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First Record of *Bolbitius reticulatus* (Agaricales: *Bolbitiaceae*) in Turkey, with Nuclear ITS and LSU rDNA Sequences Data

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Abstract: *Bolbitius reticulatus*, which is pertained to European region, is reported here for the first time from Black Sea Region, north-eastern Turkey based on both morphological characters and constructed multilocus dataset (nrITS, nrLSU rDNA). A complete macro- and micro-morphological description of the species is provided along with the photographs of fresh basidiomes in natural habitat and the line drawings of its microscopic stuctures. Comparisons of the presented species with phenotypically and molecularly related taxa are elucidated and discussed in detail.

Keywords: Basidiomycota, New record, Phylogeny, Taxonomy, Turkey

Bolbitius reticulatus'un (Agaricales: *Bolbitiaceae*) Nükleer ITS ve LSU rDNA Sekans Verileriyle Türkiye'deki İlk Kaydı

Öz: Avrupa bölgesine ait olan *Bolbitius reticulatus*, hem morfolojik karakterlere hem de yapılandırılmış çok lokuslu veri setine (nrITS, nrLSU rDNA) dayalı olarak, Türkiye'nin kuzey doğusundaki Karadeniz Bölgesi'nden ilk kez rapor edilmektedir. Türün makro ve mikro morfolojik deskripsiyonu, doğal ortamdaki taze bazidiomların fotoğrafları ve mikroskobik özelliklerinin çizimleri eşliğinde sağlanmaktadır. Sunulan türün fenotipik ve moleküler olarak ilişkili taksonlarla karşılaştırılması aydınlatılmış ve detaylı olarak tartışılmıştır.

Anahtar kelimeler: Bazidiyomikota, Yeni kayıt, Filogeni, Taksonomi, Türkiye

Introduction

Currently, *Bolbitiaceae* Singer is composed of six genera, namely *Bolbitius* Fr., *Conocybe* Fayod, *Descolea* Singer, *Galerella* Earle, *Gastrocybe* Watling, and *Pholiotina* Fayod. *Bolbitius* is a small and worldwide distributed genus in the family *Bolbitiaceae*. Above 70 species of *Bolbitius* are known to occur in the world (www.indexfungorum.org). The genus is characterized by mostly fragile basidiomata with a brightly-colored, glutinous and viscid pileus surface, free lamellae with ochraceous to rusty brown, a brown spore-print and a hymeniderm pileipellis (Singer, 1951; Arnolds, 2003, 2005; Malysheva et al., 2015). The members of the genus grow on manure, humus sawdust, rotten wood or soil (Watling, 1982; Enderle et al., 1985; Singer, 1986; Pegler, 1986; Arnolds, 2003; Hausknecht et al., 2007; Malysheva et al., 2015). Recent molecular analysis have verified the monophyletic position of *Bolbitius* and its species which form a well supported sister clade to *Pholiotina* (Moncalvo et al., 2002; Matheny et al., 2006; Tóth et al., 2013).

Recently, numerous studies have been done to contribute to the fungal diversity in Turkey (Bozok et al., 2018; Akata and Erdoğdu, 2020; Akata et al., 2020; Çağli and Öztürk, 2020; Keleş, 2020; Kocakaya et al., 2020; Sesli, 2020; Sesli et al., 2020; Şengül et al., 2020; Acar et al., 2021; Doğan, 2021; Kaplan et al., 2021; Keleş and Kaya, 2021; Sesli, 2021; Şengül et al., 2021; Uzun, 2021). The aim of this study is to determine the taxonomic position of *Bolbitius reticulatus* (Pers.: Fr.) Ricken, the first time reported from Turkey, based on both morphological and multilocus dataset, and also to compare it with phenetically similar and phylogenetically allied species.

Material and Methods

Morphology

The basidiomata were collected from the Black Sea Region, during a field survey in the Hopa district of Artvin Province, located in northeastern Turkey.

The macro-morphological characters were described based on the fresh basidiomata. Micro-characters were studied from fresh and dried material

stained in 1% Congo red or mounted in 3% potassium hydroxide (KOH). Microscopical observations were made on a Leica DM500 light microscope (Leica Microsystems, Wetzlar, Germany), after that the line drawings were made. Among the following abbreviations used: L_m and W_m indicate the average length and width of basidiospores, Q shows the ratios of length/width and Q_m presents the average quotient of the measured basidiospores or pileipellis elements (Kaygusuz et al., 2021a).

Reconstraction of the phylogeny

DNA isolation, polymerase chain reaction (PCR) amplifications and sequencing methods were carried out according to the Ref. (Kaygusuz et al., 2021b). The following primer pairs ITS1F and ITS4 were used for the amplification of nrDNA ITS region (White et al., 1990; Gardes and Bruns, 1993), while LR0R and LR5 were used to amplify the nrLSU region (Vilgalys and Hester, 1990). PCR products were purified and sequenced by Sanger DNA sequencing service (Source Bioscience, Berlin, Germany). The newly generated ITS/LSU sequences were deposited in GenBank. Multiple sequence alignment was aligned using MAFFT v7.110 (Katoh and Standley, 2013) and manually edited using BioEdit v7.0.5 (Hall, 1999). *Pholiota baeosperma* Singer was used as the outgroup taxa.

For phylogenetic analyses, Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were used. The ML analysis was executed using RAxML v8.2.10 (Stamatakis, 2014) under the GTRGAMMA model of the nucleotide substitution and bootstrap test was done with 1.000 replicates. The BI analysis was conducted with MrBayes 3.2.2 (Ronquist et al., 2012) using Markov chain Monte Carlo (MCMC) method. Four Markov chains were burn-in each for 1.000.000 generations, with sampling every 100 generations. Phylogenetic trees were exhibited in FigTree v1.4.3 (Rambaut, 2016).

Results

Molecular studies

For the *Bolbitius reticulatus* study, two nrITS and two nrLSU sequences were generated. Another 57 related sequences (30 nrITS and 27 nrLSU) were taken from GenBank and UNITE databases. The combined data matrix (nrITS and nrLSU) contained 61 sequences with 1045 nucleotide sites. The ML and BI phylogenetic results showed similar topologies and only ML phylogenetic tree with both MLB and BPP values was chosen as the backbone phylogeny (Figure 1). A phylogenetic analysis of a combined data set demonstrated that all sequences of *Bolbitius reticulatus* formed a monophyletic lineage with strong support (MLB = 80%, BPP = 0.90, Figure 1).

Taxonomy

Bolbitiaceae Singer

Bolbitius reticulatus (Pers.: Fr.) Ricken, Die Blätterpilze 1: 68 (1915) (Figure 2 and 3)

Synonyms: *Bolbitius aleuriatus* (Fr.) Singer, Lilloa 22: 490 (1951), *Bolbitius reticulatus* f. *aleuriatus* (Fr.) Enderle, Ulmer Pilzflora 4: 50 (1996), *Bolbitius reticulatus* var. *aleuriatus* (Fr.) Bon, Docums Mycol. 20(no. 78): 39 (1990), *Bolbitius reticulatus* var. *australis* (E. Horak) Garrido, Biblthca Mycol. 99: 27 (1985), *Bolbitius reticulatus* var. *reticulatus* (Pers.) Ricken, Die Blätterpilze 1: 68 (1915), *Galera reticulata* (Pers.) P. Kumm., Der Führer in die Pilzkunde: 76 (1871), *Pluteolus aleuriatus* var. *reticulatus* (Pers.) J.E. Lange, Dansk botanisk Arkiv 9 (6): 49 (1938), *Pluteolus reticulatus* (Pers.) Gillet, Hyménomycètes (Alençon): 373 (1876), *Pluteus phlebophorus* var. *reticulatus* (Pers.) Cooke: tab. 422 (1886).

Pileus 8–15 mm diam., subglobose to convex while young, then convex and finally flattened, slightly depressed at center, surface viscid to glutinous, glabrous, not hygrophanous, white pink or greyish beige to white grey, striate margin over one half of the radius in mature. Lamellae moderately crowded, free, thin, pale yellow pink or yellow brown, with flocculose whitish margin. Stipe 10–25 × 1–2 mm, delicate and very fragile, cylindrical or slightly tapering upwards, without bulb, white to pale yellowish, minutely flocculose to pruinose. Context fragile, whitish at the pileus and stipe. Neither the odor nor the taste are distinguishable.

Basidiospores (8.5–)9.6–11.8(–12.7) × (5.0–)5.5–6.5(–7.2) µm, $L_m \times W_m$ = 10.7 × 6.1 µm, Q = 1.5–2.2, Q_m = 1.8, elipsoid to oblong, golden brown or light honey brown in KOH, slightly thick-walled. Basidia (14.5–)16.0–20.5(–23.5) × (8.0–)8.5–9.0(–9.5) µm, clavate to broadly clavate, 4-spored, hyaline, thin-walled. Pleurocystidia absent. Cheilocystidia (25.0–)25.5–38.5(–47.5) × (6.5–)10.5–16.5(–17.0) µm, numerous, polymorphic, shape ranging between narrowly utriform to broadly utriform or lageniform with rounded apex, fusiform or rarely clavate, thin-walled, hyaline in KOH. Pleurocystidia absent. Pileipellis hymeniform, made up of clavate, subglobose or globose elements, 15.0–50.0 × 10.0–30.0 µm, often in chains, thin-walled, hyaline in KOH. Stipitipellis a cutis, consisting of 5.0–20.0 µm wide hyphae, smooth and thin-walled, hyaline in KOH.

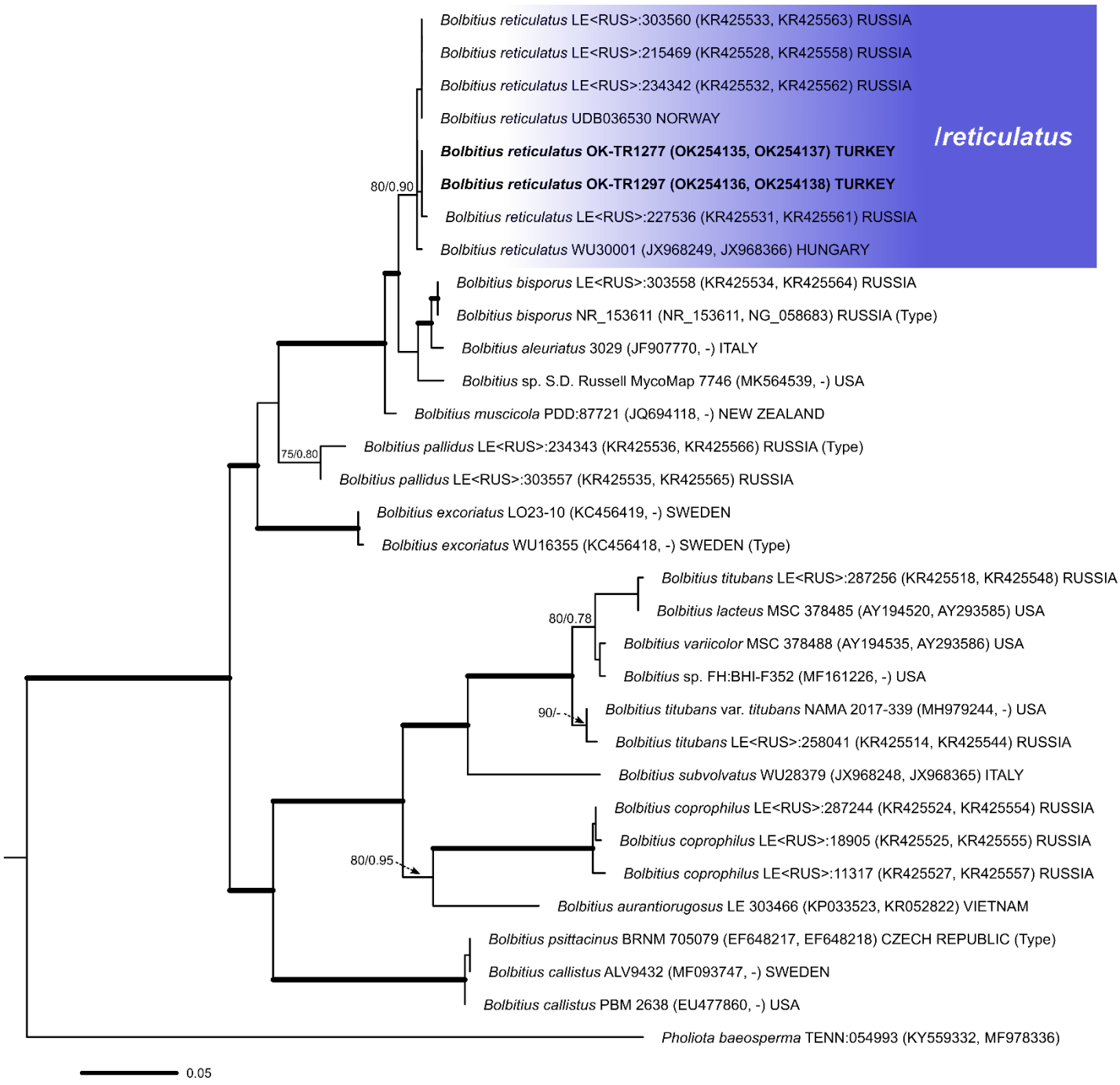

Figure 1. Phylogenetic tree of *Bolbitius* species inferred from the combined dataset (nrITS and nrLSU) using Maximum-likelihood. Maximum-likelihood bootstrap (MLB) values ≥ 75% and Bayesian posterior probabilities (BPP) ≥ 0.78 are shown on the branches. Bold branches represent MLB ≥ 90% and BPP ≥ 0.95. The generated sequences are shown in bold. Bar indicates 0.05 anticipated changes per site per branch.

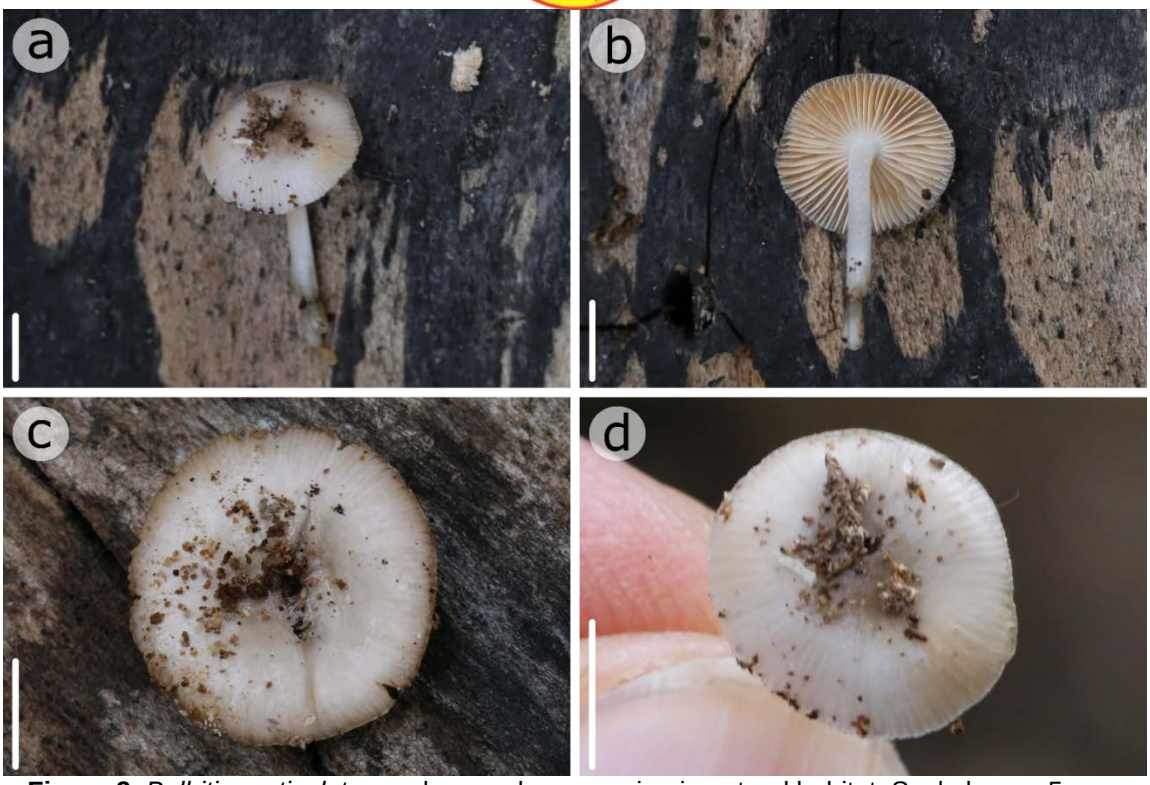

Figure 2. *Bolbitius reticulatus*: a-d carpophore growing in natural habitat. Scale bars = 5 mm.

Caulocystidia 35.0–46.5 × 10.5–25.5 µm, as variable as cheilocystidia, clavate to broadly clavate, narrowly utriform or narrowly lageniform, thin-walled, hyaline in KOH. Clamp connections absent in all parts examined.

Habit, habitat and distribution: Saprotrophic, solitary or sometimes in small groups, present at elev. 1500 m, on rotten wood of fallen branch of *Fagus orientalis* L., on mountain slope, in humid, shady and cool places, on moist, weakly acid soils, which are rich in humus.

Specimens examined: TURKEY, Artvin Province, around Borçka town, on decayed wood of *Fagus orientalis*, alt. 1500 m, 11 October 2015, O. Kaygusuz, OKA-TR1277; GenBank: OK254135 for nrITS, OK254137 for nrLSU; *ibid.,* on wood and fallen branch of *F. orientalis*, alt. 1510 m, 15 October 2015, O. Kaygusuz, OKA-TR1297; GenBank: OK254136 for nrITS, OK254138 for nrLSU.

Discussion

Bolbitius reticulatus is a widely distributed but inconspicuous woodland species. It is characterized by delicate and sticky when moist basidiocarps with greyish, greyish-purple or brownish pileus surface, free and ochre-brown lamellae, pruinose to furfuraceous stipe, and habitat on woody substrates (Arnolds, 2003, 2005; Malysheva et al., 2015).

Morphologically, *B. reticulatus* is closely related to *B. coprophilus* (Peck) Hongo, *B. demangei* (Quél.) Sacc. & D. Sacc., *B. excoriatus* Dähncke, Hauskn., Krisai, Contu & Vizzini, *B. ferrugineus* Arnolds, and *B. incarnatus* Hongo. *Bolbitius coprophilus* can be distinguished from *B. reticulatus* by a pale pink or pale orange pileus and slightly larger basidiospores size (11.5–16.5 × 8.0–11.0 µm) (Arnolds, 2003). *B. demangei* is separated by a larger basidiocarps (70 mm broad) and pale to dark violaceous grey pileus (Arnolds, 2005; Hausknecht and Contu, 2006; Malysheva et al., 2015). *B. excoriatus*, originally described from Spain, has a smaller basidiospores size (8.5–10.0 × 6.0–7.0 µm) and mostly clavate pileipellis (Hausknecht et al., 2010). *B. ferrugineus* differs from the alike *B. reticulatus* by orange-brown pileus colour, slightly larger basidiospores size (7.5–10.5 × 4.5–6.0 µm), clavate pileipellis elements, present clamp-connections and habitat on soil (Arnolds, 2003). *B. incarnatus*, initially presented from Japan, has a pink-coloured pileus, a slightly pinkish stipe and larger basidiospores size (up to 16.0 µm long) (Arnolds, 2003; Hausknecht et al., 2007; Malysheva et al., 2015).

Finally, *Bolbitius reticulatus* also can be morphologically confused with *Pluteus longistriatus* (Peck) Peck because of its small wood-rotter with a striate margin and completely free lamellae. However, viscid pileus, lamellae which are hazel, orange-brown or rusty

brown at maturity in addition to some microscopic features distinguish *B. reticulatus* from *P. longistriatus*.

As could be seen from the phylogenetic tree based on the combined nrITS/nrLSU dataset, two collections of *B. reticulatus* from Turkey and six samples from Hungary, Norway and Russia grouped together in a distinct lineage (Figure 1).

Furthermore, *B. reticulatus* formed a sister relationship with *B. bisporus* E.F. Malysheva and *B. aleuriatus* (Fr.) Singer from the European region. However, although *Bolbitius reticulatus* is closely related to *B. bisporus* and *B. aleuriatus,* it is apparently clustered on the separated branch on the phylogenetic tree.

Acknowledgement

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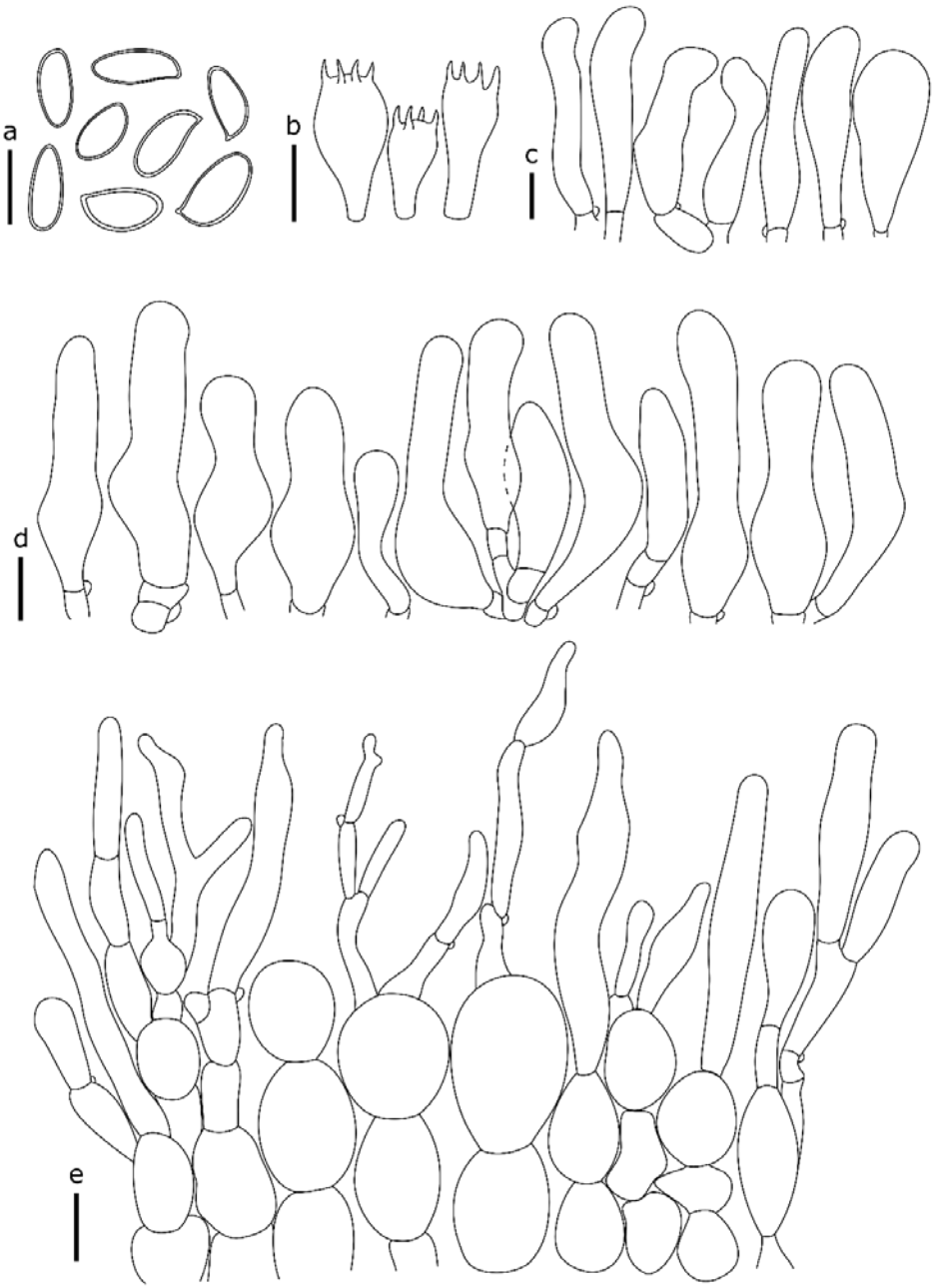

Figure 3. Microcharacters of *Bolbitius reticulatus*: a- basidiospores, b- basidia, c- caulocystidia, d- cheilocystidia, e- pileipellis elements. Scale bars = 10 µm.

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A Novel Yeast Isolated From Olive Mill Waste Candida tropicalis; Optimization of Medium Composition For Lipase Production

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Abstract: A novel microorganism that was isolated from an olive mill waste sample was screened for lipase production. This novel strain was identified and determined by 18S rDNA analysis and it was detected that the strain was 92% *Candida tropicalis* in ratio. Optimization of lipase production was carried out by the addition of nitrogen and carbohydrate sources into the lipase production medium as well as the effect of pH and temperature parameters were studied to increase the lipase production. Maximum growth conditions for the strain were detected at 4.0 pH medium and 30°C growth temperature. The effect of various nitrogen sources on lipase production showed that ammonium sulfate increased lipase production whereas urea, peptone and casein did not show a distinct effect. In addition presence of various sugars in the lipase production medium did not increase the lipase production efficiently although some oils did. The highest lipase activity was determined as 10.67 U/ml, with the addition of 1% ammonium sulfate and 1% olive oil into the production medium.

Keywords: *Candida tropicalis*, Lipase Production, Optimization

Zeytin Değirmeni Atıklarından Yeni İzole Edilen Bir Maya *Candida tropicalis*; Lipaz Üretimi İçin Besiyeri Kompozisyonun Optimizasyonu

Öz: Bu çalışmada bir zeytin değirmeni atık örneğinden yeni izole edilen bir mikroorganizma lipaz üretimi için taranmıştır. Bu yeni suş 18S rDNA analizi ile tanımlanmış ve suşun %92 oranında *Candida tropicalis* (yuvarlakmaya) olduğu tespit edilmiştir. Lipaz üretim ortamına azot ve karbonhidrat kaynakları ilave edilerek lipaz üretiminin optimizasyonu gerçekleştirilmiş, ayrıca lipaz üretimini artırmak için pH ve sıcaklık parametrelerinin etkisi incelenmiştir. Suş için maksimum büyüme koşulları 4.0 pH ortamında ve 30°C büyüme sıcaklığında tespit edilmiştir. Çeşitli azot kaynaklarının lipaz üretimi üzerindeki etkisi araştırıldığında, amonyum sülfatın lipaz üretimini arttırdığını, üre, pepton ve kazeinin ise belirgin bir etki göstermediğini saptanmıştır. Birtakım yağların artırmasına rağmen lipaz üretim ortamında çeşitli şekerlerin bulunması lipaz üretimini verimli bir şekilde artırmamıştır. En yüksek lipaz aktivitesi, üretim ortamına %1 amonyum sülfat ve %1 zeytinyağı ilavesiyle 10.67 U/ml olarak belirlenmiştir.

Anahtar kelimeler: *Candida tropicalis*, Lipaz üretimi, Optimizasyon

Introduction

According to Markets and Markets report on the industrial enzymes market, published in October 2016, the industrial enzymes market was estimated to be valued at USD 4.61 Billion in 2016, and the global industrial enzymes market is projected to reach USD 6.30 Billion by 2022 in terms of value, at a CAGR of 5.8% from 2017. This huge market size led researchers to show interest in the production of enzymes. Lipases (E.C. 3.1.1.3) are placed only after proteases and carbohydrases in the world enzyme market and share about 5% of it by the year 2006 (Vakhlu and Kour, 2006) and it still keeps it's leading place in 2017. These enzymes can be defined as carboxylesterases that catalyze the hydrolysis of long-

chain acylglycerols to glycerol, free fatty acids, and mono- and diglycerides (Aehle, 2007). Therefore they also can be named triacylglycerol acyl hydrolases. The hydrolysis of fats occurs at the water/lipid interface in aqueous media, while in non-aqueous media, the biochemical reactions driven by lipases include hydrolysis, interesterification, alcoholysis, acidolysis and esterification (Yu et al, 2016). Lipases also catalyze aminolysis in addition to the hydrolytic activity on triglycerides (Joseph et al, 2008). They hydrolyze esters preferentially at the interface between lipid and water in heterogeneous systems (Corzo and Revah, 1999). Furthermore, lipases have chemo-, region- and stereo-selective properties, which make lipases one of the most desirable enzymes for many industries (Haki and Rakshit, 2003). One of the most important classes of industrial enzymes are lipases (Babu and Rao, 2007) and they find immense applications in food, dairy, detergent and pharmaceutical industries. Novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agro-chemicals, and flavour compounds (Jaeger and Eggert, 2002)).

Lipases are by and large produced from microbes (Gupta et al, 2004) According to a report by Business Communications Company, Inc. in 2007, lipases are forecasted as the fastest growing class of enzyme (Gupta et al, 2015). Since lipases are physiologically necessary for living organisms, they are ubiquitous and can be found in diverse sources, such as plants, animals and microorganisms (Rahman et al, 2006). More abundantly, however, they are found in bacteria, fungi and yeasts (Haki and Rakshit, 2003). The major sources include microbial lipases; among these yeast and fungal lipases are of special interest because they can carry out various stereoselective reactions. These lipases are highly diverse and are categorized into three classes based on oxyanion hole: GX, GGGX and Y. The detailed phylogenetic analysis showed that the GX family is more diverse than GGGX and Y families (Gupta et al, 2015). Microbial lipases have a great potential for commercial applications due to their stability, selectivity and broad substrate specificity because many unnatural acids, alcohols or amines can be used as substrates. There are also a certain number of lipases produced by yeasts, most of them belonging to the *Candida* genus, that have been used for the biotechnological purpose (Cardenas et al, 2001).

This work was undertaken to optimize lipase production by a novel yeast, *Candida tropicalis* (Sesli et al, 2020). For this purpose soil samples were collected from olive mill wastes and various microorganisms were isolated. A yeast which was then identified as *Candida tropicalis* by 18S rDNA analysis, showed the highest lipase activity and was selected for the optimization of lipase production. The pH optima of lipase production medium as well as optimum temperature and stirring speed, were studied. However effect of different carbon and nitrogen sources was also detected to increase lipase production

Material and Method

Isolation and Identification: Soil samples were collected into sterilized plastic bags, from an olive mill in Tarsus/Mersin regio. Under sterilized conditions 1 g of soil sample was washed with 10 ml of 0.9% NaCl solution. 1 ml of the mixed solution inoculated on modified Yeast Medium Agar plates and incubated at 30°C for 72 hours. Novel yeasts were isolated by streak-plate technique and then assayed for lipolytic activity. A strain showed the highest activity was selected. Besides biochemical and morphological tests, the novel strain then was identified by 18S rDNA phylogenetic analysis. Catalase, amilase and urease activities were examined. Also nitrate reduction of the strain were tested. Colony morphology and microscopy studies were studied.

Medium and Incubation: 1 ml of novel *Candida tropicalis* liquid sample was inoculated in lipase production medium (Hatzinikolaou et al, 1996). The lipase medium composition was (g/l): 12 NaH_2PO_4, 2 KH_2PO_4, 0.3 $MgSO_4.7H_2O$, 0.25 $CaCl_2$, 0.005 $FeSO_4.7H_2O$, 0.015 $MnSO_4.7H_2O$, 0.03 $ZnSO_4.7H_2O$, 1 peptone “as initial nitrogen source and displaced by other nitrogen sources during optimization”. Medium pH was adjusted to 4.5 and after sterilization 1% olive oil (v/v) was added. Incubation was carried out at 30ºC, 100 rpm for 72 hours in a rotary incubator.

Optimization of Lipase Production: Lipase production medium, as described above, was prepared for the optimization. Primarily initial pH was adjusted between 3-9 and optimum pH range was detected. Afterwards optimum temperature range was detected between 10-40ºC. Also stirring speed of the incubator between 100-250 rpm with 50 rpm intervals was carried out.

Carbon and nitrogen sources affecting growth and lipase production in *Candida tropicalis* were studied.

Various carbohydrates (with and without 1% olive oil) were added to the production medium individually. As carbon sources, also 1% olive oil, extra virgin olive oil, sunflower oil, corn oil, soybean oil and canola oil were added to the medium separately. For detection of nitrogen sources; 1% protease peptone, peptone, yeast extract, casein, urea, ammonium oxalate, ammonium nitrate, ammonium carbonate and ammonium sulfate were added with and without 1% olive oil. The results of the experiments were determined and computed.

Preparation of Crude Lipase: After incubation the culture media was filtered by Whatman No:1 filter paper and then centrifuged at 7200 rpm for 10 minutes to obtain the cell-free supernatant (CFS). The lipase activity was carried out from the CFS. Biomass was determined by dry weight at 30°C for 48 hours and was expressed as g of cell dry weight per 100ml.

Lipase Assay: CFS was used as an enzyme source for lipase assay. 1 ml of olive oil, 1 ml of enzyme source, 4.5 ml of 50mM asetate buffer (pH 5.6), 0.5 ml of 0.1M $CaCl_2$ were strirred gently and incubated at 30°C, 200 rpm for 30 minutes. The reaction was stopped by adding 20 ml of ethyl alcohol. Lipase activity was determined by titration of the released fatty acids with 50 mM potassium hydroxide "up to final pH=10.5" (Kamzolova et al. 2005; Sugihara et al. 1991). One unit of lipase activity was defined as the amount of enzyme that catalysed the release of 1 µmol of fatty acids per minute at 30°C under assay conditions.

Results

Soil samples were collected from an olive mill in Tarsus/Mersin regio and besides bacteria four isolated yeasts were assayed for lipolytic activity. A novel yeast strain that showed the highest activity was selected for future experiments. After biochemical and morphological tests carried out, 18S rDNA phylogenetic analysis was applied and the novel strain was identified as *Candida tropicalis* in 92% ratio [Fig. 1].

For detection of the biochemical properties of *Candida tropicalis* catalase activity was determined and was found that it was catalase positive. Also biochemical tests showed that nitrate reduction of the strain was negative, it's amilase activity was negative and urease activity was positive.

Colony morphology of the strain were also determined as S type colony. The strain was dyed by methylene blue. At microscopy examination no pseudohyphae or hyphae were detected. 18 RNA analysis and identification was carried out by a private biotechnology company located in Ankara.

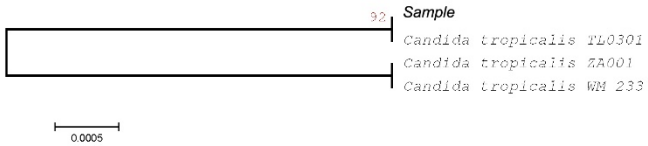

Figure 1. Identification of novel yeast strain by 18S rDNA analysis.

The strain's optimum pH range of growth was 3-7 and maximum was 4. The optimum pH range on lipase production was 3-5 and maximum were at 4 [Fig. 2].

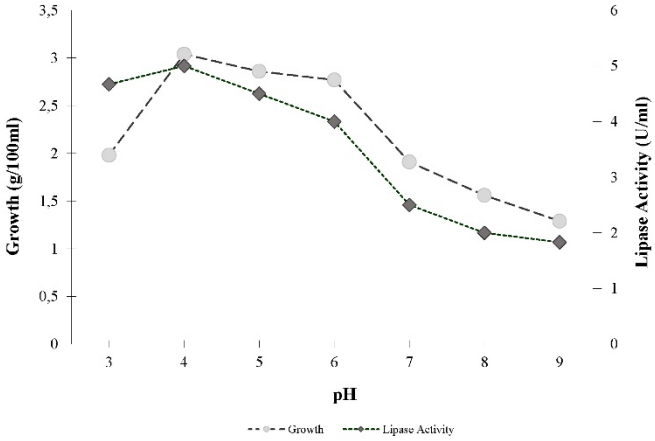

Figure 2. Effect of pH on growth and lipase production by *Candida tropicalis*.

The growth temperature was also detected and maximum growth and lipase production was determined at 30°C [Fig. 3].

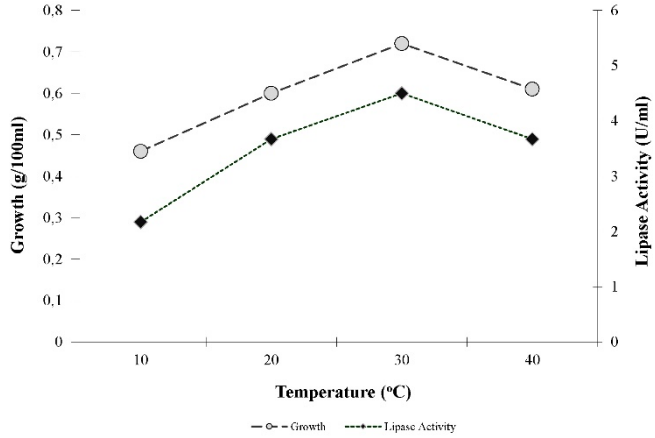

Figure 3. Effect of temperature on growth and lipase production by *Candida tropicalis*.

Stirring speeds between 100-250 rpm were studied for growth and lipase production. It showed that maximum growth and lipase production were detected at 100 rpm stirring speed [Fig. 4].

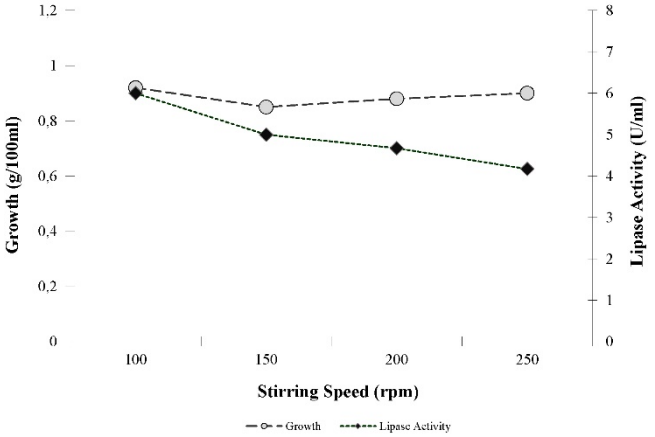

Figure 4. Effect of stirring speed on growth and lipase production by *Candida tropicalis*.

The addition of carbon and nitrogen sources into the lipase production medium showed that carbohydrates used as carbon sources did not give a significant effect on lipase production, however, with olive oil growth increased significantly [Fig. 5 and Fig. 6].

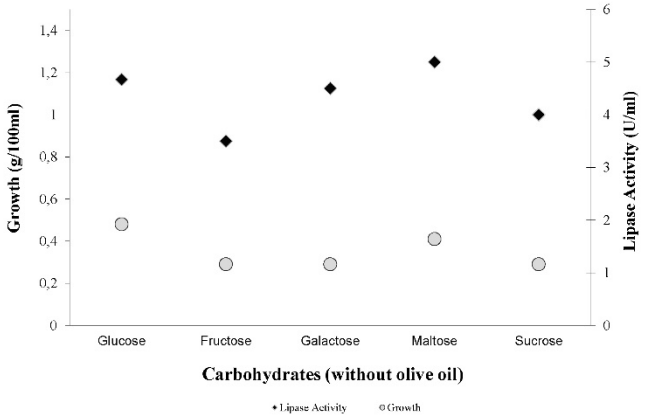

Figure 5. Effect of carbohydrates without olive oil on growth and lipase production by *Candida tropicalis*.

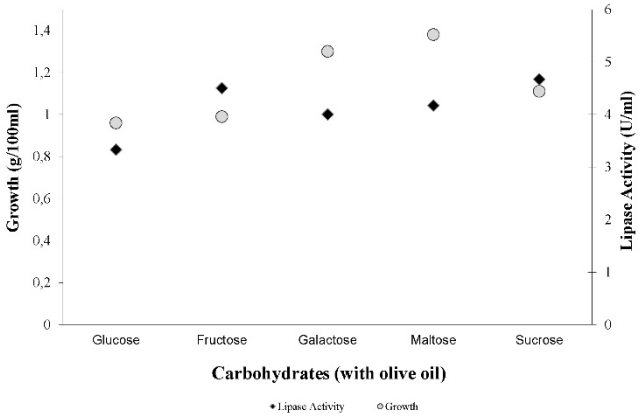

Figure 6. Effect of carbohydrates with olive oil on growth and lipase production by *Candida tropicalis*.

Furthermore effect of oils was determined. It was obvious that oils increased lipase production and growth according to carbohydrate additives. Maximum lipase activity was detected in extra virgin olive oil, corn oil, soybean oil and canola oil as 5.17 U/ml (Fig. 7).

Figure 7. Effect of oils on growth and lipase production by *Candida tropicalis*.

Although carbon sources did not affect lipase production as expected, the addition of nitrogen sources did. Ammonium sulfate increased lipase production more than other nitrogen and carbon sources, with olive oil lipase production was determined as 10.67 U/ml although without olive oil it was 10.33 U/ml [Fig. 8 and Fig. 9]. Ammonium oxalate and ammonium nitrate also showed a distinct effect on lipase production.

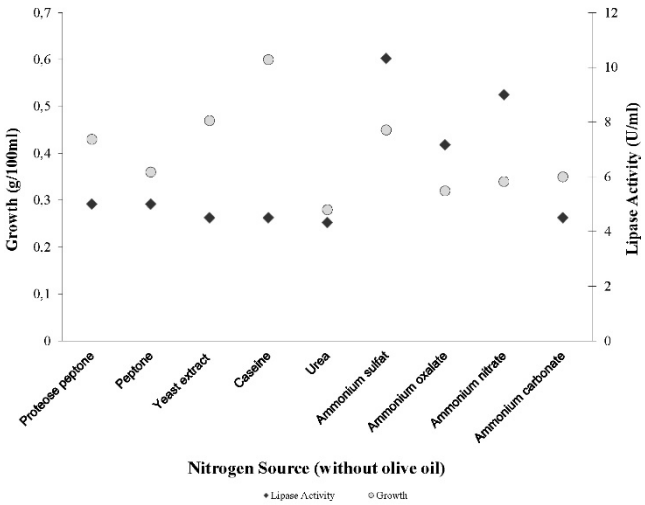

Figure 8. Effect of nitrogen sources with olive oil on growth and lipase production by *Candida tropicalis*.

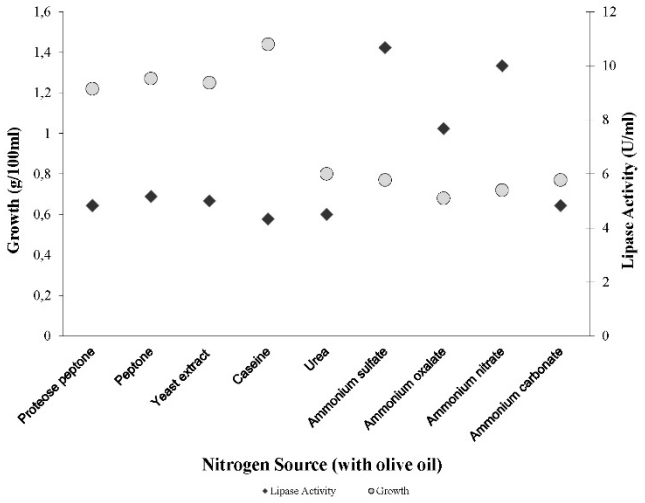

Figure 9. Effect of nitrogen sources without olive oil on growth and lipase production by *Candida tropicalis*.

Discussions

Only about 2% of the world's microorganisms have been tested as enzyme sources (Hasan, 2006). Companies of the enzyme industry continue searching for economical sources of high activity lipases also with simple growth and lipase producing conditions. New lipases from microbial sources have been reported sporadically. The need for novel lipases is obvious, but little effort has been made for conducting a large-scale systematic screening for new lipases (Hou, 1997). *Candida* sp. is the most potential lipase producer from yeasts reported in the literature (Treichel, 2010). According to Vakhlu and Kour (2006), the main terrestrial species of yeasts that were found to produce lipases are: *Candida tropicalis*, *C. rugosa*, *C. antarctica*, *Candida deformans* and *Yarrowia lipolytica*, et al.

Microbial lipases are mostly extracellular and their production is greatly influenced by medium composition besides physicochemical factors (Treichel, 2010). Therefore many researchers aimed to change the composition of the medium by addition of different carbon and nitrogen sources and changing physicochemical factors such as temperature, pH, and dissolved oxygen (Rajendrean and Thangavelu, 2007; Treichel, 2010). The major factor for the expression of lipase activity has always been reported as the carbon source, since lipases are inducible enzymes. These enzymes are generally produced in the presence of a lipid such as oil or any other inducer (Sharma et al, 2001; Gupta et al, 2004; Treichel, 2010). However, nitrogen sources and essential micronutrients should also be carefully considered for growth and production optimization (Treichel, 2010).

Different carbon sources affecting growth and lipase production in *Candida rugosa* were studied on a defined medium (Dalmau et al, 2000). And carbohydrates and acids non-related to fats did not induce lipase production. A present study was therefore undertaken to investigate the effect of different growth media (with and without olive oil) supplemented with various nitrogen (yeast extract, tryptone and proteose-peptone) and carbon sources (glucose and fructose) on lipase production by *C. rugosa* (Fadıloğlu and Erkmen, 2002). In this study high yields of the enzyme were obtained with yeast extract and proteose-peptone in the medium with olive oil.

The initial pH of the growth medium and temperature are important for lipase production therefore culture pH and growth temperature should be assayed for lipase production optima. Researchers studied the optimization of lipase production generally adjusted culture pH between 3-11 and growth temperature between 10-70ºC. Stirring speed is as important as carbon and nitrogen sources, the culture pH and growth temperature. Alonso et al, (2005), studied lipase production by a Brazilian wild strain of *Yarrowia lipolytica* (formerly *Candida lipolytica*) at different stirring speeds and air flow rates and maximum lipase activity was detected in the late stationary phase at 200 rpm.

In this study a novel yeast isolated from olive mill soil was identified as *Candida tropicalis* by 18S rDNA

analysis. Optimization of lipase production assayed by carbon and nitrogen sources, culture pH, growth temperature and stirring speed conditions. According to the results obtained, the lipase production medium was modified as (g/l): 12 NaH_2PO_4, 2 KH_2PO_4, 0.3 $MgSO_4.7H_2O$, 0.25 $CaCl_2$, 0.005 $FeSO_4.7H_2O$, 0.015 $MnSO_4.7H_2O$, 0.03 $ZnSO_4.7H_2O$ and %1 ammonium sulfate, initial pH adjusted to 4.5, and after sterilization 1% olive oil (v/v) added. Maximum lipase production assayed at 30ºC growth temperature, 100 rpm stirring speed at 72 hours incubation.

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In vitro Prebiotic Activity of Polysaccharides Extracted from Edible / Medicinal Macrofungi Species

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Abstract: In this study, the extracted polysaccharides of *Agaricus bisporus* (Kültür mantarı; white and cream lines), *Boletus edulis* (Çörek mantarı), *Cantharellus cibarius* (Sarıkız mantarı), *Ganoderma lucidum* (Reyşi), *Pleurotus ostreatus* (İstiridye mantarı) and *Trametes versicolor* (Hindi kuyruğu) were investigated in terms of their *in vitro* prebiotic potential. For this purpose, the growth of *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Escherichia coli* were observed as nephalometrically in presence of polysaccharides at 0.25%, 0.5%, 1.0% and 2% concentrations for 24 hours. *Trametes versicolor* and *Ganoderma lucidum* have presented the highest (5.59%) and lowest (0.31%) polysaccharide yields, respectively. The higher polysaccharide concentrations have inhibited to proliferation of all bacterial strains. Among the polysaccharides, efficient ones as potential prebiotics were *Agaricus bisporus* (white line), *Trametes versicolor*, *Cantharellus cibarius*, *Boletus edulis*, and *Pleurotus ostreatus*.

Keywords: Macrofungi, Prebiotic, Microbiota, Nephelometre, *In vitro*.

Yenebilir / Tıbbi Önemi Olan Makrofunguslardan Ekstrakte Edilen Polisakkaritlerin *in vitro* Prebiyotik Aktivitesi

Öz: Bu çalışmada *Agaricus bisporus* (Kültür mantarı; beyaz ve kestane formu), *Boletus edulis* (Çörek mantarı), *Cantharellus cibarius* (Sarıkız mantarı), *Ganoderma lucidum* (Reyşi), *Pleurotus ostreatus* (İstiridye mantarı) ve *Trametes versicolor* (Hindi kuyruğu) türlerinden ekstrakte edilen polisakkaritler *in vitro* prebiyotik potansiyelleri açısından araştırılmıştır. Bu amaçla %0.25, %0.5, %1 ve %2 konsantrasyonlarda polisakkarit içeren ortamda *Lactobacillus plantarum*, *Lactobacillus acidophilus* ve *Escherichia coli* bakterilerinin üremesi 24 saat boyunca nefalometrik olarak belirlenmiştir. *Trametes versicolor* ve *Ganoderma lucidum* en yüksek (%5.59) ve en düşük (%0.31) polisakkarit verimi veren türler olmuştur. Yüksek polisakkarit konsantrasyonlarının tüm bakterilerin üremesini ihhibe ettiği görülmüştür. *Agaricus bisporus* (beyaz form), *Trametes versicolor*, *Cantharellus cibarius*, *Boletus edulis* ve *Pleurotus ostreatus* türlerinden elde edilen polisakkaritlerin potansiyel prebiyotik olarak kullanılabileceği belirlenmiştir.

Anahtar kelimeler: Makrofungus, Prebiyotik, Mikrobiyota, Nefalometre, *In vitro*.

Introduction

Prebiotics are oligosaccharides and polysaccharides that can not be digested by digestive system enzymes or can not be metabolized by the host (Prathumpai et al., 2019). Therefore, they can reach to the colon, can stimulate the growth of probiotic bacteria, and can prevent the growth of non-probiotic or pathogenic microorganisms such as *Escherichia coli* and *Salmonella* spp. in the gastrointestinal track (Pompei et al., 2008). Thus, the prebiotics can regulate the microbiota balance in favor of the host live. Different plants and/or their products such as garlic, wheat bran, bananas, onions, leeks, almond, lentil, and grain legumes such as lupin and chickpea are well known prebiotic sources (Swennen et al., 2006; Dwivedi et al., 2014).

Macrofungi are also contain non-digestible fibers such as chitin, α and β glucans, xylans, mannans, and galactans (Aida et al., 2009) Among them particularly β glucans are of considerable interest because of their immune-enhancing, anticancer, antibacterial, antifungal and antioxidant effects (Khan et al., 2018). In addition, it is reported by Nowacka-Jechalke et al. (2018) that the growth of *Lactobacillus* strains can be stimulated by fungal polysaccharides better than commercially available prebiotics like inulin or fructooligosaccharides (FOS) and macrofungal polysaccharides subjected to artificial human gastric juice have remained undigested in more than 90%. It means that these polysaccharides can be a good candidate as prebiotic and can maintained their properties to stimulate the beneficial probiotic microrganisms. *In vitro* prebiotic activities of polysaccharidic fractions of different macrofungi species have been reported in last years such as *Auricularia nigricans* (Karakulak mantarı, Nasution et al., 2018), *Cyclocybe cylindracea* (Çizgili metelik; Mitsou et al., 2020), *Hericium erinaceus* (Tülübüzük; Mitsou et al., 2020), *Lactifluus volemus* (Tirmit; Huang et al., 2020), *Lignosus rhinocerus* (Gao et al., 2009), *Ophiocordyceps dipterigena* (Prathumpai et al., 2019), *Ophiocordyceps sinensis* (Song et al., 2019), *Pleurotus* spp (İstiridye mantarı; Synytsya et al., 2009, Zhao and Cheung, 2011, Nasution et al., 2018, Mitsou et al., 2020 , Ogidi et al., 2020), and *Wolfiporia cocos* (Gao et al., 2009). Besides *in vitro* prebiotic activity of polysaccharide fractions of 53 wild growing macrofungi species has presented by Nowak et al. (2018). In the presented study, *in vitro* prebiotic potential of polysaccharide fractions of fruiting body samples of six edible/medicinal macrofungi species was reported.

Material and Metod

Materials

The fruiting body samples of *Agaricus bisporus* (Kültür mantarı; white line, AB(W)), *Agaricus bisporus* (cream line; AB(C)) and *Pleurotus ostreatus* (İstiridye mantarı; PO), was purchased from MÜPA (Kocaeli). *Trametes versicolor* (Hindi kuyruğu; TV) and *Ganoderma lucidum* (Reyşi; GL) fruiting body samples were obtained from AGROMA (Denizli). *Boletus edulis* (Çörek mantarı; BE) fruiting body samples were obtained from Çalışkan Mantar (Denizli) *Cantharellus cibarius* (Sarıkız mantarı; CC) fruiting body samples were donated by Aysun Pekşen (Ondokuz Mayıs Üniversity, Samsun, Turkey).

Escherichia coli (ATCC 25922) and two *Lactobacillus* species (*L. acidophilus* (B 4495) and *L. plantarum* (B 227) from Agricultural Research Service Culture Collection, NRRL, USA) were used as test organisms. Inulin was purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). Ethyl alcohol, trichloroacetic acid, and all culture media were purchased from Merck KGaA (Darmstadt, Germany).

Polysaccharides extraction of macrofungi materials

The studied macrofungi samples were cleaned, dried overnight at 60°C, and blended. The prepared samples were pulverized, and boiled twice in distilled water at 1:10 (w/v) for 1 h. Then, the extract solutions were treated with an equal volume of 0.8 M trichloroacetic asid at 4°C for 3 h to deproteinization. The obtained solutions were centrifuged for 10 min at 5000 g. The polysaccharide fraction in the supernatant was precipitated with 95% cold ethanol at a ratio of 1:4 (v/v) and kept overnight at 4°C. The obtained precipitates were collected by centrifugation for 10 min at 5000 g and lyophilized using a shelf freeze-drying model (Christ Alpha 1-2 LD). The percentage polysaccharide yields (%) were calculated by the following equaition (Chou et al., 2013);

Yield (%;w/w) = (Weight of extracted polysaccharide / Weight of dried macrofungi material) ×100

Preparation of bacterial suspension and culture conditions

Lactobacillus strains (*L. acidophilus* (B 4495), *L. plantarum* (B 227)) as probiotic bacteria and *E. coli* ATCC 25922 as pathogenic bacterium were used in this study. The cultures of *E. coli* and *Lactobacillus* spp. were grown in Luria Bertani broth and MRS broth and incubated at 37°C, for 24 h at anaerobic and aerobic conditions, respectively. For culture control of *Lactobacillus* bacteria, the catalase test and gram strains were performed. The suspensions of bacteria were prepared according to McFarland standard 0.5 as inoculum that contains 1.5 × 10^8 CFU/mL.

Determination of macrofungi polysaccharides on growth of the test microorganisms

The tested bacteria were cultivated in presence of the fungal polysaccharides as carbon source in comparison to glucose and inulin. The bacterial growth was monitored by microplate laser nephelometry (MLN) using the NEPHELOstar Galaxy (BMG, Offenburg, Germany) (Finger et al., 2013). A 100 µl appropriate double strenght medium for the test microorganisms were put in triplicate into the wells of sterile 96-well microplate (GreinerBioOne, Frickenhausen, Germany). The media were supplemented with 100 µl polysaccharide fraction at different concentrations (0.25, 0.5, 1, and 2%). Each well was inoculated with 5 µl bacterial suspension. Microplates were covered with a clear adhesive film and placed to the microplate laser nephelometer, and incubated 37°C, for 24 hours. The used nephelometer posses a 635-nm laser as radiating source with a laser beam focus 2 mm. The plates were shaken in 3 mm orbital shake witdth. Each well was measured for 1.0 s during the hourly measurement. The reproduction of bacteria is expressed in the RNU (Relative Nefalometric Unit) unit (Finger et al., 2012). Density adjusted to McFarland 0.5 was accepted as 0 (zero) in the initial RNU. Commercial prebiotic inulin and glucose were used as controls. The medium including polysaccharide was used as blank for each polysaccharide concentrations.

Statistical analysis

Statistical analysis of polysaccharide fractions of the macrofungi species on growth of the test microorganisms were performed after incubation period. Three independent experiments were performed and each sample was measured in three replicates. Data are presented as mean ± standard deviation. The obtained results were evaluated by analysis of variance. The differences were statistically significant when p≤0.05.

Results and Discussion

Extraction Yield

Apparent differences were found between the polysaccharide yields of the macrofungi species in this study (Table 1). The highest and lowest polysaccharide yields were obtained in *Trametes versicolor* and *Ganoderma lucidum*, respectively. Since a standard method was used in the polysaccharide production stage (Chou et al., 2013), it is thought that these yield differences are due to the difference in the specific polysaccharide ratio, which is included in the macrofungi species/strain rather than deviations from the method. The differences between the polysaccharide yields of macrofungi basidiocarps can result from the difference in polysaccharide solubility, boiling time, temperature, the water/solid ratio, the number of extraction, and especially macrofungi species/strain. For example, soluble polysaccharide yield of *Ganoderma lucidum* by hot water extraction could vary from 0.4% (Chang and Lu, 2004) to 3.7% (Pang et al., 2007). On the other hand, following the hot water extraction, very low (0.022%) and moderate (3.7%) polysaccharide yield values were obtained from *Trametes versicolor* (Kozarski et al., 2012; Nowak et al., 2018).

Table 1. The polysaccharide yields of macrofungus species

Macrofungi species	Yields (%; w/w)
*Agaricus bisporus (*white line)	0.81
*Agaricus bisporus (*cream line)	3.17
Boletus edulis	2.40
Cantharellus cibarius	1.39
Ganoderma lucidum	0.31
Pleurotus ostreatus	1.41
*Trametes versicolo*r	5.59

Determination of macrofungi polysaccharides on growth of the test microorganisms

Investigating the fermentability of potential prebiotics in human or animal model is time consuming, labor-intensive, expensive, and difficult *in vivo* conditions. Therefore, *in vitro* screening studies are presents valuable data for the determination of novel prebiotics. In this study, the effect of polysaccharide fractions obtained

from fruiting body samples of 7 strains belonging to 6 edible/medicinal macrofungus species on the proliferation of 3 bacterial species was investigated nephelometrically.

To promote and to inhibit the growth of Lactobacilli and *E.coli*, respectively, the ability of the seven macrofungi polysaccharides was investigated in four different concentrations during incubation period. In general, the growth of all reference microbial strains has decreased dose dependently in presence of almost all macrofungi polysaccharides. The microbial growth in presence of higher polysaccharide concentrations in all experimental polysaccharide groups were lower than those of negative and positive controls, glucose and inulin, respectively (Figures 1-3). It means that the studied macrofungi polysaccharides have antimicrobial activities in higher concentrations and should be used as prebiotic in lower concentrations such as 0.25% for the studied macrofungi species.

The influence of fungal polysaccharides at four concentrations on the proliferation of *L. plantarum* after 24 hours is presented in Fig 1. The maximum growth of the bacterium was obtained at the 0.25% concentration in all fungal polysaccharides. In this concentration, five of the studied polysaccharides (TV, PO, CC, BE, AB(W)) have stimulated the growth of the *L. plantarum* according to glucose control (p≤0.05). This situation confirms the potential of these polysaccharides as prebiotics and could be good substrate for *Lactobacillus plantarum*. However, none of them did not stimulate the growth of *L. plantarum* as inulin (p≤0.05). The growth inhibiting activity of GL and AB(C) polysaccharides for *L. plantarum* were distict in all concentrations.

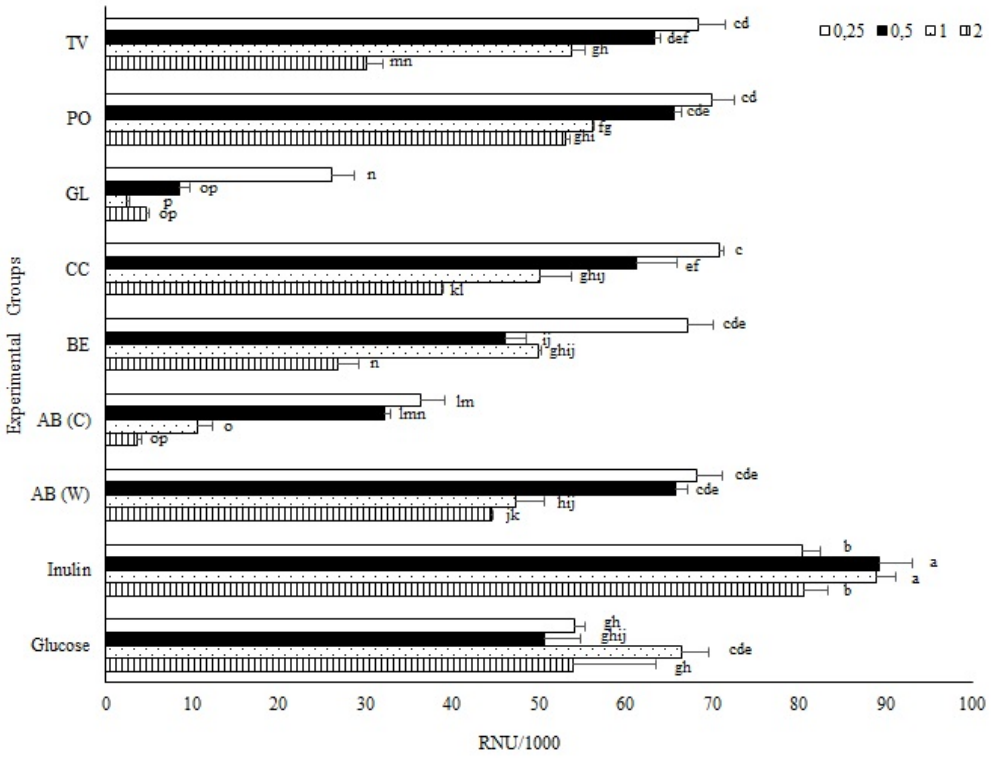

Fig 1. The influence of fungal polysaccharides on the growth of *Lactobacillus plantarum*. Same letters denote statistical equivalence between groups

Only two (PO and AB(W)) polysaccharides at the 0.25% concentration have increased the growth intensity of the other *Lactobacillus* species, *L. acidophilus*, in statistically significant level (p≤0.05) (Fig 2) according to inulin. Similar to *L. plantarum*, GL and AB(C) have presented the lowest growth rates in *L. acidophilus*, also.

Four (TV, PO, CC, BE) and two (GL, AB(C)) of the seven macrofungi polysaccharides were found to be statistically significant in enhanging and decreasing the growth of the *E. coli* according to glucose at the concentration of 0.25%, respectively. The only polysaccharide presented similar growth with glucose was AB(W) (p≤0.05) (Fig 3). The influence of TV, CC, and BE polysaccharides on the growth of *E. coli* was statistically similar to inulin (p≤0.05). On the other hand, the growth of *E.coli* was lower in presence of AB(W) polysaccharide according to inulin.

The time dependent proliferation studies with *Lactobacillus plantarum* demonstrated that AB(W), TV, CC, and BE polysaccharides after 24 hours were very similar which were lower and higher than inulin and glucose, respectively ($p<0.05$). The highest proliferative effects on the bacterium was presented by the polysaccharides of TV and CC during almost all incubation period which were higher or very similar to that of inulin except last 4 hours (Fig 4). These results indicates that all of the polysaccharide fractions of the tested macrofungi species could be good substrates for *Lactobacillus plantarum* than glucose and could be compete with inulin. The positive control, inulin, and the most of the macrofungi polysaccharides demonstrated similar prebiotic activity on *L. acidophilus* after 24-h of incubation (Fig 5). It is worth mentioned that only AB(W) resulted in significantly higher proliferative effect compared to inulin during in all incubation period. On the other hand, glucose was the best substrate for *L. acidophilus*. Besides the proliferation of *E. coli* was not promoted by AB(W) during 24-h of incubation period (Fig 6). Commercial prebiotic, inulin, had lower inhibition activity for *E. coli* than most of the studied polysaccharides.

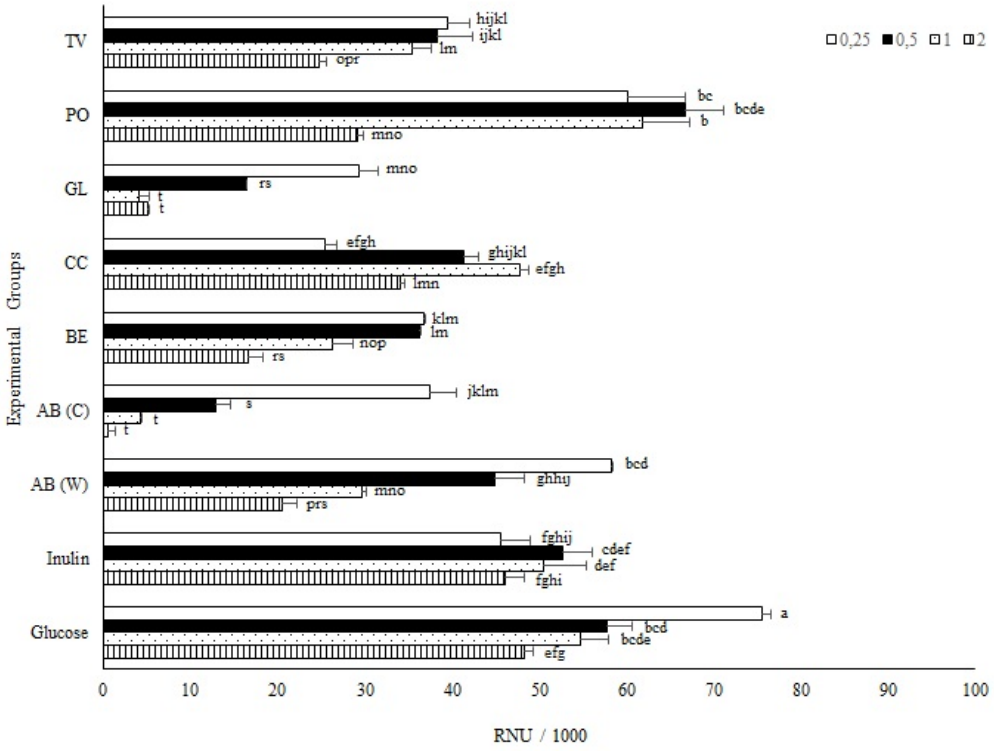

Fig 2. The influence of fungal polysaccharides on the growth of *Lactobacillus acidophilus*. Same letters denote statistical equivalence between groups.

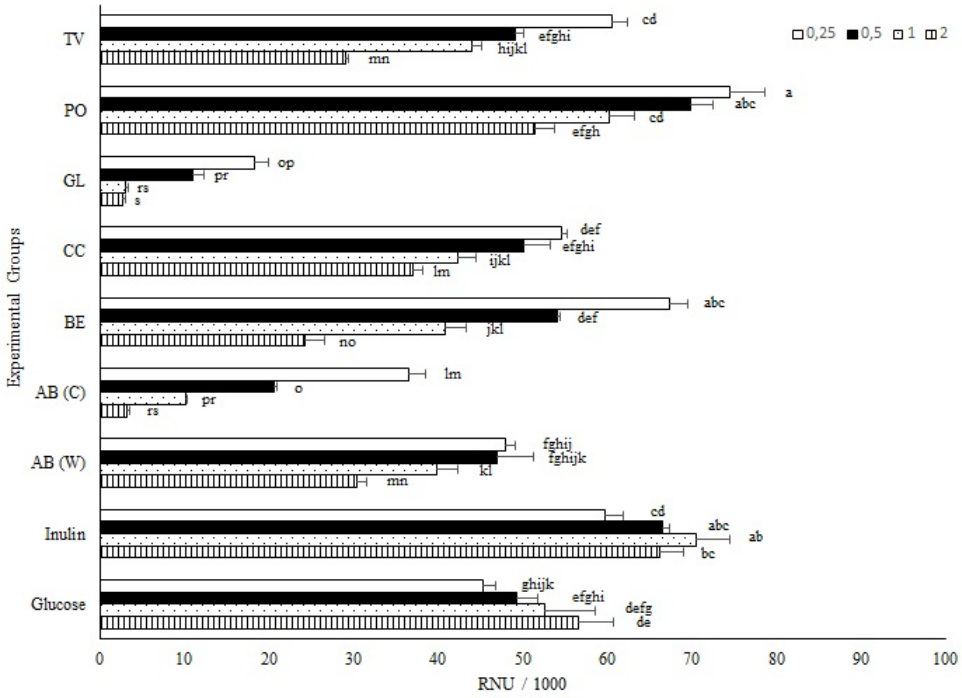

Fig 3. The influence of fungal polysaccharides on the growth of *Escherichia coli*. Same letters denote statistical equivalence between groups.

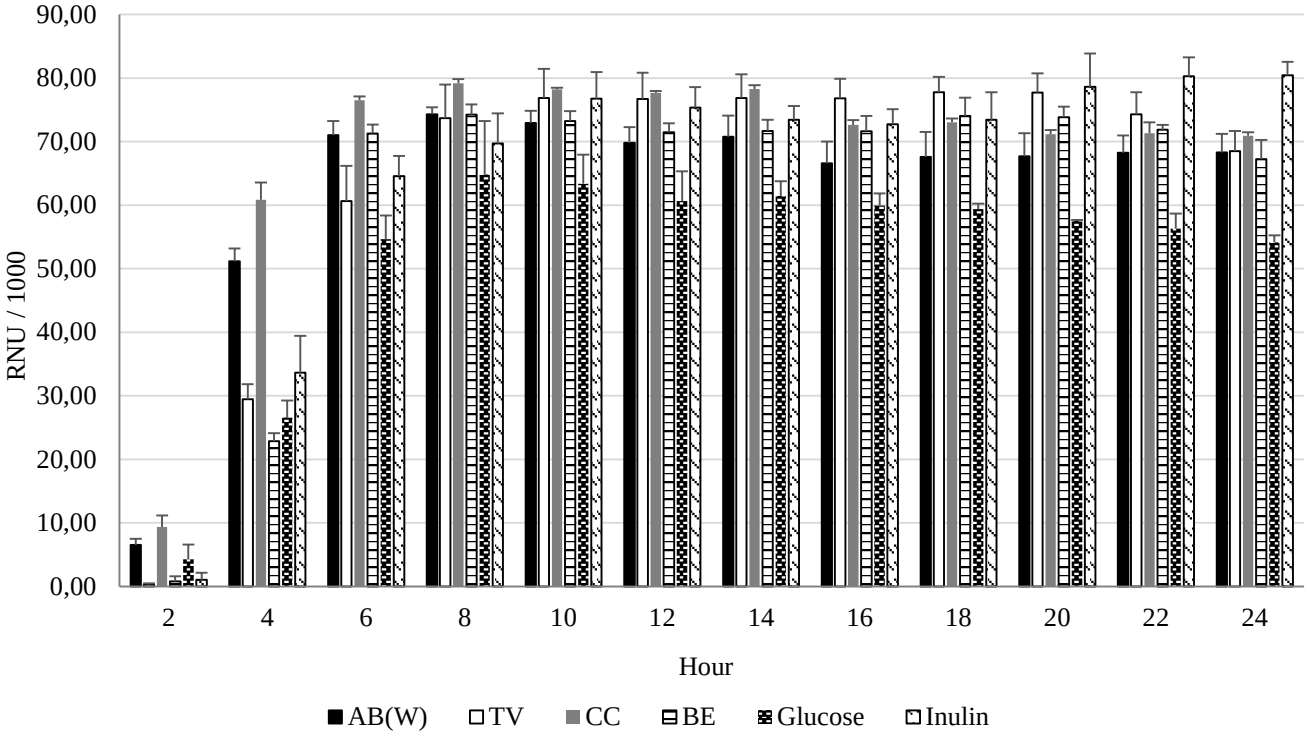

Fig 4. Time dependent growth of *Lactobacillus plantarum* in presence of 0.25% fungal polysaccharides.

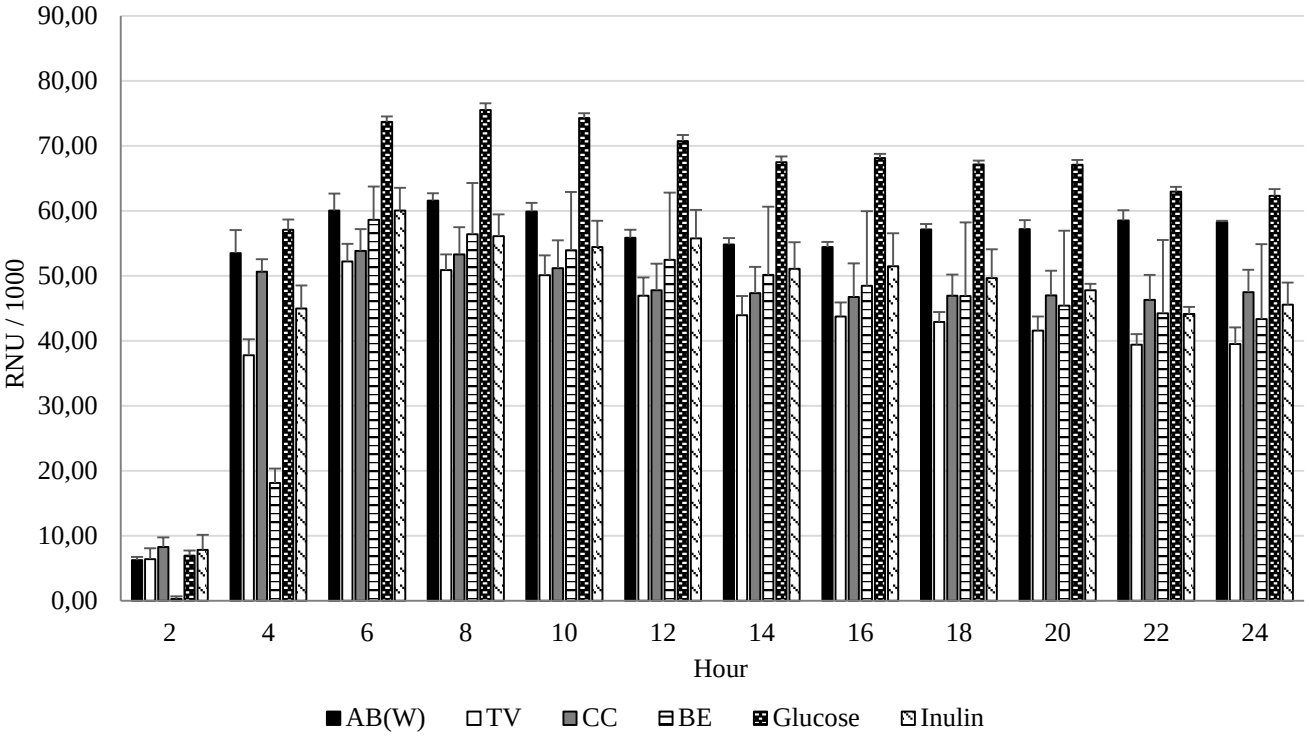

Fig 5. Time dependent growth of *Lactobacillus acidophilus* in presence of 0.25% fungal polysaccharides

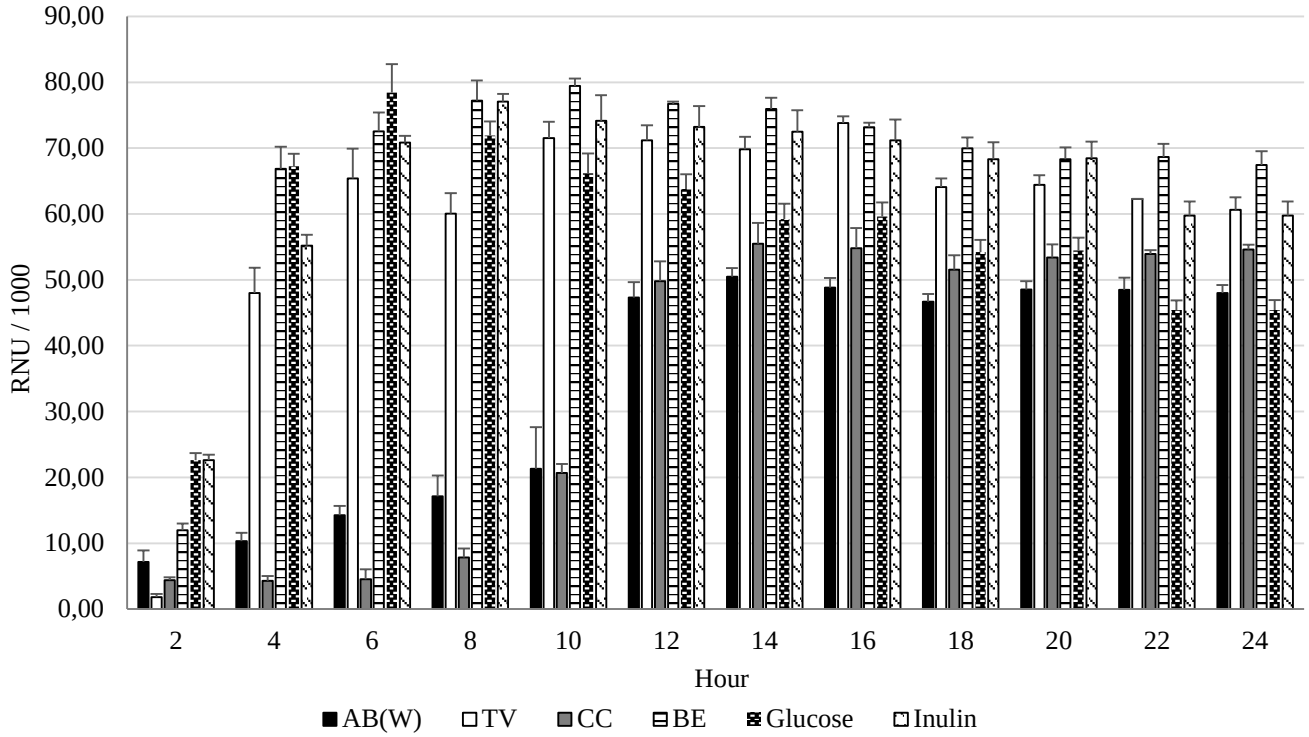

Fig 6. Time dependent growth of *Escherichia coli* in presence of 0.25% fungal polysaccharides

As a result of detailed examination of the data presented in Figure 4, it was determined that *Agaricus bisporus* (white form) polysaccharide (ABP) increased the growth of *L. plantarum* at 0.25% concentration according to glucose, but lower than the inulin used as a positive control. On the other hand, 0.25% ABP increases the growth of *L. acidophilus* compared to inulin, although it offers lower values than glucose. Briefly, the stimulation of probiotic bacterial strains in the presence of AB(W) supports the fact that AB(W) has a prebiotic feature which is in agreement with Giannenas et al. (2010). As an important finding, it was determined that growth of *E. coli* is not stimulated in the presence of AB(W). The enhanging of the *Lactobacillus* spp. and *E. coli* amouth in gastrointestinal track is and is not demanded, respectively. Therefore, it can be argued that AB(W) polysaccharide has presented the highest prebiotic activity and in particular AB(W) has potential as prebiotic and nutraceutical. TV, CC, and BE polysaccharides have also prebiotic activity potential. Among them, the prebiotic activity of CC was confirmed by our group at *in vivo* conditions (Türsen Uthan et al., 2021)

Agaricus bisporus (white form) is a fungal species cultivated worldwide. It has high polysaccharide and fiber content. In addition, its protein, vitamin and mineral ratio is high (Giannenas et al., 2010). It is emphasized that this macrofungus polysaccharide has prebiotic activity due to heteropolysaccharide, D-galactose, D-mannose, D-xylose, L-fructose, L- (or D) -arabinose, xylose, fructose, glucose, sucrose and trehalose (Aida et al., 2009).

Mitsou et al. (2020) confirmed that there are limited number of study examined the *in vitro* prebiotic effets of edible muhroom species. The previous reports have demonstrated that prebiotic activities of the macrofungi are species/strain dependent, very variable (Table 2), and only some of them can be used as nutraceuticals.

Based on the results, it could be speculated that the polysaccharide of especially AB(W), TV, CC, and BE could be used as substrate to promote the growth of the *Lactobacillus* species, selectively. Nevertheless, our further *in vivo* studies will be focused on to clarify the exact mechanism amd to perform microbiome analysis.

Table 2. Growth stimulation activities of different macrofungi species for probiotic bacteria

Species	Polysaccharide	Source	PS (%, w/v)	Probiotic taxon	Growth Conditions	Medium	GOS	FOS	İnulin	Glucose	Reference
Agaricus bisporus (Cream line)	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	82.04 24.48 8.31 1.30 [1]	60.00 22.35 7.66 1.24 [2]	This study
Agaricus bisporus (Cream line)	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	45.12 36.15 11.92 4.47 [1]	67.02 63.74 15.93 6.66 [2]	This study
Agaricus bisporus (White line)	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	84.89 73.70 53.19 55.40 [1]	93.53 77.93 54.01 42.39 [2]	This study
Agaricus bisporus (White line)	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	127.88 85.35 58.59 44.50 [1]	126.11 129.94 71.09 82.56 [2]	This study
Agaricus bisporus	Crude PS	Fruiting body	3.12 6.25 12.50 25.00	*Lactobacillus casei*	37 °C 24 h	MRS Broth	ND	ND	ND	4.51 29.65 58.28 192.44 [2]	Nasution et al., 2018
Auricularia nigricans	Crude PS	Fruiting body	3.12 6.25 12.50 25.00	*Lactobacillus casei*	37 °C 24 h	MRS Broth	ND	ND	ND	64.69 80.56 103.89 110.91 [2]	Nasution et al., 2018
Boletus edulis	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	80.50 68.88 51.86 36.29 [1]	58.88 62.89 47.81 34.57 [2]	This study

Boletus edulis	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	83.52 51.68 56.12 33.29 [1]	124.08 91.11 74.99 49.61 [2]	This study
Cantharellus cibarius	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	55.93 78.37 94.42 73.99 [1]	40.91 71.55 87.04 70.48 [2]	This study
Cantharellus cibarius	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	88.12 68.71 56.46 48.20 [1]	130.91 121.15 75.44 71.83 [2]	This study
Cantharellus cibarius	Crude PS	Fruiting body	1.50	*L. acidophilus ATCC 4356*	48 h	Rogosa Broth without glucose	ND	11.53 [2]	11.34 [2]	20.3 [2]	Nowacka-Jechalke et al., 2018
Cantharellus cibarius	Crude PS	Fruiting body	1.50	*L. rhamnosus-1*	48 h	Rogosa Broth without glucose	ND	11.71 [2]	12.72 [2]	33.85 [2]	Nowacka-Jechalke et al., 2018
Cantharellus cibarius	Crude PS	Fruiting body	1.50	*L. rhamnosus-2*	48 h	Rogosa Broth without glucose	ND	8.87 [2]	11.34 [2]	0.00 [2]	Nowacka-Jechalke et al., 2018
Ganoderma lucidum	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	64.27 31.31 8.06 10.93 [1]	47.01 28.59 7.43 10.41 [2]	This study
Ganoderma lucidum	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	32.44 9.64 2.70 5.84 [1]	48.19 17.00 3.61 8.70 [2]	This study
Ganoderma lucidum	Crude PS	Fruiting body		*Bifidobacterium spp.*	37 °C 24 h	Human feces	ND	15.28	ND	4.17 [4]	Yamin et al., 2012

Ganoderma lucidum	PS Fraction	Fruiting body		*Bifidobacterium spp.*	37 °C 24 h	Human feces	ND	15.28	ND	9.72 [4]	Yamin et al., 2012
Ganoderma lucidum	Crude PS	Fruiting body		*Lactobacillus spp.*	37 °C 24 h	Human feces	ND	27.69	ND	10.77 [4]	Yamin et al., 2012
Ganoderma lucidum	PS Fraction	Fruiting body		*Lactobacillus spp.*	37 °C 24 h	Human feces	ND	27.69	ND	15.38 [4]	Yamin et al., 2012
Hypholoma capnoides (Al sarıpapak)	Crude PS	Fruiting body	1.50	*L. rhamnosus 1*	37 °C 72 h	Rogosa broth	ND	11.71 [2,5]	12.72 [2,5]	56.63 [2,5]	Nowak et al., 2018
Hypholoma capnoides	Crude PS	Fruiting body	1.5	*L. rhamnosus 2*	37 °C 72 h	Rogosa broth	ND	11.34 [2,5]	8.87 [2,5]	17.84 [2,5]	Nowak et al., 2018
Lactifluus volemus	Crude PS	Fruiting body	0.50 1.00 1.50	*Lactobacillus plantarum DMDL 9010*	4 °C 28 day	Yoghurt	ND	ND	ND	9.83 10.91 13.07 [2]	Huang et al., 2020
Lactifluus volemus	Crude PS	Fruiting body	0.50 1.00 1.50	*Lb. casei subsp. rhamnosus 6013*	4 °C 28 day	Yoghurt	ND	ND	ND	6.85 9.02 9.89	Huang et al., 2020
Lactifluus volemus	Crude PS	Fruiting body	0.50 1.00 1.50	*Lactobacillus acidophilus 1.1878*	4 °C 28 day	Yoghurt	ND	ND	ND	6.67 8.61 10.22 [2]	Huang et al., 2020
Lignosus rhinocerus	β-glucan rich nondigestible carbohydrates	Sclerotium	1.00	*Bifidobacterium longum (JCM 1217)*	37 °C 100 rpm 24 h	Basal medium	ND	ND	ND	14.3 [4]	Gao et al., 2009
Lignosus rhinocerus	β-glucan rich nondigestible carbohydrates	Sclerotium	1.00	*Lactobacillus brevis (JCM 1059)*	37 °C 100 rpm 24 h	Basal medium	ND	ND	ND	18.9 [4]	Gao et al., 2009
Ophiocordyceps sinensis	Crude EPS	Submerged culture	0.50	*Bifidobacterium adolescentis (CICC 6070),*	4 °C 28 day	Water	0.70 3	ND	25.30 [3]	49.90 [3]	Song et al., 2019
Ophiocordyceps sinensis	Crude EPS	Submerged culture	0.50	*Bifidobacterium infantis (CICC 6069)*	4 °C 28 day	Water	0.40 3	ND	-1.30 [3]	65.80 [3]	Song et al., 2019

Ophiocordyceps sinensis	Crude EPS	Submerged culture	0.50	*Bifidobacterium infantis (R33)*	4 °C 28 day	Water	35.40 3	ND	34.60 [3]	71.80 [3]	Song et al., 2019
Ophiocordyceps dipterigena	(1, 3)-β-D-glucan	Submerged culture	1.00	*Lactobacillus acidophilus BCC 13938*	37 °C 150 rpm 48 h	MRS Medium	400 [2]	400 [2]	ND	ND	Prathumpai et al., 2019
Ophiocordyceps dipterigena	(1, 3)-β-D-glucan	Submerged culture	1.00	*Bifidobacterium animalis ATCC 25527*	37 °C 150 rpm 48 h	Reinforced Clostridial Medium	0 2	0 2	ND	ND	Prathumpai et al., 2019
Paxillus involutus (Ağulu iztutan)	Crude PS	Fruiting body	1.50	*Lactobacillus acidophilus ATCC 4356*	37 °C 72 h	Rogosa broth	ND	11.53 [2,5]	11.34 [2,5]	37.96 [2,5]	Nowak et al., 2018
Pleurotus cystidiosus	Crude PS	Fruiting body	3.12 6.25 12.50 25.00	*Lactobacillus casei*	37 °C 24 h	MRS Broth	ND	ND	ND	65.31 134.81 244.50 287.32 [4]	Nasution et al., 2018
Pleurotus ostreatus	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	131.83 126.57 122.33 63.19 [1]	103.58 115.56 112.77 60.19 [2]	This study
Pleurotus ostreatus	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	86.88 73.65 63.32 65.93 [1]	129.06 129.84 84.61 98.24 [2]	This study
P. pulmonarius (Yaz istiridyesi)	Crude EPS	Submerged culture	1.00	*Lactobacillus delbrueckii*	37 °C 48 h	MRS Broth	ND	23.41 [2]	ND	26.98 [2]	Ogidi et al., 2020
P. pulmonarius	Crude EPS	Submerged culture	1.00	*Streptococcus thermophiles*	37 °C 48 h	MRS Broth	ND	19.81 [2]	ND	20.77 [2]	Ogidi et al., 2020
P. tuber-regium	β-glucan	Sclerotium	0.50	*B. infantis (JCM 1222)*	37 °C 100 rpm 24 h	Medium for colonic bacteria (MCB)	ND	ND	ND	67.06 [5]	Zhao and Cheung, 2011

P. tuber-regium	β-glucan	Sclerotium	0.50	*B. longum (JCM 1217)*	37 °C 100 rpm 24 h	Medium for colonic bacteria (MCB)	ND	ND	ND	30.02 [4]	Zhao and Cheung, 2011
P. tuber-regium	β-glucan	Sclerotium	0.50	*B. adolescentis (JCM 1275)*	37 °C 100 rpm 24 h	Medium for colonic bacteria (MCB)	ND	ND	ND	51.36 [4]	Zhao and Cheung, 2011
Trametes versicolor	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	86.64 72.68 70.07 53.67 [1]	63.37 66.35 64.60 51.13 [2]	This study
Trametes versicolor	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	85.01 71.07 60.50 37.37 [1]	126.29 125.30 80.85 55.68 [2]	This study
Trametes versicolor	Polysaccharopeptide (PSP)	Cultured mycelium	0.50	*Bifidobacterium spp.*	37 °C 24 h	Sythetic medium	ND	29.66 [2]	ND	23.28 [2]	Yu et al., 2013
Trametes versicolor	Polysaccharopeptide (PSP)	Cultured mycelium	0.50	*Lactobacillus spp.*	37 °C 24 h	Sythetic medium	ND	17.57 [2]	ND	41.89 [2]	Yu et al., 2013
Wolfiporia cocos	β-glucan rich nondigestible carbohydrates	Sclerotium	1.00	*Bifidobacterium longum (JCM 1217)*	37 °C 100 rpm 24 h	Basal medium	ND	ND	ND	6.74 [4]	Gao et al., 2009
Wolfiporia cocos	β-glucan rich nondigestible carbohydrates	Sclerotium	1.00	*Lactobacillus brevis (JCM 1059)*	37 °C 100 rpm 24 h	Basal medium	ND	ND	ND	11.9 [4]	Gao et al., 2009

PS: Polysaccharide, EPS: Exopolysaccharide, FOS: Fructooligosaccharide, GOS: Galactooligosaccharide

[1] Growth stimulation activity (%) according to inulin (taken as 100%).

[2] Growth stimulation activity (%) according to glucose (taken as 100%).

[3] Reduction of bacterial death rate (%)

[4] Ratio of number of bacterial populations at 24 hours compared to that at 0 hours.

[5] The highest value among 53 wild-growing macrofungi for the studied probiotic bacterium.

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Egzotik Bazı Mantarların (*Pleurotus ostreatus*, *Pleurotus eryngii*, *Hericium erinaceus*) Fizikokimyasal, Biyoaktif ve Duyusal Özelliklerinin Belirlenmesi

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Öz: Besin değerinin yüksek olması yanında içerdikleri biyoaktif maddeler bakımından zengin gıda kaynaklarına olan ilgi her geçen gün artmaktadır. Bu arayışta kültüre alınmış mantarlar son derece dikkat çekicidir. Bu çalışmada; ülkemizde üretim ve tüketim potansiyeli yüksek olan egzotik mantarlardan, *Pleurotus ostreatus*, *Pleurotus eryngii* ve *Hericium erinaceus* mantarlarının fizikokimyasal, biyoaktif ve duyusal özellikleri irdelenmiştir. Sonuçlar mantarların yüksek protein ve kül içermesine karşın düşük yağ ve kalori değerlerine sahip olduğunu göstermiştir. En yüksek protein değeri (23.31±0.1 g/100 g kurumadde) *H. erinaceus* da olmasına rağmen, mantarların protein değerleri arasındaki fark istatistiksel olarak önemsiz bulunmuştur ($p>0.05$). Ayrıca biyoaktif özellikler bakımından da en yüksek toplam fenolik madde miktarı (5.06±0.21 mg GAE/g) ve antioksidan aktivite (43.11±2.74 µmol TE/g) *H. erinaceus* da gözlemlenmiştir. *Pleurotus* cinsi mantarlar arasında *P. eryngii* daha yüksek biyoaktivite sergilemesi yanında istatistiksel açıdan da farklılık göstermiştir ($p<0.05$). Duyusal açıdan ise en yüksek kabul edilirliği *P. eryngii* mantarı göstererek diğer mantar çeşitlerinden ayrılmıştır ($p<0.05$). Bu çalışma da yaygın tüketimi bulunan bazı egzotik mantarlar, çeşitli karakteristik özellikleri açısından karşılaştırılarak verilmiş olup üretici, tüketici ve literatüre katkı sağlaması hedeflenmiştir.

Anahtar kelimeler: *Pleurotus ostreatus*, *Pleurotus eryngii*, *Hericium erinaceus*, Egzotik mantarlar, Biyoaktivite, Sous vide.

Determination of Physiochemical, Bioactive and Sensory Characteristics of Some Exotic Mushrooms (*Pleurotus ostreatus*, *Pleurotus eryngii*, *Hericium erinaceus*)

Abstract: In addition to its high nutritional value, the interest in food sources which is containing bioactive products is steadily increase nowadays. In respect to cultivated mushrooms, they excite world wide interest. In this study; the physicochemical, bioactive, and sensory properties of *Pleurotus ostreatus*, *Pleurotus eryngii*, and *Hericium erinaceus* from the exotic mushrooms, which have a high production and consumption potential in our country, were examined. The results showed that although mushrooms contain high protein and ash, they have low fat and calorie values. Although the highest protein value (23.31±0.1 g/100 g dry matter) was determined in *H. erinaceus,* it was not found differences among mushroom species ($p>0.05$) used in this study. In addition, in bioactive properties, the highest amount of total phenolic substance (5.06±0.21 mg GAE/g) and antioxidant activity (43.11±2.74 µmol TE/g) were observed in *H. erinaceus* mushrooms. Among the mushrooms of the *Pleurotus* species, *P. eryngii* showed higher bioactivity as well as a statistically significant difference ($p<0.05$). In this study, various characters of some exotic mushrooms, which are widely consumed, are examined. it is aimed that results will contribute to the producer, consumer, and literature.

Key words: *Pleurotus ostreatus*, *Pleurotus eryngii*, *Hericium erinaceus*, Exotic mushrooms, Bioactivity, Sous vide

Giriş

Doğada kendiliğinden yetişen mantarlar, insanlar tarafından yüzyıllardır gıda maddesi olarak kullanılmaktadır. Ancak doğadan toplanan mantarların zehirli olanları, fiziksel özelliklerine bakılarak ayırt edilememekte ve bu durum önemli tehlikelere yol açmakta, hatta bazen ölümle bile sonuçlanmaktadır. Bu yaşananlar, mantar tüketimi üzerinde olumsuz bir etki yaratmaktadır. Günümüzde kültür mantarı yetiştiriciliğinin yaygınlaşması, tüketiciler üzerindeki bu olumsuz etkinin azalmasını ve mantar tüketiminin artmasını sağlamıştır. Mantarların büyük çoğunluğu heterotrof canlılar olup gelişmeleri ve çoğalmaları için gerekli besin maddelerini cansız materyallerden ve organik bazı bileşiklerden temin ederler. Dolayısı ile kültür mantarı üretiminde, mantarlar aynı cins ve türe ait olsa dahi farklı yetiştirme ortamlarında üretilebilmektedirler. Mantar üreticiliği yapılan bölgelerdeki atık materyallerin değerlendirilmesi, ülke ekonomisi açısından son derece önemlidir. Dünya kültür mantarı yetiştiriciliğinde üretimin yapıldığı bölgedeki tarımsal ve endüstriyel ürünlere göre; kavak, meşe, çam, kayın, akçaağaç, huş gibi ağaç türlerinin talaşı, hububat samanı, fındık zurufu mısır koçanı, yer fıstığı kabukları, çay artığı, kahve pulpu, ayçiçeği tohum kabuğu, pamuk tohumu atıkları gibi birçok tarımsal atığın yetiştirme ortamı olarak kullanıldığı görülmektedir. (Philippoussis ve ark., 2000).

Mantarların benzersiz tadı yanında, protein (Shah ve ark., 1997), mineraller (Mallikarjuna ve ark., 2013) ve β-glukan içeriği (Khan ve ark., 2018) bakımından son derece zengin olması ve % 2-6 yağ oranı ile kalorisi düşük gıdalar arasında sayılabilir. Yağda çözünen vitaminler ve ergosterol içeriği bakımından zengin olan mantarların, vejeteryanlar için önemli bir D vitamini kaynağı olduğu düşünülmektedir (Rathore ve ark., 2017). Ayrıca mantarların antiviral (Seo ve Choi, 2021), antifungal (Park ve ark., 2009) antibakteriyel (Schillaci ve ark., 2013), antikolesterol (Khatun ve ark., 2007), antienflamatuar ve antikarsinojenik (Ma ve ark., 2013), antihipertansif (Miyazawa ve ark., 2008), antidiyabetik ve antioksidan özellikleri (Doğan ve Doğan, 2021) gibi tıbbi faydaları ile de destekleyici bir gıda olduğu bilinmektedir.

Agaricus bisporus (J.E. Lange) Imbach (Kültür mantarı) dünyada üretimi yapılan mantar türleri arasında birinci sırada, *Pleurotus ostreatus* (Jacq.) P. Kumm. (İstiridye mantarı) ise ikinci sırada yer almaktadır (Öztürk ve Çopur, 2009). *Pleurotus* cinsi mantarlar arasında da *Pleurotus ostreatus* ve *Pleurotus eryngii* (DC.) Quél. (Çakşır mantarı), lezzetli ve ekonomik değeri en yüksek olanları olarak kabul edilirler. Son zamanlarda dünyada popülaritesi artan ancak doğada nadir olarak bulunan *Hericium erinaceus* (Bull.) Pers. (Tülübüzük) (Sesli ve ark., 2020) mantarının kültivasyonu da ülkemizde de hız kazanmıştır. Türkiye florasında da kayıtlı olan *H. erinaceus* özellikle uzak doğuda sevilerek tüketilen ve birçok hastalığın tedavisinde kullanımı ile dikkat çeken bir mantar çeşididir (Kawagishi ve ark., 1992; Mizuno, 1999). Ülkemizde kültür mantarlarına talep gün geçtikçe artmakta olup, üretimi de buna paralel olarak hızlı bir büyüme içerisindedir. Kültüre alınmış mantarların üretiminin mevsime bağlı olmayışı, besleyici ve tıbbi özelliklerinin yüksek olması açısından gıda sanayiine ve bu sanayinin gelişmesine büyük katkı sağlayabileceği düşünülmektedir.

Bu çalışmada, ülkemizde üretim ve tüketim potansiyeli yüksek olan egzotik mantarlardan, *P. ostreatus*, *P. eryngii* ve *H. erinaceus* mantarlarının fizikokimyasal, biyoaktif ve duyusal özelliklerinin belirlenmesi amaçlanmıştır.

Materyal ve Metot

Materyal

Mantarların üretiminde kullanılan sıvı kültür ve misel Yozgat Bozok Üniversitesi, Boğazlıyan Meslek Yüksekokulu Kültür Bankasından temin edilmiştir. Sıvı kültür, misel ve mantar üretiminde kullanılan malt özütü, kavak talaşı, maya, yulaf, K_2PO_4, $MgSO_4$, kepek, kavak talaşı, $CaSO_4$ ve $CaCO_3$ yerel tedarikçilerden, diğer kimyasallar aksi belirtilmedikçe Merck/Sigma-Aldrich (Darmstadt, Almanya)' den temin edilmiştir.

Metot

Çalışma kapsamında kullanılan mantarların, sıvı kültürlerin ve misellerin üretim şekli önceki çalışmalarımızda detaylarıyla anlatılmıştır (Doğan ve Doğan, 2021; Doğan ve ark., 2021). *P. ostreatus*, *P.eryngii* ve *H.erinaceus* mantarları için sırasıyla; yaprak çapı 5 cm, fruktifikasyon yapısı (sap ve şapka dahil) 9 cm ve fruktifikasyon çıkıntıları 2 cm olduğunda toplanmıştır. Toplanan örnekler taze iken duyusal olarak değerlendirilmiştir. Diğer analizler için mantarların raf ömürlerinin düşük olması sebebiyle kurutma işlemi gerçekleştirilmiştir. Bunun için, 0.5 cm kalınlığında kesilen mantarlar etüvde 40°C de 12 saat kurutulmuştur. Nem kayıpları not edildikten sonra kuru mantarlar bir parçalayıcı (Bosch MKM6000, Almanya) kullanılarak toz haline getirilmiştir. Örnekler -18°C de 3 aydan fazla olmamak koşuluyla analiz edilinceye kadar depolanmıştır.

Fizikokimyasal Analizler ve Enerji Değeri

Mantarların temel bileşim analizleri (protein, yağ, kurumadde, kül) William (2000) tarafından belirtilen

metotlar modifiye edilerek uygulanmıştır. Ham protein Kjeldahl metodu ile yakma, distilasyon (Velp Scientifica, UDK 132, Italy) ve titrasyon ile gerçekleştirilmiştir. Elde edilen Azot (N) değeri 4.38 katsayısı ile çarpılarak protein miktarı hesaplanmıştır. Yağ içeriği, solvent ekstraksiyonu yöntemine göre yapılmıştır. Bu amaçla mantarlar otomatik soxhlet cihazı (Büchi, Universal Extraction Unit B-811, İsviçre) ile petrol eteri kullanılarak ekstraksiyona tabi tutulmuştur. Örneklerin protein, yağ ve kül değerleri karşılaştırmanın daha iyi verilebilmesi amacı ile kurumadde cinsinden verilmiştir. Mantarın kurumadde miktarları 105±3 ºC'ye ayarlı fırında (Daihan, Korea) sabit tartıma (>3 saat) gelinceye kadar kurutulması ile tespit edilmiştir. Taze örneğin nem değerini hesaplamak amacı ile başlangıçta kaydedilen nem değeri de hesaplamaya dahil edilmiştir.

Örneklerin karbonhidrat ve enerji değerleri sırası ile aşağıdaki eşitlikler kullanılarak elde edilmiştir (Falch ve ark., 2009).

$$K = 100 - (Kül + Protein + Yağ)$$

$$E = [(P \times 4) + (Y \times 9) + (K \times 4)]$$

E enerji değeri (kcal/100 g taze örnek) iken, P, Y ve K ise sırasıyla protein, yağ ve karbonhidrat değerini ifade etmektedir.

Ekstraksiyon ve Biyoaktivite

1 gram kuru örnek, %80 konsantrasyona sahip 20 mL metanol çözeltisi ile karıştırılarak 45°C'de 60 dakika inkübe edilmiştir. Süpernatant, çökeltiden ayrıldıktan sonra analiz edilmiştir. Örneklerin biyokaktif özellikleri toplam fenolik madde miktarları ve antioksidan aktiviteleri dikkate alınarak belirlenmiştir.

Toplam Fenolik Madde Miktarı (TFMM)

TFMM'nin belirlenmesinde Singleton ve ark. (1999)'nın metotu esas alınmıştır. 0.4 ml seyreltilmiş ekstrakt, 2 ml Folin & Ciocalteu reaktifi ve 1.6 mL %7.5 Na2CO3 çözeltisi ile karıştırıldıktan sonra karanlık bir ortamda 60 dakika bekletilmiş ve spektrofotometrede (Shimadzu UV-1700, Kyoto, Japonya) 765 nm dalga boyunda absorbansı okunmuştur. Sonuçlar gallik asit eşdeğeri (GAE) olarak ifade edilmiştir.

Antioksidan kapasite

Antioksidan kapasite tayini 2,2-diphenyl-1-picrylhydrazyl (DPPH) yöntemine göre belirlenmiştir. Bu bağlamda 0,1 mL ekstrakt 3.9 mL (25 mg/L) metanolik DPPH solüsyonu ile karıştırıldıktan sonra karanlık bir yerde oda sıcaklığında 30 dakika inkübe edilmiş ve 515 nm'de absorbans kaydedilmiştir (Brand-Williams ve ark., 1995). Sonuçlar Trolox eşdeğeri (µM TE/g örnek) olarak ifade edilmiştir.

Duyusal Değerlendirme

Mantar örnekleri, gıda endüstrisinde yaygın olarak kullanılan ve kontrol edilebilir bir pişirme yöntemi olan sous-vide yöntemi ile hazırlanmıştır. Bu amaçla mantarlar temizlendikten sonra 1-2 cm kalınlığında dilimlenerek sous vide için uygun plastik torbalara 200 g olarak konulmuş ve bir vakum kapatma makinesi (QH-02, White dolphin, Çin) ile vakumlanmıştır. Vakumlanan torbalar 80°C' deki su banyosunda (Wisd, Korea) 10 dakika süreyle tutulmuştur. Isıl işlemin akabinde önce 2 dakika boyunca 20°C'nin altında ve ardından 5 dakika buzlu su banyosunda (5–9°C) tutulmuştur. Soğutulduktan sonra, torbalar -18° C'de depolanmıştır (Aisala ve ark., 2018). Örneklerin duyusal değerlendirmesi kabul edilebilirlik testi ile yapılmıştır (Stone ve ark., 2020). Yozgat Bozok ve Erciyes Üniversitesinde görevli 20 panelistten 1 (en az tercih edilen) -7 (en çok tercih edilen) arasında bir hedonik ölçek skalasına göre her bir mantar örneği için puan vermeleri istenmiştir. Puanların aritmetik ortalaması genel kabul edilebilirlik olarak değerlendirilmiştir.

İstatistiksel Analiz

Veriler arasındaki farkların önemini belirlemek için SPSS 22.0 istatistik paketi (SPSS Inc., Chicago, IL) kullanılmış olup, duncan çoklu karşılaştırma testi ile grup ortalamaları karşılaştırılmıştır.

Bulgular ve Tartışma

Fizikokimyasal Analizler ve Enerji Değerleri

Mantar örneklerine ait temel bileşim analizleri ve enerji değerleri Tablo 1 de verilmiştir.

Tablo 1. Mantarların temel bileşim analizleri ve enerji değerleri

Örnek	Nem (g/100 g taze örnek)	Protein (g/100 g kurumadde)	Kül (g/100 g kurumadde)	Yağ (g/100 g kurumadde)	Karbonhidrat (g/100 g kurumadde)	Enerji değeri (kcal/100 g taze örnek)
Pleurotus ostreatus	91.16±1.12^{a}	22.08±0.1^{a}	7.29±0.1^{b}	1.68±0.5^{ab}	68.94±0.1^{a}	33.52±0.8^{b}
Pleurotus eryngii	89.72±0.7^{a}	22.39±0.4^{a}	7.54±0.1^{b}	1.95±0.3^{a}	68.12±1.02^{a}	39.03±0.4^{a}
Hericium erinaceus	92.48±0.3^{a}	23.31±0.1^{a}	8.64±0.3^{a}	1.58±0.3^{b}	66.47±0.9^{a}	28.08±0.8^{c}

$^{a-c}$Aynı sütundaki farklı harfler örneklerin istatistiksel olarak farklı olduğunu göstermektedir ($p<0.05$).

Mantar örneklerinde en yüksek nem %92.48 g/100 g taze örnek ile *H. erinaceus* da, en düşük nem ise %89.72 g/100 g taze örnek ile *P. eryngii* de tespit edilmiştir. Ancak her 3 mantar arasında nem içeriği açısından önemli bir fark görülmemiştir ($p>0.05$). Nem içeriklerindeki bu değişiklik, mantar cinsine ve türüne, sıcaklık, nisbi rutubet ve metabolik su ilişkisi gibi çevresel faktörlere bağlı olmakla birlikte raf ömrü üzerinde önemli bir parametredir (Van Loon ve ark., 2000).

Protein içerikleri genel anlamda birbirine yakın olsa da en yüksek protein değeri %23.31 g/100 g kurumadde ile *H. erinaceus* da, en düşük protein değeri ise %22.08 g/100

g kurumadde ile *P. ostreatus* mantarında gözlenmiştir. Mantarlarının protein değerleri arasındaki fark istatistiksel olarak önemsiz bulunmuştur (p>0.05). Mantarların iyi bir protein kaynağı olduğu bilinmekle birlikte, bazı araştırmacılara göre mantar bileşimlerindeki aminoasit profilinin hayvansal proteinlerle karşılaştırılabilir nitelikte olduğu düşünülmektedir (Longvah ve Deosthale, 1998).
H. erinaceus %8.64 g/100 g kurumadde ile en yüksek kül değerine sahip iken, *P. ostreatus* %7.29 g/100 g kurumadde ile en düşük kül değerine sahiptir. *Pleurotus* cinsi mantarların kül içeriği birbiri ile istatistiksel olarak farklılık göstermese de (p>0.05), *Hericium* cinsi ile farklılık göstermiştir (p<0.05). Önceki çalışmadan elde edilen kül değerleri ile bu çalışmadan elde edilen kül değerleri arasında farklılıklar olsa da genel anlamda uyumlu olduğu görülmektedir (Mattila ve ark., 2002).
Mantarların yağ içeriği %1.58-%1.95 g/100 g kurumadde arasında değişim göstermiş olup, *H. erinaceus* en düşük yağ içeriğine sahip mantar olmuştur. Çalışmadan elde değerler önceki çalışmalarla kısmi farklılıklar olsa da uyum içindedir (Crisan ve Sands, 1978). Mantarların içermiş olduğu yağ miktarının kimyasal kompozisyonuna bakıldığında linoleik asit ve oleik asit açısından son derece zengin ve bazı mantar türlerinin de yüksek miktarda ergosterol gibi sterolleri içermesi ile dikkat çekicidir (Krzyczkowski ve ark., 2009; Yılmaz ve ark., 2006).
100 g taze mantar örneklerinde en yüksek kalori 39.03 kcal ile *P. eryngii* de, en düşük ise 28.08 kcal ile *H. erinaceus* da hesaplanmıştır. Mantarların kalori değeri genel anlamda düşük olmakla birlikte, toplam kalorinin büyük kısmının karbonhidratlardan ve özelinde diyet liflerden kaynaklandığı bilinmektedir.

Biyoaktif Özellikler

Mantarların TFMM ve antioksidan kapasite değerleri Tablo 2 de verilmiştir.

Tablo 2. Mantarların toplam fenolik madde miktarı ve antioksidan kapasitesi

Örnek	Toplam Fenolik Madde Miktarı (mg GAE/g)	Antioksidan kapasite (µmol TE/g)
P. ostreatus	3.01±0.89^{c}	20.43±1.21^{c}
P. eryngii	4.79±0.48^{b}	26.32±1.76^{b}
H. erinaceus	5.06±0.21^{a}	43.11±2.74^{a}

$^{a-c}$Aynı sütundaki farklı harfler örneklerin istatistiksel olarak farklı olduğunu göstermektedir (p<0.05).

H. erinaceus, P. eryngii ve *P. ostreatus* için sonuçlar sırasıyla; TFMM (5.06±0.21 mg GAE/g, 4.79±0.48 mg GAE/g, 3.01±0.89 mg GAE/g) ve antioksidan kapasitesi (43.11±2.74 µmol TE/g, 26.32±1.76 µmol TE/g, 20.43±1.21 µmol TE/g) olarak bulunmuştur. Örnekler arasında ne yüksek TFMM değeri 5.06±0.21 mg GAE/g ile *H. erinaceus* da en düşük ise 3.01±0.89 mg GAE/g ile *P. ostreatus* da bulunmuştur. Antioksidan kapasite değerleri de TFMM ile pozitif korelasyon göstererek en yüksek 43.11±2.74 µmol TE/g ile *H. erinaceus* da en düşük ise 20.43±1.21 µmol TE/g ile *P. ostreatus* da bulunmuştur. Elde edilen sonuçlar, bazı çalışmalar ile uyum içinde olduğu görülmektedir (Cheung ve ark., 2003; Doğan ve ark., 2020). Fenolik bileşikler kolayca okside olabilme özelliği dolayısıyla antioksidan aktiviteye sahiptirler (Carabias-Martínez ve ark., 2005). Mantarlar içermiş olduğu fenolikler sayesinde yüksek antioksidan özellik gösterirler (Doğan ve ark., 2020). *P. ostreatus* mantarında 1-6 mg fenolik madde/1 g mantar tozu tespit edilmiş olup, lipid oksidasyonunu %36 engellediği ve bu özelliğinden dolayı antioksidan kapasitesinin yüksek olduğu bildirilmiştir (Palacios ve ark., 2011). *P.eryngii* de içermiş olduğu polisakkaritler, polifenoller ve flavonoidler ile yüksek antioksidan aktivite göstermektedir (Dubost ve ark., 2007). Mau ve ark. (2002) tarafından yapılan çalışmada *H. erinaceus* mantarının metanolik ekstraktına geçen polifenollerin; mükemmel indirgeyici, süpürücü ve demir iyonlarını şelatlayıcı etkiye sahip, doğal antioksidanlar olduğu belirlenmiştir.

Duyusal Değerlendirme

Mantar örneklerinin duyusal değerlendirmesi, kabul edilebilirlik testi ile belirlenmiş olup, panelistlerin örneklere vermiş olduğu puanların aritmetik ortalaması genel kabul edilebilirlik olarak değerlendirilmiştir (Tablo 3).

Tablo 3. Mantarların duyusal değerlendirilmesi

Örnek	Genel Kabul Edilirlik
Pleurotus ostreatus	6.55 ±0.5^{b}
Pleurotus eryngii	6.91 ±0.1^{a}
Hericium erinaceus	6.42 ±0.3^{c}

$^{a-c}$Aynı sütundaki farklı harfler örneklerin istatistiksel olarak farklı olduğunu göstermektedir (p<0.05).

Souse-vide yöntemi ile pişirilen mantar çeşitleri arasında en beğenilen mantar 6.91 lik puan ile *P.eryngii* olmuş olup, bunu sırasıyla 6.55 ile *P. ostreatus* ve 6.42 ile *H. erinaceus* takip etmiştir. Panelistlere göre mantar çeşitleri, genel kabul edilebilirlik açısından birbirine yakın skorlar almışsa da istatistiksel olarak farklılık göstermiştir (p <0.05).
Bir gıda ürünü her ne kadar besinsel ve biyoaktif özellikler açısından üstün olsa da gıda mahiyetinde kullanılabilmesi, gastronomik açıdan geçer not alabilmesine bağlıdır. Özellikle tüketim aşinalığı olmayan ürünlerin günlük diyette yer alması, öncelikli olarak tüketiciye duyusal olarak hitap etmesine bağlıdır. Gıdalarda duyusal özelliklerinin bu denli önemli olması son zamanlarda revaçta olan moleküler gastronomiyi gündeme taşımıştır. Moleküler gastronomide kullanılan pişirme tekniklerinden biri de souse-vide pişirme yöntemidir. Sous-vide pişirme, ürünü tekstürel ve besinsel özelliklerine en azan zarar veren yöntemlerden biri olup, kontrollü sıcaklık/sürede vakum ambalaj içerisinde soslu ve/veya sossuz ortamda çeşitli gıdaların hazırlanmasına olanak sağlar. Geleneksel pişirme yöntemlerine göre besin değerlerinde daha az kayıp, homojen ısı dağılımı, daha az nem kaybı, pişerken başında beklememe, kolay

servis ve muhafaza, aroma bileşenlerinde daha az kayıp gibi çeşitli avantajlara sahiptir (Ghazala ve ark., 1996). Günümüzde yenilebilir mantarlar yüksek besin değerleri, benzersiz lezzetleri ve biyoaktif madde içerikleri gibi çeşitli özelliklerinden dolayı tercih edilmektedir. *P. eryngii* yüksek besin, kanıtlanmış tıbbi özellikleri ile bilindiği kadar üstün lezzeti ile de bilinen bir mantar çeşitidir. Kral mantarı olarak da bilinen lezzeti ve gastronomik üstünlüklerinden dolayı *P.* eryngii'ye olan talebin gün geçtikçe arttığı bilinmektedir (Ohga ve Royse, 2004). Çalışma bulgularına göre de en beğenilen mantar türünün *P. eryngii* olması da literatürü destekler niteliktedir.

Dünya nüfusunun artmasına karşın, tarım alanlarının azalması ve toprağın verimsizleşmesi, iklime bağlı olmadan ürün yetiştirme isteği ve tarım ve orman ürünlerindeki endüstrileşme ile atık ve/veya artık ürünlerin artması, insanoğlunu farklı gıda kaynakları üretimine sevk etmiştir. Tüm bu ihtiyaçları karşılama noktasında kültür mantarları çare olabilir. Ülkemizde kültür mantarları üretim ve günlük diyette tüketiminin arttırılması son derece önemlidir. Bu konuda edinilen veriler ivmenin pozitif yönde iyileştiğini göstermektedir. Mantarlar taze ve konserve olarak direk kullanılabildiği gibi kurutulmuş ve ekstrakt formlarında da çeşitli gıda formülasyonlarında halihazırda kullanılmaktadır. Çalışmada kapsamında egzotik mantarlardan *P. ostreatus*, *P. eryngii* ve *H. erinaceus* mantarlarının fizikokimyasal, biyoaktif ve duyusal özellikleri ortaya konulmuştur. Sonuçlar, örneklerin yüksek miktarda protein ve kül içermesine karşın düşük oranda yağ ve kalori değerlerine sahip olduğunu göstermiştir. Bu yaklaşımla mantarların protein değerinin yüksek olması vejeteryan/vegan beslenme de avantaj sağlamaktadır. Mantarlar, kalorisinin düşük olması yanında sahip oldukları kalorinin önemli bir kısmının da içermiş oldukları diyet liflerden kaynaklanması, kalorisi azaltılmış yüksek protein diyetlerinde aranan bir girdi olacaktır. Mantar çeşitlerinin biyoaktivitesinin de yadsınamayacak oranda yüksek olduğu görülmektedir. Ayrıca, besinsel ve biyoaktivite açısından son derece yüksek olması yanında gastronomik açıdan uygunluğu bu mantarların üretim ve tüketimini cazip hale getirmektedir. Bu çalışma kapsamında egzotik mantarlardan yalnızca 3 tanesi çalışılmıştır. Ancak ülkemizde, küresel çapta değerli olan yenilebilir mantarlar üzerine de çalışmalar yapılması ve literatüre kazandırılmasının ileri çalışma konuları arasında değerlendirilmesi gerektiği düşünülmektedir.

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Investigation of Antioxidant Activities of *Agrocybe praecox* Fungus

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Abstract: Mushrooms have been consumed as foodstuffs since time very old. From a nutritional point of view; In addition to containing low calories, it has a rich content in essential amino acids, carbohydrates, fibers, important vitamins and minerals. Antioxidants are one of the most studied topics in recent years and can be defined as a whole of systems that neutralize toxic products that occur as a result of cell metabolism. Antioxidant activities of water extracts of *Agrocybe praecox* (Pers.) Fayod mushrooms were obtained according to total phenolics, total flavonoids, metal chelation and ABTS methods. It was observed that some compounds were found in the extracts used by the analyzes performed in this study and the mushroom had antioxidant properties. In this study, the total amount of phenol contained in *A. praecox* mushroom was detected to be 52.7 mg GAE/g. The amount of quercetin was found to be 1.46 mg QE/g, catechin 23.2 mg CA/g it was found to have total flavonoid properties. The IC50 of the metal chelating activity was calculated as 5.74 mg. The ABTS+ radical scavening activity was calculated as 12.5 mg.

Creating new products by using the compounds present and detected in the fungus or isolating the enzymes with high activity in them and making them more useful in vitro will be an important medical contribution. In addition, determination of its cytotoxic feature will pave the way for new studies in pharmaceutical and medical fields.

Keywords: *Agaricales*, Edible mushroom, Phenolic compounds, Van

Agrocybe praecox Mantarının Antioksidan Aktivitelerinin Araştırılması

Öz: Mantarlar, çok eski zamanlardan beri gıda maddesi olarak tüketilmektedir. Beslenme açısından bakıldığında; Düşük kalori içermesinin yanı sıra esansiyel amino asitler, karbonhidratlar, lifler, önemli vitaminler ve mineraller açısından zengin içeriğe sahiptir. Antioksidanlar, son yıllarda en çok çalışılan konulardan biridir ve hücre metabolizmasının bir sonucu olarak ortaya çıkan toksik ürünleri nötralize eden bir sistem bütünü olarak tanımlanabilir. *Agrocybe praecox* (Pers.) Fayod mantarlarının su ekstraktlarının antioksidan aktiviteleri total fenolikler, total flavonoidler, metal şelasyon ve ABTS yöntemlerine göre elde edildi. Bu çalışmada yapılan analizlerde kullanılan ekstraktlarda bazı bileşiklerin bulunduğu ve mantarın antioksidan özelliklere sahip olduğu görülmüştür. Çalışmanın sonunda *A. praecox* mantarında bulunan toplam fenol miktarı 52.7 mg GAE/g olarak tespit edildi. Kuersetin miktarı 1.46 mg QE/g, kateşin 23.2 mg CA/g toplam flavonoid özelliklere sahip olduğu hesaplandı. Metal şelatlama aktivitesinin IC50'si 5.74 mg olarak belirlendi. ABTS+ radikal süpürme aktivitesi 12.5 mg olarak hesaplandı.

Mantardan tespit edilen bileşiklerin kullanılması ve yüksek aktiviteye sahip enzimlerin izole edilmesiyle elde edilebilecek yeni ürünler önemli bir tıbbi katkı olacaktır. Ek olarak, sitotoksik özelliğinin belirlenmesi farmasötik ve tıbbi alanlarda yeni çalışmaların önünü açacaktır.

Anahtar Kelimeler: *Agaricales*, Yenilebilir mantar, Fenolik bileşikler, Van

Introduction

Mushrooms have been consumed as foodstuffs since time very old. From a nutritional point of view; In addition to containing low calories, it has a rich content in essential amino acids, carbohydrates, fibers, important vitamins and minerals. Fungi are used effectively in the treatment of many diseases with the active ingredients found in their composition (Öztürk et al., 2009, Sevindik et al., 2021). Mushrooms are one of the sources of powerful new pharmaceutical products that are wide and have not yet been largely addressed. Mushrooms are used as medicines and foods because they have rich protein and carbohydrate content all over the world (Wasser, 2002, Eraslan et al., 2021). Active ingredients found in fungi include pheasants; they have attracted a lot of attention due to their antioxidant and anti-inflammatory effects (Cayan et al., 2018, Bal et al., 2019).

A nutraceutical is defined as a substance that provides medical or health benefits for the prevention and treatment of the disease as part of a food or food. Nutraceuticals extend from isolated nutrients and nutritional supplements to genetically modified design foods, plant products and 4 processed products such as cereals, soups and beverages. Some examples of nutritious nutraceuticals or functional foods are dietary fibers, polyunsaturated fatty acids, proteins, peptides, amino acids, ketocytes, minerals, antioxidative vitamins, and other antioxidants such as glutathione, selenium, etc. (Andlauer et al., 2002; Kruger et al., 2003). The presence of specific bioactive compounds makes fungi therapeutically valuable by strengthening the immune system as well as preventing and treating life-threatening diseases such as heart diseases, hypertension, cerebral stroke and cancer. Fungi are known to exhibit antifungal, anti-inflammatory, antiviral, antibacterial, hepataprotective, antidiyabetic, hypolypydemic and hypotensive activities (Rathore et al., 2017).

The human relationship with mushrooms is fascinating as it has been used as both food and medicine for the past 20 years. The use of mushrooms has expanded not only as food, but also in the fields of pharmaceuticals, nutraceuticals and cosmetics (Rathore et al., 2017). Mushrooms have been consumed and appreciated as food for their exquisite flavors, economic and ecological values and medicinal properties for years. Overall, mushrooms contain 90% water and 10% dry matter (Sánchez, 2010). For this reason, the life expectancy is short and immediately rots. Nutritional values are comparable to eggs, milk and meat and in addition to their nutritional value, they are considered functional foods due to their health benefits (Rathee et al., 2012).

Mushrooms contain vitamins (thiamine, riboflavin, ascorbic acid, ergosterol and niasin as well as plenty of essential amino acids. Mushrooms also contain proteins, fats, ash and glucosites. Essential oils, tocopherols, phenolic compounds, flavonoids, carotenoids, folates, organic acids, etc. are other components of fungi (Sánchez, 2004; Patel et al., 2012). Mushrooms are used as a traditional drug to prevent and cure various diseases due to their lack of side effects, and their use is increasing day by day. Among natural products, mushrooms are seen as the most potential candidate in clinical trials due to the inexpensive ability to obtain easily and abundantly. Antibiotics of fungal origin are used today for bacterial infections. Research has focused on the antiquated nature of the fungus through antifungal carbohydrates, especially its effect on lung cancer. The use of fungi as a treater by various tribes from a long time ago revealed the importance of their medical potential and led researchers to emphasize their views on their modern medical potential. Mushrooms were also mixed with other fungi or herbs in societies where natural treatment was applied, increasing/reducing their bioactivity or preventing side effects (Blackwell, 2010). Many researchers have found that edible mushrooms are the source of various nutraceutical compounds, including polysaccharides (β-glucan), dietary fibers, terpenes, peptides, glycoproteins, alcohols, mineral elements, unsaturated fatty acids and antioxidants such as phenolic compounds, tocopherols and ascorbic acid (Pardeshi et al., 2009).

Today many types of fungi are used for medical purposes due to their antibiotic, antiquated, immune-regulatory, cardiovascular and antumor properties. Mushrooms contain phenolic compounds, antioxidants and important in terms of specific amino acids. In addition, the protein deficit that cannot be obtained from animal foods. It is an extremely delicious food product that can be eliminated (Çavuşoğlu et al., 2018; Sevindik et al., 2018). Antioxidants are one of the most studied topics in recent years and can be defined as a whole of systems that neutralize toxic products that occur as a result of cell metabolism. Water extracts of dried wild edible mushrooms have been analyzed in different experiments in terms of antioxidant activity, ferric antioxidant reduction power (FRAP), cleansing activity on 1.1-difenil-2-picrilhydrazyl (DPPH) radicals, and have been found to have the potential for natural antioxidants (Keleş et al., 2011).

A. praecox (Bahar meteliği) (Sesli et al., 2020) *(Basidiomycetes, Agaricales, Strophariaceae*) has several features that initiate biosystematic research. Members of this group are phenotypically polymorphic and contain a variety of woody substrates. As a result, taxa identified as *A. praecox* are often confused with morphologically different taxa such as *A. molesta* (Lasch) Singer (Şen metelik) (Sesli et al., 2020) and additional taxa are often identified from a single collection. Variable criteria used to identify taxa have formed a nomenclature (Flynn,1986).

The genus *Agrocybe* produces various growth regulators such as growth stimulation and suppression on

plants. *A. praecox* is an edible mushroom species worldwide, and northern temperate is fairly common. These fungi are non-specific enzymes that grow in the soil. Enzymes make the monomers and oligomers in the plant usable. The fungus is seen as a focal point for bioremediation and hydrolytic and ligninolytic enzymatic activities (Fushimi et al., 2012).

Materials and Methods

Collection of mushroom samples

The fungal samples used in the study were collected from the Campus of Van Yuzuncu Yıl University and examined in the research laboratory of the Faculty of Science, Department of Molecular Biology and Genetics. Mushroom diagnosis was made by Dr. İsmail ACAR.

Mushroom extraction

Collected mushroom samples were kept at -20°C until experiments were carried out. The mushrooms, which were then ground, weighed 10g on a delicate scale. 150 mL of hexane was placed on it and left in a magnetic mixer with a heater for 8 hours. From the samples, hexane was filtered and 150 mL of ethanol was added to the remaining dry matter and left for incubation for 48 hours. After 48 hours, ethanol was filtered and ethanol was flown, 150 mL of water was added to the extract and incubated for 48 hours. After the water was filtered from the mixture and taken into 50 mL falcon tubes, it was placed horizontally at -80 °C to stay for 1 night and left in the lyophilizer for 72 hours to remove the water from the samples. The efficiency of the dried samples was calculated by the following equation.

Extraction(w/w)=(mass of dried extract)/(total sample mass)x1

Determination total phenol capacity

Total phenol capacity was performed according to the optimized form of by using the Folin-Ciocalteu method of Singleton and Rossi (1999) technique. After the fungal extract was dissolved in pure water and mixed with the extract Folin-Ciocalteu's reagent and added %10 sodium carbonate on top, the mixture was added to the microbe wells and piped and left in the dark at room temperature for 30 minutes. The absorbances of the samples were read at 750 nm. Gallic acid was used as standard. The experiment was run/studied in 3 replicates.

Determination total flavonoid content

Flavonoid content was performed according to the optimized form of by Zhishen et al, (1999) technique. Quercetin and Catechin were used as standard. Different concentrations were obtained by serial dilution from the stock standard. After the fungal extract was dissolved in 1 mL of pure water and added to the microplate wells, pure water and sodium nitrite were added for 5 minutes and then aluminum chloride was added to it and pure water was added for 6 minutes and the wells were completed to 200 µl and measurements were taken at 415 nm and 510 nm. Experiment 3 was studied to happen again.

Determination metal chelating activity

Metal chelating activity was performed according to the optimized form of by Dinis et al., (1994) technique. EDTA (Etilendiamintetraacetic acid) was preferred as standard. By preparing the stock concentration and obtaining different concentrations with serial dilution, extracts of this different concentration were added to the microplate wells and ferrozin, iron (II) chloride and methanol were added to it and 562 nm reading was performed after incubation at room temperature of 10 minutes.

Determination ABTS radical scavening activity

ABTS radical sweeping activity was performed according to the optimized form of by Re et al., (1999) technique. For the creation of ABTS radical, 7 Mm ABTS solution 2.45 Mm potassium persulfate solution is mixed in a ratio of 1:1 and reacted. It is kept in a dark environment at room temperature for 16 hours before radical use. Extract and diluted ABTS radical are added to the plate with 96 wells, mixed and incubated at room temperature for 6 minutes. After incubation, absorbance reading is performed at 734 nm.

Results

In the study, antioxidant activities of water extracts of *Agrocybe praecox* fungus were looked at. The yield of water extracts was calculated and found to be 15%.

Antioxidant activities of *A. praecox* fungus; total phenol was determined using total flavonoid, metal chelating and ABTS methods.

Gallic Acid was used as standard to determine the total phenol content. Gallic Acid Standard Curve is given in Figure 1.

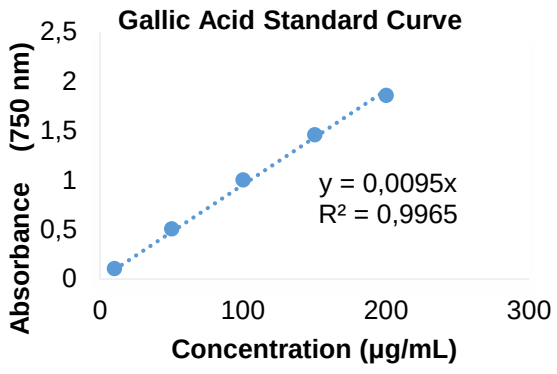

Figure 1. Gallic Acid Standard Curve, Experiments studied to be 3 repe.

The total phenolic content of *A. praecox* fungus was calculated according to 3 different concentrations. The results obtained from the fungus are given in Table 1.

For the determination of total flavonoid content Quercetin and Catechin were used as standard and standard curves were drawn. The standard curves of Quercetin and Catechin are given in Figures 2 and 3 respectively.

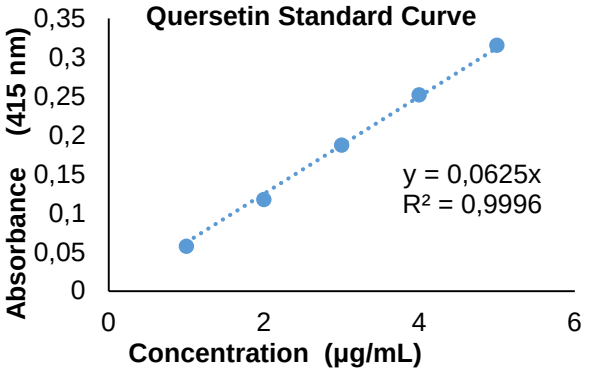

Figure 2. Quersetin Standard Curve, Experiments were studied to be 3 repe.

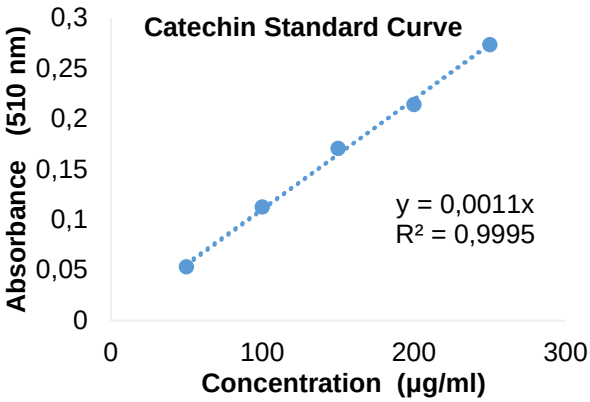

Figure 3. The Standard Curve of the Catechin was studied to be experiments 3 repe.

Total flavonoid content of *A. praecox* mushroom was calculated according to 3 different concentrations. The results obtained from mushrooms are given in Table 1.

EDTA was used as standard for determination of metal chelating activity. EDTA Standard Curve is given in Figure 4.

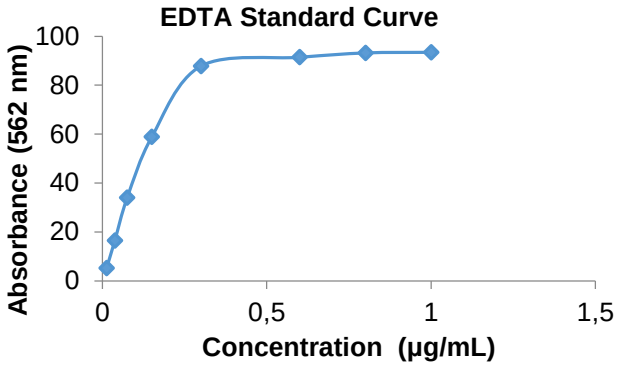

Figure 4. EDTA Standard Curve, Experiments were studied to be 3 repe. EDTA was used as standard.

Metal chelating activity of *A. praecox* mushroom was calculated according to 7 different concentrations. The results obtained from mushrooms are given in Table 1.

Trolox was used as a standard for determination of radical scavenging activity. Trolox Standard Curve is given in Figure 5.

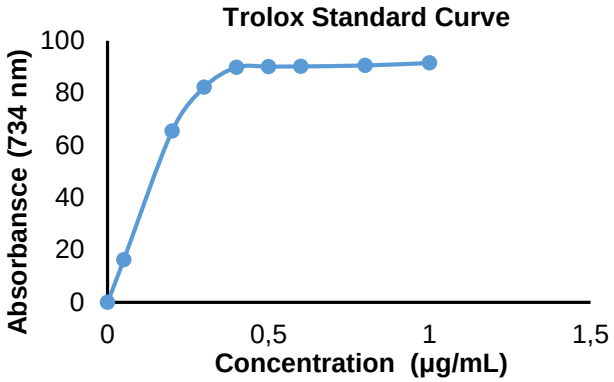

Figure 5. Trolox Standard Curve, Experiments were studied to be 3 repe another.

Radical scavenging activity of *A. praecox* mushroom was calculated according to different concentrations. The results obtained from mushrooms are given in Table 1.

Table 1. Antioxidant activities of the fungus *A. praecox*

	Agrocybe praecox
Yield	%15
Total phenol	52.7 ± 1.90 mg/mL
Catechin	23.2 ± 2.09 mg/mL
Quercetin	1.46 ± 0.3 mg/mL
Metal chelating	5.74 ± 0.09 mg/mL
ABTS	12.5 ± 0.35 mg/mL

*Values are presented as mean±SD; Experiments were made in 3 pere.

In this study, *A. praecox* fungus samples were collected and powdered and the antioxidant activities of the obtained water extracts were examined. Water extract yield is given in Table 1. Antioxidant activity of *A. praecox* fungus was investigated using total phenol, total flavonoid, metal chelation and ABTS methods.

The total phenolic activity of the fungus used in the study was determined as 52.7 GAE/mg crude extract and the phenolic compounds it contained were found to be quite high. Total flavonoid activity was calculated as 1.46 QE/mg and 23.2 CE/mg. It may be where it is more effective than the standard catechin quercetin. It is well known that fungi absorb or store heavy metals in the environment. Based on this, the metal chelating activity of *A. praecox* fungus was examined and by calculating the IC_{50}, the percentage inhibition of the fungus was determined to be 5.74 mg when the chelating activity of heavy metals was high. The damage of free radicals to the body has been supported by research and the presence of antioxidants must be present in order to cleanse the radicals mushroom. Write with the study that we have antioxidant properties of *A. praecox* fungus.

Discussion and Conclusion

Mushrooms are an easily digestible protein source that contains less than animal foods but more protein than many plant foods (Andlauer et al., 2002; Kruger et al., 2003). Fungi contain all the essential amino acids but small amounts of methionine, cysteine and sulfuric amino acids. Fungi contain all minerals at all stages of their development including abundant P and K to a lesser extent Fe and Ca (Andlauer et al., 2002; Kruger et al., 2003). They are also a source of vitamins such as thiamine, riboflavin, niacin, biotin and ascorbic acid (Andlauer et al., 2002; Kruger et al., 2003). In addition, since fungi are rich sources of folic acid they can be used in the treatment of anemia (Schwartz., 1950). Some types of fungi contain high levels of β-carotene and ergosterol. These compounds are converted to active vitamin D when exposed to UV rays (Breene, 1990). In addition, chanterelle (2.9-5.8 µg / 100 µg) contains high amount of vitamin D2 (Mattila et al., 2000). Mushrooms; fatty acids, glycerides (mono-, di- and tri-), sterols, sterol esters and phospholipids etc. It contains all the main lipid components. However, their oil content is generally low, about 2-8% in dried mushrooms (Öztürk et al., 2009). Fungi contain many biochemical substances with therapeutic activity such as protein polysaccharide compounds (polysaccharide-K, polysaccharide peptide and lentinan), secondary metabolites (terpenes, alkaloids and lactones) and enzymes (lactase, glucose oxidase and peroxidase (Smith et al., 2002).

Fungi can synthesize some phenolic compounds, flavanoids, tocopherols, ascorbic acid, quinones, terpenoids and phenyl propanoid derivative compounds with antioxidant effect. The compounds calvacin, volvotoxin, flammutoxin, lentinan and poricin, which have antitumor effect are very important antioxidant substances isolated only from macro fungi. These compounds also show antiviral properties (Smith et al., 2002).

Numerous medicinal effects of commercial mushrooms have been discovered as well as their nutritional properties (Türkoğlu et al., 2006). Recently, a large number of fungi used in scientific studies on new treatment methods; It has been found to have many therapeutic effects such as antitumor, anti-inflammatory, immunosupporting and antibiotic effects. In the literature study, there is no information showing that the mushroom sample used in this study has antioxidant properties. Only a few studies have noted that this mushroom has antitumor properties (Rathore et al., 2017).

Thanks to our study, it has been proven that *A. praecox* edible fungi have antioxidant properties and it has been one of our aims to add new information to the literature and to enable researchers to benefit from this information. It is obvious how important fungi are used as food supplements among the people. It reveals the importance of their use and importance in terms of the demands placed on them since ancient times. With further studies a more comprehensive study will be conducted by making antitumor, antibacterial and cytotoxicity analyzes of this species. Gallic acid, quercetin, catechin, chelating activity and radical scavenging activity values of the water extract of the *A. praecox* mushroom used in the study were found to be quite high. Similar results were obtained in other studies

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Isolation and Identification of *Penicillium toxicarium*: A New Record for Turkey

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Abstract: In this study *Penicillium toxicarium* was cited for the first time as a new record of *Penicillium* in Turkey. This species was isolated from decaying wood samples in Usak province, Turkey. Morphological, colonial and molecular identification were performed. Fungal DNA Isolation Kit and universal internal transcribed spacer (ITS) primers were used for molecular identification.

Keywords: *Penicillium toxicarium* , *Penicillium* ,Turkey, Fungi, Mycobiota ,Record

Penicillium toxicarium'un İzolasyonu Ve İdentifikasyonu: Türkiye İçin Yeni Kayıt

Öz: Bu çalışmada *Penicillium toxicarium* Türkiye'de ilk kez yeni bir *Penicillium* türü olarak gösterildi. Bu tür, Türkiye'nin Uşak ilinde çürüyen ağaç örneklerinden tespit edilmiştir. Makroskobik ve mikroskobik morfolojiksine göreve moleküler tani yöntemlerine gore tanımlama yapıldı. Fungal DNA İzolasyon Kiti ve evrensel primerler kullanılarak ITS bölgsi dizi analizleri moleküler tanımlama için kullanıldı.

AnahtarKelimeler: *Penicillium toxicarium, Penicillium*, Türkiye, Mantarlar, Mikobiyota, Kayıt

Introduction

Traditionally, fungal species determination depends on media culturing and macro or microscopic appearances identification.

However, these methods have disadvantages, as the evaluation of fungal species according to the existence or not of fruiting structures, the incapacity of cultural growth of specific types, and the uneasy recognizing to the species level depending on microscopic appearances. Hence, molecular methods have been found as rapid and easy methods for identifying of fungi to species level and found as a dependable replacement to conventional techniques (Kiraz N, 2015).

Generally the recognition of fungal species molecularly depends on the amplification and sequencing of (ITS) region of the genetic material of fungi, which shows greatliability to vary or change with in the species or even in community of the similar species. ITS primers (ITS1 and ITS4) have been used widely in molecular studies of fungi identification (Irinyi et al., 2015).

Penicillium genus includes a wide range of species that look similar. Morphology, colonyforms, metabolite description and molecular informations have been used recentlyfor taxonomy of this genus.

Molecular phylogenetic examinations depending on (ITS) have been applied for distinguishing of the *Penicillium* species (Berbee et al.,1995; Peterson, 2000b).

There are over 250 species of *Penicillium* genus (Visagie et al., 2014; Abastabar et al., 2016). Many of *Penicillium* species had been isolated and identified from the different places of Turkey. A check list documented by Asan, (2000) gave about 159 species of *Penicillium* in 2000, and later data base added 66 species to the earlier list, to reach the total number of *Penicillium* species isolated in Turkey to 225 as of February 10, 2015 (Asan, 2004).

After the set wo checklist, the number of novel *Penicillium* species isolated in Turkey continued in increase (Çakır and Maden, 2015; Kolanlarli et al., 2019). Recent check list that published in 2020 have been included a huge list of certain fungi found inTurkey (Sesli et al., 2020).

Although many of published articles and variety of *Penicillium sp.* that had been isolated in Turkey, up to date we couldn't find one published article about isolation of *Penicillium toxicarium* I. Miyake ex C. Ramírez in Turkey.

So the purpose of this paper is to record for the first time the isolation and identification of *Penicillium toxicarium* in Turkey.

Materials and Methods

Materials

For fungus isolation process two media have been used which are Potato Dextrose Agar (PDA) (Merck 110130) and Rose Bengal Agar (RBA). Czapek-Dox Agar (CDA) and Malt Extract Agar (MEA) (Merck 105398) have been used for macroscopic and microscopic identification.

Decaying wood samples around Uşak University 1 Eylül Campus were collected for the isolation of the species reported in the study (Figure 1).

The collected *Pinus nigra* Arnold. *subsp. Pallasiana* (Lamb.) Holmboe wood samples were diluted with distilled water. Rose Bengal Agar (RBA) and Potato Dextrose Agar (PDA) were inoculated by the samples and left to incubate for a week in the dark environment at 25 °C. At the end of one week incubation, colonies were selected and stored in pure culture at + 4℃ in RBA. Macroscopic and microscopic descriptions of the isolated fungi were made. Fungi were cultured on CDA and MEA at 25 °C in 14 days for identification. In macroscopic identification of fungi, colony growth pattern, surface topography surface texture smell, pigmentation, mycelium and sporulation pattern were assessed. In microscopic identification, measurements were made from slide preparations stained with lactophenol-aniline blue. Microscopic identification is based on the branching shape of conidiophores, the shape and emergence of phialids, as well the shape, color and wall characteristics of the conidia (Hasenekoğlu, 1991*;* Singh, 2014).

Penicillium toxicarium I. Miyake ex C. Ramírez, Manual and Atlas of the *Penicillia* (Amsterdam): 125 (1982) Specimen examined: Colonies on CDA were 40 mm in diameter.

Moderately deep, dense and velutinous to lanose, radially sulcate and often centrally wrinkled; the margins were narrow with mycelium that were white; sporulation moderate, deep blue or blue-green in color; without exudates; soluble pigment pale amber; without odor; a reverse coloration of salmon pale.

Figure 1**:** Uşak University 1 Eylül Campus where *Pinus nigra* Arnold*. subsp. pallasiana* decaying wood sample has been collected (from Google maps)

Colonies on MEA were 20~25 mm in diameter, dense and velutinous, plane to radially sulcate; margins entire or ırregular; mycelium white, becoming yellow or green; sporulation moderate to danse; exudates yellow; soluble pigment brown (Figure 2). Conidiophores monoverticillate, stipes delicate 50~10 ×2.0~2.5 µm, smooth walled; phialides phialides ampulliform (7.5~10.0)×(2.3~3.5) µm, in verticils of 3~6,; Conidia, globose 2~2.5 µm, slightly rough, borne in short disordered chains (Figure 3).

PCR reaction was carried out with Solis Biodyne (Estonia) FIREPol® and DNA Polymerase taq polymerase enzyme. After PCR, a single band was obtained in agarose gel using 100 bp DNA Ladder Ready toLoad (SolisBioDyne) marker, and it was observed that the PCR process was successful. Colony characteristics and micro-morphology of the fungus were similar to thedescription of P. toxicarium (Pitt,1979)

Molecular identification has been performed by culturing of the isolated fungus on potato dextrose agar (PDA). The EurXGene MATRIX Plant and Fungi DNA isolation kit was used for DNA isolation from fungus (https://eurx.com.pl/docs/manuals/en/e3595.pdf.). Spectrophotometric measurement (Thermo Scientific Nanodrop 2000 USA) was carried out in order to control the amount and purity of DNA obtained after DNA isolation.

In the PCR study, gene regions targeted for species identification were amplified using fungal specifically universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Gardes and Bruns, 1993).

PCR reaction was carried out with Solis Biodyne (Estonia) FIREPol® and DNA Polymerase taq polymerase enzyme. After PCR, a single band was obtained in agarose gel using 100 bp DNA Ladder Ready toLoad (SolisBioDyne) marker, and it was observed that the PCR process was successful.

ExoSAP-IT ™ for single band samples was obtained in the PCR product purification stage. The PCR product was purified using a PCR Product Clean up Reagent (Thermo Fisher Scientific, USA) and was performed according to the procedures of the kit (www.thermofisher.com). For Sanger sequencing, the ABI 3730XL Sanger sequencer (Applied Biosystems, Foster City, CA) and Big Dye Terminator v3.1 Cyclesequencing kit have been used. Reads obtained with the ITS primers were contiguous to form a consensus sequence. CAP contig assembly in BioEdit software to perform this operation algorithm is used.

Species (OK037182) determination result according to the nearest species *Penicillium toxicarium* on NCBI Showed the following data:

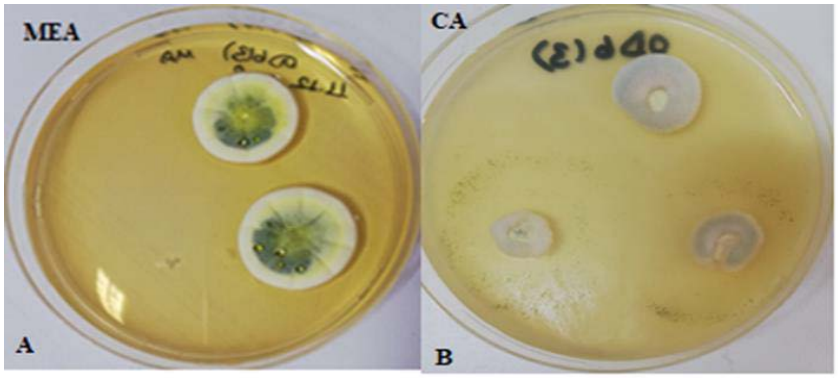

Figure 2: Colonies appearance of *P.toxicarium* on (A). MEA and (B) on CDA (Korcan vd, 2021)

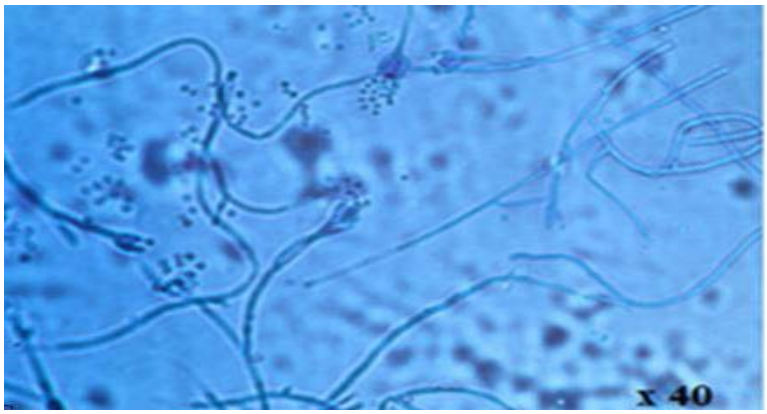

Figure 3: Microscope images of *P. Toxicarium* under High Power Objective Lens (40x) (Korcan et al., 2021)

Penicillium toxicarium

Total Base Number: 532

SimilarityScore: 983

SequenceMatch Rate: 100%

Similarity Rate: 100% (Korcan et al., 2012).

The FASTA format revealed the following data:

>OK037182.1 *Penicillium toxicarium* strain KJ173540.1 internaltranscribedspacer 1, partialsequence; 5.8S ribosomal RNA gene andinternaltranscribedspacer 2, completesequence; andlargesubunitribosomal RNA gene, partialsequence

GGTCACCTCCCACCCGTGTTTATCGTACCTTGTTGCT
TCGGCGGGCCCGCCGCAAGGCCGCCGGGGGGCA
TCTGCCCTCTGGCCCGCGCCCGCCGAAGACACCATTG
AACGCTGTCTGAAGATTGCAGTCTGAGCAATTA

GTTAAATAACTTAAAACTTTCAACAACGGATCTCTTGGTTC
CGGCATCGATGAAGAACGCAGCGAAATGC
GATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGT
CTTTGAACGCACATTGCGCCCCCTGGTAT
TCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAA
GCACGGCTTGTGTGTTGGGCTCCGTCCTC
CTTCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGT
CCGGTCCTCGAGCGTATGGGGCTTCGTCA
CCCGCTCTGCAGGCCCGGCCGGCGCTTGCCGACACATCAAT
CTTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGC
TGAACTTAAGCATATCAATAAGCGGAGGAA

Methods

Obtained sequences have been matched to the ID region presenting the Genbankdata base using the BLAST application of the GenBank NCBI data base. Sequences generated from materials in this study and extracted from GenBank were initially aligned by clustal W using the MegaX program and the phylogenetic tree was created using the neighbor joining, UPGMA and Neighbor-Joining phylogenetic analysis (Saitou and Nei, 1987).

Results

It is widely used in species identification and interspecies relations in taxonomic studies. ITSs regions have become an accepted official molecular barcode in the taxonomy of fungi,

In this study, phylogenetic trees were drawn by including the ITS region analyzed. Accession Numbers of

DNA sequences used in constructing phylogenetic trees are given in the table 1.

The phylogenetic tree deduced from the ID region showed similarity to *P. toxicarium* 100% *Penicillium toxicarium* KJ173540.1, *Penicillium toxicarium* strain EIODSF017, *Penicillium toxicarium* strain CNU 06007, *Penicillium toxicarium* strain NRRL 6172, *Penicillium toxicarium* strain NRRL 29751 and *Penicillium toxicarium* strain NRRL 2047 (Figure 4-6).

Discussion:

Penicillium is a genus that often found and isolated from different habitats like, soil ,food ,indoor habitat, and decaying wood .Being as decompose ragent making this fungi play an important role ecologically (Pitt, 1979; Visagie et al., 2014; Peterson, Bayer et al., 2004).

Penicillium species have been isolated as endophytes of large variety of plants (Nicoletti et al., 2014). It has been found that through living as endophytes, *Penicillium* species can provide plants protection against biotic stresses and pathogen attacks as well enhance their growth (Waqas, 2015; Hassan, 2017). Many endophytic *Penicillium* sp. Have been distinguished as biocatalysts, promoters of plant growth, phytoremediators, and producers of enzyme (Toghueo and Boyom, 2020). (Kim et al., 2008) recognized many species of *Penicillium*, through is work on the diversity of endophytic fungi from needles of pine trees in Korea, Two species which were new to Korea has been identified in this study and *Penicillium toxicarium* was one of them (Kim et al., 2008).

Recent technologies have showed characters, which were earlier not indicated in some species (Paterson et al., 2003). Analyses of .Rdna sequence revealed that the subgenus *Penicillium* is mainly monophyletic, and present species may be diversities (Peterson, 2000a).

So depending on the novel molecular techniques as well morphological characteristics, has been participated in the isolation of new species of *Penicillium* which have not been reported in certain regions before (Deng et al., 2012).

(Sugiura et al., 2020) stated that *Penicillium citreonigrum* NBRC 4692 was originally isolated as the toxigenic fungus responsible for the yellow rice incident in Japan in 1937 and named *Penicillium toxicarium* by I. Miyake, which was considered invalid due to the lack of a Latin diagnosis. Later *P. toxicarium*, was validated by C. Ramírez in 1982 with a Latin diagnosis and type designation. But *P. toxicarium* was assigned to *Penicillium trzebinskii* by Houbraken et al. in 2014. Later *Penicillium toxicarium* was treated as synonym of *Penicillium citreosulfuratum* based on the conclusion of molecular phylogenetic analysis by Visagie et al. 2016.

(Sugiura et al.2021) discovered the taxonomic and nomenclatural short communication (in Japanese) by I. Miyake in 1947 on *P. toxicarium* sp. nov. with its Latin description and four illustrations but lacking the type designation.

They determined that phylogenetic analysis revealed that the NBRC strain belongs to a unique clade, different from the clade comprising *P. citreosulfuratum* strains. Consequently, *P. toxicarium* I. Miyake (1947) was reinstated as a correct name with the lectotype designation by Sugiura et al. 2020.

Many articles regarding flora studies of mycobiota in Turkey have been reported. In 2000 a check list documented most of these articles since 1914 and reported about 159 of *Penicillium* species (Asan, 2000).

In 2004 another check list swhich was updated in 2015, added 66 species to the earlier list, increasing the number of isolated in Turkey to 225 (Asan, 2004).Up to date more than this number are found since reporting of new record of *Penicillium* species is in continue (Çakır and Maden, 2015, Kolanlarli et al., 2019).

Recent check list about certain fungi found in Turkey has been also published in 2020 (Sesli et al., 2020).

Despite of all these articles and the huge number of *Penicillim* species isolated in Turkey, up to the writing of this paper we couldn't find one published article reporting the isolation of *Penicillium toxicrium* in Turkey. So were corded for the first time the isolation of *Penicillium toxicarium* in Turkey.

Table 1 BLAST analysis results by ITS gene region

Species Accession Number	Matching species	Matching species rate (MI)	Matching species Accession Number
OK037182.1	*Penicillium toxicarium* strain EIODSF017	532/532(100%)	KJ173540.1
OK037182.1	Fungal sp. strain Xmf132	532/532(100%)	KX098096.1
OK037182.1	*Penicillium citreosulfuratum*	528/528(100%)	NR153252.1
OK037182.1	*Penicillium toxicarium* strain NZD-mf65	527/530(99%)	KM278076.1
OK037182.1	*Penicillium sp.*isolate MG-09 i	532/533(99%)	MK788347.1
OK037182.1	*Penicillium toxicarium* strain NZD-mf144	527/527(100%)	KM278008.1
OK037182.1	*Penicillium toxicarium* strain	532/532(100%)	KJ173540.1
OK037182.1	*Eurotiales sp.*	516/516(100%)	MG437224.1
OK037182.1	*Penicillium fundyense* strain KAS 2174	524/527(99%)	KT887853.1
OK037182.1	*Phialocephala fortinii* strain PPE5	525/527(99%)	KM042212.1
OK037182.1	Penicillium sp. CBS 140612	525/528(99%)	KX961207.1
OK037182.1	*Penicillium toxicarium* strain S4-M-3-10	528/528(100%)	KP216896.1
OK037182.1	*Penicillium toxicarium* strain CNU 060075	528/528(100%)	FJ557247.1
OK037182.1	*Penicillium toxicarium* strain NRRL 6172	528/528(100%)	EF198650.1
OK037182.1	*Penicillium toxicarium* strain NRRL 29751	528/528(100%)	EF198654.1
OK037182.1	*Penicillium toxicarium* strain NRRL 2047	528/528(100%)	EF198648.1
OK037182.1	Penicillium sp. strain Eef-3	527/527(100%)	MK120856.1
OK037182.1	*Penicillium toxicarium* strain NRRL 35628	527/528(99%)	EF198662.1
OK037182.1	*Penicillium toxicarium* strain NRRL 29679	528/529(99%)	EF198652.1
OK037182.1	*Penicillium toxicarium* strain R6-1-1	527/529(99%)	HM042308.1
OK037182.1	*Penicillium toxicarium* isolate 97	528/531(99%)	KP794065.1
OK0371821	*Penicillium sp. strain MG-02*	503/524(96%)	MK788349.1

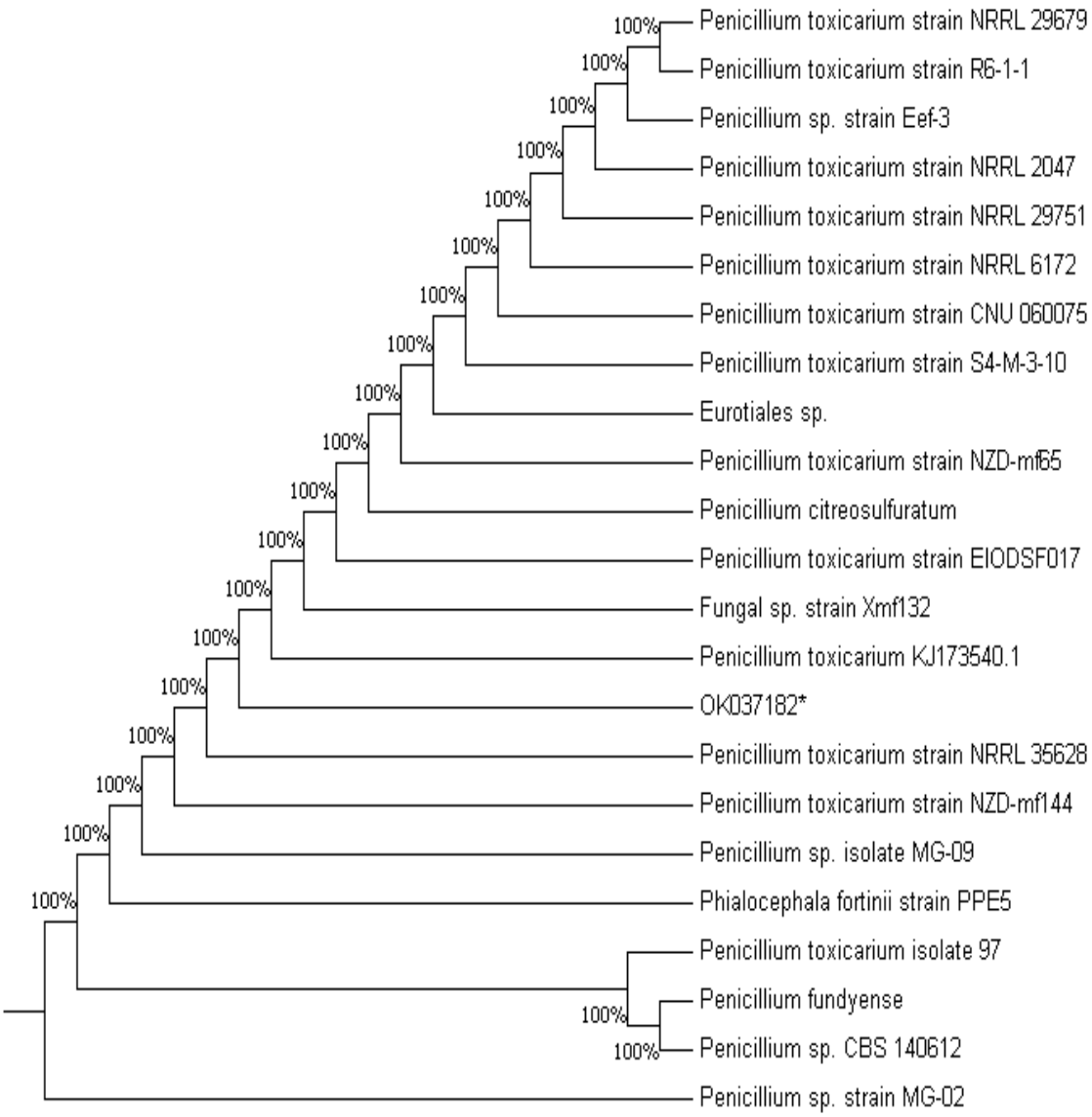

Figure 4: UPGMA phylogenetic analysis of OK037182.1

UPGMA tree shows that isolated strain in our study belongs to *P. toxicarium*. Branching of *Penicillum* species, which were molecularly identified according to ITS gene region, in the UPGMA phylogenetic tree Reference strain sequences were obtained from NCBI Genkbank.

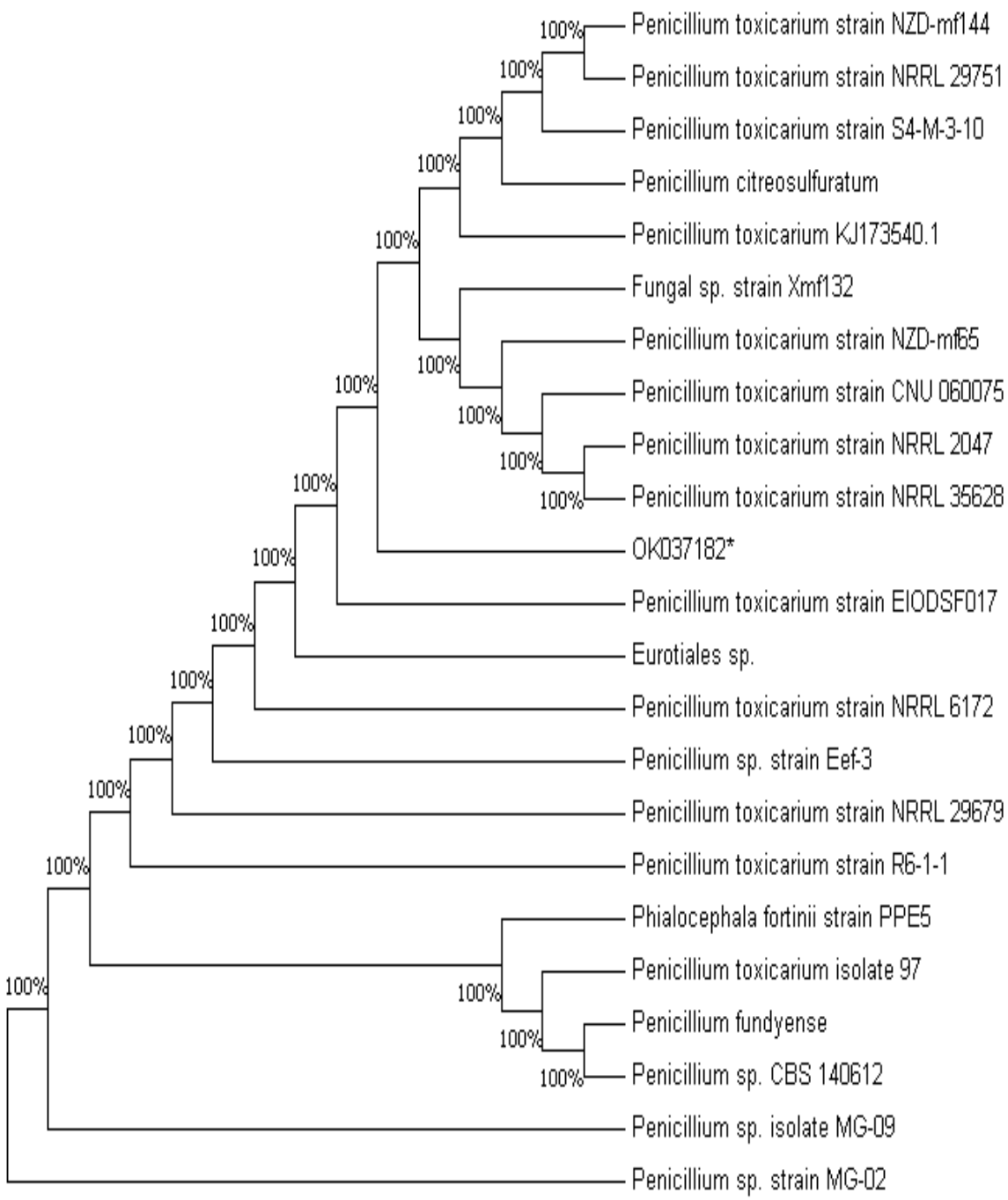

Figure 5 Neighbor-Joining phylogenetic analysis of OK037182.1

The phylogenetic tree deduced from the ID region showed 100% similarity to *P. toxicarium* Neighbor-Joining phylogenetic analysis of *Penicillium* species diagnosed molecularly according to ITS gene region. Reference strain sequences from NCBI Genkbank taken.

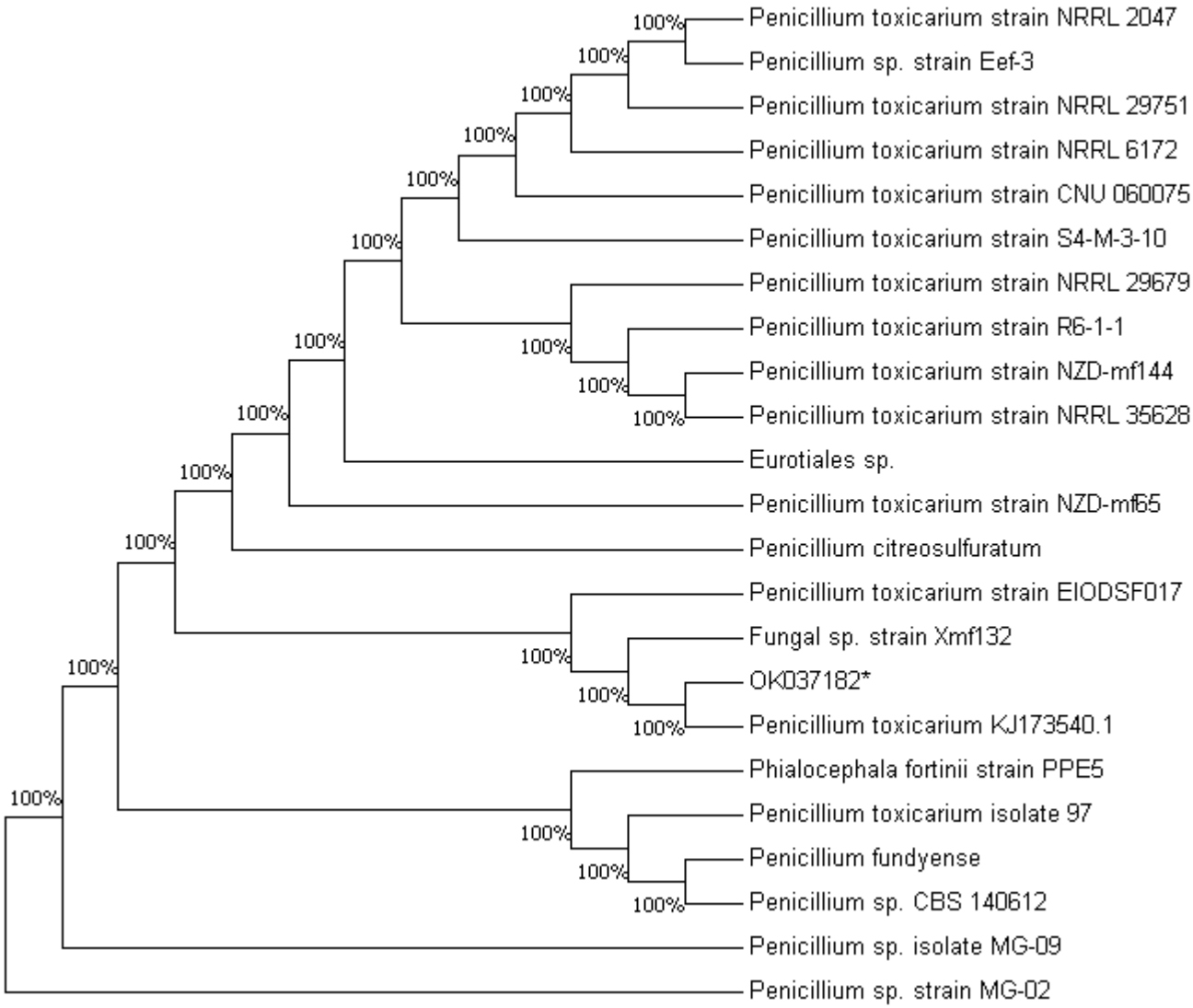

Figure 6. Maximum Likelihood phylogenetic analysis of OK037182.1

The presence of this species in Turkey opens the door for further studies to discover the facts behind the appearance of this unfamiliar species in the ecology of Turkey. Dispersal of this species to other regions of Turkey must also be studied and founding weather this species is originally one of the mycobiota of Turkey or being transported from other regions especially that the isolation of this species in this study was conducted in university campus area. Habitat so ther than decaying wood, more morphological and biochemical studies as well environmental, physiochemical and other factors should all be investigated in future studies.

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Comparing Cellulotic Enzyme Activities of *Neocallimastix* sp. in Orpin's and Menke's Media

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Abstract: *Neocallimastix* sp. is a member of the Anaerobic Gut Fungi (AGF) family with unique lignocellulotic enzymes, and it is usually grown in two basic media *in vitro*. Orpin's mediais preferred for the detection of enzymatic activites while Menke's is mostly chosen for evaluating the gases produced during the fermantation. Although these two media were shown to be effective for their targeted purposes, no attempt has been made to compare the activities of various cellulotic enzymes in them. In this study, we measured the growth rate of *Neocallimastix* sp. in these two media up to 7 days and A similar progress was observed in both ($p>0.05$). We also discovered that Menke's media was better for the 5th ($p<0.001$) and 7th ($p<0.0.1$) days of Avicellase and for up to 5 days [0 ($p<0.001$), 3 ($p<0.01$), 5 ($p<0.0001$)] of Xylanase activity. Orpin's media, on the other hand, displayed superior CMCase activity in all time points[0 ($p<0.001$), 3 ($p<0.0001$), 5 ($p<0.05$, 7 ($p<0.001$)]. As for Cellulase, the activities were measured virtually the same for the both media on the 0th and the 3rd days whereas they were higher in Menke's ($p<0.0001$) on the 5th, and in Orpin's on the 7th day ($p<0.01$). As a result, in Xylanase enzyme studies, it has been determined that menke media gives better results.

Keywords: Anaerobic fungi, Enzyme activity, Orpin, Menke

Orpin ve Menke Besi Ortamlarında *Neocallimastix* sp'nin Selülotik Enzim Aktivitelerinin Karşılaştırılması

Öz: *Neocallimastix* sp., benzersiz lignoselülotik enzimlere sahip Anaerobik Gut Fungusları (AGF) ailesinin bir üyesidir ve genellikle in vitro olarak iki temel besi ortamında da yetiştirilir. Enzimatik aktivitelerin tespiti için Orpin besi ortamı tercih edilirken, fermantasyon sırasında oluşan gazların değerlendirilmesi için daha çok Menke besi ortamı tercih edilmektedir. Bu iki ortamın hedeflenen amaçlar için etkili olduğu gösterilmiş olmasına rağmen, bu besi ortamlarındaki çeşitli selülotik enzimlerin aktivitelerini karşılaştırmak için hiçbir girişimde bulunulmamıştır. Bu çalışmada *Neocallimastix* sp.'nin bu iki besiyerinde de 7 güne kadar büyüme hızı ölçülmüş ve her ikisinde de benzer bir gelişim gözlenmiştir ($p>0.05$). Ayrıca Menke besi ortamının Aviselaz'ın 5. ($p<0.001$) ve 7. ($p<0.0.1$) günleri ve 5. güne kadar [0 ($p<0.001$), 3 ($p<0.01$), 5 ($p<0.0001$)] Ksilanaz aktivitesi için daha iyi olduğu belirlenmiştir. Orpin besi ortamı ise tüm zaman noktalarında [0 ($p<0.001$), 3 ($p<0.0001$), 5 ($p<0.05$, 7 ($p<0.001$)] üstün KMSaz aktivitesi sergilemiştir. Selülaz için,

aktiviteler her iki ortamda 0. ve 3. günlerde hemen hemen aynı ölçülürken, Menke'de ($p<0.0001$) 5. günde ve Orpin'de 7. günde ($p<0.01$) daha yüksek olduğu görülmüştür. Sonuç olarak Ksilanaz enzim çalışmalarında menke besi ortamınız daha iyi sonuç verdiği tespit edilmiştir.

Anahtar kelimeler: Anaerobik fungus, Enzim aktivitesi, Orpin, Menke

Introduction

Ruminants are mainly fed on herbal materials (Canbolat, 2012), yet they cannot produce cellulolytic or hemi-cellulolytic enzymes to digest them. This task is accomplished by the microorganisms in their gut with which they are in a symbiotic relationship (Vinzelj et al., 2020). Polymeric components in plant cell walls such as hemicellulose, cellulose and lignin make up lignocellulosic biomass (Bobleter, 1994). Anaerobic gut fungi (AGF) are one of the most important microorganisms that form lignocellulosic by-products in the rumen ecosystem (Kamra, 2005; Yazdic et al., 2021). By rapidly colonizing plant cell walls, rumen fungi can break down cell wall carbohydrates (Grenet et al., 1988) thanks to a group of highly active enzymes such as cellulases, xylanases, glycosidases and xylosidases (Comlekcıoglu et al., 2010). As one of the most widely studied AGF's, *Neocallimastix* sp., has a wide range of extremely promising and largely undiscovered enzymes for the complete and efficient break down of lignocellulosic bio mass that can be used for industrial applications (Dagar et al.,2018; Banerjee et al., 2010). Appropriate media is needed to study the growth of microorganisms in vitro. Orpin's medium is generally used in in vitro studies for the determination of the activity of lignocellulosic enzymes (Orpın, 1976). However, Menke's medium, which is thought to represent the rumen environment as Orpin's does (Menke et al.; 1979), has not been utilized for such a purpose. Menke medium is a preffered for the in vitro analysis of gases such as CO_2 and CH_4 formed as a result of fermentation in the rumen (Sanni et al.,2020). Although the two media have been widely used for different purposes, there have been no study to investigate the growth rate of *Neocallimastix* sp. in these media and how the lingocellulosic enzyme producing potential of the organism is affected by them. Here we compared not only the number of viable individuals in Orpin's and Menke's media, but also the activities of Xylanase, Avicelase, Cellulase and Carboxy Methyl Cellulase (CMCase) enzymes per individual on the 0th, 3rd, 5th and 7th days of inoculation. It has been studied to minimize the starter cost of commercially used enzymes such as xylanase.

Material and Metod

Revitalization of anaerobic gut fungi

The Anaerobic Gut Fungi (AGF), namely GMLF 35 (*Neocallimastix* sp.), was obtained from the culture collection of Kahramanmaraş Sütcü Imam University, Faculty of Agriculture, Department of Animal Science, Biotechnology and Gene Engineering Laboratory (BIGEM).It was taken from liquid nitrogen (-196°C), thawed at room temperature and placed into hay-fattening environment under 2 atm carbondioxide (CO_2) pressure (Balch and Wolfe, 1976). Antibiotic mixture (chloromphenicol, amphicillin, streptomycin, erythromycin) was added into the media to prevent bacterial growth. It was then left in incubation (38°C) and its development was observed on the 0th, 24th, 48th and 72nd hours.

Classical fattening medium: This medium was prepared based on Orpin (1976). Glucose was added to the medium as a source of energy, with the final concentration of 0.5 (w/v) %. Then the medium was boiled and saturated with CO_2 until its color is pale yellow. Later, it is transferred into hungate tubes (8 mL) under 2 atm CO_2 pressure and autoclaved at 110°C for 10 minutes. The basal medium content required for the development of AGFs in vitro is given in the table 1. Therewithal, while preparing the anaerobic medium, L-Cystein is added for oxygen induction and Resazurin is added for the oxygen indicator in the medium.

Modified fattening environment: This environment was inspired by the fattening environment prepared by Menke (1979), yet some modifications on the content of the media were performed for the study. After artificial saliva was prepared as Menke described, and rumen fluid and glycose with a final concentration of 0.5 (w/v) % were added to it. The environment was then saturated with CO_2 and distributed in hungate tubes. The tubes were autoclaved at 110 °C for 10 minutes and let it cool before use (Table 2).

Table.1. Medium Content (Orpin,1975)

Content	Liquid Media
Mineral Solution-1 [K_2HPO_4-3g/L]	150ml/L
Mineral Solution-2 [KH_2PO_4-3g/L, $(NH_4)SO_4$-6g/L, $MgSO_4.7H_2O$-0,6g/L, $CaCl_2$-0,4g/L, NaCl-6g/L]	150ml/L
Rumen fluid	150ml/L
$NaHCO_3$	6 g/L
Yeast Extract	2,5g/L
dH_2O	550ml/L

Preparation of substrates

All substrates used in the study [Carboxy methyl cellulose (CMC), Cellulose, Avicel and Xylane] were purchased from Sigma (UK). 500 mg of each was weighed and dissolved in 100 mL (50 mM) of sodium phosphate buffer (pH:6.0).

Preparation of enzyme extracts

0.5 mL of AGFs were added to two different medium prepared and incubated for 0, 24, 48 and 72 hours. At the end of each time point, tubes were centrifuged at 1200 g for 10 minutes and the supernatants were transferred to a new tube to store at -20 °C for later use.

Determination of enzyme activities

Enzyme activities of supernatants in two different fattening environents were determined by the method described by Miller (1959). Briefly, each substrate [Carboxy methyl cellulose (CMC), Cellulose, Avisel and Xylane] was treated with all supernatants. Samples were incubated at 50°C for 45 minutes. By adding DNS to them, enzyme activity was stopped and the mixtures were boiled for 5 minutes. Absorbances were read at 545 nm. The standard graph for calculating enzyme activities was obtained using glucose. One unit of enzyme activity was defined as the mmol amount of reducing sugar released within 1 min. Protein concentrations were determined by Bradford Assay.

Table 2. Medium Content (Menke,1979)

Content	Liquid Media
Solution A	13,2g/L
$CaCl_2.H_2O$	10g/L
$MnCl_2.4H_2O$	1g/L
$CaCl_2.6H_2O$	0,8g/L
$FeCl_3.6H_2O$	Liquid Media
Solution B (Buffer)	35g/L
$NaHCO_3$	Liquid Media
Solution C	5,7g/L
Na_2HPO_4	6,2g/L
KH_2PO_4	0,6g/L
$MgSO_4.7H_2O$	13,2g/L

Determination of population quantity

The most probable number (MPN) is one of the best methods for calculating the population density of Anaerobic Gut Fungi (AGF) in vitro and in this method, which is used to calculate the number of viable individuals in the population of fibrolytic organisms, the thallus forming unit (tfu) is preferred as the unit. (Theodorou et al., 1990). In this study, MPN table (De Man,1975) and 'most probable number calculator.epa.gov' database were used to calculate tfu amount. To determine the number of individuals grown in the fungal population, 1 mL of fungi was taken and inoculated into 9 mL of

fattening medium. This process was repeated several times and dilution was performed (10^{-1},10^{-2},10^{-3},10^{-4},10^{-5}). The tubes were kept in incubation at 38°C for 15 days and the tubes were checked every day. The tubes in which the fungi were develop were marked with '+' while the ones without any growth were marked with '-' .Each condition was prepared in triplicates.

Enzymatic activity per cell

The enzyme activity value per individual was calculated by the division of the total enzyme activity values found through Miller's method (1959) by the number of individuals in the population determined through MPN table (De Man, 1975) and database calculate programs.

Statistical analysis

Enzyme acitivites were calculated and graphs were drawn by using GraphPad Prism (CA, USA). Activities of the same enzyme in two different media were compared via Two-Way ANOVA, and the difference was considered statistically significant if the P value for interaction was equal to or lower than 0.05. For any specific time point, enzyme acitivties were compared by student t-test. Again, P values which were equal to or lower than 0.05 indicated a statistical significance.

Results

According to the counts made with the help of MPN technique and database calculator, it has been revealed that *Neocallimastix* sp. was able to grow in the nutrient medium of Orpin and Menke. However, it was also realized that the growth rate of *Neocallimastix* sp. in the nutrient medium belonging to Orpin appeared to beslightly higher than it was in the culture medium of Menke even though no statistical analysis could be performed because of the difficulty of having biological replicates. Yet, it was still quite observable that number of individuals in both environments increased until the 5th day and decreased on the 7th day.

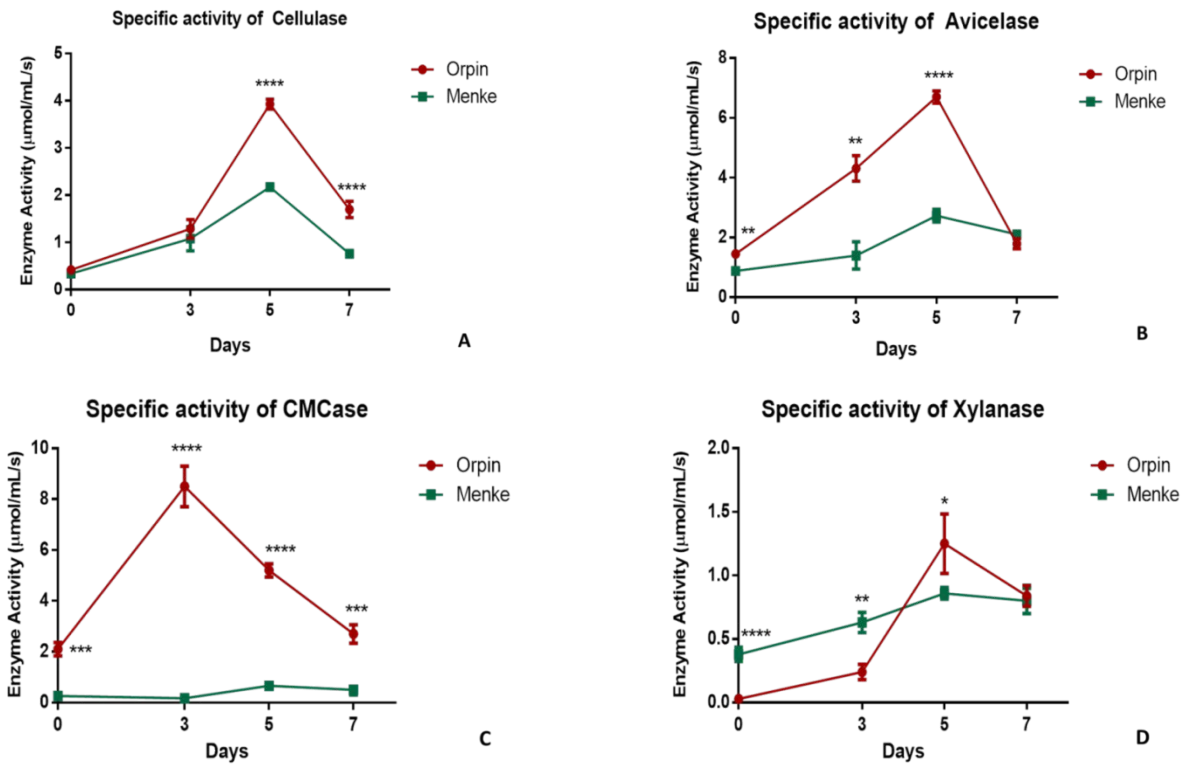

Figure.1. Specific enzyme activity of A) Cellulase B) Aviselase C) CMC D) Xylanase. Specific activites of all enzymes at four different time points. Red line respresents Orpin's media while the green one is for Menke's. Asterisks were placed (*: P <0.05, **: P <0.01, ***: P <0.001, ****: P <0.0001)

To calculate the extra cellular enzyme activity of *Neocallimastix* sp. the microorganism was left to incubate for 0, 3, 5 and 7 days. At the end of each incubation, the supernatant of the culture was separated from the fungal biomass and the Xylanease, Avicelase, CMCase and Cellulase activities were determined. According to the results obtained, the CMCase enzyme activity of *Neocallimastix* sp. grown in Orpin's medium was determined to have the highest activity on the 3rd day where all other enzyme in both media were at their maximum capacity on the 5th day. The results obtained were presented in Figure 1.

As it can be seen in Figure 1, When 2-way ANOVA analysis were performed on specific activities of all

enzymes studied, Orpin and Menke's media were observed to cause a significant change in all. In general, Orpin's media seemed to be superior to Menke's for each condition examined.

Later, specific enzyme activities were normalized to the actual numbers of individual *Neocallimastix* sp.in each case, and distinct patterns were emerged for all enzymes compared to their specific activities. Considering all times for CMCase enzyme activity, Orpin's medium gives information that it will be a suitable medium for this enzyme study (Figure 2A). For cellulase enzyme activity, the activity of the first 3 in Orpin's nutrient medium and Menke's nutrient medium was found to be parallel to each other. However, it was observed that the enzyme activity on the 5th day increased in Menke's broth (Figure 2 B). It was observed that Menke's medium was more suitable for avicelase and xylanase enzyme activity of *Neocallimastix* sp, which was grown on two different media Figure 2C-D). It was observed that xylanase enzyme activity was more effective in Menke's medium than in Orpine's medium in all preferred times throughout the study (Figure 2D).

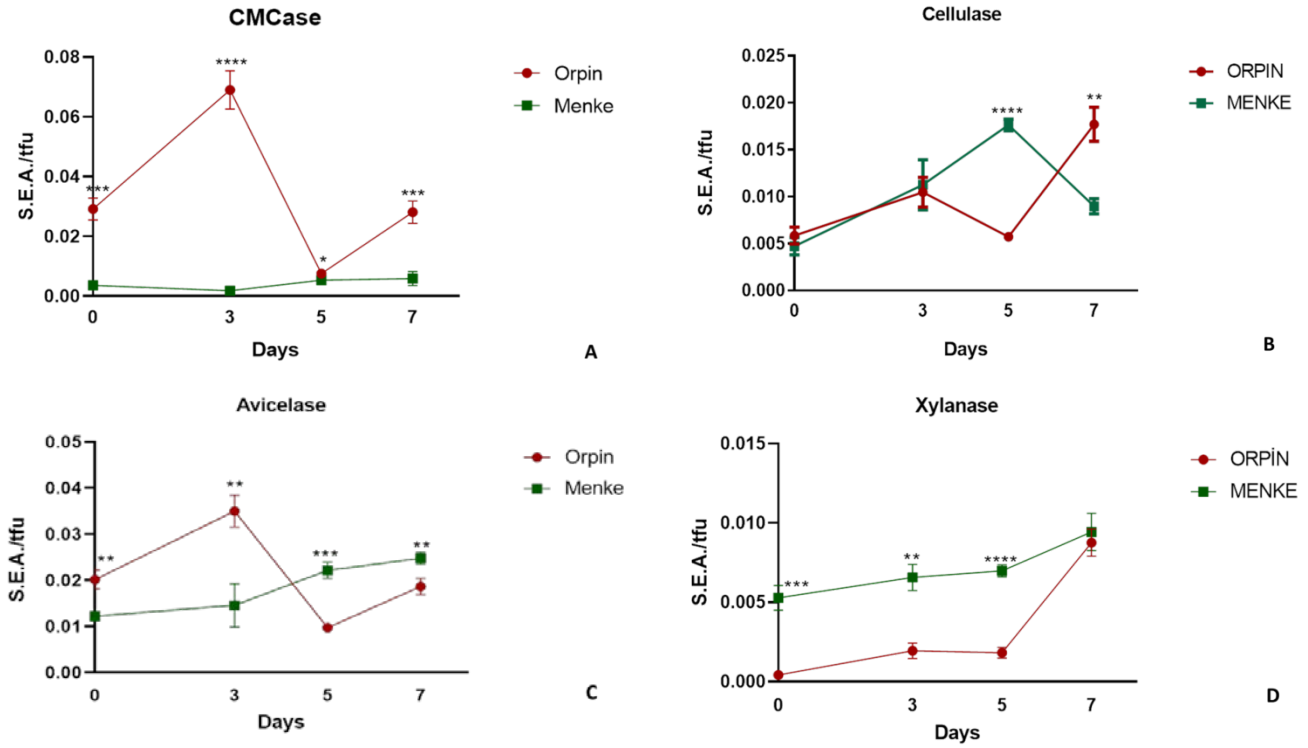

Figure.2. Enzyme activities tfu (thallus forming unit) of AGFs that have completed their incubation in the nutrient medium of Menke and Orpin. A) Avicelase B) CMCase C) Xylanase D) Cellulase. Specific enzymatic activities were divided by the number of the individuals in each condition. Results for each enzyme at each time point were compared through unpaired t test. Asterisks were placed when the differences were statistically significant (*:P <0.05, **: P <0.01, ***: P <0.001, ****: P <0.0001).

Discussions

It has been known that the cellulotic enzyme activity of AGFs is more effective than other bacteria and anaerobic fungi that synthesize these enzymes (Steenbakkers et al., 2003). In this study, we culture done of the most widely studied AGF, *Neocallimastix* sp, in the media suggested by Orpin (1976) and Menke (1979) to measure their growth rates as well as specific and individualistic activites of certain fibrolitic enzymes.

Firstly, we observed that *Neocallimastix* sp. has grown in Menke's almost as sufficiently as it has in Orpin's. As seen in Table 1., the maximum growth of the species was detected on the 5th day in Orpin's medium, which was expected because there a great number of studies reporting a similars observation (Dagar et al., 2018). Menke's, in spite of presenting a similar trend with Orpin's (i.e., showing the highest number on the 5th day), appeared to slightly lower the growth rate of the species. Unfortunately; however, the data has not allowed us to perform a statistical analysis, therefore, our conclusion remained only suggestive.

Although there have been many studies in which Orpin was declared as the choice of medium for *Neocallimastix* sp.culturing (Comlekcıoglu et al., 2017), the number of the studies investigating the growth rate of the species in Menke's has been rather limited. Menke's

medium, on the other hand, has been prefered in calculating the fermantation rate of various anaerobic microorganisms (Totakul et al., 2020; Sarnataro et al., 2020) including *Neocallimastix* sp. (Cao and Yang, 2011).

Secondly, when we measured the specific activities of four fibrolitic enzymes (Avicelase, CMCase, Cellulase, Xylanase) in both medium, we found that the maximum enzymatic activites were determined on the 5th day in all conditions except for CMCase measured in Orpin's, whose highest efficiency was obtained on the 3rd day (Figure 1). Here, the results presented for Orpin were quite in agreement with the previous results in the literature (Comlekcıoglu et al., 2012) even though, for Menke's, no such study has been found to compare our results to.

Later on, however, by considering the findings in Figure 1 along with the fact that the species had its highest growth also on the 5th day, we suggested that the specific activities of these enzymes could be highly depending on the cell number. To investigate this assumption, we divided the values of the specific enzyme activity by the number of the viable cells in each condition and labeled them as individualistic enzyme activity (Figure 2). Again in most cases, the values were higher for the cells cultured in Orpin's. For the individualistic activities of Xylanase and Avicelase; however, the results were pointing another direction. Individualistic Xylanase activities were detected to be higher in Menke's than Orpin's at virtually all time points ($p<0.05$) whereas Avicelase presented a significantly higher individualistic enzyme activity in Menke's ($p<0.05$) at the longest time point (7 days). Convientiently, our results for Xylanase were in accordance with the report of (Cao and Yang 2011), who demonstrated a significant activity of Xylanase in Menke's medium.

In a closer examination of the graphs in Figure 2, we realized that all individualistic enzyme activites were at their highest on the 0th day. Then we reflected that this fact might be caused by a possible back-up mechanism, where the amount of secreted enzymes could be kept at a certain range regardless of the viable cells present. However, since we have not performed any experiment nor have we encountered such an observation in the literature, this possiblity must be considered as a bare speculation. As far as our knowledge is concerned, the present study is the first one on the comparison of the activites of any cellulotic enzymes in Menke's and Orpin medium. Therefore, not only does it provide a novel set of data and a fresher look to the field but it aslo provokes many questions for further evaluations.

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Chlorophyllum hortense, A New Record for Turkish Mycobiota

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Abstract: *Chlorophyllum hortense* (Murrill) Vellinga is reported as a new record from Turkey, based on the identification of the samples collected from Rize province. A brief description of the species is provided together with the photographs, related to the macroscopy and microscopy.

Keywords: Biodiversity, *Agaricaceae,* New record, Turkey

Chlorophyllum hortense, Türkiye Mikobiyotası İçin Yeni Bir Kayıt

Öz: *Chlorophyllum hortense* (Murrill) Vellinga., Rize'den toplanan örneklerin teşhis edilmesiyle, Türkiye'den yeni kayıt olarak rapor edilmiştir. Türün kısa bir betimlemesi makroskobi ve mikroskobisine ilişkin fotoğrafları ile birlikte verilmiştir.

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

Introduction

Chlorophyllum Massee is a genus of the family *Agaricaceae*. It was first erected as an agaricoid genus by the typification of *Chlorophyllum molybdites* (G. Mey.) Massee. Based on molecular data and some morphological similarities, some other species belonging to the genera *Endoptychum* Czern., *Lepiota* (Pers.) Gray and *Macrolepiota* Sing. were transferred to the genus (Carlavilla et al., 2018; Ge et al., 2018; Alves et al., 2019). Currently the members of the genus are characterized by agaricoid to secotioid, or gasteroid habit; white, green, brownish or brown spore deposit; basidiospores without germ pore or with a germ pore caused by a depression in the episporium without a hyaline covering (Ge and Yang, 2006; Crous et al., 2015, Carlavilla et al., 2018; Ge et al., 2018; Alves et al., 2019).

Though the existing number of *Chlorophyllum* was presented as 16 by Kirk et al. (2008), Index Fungorum (Accessed 15 November 2021) currently lists 26 conformed species of the genus.

Currently 5 species of the genus are known to exist in Turkey. Uzun and Kaya (2022) prepared a synoptic key for Turkish *Chlorophyllum* species while presenting *Chlorophyllum lusitanicum* G. Moreno, Muñ.-Moh., Manjón as new record for Turkey.

According to the current checklist (Sesli et al., 2020) and the latest contributions to Turkish higher fungi (Akçay, 2020; Çağli and Öztürk, 2020; Işık, 2020; Sesli, 2020; Uzun et al., 2020; Acar et al., 2021; Çetinkaya and Uzun, 2021; Doğan et al., 2021; Kaygusuz et al., 2021; Şelem et al., 2021; Uzun, 2021; Uzun and Kaya, 2022) *C. hortense* (Murrill) Vellinga, hasn't been reported before from Turkey.

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

Material and Method

The fruit bodies of *Chlorophyllum hortense* were collected from İyidere district of Rize province, in 2015, during a routine field study. Fruit bodies were photographed at their natural habitats, and ecological characteristics and geographic position were noted. Then the samples were transferred to the fungarium in a paper box. After letting them dry in an air conditioned room, they

were prepared as fungarium material. Microscopic characteristics were based on dry samples. Microscopic investigations were carried out under a trinocular light microscope. Photographs related to micromorphology were obtained with the aid of a digital camera. The sample was identified with the help of Akers and Sundberg (1997), Ge and Yang (2006), Nascimento and Alves (2014), Vizzini et al. (2014) and Ge et al. (2018).

The specimen is kept at Van Yüzüncü Yıl University, Science Faculty, Department of Biology.

Results

Fungi R.T. Moore

Basidiomycota R.T. Moore

Agaricales Underw.

Agaricaceae Chevall

Chlorophyllum hortense (Murrill) Vellinga, Mycotaxon 83: 416 (2002)

Syn: [*Lepiota hortensis* Murrill, *Leucoagaricus hortensis* (Murrill) Pegler]

Macroscopic and microscopic features: Pileus 35-80 (90) mm in diam., ovoid when young, then conic-campanulate, finally expanding to plano-convex with an umbo, whitish to creamy with a yellowish umbo, surface breaking up into flat, appressed, irregularly shaped scales on a whitish background, scales radially oriented and more densely packed toward the center while scattered toward the margin, margin thin, sub-striate to striate (Figure 1). Flesh thin, whitish. Lamellae free, crowded, whitish, becoming dirty white to pale ocher. Stipe 40-80 × 4-8 mm, central, cylindrical, with an equal to sub-bulbous base, surface whitish, glabrous, staining reddish upon bruising, annulus persistent, membranous, whitish, spore print white. Basidia 25-35 x 8-10 µm, clavate to narrowly clavate, hyaline, 2-spored. Cheilocystidia 30-50 × 8-10 (12) µm, cylindrical to narrowly clavate, sometimes constricted, hyaline. Basidiospores (7.8)8.5-11 (12) × 6.5-8.5 µm, ellipsoid to broadly ellipsoid, rarely ovoid, hyaline, thick-walled, with an apiculus and a single large oil drop, without a germ pore (Figure 2).

Figure 1. Basidiocarps of *Chlorophyllum hortense*

Chlorophyllum hortense was reported to grow as solitary or scattered on organic rich soil, fertilized especially with cattle feces and grasses (Akers and Sundberg, 1997; Ge and Yang, 2006; Nascimento and Alves, 2014; Vizzini et al., 2014; Ge et al., 2018).

Specimen examined: Rize, İyidere, Denizgören village, on soil composted with processed *Camellia sinensis* (L.) Kuntze remains, 40°58′N, 40°22′E, 20 m, 23.10.2015., AK 3077.

Discussions

Chlorophyllum hortense was added as new record for the mycobiota of Turkey. This is the sixth member of the genus to be reported in Turkey. Macro and micromorphological characteristics of the studied collection are in agreement with Ge and Yang (2006), Nascimento and Alves (2014) and Vizzini et al. (2014).

Chlorophyllum hortense is characterized by medium to large-size, whitish basidiomes with whitish to creamy or ocher-yellow squamules on the pileus and a yellowish umbo, stipe's reddening reaction when injured, a double annulus, spores without a germ pore, 2-spored basidia and cylindrical cheilocystidia (Akers and Sundberg, 1997; Vellinga, 2003; Nascimento and Alves, 2014; Ge et al., 2018). It shares some morphological features with *Leucoagaricus carminescens* Heinem., but tetrasporic basidia of the latter species differs the two species from each other. *Chlorophyllum africanum* Z.W. Ge & A. Jacobs is also a similar species to *C. hortense* in terms of basidiocarp morphology, ellipsoid basidiospores and subcylindrical cheilocystidia, but the whitish context of the stipe becoming reddish where bruised and 2-spored basidia differs *C. hortense* from *C. africanum*.

By the addition of *C. hortense,* current taxa number of the genus *Chlorophyllum* in Turkey increased to six.

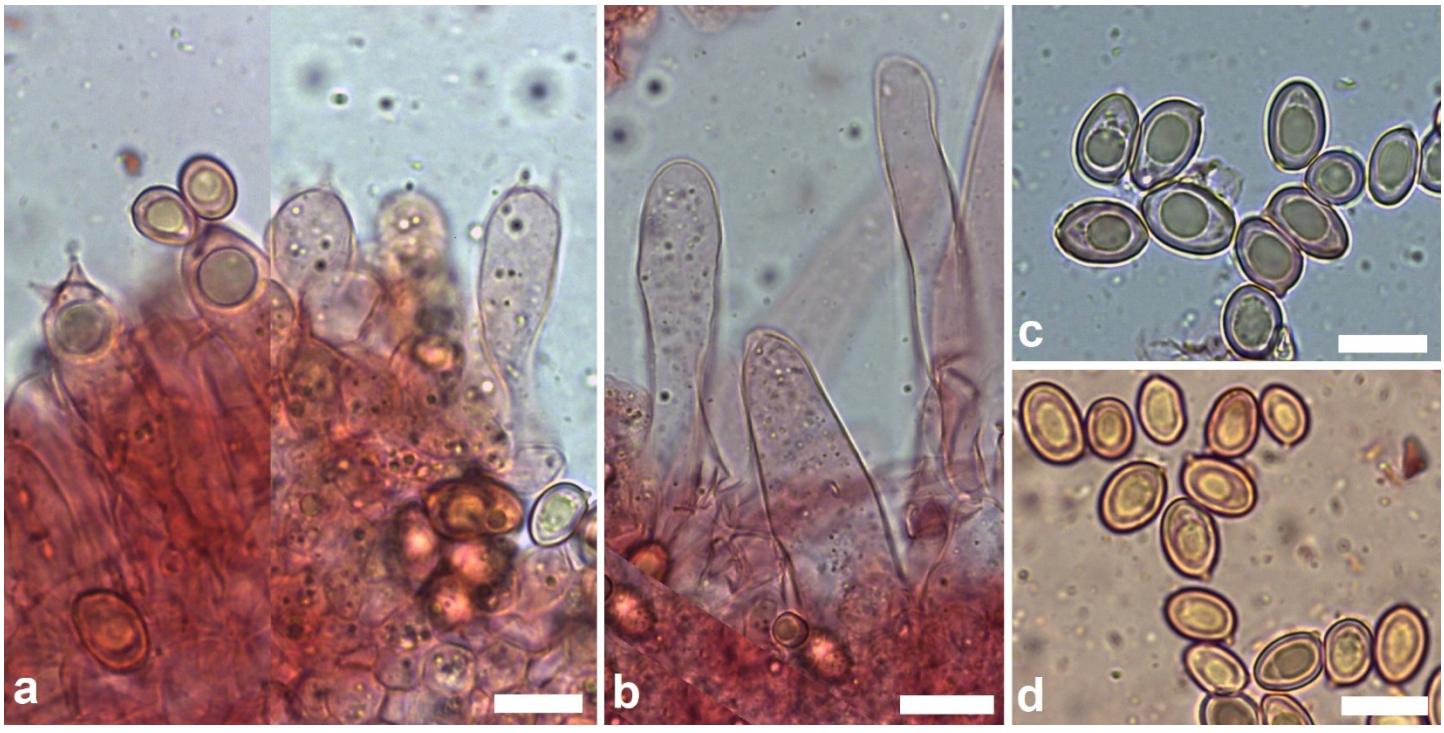

Figure 2. Basidia (a), cheilocystydia (b) and basidiospores (c,d) of *Chlorophyllum hortense* (bars- a-d: 10 µm) (a,b,d in Congo-Red, c in water)

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Suillus mediterraneensis (Jacquet. & J. Blum) Redeuilh (Suillaceae): Türkiye Mikotası İçin Yeni Bir Kayıt

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Öz: *Suillus mediterraneensis*'in meyvemsileri Türkiye'den ilk kez toplanmış, burada ilgili resimler ve kısa bir tartışma ile birlikte sunulmuştur. Türün teşhisi araziden elde edilen veriler ve mikroskobik inceleme sonuçlarına göre yapılmıştır. Sarımsı içeriği, kozalaklı ağaç ormanlarında yayılış göstermesi, tadı ve kokusunun belirsiz oluşu ve 7.5−10.5 × 3−4 µm bazidiyosporları ile yakın türlerden ayrılır.

Anahtar kelimeler: Etli mantar, Bazidiyomikota, Porçini mantarları, Trabzon

Suillus mediterraneensis (Jacquet. & J. Blum) Redeuilh (Suillaceae): A New Record For The Turkish Mycota

Abstract: The fruit bodies of *Suillus mediterraneensis* were collected for the first time from Turkey and are presented here with relevant pictures and a short discussion. The identification of the species was made according to the data obtained from the field and the results of microscopic examination. It is distinguished from related species by its yellowish content, distribution in conifer forests, unclear taste and odor, and basidiospores of 7.5−10.5 × 3−4 µm.

Keywords: Fleshy fungi, Basidiomycota, Porcini mushroom, Trabzon

Giriş

"*Suillus*" ismi ilk kez 1729 yılında İtalyan Botanikçi Pier Antonio Micheli tarafından verilmiş ve dünyanın çeşitli yörelerinde çalışan araştırmacıların katkıları sayesinde bu cins günümüzde yaklaşık 500 tür ile temsil edilmektedir (Kirk ve ark., 2008). Bu grup üyelerinde silindir biçiminde, dolu veya boş, yüzeyi tanecikli bir sap, boletoyit görünümde, yarım küre, yastıkçık, konik veya konveks, yüzeyi yapışkan ve jelatinli bir şapka, süngerimsi doku ve sarımsı veya turuncumsu, üreyimli tüpsü tabaka, iğ biçiminde, kahverengimsi ve yakın gruplara göre daha küçük bazidiyosporlar ve zeytini sarımsı veya kahverengimsi bazidiyospor izi dikkat çeker. Çoğu yenilebilen ve besin değeri yüksek mantarlardır (Engel ve ark., 1996; Muñoz, 2005; Knudsen ve Vesterholt, 2008; Šutara ve ark, 2009). Grubun en çok tanınan ve ülkemizde de yayılış gösteren türleri, *S. amygdalinus* (Thiers) Vizzini, Simonini & Gelardi (Acar ve ark., 2019), *S. bellinii* (Inzenga) Watling, *S. boudieri* (Quél.) Marchand, *S. bovinus* (L.: Fr.) Roussel, *S. collinitus* (Fr.) Kuntze, *S. flavidus* (Fr.) Singer, *S. granulatus* (L.: Fr.) Roussel, *S. grevillei* (Klotzsch: Fr.) Singer (Sesli ve ark., 2020), *S. lakei* (Murrill) A.H. Sm. & Thiers (Akata ve ark., 2018; Uzun ve Kaya, 2022), *S. luteus* (L.: Fr.) Roussel, *S. pictus* (Peck) A.H. Sm. & Thiers., *S. placidus* (Bonord.) Singer, *S. pulchrotinctus* (Alessio) Blanco–Dios (Sesli ve ark., 2020), *S. queletii* (Schulzer) Vizzini, Simonini & Gelardi (Doğan ve ark., 2021), *S. rhodoxanthus* (Krombh.) Blanco-Dios (Sadullahoğlu ve Uzun 2019), *S. rubrosanguineus* (Cheype) Blanco-Di (Doğan ve ark., 2021), *S. tomentosus* Singer (Oruç ve ark., 2021) ve *S. variegatus* (Sw.) Richon & Roze'dir (Kaşık ve ark., 2000; Keleş ve ark., 2014; Sesli ve ark., 2020). Trabzon il sınırları içerisinde daha önceden çeşitli çalışmalar gerçekleştirilmiştir (Akata ve ark., 2014). Bu çalışmanın amacı meyvemsi yapıları Türkiye'den ilk kez toplanarak teşhisi yapılan *Suillus mediterraneensis'in* makroskopik ve mikroskobik özelliklerini tanıtmaktır.

Materyal ve Metot

Araştırmanın materyalini oluşturan meyvemsi yapılar 29.07.2009 tarihinde Trabzon ili Akçaabat ilçesi kırsal kesiminde saptanmış, fotoğrafları çekilmiş, kaba morfolojik özellikleri ve koordinatları not edilmiş, standart yöntemlerle toplanarak laboratuvara getirilmiş, bazidiyospor izleri elde edildikten sonra kurutulup etiketlenerek fungaryum dolabına yerleştirilmiştir. Bazidiyum, bazidiyospor ve şapka derisinin hücresel yapısını görüntülemek için keskin jiletle mikroskop altında kesitler alınmıştır. Bazidiyosporların görüntülenebilmesi için meyvemsiden bir parça kesilerek 2 dakika % 5'lik amonyak çözeltisi içerisinde tutulmuş, daha sonra bir pens yardımı ile lam üzerine alınmış ve hücreler lam üzerine düşünceye kadar sıkılıp bırakılmıştır. Kesitler saf su ve % 5'lik amonyak çözeltisi ile işlemden sonra Zeiss Axio Imager A2 araştırma mikroskopu ile incelenmiş, hücresel yapıların ölçümleri yapılmış ve fotoğrafları çekilmiştir. Boyutların belirlenebilmesi için yaklaşık 40 civarında ölçüm yapılmıştır. Teşhisler arazi gözlemleri, makroskobik ve mikroskobik veriler ve ölçüm sonuçlarının ilgili literatür ile karşılaştırılması sonucunda yapılmıştır (Engel ve ark., 1996; Muñoz, 2005; Knudsen ve Vesterholt, 2008; Šutara ve ark, 2009). Kurutulmuş örnekler Trabzon Üniversitesi Fatih Eğitim Fakültesi'ndeki kişisel fungaryumda saklanmaktadır.

Bulgular

Basidiomycota R.T. Moore / **Topuzlu mantarlar** **Boletales** E.-J. Gilbert

Suillaceae Besl & Bresinsky / **Süngermantarıgiller**

Suillus mediterraneensis (Jacquet. & J. Blum) Redeuilh, Docums Mycol. 22(no. 86): 40 (1992) / ***Ak süngermantarı*** (Şekil 1)

Şapka yarım küre, konveks veya yastıkçık biçiminde, 50−120 mm, sarımsı, donuk sarı, turuncumsu, bakır rengi, kahverengimsi, toprak rengi veya zeytini kahverengi, lekeli, yüzeyi yapışkan, yayvan tepe çıkıntılı ve kenarı içeriye kıvrıktır. Tüpler sapa uzunlukları ölçüsünde birleşmiş, beyazımsı, krem rengi, açık sarımsı, sarımsı yeşil, zeytin sarısı veya sarımsı kahverengidir. Sap silindirik, tabana doğru daha ince, kahverengi ve küçük kesecikler veya pullarla kaplı, beyazımsı veya krom sarısı, tabanda genellikle morumsu pembe ve ipliksi miselyumlu ve 50−75 × 10−20 mm'dir. Eti hafif tatlı ve hoş, süngerimsi, beyazımsı, merkezde daha kalın, sarımsı veya krom rengidir. Bazidiyumlar iğ veya çomakçık biçiminde, 4 sporlu, bazen iki sporlu ve 20−30 × 5−7 µm'dir. Bazidiyosporlar uzun eliptik veya silindirik, açık sarı, toprak veya zeytin sarısı ve 7.5−10.5 × 3−4 µm'dir. Çeylosistityumlar çomakçık biçiminde ve 35−55 µm'dir. Şapka derisi soyulabilir özellikte, az veya çok paralel veya iç içe geçmiş hiflerden yapılmıştır. Yaz aylarından sonbahar sonlarına kadar çoğunlukla kireçli topraklarda, iğne yapraklı ağaç ormanlarında tek tek veya küçük gruplar halinde yetişen mikorizal bir mantardır.

İncelenen örnekler: Türkiye, Trabzon, Akçaabat, 40°58'03.72'' K ve 39°32'15.88'' D, 313 m, 28.07.2009, gruplar halinde, çam altında, E. Sesli 2641.

Tartışma

Suillus cinsi (Sünger mantarı: Güner ve ark., 2020) mantarlardan *S. granulatus* (*Damlamantar*), *S. collinitus* (*Benekli süngermantarı*), *S. bellinii* (*Gâvur süngermantarı)* ve *S. mediterraneensis* (*Ak süngermantarı*) morfolojik olarak birbirine yakındır. Çalışmamızın konusu olan *S. mediterraneensis* sarımsı içeriği, çam ve ladin ormanlarında yayılış göstermesi, tadı ve kokusunun hoş oluşu ve 7.5−10.5 × 3−4 µm bazidiyosporları ile yakın türlerden ayrılır. *Suillus granulatus* kırmızımsı veya turuncumsu ve fazlaca yapışkan şapkası ve sapı üzerinde görülen beyazımsı damlacıklar ile fark edilir. *Suillus collinitus fazlaca* lifli, kırmızımsı veya kestane rengi şapkası, daha kısa ve geniş bazidiyosporları, tabanda daha kalın ve kırmızımsı kahverengi sapı ile diğer türleden ayrılır. *Suillus bellinii* daha büyük ve açık renkli şapkaya, beyazımsı olan fakat kırmızımsı süt salan tüpsü yapılara ve beyazımsı zemin üzerinde kırmızımsı benekli sapa sahiptir. Bu türlere yakın *S. flavidus* daha küçük şapkaya, sapa ve bazidiyosporlara sahiptir. Grup üyeleri depoladıkları madde miktarına bağlı olarak az ya da çok ishal ve zehirlenme yapabilir. Ölçülü oranda toplanır ve tüketilirler. *Suillus mediterraneensis* (Ak süngermantarı) birçok ülkede toplanıp tüketilmesine rağmen araştırma alanında tanınmamaktadır (Blum, 1969; Engel ve ark.,1996; Muñoz, J.A., 2005; Knudsen ve Vesterholt, 2008; Šutara ve ark, 2009).

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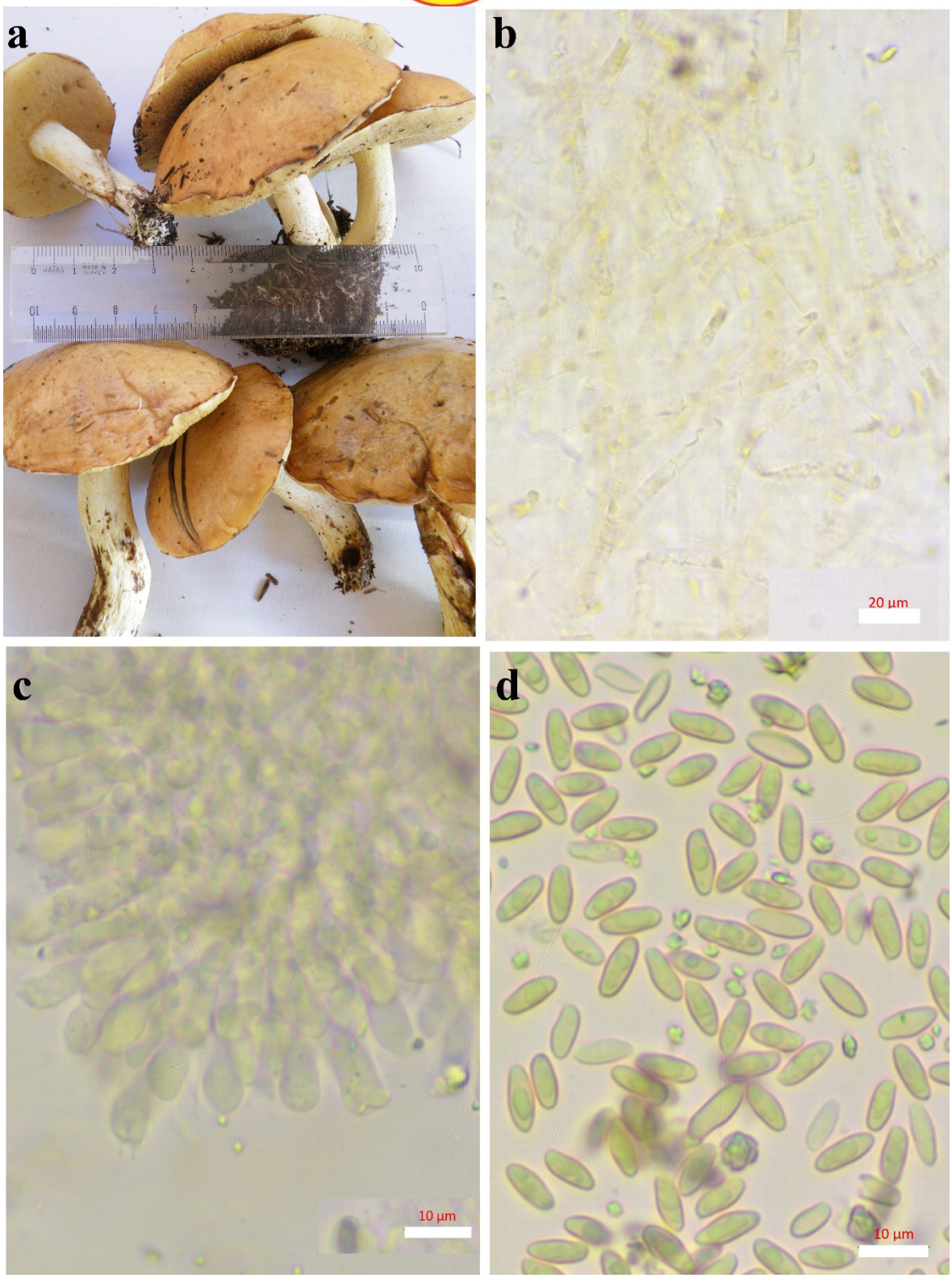

Şekil 1. *Suillus mediterraneensis*: a- meyvemsiler, b- şapka derisi kesiti, c-bazidiyumlar, d- bazidiyosporlar (ölçek çubukları: b: 20 µm, c ve d: 10 µm)

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Temporal Distribution of *Cladosporium* and *Alternaria* Spores in the Atmosphere of Gelibolu (Çanakkale), Turkey

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Abstract: This aeropalynological study aims to determine the inter-annual and seasonal variation of atmospheric spores belonging to *Cladosporium* Link and *Alternaria* Nees genera during the three years. *Cladosporium* and *Alternaria* spores are generally recorded as dominant in many atmospheric fungal spore studies and have high allergic effects on susceptible individuals. The species belonging to these two genera may also live as pathogens on the plants and cause low yield. This study was carried out for three years, between January 2018 to December 2020. Durham sampler, which is the device of the gravimetric method, was used in the research. A total of 17399 *Cladosporium* and *Alternaria* spores were recorded in the Gelibolu (Çanakkale) atmosphere for three years. The total numbers of *Cladosporium* spores were found about five times more than the total numbers of *Alternaria* spores. The maximum spore levels were recorded in June for both spore types. Summer months can be reported as a risky period in terms of *Alternaria* and *Cladosporium* pathogenicity and atmospheric fungal spore allergy in the region.

Keywords: Atmospheric fungal spores, Aeromycology, Allergy, Northwest Turkey.

Gelibolu (Çanakkale), Türkiye Atmosferindeki *Cladosporium* ve *Alternaria* Sporlarının Zamansal Dağılımı

Öz: Bu aeropalinolojik çalışma, *Cladosporium* Link (Havaküfü) ve *Alternaria* Nees (Arıküfü) cinslerine ait atmosferik sporların üç yıl süre zarfındaki yıllar arası ve mevsimsel değişimlerini belirlemeyi amaçlamaktadır. *Cladosporium* ve *Alternaria* Nees sporları birçok atmosferik mantar sporu çalışmasında dominant olarak kaydedilmiştir ve duyarlı bireyler üzerinde yüksek allerjik etkilere sahiptirler. Ayrıca bu iki cinse ait türlerin sporları, bitkiler üzerinde patojen olarak yaşayabilmekte ve bitkilerde verim kaybına da neden olmaktadılar. Bu çalışma Ocak 2018 – Aralık 2020 tarihleri arasında üç yıl süreyle gerçekleştirilmiş olup araştırmada gravimetrik yönteme dayalı Durham cihazı kullanılmıştır. Üç yıl boyunca, Gelibolu (Çanakkale) atmosferinde toplam 17399 adet *Cladosporium* ve *Alternaria* sporu kaydedilmiş ve *Cladosporium* sporlarının toplam sayısı, *Alternaria* sporlarının toplam sayısından yaklaşık beş kat daha fazla bulunmuştur. Her iki spor türü için de maksimum spor mikatarınna Haziran ayında ulaştığı kaydedilmiştir. Yaz ayları bölgede *Alternaria* ve *Cladosporium* patojenitesi ve atmosferik fungal spor alerjisi açısından riskli dönemler olarak bildirilebilir.

Anahtar Kelimeler: Atmosferik mantar sporları, Aeromikoloji, Allerji, Kuzeybatı Türkiye.

Introduction

Fungi are a large kingdom with a wide distribution on the earth, can live in a wide variety of habitats, and contain many species (Gregory, 1961; Blackwell, 2011). These organisms can produce large numbers of spores, and these spores are mostly dispersed into the atmosphere, generally with the help of wind (Money, 2015). The most abundant spores in the atmosphere

influence various aspects of human health and agriculture (Caretta, 1992; Anton et al., 2021).

Fungal spores, especially *Cladosporium* (Havaküfü) and *Alternaria* (Arıküfü) spores, cause many allergic reactions on susceptible individuals and damage plants and animals. Due to their presence in the air, we breathe, they can cause respiratory diseases such as asthma and allergic rhinitis in people and may cause skin diseases in animals and yield losses in plants (D'Amato et al., 1984; Buck and Levetin, 1985; Vjay et al., 1991; Angulo-Romero et al., 1999; Sesli et al., 2020).

Since the sporulation times are different for each fungus, the periods and the number of spores of these fungi also differ in the atmosphere. The types and densities of atmospheric spores may vary according to geographical, ecological, meteorological factors and the floristic structure of the region. For this reason, it is necessary to find out together with spore calendars as a result of long-term studies by comparing the atmospheric spore types and their distribution during the year with meteorological factors in regions with topography and especially climate difference.

The first aeromycological study of Turkey was conducted by Özkaragöz in 1969 with the open Petri method to determine atmospheric fungal spores in Ankara. Atmospheric fungal spore studies have been continuing since 1969 in the different cities in Turkey and in the world (Özkaragöz, 1969; Gioulekas et al., 2004; Oliveira et al.. 2010; Mallo et al., 2011; Grinn-Gofron et al., 2016; Kasprzyk et al., 2016; Akgul et al., 2016; Ding et al., 2016; Saatcioglu et al., 2016; Yılmazkaya et al. 2019; Kilic et al., 2020; Bekil et al., 2021; Sevindik et al. 2021).

The aims of this study can be summarized as follows: (i) to contribute to taking the necessary measures to prevent yield losses in the economically important plants growing in the region; (ii) to benefit the people of the region and allergologists in the treatment process and in taking precautions to prevent allergies in risky periods; (iii) to investigate the annual variation of atmospheric *Cladosporium* and *Alternaria* spores and the effects of meteorological factors (Mean temperature, relative humidity, total rainfall, and wind speed) on them in the Gelibolu (Çanakkale) atmosphere.

Material and Method

The description of the Study area, Climate, and the Floristic Structure

Gelibolu is derived from its old name "Gallipoli", which the ancient Greeks gave, and it means good/beautiful city. Its history dates back to before Christ. The Gelibolu is a peninsula, stretches between the Çanakkale Boğazı “Dardanelles Strait” and the Saroz Körfezi “Saros Gulf”, expanding to the south. Located in the Northwest of Turkey, the peninsula is also the last piece of land in the South-East of the European continent. The district of Gelibolu is situated on the North-Eastern coast of the peninsula with the same name, at the point where the Dardanelles Strait opens to the Sea of Marmara (40° 27' 44.38" N–26° 37' 54.30" E). The district has an area of 825 km^2, and the altitude is at sea level (Figure 1).

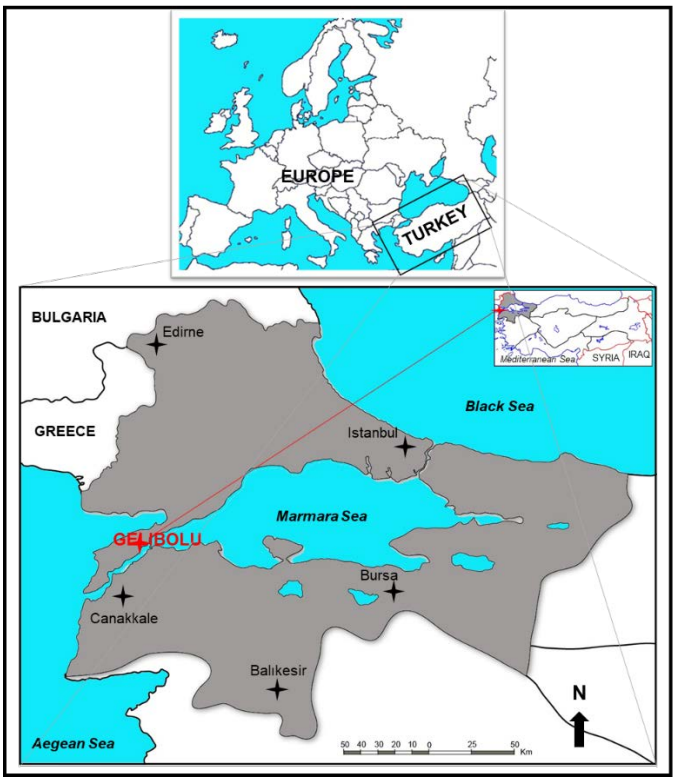

Figure 1. Location of study area (Gelibolu).

According to meteorological data provided by the Turkish State Meteorological Service, for three studied years (2018-2020), the annual average temperature was 16.8 °C (min. temperature 6.01°C - in January, max. temperature 25.95°C - in August). The total amount of precipitation was 1655.8 mm; the annual average relative humidity is 76.7%, and the annual average wind speed is 4.4 m/s. Since the district is located on the coast of the Dardanelles strait, it is under the influence of continuous air currents in all four seasons of the year.

The region is under the influence of the Mediterranean climate. *Pinus brutia* Ten. is the dominant species in the forest formation in the region, and there are *Pinus nigra* Arn., *Fagus* L., *Carpinus* L. pure or mixed forests in the higher parts of the region, and maquis

communities took place in areas where forests were destroyed (Turrill, 1924). *Populus* L. and *Quercus* L. species are primarily found in the valleys and *Cupressus* L. species in graveyards. On the side of the peninsula facing the Dardanelles strait, at an altitude of 150-200, species belonging to the genus *Quercus*, *Pinus* L., *Juniperus* L., *Arbutus* L., *Myrtus* L. on the steep slopes. A vegetation type consisting of grasses and scrub bushes covers the soil-poor and stony slopes of the peninsula, such as *Quercus* L., *Cistus* L., *Erica* L., *Astragalus* L., *Tymelaea* Mill. (Turrill, 1924; Şahin and Kartepe, 2020). The main species of the urban flora are *Pinus brutia*, *Pinus pinea* L., *Pinus pinaster* Ait., *Pinus nigra*, *Carpinus orientalis* Mill., *Quercus coccifera* L., *Quercus ithaburensis* subsp. *macrolepis* (Kotschy) Hedge & Yalt., *Juniperus oxycedrus* L., *Arbutus andrachne* L., *Arbutus unedo* L., *Olea europea* L., *Erica arborea* L., *Myrtus communis* L., *Pistacia terebinthus* L. and *Laurus nobilis* L. (Bağcı et al., 2004). The field crops grown around are; *Helianthus* L., *Triticum* L., *Hordeum* L., *Avena* L. *Sesamum* L., *Nicotiana* L. *Phaseolus* L. species.

Aeromycological study

This study was conducted between January 2018 to December 2020. Durham sampler, the device of the gravimetric method used in the research, was placed at the top roof of a building, 9 meters above ground level.

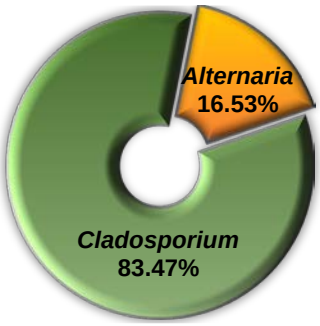

Figure 2. Percentage distribution of *Alternaria* and *Cladosporium* spores detected in Gelibolu atmosphere (three-year average of 2018-2020).

Before being placed on the device, slides were covered with glycerin-jelly (Charpin et al., 1974) and were changed weekly. Counting was conducted on a 24 x 24 mm area of the slide extrapolated to 1 cm^2 later; all spore numbers were given for per cm^2.

Results

In studied years, a total of 17399 spores were recorded, 14533 spores (83.47%) belong to the *Cladosporium* genus, and 2876 spores (16.53%) belong to the *Alternaria* genus (Figure 2).

4086 spores (79.69% *Cladosporium* – 20.31% *Alternaria*); 4843 spores (81.88% *Cladosporium* –18.12% *Alternaria*); 8509 spores (86.19% *Cladosporium* –13.81% *Alternaria*) (Table 1,2) counted in order between 2018 - 2020. The total amounts of *Cladosporium* spores have been found higher than the amounts of *Alternaria* spores in each of the studied years.

Considering the average values of three years, the months that reached the highest spore levels were May, June and July. The months with the lowest amount of spores are December, January and February. Both *Cladosporium* and *Alternaria* spores have reached their maximum level in June every three years (Figure 3). Two peaks occurred in the total number of *Cladosporium* and *Alternaria* spores each year. The first peak occurred in June in all three years. The second peak occurred in October in 2018 and 2020, and November in 2019. These three years' the first peaks have been found larger than the second peaks. Both peaks have been higher in 2020 particularly the second peak showed a greater difference (Figure 3).

The atmospheric amount of *Cladosporium* has reached the lowest level in November in 2018 and 2020, and in January in 2019. The lowest amount of *Alternaria* spores have been found in May in 2018, in February in 2019, and in January in 2020 (Figure 4, Table 2).

In May, June and July, which the months that reached the highest spore levels, the temperature has been rising, and humidity has fallen every three years. In December, January, and February, which have the lowest amount of spores, the temperature has decreased, and the rainfall and humidity have increased in all these three years. In June, the temperature has increased but has not reached its maximum level and humidity has decreased, but not to its minimum level (Figure 5).

In this study, when the total number of *Cladosporium* and *Alternaria* spores in 3 years is examined seasonally, summer was recorded as the season with the most spores, and winter was recorded as the season with the fewer spores. 47.69% of *Cladosporium* spores and 10.17% of *Alternaria* spores were recorded in the summer and 2.97% of *Cladosporium* spores and 0.43% of *Alternaria* spores were recorded in the winter (Figure 6). On the other hand, the lowest amount of humidity and the highest amount of

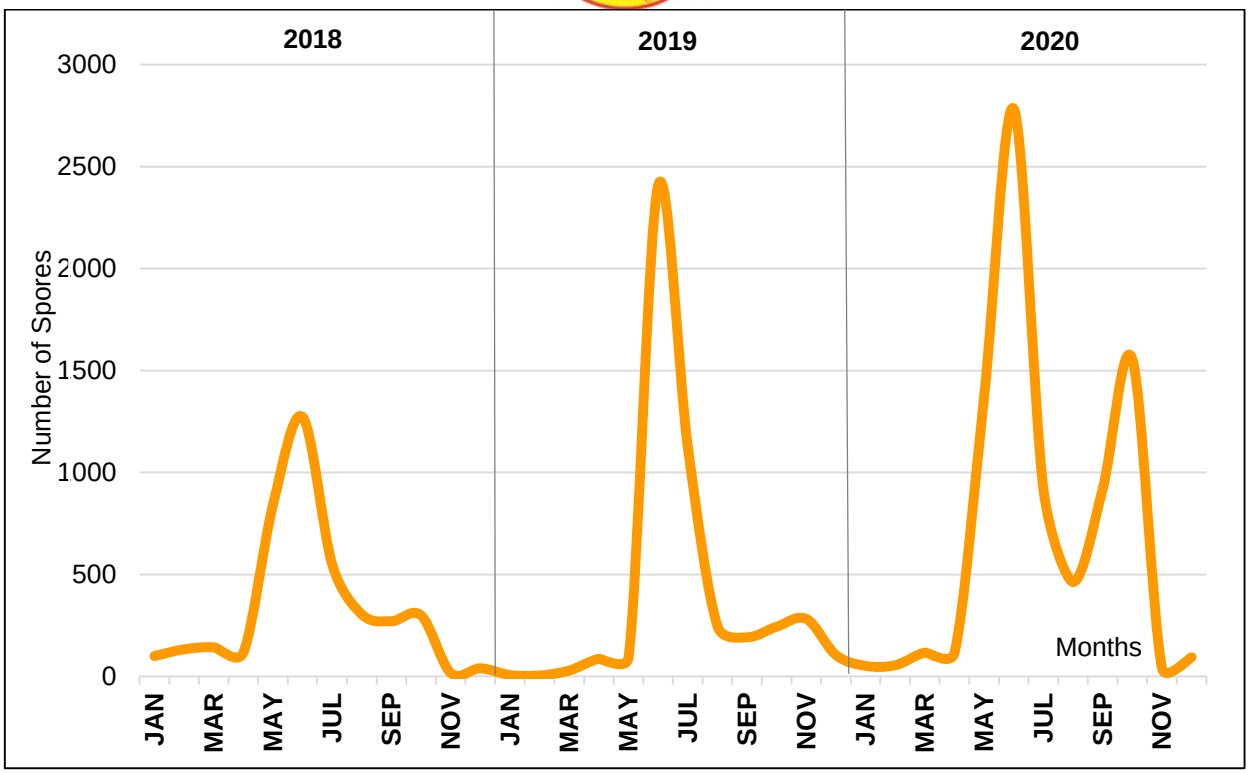

Figure 3. Monthly variation of total spore amounts of *Alternaria* and *Cladosporium* during 2018-2020.

Table 1**:** Total amounts of *Cladosporium* and *Alternaria* spores in Gelibolu atmosphere in years 2018-2020 (mean and percentage values).

	2018	***%***	**2019**	***%***	**2020**	***%***	**MEAN**	**TOTAL**	***%***
Cladosporium	3256	*79.69*	3950	*81.88*	7317	*86.19*	4841.00	14523	*83.47*
Alternaria	830	*20.31*	874	*18.12*	1172	*13.81*	958.67	2876	*16.53*
TOTAL	**4086**	***100***	**4824**	***100***	**8489**	***100***	**5799.67**	**17399**	***100.00***

temperature was recorded in the summer and the opposite was observed in the winter (Figure 4).

When the total number of *Cladosporium* and *Alternaria* spores were examined weekly, the week with the highest amount of spores was recorded at 24th week in 2018, at 26th week in 2019, and 41st week in 2020. The lowest amount of spores was recorded at 17th and 44th weeks in 2018, at 9th and 12th weeks in 2019, and at 2nd week in 2020 (Figure 7).

Discussions

In Turkey and worldwide spores of *Cladosporium* and *Alternaria* are often recorded as the dominant airborne spore types many researchers relate the occurrence of respiratory allergy symptoms with the presence of these spores in the ambient air (Kauffman et al., 1995; Downs et al., 2001).

In this study, the total number of *Cladosporium* spores was recorded much more than *Alternaria* spores (Figure 2, Table 1). *Cladosporium* is the dominant airborne fungal spore type observed in many countries (Katial et al., 1997; Bustos et al., 2001; Henriguez et al., 2001; Al-Subai, 2002; Pepeljnjak and Segvi, 2003; Gioulekas et al., 2004, Mitakakis et al., 2005; Asan, 2015). This could be due to many factors; as seen in many fungal spore studies, *Cladosporium* forms more

colonies than *Alternaria,* and the number of species belonging to *Cladosporium* genus is higher than that of *Alternaria* as observed in many systematic studies (Şen and Asan, 2001; Dugan et al., 2004; Asan,2015; Kireçci and Alagöz, 2019).

The annual total number of spores in 2020 (8489 spores) has been nearly doubled compared to 2019 (4824 spores) and 2018 (4086 spores), and this is why related to meteorological factors (Figure 3). In many studies, it has been determined that the weather conditions have the most important and greatest effect on airborne spore concentration (Hjelmroos, 1993; Ianovici, 2016; Grinn-Gofron et al., 2016; Olsen et al., 2020). Meteorological factors need to be examined to explain the difference in spore amounts between 2020, 2019, and 2018 (Figure 3, Table1-2). The total amount of rainfall in 2020 was lower than in 2018 and 2019 (Figure 5). The amount of precipitation is one factor influencing the concentration of spores in the atmosphere. It's known that annual variation in climate, particularly rainfall, can determine the fungal spore concentration in the atmosphere, and in dryer years, higher fungal concentration was observed in different studies (Kendrik 2001; Pakpour et al., 2014). Also, precipitation may clean the air by forcing fungal propagules back to the ground or onto other surfaces (Katial et al., 1997; Polymenakou, 2012).

One of the other important factors affecting spore concentration is relative humidity, and many researchers reported a negative correlation between atmospheric fungal spore concentrations and relative humidity (Kurkela, 1997; Stepalska and Wolek, 2005; Oliveira et al., 2007). In Gelibolu, relative humidity was recorded in 2020 (74.74%) is lower than in 2019 (77.28%) and 2018 (78.05%) and there was not much difference between mean temperature and wind speed for these three years (Figure 5).

When the total number of spores in these three years for both *Cladosporium* and *Alternaria* is examined, the maximum number of spores belong to summer, and the minimum number of spores belong to the winter (Figure 6). *Cladosporium* and *Alternaria* were reported as the most prevalent fungal spore type during summer (Ianovici 2017; Oliveira et al., 2005). They are classified as dry-air spores, too, and for this reason, they are found in the greatest abundance in the atmosphere with high temperatures. Low humidity and these conditions are most visible in the summer (Peternel et al., 2004). In each of these three years, it was observed that the temperature has increased and humidity has decreased in summer (June, July, August) and the temperature has decreased, and humidity and precipitation have increased in winter (December, January, February) (Figure 5).

Temperature is stated as one of the most influential and important factors on fungal spore concentration in the atmosphere and has a positive effect on spore concentration in the air (Hjelmroos, 1993). The positive impact of the high temperature on the spore amounts was observed in the summer in Gelibolu (Figure 5). Similar results have been obtained in other atmospheric fungal spore studies with similar climatic conditions (Şakıyan and İnceoğlu, 2003; Kizilpinar and Dogan, 2011; Gioulekas et al., 2004). As mentioned before many studies were reported a negative correlation between atmospheric fungal spores and relative humidity. Another reason why the summer has the maximum number of spores is the low relative humidity in the summer. In addition to low temperature and high humidity in winter, both of which have a negative effect on the atmospheric amount of spores in the air, the amount of rainfall is another effective factor. Some researchers have considered that the rain removes ambient fungal spores by both rain-out and wash-out effects (Magyar et al., 2009; Artac et al., 2014).

Two peaks occurred in the total number of *Alternaria* and *Cladosporium* spores in each of these three years (Figure 3). The first peak has occurred in June, same this in Timisoara (Romania) maximum spore amounts (first peak) were determined in June too (Ianovici, 2016). The second peak occurred in October in 2020 and 2018 and in November in 2019 (Figure 3). The first peak was more significant than the second peak in all three years and had a more significant number of spores; this is related to the conditions in June (Figure 3, 5). High temperature and low humidity in June can be seen as an optimum sporulation conditions for *Cladosporium* and *Alternaria*. Although it has been mentioned before that precipitation has a negative effect on the spore concentration, much rainfall was recorded in June (Figure 5); this is probably because of different rainfall types, which have different effects on atmospheric spore amounts. Heavy rain such as rain storms and thunderstorms may increase the spore concentration in the atmosphere because the spore of fungi such as *Cladosporium* and *Alternaria,* which can live on plants or in the soil, can be separated from the conidium by force and dispersed into the atmosphere but the light rain decrease spore concentration in the air (Rich and Waggoner, 1962). Besides, rainfall may cause a more accessible release of fungal spores to the atmosphere by splash and tap-and-puff mechanisms (Ho et al., 2005).

In July, the spores of *Cladosporium* and *Alternaria* spores started to decrease rapidly, which may be due to the extreme temperature in August (Figure 4, 5). Several authors found a negative correlation between fungal spores and temperature (Aira et al.,2003; Calderon et al., 1997). Common fungal spore only develops under a certain temperature threshold; therefore, with extremely high or low temperature, the spore concentration decreases (Oliveira et al., 2005). In this study was observed that the high temperature of August and the cold weather of January correspond to a decrease in the spore amounts. Same this was observed in another study in Porto city (Oliveira et al., 2005) was reported the exceedance of the higher temperature limit for growth might kill fungi. In contrast, temperatures below the lower limit are less lethal (Eduard, 2009). It is known that, for each fungal species, there is an optimal temperature range for growth to occur; outside of that optimal temperature range, more water is necessary for growth (Ianovici, 2016). Another reason for decreasing the number of *Cladosporium* spores sharply in July may be the excessive humidity reduction. It is well known that fungi typically require moisture to grow and sporulate, many spores eventually become airborne (Kendrik, 2001, Tilak, 2009) and low relative humidity and extreme temperature inhibit growth and spore germination (Talley et al., 2002), as seen in our study.

When the total number of *Cladosporium* and *Alternaria* spores was examined weekly, in the week with the highest number of spores in each of the three years, the relative humidity and temperature were recorded at moderate levels, neither maximum nor minimum (Figure 7). And this is because as has been mentioned common fungal spore only develops under a certain temperature threshold; therefore, with extremely high or low temperature, the spore concentration decreases (Oliveira et al., 2005). In all three years in the weeks when the lowest number of spores was recorded, the total precipitation was also low (Figure 5). The low amount of precipitation negatively affected the number of weekly spores. Rainfall may cause a more accessible release of fungal spores to the atmosphere by splash and tap-and-puff mechanisms (Ho et al., 2005).

The formation of the peaks in our study may have many reasons, as Dugan et al. stated earlier (2004); related meteorological factors may have been influential for sporulation and sporulation times of different species belonging to both genera. Also, the second peak in the fall can be related to decaying leaves from the plants, which constitute a substrate where fungal spores can grow, as shown by Oliveira et al. (2005). Also, one factor that increases *Cladosporium* and *Alternaria* spores in summer and autumn can be related to agricultural activities. Harvest time corresponds to the June-October term depending on the plants that *Cladosporium* and *Alternaria* can live on as pathogens and that can be released and increase the spore concentration in the atmosphere (Landecker, 1996). The seasonal pattern of *Cladosporium* generally follows the life cycle of the local vegetation (Mitakakis et al., 1997). Many factors affect the concentration of *Cladosporium* and *Alternaria* spores in the atmosphere, including geobotanical characteristics of the region, vegetation, climate, the types of fungal spores, proximity and abundance of the source of fungal spore and sporulation periods of them (Hjelmroos, 1993; Şakıyan and İnceoğlu, 2003; Ianovici, 2016; Sindt et al., 2016; Grinn-Gofron et al., 2016; Olsen et al., 2020; Damialis and Gioulekas, 2006). And many authors indicate that airborne fungal spore levels are dependent on crop production and proximity of grassland areas (Solomon, 1978; Mitakasis et al., 2001; Corden et al., 2003; Pepeljnjak and Segvic, 2003; Damialis and Gioulekas, 2006). Among these, the weather condition has the most important and greatest effect on spore concentration (Hjelmroos, 1993).

The atmospheric concentrations of *Cladosporium* and *Alternaria* are very important for many reasons: *Cladosporium* and *Alternaria* spores have been in the atmosphere for a long time, and they can be a factor in the emergence of disease symptoms such as asthma, allergic rhinitis, and conjunction by entering the body through ways such as eye conjunctiva, skin, respiratory and nasal mucosa (Chakrabarti et al., 2012). And also have pathogenic effects on many plants of economic importance. *Cladosporium* and *Alternaria* spores may cause skin diseases in animals and yield losses in plants (D'Amato et al. 1984, Buck and Levetin 1985, Vjay et al. 1991, Angulo-Romero et al. 1999).

Conclusions

In this study, the atmospheric amounts of *Cladosporium* and *Alternaria* spores and the effect of meteorological factors were investigated in the Gelibolu atmosphere for three consecutive years (2018-2020). In the Gelibolu atmosphere, the temperature was positive,

Table 2. Monthly variation of *Cladosporium* and *Alternaria* spores in Gelibolu atmosphere during the years 2018-2020

TAXA/MONTHS		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	TOTAL
Cladosporium	**2018**	90	116	141	108	704	1045	459	253	59	235	9	37	**3256**
	%	*2.20*	*2.84*	*3.45*	*2.64*	*17.23*	*25.58*	*11.23*	*6.19*	*1.44*	*5.75*	*0.22*	*0.91*	***79.69***
	2019	2	5	28	83	81	2004	887	181	123	211	254	91	**3950**
	%	*0.04*	*0.10*	*0.58*	*1.72*	*1.68*	*41.54*	*18.39*	*3.75*	*2.55*	*4.37*	*5.27*	*1.89*	***81.88***
	2020	46	48	108	103	1328	2450	657	363	699	1413	21	81	**7317**
	%	*0.54*	*0.57*	*1.27*	*1.21*	*15.64*	*28.86*	*7.74*	*4.28*	*8.23*	*16.65*	*0.25*	*0.95*	***86.19***
	Mean	46.00	56.33	92.33	98.00	704.33	1833.00	667.67	265.67	293.67	619.67	94.67	69.67	**4841.00**
	%	*0.93*	*1.17*	*1.77*	*1.86*	*11.52*	*31.99*	*12.45*	*4.74*	*4.08*	*8.92*	*1.91*	*1.25*	***82.59***
Alternaria	**2018**	10	17	2	9	138	227	89	51	212	64	8	3	**830**
	%	*0.24*	*0.42*	*0.05*	*0.22*	*3.38*	*5.56*	*2.18*	*1.25*	*5.19*	*1.57*	*0.20*	*0.07*	***20.31***
	2019	5	0	1	4	12	405	238	65	69	33	28	14	**874**
	%	*0.10*	*0.00*	*0.02*	*0.08*	*0.25*	*8.40*	*4.93*	*1.35*	*1.43*	*0.68*	*0.58*	*0.29*	***18.12***
	2020	6	7	9	13	46	334	261	100	220	143	20	13	**1172**
	%	*0.07*	*0.08*	*0.11*	*0.15*	*0.54*	*3.93*	*3.07*	*1.18*	*2.59*	*1.68*	*0.24*	*0.15*	***13.81***
	Mean	7.00	8.00	4.00	8.67	65.33	322.00	196.00	72.00	167.00	80.00	18.67	10.00	**958.67**
	%	*0.14*	*0.17*	*0.06*	*0.15*	*1.39*	*5.96*	*3.40*	*1.26*	*3.07*	*1.31*	*0.34*	*0.17*	***17.41***
TOTAL	**2018**	100	133	143	117	842	1272	548	304	271	299	17	40	**4086**
	%	*2.45*	*3.26*	*3.50*	*2.86*	*20.61*	*31.13*	*13.41*	*7.44*	*6.63*	*7.32*	*0.42*	*0.98*	***100.00***
	2019	7	5	29	87	93	2409	1125	246	192	244	282	105	**4824**
	%	*0.15*	*0.10*	*0.60*	*1.80*	*1.93*	*49.94*	*23.32*	*5.10*	*3.98*	*5.06*	*5.85*	*2.18*	***100.00***
	2020	52	55	117	116	1374	2784	918	463	919	1556	41	94	**8489**
	%	*0.61*	*0.65*	*1.38*	*1.37*	*16.19*	*32.80*	*10.81*	*5.45*	*10.83*	*18.33*	*0.48*	*1.11*	***100.00***
	Mean	53.00	64.33	96.33	106.67	769.67	2155.00	863.67	337.67	460.67	699.67	113.33	79.67	**5799.67**
	%	*1.07*	*1.34*	*1.83*	*2.01*	*12.91*	*37.95*	*15.85*	*6.00*	*7.15*	*10.24*	*2.25*	*1.42*	***100.00***

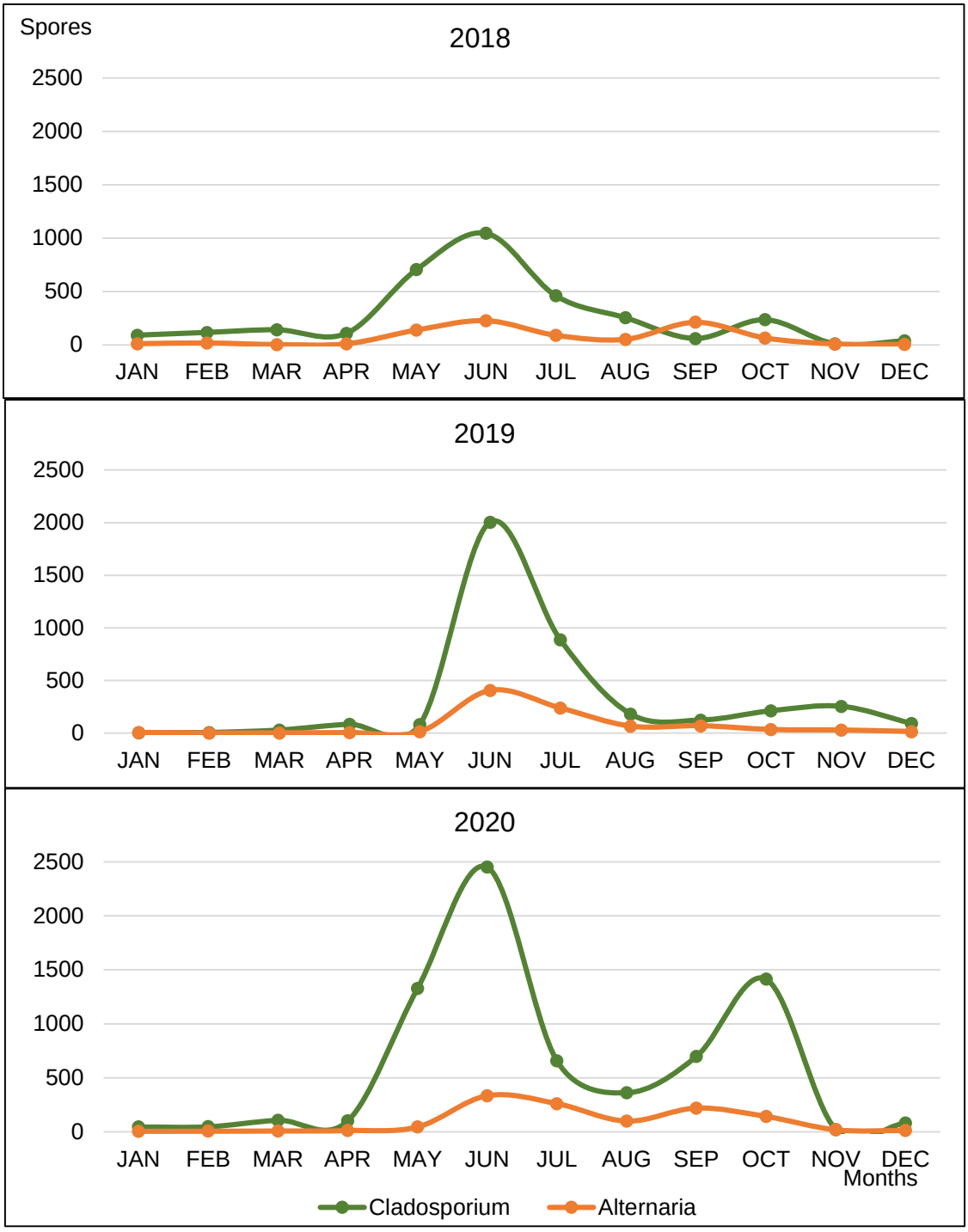

Figure 4. Monthly amounts of *Cladosporium* and *Alternaria* spores in Gelibolu atmosphere in the studied years (2018-2020).

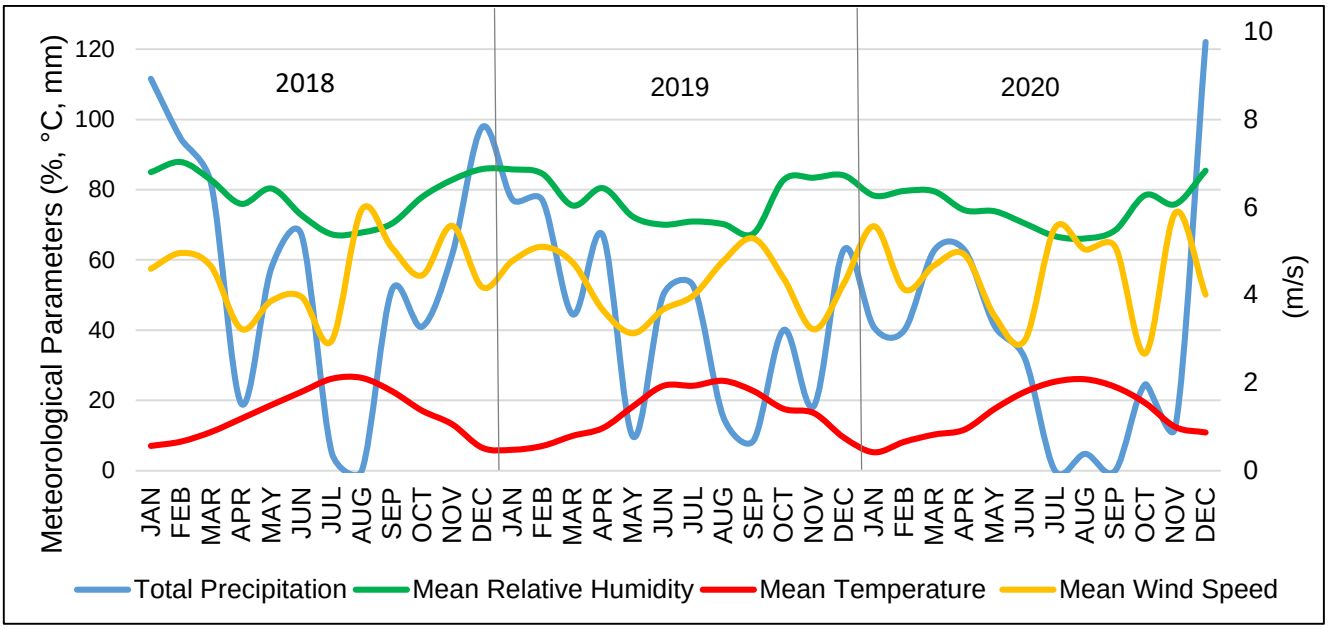

Figure 5. Monthly variation of meteorological parameters in Gelibolu during the years 2018-2020.

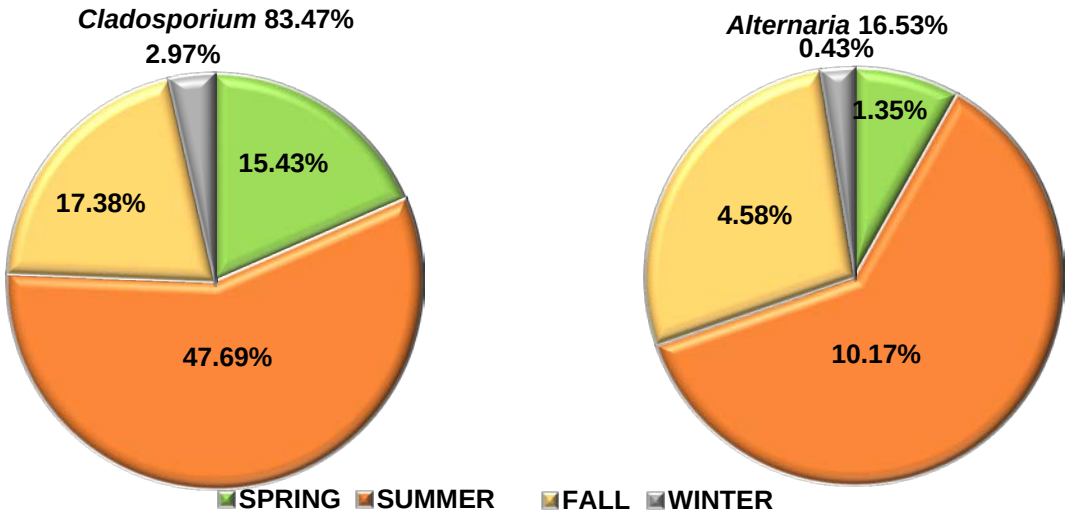

Figure 6. Seasonal dispersion of *Cladosporium* and *Alternaria* spores in Gelibolu atmosphere.

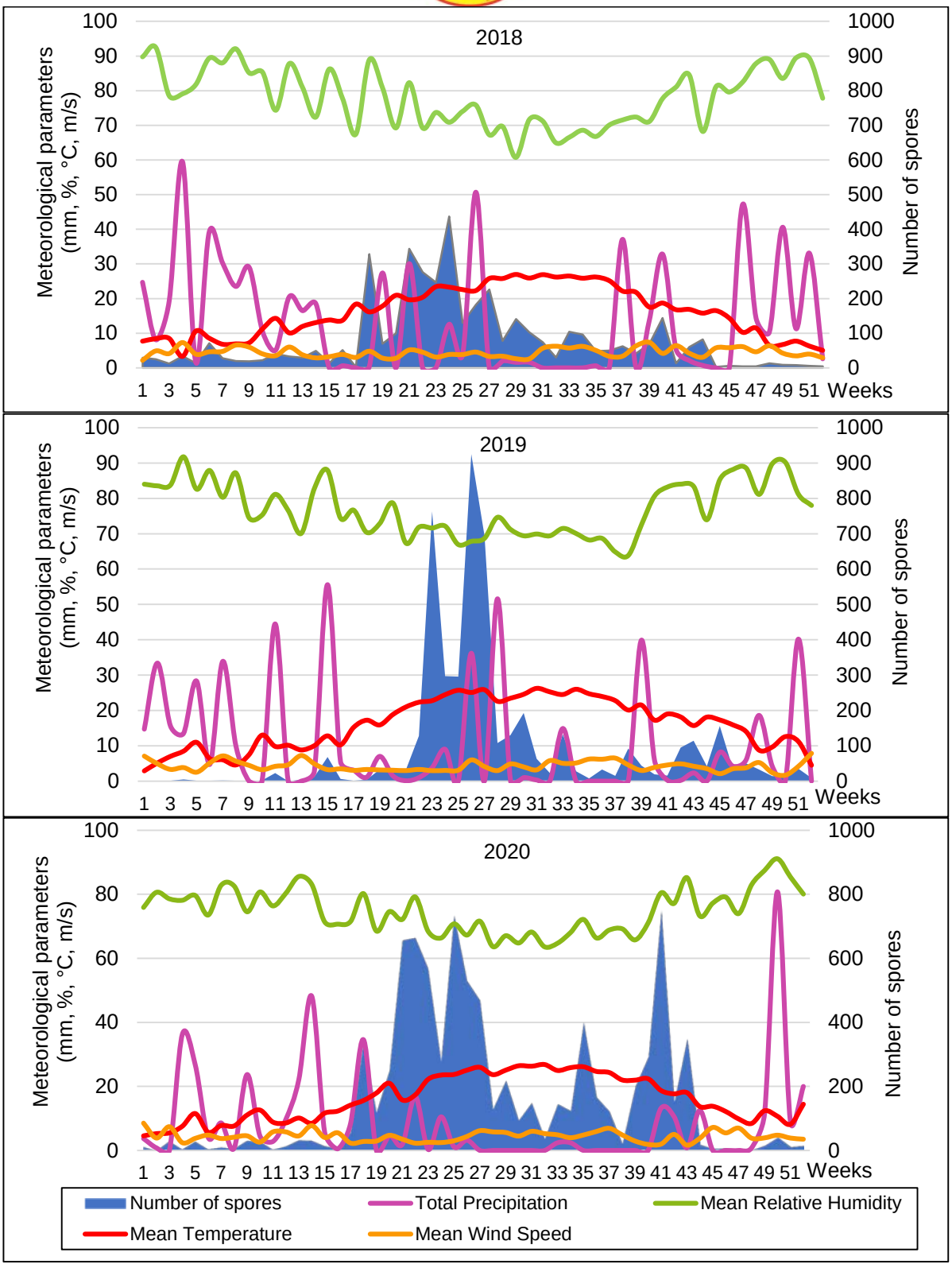

Figure 7. Weekly variation of meteorological parameters and number of spores in Gelibolu during the years 2018-2020.

and relative humidity and rainfall negatively affected spore amounts. Still, there could not be found any relation between wind speed and spore amounts. In all years, the highest spore amounts were recorded in the summer, and the total number of spores was the highest, especially in June. June had provided optimal conditions (high temperature and low humidity) for both *Cladosporium* and *Alternaria* sporulation. Therefore, June can be very risky for susceptible individuals, and conducting such studies can be helpful to doctors in the diagnosis, identification, and treatment of allergic patients.

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Influence of Magnetic Field on the Mycelial Growth Rate of *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. and *Lentinula edodes* (Berk.) Pegler

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Abstract: The effects of magnetic field application on the mycelial growth rate of oyster (*Pleurotus ostreatus*) and shiitake (*Lentinula edodes*) mushrooms were studied. 30 ml, %2 Malt Extract Agar (MEA) which autoclaved at 121°C for 30 minutes was placed in 9 cm diameter sterilized Petri dishes. Then, the mycelia of both fungi were planted in the Petri dishes under sterilized conditions, and magnetically exposed to one of seven magnetic field strengths, 0.25, 0.5, 1, 5, 10, 50, and 100 mT for different periods of time (5, 10, 15, and 30 min) using a solenoid., The cultures were conserved at room temperature (approximately 23°C). Once every two days, the growth rates of the mycelia were measured for a period of 20 days. A two-way analysis of variance was performed with SPSS18 for different magnetic field strengths and intervals of application. The LSD test was performed to show the differences. The following results were found: 1) Magnetic field application had a significant effect on the mycelial growth rate of shiitake mushroom, but exposure time and magnetic field-exposure time interaction had not any influence on the growth. In this study, it was concluded that 5 mT and 10 mT magnetic field exposure may be efficient for the development of shiitake mushroom mycelia. 2) Mycelial growth rate of oyster mushroom was not significantly affected by a magnetic field, exposure time, and magnetic field-exposure time interaction.

Keywords: *Pleurotus ostreatus, Lentinula edodes*, Magnetic field, Mycelial growth rate.

Manyetik Alanın *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. ve *Lentinula edodes* (Berk.) Pegler 'in Misel Büyüme Hızına Etkisi

Öz: Bu çalışmada, manyetik alan uygulamasının istiridye (*Pleurotus ostreatus*) ve şitake (*Lentinula edodes*) mantarları misel büyüme hızına etkileri incelenmiştir. 121°C'de 30 dakika otoklavlanan 30 ml, %2 Malt Extract Agar (MEA), 9 cm çapında sterilize edilmiş petri kaplarına dökülmüştür. Daha sonra, her iki mantarın miselleri steril koşullar altında petri kaplarına ekilmiş ve bir solenoid kullanılarak farklı sürelerde (5, 10, 15 ve 30 dak.) 0.25, 0.5, 1, 5, 10, 50 ve 100 mT olmak üzere yedi manyetik alan kuvvetinden birine maruz bırakılmıştır. Kültürler oda sıcaklığında (yaklaşık 23°C) muhafaza edilmiştir. Her iki günde bir, misellerin büyüme oranları 20 günlük bir süre boyunca ölçülmüştür. Farklı manyetik alan güçleri ve uygulama aralıkları için SPSS18 ile iki yönlü varyans analizi yapılmıştır. Manyetik alan ve uygulama süreleri arasındaki farkı göstermek için LSD testi kullanılmıştır. Yapılan analizler sonucunda şu sonuçlar bulunmuştur: 1) Manyetik alan uygulaması, şitake mantarının misel büyüme hızı üzerinde önemli bir etkiye sahip olmuştur, ancak maruz kalma süresi ve manyetik alan-maruz kalma süresi etkileşiminin büyüme üzerinde herhangi bir etkisi görülmemiştir. Bu çalışmada, şitake mantarı

misellerinin gelişimi için 5 mT ve 10 mT manyetik alan maruziyetinin etkili olabileceği sonucuna varılmıştır. 2) İstiridye mantarının misel büyüme hızı, manyetik alan, maruz kalma süresi ve manyetik alan-maruz kalma süresi etkileşiminden önemli ölçüde etkilenmemiştir.

Anahtar kelimeler: *Pleurotus ostreatus, Lentinula edodes*, Manyetik alan, Misel büyüme oranı.

Introduction

In recent years, there has been a growing concern for food production due to the increasing population (Fujimaki and Kikuchi, 2010). For this reason, scientists are trying to find production methods that are effective, environmentally friendly, and inexpensive. Biological, chemical, and physical methods are being used to achieve higher growth and yield. However, although chemical methods are effective in increasing growth and yield, it has been observed that they can be harmful in the later stages of development. Researchers working on magnetic field applications have reported high performance in terms of growth and yield in many vegetables, fruits, and mushrooms (Jamil et al., 2012).

Mushrooms are one of the oldest known foods in the world. Mushrooms are important because they are low in calories, high in nutritional value, rich in protein and vitamins. Also, mushrooms are easy to produce and their production costs are low (Ali et al., 2007).

Nowadays oyster and shiitake mushrooms are among the popular mushrooms produced in the world.

Shiitake (*Lentinula edodes* (Berk.) Pegler) are an edible mushroom species that can be found naturally in the forest or being done culture on logs in China, Korea, Japan, Singapore, Thailand, and other Asian countries. Not only these countries, but it is also now grown in many other countries, such as Brazil, Canada, the Netherlands, the United Kingdom and the United States (Slee 1991; Ciesla,2002; Aji,2009, Sesli et al., 2020). Chittaragi et al., (2018) pointed out that shiitake mushroom is cultivated throughout the world and contributes around 25 percent of the aggregate yearly generation of mushrooms. Shiitake mushroom is a nonpathogenic white-rot fungus. It secretes a class of lignocellulolytic enzymes, which permit it to grow on lignocellulosic substrates rich in lignin (Leatham, 1986).

Zembron-Lacny et al. (2013) indicated that compounds produced by shiitake which are attributed to have antioxidant, antimicrobial, antilipidemic, anticancer, anticarcinogenic, and immunoregulatory activities include lentinan, eritadenine, ergothioneine as well as vitamins, especially provitamin D2 (ergosterol and calciferol), vitamins B (thiamine, riboflavin, and niacin) and pantothenic acid. Magnesium, nickel, copper, phosphorus, strontium, and zinc are the minerals found at high concentrations.

Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm.called oyster mushroom , is edible fungi cultivated throughout the world, particularly in Southeast Asia, India, Europe, and Africa (Eşitken, 2003; Tesfaw et al., 2015; Sesli et al., 2020). Oyster mushrooms are the second-largest (Debeaujon, 2000) next to *Agaricus bisporus* (J.E.Lange) Imbach, Mitt. Naturf. Ges. Luzern 15:15 (1946) or the third-largest (Bhatnagar and Deb, 1977) commercially produced mushroom in the world (Tesfaw et al., 2015). Yıldız et al. (2002) addressed that recently some species of *Pleurotus* are cultivated commercially because of their rich mineral contents and medicinal properties, short life cycle, reproducibility in the recycling of certain agricultural and industrial wastes, and low demand on resources and technology.

Improving the performance of mushroom mycelia is very important in terms of cost reduction and time-saving. Magnetic field application is one of the important factors that affect mycelial growth (Sarıtaş, 2015). The influence of magnetic field application on living organisms has been studied by some researchers focusing on germination, vigour as well as growth at later stages of development (Flórez et al., 2007; Marks and Szecówka, 2010; Jamil et al. 2012). Each living organisms have a particular reaction to the electromagnetic field (Ružič et al., 1997) including mushroom mycelia. Shams et al. (2013) indicated that the effects of electromagnetic energy on living tissues are the reason why they are used for agricultural development. Effects of such energy depend on the type, seasonal life spans, field intensity, and duration of treatment (Piacentini et al., 2001).

There are several studies on the effects of magnetic field applications on the mycelia of *Pleurotus* species (Peláez et al., 2013; Mosa et al., 2018). However, there are no previous studies in the literature about the effects of magnetic field application on the mycelia of shiitake. In this study, we examined the impact of various magnetic field strengths (0.25, 0.5, 1, 5, 10, 50, and 100

mT) and exposure durations (5, 10, 15, and 30 min) on the development of the mycelia of oyster and shiitake mushrooms.

Material and method

Shiitake and oyster mushroom mycelia, and the medium for mycelial growth, malt extract agar (MEA), were used in this study. The mycelia of both kinds of mushroom were obtained from a company

in Bursa. A solenoid and strong magnets were used to expose the fungal mycelia to the magnetic fields. A Gauss/Teslameter magnetic field measuring device (Me Magnet-Phy Dr. FH 51 Steingroev GmbH, Art No: 2000510 and serial number: 113 592 CE) was used to determine the effect of the magnetic field on the mycelial development of shiitake and oyster mushrooms. Measurements were performed with a digital multimeter.

The MEA medium (2%), which was used for the mycelial growth, was autoclaved at 121°C for 30 min. Then approximately 30 ml of sterilized medium was poured into plastic Petri dishes with a diameter of 9 cm. A total of 96 shiitake mycelium pieces and 96 oyster mushroom mycelium pieces were placed on the MEA plates (Figure 1). Powerful magnets and a solenoid for low magnetic field strengths were used to generate the necessary magnetic fields for the experiment. The magnetic field was applied to the mycelia at various doses (0.25, 0.5, 1, 5, 10, 50, and 100 mT) and durations (5, 10, 15, and 30 min). The control groups were not subjected to magnetic field treatment. Three replicates were done for each treatment. The growth rates of fungal mycelia were measured once every two days for 20 days. Then, variance analysis (two-way ANOVA) was performed to determine significance. LSD (Least significance difference) was used for multiple comparisons of means. The significance level was set at $p < 0.05$ for all analyses. All statistical analyses were performed using SPSS22.

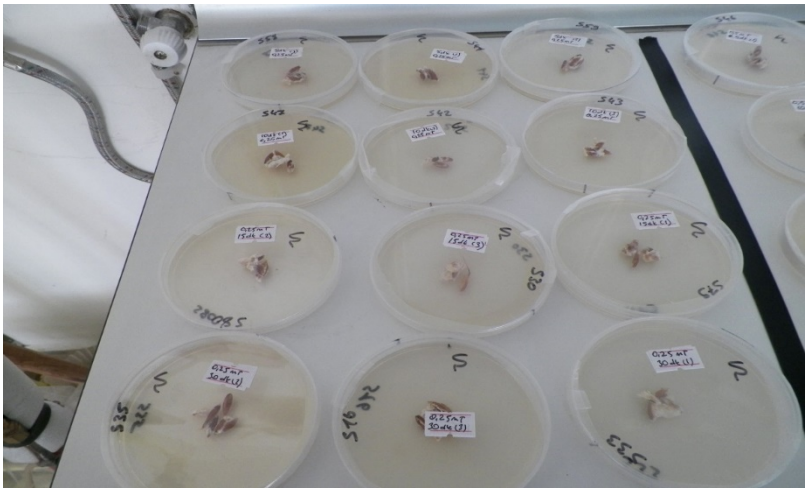

Figure 1. A magnetic field applied shiitake mushroom mycelia

Results and Discussions

Shapiro-Wilk test 'test of normality test' was done whether there was a normal distribution for two types of mushroom mycelium (Table1 and Table 2). Analysis of data showed that the variables were not normally distributed ($p<0.05$). A logarithmic transformation was applied to eliminate the skewness of the original data distributions.

Table 1. Kolmogorov-Smirnov and Shapiro-Wilk tests of normality for shiitake mushroom mycelium

Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
0.193	96	0.000	0.756	96	0.000

Table 2. Kolmogorov-Smirnov and Shapiro-Wilk tests of normality for oyster mushroom mycelium

Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
0.187	96	0.000	0.843	96	0.000

Two-way ANOVA was performed to determine the effect of density and exposure duration of the magnetic field on mycelial growth. Table 3 showed that various magnetic field strengths significantly affected the development of shiitake mycelium ($p < 0.05$), whereas no effect of exposure durations on the mycelial growth ($p > 0.05$) was observed.

Table 3. Two-way ANOVA test results of mycelial growth mean between magnetic field strengths and exposure time for shiitake mushroom mycelium.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	0.021[a]	31	0.001	1.260	0.216
Intercept	81.660	1	81.660	151236.246	0.000
MA	0.009	7	0.001	2.470	**0.026***
Time	0.001	3	0.000	0.548	0.651
MA * Time	0.011	21	0.001	0.958	0.524
Error	0.035	64	0.001		
Total	81.716	96			
Corrected Total	0.056	95			

LSD (Least significance difference) multiple comparison tests were performed to compare the mean values of mycelial growth of shitake mushroom according to the various magnetic field strengths ($p < 0.05$) (Table 4). The magnetic field treatment of 5 mT resulted in a significant increase in mycelial growth in comparison with the 50 mT, 100 mT, and control group. In the same way, mycelia exposed to a magnetic field of 10 mT grew higher than 50 mT and the control group (Table 4). It was reported that static and pulsed magnetic fields affected the growth and enzymatic activity of fungi (Owen, 1998; Nagy and Fischl, 2002).

Table 4. Effect of magnetic field exposure on the mycelial growth rate of shiitake mushroom

(I) MA	(J) MA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.25mT	0.5mT	.0004	.00949	.966	-.0185	.0194
	1mT	.0027	.00949	.775	-.0162	.0217
	5mT	-.0121	.00949	.207	-.0310	.0069
	10mT	-.0062	.00949	.517	-.0251	.0128
	50mT	**.0208***	**.00949**	**.032**	.0018	.0397
	100mT	.0085	.00949	.373	-.0104	.0275
	Control	.0133	.00949	.165	-.0056	.0323
0.5mT	0.25mT	-.0004	.00949	.966	-.0194	.0185
	1mT	.0023	.00949	.808	-.0166	.0213
	5mT	-.0125	.00949	.193	-.0314	.0065
	10mT	-.0066	.00949	.490	-.0255	.0124
	50mT	**.0204***	**.00949**	**.036**	.0014	.0393
	100mT	.0081	.00949	.396	-.0109	.0271
	Control	.0129	.00949	.179	-.0061	.0319
1mT	0.25mT	-.0027	.00949	.775	-.0217	.0162
	0.5mT	-.0023	.00949	.808	-.0213	.0166
	5mT	-.0148	.00949	.124	-.0338	.0041
	10mT	-.0089	.00949	.352	-.0279	.0100
	50mT	.0180	.00949	.062	-.0009	.0370

	100mT	.0058	.00949	.544	-.0132	.0247
	Control	.0106	.00949	.269	-.0084	.0295
5mT	0.25mT	.0121	.00949	.207	-.0069	.0310
	0.5mT	.0125	.00949	.193	-.0065	.0314
	1mT	.0148	.00949	.124	-.0041	.0338
	10mT	.0059	.00949	.536	-.0130	.0249
	50mT	**.0328***	**.00949**	**.001**	.0139	.0518
	100mT	**.0206***	**.00949**	**.034**	.0016	.0395
	Control	**.0254***	**.00949**	**.009**	.0064	.0443
10mT	0,25mT	.0062	.00949	.517	-.0128	.0251
	0,5mT	.0066	.00949	.490	-.0124	.0255
	1mT	.0089	.00949	.352	-.0100	.0279
	5mT	-.0059	.00949	.536	-.0249	.0130
	50mT	**.0269***	**.00949**	**.006**	.0080	.0459
	100mT	.0147	.00949	.126	-.0043	.0336
	Control	**.0195***	**.00949**	**.044**	.0005	.0384
50mT	**0,25mT**	**-.0208***	**.00949**	**.032**	-.0397	-.0018
	0,5mT	**-.0204***	**.00949**	**.036**	-.0393	-.0014
	1mT	-.0180	.00949	.062	-.0370	.0009
	5mT	**-.0328***	**.00949**	**.001**	-.0518	-.0139
	10mT	**-.0269***	**.00949**	**.006**	-.0459	-.0080
	100mT	-.0123	.00949	.201	-.0312	.0067
	Control	-.0075	.00949	.435	-.0264	.0115
100mT	0,25mT	-.0085	.00949	.373	-.0275	.0104
	0,5mT	-.0081	.00949	.396	-.0271	.0109
	1mT	-.0058	.00949	.544	-.0247	.0132
	5mT	**-.0206***	**.00949**	**.034**	-.0395	-.0016
	10mT	-.0147	.00949	.126	-.0336	.0043
	50mT	.0123	.00949	.201	-.0067	.0312
	Control	.0048	.00949	.615	-.0142	.0238
Control group	0,25mT	-.0133	,00949	,165	-,0323	,0056
	0,5mT	-.0129	.00949	.179	-.0319	.0061
	1mT	-.0106	.00949	.269	-.0295	.0084
	5mT	**-.0254***	**.00949**	**.009**	-.0443	-.0064
	10mT	**-.0195***	**.00949**	**.044**	-.0384	-.0005
	50mT	.0075	.00949	.435	-.0115	.0264
	100mT	-.0048	.00949	.615	-.0238	.0142

* indicates significant difference at p<0.05 level (LSD multiple comparison test

The growth rate was characterized with respect to the magnitude of the applied magnetic field, ignoring the time interval of application. At first (between days 2 and 8), the control showed a good growth rate but the growth rate was later (between days 10 and 20) found to be best at 5 and 10 mT (Figure 2). The mycelial growth rate was highest under the magnetic field of 5 mT and 10 mT, respectively, and least at the control group and 50 mT (Figure 2).

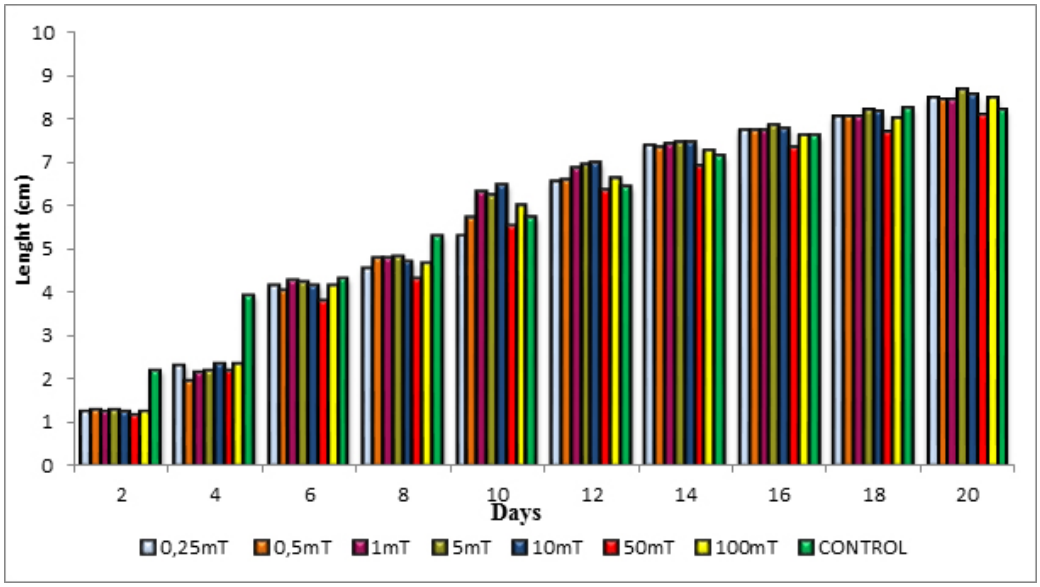

Figure 2. The developmental course of shiitake mushroom mycelium in the different magnetic field strengths

Table 5 showed that the magnetic field strength and exposure durations had no effect on the development of the oyster mushroom mycelium (p>0.05) (Table 5).

The magnetic field applied *Pleurotus* mycelia and non-exposed control group was examined in terms of mycelial growth rate ignoring the exposure time (Figure 3). No significant differences (p>0.05) were found among the magnetic field strengths. At the end of the 20th day, the best mycelial growth rate result was however obtained with the 0.5 mT and the worst was the 100 Mt (Figure 3).

Table 5. Two-way ANOVA test results of mycelial growth mean between magnetic field strengths and exposure time for oyster mushroom mycelium

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	.111[a]	31	.004	.733	.828
Intercept	74.352	1	74.352	15278.158	.000
MA	.039	7	.006	1.142	.349
Time	.021	3	.007	1.469	.231
MA * Time	.050	21	.002	.491	.964
Error	.311	64	.005		
Total	74.774	96			
Corrected Total	.422	95			

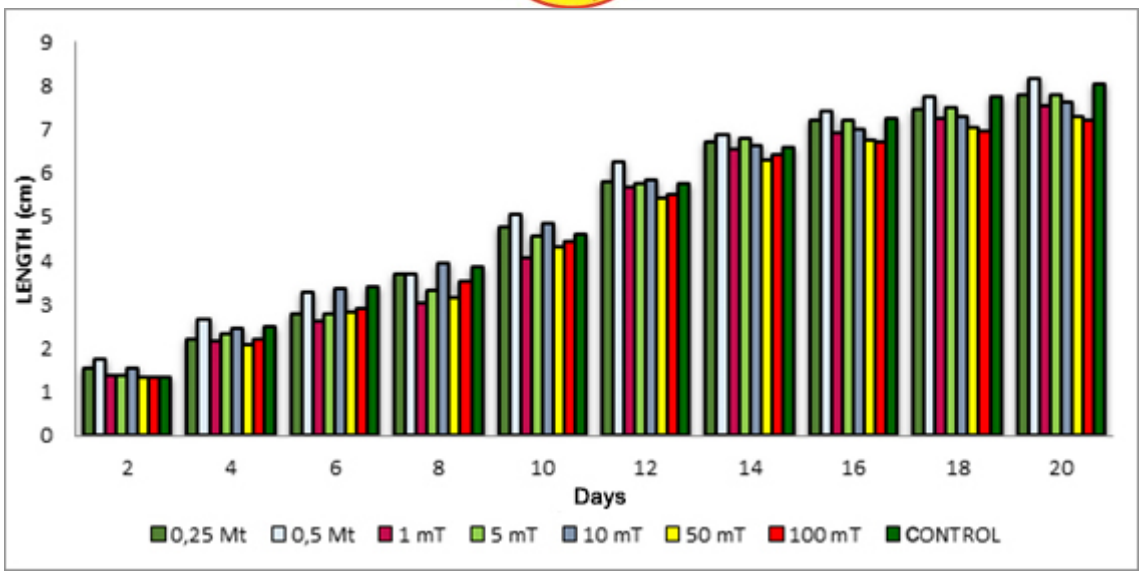

Figure 3. The developmental course of oyster mushroom mycelium in the different magnetic field strengths

Mycelial growth data were taken on 7th day and it was observed that the magnet treatment of 250 mT with the exposure period of 7 days showed the greatest mycelial diameter (8.8 cm), with the indication that this treatment showed the lowest variation compared to the others. In the Dunnett post-Anova test, the treatment of 125 mT with 7 days of exposure was the only one that showed significant differences compared with the control group. It was concluded that the application of magnetic fields has a positive growth effect on the fungus (Pelaez *et al.*, 2013).

In a similar study conducted by Mosa *et al.* (2018), different magnetic field strengths (2, 25, 50, 100, and control) were applied on *P. ostreatus* mycelia. In this study, there was no significant difference in mycelial growth rate under the 50 and 100 mT magnetic fields. It was consistent with our study.

Conclusion

In this study, the development of the mycelia of shiitake and oyster mushrooms was examined under various magnetic field strengths and application intervals. Shiitake mushroom mycelia showed better development under magnetic fields of 5 and 10 mT as compared with the control group. However, no effect was observed for the application interval. According to the results obtained from this study, it can be concluded that the magnetic field exposure of 5 mT and 10 mT may be beneficial for the development of shiitake mushroom mycelia. The magnetic field strength and exposure durations had no effect on the development of the oyster mushroom mycelium.

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