizler** 12-15 µm geniştiğinde, silindir veya çomak şeklinde, bölmeli ve renksizdir. **Askosporlar** 23-26 × 13.5-15 µm, eliptik veya genişçe eliptik, pürüzsüz, kalın duvarlı, renksiz ve inamiloittir. **Akroparafizler** 90–140 × 22–38, çomak şeklinde, kalın duvarlı ve 2-3 bölmelidir. GenBank numaraları: MT435016 ve MT430967.

3. *Morchella dunensis* (Castañera, J.L. Alonso & G. Moreno) Clowez (2012) (Şekil 4).

Syn.: *Morchella esculenta* f. *dunensis* Castañera, J.L. Alonso & G. Moreno (1996).

Makroskobik ve mikroskobik özellikler

Askokarp 75-100 mm boyunda, şapka ve saptan meydana gelmiştir. **Şapka** 50-60 × 25-40 mm, düzensiz yumurta veya küre şeklinde, düzensiz ve kaburga benzeri çıkıntılara sahip, açık kahverengi, grimsi kahverengi veya sarımsı kahverengidir. **Sap** 25-30 × 20-25 mm, silindir şeklinde ve taban kısmı şişkinleşmiş, içi boş, beyazdan sarımsı kahverengiye değişen tonlardadır. **Etli kısım** sert,

elastik ve kalındır. **Askuslar** 280-310 × 21-23 µm, silindir veya çomak şeklinde, sekiz sporlu, renksiz ve inamiloittir. **Parafizler** 10-13 µm geniştiğinde, silindir veya çomak şeklinde, bölmeli ve renksizdir. **Askosporlar** 21- 23.5 × 12.5-15 µm, eliptik, pürüzsüz, kalın duvarlı, renksiz ve inamiloittir. GenBank numaları: MT435008 ve MT430911.

4. *Morchella eximia* Boud. (1910) (Şekil 5).

Syn.: *Morchella conica* var. *acuminata* J. Kickx f. (1867), *M. conica* subsp. *acuminata* (J. Kickx f.) Sacc. (1889), *M. costata* var. *acuminata* (J. Kickx f.) Boud. (1897), *M. eximia* f. *schizocostata* Jacquet. (1985), *M. eximia* f. *acuminata* (J. Kickx f.) Clowez (2012), *M. eximia* f. *multiformis* Clowez (2012).

Makroskobik ve mikroskobik özellikler

Askokarp 60-80 mm boyunda, şapka ve saptan meydana gelmiştir. **Şapka** 40-50 × 30-40 mm, silindir veya koni şeklinde, üzerinde çukurlar ve kaburga benzeri çıkıntılar mevcut, sarımsı, solgun kahverengiden koyu kahverengiye değişen tonlardadır. **Sap** 20-30 × 15-25 mm, silindir şeklinde ve taban kısmı hafifçe şişkinleşmiş, içi boş, beyazdan sarımsı kahverengiye değişen tonlardadır. **Etli kısım** sert, elastik ve kalındır. **Askuslar** 260-300 × 20-23 µm, silindir şeklinde, sekiz sporlu, renksiz ve inamiloittir. **Parafizler** 10-14 µm geniştiğinde, silindir veya çomak şeklinde, bölmeli ve renksizdir. **Askosporlar** 20- 23 × 12-14 µm, pürüzsüz, eliptik, kalın duvarlı, renksiz ve inamiloittir. GenBank numaraları: MT435009 ve MT430984.

5. *Morchella importuna* M. Kuo, O'Donnell & T.J. Volk (2012) (Şekil 6).

Makroskobik ve mikroskobik özellikler

Askokarp 130-160 mm boyunda, şapka ve saptan meydana gelmiştir. **Şapka** 80-100 × 35-45 mm, koni veya genişçe koni şeklinde, üzerinde grimsi kahveringiden koyu kahverengiye değişen tonlarda, birincil dikey, çok sayıda yatay kaburga benzeri çıkıntılar ve griden gri-kahverengiye değişen tonlarda çukursu yapılar mevcuttur. **Sap** 50-60 × 25-40 mm, çomak şeklinde ve taban kısmı şişkinleşmiş, içi boş, beyazdan açık kahverengiye değişen tonlardadır. **Etli kısım** sert, elastik ve kalındır. **Askuslar** 260-300 × 18-24 µm, silindir şeklinde, sekiz sporlu, renksiz ve inamiloittir. **Parafizler** 8-13 µm geniştiğinde, silindir veya çomak şeklinde, bölmeli ve renksizdir. **Askosporlar** 21-23 × 12-13 µm, pürüzsüz, eliptik, kalın duvarlı, renksiz ve inamiloittir. GenBank numaraları: MT435015, MT435012, MT430989, MT430969, MT430941 ve MT430882.

6. *Morchella mediterraneensis* H. Taşkın, Büyükalaca & H.H. Doğan (2016) (Şekil 7).

Makroskobik ve mikroskobik özellikler

Askokarp 50-60 mm boyunda, şapka ve saptan meydana gelmiştir. **Şapka** 30-35 × 20-25 mm, koni veya yumurta şeklinde, üzerinde çukurlar ve kaburga benzeri çıkıntılar mevcut, sapa belirgin dar bir girintiyle bağlanır, başlangıçta menekşemsi siyah, gelişme ilerledikçe koyu siyaha döner. **Sap** 20-25 × 10-20 mm, silindir şeklinde ve taban kısmı şişkinleşmiş, içi boş, beyazdan açık bal kahverengisine değişen tonlardadır. **Etli kısım** sert, elastik ve kalındır. **Askuslar** 280-300 × 20-22 µm, silindir şeklinde, sekiz sporlu, renksiz ve inamiloittir. **Parafizler** 10-13 µm geniştiğinde, silindir şeklinde, bölmeli ve renksizdir. **Askosporlar** 21-23 × 12-13 µm, pürüzsüz, eliptik, kalın duvarlı, renksiz ve inamiloittir. GenBank numaraları: MT435014, MT435011, MT435010 ve MT435007.

7. *Morchella tridentina* Bres. (1892) (Şekil 8).

Syn.: *Morchella elatoides* Jacquet. (1984), *M. elatoides* var. *elegans* Jacquet. (1984), *M. conica* var. *pseudoeximia* Clowez (2012), *M. quercus-ilicis* Clowez, L. Ballester & L. Romero, in Clowez (2012), *M. frustrata* M. Kuo, in Kuo, Dewsbury, O'Donnell, Carter, Rehner, Moore, Moncalvo, Canfield, Stephenson, Methven & Volk (2012).

Makroskobik ve mikroskobik özellikler

Askokarp 100-120 mm boyunda, şapka ve saptan meydana gelmiştir. **Şapka** 60-70 × 25-40 mm, koni veya genişçe koni şeklinde, üzerinde az veya çok aralıklı parallel kaburga benzeri birincil çıkıntılar, merdiven benzeri bir desen oluşturan ikincil enine çıkıntılar ve bu çıkıntılarla uyumlu çukurlar bulunmakta, gençken grimsi tonlarda, gelişme ilerledikçe grimsi kahverengi, grimsi bej tonlarda ve yer yer kırmızımsı veya turncu lekere sahiptir. **Sap** 40-50 × 20-25 mm, silindir şeklinde ve taban kısmı şişkinleşmiş, içi boş, kirli beyazdan açık sarımsı kahverengiye değişen tonlarda, üzerinde yer yer kırmızımsı lekeler mevcuttur. **Etli kısım** sert, elastik ve kalındır. **Askuslar** 280-310 × 20-24 µm, silindir şeklinde, sekiz sporlu, renksiz ve inamiloittir. **Parafizler** 12-15 µm genişliğinde, silindir şeklinde, bölmeli ve renksizdir. **Askosporlar** 21-23 × 12-13 µm, pürüzsüz, genişçe eliptik, kalın duvarlı, renksiz ve inamiloittir. GenBank numaraları: MT430968 ve MT430881.

Çalışmada, toplanan ve 50 üyeden oluşan *Morchella* koleksiyonundaki tüm örnekler, öncelikle ITS rDNA gen bölgesi ile taranmış ve Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI) programı kullanımı ile %100'lük benzerlik oranında karşılaştırılmışlardır. Bu karşılaştırma sonucunda, %100 benzer olan örneklerden seçilen örneklerle (10 adet) (Tablo 2), ITS rDNA ve 28S rDNA (LSU) gen bölgelerinin birleştirilmiş şekilde Maksimum Likelihood (ML) analizi ile MEGA 7.0 programında filogenetik ağaç oluşturulmuştur. Grubu temsilen seçilen örneklerin, yanıltıcı olmaması için, DNA dizilerinin hem ileri hem de geri primer ile kaliteli çıktığı koleksiyonların seçimine dikkat edilmiştir. Değerlendirilen koleksiyondaki tür tahminleri de Tablo 3'de sunulmuştur.

Moleküler olarak yapılan değerlendirmelerde, Şekil 1'de de görüldüğü gibi, her bir klatta kümelenen türler yüksek bootstrap değeri almıştır. Bootstrap değerinin %70'in üzerinde olması örneklerin topolojik olarak benzerliklerini göstermektedir. Böylelikle analiz edilen bütün örneklerin bootstrap değerinin yüksek olması, bu

türlerin filogenetik ağaçta güvenilir bir şekilde kümelendiğini göstermektedir. Ağacın en yüksek log likelihood değeri -2584.60 olmuştur. Bölgeler arasındaki evrimsel hız farklılıklarını modellemek için ayrık bir Gama dağılımı kullanılmıştır (5 kategori (+G, parametre = 2.5104)). İlişkili taksonun birlikte kümelendiği ağaçların yüzdesi, dalların yanında gösterilmektedir. Analiz, 19 nükleotid dizisini içermektedir. Boşluklar ve eksik veriler içeren tüm pozisyonlar ortadan kaldırılmıştır. Bu çalışma kapsamında Genbankasına yüklenen sekanslar, toplayıcı numarası ile birlikte koyu harflerle gösterilmiştir. Dış grup olarak *Gyromitra gigas* kullanılmıştır.

Tartışma

Çalışma sonuçlarına göre; HT554 nolu örnek *M. dunensis*, HT593 nolu örnek *M. tridentina*, HT628, HT669 ve HT670 nolu örnekler *M. importuna*, HT630 nolu örnek *M. eximia*, HT667 nolu örnek *M. dunalii*, HT693 ve HT698 nolu örnekler *M. mediterraneensis* ve HT690 nolu örnek *M. deliciosa* olarak görünmektedir (Şekil 1).

Morchella deliciosa Fr. (*Mel*-26), Taşkın ve ark. (2010, 2012) tarafından yapılan Türkiye *Morchella* cinsi çeşitliliğinin çoklu gen bölgeleri DNA dizi analizlerine dayalı yöntemlerle araştırılması çalışmasında, filogenetik adı *Mel*-26 olarak, 819-1593 m yükseltiler arasında; *Pinus brutia*, *Pinus nigra* ve *Pinus sylvestris* ormanlarından toplanılmıştır. Çalışma süresince, 500'e yakın örnek içeren koleksiyondan sadece 9 adet örnek *M. deliciosa* olarak tanımlanmıştır. Richard ve ark. (2015) tarafından yapılan çalışmada, ITS gen bölgesi DNA dizi analizleri ile *M. deliciosa*, *Mel*-13'den ayırt edilememiştir. Ancak, çok genli filogenetik analizlerle ayrım yapılabilmiştir. Araştırıcılar, *Mel*-26'ya ait DNA dizilerinin, sadece Türkiye'den yayınlandığını ve yaptıkları çalışma ile Fransa'dan elde edilen DNA dizilerinin de eklendiğini bildirmişlerdir. Richard ve ark. (2015)'nın çalışmalarında bu tür, *Buxus sempervirens*'e yakın *Picea abies* altında, yaşlı *P. abies*, *Larix decidua* ve *Fraxinus excelsior* altında kaydedilmiştir. Loizides (2017) bu türün kayıtlı olduğu ülkeleri; Fransa, İsveç ve Türkiye olarak belirtmiştir. Bu çalışmada, bu türe giren örneklerin tamamı hem 2017 hem de 2018 yılında toplama yapılan Isparta ilinin 986-1734 m yükseltileri arasında, *Juniperus* spp. ve *Abies* spp. ormanlarından kaydedilmiştir (Şekil 2). Daha önce Türkiye'de Taşkın ve ark. (2010, 2012) çalışmalarında, daha çok *Pinus* spp. ormanlarında, bu türün varlığı bildirilmiştir.

Morchella dunalii Boud. (*Mel*-25), Boudier (1887) tarafından, Montpellier-Fransa'da, bir Akdeniz koleksiyonunun su boyamaya dayalı teknikle tanımlanan ilk *Morchella* türü olmuş ve Moreau ve ark. (2011) tarafından yayınlanmıştır. Richard ve ark. (2015) tarafından, Fransa ve İspanya'da bu türün tipik olarak kireçli topraklarda *Quercus ilex* altında kaydedildiği, ancak Taşkın ve ark. (2012) çalışmalarında *Mel*-25 olarak *Pinus* spp. ormanlarından toplanıldığı bildirilmiştir. Loizides ve ark. (2016) bu türü Kıbrıs'da *Pinus brutia*, *Cistus parviflorus*, *C. salvifolius*, *C. creticus*, *Quercus coccifera* spp. *calliprinos*, *Arbutus andrachne*, *Mirtus communis* ve *Olea europaea* ormanlarında kaydetmişlerdir. Loizides ve ark. (2016) tarafından, genellikle Şubat ayının sonundan Nisan ayının başlarına kadar çıkan ilk kuzugöbeklerinden olarak, genellikle 200-1000 m arasında değişen yükseltilerde; nemli, yosunlu, *Pinus brutia* ormanlarında, bazen de herdem yeşil *Quercus* standlarında veya *Cistus* alanlarında, kireçli topraklarda rapor edilmiştir. Moreau ve ark. (2011) tarafından, bu yaygın ve sık rastlanılan Akdeniz türünün, yüksek oranda ekolojik ve morfolojik değişimler göstermesi nedeni ile sıklıkla *M. conica*, *M. delicosa* ve *M. purpurascens* olarak yanlış adlandırıldığı kaydedilmiştir (Loizides ve ark., 2016). Taşkın ve ark. (2010, 2012) tarafından yapılan çalışmalarda, bu tür Türkiye'de çalışma süresince en fazla rastlanılan üçüncü tür olmuş (*M. tridentina* ve *M. mediterraneensis*'den sonra), 18-1048 m yükseltiler arasında; *Pinus brutia*, *Pinus nigra*, *Pinus sylvestris* ve *Quercus* spp. karışık ormanlarından toplanılmıştır. Loizides (2017) bu türün kayıtlı olduğu ülkeleri; Kıbrıs, Fransa, İspanya ve Türkiye olarak bildirmiştir. Bu çalışma süresince incelenen koleksiyonda bu türe giren örnekler, Çanakkale ve Balıkesir illerinde, 407 m yükseltide *P. brutia* ve *Cistus* spp. ormanından kaydedilmiştir (Şekil 3).

Morchella dunensis (Castañera, J.L. Alonso & G. Moreno) Clowez, ilk defa *Morchella esculenta* olarak Castañera ve Moreno (1996) tarafından keşfedilmiş, sonrasında Clowez (1997) tarafından farklı bir tür olarak tanımlanmıştır. Richard ve ark. (2015) tarafından bu tür *Morchella vulgaris*'e filogenetik yakınlık nedeni ile konspesifik olarak tanımlanmıştır. Loizides ve ark. (2016) daha ileri moleküler analizler ve örneklemeler sonucu ise iki tür içerisindeki ilişkilerin yakın olduğunu, ancak filogenetik farklılıklar olduğunu rapor etmişlerdir. Aynı zamanda, *Morchella andalusiae*'nın filogenetik olarak *dunensis* ile sinonim olabileceğini bildirmişlerdir. Bu tür, ilk çok genli filogenetik analizlerde *Mes*-17 olarak isimlendirilmiştir. Taşkın ve ark. (2010, 2012) tarafından yapılan çalışmalarda, *Mes*-17 türüne rastlanmış ve *M. vulgaris* olarak isimlendirilmiştir. Sonrasında Loizides ve ark. (2016) tarafından yapılan çalışmada, Türkiye örneklerinin *M. dunensis* ile daha yakın olduğu tespit edilmiştir. Bu iki tür arasındaki ilişkiler, karışık görünmektedir. Bu çalışma için oluşturulan koleksiyonda da *Mes*-17 türüne rastlanmış ve *M. dunensis* olarak değerlendirilmiştir. Taşkın ve ark. (2010, 2012) tarafından yapılan çalışmada, bu türe ait 17 örnek toplanmış ve filogenetik olarak değerlendirilmiştir. Örneklerin çoğu Adana ve Antalya illerinden, yani Akdeniz Bölgesi'nden toplanmıştır. Sadece bir örnek Çanakkale ilinden kaydedilmiştir. Yükselti 248 ile 893 m arasında olmuş ve tüm örnekler *Pinus brutia* ormanlarından toplanılmıştır. Richard ve ark. (2015) çalışmasında bu türe yakın ağaç

türleri; *Fraxinus excelsior*, *Ranunculus ficaria*, *Ammophila arenaria*, *Hedera helix*, *Robinia pseudoacacia*, *Crataegus oxyacantha*, *Sorbus aucuparia*, *Ulmus laevis*, *U. minör*, *Ribes nigrum*, *Abies concolor*, *Acer pseudoplatanus*, *Fraxinus angustifolia*, *Castanea sativa* ve *Populus nigra* olarak sunulmuştur. Sunulan bu çalışmada incelenen örnek, Çanakkale ilinden toplanılmıştır (Şekil 4). Daha önce Taşkın ve ark. (2010, 2012) çalışmalarında da incelenen örnekler, *P. brutia* ormanlarından kaydedilmiştir.

Morchella eximia Boud. (*Mel*-7), Taşkın ve ark. (2010, 2012) tarafından yapılan çalışmalarda, filogenetik adı *Mel*-7 olarak, 180-761 m yükseltiler arasında, sadece yanmış alanlardan toplanılmıştır. Çalışma süresince, 500'e yakın örnek içeren koleksiyondan sadece 28 adet örnek *M. eximia* olarak tanımlanmıştır. Richard ve ark. (2015) tarafından yapılan çalışmada da bu tür, yangın sonrası mantarı (post-fire) olarak tanımlanmış ve yanmış *Pinus nigra* subsp. *laricio*, *Pinus pinaster*, *Arbutus unedo*, *Eucalyptus diversicolor*, Pinaceae ve *Thuja plicata* ormanlarından kaydedilmiştir. Türkiye'de ise yanmış *Pinus brutia*, *Pinus nigra* ve *Quercus* spp. ormanlarından toplanılmıştır. Bu tür; Arjantin, Avustralya, Kanada, Çin, Kıbrıs, Fransa, Kuzey Amerika, İspanya ve Türkiye'de kaydedilmiştir (Loizides ve ark., 2016; Loizides, 2017). Yine Kıbrıs'da 2 yıl önce yanmış *P. brutia* ormanlarında rapor edilmiştir (Loizides ve ark., 2016). Bu çalışmada bu türe giren örnekler, Taşkın ve ark. (2010, 2012) çalışmaları ve tüm dünya ile uyumlu olarak sadece yanık alanlarda, Edirne ilinde 48-90 m yükseltiler arasında ve Kars ilinde kaydedilmiştir (Şekil 5).

Morchella importuna M. Kuo, O'Donnell & T.J. Volk (*Mel*-10), Kuo ve ark. (2012) tarafından yapılan bir çalışmada isimlendirilmiştir. Kuo ve ark. (2012), Kuzeybatı Pasifik ve Kuzey Kaliforniya'daki bahçelerde, yetiştiricilerde, ağaç yonga yataklarında ve kentsel peyzaj düzenlemelerinde, Mart-Mayıs aylarında kaydedildiğini bildirmişlerdir. Bu tür; *Morchella elata* Fr., *Morchella vaporaria* Bartayrès ex Brond. ve *Morchella hortensis* ile sinonim olarak düşünülmektedir (Richard ve ark., 2015). Loizides ve ark. (2016)'na göre *M. importuna*; Kanada, Çin, Kıbrıs, Finlandiya, Fransa, Almanya, Kuzey Amerika, İspanya, İsviçre ve Türkiye'de kaydedilmiştir. Kıbrıs'da *Malus domestica* bahçesinden toplanmıştır (Loizides ve ark., 2016). Richard ve ark. (2015)'nın çalışmalarında; *Pyrus*, *Malus* ve *Cydonia oblonga* altında kayıtlar rapor edilmiştir. Taşkın ve ark. (2010, 2012) tarafından yapılan çalışmalarda, bu tür Türkiye'de çalışma süresince en fazla rastlanılan beşinci tür olmuş (*M. tridentina*, *M. mediterraneensis*, *M. dunalii* ve *M. purpurascens*'den sonra), hem yanmış hem de yanmamış alanlarda, 180-1449 m yükseltiler arasında; *Pinus brutia*, *Pinus nigra*, *Pinus sylvestris*, *Quercus* spp., *Populus* spp. ve *Abies* spp. karışık ormanlarından toplanmıştır. Diğer çalışmalarda olduğu gibi, Türkiye'de de meyve bahçelerinde (*Malus communis* ve *Citrus* spp. gibi) sıkça rastlanılmaktadır. Bu türün saprop olduğu düşünülmektedir (Richard ve ark., 2015). Bu çalışmada incelenen koleksiyon içerisindeki bu türe giren örnekler, daha önce Taşkın ve ark. (2010, 2012) çalışmalarında da olduğu gibi hem yanık hem de yanık olmayan alanlardan toplanılmıştır. Yanık olmayan sahadan kaydedilen örnekler, Adana ili Feke ilçesinden, 625-741 m yükseltilerden *Pinus brutia* ve *Arbutus* spp. ormanlarından toplanmıştır. Yanık sahadan elde edilen örnekler ise Adana, Edirne (48-93 m yükseltilerden) ve Balıkesir (195-208 m yükseltilerden, yangın öncesi *P. brutia* varlığı bilinmektedir) illerinden kaydedilmiştir (Şekil 6). Ayrıca bir örnekte, Balıkesir'de bir evin bahçesinden toplanmıştır. Yukarıda sunulan bilgilerden, ev bahçelerinde ve meyve bahçelerinde sıkça karşılaşılan bir tür olduğu açıkça görülmektedir.

Morchella mediterraneensis Taşkın, Büyükalaca & H.H. Doğan (*Mel*-27), Taşkın ve ark. (2016) tarafından çoklu gen bölgeleri DNA dizi analizlerine dayanarak yeni bir tür olarak tanımlanmıştır. Taşkın ve ark. (2010, 2012) tarafından yapılan çalışmalarda, bu tür Türkiye'de çalışma süresince *M. tridentina*'dan sonra en fazla rastlanan tür olmuştur. Türkiye'de bu tür, 1335-1685 m yükseltiler arasında; *Pinus brutia*, *Pinus nigra*, *Juniperus* spp., *Abies* spp., *Cedrus* spp., *Populus* spp., *Quercus* spp. karışık ormanlarında, çoğunlukla Akdeniz Bölgesi'nde tespit edilmiştir. Loizides (2017), bu türün İspanya'daki varlığını da rapor etmiştir. Kıbrıs ve Yunanistan'da da varlığı bilinmektedir. Bu çalışmada incelenen koleksiyonda bu türe giren örnekler; Isparta ve Adana illerinden 1186-1719 m yükseltilerde, *Pinus brutia*, *Pinus nigra*, *Juniperus* spp. ve *Abies* spp. ormanlarından toplanmıştır (Şekil 7). Yani yine Akdeniz Bölgesi koleksiyonları, bu tür içerisine yerleşmiştir.

Morchella tridentina Bres. (*Mel*-2), Kuo ve ark. (2012) tarafından, çoklu gen bölgeleri DNA dizi analizlerine göre, *Morchella frustrata* olarak tanımlanmıştır. Sonrasında, Loizides ve ark. (2015) tarafından yapılan bir çalışmada, ITS, TEF1 ve RPB2 gen bölgelerinin sekans analizlerine göre, *M. tridentina* ile *M. elatoides*, *M. quercus-ilicis* ve *M. frustrata* sinonim olarak önerilmiştir. Richard ve ark. (2015) tarafından yapılan çalışmada da *M. elatoides* ve *M. elatoides* var. *elegans* (Clowez, 2012), *M. frustrata* (Kuo ve ark., 2012) ve *M. tridentina* sinonim olarak kaydedilmiştir. Loizides ve ark. (2016); Arjantin, Ermenistan, Şili, Kıbrıs, Fransa, Hindistan, İtalya, Kuzey Amerika, İspanya ve Türkiye'de bu türün varlığını bildirmişlerdir. Taşkın ve ark. (2010, 2012) tarafından yapılan Türkiye *Morchella* cinsi çeşitliliğinin çoklu gen bölgelerinin DNA dizi analizlerine dayalı yöntemlerle araştırılması çalışmasında, bu tür Türkiye'de çalışma süresince en fazla rastlanan tür olmuş ve yoğunluklu olarak Akdeniz Bölgesi'nde kaydedilmiştir. Loizides ve ark. (2015) bu türü genellikle Akdeniz'de, 500 m'den fazla yükseklikte, geniş yapraklı, karışık ve iğne yapraklı ormanlık alanlarda, kireçtaşı, nötr veya hafif asitli

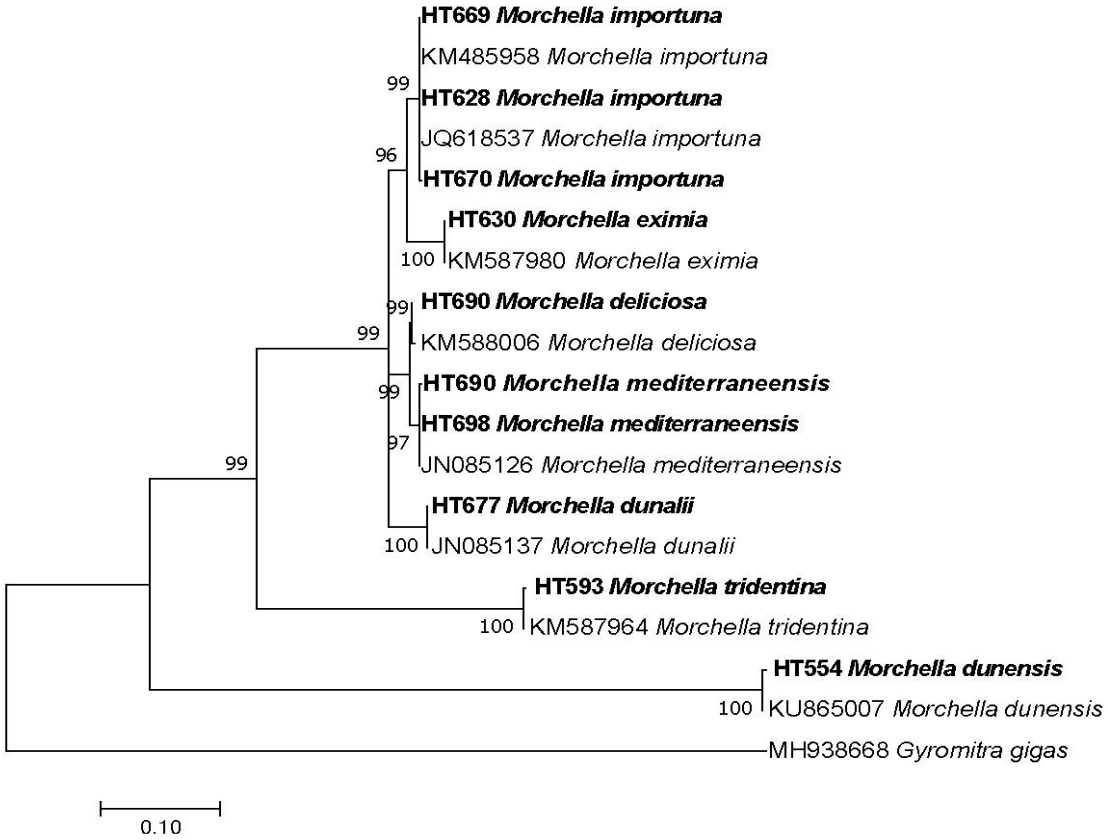
topraklarda, kalkerli alanlarda, kum ve ayrıca orman humusu üzerinde Mart ayının sonuyla Mayıs ayının başları arasındaki ılık dönemlerde tespit etmişlerdir. Aynı zamanda çalışmalarında; *Quercus*, *Arbutus*, *Olea*, *Abies*, *Pinus*, *Alnus*, *Pseudotsuga*, *Corylus*, *Fraxinus*, *Castanea* vb. yakınlarında rapor edildiğini de eklemişlerdir. Kuo ve ark. (2012) tarafından ise farklı yükseltilerde; *Arbutus menziesii* Pursh, *Quercus* spp., *Pseudotsuga menziesii* (Mirb.) Franco, *Pinus ponderosa* Laws., *Pinus lambertiana* Dougl. ve *Abies concolor* (Gord. & Glend.) Lindl. gibi farklı türlerin dominant olduğu karışık ormanlarda Nisan ayında, ABD'de Kaliforniya ve Oregon'da tespit edilmiştir. Türkiye'de ise farklı lokalitelerde, 241-1484 m yükseltilerde, *Pinus brutia*, *Pinus nigra*, *Quercus* spp., *Cedrus* spp., *Abies* spp., *Castanea sativa* ve *Juniperus* spp. ormanlarında tespit edilmiştir (Taşkın ve ark., 2010, 2012). Bu çalışmada, bu tür içerisinde incelenen örnekler ise Çanakkale ilinden ve Isparta ilinden *Juniperus* spp. ve *Abies* spp. ormanlarından, 386-1694 m yükseltilerden toplanmıştır (Şekil 8).

Çalışma ile tespit edilen bu türler, daha önce Taşkın ve ark (2010, 2012) çalışmalarında da tespit edilen türler olmuşlardır. Yeni koleksiyonların moleküler kanıtlı bir şekilde eklenmesiyle, incelenen örnek sayısı artırılmıştır. *M. mediterraneensis*, zaten Taşkın ve ark (2016) tarafından isimlendirilmiş bir tür olup, yoğun olarak Akdeniz ülkelerinde tespit edildiği için, "mediterraneensis" adı verilmiştir. Loizides (2017) tarafından bu türün Türkiye ve İspanya'dan kaydı bildirilmiş ve habitatı çoğunlukla kozalaklı ağaçlı ormanlar olarak tanımlanmıştır. Son zamanlarda bu türün Kıbrıs ve Yunanistan gibi diğer Akdeniz ülkelerinde de varlığı bilinmektedir. *M. tridentina* ve *M. frustrata* türleri sinonim türler olup (Kuo ve ark., 2012; Richard ve ark., 2015), olgun halinde moleküler destek olmadan da tespit edilebilen nadir türlerdendir. Elata (siyah) *Morchella* grubunda yer alan bu tür, diğer siyah grup türlerinin tersine koyulaşmış sırta sahip olmayan tek tür olarak gözlemlenmiştir (Loizides ve ark., 2015, 2016; Loizides, 2017). Türün diğer sinonim isimleri, Loizides (2017) tarafından *M. quercus-ilicis, M. frustrata, M. elatoides, M. elatoides* var. *elegans*, *M. conica* var. *pseudoeximia* olarak belirtilmiştir. Tür aynı zamanda Lozides (2017) tarafından, büyük olasılıkla biotropik veya iç simbiyotik (endofitik) olarak tanımlanmıştır. Daha önce Taşkın ve ark (2010, 2012) çalışmalarında *M. tridentina*, Türkiye'de en fazla rastlanan kuzugöbeği türü olmuştur. *M. importuna*'da ülkemizde daha önce yoğun tespit edilen türlerden birisi olmuştur. Bu türün en ilginç özellikleri; ev bahçeleri, yol kenarları, meyve bahçeleri gibi ormanlık alanlar dışında en fazla rastlanan tür olması ve aynı zamanda hem yangın görmüş hem de görmemiş alanlarda görülmesidir. Ülkemiz koleksiyonunda da hem yangın görmüş hem de görmemiş alanlardaki örnekler bu grupta yer almış ve ev bahçeleri ve peyzaj alanlarından toplanan türlerde, yine bu gruba yerleşmiştir. Loizides (2017) tarafından bu türün sinonimleri; *M. elata, M. hortensis, M. pragensis* ve *M. vaporaria* olarak rapor edilmiş, görüldüğü habitat ise fakültatif, yakılmış zeminde gelişebilen (pirofilik), 1-2 yaşındaki yanmış ormanlarda, zarar görmüş alanlarda, odunluk alanlarda, aynı zamanda *Malus* altında olarak tanımlanmıştır. Son zamanlardaki moleküler veriler eşliğinde, *M. elata* olarak bilinen türün bu tür olduğuna dair önemli kanıtlar sunulmuştur (Richard ve ark., 2015; Loizides ve ark., 2016; Loizides, 2017). *M. eximia*, İngilizce "post-fire" olarak adlandırılan yangın sonrası mantarı olarak da bilinmektedir. Loizides (2017) tarafından sinonimleri *M. anthracophila, M. carbonaria* ve *M. septimelata*; habitatı ise kesinlikle zorunlu olarak yakılmış zeminde gelişebilen (pirofilik), 1-2 yaşındaki yanmış kozalaklı ormanlar olarak tanımlanmıştır. Kuzugöbeği mantarı türlerinde yangın sonrası çıkışın mekanizması tam olarak çözülmemiş olmakla birlikte, bazı tahminler bulunmaktadır. Bu tahminlerden bazıları, yangın sonrası steril alanların elde edilmesi ve ölmek üzere olan ağaçlarla bağlantıdır (Greene ve ark., 2010; Loizides, 2017). Bu durum, Elata (siyah) *Morchella* grubunda görülürken, Esculenta (sarı) grupta görülmemektedir. Loizides (2017), Du ve ark. (2012b) ve Loizides ve ark. (2016) çalışmalarına atfen, siyah grupta görülen yangın sonrası çıkışın, orman yangınları gibi rahatsızlık fenomeni ile başa çıkmak için, sıcak ve kuru bölgelerde siyah kuzugöbeklerinin adaptasyonları kaynaklı olabileceğini bildirmiştir. Türkiye'de de bu türe giren tüm örnekler, yangın görmüş alanlardan toplanılmıştır (Taşkın ve ark., 2010, 2012; bu çalışma). *M. dunensis*, Taşkın ve ark. (2010, 2012) çalışmalarında filogenetik adı ile *Mes*-17 olarak kaydedilmiş ve sonrasında *Mes*-17, *M. vulgaris* olarak adlandırılmıştır. Ancak daha sonra yapılan çalışmalarda, Türkiye'deki örneklerin *M. dunensis* olduğu bildirilmiştir (Loizides, 2017). Loizides (2017) bu türün sinonimlerini; *M. esculenta* f. *dunensis, M. esculenta* f. *sterilis* ve *M. andalusiae*; habitatını ise muhtemelen saprofit, aynı zamanda fakültatif biotrofik veya iç simbiyotik (endofitik), *Malus* altında ve kum tepeleri olarak rapor etmiştir. Kıbrıs, Türkiye ve İspanya'da kaydedilen *M. dunalii* bir Akdeniz türü gibi görünmektedir. Türkiye'de de yaygın rastlanan bir tür olmuştur (Taşkın ve ark., 2010, 2012). *M. deliciosa*'nın sinonim türleri Loizides (2017) tarafından; *M. deliciosa* var. *elegans*, *M. deliciosa* var. *incarnata*, *M. conica* var. *flexuosa*, *M. conica* var. *nigra*, *M. conica* var. *violeipes* ve *Morilla deliciosa*; habitatı ise kozalaklılarla ilişkili, çoğunlukla *Larix*, *Picea* ve *Pinus* olarak tanımlanmıştır. *M. pulchella'*nın habitatı Loizides (2017) tarafından, hem geniş yapraklılar hem de kozalaklı ağaçlar olarak rapor edilmiş; Çin, Fransa ve Türkiye'den kaydı bildirilmiştir. Bu türlerin tamamı zaten Türkiye'de bilinmekle birlikte, DNA dizi analizleri içeren ek koleksiyonlar sağlanmış ve ileride yapılacak çalışmalar için saklanmaktadır. Aynı zamanda, bu çalışma ile bu türlerin Türkiye'nin farklı bölge ve illerindeki kayıt sayısı

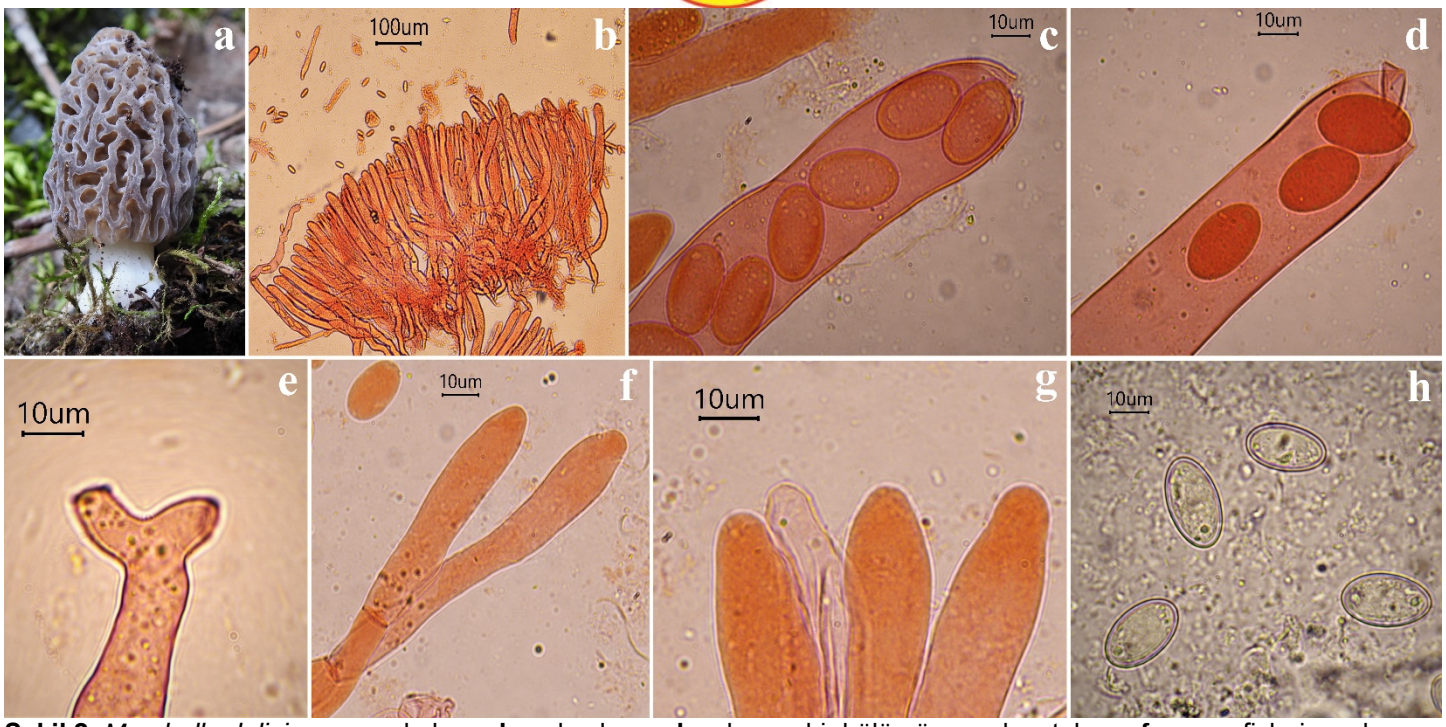
da artırılarak ülkemiz envanter kaydı çalışmalarına katkı sağlanmıştır.

Teşekkür

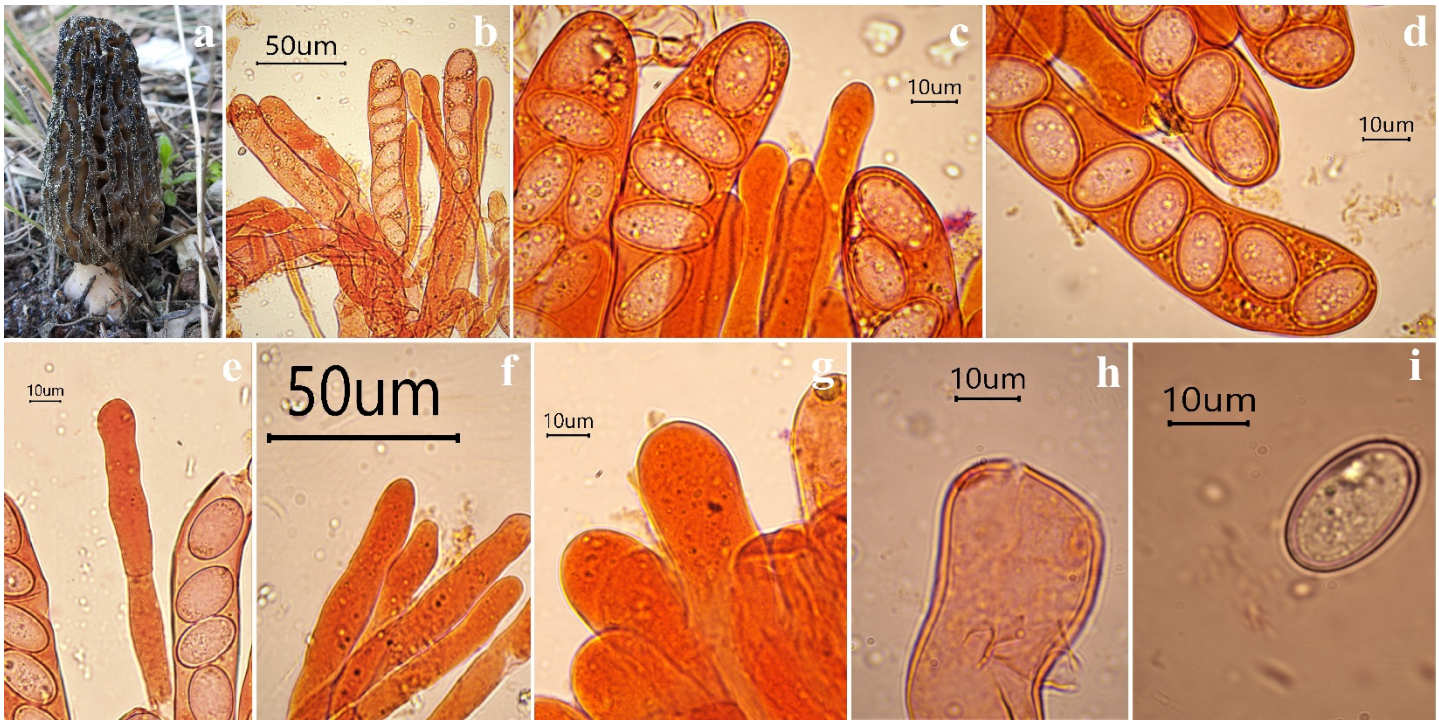
Bu çalışma, Çukurova Üniversitesi Bilimsel Araştırma Projeleri Birimi (Proje No: FYL-2018-10495) tarafından desteklenmiştir.



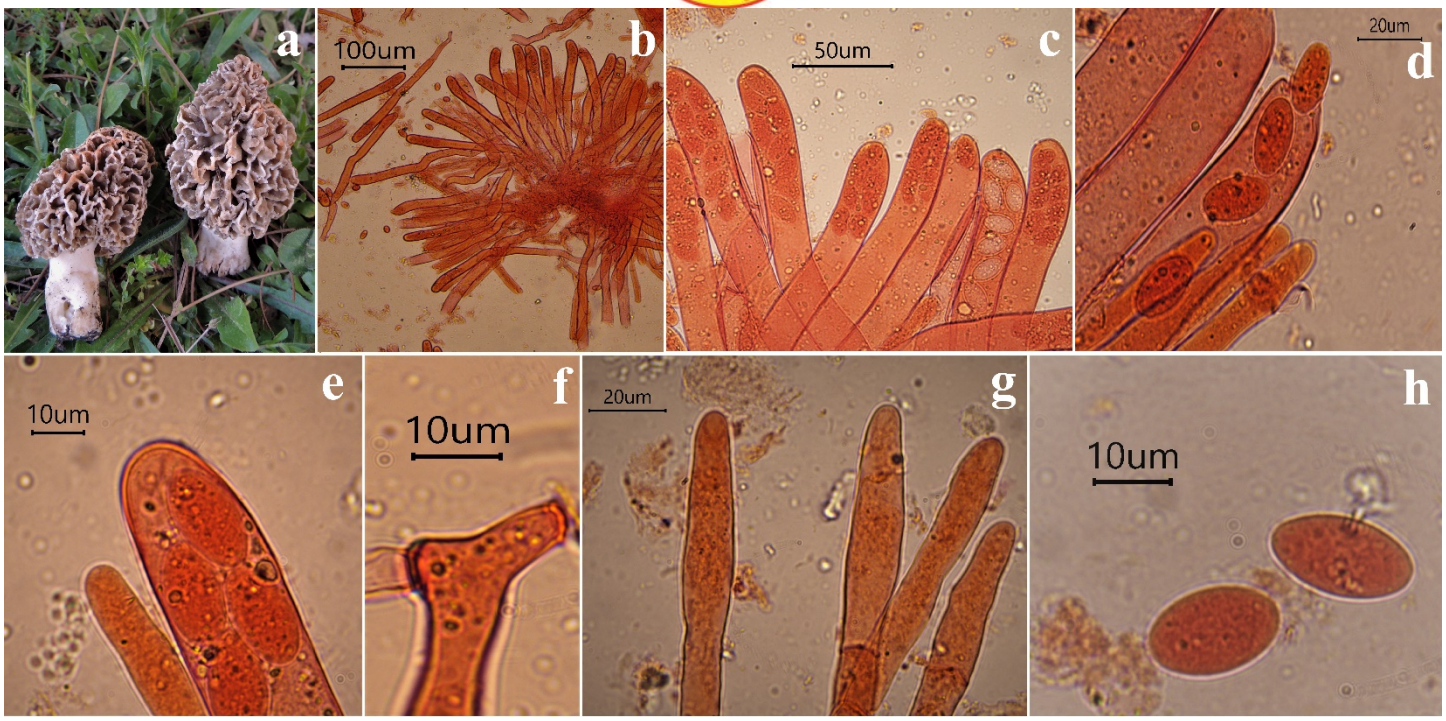
Şekil 1. Kimura 2-parametre modeline dayanan Maximum Likelihood (ML) analizi ile çizilen *Morchella* cinsine ait bazı türler için ITS ve LSU rDNA gen bölgelerini içeren filogenetik ağaç.



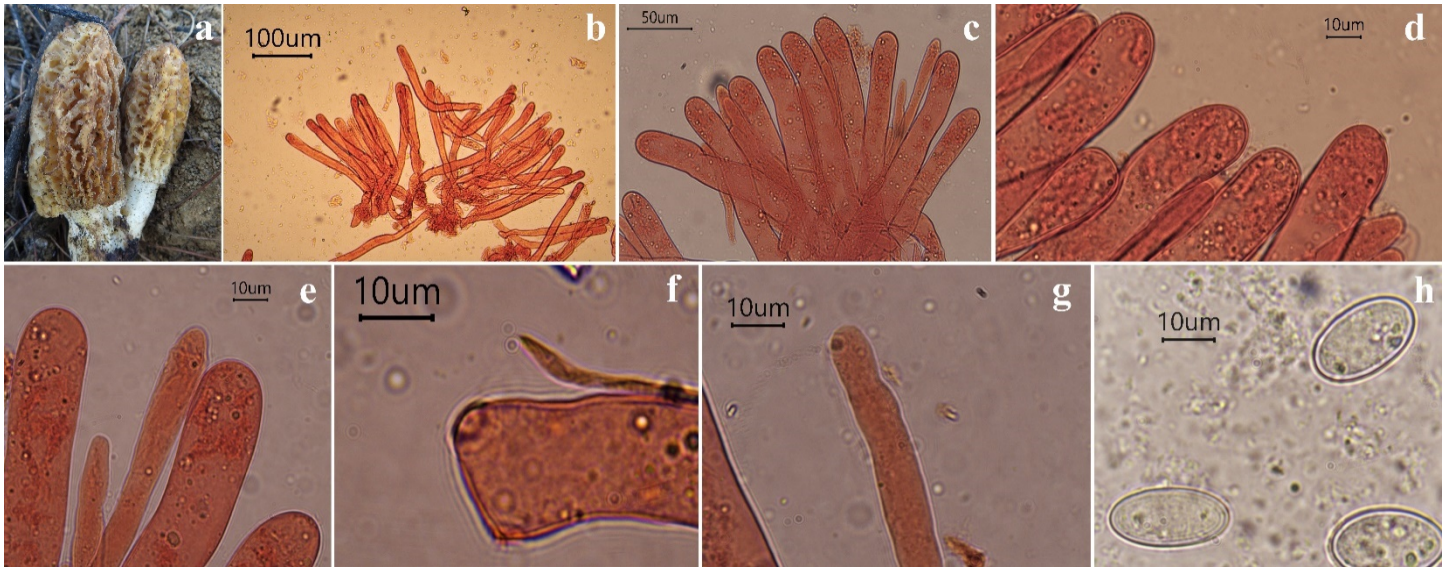
Şekil 2. *Morchella deliciosa*: **a.** askokarp, **b.** askuslar, **c-d.** askusun bir bölümü, **e.** askus tabanı, **f-g.** parafizlerin uç kısmı, **h.** sporlar.



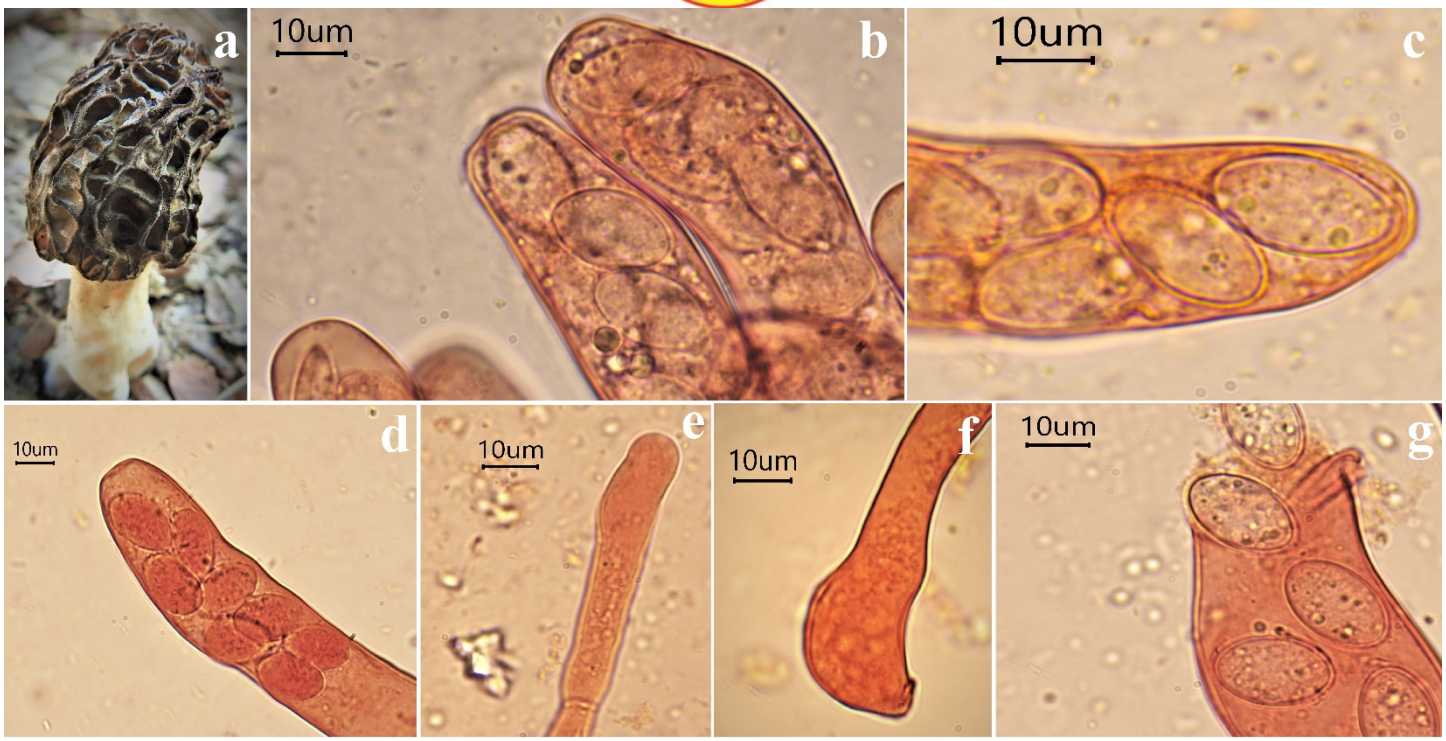
Şekil 3. *Morchella dunalii*: **a.** askokarp, **b-e.** askuslar ve parafizler, **f.** parafizlerin uç kısmı, **g.** akroparafiz, **h.** askus tabanı, **i.** spor.



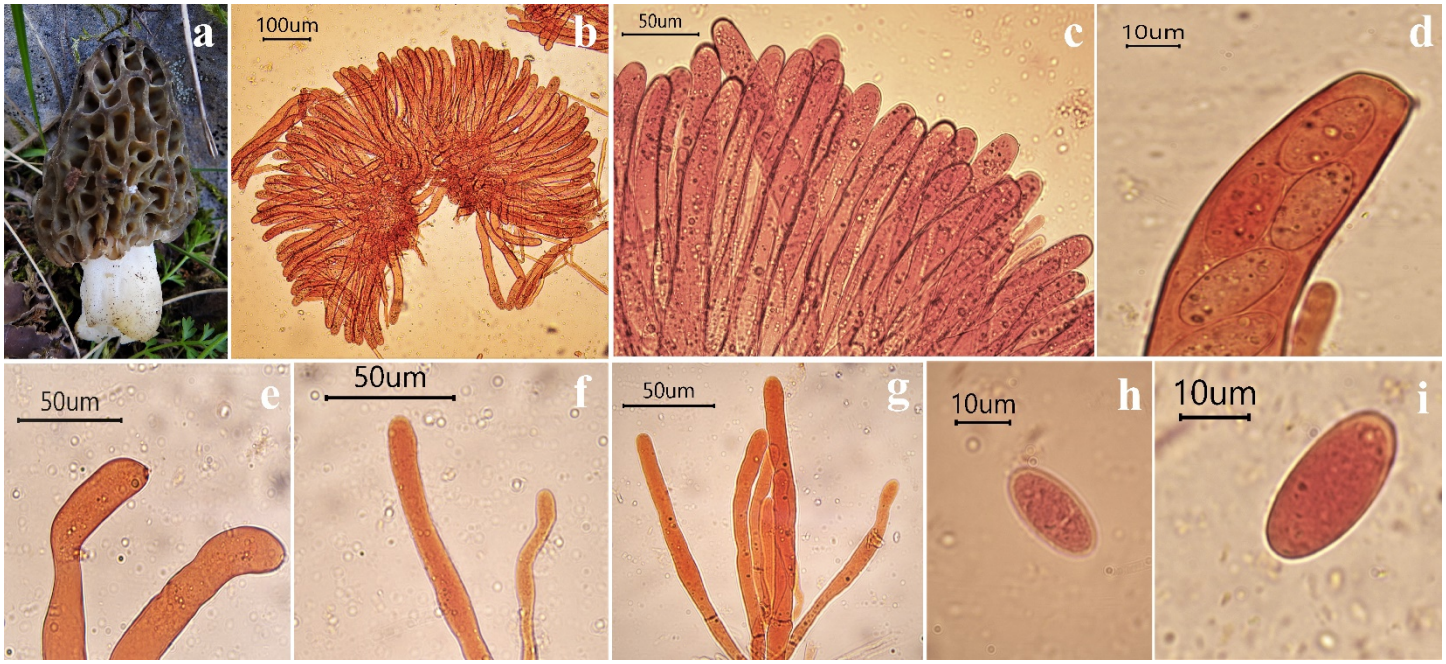
Şekil 4. *Morchella dunensis*: **a.** askokarp, **b-e**. askuslar ve parafizler, **f.** askus tabanı, **g.** parafizlerin uç kısmı, **h.** sporlar.



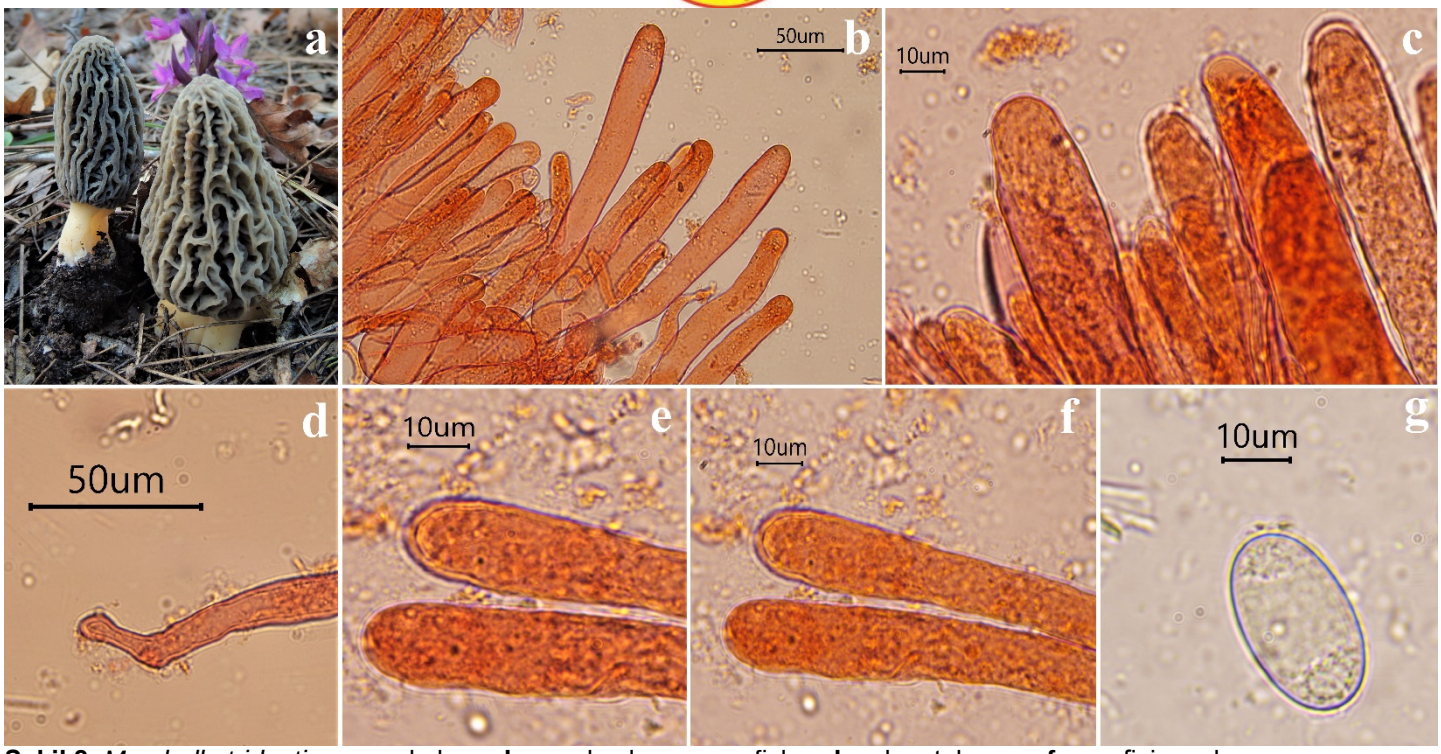
Şekil 5. *Morchella eximia*: **a.** askokarp, **b-e.** askuslar ve parafizler, **f.** askus tabanı, **g.** parafizin uç kısmı, **h.** sporlar.



Şekil 6. *Morchella importuna*: **a.** askokarp, **b-d.** askuslar, **e.** parafizin uç kısmı, **f.** askus tabanı, **g.** askus içinde sporlar



Şekil 7. *Morchella mediterraneensis*: **a.** askokarp, **b-c.** askuslar ve parafizler, **d.** askusun bir bölümü, **e.** askus tabanı **f-g.** parafizin uç kısmı, **h-i.** spor



Şekil 8. *Morchella tridentina*: **a.** askokarp, **b-c.** askuslar ve parafizler, **d.** askus tabanı, e-**f.** parafizin uç kısmı, **g.** spor

Tablo 2. ITS rDNA+28S rDNA gen bölgeleri DNA dizi analizlerine göre filogenetik ağaç için seçilen örnekler ve aynı gruba giren diğer örnekler

Seçilen koleksiyon numaraları	Gruba ait diğer örnekler
HT690 (90)	HT682 (17), HT683 (42), HT685 (22), HT687 (105), HT690 (90), HT694 (3), HT697 (86), HT707 (56)
HT677 (80)	HT559 (10), HT562 (52), HT567 (104), HT587 (14), HT677 (80)
HT554 (44)	HT554 (44)
HT630 (94)	HT630 (94), HT631 (35), HT633 (81), HT715 (103)
HT628 (84), HT669 (98), HT670 (25)	HT560 (15), HT628 (84), HT629 (110), HT640 (76), HT636 (75), HT637 (71), HT641 (41), HT643 (39), HT644 (107), HT647 (96), HT648 (73), HT650 (49), HT651 (106), HT653 (95), HT654 (72), HT655 (47), HT668 (37), HT669 (98), HT670 (25), HT671 (55), HT672 (51), HT678 (74), HT679 (93), HT680 (40)
HT693 (4), HT698 (83)	HT605 (30), HT688 (24), HT689 (5), HT693 (4), HT698 (83),
HT593 (66)	HT593 (66), HT601 (65), HT691 (63)

Tablo 3. ITS rDNA + 28S rDNA gen bölgeleri sonuçlarına göre tür tahminleri

Kolleksiyon Numaraları	Türler	GenBank Numaraları	Benzerlik	Referans
HT690 (90)	*M. deliciosa*	KM588006	%100	Richard ve ark. (2015)
HT677 (80)	*M. dunalii*	JN085137	%100	Taşkın ve ark. (2012)
HT554 (44)	*M. dunensis*	JQ723102 KU865007	%99.82 %99.71	Du ve ark. (2012b) Loizides ve ark. (2016)

HT630 (94)	*M. eximia*	KM587980	%99.86	Richard ve ark. (2015)
HT628 (84)	*M. importuna*	KM485958 JQ618537	%99.31 %100	Yayınlanmamış Du ve ark. (2012b)
HT669 (98)	*M. importuna*	KM485958 JQ618537	%99.31 %99.86	Yayınlanmamış Du ve ark. (2012b)
HT670 (25)	*M. importuna*	KM485958 JQ618537	%99.31 %99.85	Yayınlanmamış Du ve ark. (2012b)
HT693 (4)	*M. mediterraneensis*	JN085126	%100	Taşkın ve ark. (2012)
HT698 (83)	*M. mediterraneensis*	JN085126	%100	Taşkın ve ark. (2012)
HT593 (66)	*M. tridentina*	KM587964	%99.88	Richard ve ark. (2015)

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Phylloscypha boltonii, A New Record for the Mycobiota of Turkey

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Abstract: *Phylloscypha boltonii* is reported for the first time for the mycobiota of Turkey. This species is the second member of the genus *Phylloscypha* Van Vooren in Turkey. A brief description of the taxon is given together with the photographs related to its macro and micromorphologies.

Key words: *Ascomycota,* Biodiversity, Mersin, *Pezizales,* Taxonomy

Phylloscypha boltonii, Türkiye Mikobiyotası İçin Yeni Bir Kayıt

Öz: *Phylloscypha boltonii* Türkiye mikobiyotası için ilk kez rapor edilmiştir. Bu tür *Phylloscypha* Van Vooren cinsinin Türkiye'deki ikinci üyesidir. Taksonun kısa bir betimlemesi, makro ve mikromorfolojilerine ait fotoğrafları ile birlikte verilmiştir.

Anahtar kelimeler: *Ascomycota,* Biyoçeşitlilik, Mersin, *Pezizales,* Taksonomi

Introduction

Phylloscypha Van Vooren is an ascomycete genus within the family *Pezizaceae*. It is a newly erected genus and proposed by Van Vooren (2020). Based on both morphological characters and molecular data obtained from databases, he transferred five *Peziza* Dill. Ex Fr. species with new names *Phylloscypha boltonii* (Quél.) Van Vooren & Hairaud, *P. coquandii* (Donadini) Van Vooren, *P. labessiana* (Boud.) Van Vooren, *P. phyllogena* (Cooke) Van Vooren and *P. retrocurvatoides* (Van Vooren) Van Vooren) with the type species *P. phyllogena* (Van Vooren, 2020; Index fungorum, accessed 28 August 2020). Members of the genus are characterized by epigeous, sessile, cupulate ascomata witih a distinctly furfuraceous or pustulate external surface; a purplish-coloured flesh without latex; an operculate, 8-spored asci with wall diffusely bluing in iodine solution; hyaline paraphyses; eguttulate spores with small polar granules and warty ornamentation.

The checklists (Sesli and Denchev, 2014; Solak et al., 2015) don't contain any member of the genus *Phylloscypha.* But three papers (Acar et al., 2015; Türkekul and Işık, 2016; Sadullahoğlu and Uzun, 2020) reported the existance of *P. phyllogena* in Turkey. According to the current checklists (Sesli and Denchev, 2014; Solak et al., 2015) on Turkish macromycota and the later contributions (Kaşık et al., 2017; Akçay et al., 2018; Işık and Türkekul, 2018; Uzun et al., 2018, 2020; Acar et al., 2019; Çağli et al., 2019; Keleş, 2019; Sesli, 2019; Yakar et al., 2019; Yıldız et al., 2019), *P. boltonii* hasn't been reported from Turkey before.

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

Material and Method

Fruit bodies of *Phylloscypha boltonii* were collected in 2019 during a routine field trip in Silifke district of Mersin Province. First they were photographed at their natural habitats, and notes were taken related to their ecology, morphology and geographic position etc. The collected fruit bodies were put in paper boxes and transferred to the fungarium. The samples were dried in an air conditioned room. Microscopic investigations were carried out on dry samples. A Nikon Eclipse Ci-S trinocular microscope was

used for microscopic investigation and a DS-Fi2 digital camera was used to obtain microstructural photographs. The samples were identified according Seaver (1942); Hohmeyer (1986); Lantieri (2004, 2005); Medardi (2006) and Pancorbo and Ribes (2010). The specimens are kept at Karamanoğlu Mehmetbey University, Kâmil Özdağ Science Faculty, Department of Biology.

Results

Ascomycota Caval-Sm

Pezizomycetes O.E.Erikss. & Winka

Pezizales J.Schröt.

Pezizaceae Dumort.

Phylloscypha boltonii (Quél.) Van Vooren & Hairaud

Syn: [*Aleuria boltoni* (Quél.) Gillet; *Galactinia boltonii* (Quél.) Boud.; *Peziza boltonii* Quél.]

Macroscopic and microscopic features: Apothecia 20-40 mm in diameter, sessile, cup to saucer shaped, edges irregular, wavy and generally inrolled, hymenial surface smooth, dark purplish to dark violet when young, more brown when at maturity, outer surface concolorous with the hymenial surface or lighter brown, completely covered with small violet-black furfurations (Fig. 1). Flesh brittle, dark purple.

Asci 245-275 × 15-18 µm, cylindrical, operculate, amyloid, uniseriate, eight-spored (Fig. 2a-e). Paraphyses cylindrical, multi-septate (Fig. 2b,c), some slightly thickened at the apex. Ascospores 15-18 × 7.5-9 µm, ellipsoidal, hyaline, biguttulated to without guttules, ornamented with small warts (Fig. 2d-f).

Phylloscypha boltonii was reported to grow as solitary or gregariously on sandy soil, dune with *Pinus* spp., broadleaved shrubs (Lantieri, 2004; Medardi, 2006; Pancorbo and Ribes, 2010), rarely on mosses and burnt soil (Dougoud 2001; Lantieri et al., 2009).

Specimen examined: Mersin, Silifke, Kargıcak Village, Göksu river bank, on sand and sandy soil, 36°26′N-33°38′E, 110 m, 29.10.2019, D.Kap.252; 09.11.2019, D.Kap.296.

Figure 1. Ascocarps of *Phylloscypha boltonii* on sand and sandy soil

Discussions

Phylloscypha boltonii is reported for the first time for the mycobiota of Turkey. Macroscopic and microscopic characteristics of Turkish collection are in accordance with those given in literature (Seaver, 1942; Hohmeyer, 1986; Lantieri, 2004, 2005; Medardi, 2006; Pancorbo and Ribes, 2010).

Phylloscypha boltonii is generally linked with sandy soils but may also grow on burnt places. Though it has some very marked macroscopic characteristics, it is possible to confuse *P. boltonii* with numerous macroscopically similar *Peziza* species like *Pz. gerardii* Cooke, *Pz. lividula* W. Phillips, *Pz. lobulata* (Velen.), *Pz. moseri* Svrcek Aviz.-Hersh. & Nemlich, *Pz. pseudoampelina* Donadini, *Pz. tenacella* W. Phillips and *Pz. violacea* Pers. But the microscopic characters of all these taxa differentiate them from *P. boltonii.* Among them *Pz. gerardii* and *Pz. lividula* has fusiform ascospores while *Pz. lobulata* and *Pz. moseri* have smooth ascospores. Like *P. boltonii, Pz. pseudoampelina* and *Pz. tenacella* also have ornamented ascospores but the prior species has quite larger ascospores (20-25 x 9.5-12 µm), and the latter one has smaller ascospores (11-14 x 6-8 µm) compared to that of *P. boltonii.* On the other hand *Pz. violacea* has smaller and finely warty ascospores. Violet coloration of *Pz. violacea* is also another differentiating character between this species and *P. boltonii* (Lantieri, 2005; Medardi, 2006; Pancorbo and Ribes, 2010).

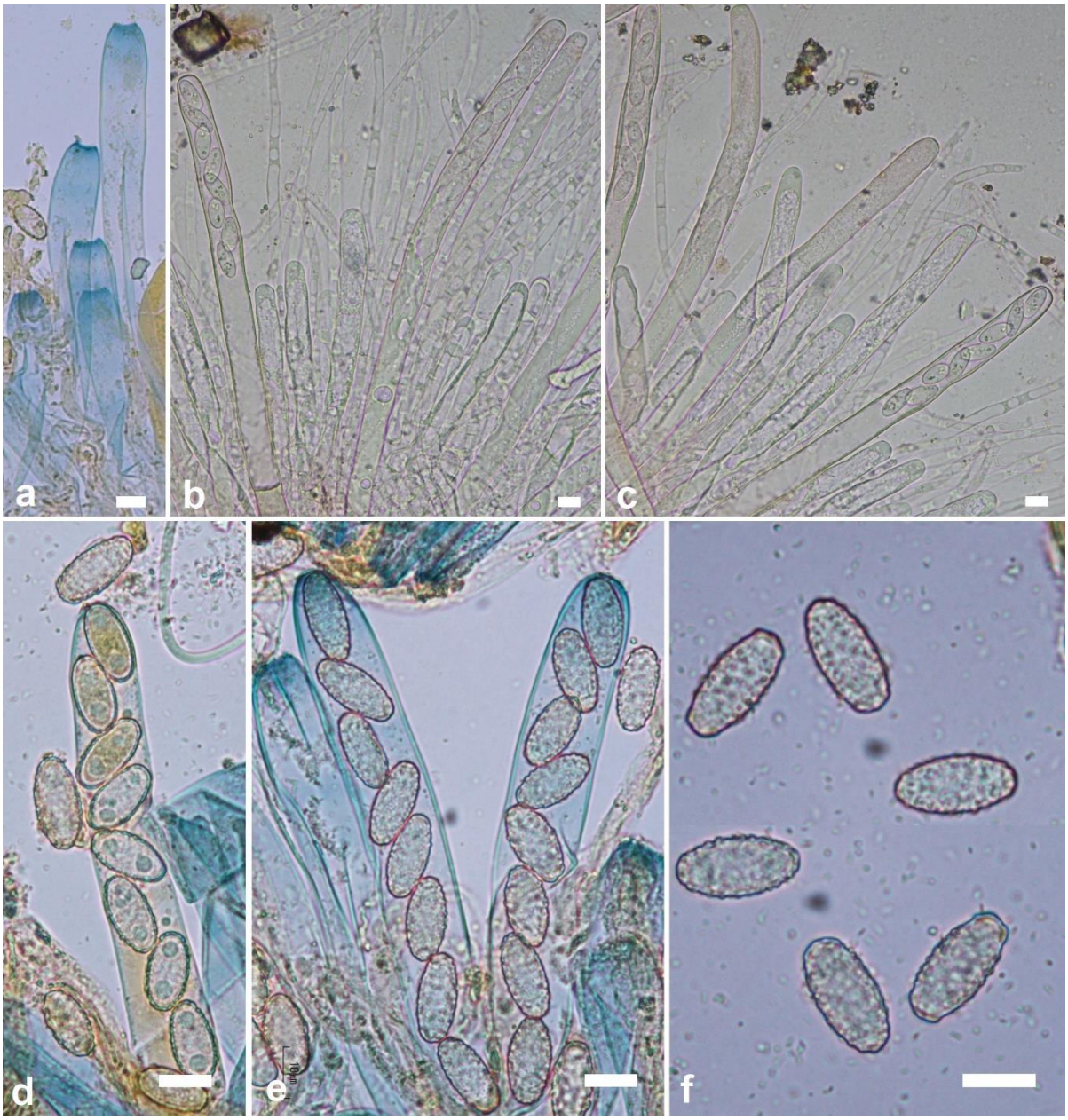

Figure 2. Asci (a-e), paraphyses (b,c) and ascospores (d-e) of *Phylloscypha boltonii* (bars: 10 µm; a,d,e,f in Melzer)

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Pleurotus ostreatus (Jacq.) P. Kumm. Extract Alters the Expression of Some Apoptosis Related Genes

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Abstract: Mushrooms have been used for food and medicinal purposes since ancient times. Especially mushrooms with therapeutic effects attract the attention of many research groups. Besides, it is thought that the active compounds derived from fungi could potentially be a valuable source of new anticancer agents. This study aims to evaluate the effects of methanolic extract of *Pleurotus ostreatus* (Jacq.) P. Kumm. on the expression levels of some genes important in the intrinsic pathway in apoptosis. For this purpose, after the mushroom samples were dried without sunlight, extracts were prepared via Soxhlet apparatus by using methanol. Cytotoxic effects of the extracts were evaluated with the MTT test. Real-time PCR was performed to evaluate the expression levels of the four apoptotic genes (*Hrk*, *Bax*, *Apaf1* and *casp3*). The results of the MTT assay showed that the extracts obtained show a cytotoxic effect in a dose and time-dependent manner. Also, methanolic extracts from *P. ostreatus* were found to cause upregulation in expression levels of genes which related apoptotic cell death. In conclusion, this study shows that *P. ostreatus* has a potential therapeutic effect on colorectal cancer and is compatible with other studies of different types of cancer and cell lines. This study is a pioneering study for future studies that will continue to identify the active substances in the extract and find the molecular pathways of cell death.

Key words: cDNA, MTT, RT-PCR, Oyster mushroom, Turkey.

Pleurotus ostreatus (Jacq.) P. Kumm. Ekstraktı Bazı Apoptotik Genlerin İfadesini Değiştirir

Öz: Mantarlar, eski çağlardan beri yiyecek ve tıbbi amaçlarla kullanılmıştır. Özellikle tedavi edici etkisi olan mantarlar birçok araştırma grubunun ilgisini çekmektedir. Aynı zamanda, mantarlardan türetilen aktif bileşiklerin potansiyel olarak yeni antikanser ajanların değerli bir kaynağı olabileceği düşünülmektedir. Bu çalışmanın amacı, *Pleurotus ostreatus* (Jacq.) P. Kumm'un metanolik ekstraktının apoptozda intrinsik yolda önemli olan bazı gen bölgelerinin ekspresyon seviyeleri üzerindeki etkilerini değerlendirmektir. Bu amaçla mantar örnekleri güneş ışığı görmeden kurutulduktan sonra Soxhlet cihazı ile metanol kullanılarak ekstraktlar hazırlandı. Ekstrelerin sitotoksik etkileri MTT testi ile değerlendirildi. Gerçek zamanlı PCR ile, dört apoptotik gen bölgesinin (*Hrk*, *Bax*, *Apaf1* ve *casp3*) ekspresyon seviyelerini değerlendirildi. MTT testinin sonuçları, elde edilen ekstrelerin doza ve zamana bağlı bir şekilde sitotoksik bir etki gösterdiğini göstermiştir. Bununla birlikte *P.ostreatus*'tan elde edilen metanolik özütlerin apoptotik hücre ölümüyle ilişkili gen bölgelerinin ekspresyon seviyelerinde upregülasyona neden olduğu bulundu. Sonuç olarak, bu çalışma ile, *P. ostreatus*'un kolorektal kanser üzerinde potansiyel bir terapötik etkiye sahip olduğunu ve bu durumun farklı kanser türleri ve hücre dizileri ile ilgili diğer çalışmalarla uyumlu olduğunu göstermektedir. Bu çalışma, ekstraktaki aktif maddeleri tanımlamaya ve hücre ölümünün moleküler yollarını bulmaya devam edecek gelecekteki çalışmalar için öncü bir çalışmadır.

Anahtar kelimeler: cDNA, MTT, RT-PCR, Kavak Mantarı, Türkiye.

Introduction

Nature has been an important material and source of inspiration for medicine since ancient times. Throughout evolution, it produces, among other things related to cancer treatment, a wide variety of biologically active substances with therapeutic potential (Blagodatski et al. 2018). Recently, mushrooms have come to the fore as an excellent antiinflammatory, antioxidant, antidiabetic, anticancer, antimicrobial, prebiotic and immunomodulatory resources (Barros et al. 2007; Kim et al. 2007; Sarıkurkcu et al. 2008; Synytsya et al. 2009).

Cancer is one of many types of diseases that cause death and can occur in humans regardless of age group, gender or race. Current anticancer drugs on the market highlight the urgent need for new, effective and less toxic therapeutic approaches, leading to various side effects and complications in the clinical management of various types of cancer. In this context, the fungal treatment of cancer is a hopeful scientific area dealing with antitumor agents produced from fungi and has been a complementary part of traditional medicine since ancient times (Xu et al. 2012). *P. ostreatus* is one of the medicinal mushrooms and has various benefits such as antioxidant, anticancer, blood pressure lowering and cholesterol (Wasser, 2002; Lindequist et al. 2005; Fan et al. 2006). Various studies have demonstrated the antiproliferative and proapoptotic effects of *P. ostreatus* on leukaemia, breast, cervical, colon and prostate cancer cell lines (Lavi et al. 2006; Gu &Sivam, 2006; Polyakov et al. 2007; Jedinak and Sliva, 2008; Ekowati et al. 2017).

Apoptosis is programmed cell death and the applied extract or active substance is desired/expected to direct the cancer cells to programmed cell death. This process is controlled by intracellular and extracellular pathways. The Bcl-2 family is the oncoprotein group, which consists of antiapoptotic and proapoptotic members and has the most important role in regulating apoptosis (Altunkaynak & Özbek 2008). Bcl-2 family consists of two groups with opposite effects. One of these groups is antiapoptotic (such as Bcl-XL and Bcl-2), the other is pro-apoptotic (such as Hrk, Bid, Bax, Apaf1, Puma and Noxa). The sensitivity of cells to apoptotic stimulation depends on the balance between pro-apoptotic and antiapoptotic Bcl-2 proteins (Burlacu, 2003). The aim of this study was to evaluate the effects of *P. ostreatus* methanolic extract on expression levels of Hrk, Bax, Apaf1 and casp3 genes, which are important in the intrinsic pathway in apoptosis, on the DLD1 cell line.

Material and Method

Material

In our study, the studied mushroom samples were provided by Dr Sinan AKTAŞ. Samples were dried without sunlight at room temperature. The dried material was pulverized and prepared for extraction process.

Preparation of extracts

P. ostreatus were extracted in absolute methanol for 6-8h via Soxhlet apparatus then the extract was filtered. The extract was concentrated using a rotary evaporator. The extracts were stored at -20 °C until use. The extract coded as POE.

Cell line and culture

DLD1 (human colorectal adenocarcinoma) cells were obtained from ATCC and maintained in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1 % (v/v) penicillin-streptomycin. Cells were incubated at 37 °C under 5 % CO_2 conditions.

Cell Viability Assay

Cell viability was determined via MTT assay. Cells were seeded into a 96-well plate and treated with *P. ostreatus* extracts (0–5 mg/ml) and then the plates were incubated for 24 -48 hours. The optical density of the plates was measured using the Elisa microplate reader at 540 nm. Each experiment was performed three times and mean values were taken into consideration.

RNA isolation and Real-Time PCR

The DLD1cells (1 × 10^6 cells/mL) were seeded in six-well plates and treated with different concentrations of *P. ostreatus* extracts (0-1mg/ml) for 24h at 37°C and 5% CO_2. Total RNA was isolated using Bio-Rad Aurum Total RNA Isolation kit and cDNA was synthesized using Bio-Rad cDNA synthesis kit. The expression levels of apoptotic genes (*Hrk*, *Bax*, *Apaf1* and *casp3*) were evaluated via Real-Time PCR. Amplifications were

performed using the Bio-Rad CFX Connect system. The data were analysed by the comparative CT method and the fold change was calculated by $2^{-\Delta\Delta Ct}$ method. To confirm the specificity of PCR products melting curve analysis was performed. Results were determined by three independent experiments and graphs were created using mean values.

Evaluation of data

For evaluation of the data, multiple comparisons were made using one-way variance analysis followed by Dunnett's test for post hoc analysis. The differences in $p < 0.05$were considered statistically significant.

Results

Cytotoxicity results

In this study, the cytotoxic activity of *P. ostreatus* methanol extracts were determined by using MTT test in DLD1 cell line exposed to 0.3125-5 mg/ml extracts in two 24- and 48-hours incubation periods. Cell survival analysis showed that *P. ostreatus* extract caused cell death of DLD1 cells in a dose and time-dependent manner. The graphics of the MTT assay were given in **Figure.1.** After 48 hours incubation, the extract showed the most cytotoxic activity at the highest concentration (5000 µg / ml) with 98% inhibition of cell growth. The IC_{50} dose for 48h was calculated as 1263 µg /ml. These results show that methanolic extracts of *P. ostreatus* have a cytotoxic effect on colon cancer cells and suppress the proliferation.

Gene expression results

The mRNA levels of four genes involved in different steps of apoptosis were studied by Real-Time PCR. Gene expression changes were given in **Table 1** and **Figure 2**. First, when the expression level of the Hrk gene located at the beginning of apoptosis was evaluated, no difference was observed at low doses compared to the control, while a 1.2 and 2-fold increase was observed at 0.5mg and 1mg / ml doses, respectively. Another gene, Bax, was evaluated, a concentration-dependent increase in Bax gene expression was observed at all doses administered. Bax expression increased 2.41-7.54-fold in the applied dose range. Looking at the Apaf1 gene expression, it was found that the Apaf1 gene expression reached its highest value at a dose of 0.5 mg/ml and this increase was approximately 6.8-fold. Finally, when the casp3 gene expression in the last step of apoptosis was evaluated, a concentration-dependent increase was observed like in the Bax gene expression. This increase was calculated as 1.35-4.05-fold respectively. In general, we can clearly say that POE induces apoptosis-related gene expressions and the optimum concentration for gene expressions is 0.5mg / ml.

Table.1. Gene expression fold changes by $2^{-\Delta\Delta Ct}$ calculation (st: stable)

POE extract conc.	Gene expression fold change			
	Hrk	**Bax**	**Apaf1**	**casp3**
1mg/ml	2.05	7.54	2.31	4.05
0.5mg/ml	1.2	6.38	6.81	2.5
0.25mg/ml	st	2.78	st	2.18
0.125mg/ml	st	2.41	st	1.35

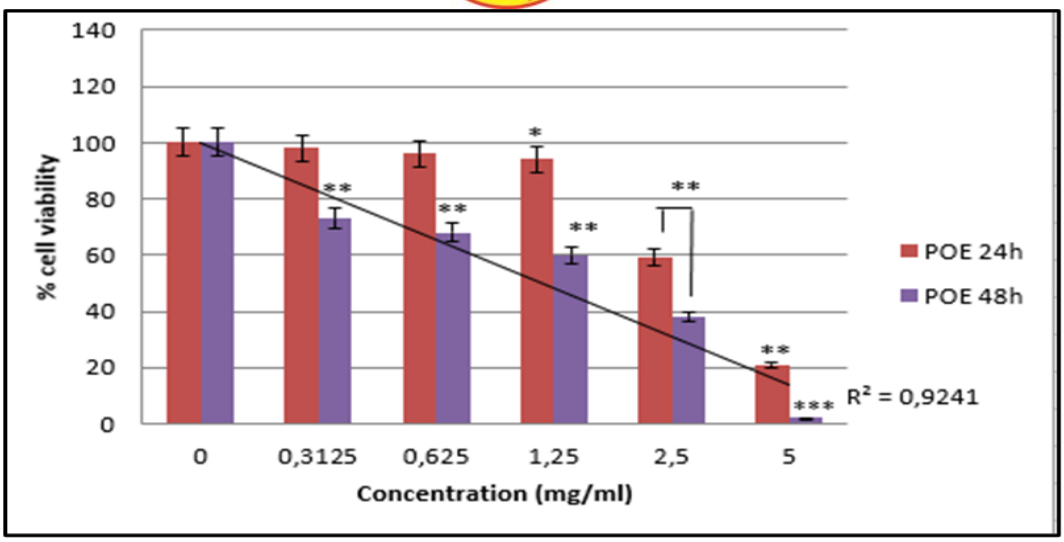

Figure 1. The viability plot based on MTT assay results (*p <0.05, **p <0.01 and ***p <0.001)

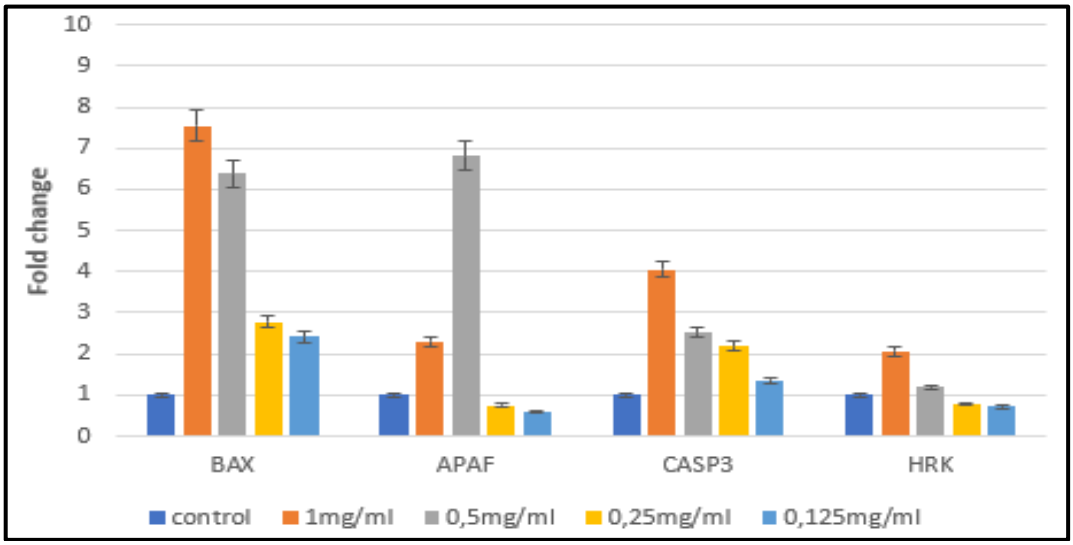

Figure 2. Relative expression levels of apoptosis-related genes

Discussion

Triggering apoptosis in cancer cells is a valuable therapy and it has been recognized that natural substances derived from various sources can induce apoptosis in various tumour cells.In this context, mushrooms play a very important role as antitumour agents. Edible mushrooms have been used for several centuries as healthy nutritional supplements and as a complementary therapy in chemotherapy and radiation therapy against cancer side effects (Mizuno et al. 1995). This study aims to determine the effects of methanolic extract of *P. ostreatus* on the expression levels of some genes that are important in the intrinsic pathway in apoptosis. POE significantly induces the expression of the four apoptotic genes in a concentration-dependent manner. Our results show that the treatment of cancer cells with POE leads to changes in the expression pattern of genes associated with apoptosis.

Expression changes in pro-apoptotic Bcl-2 members and mitochondrial status of tumour cells are an important indicator of their response (Czabotar et al. 2014; Montero et al. 2015). Hrk is an important component in the regulation of apoptosis in tumor cells, contains BH3 and also regulates apoptosis by interfering with antiapoptotic Bcl-xL and Bcl-2 proteins and blocking their functions (Inohara et al.1997). In this study, POE upregulated Hrk expressions compared to untreated cells. This may indicate that Bcl-2 gene expression is suppressed. It is

desirable that the Bax / Bcl-2 ratio, which is the key to apoptosis, shifts to Bax. Bax is a pro-apoptotic gene homologous to Bcl-2 (Oltvai et al. 1993). However, Bax works as an apoptosis enhancer unlike Bcl-2, which has antiapoptotic properties. In our study, Bax expression levels increased depending on the concentration, indicating that apoptosis was triggered in the intrinsic pathway and correlated with Hrk gene expression. In previous studies, it has been reported that extracts obtained from *P. ostreatus* using different solvents increase Bax expression, but the cell lines used (HT-29, COLO-205 and KG-1) and the preparation method of the extracts are different, our results are consistent with these studies in terms of triggering Bax expression (Lavi et al. 2006; Arora &Tandon 2015; Ebrahimi et al. 2018). In the mitochondrial pathway of apoptosis, the main soluble receptor is Apoptotic protease-activating factor 1 (Apaf1) (Fulda& Debatin 2006). Apaf1 is the central component of the apoptosome, which activates procaspase-9 following cytochrome c release from mitochondria in the intrinsic pathway of apoptosis (Gortat et al 2015). In our study, Apaf1 expression was upregulated, indicating that the apoptotic process is irreversible. The last gene caspase3 is a ruling caspase that is activated by the promoter caspases and impairs the survival and integrity of proteins. Death signals lead to the establishment of the Bcl-2 family of apoptosis proteins, particularly Bax. These proteins cause cytochrome c to be released out of the mitochondria. The release of mitochondrial cytochrome c into the cytoplasm and its subsequent association with the Apaf-1 protein is thought to be an absolute requirement for the activation of caspase-9, the apical caspase in the mitochondrial pathway of apoptosis (Nagata 2000; Spanos et al. 2002). According to the results of this study, the caspase3 expression up-regulated concentration-dependent manner. This suggests that POE reduces mitochondrial membrane potential and increases caspase-3 gene expression. As a general conclusion, when analysing the results of RT-PCR, it was found that the methanolic extracts from *P.ostreatus* caused an increase in the expression levels of the genethat regulate apoptosis at four different apoptotic stages. Although this study reveals differences at the gene level, future studies at protein level are needed. Many clinical studies are confirming the efficacy of fungi and their extracts from fungi as components of modern anticancer therapy. However, the complex mechanisms of action and molecular pathways and the precise structures of the active ingredients obtained from these fungi still need to be studied in more detail. As a conclusion, although strong progress has been made in the field of medicinal mushroom research in anticancer drug development, more research is needed in this area. Our studies will focus on elucidating the molecular targets of medicinal mushrooms, gene expression as well as changes in protein level and determination of active substance or substances.

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Türkiye Mikotası İçin Yeni Bir Cins (*Gerronema* Singer) Kaydı

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Öz: *Gerronema* Singer, *G. nemorale* Har. Takah. örneklerinin toplanıp teşhis edilmesine bağlı olarak Türkiye'den ilk kez rapor edilmiştir. Türü temsil eden örneklerin fotoğrafları ve kısa betimlemesi kısa bir tartışmayla birlikte verilmiştir.

Anahtar kelimeler: Biyoçeşitlilik, *Basidiomycota,* Taksonomi, Rize

A New Genus (*Gerronema* Singer) Record for Turkish Mycota

Abstract: *Gerronema* Singer were reported for the first time from Turkey based on the collection and identification of *G. nemorale* Har. Takah. samples. The photographs and a brief description of the species were given with a short discussion.

Key words: Biodiversity, *Basidiomycota,* Taxonomy, Rize

Giriş

Gerronema Singer, Marasmiaceae familyasına ait bir cinstir ve ilk kez 1951 yılında yayınlanmıştır. Singer (1986) ve Latha ve ark. (2018) cinsin dünya genelinde 57 türle temsil edildiğini belirtmişlerdir. Ancak Index Fungorum veri tabanında cinse ait 59 tür isminin var olduğu görülmektedir (giriş tarihi 25 Haziran 2020). *Gerronema* cinsine ait türler saprotrofit mantarlardır (Pegler 1983) ve genel olarak küçük, omphalinoid veya clitocyboid fruktifikasyon organları, pigmentsiz veya bütünüyle pigmentli umbilikat, umbonat veya infundibuliform şapka, dekurrent veya arkuat tarzda bağlı lameller, bazı tropik türler haricinde gerçek sistidinin yokluğu, velum artığı taşımayan merkezi veya eksentrik iyi gelişmiş bir sap, pürüzsüz, ince duvarlı inamyloid sporlar, sarkodimitik trama dokuları, cutis-tipi pileipellis ile karakterizedir (Singer, 1986; Norvell ve ark., 2014; Latha ve ark., 2018). Cins üyelerinin geniş bir yayılışının olduğu bildirilmiştir (Singer, 1986; Latha ve ark., 2018).

Türkiye mikobiotasına katkıda bulunulması amaçlanan bu çalışmada *G. nemorale* ülke mikotası için yeni kayıt olarak verilmiştir.

Materyal ve Metot

Araştırma materyalini oluşturan örnekler 2015 yılında Rize ili İyidere ilçe merkezi Leylekboğazı mevkiinden toplanmıştır. Arazi çalışması esnasında toplanan örneklerin ekolojik özellikleri kaydedilip doğal ortamlarında makroskobik fotoğrafları çekilmiştir. Toplanan örnekler kâğıt kutular içinde fungaryuma taşınmıştır. Burada hava sirkülasyonu sağlanmış ortamda kurutulan örnekler polietilen torbalara konarak fungaryum materyali haline getirilmiştir. Mikroskobik incelemelerde kuru materyaller kullanılmıştır. Mikroskobik yapıların gözlem ve ölçümleri için Leica DM500 ışık mikroskobu kullanılmış, mikromorfolojiye ilişkin çekimler de bu mikroskoba monte edilen Leica ICC50 HD kamera ile yapılmıştır. Mikroskobik yapıların incelenmesi %5 KOH ortamında Kongo kırmızısı ile boyanarak yapılmıştır. Arazi ve laboratuvar çalışmaları sonucunda elde edilen veriler ilgili literatür (Takahashi, 2000; Antonin ve ark., 2008; Latha ve ark., 2018) ile karşılaştırılarak örneklerin teşhisi yapılmıştır. Örnekler Van Yüzüncü Yıl Üniversitesi'ndeki (VANF) fungaryumunda saklanmaktadır.

Bulgular

Türkiye makromikotasına yönelik kontrol listeleri (Sesli ve Denchev, 2014; Solak ve ark., 2015) ve bu listeler sonrasında yapılan çalışmalar (Uzun ve Kaya, 2018; Sesli ve ark., 2018; Akçay ve ark., 2018; Uzun et al., 2018)'ın incelenmesi sonucunda *Gerronema* cinsinin ve *G. nemorale* türünün daha önce Türkiye'den rapor edilmediği belirlenmiştir.

Marasmiaceae Roze ex Kühner

Gerronema nemorale Har. Takah.

Macroscopic and microscopic features: Şapka 7-14 mm. çapında, gençken yarım küre şeklinde yüzeyi düz, pürüzsüz, zeytinimsi kahverengi renkte, olgunlarda ise dış bükey şekilde, yüzeyi radyal fibrilli, grimsi yeşil ve en sonunda ise donuk sarı renktedir. Etli kısım ince, soluk sarı renkte, koku ve tadı belirsizdir. Lameller sapa dekurrent tarzda bağlı, kısmen geniş yapılı ve soluk sarı renklidir (Şekil 1). Sap 18-35 × 1-2 mm boyutlarında, silindirik, hemen hemen eşit yapılı fakat taban kısmı genişçe, yüzeyi düz, içi boş, soluk sarı renkte ve tabanda beyaz misel yapıları mevcuttur. Spor baskısı beyazdır. Sporlar 8.3-10.3 × 4.7-6 µm, genişçe ellipsoid, yüzeyleri düz, renksiz ve ince çeperlidir (Şekil 2c). Bazidiumlar 20-40 × 4-9 µm, silindirik ve 4 sporludur (Şekil 2a). Kleosistidyumlar 35-50 × 6-7 µm, klavat, sub-silindirik, lageniform veya fusoid yapıda ve ince çeperlidir. Pleurosistidyumlar gözlenmemiştir. Pileipellis silindirik, radyal şekilde organize, ince veya hafifçe kalın çeperli, düz veya divertikulat, 8 µm 'ye kadar genişliğe ulaşan hiflerden oluşan cutis tipindedir (Şekil 2b). Caulosistidyumlar 20–45 × 6.0–9 µm, silindirik veya klavat, bazen düzensiz şekillidir.

İncelenen Örnek: Rize, İyidere, Merkez Leylekboğazı mevkii, kuru kızılağaç (*Alnus glutinosa* (L.) Gaertn.) dalı üzer, 41°56′N-40°21′E, 45 m, 15.07.2015, AK.2992.

Şekil 1. *Gerronema nemorale*'nin bazidiyokarpları

Tartışma

Japonya, Meksika ve Kore'den rapor edilen, ve Kore ve Doğu Asya'da geniş yayılışa sahip olabileceği belirtilen (Antonin ve ark., 2008). *Gerronema nemorale* Türkiye'den ilk kez rapor edilmektedir. Tür *Gerronema* cinsinin ilgili ülkemizde tespit edilen ilk üyesidir.

Teşhis edilen türe ait özellikler genel olarak literatür (Takahashi, 2000; Antonin ve ark., 2008; Latha ve ark., 2018) verileriyle uyumludur. Literatürde verilen örnekler *Pasania edulis* Makino, *Quercus myrsinaefolia* Blume, *Ligustrum japonicum* Thunb. ve teşhişsiz yayvan yapraklı ağaç kalıntıları üzerinden (Takahashi, 2000; Antonin ve ark., 2008) rapor edilmesine karşın bizim örneğimiz kuru *A. glutinosa* dalı üzerinden toplanmıştır.

Gerronema nemorale ve *G. kuruvense* K.P.D.Latha & Manim'nin bazı benzerlikleri (şapka yapısı, lamel yapısı, bazidiyor spor büyüklüğü, pleurosistidiyum yokluğu) olmasına karşın, *G. nemorale* daha geniş şapka çapı, şapka rengi, 4-sporlu basidiyumları ve bol kleiosistidiyumlu lamel kenarları ile diğer türden ayırt edilmektedir (Latha ve ark., 2018). *Gerronema nemorale* ophalinoid fruktifasyon yapısı, düzensiz silindirik kleiosisditiyumları ve habitatı itibariyle *G. icterinum* (Singer) Singer, *G. tenue* Dennis ve *G. citrinum* (Corner) Pegler türlerine de benzerlik göstermektedir. Ancak kleiosistidiumsuz fertil lamel kenarları bu türleri G. nemarale'den ayırmaktadır. *Gerronema citrinum* ilave bazı özellikleriyle (daha geniş şapka çapı, merkeze doğru grimsi kahverengi şapka rengi ve iki sporlu bazidiyumda gelişen daha küçük bazidiyosporlar (6-7.5 x 3.5-4 µm)) *G. nemorale*'en ayrıca farklılık göstermektedir (Tkahashi, 2000).

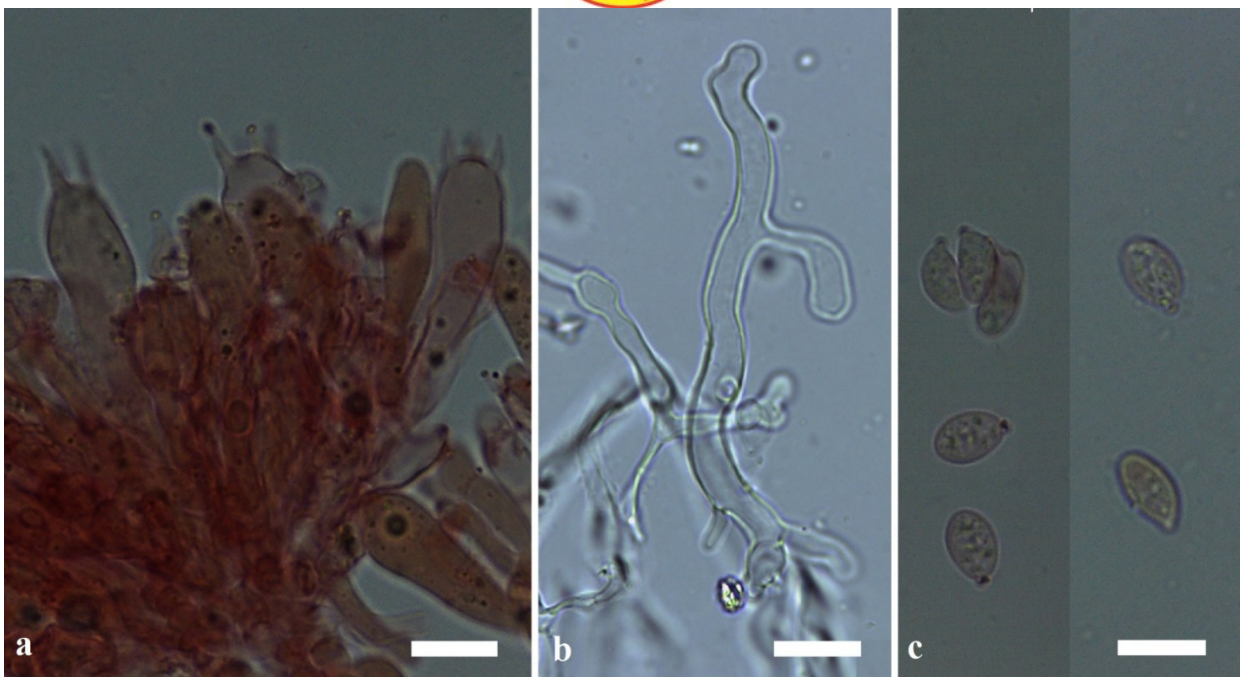

Figure 2. *Gerronema nemorale*'nin bazidiyumları (a), pleipellis yapısı (b) ve bazidiyosporları (c) (barlar: 10 µm)

Teşekkür

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Kültüre Alınmış *Pleurotus ostreatus*'un İnsan Respiratuvar Sinsityal Virüsü (HRSV)'ne Karşı İn Vitro Antiviral Etkisinin Değerlendirilmesi

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Öz: İnsan respiratuvar sinsityal virüsü (HRSV), bebekler, çocuklar, yaşlı yetişkinler ile solunum, kalp veya immun sistem yetersizliği olan her yaştan insanlarda ciddi üst ve alt solunum yolu enfeksiyonunun önde gelen nedenlerindendir. Virüsün kontrolü ve önlenmesine yönelik yeni yöntemlere olan gereksinim, HRSV'den etkilenen insanların sayısını azaltmak için son derece önemlidir. Mantarlar, yeni antiviral etki mekanizmalarına sahip ilaçların öncü bileşiklerini içerirler. Nihai hedefi anti-HRSV ilaç adaylarını tespit etmek olan bu çalışma, *Pleurotus ostreatus* (Jacq.) P. Kumm'dan elde edilen metanol, etanol ve su ekstrelerinin HRSV'ye karşı antiviral aktiviteye sahip olup olmadığını belirlemek amacıyla yapılmıştır.

Ekstrelerin HRSV'ye karşı antiviral aktivitesi kolorimetrik XTT testi ile değerlendirilmiştir. Ekstrelerin HRSV'ye karşı %50 koruma sağlayan konsantrasyonu EC_{50} olarak tanımlanmış ve CC_{50} (%50 hücresel sitotoksisite gösteren konsantrasyon)'nin EC_{50}'ye oranından da seçicilik indeksi (SI) belirlenmiştir. Ribavirin (RBV) anti-HRSV aktiviteye yönelik pozitif kontrol olarak kullanılmıştır.

Sitotoksite sonuçlarına göre, en toksik ekstre 12500 µg/mL MNTC (Maksimum non toksik konsantrasyon) ve 23811.05 CC_{50} ile metanol olup, bunu 3125 µg/mL MNTC, 5967.67 CC_{50} değerleriyle etanol ve 3125 µg/mL MNTC, 6622.28 CC_{50} değerleriyle su ekstresi izlemiştir. RBV için ise bu değerler 0.98 µg/mL MNTC ve 117.00 CC_{50} dir.

Araştırma sonucunda, HRSV'ye karşı en güçlü antiviral aktiviteyi 2910.57 µg/mL EC_{50} ve 8.18 SI değeri ile metanol ekstresi göstermiş, bunu sırasıyla, etanol (EC_{50}: 900.71 µg/mL, SI: 6.63) ve su (EC_{50}: 1654.55 µg/mL, SI: 4.00) ekstreleri takip etmiştir. RBV için bu değerler 4.19 EC_{50} ve 27.92 SI tir.

Sonuç olarak, *P. ostreatus* ekstrelerinin HRSV'ye karşı klinikte kullanılan ilaçlara karşı bir alternatif olarak geliştirilebilmesi için daha ileri çalışmalara layık olduğunu söyleyebiliriz. Bu çalışma, *P. ostreatus*'un anti-HRSV aktivitesine yönelik ilk rapordur.

Anahtar kelimeler: Antiviral aktivite, İnsan respiratuvar sinsityal virüsü, Kolorimetrik XTT testi, *Pleurotus ostreatus*.

Evaluation of in Vitro Antiviral Activity of Cultured *Pleurotus ostreatus* Against Human Respiratory Syncytial Virus (HRSV)

Abstract: Human respiratory syncytial virus (HRSV) is the leading cause of serious upper and lower respiratory infections in infants, children, elderly adults and people of all ages with respiratory, heart or immunodeficiency. The requirement for new methods of virus control and prevention is crucial to reducing the number of people affected by HRSV. Fungi contain the precursor compounds of drugs with novel mechanisms of antiviral action. This study, whose ultimate goal is to identify anti-HRSV drug candidates, was conducted to determine whether methanol, ethanol and aqueous extracts from *Pleurotus ostreatus* (Jacq.) P. Kumm having antiviral activity against HRSV. Antiviral activity of extracts against HRSV was assessed by

colorimetric XTT test. The concentration of extracts providing 50% protection against HRSV was defined as EC_{50} and the selectivity index (SI) was determined from the CC_{50} (concentration showing 50% cellular cytotoxicity) to EC_{50}. Ribavirin (RBV) was used as a positive control for anti-HRSV activity.

According to cytotoxicity results; the most toxic extract was methanol with the values of 12500 µg/mL for MNTC and 23811.05 for CC_{50}, and it was followed by ethanol (3125 µg/mL for MNTC and 5967.67 for CC_{50}) and aqueous extract (3125 µg/mL for MNTC, 6622.28 for CC_{50}). These values for RBV was 0.98 µg/mL MNTC and 117.00 CC_{50}.

As a result of the research, the strongest antiviral activity against HRSV showed methanol extract with 2910.57 µg/mL EC_{50} and 8.18 SI values, followed by ethanol (EC_{50}: 900.71 µg/mL, SI: 6.63) and water (EC_{50}: 1654.55 µg/mL, SI: 4.00) extracts, respectively.

In conclusion, we can say that *P. ostreatus* extracts are worthy of further work in fighting HRSV infection (detection of the active compound/compounds responsible for anti-HRSV activity). This study is the first report of *P. ostreatus* for anti-HRSV activity.

Key words: Antiviral activity, Human respiratory syncytial virus, Colorimetric XTT test, *Pleurotus ostreatus*.

Giriş

HRSV tüm yaşlarda görülebilen en önemli respiratuvar hastalık sebebidir. Bebeklerde ve 4 yaşın altındaki çocuklarda en sık görülen şiddetli solunum yolu infeksiyonlarının nedenidir. Yaşlılarda ve bağışıklık sistemi zayıf olan hastalarda ciddi problemlere yol açan bu virüsün daha büyük çocuklar ve yetişkinlerdeki infeksiyonu hafif seyirlidir. Bebek ve küçük çocuklarda alt solunum yolu infeksiyonlarının en yaygın sebebi olan bu virüs yüksek riskli pediatrik popülasyonda (konjenital kalp hastalığı, kronik akciğer hastalığı, prematüre bebekler, immün yetmezlik) önemli oranda mortalite ve morbiditeye yol açar. Ayrıca bebeklikte geçirilen HRSV sonraki yıllarda reaktif hava yolu hastalığı gelişimine sebep olabilir (Hammer ve ark.,1995; Rota 1999; Anonim, 2003).

Güvenli ve etkili HRSV aşısının elde edilmesinde birtakım güçlükler karşımıza çıkmaktadır. Ciddi seyirli HRSV infeksiyonları hayatın ilk 7 aylık döneminde daha sık görüldüğü için bu aşının da bebeklere hayatın ilk haftalarında uygulanması gerekmektedir. Ancak hayatın ilk dönemlerindeki bebeğin immünolojik immatüritesi ve anneden geçen nötralizan HRSV antikorlarının immünosupresif etkileri ilk ayda uygulanacak immünizasyona engeldir. Uygulanacak aşı doğal infeksiyonunkine oranla daha uzun süreli bağışıklık sağlamalıdır (Collins ve ark., 1996; Ball, 1994).

HRSV'ye karşı ilk aşı 1960'lı yıllarda geliştirilmiştir. 1960-1980 yılları arasında geliştirilen inaktif HRSV aşıları antikor oluşturmalarına karşın, sonrasında vahşi virüs enfeksiyonunu takiben ağır hastalık tabloları geçirilmesine neden olmuş ve bu durum geçici bir süreyle aşı çalışmalarını sekteye uğratmıştır. Bunun sonucunda HRSV için attenue aşı çalışmaları başlamış olup bunlar; subunit aşılar (F; füzyon proteini kullanılarak), immün aktivasyon yapan anjuvanlarla kombine subunit aşılar, canlı zayıflatılmış aşılar, genetik mühendisliği yoluyla yapılan aşılar ve polipeptit aşılardır. Ancak günümüzde henüz etkili ve rutin bir aşı yoktur (Ball, 1994, Mulholland ve ark., 1996).

Ribavirin, birçok DNA ve RNA virüsüne karşı in vitro etkili, viral çoğalmayı azaltan, sentetik bir nükleozid analoğudur. Ciddi HRSV enfeksiyonlarına karşı 1993 yılında tek lisanslı ilaç olarak kabul görse de; uzun süreli aerosol uygulama ve hastaneye yatış gerekliliği, zehirlenme potansiyeli (kemik iliği baskılanması, karsinojenisite), gebelikte teratojenik etki ve yüksek maliyet gibi nedenlerle APA tarafından rutin kullanımı önerilmemektedir. HRSV enfeksiyonları için aşının henüz geliştirilmemiş olması ve ciddi enfeksiyonlarda kullanılan tek bir antiviral ilacı ribavirinin oluşu, çocuk doktorlarında sıkıntılara sebebiyet vermektedir. Bundan kaynaklanan sorunlardan dolayı, oral veya parental olarak kolay uygulamalı özgün anti-HRSV ilaçların üretilmesi ve geliştirilmesi elzem hale gelmektedir (Ma ve ark., 2002).

Mantarlar, çok eski zamanlardan beri gıda olarak tüketilmektedir. Beslenme yönünden; düşük kalori içermesinin yanı sıra, esansiyel aminoasitler, karbonhidratlar, lifler, önemli vitaminler ve mineraller bakımından zengin bir içeriğe sahiptir. Mantarlar aynı zamanda doğu ülkelerinde yüzyıllardır ilaç olarak kullanılmaktadır. Son yıllarda yapılan bilimsel araştırmalar sonucunda ise bağışıklık sistemini güçlendirdiği ve sağlığı koruduğu ispatlanmıştır (Sevindik ve ark., 2018; 2016).

Tıbbi özellikleri için analiz edilen *Ganoderma lucidum* (Curtis) P. Karst. (Reishi), *Lentinus edodes*

(Berk.) Pegler (Shiitake), *Grifola frondosa* (Dicks.) Gray (Maitake), *Agaricus blazei* Murrill (Hime matsutake), *Cordyceps militaris* (L.) Fr. (Tırtıl mantarı), *Pleurotus ostreatus* (Jacq.) P. Kumm. (Kayın mantarı) ve *Hericium erinaceus* (Bull.) Pers., (Aslan yelesi) mantar türlerinin polisakkaritler, diyet lifi, oligosakkaritler, peptidler ve proteinler, alkoller ve fenoller, çinko, bakır, iyot, selenyum ve demir gibi mineral maddeler, vitaminler, aminoasitler başta olmak üzere birçok aktif bileşeni içerdiği tespit edilmiştir. Bu bileşenlerin bağışıklık sistemini güçlendirdiği, anti-kanserojen ve kolesterol düşürücü özelliğe sahip olduğu ve hepatite karşı koruyucu ajan olarak görev yaptıkları belirlenmiştir (Lakhanbal ve Rana, 2005).

Günümüzde de birçok mantar türü antibiyotik, antikanser, antiviral, antitümör özellikleri nedeniyle tıbbi amaçlı olarak kullanılmaktadır. Ayrıca tıbbi mantar türleri özellikle son yıllarda biyoteknolojik çalışmalarda da kullanılmaktadır (Carlile ve Watkinson, 1994; Denis, 1995).

Mantarların sentezlediği ve genellikle organizmalarına özgü olan bazı fenolik bileşikler, purinler, pirimidinler, kuinonlar, terpenoidler ve fenil propanoid türevi antogonistik maddeler antimikrobiyal etkiye neden olmaktadır. Antitümör etki gösteren en önemli maddeler ise kalvasin, volvotoksin, flammutoksin, lentinan ve porisin denilen yalnızca makro mantarlardan izole edilmiş maddelerdir. Bu bileşikler aynı zamanda antiviral özellik de göstermektedir (Benedict ve Brady, 1972; Conchran, 1978).

Bu çalışma, Türkiye'de doğal olarak yetişen ve yenen bir makromantar türü olan *P. ostreatus*'un kültür ortamında geliştirilen örneklerinin HRSV'ye karşı antiviral aktivitesini değerlendirmek için yapılmıştır. Kültür olarak yetiştirilen örneklerin antiviral etkisinin varlığı veya yokluğunun ortaya konmasıyla doğal gıda olarak tüketilen mantarların sağlık açısından koruyucu olup olmadığının ortaya çıkarılması amaçlanmıştır.

Materyal ve Metot

Çalışmanın materyali olan *P. ostreatus* Seydişehir'de piyasadan satın alınmıştır. Taze olarak satın alınan örnekler 45 °C de mantar kurutma dolabında kurutularak toz haline getirilmiştir. Toz haline getirilen örnekler metanol, etanol ve su ekstreleri için ayrı ayrı 30 gr tartılarak ultrasonikatörde 45 °C de 400 mL çözücü kullanılarak 1 saat bekletilmiş ve watman no:1 filtreden geçirilmiştir. Filtreden geçmeyen katı faz tekrar 4 kez sonikasyona tabii tutulup filtre edilmiştir. Toplam ekstreler düşük basınç altında 45 °C de Evaporatörde uçurulduktan sonra liyofilize edilerek + 4 °C de toz halinde saklanmıştır.

Sitotoksisite test

P. ostreatus bazidyokarplarından elde edilen metanol, etanol ve su ekstrelerinin yanısıra HRSV için pozitif kontrol olarak kullanılan RBV (ribavirin)'nin HEp-2 hücreleri üzerine sitotoksik etkileri, imalatcı firmanın (Biological Industries, Israel) talimatlarına uygun olarak XTT temelli hücre proliferasyon kiti ile ayrı ayrı incelenmiştir.

96 kuyucuklu mikropleytlerin 1. kolonu Vasat Kontrol (VK), 2. kolonu Hücre Kontrol (HK) olarak kullanılmıştır. VK olarak kullanılan 1. kolondaki kuyucuklara 150 µL EMEM (serumsuz) konulmuştur. 3. kolon hariç, geriye kalan 10 kolondaki (yani, 2-12) kuyucukların her birine 100'er µL EMEM (serumsuz) konulmuştur. 3. Kolona ekstrelerin çalışma solüsyonundan (75 mg/mL) 200 µL konulmuştur. log_2 tabanına göre sulandırmalar (75-0.146 mg/mL) hazırlanmıştır. 3-12 arasındaki kolonların kuyucuklarına ve HK olarak kullanılan 2. kolondaki kuyucuklara mililitresinde 1×10^5 hücre içeren HEp-2 hücre süspansiyonundan 50'şer µL konulmuştur. Ekstrelerin XTT boyası ile etkileşime girip girmediğini değerlendirmek amacıyla, 3-12 arasındaki kolonların F, G, H sıralarındaki kuyucuklara 50'şer µL EMEM (serumsuz) konulmuştur. Böylece ekstrelerin kuyucuklardaki son konsantrasyonları 50-0.098 mg/mL olmuştur.

RBV'nin HEp-2 hücreleri üzerine sitotoksik etkilerinin değerlendirilmesinde de, RBV'nin XTT boyası ile etkileşime girip girmediğinin değerlendirilmesi amacıyla pleytlerin bazı kuyucuklarının ayrılmaması dışında, aynı işlemler uygulanmıştır. Mikropleytlerin 1. kolonu Vasat Kontrol (VK), 2. kolonu Hücre Kontrol (HK) olarak kullanılmıştır. VK olarak kullanılan 1. kolondaki kuyucuğun her birine 150 µL EMEM (serumsuz) konulmuştur. 3. kolon hariç, geriye kalan 10 kolondaki kuyucukların her birine 100'er µL EMEM (serumsuz) konulmuştur. Üçüncü kolonda bulunan kuyucukların her birine RBV'nin çalışma solüsyonundan (750 µg/ml) 200 µL konulmuş ve log_2 tabanına göre sulandırmalar (750.00-1.46 µg/mL) hazırlanmıştır. 2-12 arasındaki kolonların kuyucuklarına mililitresinde 1×10^5 hücre içeren HEp-2 hücre süspansiyonundan 50'şer µL konulmuştur. Böylece, RBV'nin kuyucuklardaki son konsantrasyonları 500-0.98 µg/mL olmuştur. Mantar ekstrelerini ve RBV'ni içeren mikropleytler 3 gün süreyle % 5 CO_2'li nemli bir inkübatörde 37°C'de inkübe edildikten sonra, XTT ayıracının hazırlanan karışımından her kuyucuğa 50'şer µL konulmuştur. Mikropleytler, XTT formazan ürününün oluşması için 3 saat daha inkübe edilmiştir. Optik dansisite (OD)'ler, 490 nm bir test dalga boyu ve 630 nm

bir referans dalga boyunda, ELISA okuyucusunda (Multiskan EX, Labsystems) okutularak kuyucuklardan elde edilen OD ortalamaları kaydedilmiştir. Ekstrelerin XTT ile doğrudan kimyasal etkileşimlerinin ekarte edilmesi amacıyla, hücre içeren kuyucuklardaki farklı ekstre konsantrasyonlarının ortalama OD değerlerinden, hücre içermeyen aynı konsantrasyondaki ekstrelerin ortalama OD değerleri çıkarılmıştır. Testler üç kopya olarak yapılmış ve sonuçlar hücre kontrole göre ortalama sitotoksisite % oranı olarak gösterilmiştir. Sitotoksisite % oranını hesaplamak için, A'nın hücre kontrolün OD'sini, B'nin ekstre (veya RBV) ile muamele edilmiş hücrelerin OD'sini temsil ettiği aşağıdaki formül kullanılmıştır (Andrighetti-Fröhner ve ark., 2003):

$$\text{Sitotoksisite (\%)} = \frac{(A - B)}{A} \times 100$$

Hesaplanan sitotoksik etki yüzdeleri test edilen ekstrelerin (veya RBV'nin) ilgili konsantrasyonlarına karşı grafiğe dönüştürülmüştür. HK'ler ile karşılaştırıldığında ekstreler (veya RBV) ile muamele edilmiş hücrelerin OD'sini %50'ye kadar azaltan konsantrasyon olarak tanımlanan %50 Sitotoksik Konsantrasyon (CC_{50}) değerleri, elde edilen verilerin ışığında GraphPad Prism Version 5.03 istatistik programı yardımıyla non-linear regresyon analizi uygulanarak belirlenmiştir (Ho, 2008). HK'lerin OD'leri ile karşılaştırılarak Ekstrelerin (veya RBV'nin) MNTC (maksimum non-toksik konsantrasyon)'leri belirlenmiştir. Belirlenen bu MNTC'ler ekstrelerin ve RBV'nin antiviral aktivitesinin saptanmasında kullanılmıştır.

Antiviral test

Ribavirinin anti-HRSV aktivitesinin belirlenmesi

RBV'nin Hep-2 hücrelerine karşı belirlenen MNTC'undan (0.98 µg/mL) başlangıç alan 2 misli sulandırmaları anti-HRSV aktiviteleri yönünden Ho ve ark.'nın (2010) bildirdikleri "Cytopathic Effect (CPE) Reduction" testinin kolorimetrik XTT metoduna uyarlanması ile, 100 misli %50 doku kültürü infektif doz ($DKID_{50}$) içeren HRSV süspansiyonuna karşı kontrol edilmiştir. Pleytin kuyucuklarına (VK olarak kullanılan pleytin 1. kolonundaki kuyucuklar hariç) kuyucuk başına 100 µL hacimde (2.5×10^4 hücre/kuyucuk) ekim yapılmış ve 24 saat süreyle inkübe edilmiştir. Hücreler konfluent olduğunda, kuyucuklardaki üretme vasatı çekilerek boşaltılmıştır. HRSV stoğu, %1 FBS içeren EMEM (idame vasatı) kullanılarak 100 $DKİD_{50}$ oranında sulandırılmıştır. RBV'nin stok solüsyonundan (1000 mg/mL) daha önce belirlenen MNTC'sinden (0.98 µg/mL) konsantrasyonu 2 misli fazla olan bir sulandırma hazırlanmıştır. Pleytin 1. kolonu VK (vasat kontrol), 2. kolonu HK (hücre kontrol), 3. kolonu ise virus kontrol (VrK) olarak kullanılmıştır. VK ve HK kuyucuklarına 200'er µL idame besiyeri konulmuştur. Pleytlin virus kontrol (VrK) olarak kullanılan 3. kolonundaki kuyucuklara 100 $DKİD_{50}$ oranında sulandırılan HRSV süspansiyonundan 100'er µL ve idame besiyerinden 100'er µL eş zamanlı olarak konulmuştur. Pleytin geriye kalan kolonlarındaki (yani, 4-12. kolonlarındaki) 9 kuyucuğun her birine 100 $DKİD_{50}$ oranında sulandırılan HRSV süspansiyonundan 100'er µL konulduktan sonra üzerlerine eş zamanlı olarak RBV'nin 2 × MNTC'sinden başlangıç alan sulandırmalarından 100'er µL konulmuştur. Pleytler 37ºC'de %5 CO_2'li ortamda 3 gün süreyle inkübe edilmiştir. VrK kuyucuklarında maksimum sinsityum oluşumu gözlendikten sonra, kuyucuklardaki süpernatant boşaltılmış ve kuyucuklara 150'şer µL serumsuz EMEM konulmuştur. Daha sonra, XTT karışımdan her kuyucuğa 50'şer µL konulmuştur. XTT formazan ürününün oluşması için pleyt 3 saat daha inkübe edilmiştir. Optik dansisite (OD)'ler, 570 nm dalga boyunda bir ELISA okuyucusunda (Multiskan EX, Labsystems) okutularak 8 kuyucuktan elde edilen OD ortalamaları kaydedilmiştir. Farklı RBV konsantrasyonlarının virusa karşı koruma yüzde oranları, spektrofotometrik olarak aşağıdaki formülden hesaplanmıştır (Andrighetti-Fröhner ve ark., 2003):

Koruma %'si = [(A−B) / (C−B) x 100]

A = 8 gözdeki her bir RBV konsantrasyonu için ortalama OD

B = Virus kontrol OD'si (8 gözdeki OD değerlerinin ortalaması)

C = Hücre kontrol OD'si (8 gözdeki OD değerlerinin ortalaması)

Enfekte hücrelerin % 50'sinde koruma sağlayan RBV konsantrasyonu olarak tanımlanan EC_{50} değeri, RBV konsantrasyonlarına karşı belirlenen koruma % oranlarından yararlanılarak, GraphPad Prism Version 5.03 istatistik programı kullanılarak non-linear regresyon analiziyle belirlenmiştir. RBV'nin seçicilik indeksi (SI) ise, CC_{50}/EC_{50} oranından hesaplanmıştır.

Mantar ekstrelerinin anti-HRSV aktivite testi

P. ostreatus bazidyokarplarından hazırlanan ekstrelerin HEp-2 hücrelerine karşı belirlenen MNTC'lerinden ($MNTC_{metanol}$: 12500 µg/mL; $MNTC_{etanol}$: 3125 µg/mL; $MNTC_{su}$: 3125 µg/mL) başlangıç alan 2 misli sulandırmaları anti-HRSV aktiviteleri yönünden Ho ve ark.'nın (2010) bildirdikleri "Cytopathic Effect (CPE) Reduction" testinin kolorimetrik XTT testine uyarlanmasıyla 100 misli %50 doku kültürü infektif doz ($DKID_{50}$) içeren HRSV süspansiyonuna karşı kontrol edilmiştir. Ekstrelerin stok solüsyonundan MNTC'lerinden

2 misli konsantre sulandırmaları (2×$MNTC_{metanol}$: 25000 µg/mL; 2×$MNTC_{etanol}$: 6250 µg/mL; 2×$MNTC_{su}$: 6250 µg/mL) hazırlanmıştır. Pleytlerinin kuyucuklarına kuyucuk başına 100 µL hacimde (2.5×10^4 hücre/kuyucuk) ekim yapılmış ve 24 saat süreyle inkübe edilmiştir. Hücreler konfluent olduğunda, kuyucuklardaki besiyeri çekilerek boşaltılmıştır. 96 kuyucuklu pleytlerin 1. kolonu HK, 2. kolonu ise VrK olarak kullanılmıştır. HK kuyucuklarına 200'er µL idame besiyeri konulmuştur. Pleytlerin VK olarak kullanılan 2. kolonlarındaki kuyucukların her birine 100 $DKİD_{50}$ oranında sulandırılan HRSV süspansiyonundan 100'er µL ve idame vasatından 100'er µL eş zamanlı olarak konulmuştur. Pleytlerin geriye kalan kolonlarındaki (yani, 3-12) kuyucuklara 100 $DKİD_{50}$ oranında sulandırılan HRSV süspansiyonundan 100'er µL konulduktan sonra üzerlerine eş zamanlı olarak ekstrelerin 2 × MNTC'lerinden başlangıç alan sulandırmalarından 100'er µL konulmuş ve 37ºC'de %5 CO_2'li ortamda 3 gün süreyle inkübe edilmiştir. VrK kuyucuklarında maksimum sinsityum oluşumu gözlendikten sonra, kuyucuklardaki süpernatant boşaltılmış ve kuyucuklara 150'şer µL serumsuz EMEM konulmuştur. Daha sonra, XTT karışımından her kuyucuğa 50'şer µL konulmuş, 3 saat daha inkübe edilmiştir. Optik dansisite (OD)'ler, 570 nm dalga boyunda bir ELISA okuyucusunda (Multiskan EX, Labsystems) okutularak kuyucuklardan elde edilen OD ortalamaları kaydedilmiştir. Farklı ekstre konsantrasyonlarının virusa karşı koruma yüzde oranları, spektrofotometrik olarak aşağıdaki formül ile hesaplanmıştır (Andrighetti-Fröhner ve ark., 2003):

Koruma %'si = [(A−B) / (C−B) x 100]

A = 8 gözdeki her bir ekstrakt konsantrasyonu için ortalama OD

B = Virus kontrol OD'si (8 gözdeki OD değerlerinin ortalaması)

C = Hücre kontrol OD'si (8 gözdeki OD değerlerinin ortalaması)

Enfekte hücrelerin % 50'sinde koruma sağlayan ekstre konsantrasyonu olarak tanımlanan EC_{50} değeri, ekstre konsantrasyonlarına karşı belirlenen koruma % oranlarından yararlanılarak, GraphPad Prism Version 5.03 istatistik programı kullanılarak non-linear regresyon analiziyle belirlenmiştir. Ekstrelerin seçicilik indeksi (SI) ise, CC_{50}/EC_{50} oranından hesaplanmıştır.

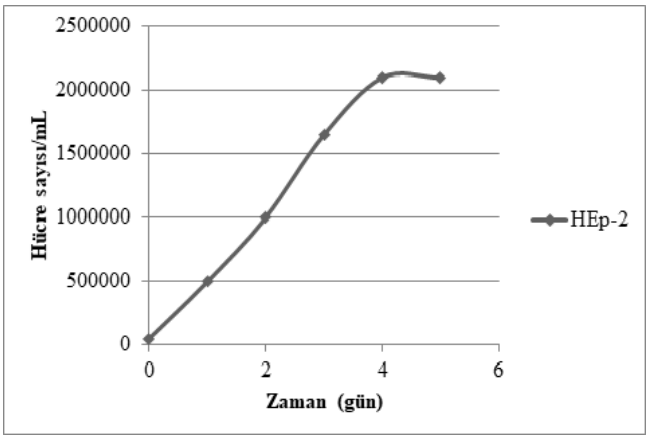
Bulgular

Hücre çoğalma eğrisi

HEp-2 hücreleri ile XTT yöntemi uygulamadan önce hücre çoğalma eğrisini çıkarmak, hücrelerin kaçıncı saatte hangi fazda olduklarını anlamak ve uygulanacak ekstrelerin (veya RBV'nin) hücre hattı ile en az kaç saat muamele edilmesi gerektiğinin anlaşılmasında önemli rol oynar. Bu amaçla ilk olarak HEp-2 hücreleri için 5 günlük çoğalma eğrisi grafiği çıkartılmış ve ikilenme zamanı "(doubling time)" elde edilmiştir. Absise zaman (gün), ordinata hücre sayıları yerleştirilerek çoğalma eğrisi oluşturulmuştur (Tablo 1, Şekil 1). HEp-2 hücrelerinin ikilenme zamanı yaklaşık olarak 2 gün (48 saat) olarak hesaplanmıştır.

Tablo.1. HEp-2 hücrelerinin zamana göre belirlenmiş hücre sayıları

Zaman (Gün)	Hücre sayısı/mL
0	50000
1	500000
2	1000000
3	1650000
4	2100000
5	2100000

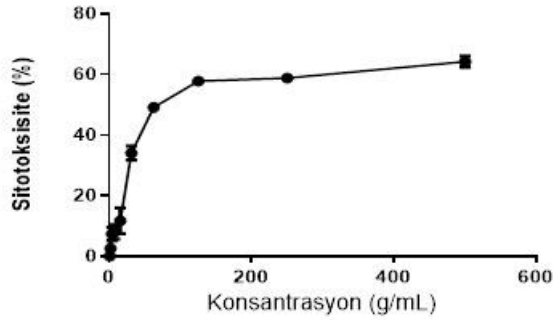


Şekil. 1. 5×10^4 hücre/mL ile başlatılan kültürlerde canlı HEp-2 hücre sayısı belirlenerek oluşturulmuş yarı-logaritmik çoğalma eğrisi (ikilenme zamanı ~2 gün = 48 saat).

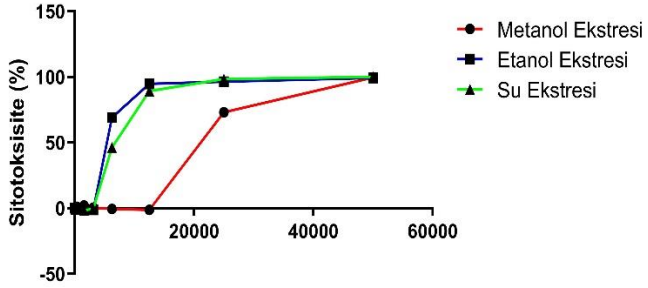
Sitotoksisite test sonuçları

Antiviral testlerin gerçekleştirilmesi için ön koşul olarak, virüs-konakçı hücrelerine (HRSV–HEp-2) karşı ekstrelerin ve HRSV'ye karşı pozitif kontrol olarak kullanılan RBV'nin sitotoksisiteleri kolorimetrik hücre canlılık testi ile araştırılmıştır. RBV'nin MNTC'si 0.98 µg/mL, CC_{50} değeri ise 117 µg/mL olarak belirlenmiştir (Şekil 2, Tablo 2). *P. ostreatus* metanol, etanol ve su

ekstrelerinin MNTC'leri sırasıyla 12500 µg/mL, 3125 µg/mL ve 3125 µg/mL dir. CC_{50} değerleri ise sırasıyla 23811.05 µg/mL, 5967.67 µg/mL ve 6622.28 µg/mL olarak belirlenmiştir (Şekil 3, Tablo 2).



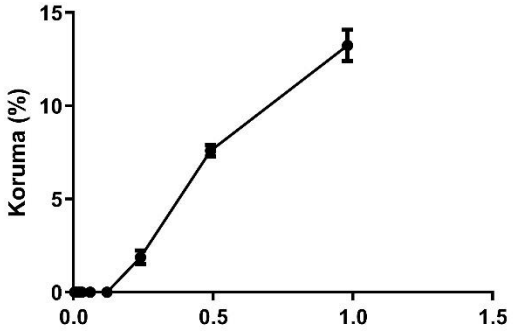
Şekil 2. RBV'nin sitotoksik aktivitesi (MNTC: 0.98 µg/mL; CC_{50} : 117 µg/mL).



Şekil 3. Hep-2 hücrelerine karşı *Pleurotus ostreatus* ekstrelerinin sitotoksisitesi. Metanol ekstresi (CC_{50} = 23811.05 µg/mL), etanol ekstresi (CC_{50} = 5967.67 µg/mL), su ekstresi (CC_{50} = 6622.28 µg/mL).

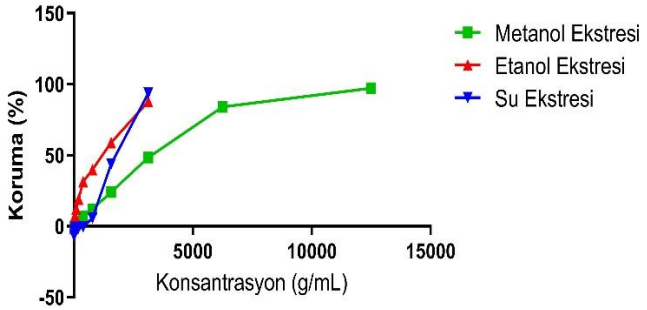
Antiviral Aktivite Test Sonuçları

HRSV inhibisyonu için pozitif kontrol olarak kullanılan RBV'nin EC_{50} (enfekte hücrelerin % 50'sinde koruma sağlayan konsantrasyon) değeri, 4.19 µg/mL olarak belirlenmiş CC_{50}'nin EC_{50}'ye oranı olarak tanımlanan seçicilik indeksi (SI) ise, 27.92 olarak belirlenmiştir (Şekil 4, Tablo 2).



Şekil 4. RBV'nin anti-RSV aktivitesi (EC_{50}: 4.19 µg/mL; SI= 27.92).

P. ostreatus metanol ekstresinın EC_{50} değeri 2910.57 µg/mL, SI değeri ise 8.18 olarak tespit edilmiştir (Şekil 5, Tablo 2). *P. ostreatus* etanol ekstresinin EC_{50} değeri 900.71 µg/mL, SI değeri ise 6.63 olarak tespit edilmiştir (Şekil 5, Tablo 2). *P. ostreatus* su ekstresinin EC_{50} değeri 1654.55 µg/ml ve SI değeri ise 4.00 olarak tespit edilmiştir (Şekil 5, Tablo 2).



Şekil 5. *Pleurotus ostreatus* ekstrelerinin anti-HRSV aktivitesi.

Tablo 2. *P. ostreatus* metanol, etanol ve su ekstrelerinin sitotoksisite ve antiviral aktivite deneyleri toplu sonuçları.

Mantar Türü	Ekstre Çeşidi	Toksisite		Anti-HRSV Aktivite	
		MNTC (µg/mL)	CC_{50} (µg/mL)	EC_{50} (µg/mL)	SI
Pleurotus ostreatus	Metanol	12500	23811.05	2910.57	8.18
	Etanol	3125	5967.67	900.71	6.63
	Su	3125	6622.28	1654.55	4.00
Ribavirin (RBV)		0.98	117.00	4.19	27.92

Tartışma

Ajanların hücreler üzerinde toksik etki sergilemesi antiviral etki göstermesi ile karıştırılabilir. Doğru bir aktiviteden bahsedebilmemiz için ajanın konak hücre sistemi üzerinde toksite etkisini belirledikten sonra antiviral aktivite deneyleri yapmak testin güvenilirliği açısından önem arz etmektedir (Dargan ve Subak Sharpe, 1986; Hu ve Hsiung, 1989). Bundan dolayı, *P. ostreatus*'un metanol, etanol ve su ekstrelerinin yanı sıra HRSV'ye karşı pozitif kontrol olarak kullanılan RBV'nin bir insan epidermal larinks karsinoma hücre hattı olan HEp-2 hücreleri üzerine sitotoksik etkileri (MNTC'leri ve CC_{50} değerleri) kolorimetrik XTT testi ile araştırılmıştır. Tablo 2'de görüldüğü gibi, ekstrelerin HEp-2 hücreleri üzerine HRSV enfeksiyonlarına karşı standart ilaç olarak kullanılan RBV'den daha çok toksik olduğu, ekstreler ve RBV'nin CC_{50} değerlerinin EC_{50} değerlerinden daha yüksek olduğu dikkat çekmektedir. Bu durum, antiviral bir ajanın güvenirliği bakımından önemlidir (Schinazi ve ark., 2009).

Sitotoksisite testlerinden sonra, ekstrelerin ve RBV'nin belirlenen MNTC'lerinden başlangıç alan sulandırmaları, anti-HRSV aktiviteleri yönünden kontrol edilmiştir. Test sonucunda, *P. ostreatus* metanol ekstresinin 2910.57 µg/mL EC_{50} değeri ve 8.18 SI değeriyle, RBV (EC_{50} = 4.19 µg/ml, SI = 27.92) ile kıyaslanabilecek oranda önemli antiviral aktiviteye sahip olduğu belirlenmiştir. Chattopadhyay ve ark. (2009), 3 ve 3'den büyük SI değerlerinin test ekstrelerinin potansiyel olarak güvenilir antiviral aktivitesinin göstergesi olarak kabul edilmesi gerektiğini bildirmişlerdir. *P. ostreatus*'un metanol, etanol ve su ekstrelerinin antiviral aktiviteye sahip olduğu belirlenmiştir.

Mantarların antiviral aktiviteleri genellikle su ekstrelerinin etkilerine bağlanmış ve sıklıkla da suda çözünür polisakkaritlerin varlığıyla ilişkili bulunmuştur (Kim ve ark., 2000; Zhang ve ark., 2004; Santoyo ve ark., 2012). *P. ostreatus* ve *P. pulmonarius* polisakkaritli su ekstreleri, herpes simpleks tip-2 virüsüne karşı aktif bulunmuştur. Mantarın su ekstrelerinin antiviral aktivitesi polisakkaritlerin varlığı ile ilişkilidir ve artan polisakkarit konsantrasyonu ile artmaktadır (Chihara ve ark., 1970). *P. tuberregium* sklerotia'dan izole edilen iki suda çözünmeyen beta-glukan, karşılık gelen suda çözünür sülfatlanmış türevleri, herpes simpleks virüsü tip 1 ve tip 2'ye karşı antiviral aktiviteler gösterir. Etki muhtemelen sülfatlanmış beta-glukanların viral partiküllere bağlanması, böylece bunların konakçı hücrelere bulaşmasını önleyerek ortaya çıkmaktadır (Zhang ve ark., 2003, 2004).

P. ostreatus'un su ve metanol ekstrelerinin antiviral özellikleri, herpes simpleks virüsü tip 1'e karşı değerlendirilmiştir. Antiviral aktivite, su ekstrelerinden daha yüksek antiviral aktivite gösteren polisakkarit fraksiyonunda bulunan beta-glukanlarla ilişkilidir (Santoyo ve ark., 2012). El Fakharany ve ark. (2010), bir lakkazın, Hepatit C virüsünün periferik kan hücrelerine ve hepatom HepG2 hücrelerine girişini inhibe edebilen ve replikasyonunu yapabilen *P. ostreatus*'tan saflaştırıldığını bildirmişlerdir. Ubikitin benzeri glikoprotein, insan immün yetmezlik virüsünün gelişimini inhibe eden *P. ostreatus*'tan izole edilmiştir (Wang ve Ng, 2000). *P. sajo-caju* ve *P. pulmonarius* sıcak su ekstreleri, insan immün yetmezlik virüsü HIV-1 ters transkriptazına karşı önleyici aktivite göstermiştir (Wang ve ark., 2007).

P. ostreatus ve *P. pulmonarius*'un su ve metanol ekstrelerinin antiviral etkisi, influenza virüsleri ile ilişkili olarak gösterilmştir. *P. pulmonarius*'un bazidyokarplarının su ekstreleri influenza virüslerine karşı yüksek aktivite göstermiştir (Kabanov ve ark., 2011).

Polisakkaritler, mantarlarda, örneğin *P. ostreatus*'ta bulunan bir çeşit makromolekül şeker polimeridir (Palacios ve ark., 2012). Antitümör, antiviral aktivite, immunomodilatasyon, antilipidemik etki ve oksidasyon koruması gibi çeşitli biyolojik özelliklere sahiptirler (Jing ve ark., 2016). *P. ostreatus*'un kimyasal bileşiminde, kuru mantarın 100 gramında 24.9 g protein, 2.08 g yağ, 61.9 g şeker, 6.32 g kül ve 1552.26 kJ enerji verdiği hesaplanmıştır (Tsai ve ark., 2009). Yapılan başka bir çalışmada mantarın şeker bileşimi arabitol, glukoz, mannitol, mannoz ve trehaloz olarak belirtilmiştir (Beluhan ve Ranogajec, 2011). *P. ostreatus*'un yağ asidi bileşimi analiz edilmiş ve çalışma sonucunda palmitik asit, palmitoleik asit, oleik asit, linoleik asit ve linolenik asit majör yağ asitleri olarak bulunmuştur (Dimau ve ark., 2002). Zhu ve ark. (2011) tarafından yapılan bir çalışmada, *P. ostreatus*'un Cu, Zn, Fe, Mn, Cd, Cr, Ni ve Pb ağır metallerini içerdiği rapor edilmiştir. Dolayısıyla çalışmamızda kullandığımız *Pleurotus ostreatus*'un metanol, etanol ve su ekstrelerinin anti-HRSV aktivitesi, metanolde, etanolde ya da suda çözünen polisakkaritlerin, yağların ya da metallerin varlığına bağlı olabilir.

Öneriler

Elde edilen sonuçlar dikkate alındığında, *P. ostreatus*'dan elde edilen metanol, etanol ve su ekstrelerinin HRSV enfeksiyonuna karşı önemli sayılabilecek antiviral aktiviteye sahip olduğu, ancak bu ekstrelerin antiviral etki mekanizması ve etkili maddelerinin henüz tespit edilmediği söylenebilir. Bu aktiviteden hangi bileşik ya da bileşiklerin sorumlu olabileceğini aynı zamanda antiviral etkiyi nasıl gösterdiklerini kanıtlamak için ilave çalışmalar gerekmektedir. Bundan dolayı ileride araştırılabilecek konuların bu araştırmadaki eksiklikleri kapatarak, *P. ostreatus*'un antiviral etkisini daha kapsamlı bir şekilde belirlenmesi sağlanabilir.

Teşekkür

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YAYIN İLKELERİ

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

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MAKALE YAZIM KURALLARI

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

(Türkçe Makaleler için);

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

(İngilizce makaleler için);

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

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

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

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

Anahtar kelimeler: 4-10 kelimeden oluşmalıdır.

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

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

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

Periyodik ise aşağıdaki örneğe uygun olarak:

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Kitap ise aşağıdaki örneğe uygun olarak:

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

Bilimsel Toplantı kitabı ise aşağıdaki örneğe uygun olarak:

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

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