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EMC'22

# 3. International Eurasian Mycology Congress

## 7- 9 SEPTEMBER 2022
## VAN TÜRKİYE

**Book of Proocedings and Abstracts**

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3rd INTERNATIONAL EURASIAN MYCOLOGY CONGRESS (EMC'22)

3. ULUSLARARASI AVRASYA MİKOLOJİ KONGRESİ (EMC'22)

# BOOK OF PROCEEDINGS AND ABSTRACTS

# TAM METİN VE ÖZET KİTABI

**September 07-09, 2022**

**Van-Türkiye**

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# 3. ULUSLARARASI AVRASYA MİKOLOJİ KONGRESİ (EMC'22)

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# 3. ULUSLARARASI AVRASYA MİKOLOJİ KONGRESİ (EMC'22)

**September 07-09, 2022**

**Wednesday, Thursday, Friday**

**in Faculty of Engineering at Van Yüzüncü Yıl University**

# Book of Proceedings and Abstracts

Van, TÜRKİYE

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Kongremize desteklerinden dolayı Van Valiliği, Van Yüzüncü Yıl Üniversitesi Rektörlüğü, TÜBİTAK Bilim İnsanı Destek Programları Başkanlığı, Van Yüzüncü Yıl Üniversitesi Eczacılık Fakültesi, Van Büyükşehir Belediyesi, İpek Yolu Belediyesi, Tuşba Belediyesi, Edremit Belediyesi, Gevaş Belediyesi, Memba Mantar işletmesi ve Yüzüncü Yıl Üniversitesi Arıcılık Uygulama ve Araştırma Merkezine teşekkürlerimizi sunarız.

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# FACE to FACE PROGRAM

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|---|---|---|
| **07.09.2022-Wednesday** | | |
| **9:00-11:00** | Registration | Faculty of Engineering |
| **11:30-12:30** | Opening Ceremony | Faculty of Engineering (Hall 1) |
| **12:30-13:30** | Lunch | University Central Restaurant |
| **Plenary Sessions** | **Chair: Prof. Dr. Younes REZAEE DANESH, Prof. Dr. Alev HALİKİ UZTAN** | |
| **Invited Speakers** | | |
| **13:30-14:00** | European Species of the Genus *Melanoleuca* Basidiomycota Agaricales<br>*Prof. Dr. Vladimír Antonín* | |
| **14:00-14:30** | Studies on Basidiomycetes from the Field to the Laboratory<br>*Prof. Dr. Ertuğrul Sesli* | |
| **14:30-15:00** | Fungal Biotechnology of Turkiye<br>*Prof. Dr. H. Halil Biyik* | |
| **15:00-15:30** | **Coffee Break** | |
| **15:30-16:00** | Revealing Soil Fungal Biodiversity in Natural and Agricultural Ecosystems: The Role of Arbuscular Mycorrhizal Fungi (AMF) and Their Potential as Bio-fertilizers<br>*Prof. Dr. Valeria Bianciotto* | |
| **16:00-16:30** | Nano-elicitor Constructed from Fungal Metabolites for Plant Immunity<br>*Assoc. Prof. Dr. Kasem Soytong* | |
| **16:30-16:45** | **Coffee Break** | |
| **Session 1 (Hall 1): Fungal Biodiversity**<br>**Chair: Prof. Dr. Celaleddin ÖZTÜRK, Prof. Dr. Tülin AŞKUN** | | |
| **16:45-17:00** | Microfungus Diversity of Salda Lake Türkiye an Alkaline Lake<br>*Semra İlhan, Emine Irdem, Omer Topyildiz* | |
| **17:00-17:15** | New Strategies for Molecular Identification of *Aspergillus* Section *nigri* Species<br>*Yüksel Gezgin* | |
| **17:15-17:30** | Contributions to the Turkish Mycobiota New Species Records<br>*Merve Ulukapı, Faruk Selcuk* | |

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| **17:30-17:45** | Contributions to the Turkish Mycobiota New Genera Records<br>*Faruk Selçuk, Merve Ulukapi* |
| **17:45-18:00** | Hakkari Turkiye Ilinden Yeni Bir Vibrisseaceous Mantar Kaydi<br>*İsmail Acar, Yusuf Uzun, Luis Quijada* |
| **18:00-18:15** | Hypogeous Sequestrate Higher Basidiomycetes of Turkiye and Their Distribution<br>*Yasin Uzun, Veysel Batur, Abdullah Kaya* |
| **Session 2 (Hall 2): Fungal Biodiversity**<br>**Chair: Prof. Dr. Faruk SELÇUK, Prof. Dr. Sevda KIRBAĞ** | |
| **16:45-17:00** | Antifungal Activity of Six Nanoparticles of Zinc Copper Cerium Oxide Silver Gold and Selenium against Clinical Isolates of *Aspergillus* Species<br>*Shaghayegh Moghadam, Behnam Azari, Hossein Zarrinfar, Majid Darroudi, Zahra Sabouri, Mohsen Dashti* |
| **17:00-17:15** | Molecular Identification of *Aspergillus* Species in Covid 19 Patients Associated with Fungal Rhinosinusitis<br>*Seyedeh Sabereh Mojtahedi, Hossein Zarrinfar, Mohammad Javad Najafzadeh, Mehdi Bakhshaee, Emane Roshanzamir, Jos Houbrakenss* |
| **17:15-17:30** | Pulmonary Candidiasis Molecular Identification and Antifungal Susceptibility Patterns<br>*Seyedeh Sabereh Mojtahedi, Hossein Zarrinfar, Shaghayegh Moghadam, Neginsadat Hosseinikarger* |
| **17:30-17:45** | Contributions to the Agaricales of Türkiye<br>*Mustafa Emre Akçay, Seyfettin Kaya, Sedat Kesici, Cemil Sadullahoğlu* |
| **17:45-18:00** | Hakkari Ilinden Turkiye Mikotasi Icin Iki Yeni Galerina Kaydi<br>*Cemil Sadullahoğlu, Sedat Kesici, Yusuf Uzun, Mustafa Emre Akcay* |
| **18:00-18:15** | Functional Properties of Iranian Agaric Mushrooms<br>*Masoomeh Ghobad Nejhad, Vladimir Antonin* |

| 08.09.2022-Thursday | |
|---|---|
| 8:00-9:30 (VAN Breakfast in Edremit Seyir Hills) | |
| Session 3 (Hall 1): Medicinal Fungi<br>Chair: Prof. Dr. Gıyasettin KAŞIK, Assoc. Prof. Dr. Mehmet AKYÜZ | |
| **10:45-11:00** | Distribution of Candida Species Isolated from Blood Cultures During the Pandemic Period and Evaluation of Antifungal Susceptibility<br>*Senanur Aydoğan, Rugiyya Samadzade, Salih Macin, Hatice Turk Dagi, Duygu Findik* |
| **11:00-11:15** | The Evaluation of Antioxidant Antimicrobial Antidiabetic Activities and Anatomical Properties of Armillaria Mellea Vahl P Kumm Physalaciriaceae<br>Ayse *Civas, Enes Tekman, Hafize Yuca, Bilge Aydin, Elif Beyza Ozer, Mohaddeseh Nobarirezaeyeh, Muhammed Ziya Sahinoz, Ilgaz Akata, Gamze Goger, Aydan Acar Sahin, Songul Karakaya, Nur Munevver Pinar, Zuhal Guvenalp* |
| **11:15-11:30** | Evaluation of In Vitro Antidiabetic Antioxidant Antimicrobial Activities and Anatomical Properties of an Edible Mushroom Macrolepiota procera Scop Singer<br>*Elif Beyza Ozer, Muhammed Ziya Sahinoz, Mohaddese Nobarirezaeyeh, Ayse Civas, Bilge Aydin, Enes Tekman, Hafize Yuca, Aydan Acar Sahin, Gamze Goger, Ilgaz Akata, Songul Karakaya, Nur Munevver Pinar, Zuhal Guvenalp* |
| **11:30-11:45** | The Evaluation of Antioxidant Antidiabetic Antimicrobial Activities and Anatomical Properties of Tricholoma terreum Schaeff P Kumm Tricholomataceae<br>*Ismail Cagri Aydin, Muhammed Sait Ertugrul, Hafize Yuca, Enes Tekman, Ayse Civas, Bilge Aydin, Aydan Acar Sahin, Gamze Goger, Ilgaz Akata, Mohaddeseh Nobarirezaeyeh, Elif Beyza Ozer, Muhammed Ziya Sahinoz, Songul Karakaya, Nur Munevver Pinar, Zuhal Guvenalp* |
| **11:45-12:00** | The Evaluation of Antioxidant Antidiabetic Antimicrobial Activities and Anatomical Properties of Clitocybe nebularis Batsch P Kumm Tricholomataceae<br>*Bilge Aydın, Mohaddeseh Nobarirezaeyeh, Ayşe Çivas, Enes Tekman, Enes Tekman, Elif Beyza Özer, Muhammed Ziya Şahinöz, Ilgaz Akata, Gamze Göğer, Aydan Acar Şahin, Songül Karakaya, Nur Münevver Pınar, Zuhal Güvenalp* |
| **12:00-12:15** | The Estimation of Antioxidant Antimicrobial Antidiabetic Activities and Anatomical Properties of an Edible Mushroom Pleurotus ostreatus Jacq P Kumm Pleurotaceae<br>*Enes Tekman, Hafiziye Yuca, Ayşe Civas, Bilge Aydın, Elif Beyza Özer, Mohaddeseh Nobarirezaeyeh, Muhammet Ziya Şahinöz, Ilgaz Akata, Gamze Göğer, Aydan Acar Şahin, Songül Karakaya, Nur Münevver Pınar, Zuhal Güvenalp* |
| **12:15-12:30** | A Case with Fungal Keratit Developed to Corneal Melting<br>*Ülkü Demir* |

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| **12:30-12:45** | Evaluation of Candida Types and Antifungal Sensitivity in Endotracheal Aspirate Samples of Icu Patients<br>*Feray Ferda Şenol, Fatma Günbey, Zülal Aşçı Toraman* |
| **12:30-13:30** | Lunch (University Central Restaurant) |
| **Session 4 (Hall 2): Medicinal Fungi**<br>**Chair: Prof. Dr. Semra İLHAN, Assoc. Prof. Dr. Hossein ZARRINFAR** | |
| **10:45-11:00** | The Assessment of Antioxidant Antimicrobial Antidiabetic Activities and Anatomical Properties of *Lepista nuda* Bull Cooke Tricholomataceae<br>*Mohaddeseh Nobarirezaeyeh, Muhammed Ziya Sahninsoz, Elif Beyza Özer, Bilge Aydın, Ayşe Civas, Enes Tekman, Hafize Yuca, Gamze Göğer, Ilgaz Akata, Aydan Acar Şahin, Songül Karakaya, Nur Munevver Pınar, Zuhal Guvenalp* |
| **11:00-11:15** | Screening of *In Vitro* Antidiabetic Antioxidant Antimicrobial Activities and Anatomical Properties of an Edible Mushroom *Lycoperdon pyriforme* Schaeff<br>*Hafize Yuca, Enes Tekman, Ayşe Civas, Bilge Aydın, Aydan Acar Şahin, Gamze Göğer, Ilgaz Akata, Mohaddeseh Nobarirezaeyeh, Elif Beyza Özer, Muhammed Ziya Şahninsöz* |
| **11:15-11:30** | Assessment of *In Vitro* Antidiabetic Antioxidant Antimicrobial Activities and Anatomical Properties of *Coprinus atramentarius* Bull Fr.<br>*Muhammed Ziya Şahinöz, Elif Beyza Özer, Mohaddeseh Nobarirezaeyeh, Ayşe Civas, Bilge Aydın, Enes Tekman, Hafize Yuca, Aydan Acar Şahin, Gamze Göğer, Ilgaz Akata* |
| **11:30-11:45** | Determination of Catalase Enzyme Activity and Reduced Glutathione Level from the Extracts Obtained from *Lepista nuda*<br>*Mustafa Bilici* |
| **11:45-12:00** | Determination of Medicinal Effects of *Ganoderma adspersum* Schulzer Donk<br>*Şule İnci* |
| **12:00-12:15** | Determination of Antimicrobial and Antioxidant Effect of *Suillus luteus* L Roussel<br>*Şule İnci, Sevda Kırbağ* |

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| **12:15-12:30** | Expression of Sap Genes 1 10 and Biofilm Formation in *Candida* spp. Comparison of Healthcare Associated Infection Isolates from Bloodstream with Isolates from Other Sources<br>*Ahmed Moustapha Nsangou, Rugiyya Samadzade, Tuğçe Duran, Salih Macin, Merve Aydın, Duygu Fındık* |
| **12:30-12:45** | The Importance of Early Diagnosis and Effective Treatment in Rino Orbito Cerebral Mucormycosis<br>*Canser Yılmaz Demir* |
| **12:30-13:30** | **Lunch (University Central Restaurant)** |

| **08.09.2022-Thursday** | |
|---|---|
| **Session 5 (Hall 1): Fungal Biotechnology and Chemistry<br>Chair: Prof. Dr. Mustafa YAMAÇ, Assoc. Prof. Dr. Hakan ALLI** | |
| **13:30-13:45** | Extraction Characterization and Biological Activity of Chitosan from a Fungal Mycelial Waste<br>*Burcu Atlı, Mustafa Yamaç, Zeki Yıldız, Zerrin Cantürk* |
| **13.45-14:00** | Fungal Pigments and Their Advantages in Industrial Biotechnology<br>*Selma Kıraç, Yüksel Gezgin* |
| **14:00-14:15** | The Biotechnological Potential of the Macrofungi Isolates Obtained from Bilecik Province<br>*Mustafa Yamaç, Hasan Hüseyin Doğan, Hakan Allı, Bahar Gülce Korkmaz Kahveci* |
| **14:15-14:30** | Turkiye Den Zehirli Iki Mantar Turu *Russula emetica* Schaeff Pers ve *Boletus satanas* Lenz in Antimikrobiyal Antioksidan Aktiviteleri ve Enzim Inhibisyonu<br>*Merve Koçak, Erdoğan Güneş, Sinan Aktaş, Habibe Ünal* |
| **14:30-14:45** | **Coffee Break** |
| **14:45-15:00** | Evaluation of B Galactosidase Enzymes Production by *Aspergillus* Section *nigri* Species Isolated from Dried Figs<br>*Yüksel Gezgin, Ayşegül Yoltaş, Ebru Kocadağ Kocazorbaz* |
| **15:00-15:15** | Kestel Bolgesinde Kadinhani Konya Yaygin Olarak Yetisen Bazi Makromantarlarin Mineral Kompozisyonu<br>*Raziye Büşra Aydemir, Gıyasettin Kaşık, Celaleddin Öztürk, Sinan Alkan* |

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| **15:15-15:30** | Mineral Compositions and Morphological Characterizations of Some Edible Natural Basidiomycota Species<br>*Sinan Alkan, Giyasettin Kaşık, İlker Akın* |
| **15:30-15:45** | Possibilities of Using Myconanotechnology in Combating *Verticillium dahliae* Fungal Pathogen<br>*Hilal Acay, Ayfer Yıldırım* |
| **15:45-16:00** | Antioxidant Antimicrobial and Cytotoxic Activities of Ganoderma Isolates from Turkiye<br>*Goksu Ceylan, Mustafa Yamac, Mustafa Kemal Soylu, Hasan Huseyin Doğan, Zerrin Canturk* |
| **Session 6 (Hall 1): Mushroom Cultivation**<br>**Chair: Prof. Dr. İbrahim TÜRKEKUL, Assoc. Prof. Dr. Şeyda ÇAVUŞOĞLU** | |
| **16:30-16:45** | Use of Biogas Waste Fermented Corn Silage as a Casing Material in the Production of Cultivated Mushrooms *Agaricus bisporus*<br>*Erkan Eren, Mehmet Çetin, Levent Türkler, Şeyda Çavuşoğlu* |
| **16:45-17:00** | A Research on the Cultivation of *Pleurotus nebrodensis* Inzenga Quel<br>*Mehmet Akyüz, Sevda Kırbağ* |
| **17:00-17:15** | Problems Detected in Truffle Cultivation in Türkiye<br>*Hakan Allı, Sevgin Ozderin* |
| **17:15-17:30** | Hasat Sonrası Kestane Mantarlara (*Agaricus campestris*) Uygulanan Melatonin ve Jelatinin Toplam Fenolik ve Antioksidan Kapasitesi İçeriği Üzerine Etkisi<br>*Şeyda Çavuşoğlu, Nurettin Yılmaz, Onur Tekin, Ramazan Ağın, Mehmet Ali Koyuncu* |
| **Session 7 (Hall 2): Fungal Taxonomy and Biodiversity**<br>**Chair: Prof. Dr. Abdullah KAYA, Prof. Dr. Makbule ERDOĞDU** | |
| **13:30-13:45** | Isolation and Identification of Microfungi with Potential Degradation Factors Detected on Historical Ruins in Izmir Archaeological Museum<br>*Niloufar Ahmari, Bengi Aldi, Alev Haliki Uztan* |
| **13.45-14:00** | Molecular Phylogeny and Morphology of *Cortinarius denseconnatus* Basidiomycota from Turkiye<br>*Ayşenur Kalmer, Sedat Kesici, Ayten Dizkırıcı, Yuzuf Uzun* |
| **14:00-14:15** | Kestel Ormani (Kadinhani-Konya) Epifitik Likenleri<br>*Gülşah Yurtyapan, Hatice Esra Akgül, Celaleddin Öztürk* |

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| **14:15-14:30** | Nehir Sularinda Rastlanan Mikrofunguslar<br>*Gulnara Hasanova* |
| **14:45-15:00** | Determination of *Pleurotus eryngii* Complex Species Grown in Eastern Region of Türkiye<br>*Mehmet Akyüz, Sevda Kırbağ,* |
| **15:00-15:15** | Molecular and Morphological Identification of *Leucoagaricus lidensis* from Turkiye<br>*İsmail Acar, Ayşenur Kalmer, Ayten Dizkırıcı* |
| **15:15-15:30** | Morpho Molecular Evidences Reveal Two Noteworthy *Hebeloma* Basidiomycota Species from Turkiye<br>*Ayşenur Kalmer, Ayten Dizkırıcı, İsmail Acar* |
| **15:30-15:45** | Identification of *Helvella* spp. with Molecular Tools Comparison of Barcode and Protein Coding Regions<br>*Şüheda S. Terman, Mustafa Emre Akçay, Ayten Dizkırıcı Tekpınar* |
| **19:30 (Gala Dinner VAN City Municipality)** | |

| 09.09.2022-Friday | |
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| **Session 8 (Hall 1): Environmental Mycology**<br>**Chair: Prof. Dr. Semra DEMİR, Prof. Dr. Younes REZAEE DANESH** | |
| **9:30-9:45** | Iranian Mycological Society Activity at a Glance<br>*Ahmad Abbasi Moghadam, Mohammad Javan Nikkhah* |
| **9:45-10:00** | A New Technique for Enumeration of Arbuscular Mycorrhizal Fungus Spores from Soil: Ultrasonication Technique<br>*Gökhan Boyno, Younes Rezaee Danesh, Emre Demirer Durak, Rojbin Çevik, Semra Demir* |
| **10:00-10:15** | Iklim Degisikliginin Funguslar Uzerindeki Etkisi<br>*Osman Alp Öztop, Senem Öztürk Köse, Hacı Halil Bıyık* |
| **10:15-10:30** | Turkiye Mikobiyotasi Icin Bir Yeni Cins Kaydi<br>*Sedat Kesici, Yusuf Uzun, Cemil Sadullahoğlu, Mustafa Emre Akcay* |
| **10:30-11:00** | **Coffee Break** |
| **11:00-12:00** | **Closing Ceremony** |
| **12:00-13:00** | **Lunch (University Central Restaurant)** |
| **14:00-17:00 (Turkish Van Cat Research Center and City Tour )** | |

# ONLINE PROGRAM

| 07.09.2022-Wednesday | |
|---|---|
| **Session 1**<br>**Chair: Prof. Dr. Hasan Huseyin DOĞAN** | |
| **14:00-14:30** | Asian Mycology in the Next Decade Challenges Solutions and Future Directions<br>*Prof. Dr. Nalin Wijayawardene* **(INVITED SPEAKER)** |
| **14:30-14:45** | Evaluation of Toxicokinetic Properties of Major Mushroom Toxins through in Silico Analysis<br>*Fadime Canbolat* |
| **14:45-15:00** | Microfungi Biodiversity of Pink Kalpli Lake in the Canakkale Ezine Dalyan Region<br>*Tülay Bican Suerdem, Gulcin Ozcan Ates* |
| **15:00-15:15** | First Report of Powdery Mildew Caused by *Erysiphe platani* on *Platanus orientalis* in Pakistan<br>*Irsa Zafar, Najam Ul Sehar Afshan, Abdul Nasir Khalid* |
| **15:15-15:30** | Biological Control of *Trichoderma* spp. against *Fusarium oxysporum* and *Rhizoctonia solani* in Mint (*Mentha multimentha* L.)<br>*Emre Demirer Durak, Füsun Gülser, Hasret Güneş* |
| **Session 2**<br>**Chair: Assoc. Prof. Ilgaz AKATA** | |
| | |
| **15:45-16:00** | Utilization of Agricultural and Forestry by Products in *Ganoderma lucidum* Curt Fr. P Karst Production<br>*Funda Atila* |
| **16:00-16:15** | A New Locality Record of *Singerocybe phaeophthalma* in Türkiye Evidence from Molecular and Morphological Data<br>*Ibrahim Türkekul, Oğuzhan Kaygusuz* |
| **16:15-16:30** | *Terfezia* species natural distributed in Türkiye and their ecologies<br>*Funda Atila, Ahmet Kazankaya* |
| **16:30-16:45** | The Evaluation of Antioxidant Antidiabetic Antimicrobial Activities and Anatomical Properties of *Lyophyllum decastes* Fr. Singer Lyophylaceae<br>*Bilge Aydin, Mohaddeseh Nobarirezaeyeh, Ayse Civas, Enes Tekman, Hafize Yuca, Elif Beyza Ozer, Muhammed Ziya Sahinoz, Ilgaz Akata, Gamze Goger, Aydan Acar Sahin, Songul Karakaya Nur Munevver Pinar, Zuhal Guvenalp* |

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|---|---|
| **16:45-17:00** | Synergistic Antifungal Activities of *P.griseofulvum* E7p Metabolites with Silver Nanoparticles<br>*Emine İrdem, Semra İlhan, Zerrin Cantürk* |
| **17:00-17:15** | Pathogenicity of Entomopathogen *Beauveria bassiana* against to the Larvae of *Cadra cautella* Lepidoptera Pyralidae and *Ephestia kuehniella* Zeller Lepidoptera Pyralidae<br>*Pınar Güner, Tülin Askun, Aylin Er, Görkem Deniz Sönmez* |
| **08.09.2022-Thursday** | |
| **Session 3**<br>**Chair: Prof. Dr. Hasan AKGÜL** | |
| **14:00-14:30** | A New Species of Genus *Bacidina* (Ramalinaceae, Ascomycota) from Pakistan<br>*Fatima Iftikhar, Najam-Ul Sehar Afshan* |
| **14:30-14:45** | Ethnomycological Studies in Anatolia from Past to Present<br>*Sanem Bulam, Aysun Peksen, Nebahat Sule Ustun* |
| **14:45-15:00** | Freshwater Fungal Diversity in Selected Anuradhapura Lakes of Sri Lanka<br>*Madhara Wimalasena, Nalin N Wijayawardene, Dong Qin Dai, T C Bamunuarachchige, Nattawut Boonyuen, R G U Jayalal* |
| **15:00-15:15** | Oyster Mushroom *Pleurotus ostreatus* as a Healthy Ingredient for Sustainable Functional Food Production<br>*Sanem Bulam, Nebahat Sule Ustun, Aysun Peksen* |
| **15:15-15:30** | Biological Degradation of Historical Buildings a Research in Teos Antique City Türkiye<br>*Hatice Yıldız Acar, Fatma Akdeniz, Hasan Akgul, Hacer Sert* |
| **15:30-15:45** | Reactions of Durum Wheat *Triticum Durum* Varieties to Yellow Rust Disease<br>*Ahmet Çat* |
| **Session 4**<br>**Chair: Prof. Dr. Füsun GÜLSER** | |
| **15:45-16:00** | *Fusarium* Species Isolated from Bozcada Cavus and Karalahna Grape<br>*Gülçin Özcan Ateş, Nukhet Nilüfer, Demirel Zorba* |

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|---|---|
| **16:00-16:15** | Kutanoz Mantar Enfeksiyonu Suphesi Olan Hastalardan Alinan Deri Kazinti Orneklerinde Dermatofit ve Diger Mantar Etkenlerinin Konvansiyonel Yontemlerle Saptanmasi<br>*Fatma Günbey, Zulal Asci Toraman, Demet Cicek, Betul Demir, Neslihan Yuksel Bozdemir* |
| **16:15-16:30** | Bronkoalveoler Lavaj Örneklerinde *Pneumocystis jirovecii* nin Gercek Zamanli Pzr Yontemiyle Arastırılması<br>*Merve Ayyıldız, Zulal Asci Toraman, Fatma Gunbey Dogukan, Faik Baytas, Feray Ferda Senol, Yuksel Akkaya* |
| **16:30-16:45** | Study on Genetic Diversity of *Fusarium oxysporum* f.sp. *dianthi* Based on Vegetative Compatibility Groups and Races in Five Provinces of Iran<br>*Nazyar Zandyavari, Nader Hasanzadeh* |
| **16:45-17:00** | Evaluation of Waste Mushroom Composts as Plant Growing Media<br>*Füsun Gülser, Coşkun Gülser* |
| **17:00-17:15** | Effects of Different Plant Wastes on Some Soil Properties<br>*Coşkun Gülser, Füsun Gülser* |
| **17:15-17:30** | Makrofunguslara Yönelik Ölçek Geliştirme ve Etnomikolojik Özelliklerinin Araştırılması Konya Modeli<br>*Mustafa Çevrim, Celaleddin Öztürk, Hakan Kurt* |
| **17:30-17:45** | Başyayla Karaman İlçesinin Miksomisetleri<br>*Gönül Eroğlu* |

# POSTERS PROGRAM

**The posters will be presented by Digital presentation system during the congress dates**

**The participants who responsible for poster presentation should register and attend in the congress, otherwise their certificates and abstracts will not be handled and published in the proceeding**

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| Identification of Subtilisin Genes in Dermatophytes in Ardabil, Iran<br>*Solmaz Basiri, Seyed Jamal Hashemi, Hossein Zarrinfar* |
| The Role of Native Preparation in the Diagnosis of Superficial Dermatophyte Infections<br>*Feray Ferda Şenol* |
| *Pneumocystis Jirovecii* Pneumonia in Children Clinical Presentation Underlying Diseases and Accurate Laboratory Diagnosis<br>*Neginsadat Hosseinikargar, Hossein Zarrinfar, Seyed Javad Seyedi, Mohammad Javad Najafzadeh, Hassan Mehrad Majd, Seyedeh Sabereh Mojtahedi, Mohsen Dashti* |
| Study of the Effect of Some Plant Essential Oils on *Verticillium dahlia* Pathogen by Mixing with Culture Medium<br>*Lida Saeedi, Mojdeh Maleki, Jalal Gholamnezhad, Samin Seddigh, Abdolbaset Azizi* |
| First Use of *Pisolithus arhizus* Scop Rauschert Mushroom in Cotton Yarn Dyeing in Türkiye<br>*Hakan Allı, Ertuğrul Kalay* |
| Determination of Fatty Acid Content of *Pleurotus eryngi* Dc Quel Growıng in the Aegean Region<br>*Hakan Allı, Sevgin Özderin, İbrahim Kıvrak* |
| Biodiversity and Ecology of Myxomycetes of Ayazma Kaz Mountains Bayramic Çanakkale<br>*Tülay Bican Suerdem, İjlal Ocak* |
| Identification of Fungi Associated With Different Pigeon Pea Seed Accessions From Iran<br>*Simin Taheri Ardestani, Ahmad Abbasi Moghadam, Mehrzad Ahmadi, Zahra Talaei* |
| Molecular Diversity on the Races of *Fusarium Oxysporum* f.sp. *ciceris* Causing Wilt of Chickpea in Kurdistan Province of Iran<br>*Tahmineh Naeim Abadi, Mohammad Ali Tajik, Ahmad Abbasi Moghadam, Vali Allah Babaeizad, Majid Hashemi* |
| Determination of *Penicillium* Biodeversity in Winter Month January, February, March 2022 of Battle Stables in Ihsangazı Dıstrıct Kastamonu<br>*Abdullah Şimşek, Gülay Giray* |

Evaluation of Biochemical Parameters in Coronavirus Disease 2019 Patients along with *Candida* infections
*Mahtab Zamanian Yazdi, Azar Khadem Razavi, Hossein Zarrinfar, Hassan Mehrad Majd*

Determination of *Aspergillus* Biodiversity in indor Air in the Winter Months January, February, March 2022 of Cattle Barns in İhsangazı District Kastamonu Which may Create Potential Infection Risk
*Gülay Giray, Abdullah Şimşek*

*Pseudohydropus floccipes* a New Record For Turkish Mycota
*Abdullah Kaya, Yasin Uzun*

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*Masoomeh Ghobad Nejhad, Vladimir Antonin*

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*Yusufjon Gafforov, Shakhnoza Teshaboeva, Tutigul Kholmuradova, Trobjon Makhkamov, Nargiza Abduboyeva, Aziz Abdurazakov, Nodirali Normakhamatov, Sylvie Rapior, Soumya Ghosh*

The Aspects of Hematological Parameters among Covid 19 Patients Affected to Yeast Infections
*Azar Khadem Razavi, Mahtab Zamanian Yazdi, Hossein Zarrinfar, Hassan Mehrad Majd*

Determination of Antifungal Activity of Different Plant Extracts
*Senem Öztürk Köse, Hacı Halil Bıyık, Esin Poyrazoğlu, Zeynep Burcu Bayrak*

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Green Synthesis of Silver Nanoparticles Using Plant Extract and Comparison Their Effect With Antifungal Drugs on the Growth of Standard Strains of *Aspergillus* and *Candida*
*Mahdi Hosseini Bafghi, Hossein Zarrinfar*

Evaluation of the Effect of Plant Essential Oils on *Verticillium dahlia* Pathogen by Paper Disk Method
*Lida Saeedi, Mojdeh Maleki, Jalal Gholamnezhad, Samin Seddigh, Abdolbaset Azizi*

İstanbulda Hava Kirliliğinin Likenler Üzerindeki Etkilerinin İncelenmesi
*Damla Yayman, Gülşah Çobanoğlu*

A Close Friendshıp in Subsoil Arbuscular Mycorrhiza and *Clonostachys rosea*
*Gökhan Boyno, Rojbin Çevik, Younes Rezaee Danesh, Semra Demir*

Phenotypic and Molecular Identification of Green Pea Powdery Mildew Pathogen from Alborz, Iran
*Simin Taheri Ardestani, Seyed Akbar Khodaparast, Ahmad Abbasi Moghadam, Mehrzad Ahmadi*

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| Morfological and Molecular Confirmation of an Edible Fungus *Agaricus arvensis* Schaeff and *Agaricus litoralis* Pilat<br>*Seyda Çomak, Şüheda S. Terman, M. Emre Akçay, Ayten Dizkırıcı Tekpınar* |
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| Yoğun Bakım Hastalarının Kan Kültüründe üreyen *Candida* Türlerinin Dağılımının Değerlendirilmesi<br>*Fatma Günbey, Zülal Aşçı Toraman, Merve Ayyıldız, Doğukan Faik Baytaş, Yasemin Bulut, Feray Ferda Şenol, Yüksel Akkaya* |
| Biyogoruntüleme için Floresan Prob olarak *Agaricus bisporus*'tan Türetilen Karbon Kuantum Noktalarının Mikrodalga Destekli Yeşil Sentezi<br>*Serdar Karakurt, Cemile Birer, Kemal Baş* |
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# FULL TEXTS

# REACTIONS OF DURUM WHEAT *TRITICUM DURUM* VARIETIES TO YELLOW RUST DISEASE

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## ABSTRACT

Wheat yellow rust, caused by obligate fungal pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a destructive disease for wheat worldwide and incessantly threatens wheat production in Türkiye, where especially in coastal and humid areas. In recent years, yellow rust disease has been observed intensively in Türkiye depending on weather conditions, however durum wheat varieties registered in Türkiye has not been evaluated on the reactions to this disease at seedling stage. Therefore, the objective of this study was to evaluate reactions of 54 durum wheat varieties to *Pst* race (virulence formula *Yr1*, *Yr6 Yr7 Yr9* and *YrSp*) at seedling stage. Seedling tests were carried out under controlled greenhouse conditions and infection types (ITs) were recorded 15-19 days post inoculation. The results showed that among 54 durum wheat varieties, 12 varieties were resistant (ITs 0-3), 32 were moderate resistance (ITs 4-6) and 10 were susceptible reaction at seedling stage according to the infection types (ITs) value. To sum up, the majority of the durum wheat varieties (81.5%) were found to be showed resistant and moderate resistance reactions against the *Pst* race used the current study at seedling stage.

***KEYWORDS*****:** Durum wheat, yellow rust, *Pst* race, seedling resistance

**Makarnalık buğday (*Triticum durum*) çeşitlerinin sarı pas hastalığına karşı reaksiyonları**

## ÖZET

Obligat fungal patojen *Puccinia striiformis* f. sp. *tritici* (*Pst*)'nin neden olduğu sarı pas hastalığı dünya genelinde buğdayın en yıkıcı hastalığıdır ve özellikle Türkiye'nin kıyı ve nemli bölgelerinde yetiştirilen buğdaylarda üretimi sınırlandırmaktadır. Son yıllarda sarı pas hastalığı iklim koşullarına bağlı olarak yoğun şekilde görülmektedir ancak Türkiye'de kayıtlı makarnalık buğday çeşitleri fide aşamasında bu hastalığa tepkiler açısından değerlendirilmemiştir. Bu yüzden, bu çalışma 54 makarnalık buğday çeşidinin fide döneminde *Pst* ırkına (virülens formülü: *Yr1, Yr6 Yr7 Yr9* ve *YrSp*) karşı tepkilerini değerlendirmek amacıyla gerçekleştirilmiştir. Fide dönemi testleri kontrollü sera koşullarında yapılmış ve inokulasyondan 15-19 gün sonra enfeksiyon tipleri (ITs) belirlenmiştir. Fide döneminde belirlenen enfeksiyon tipleri (ITs) sonuçlarına göre, 54 makarnalık buğday çeşidinden 12'sinin dayanıklı (ITs 0-3), 32'sinin orta dayanıklı (ITs 4-6) ve 10'unun hassas reaksiyon gösterdiği belirlenmiştir. Sonuç olarak, makarnalık buğday çeşitlerinin çoğunluğunun (%81,5) fide döneminde mevcut çalışmada kullanılan *Pst* ırkına karşı dayanıklı ve orta dayanıklı reaksiyonlar gösterdiği tespit edilmiştir.

***ANAHTAR KELIMELER*****:** Makarnalık buğday, sarı pas, *Pst* ırkı, fide dayanıklılığı

## Introduction

Wheat is a staple crop for human nutrition and has been widely grown around the world. Yellow rust, caused by the obligate pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most destructive fungal diseases threatening wheat production (Chen et al., 2002). The disease was first detected in Türkiye in 1991 and since then, it has been observed every year if the weather conditions are favorable. Additionally, the Seri-82 variety containing the *Yr9* resistance gene had been intensively cultivated in Türkiye but this variety has not been used in wheat production due to the yellow rust epidemic which breakdown the *Yr9* gene (Braun and Saari, 1992; Mamluk et al., 1997). Similarly, it was reported that extensively rust infection occurs in Central Anatolia (Düsünceli et al., 2000) and transition regions. Pathogen development generally occurs at high moisture and temperature. Generally, the disease is controlled by fungicide applications. However, due to its harmful effect on humans and the environment, the usage of resistant varieties prefers in the management of the disease recently (Zheng et al., 2014). These resistance varieties must contain more resistance genes in order to enable durable resistance to different races of pathogens. It has been reported the *Yr5* and *Yr10* resistance genes that provide large-scale resistance to *Pst* races have lost their effectiveness in recent years due to new virulent *Pst* races which emerged as results of the genetic variation among pathogen isolates such as sexual recombination, somatic hybridization and mutation (Sharma-Poudyal et al., 2013; Cat et al., 2021. Tekin et al., 2021). While many studies have been conducted to evaluate the reactions of registered wheat varieties commonly grown in different regions of the world against yellow rust disease at seedling stages (Li et al., 2016; Tahir et al., 2020; Hu et al., 2022) there is no study on the resistance of the seedling stages to the *Pst* race in Türkiye. In addition, registered wheat varieties must be tested against current *Pst* races due to the race changes in the disease commonly. The aim of this study was to determine the reaction of 54 durum wheat varieties to *Pst* race at the seedling stage.

## Materials and Methods

### Plant materials

Totally, 54 durum wheat (*Triticum durum*) varieties were used in the current study (Table 1). In addition, the wheat variety "Morocco" was used as a susceptible control in the seedling test.

**Table 1**. Durum wheat varieties used in this study

| No | Varieties | No | Varieties | No | Varieties |
|---|---|---|---|---|---|
| 1 | Akbaşak 073/144 | 19 | Selçuklu-97 | 37 | Şölen 2002 |
| 2 | Kunduru 414/44 | 20 | Meram-2002 | 38 | Tüten 2002 |
| 3 | Berkmen 469 | 21 | Tunca 79 | 39 | GAP |
| 4 | Çakmak 79 | 22 | Gökgöl 79 | 40 | Turabi |
| 5 | Kızıltan 91 | 23 | Diyarbakır-81 | 41 | Sham-1 |
| 6 | Altın 40/98 | 24 | Ceylan 95 | 42 | Amanos-97 |
| 7 | Yılmaz 98 | 25 | Sarı çanak 98 | 43 | Fuatbey 2000 |
| 8 | Ankara 98 | 26 | Altın toprak 98 | 44 | Sarı Başak |
| 9 | Çeşit-1252 | 27 | Aydın-93 | 45 | Akçakale-2000 |
| 10 | Mirzabey 2000 | 28 | Fırat-93 | 46 | Gündaş |
| 11 | Eminbey | 29 | Artuklu | 47 | Özberk |
| 12 | İmren | 30 | Eyyubi | 48 | Pınar-2001 |
| 13 | Kunduru 1149 | 31 | Şahinbey | 49 | Zenit |
| 14 | Altıntaş 95 | 32 | Zühre | 50 | Svevo |
| 15 | Kümbet 2000 | 33 | Güney Yıldızı | 51 | Levante |
| 16 | Yelken 2000 | 34 | Gediz-75 | 52 | Saragolla |
| 17 | Dumlupınar | 35 | Ege 88 | 53 | Maestrale |
| 18 | Fata Sel | 36 | Salihli 92 | 54 | Bisante |

**Greenhouse seedling test**

In this study, a *Pst* race known as virulence/avirulence formulae: *Yr1*, *Yr6 Yr7, Yr9*, *YrSp*/*Yr5, Yr8 Yr10, Yr15, Yr17, Yr24 Yr27, Yr32, Yr43, Yr44, YrTr1, YrExp2, YrTye*) was used in artificial inoculation to determine the reactions of durum wheat varieties. The uredospore of the *Pst* race was multiplied on susceptible wheat variety "Morocco" seedling. The seedling test was conducted in controlled greenhouse conditions according to the rust inoculation procedure reported by Chen et al. (2002). Ten seeds of each durum wheat varieties were sown in pots. Morocco seedlings at two-leaf stage were inoculated with the *Pst* race by spraying 10 mg of fresh urediniospores in 5 ml of Novec 7100 engineered fluid (3M, Company) using the airbrush spray gun connected to a vacuum pump. Inoculated seedlings were incubated at 10 °C for 24 h. Later, they were transferred to controlled greenhouse conditions. Approximately, 15-19 days post-inoculation, the durum wheat plants were scored for infection types (ITs) according to the scale described by McNeal et al. (1971)

**Data analyses**

Reactions evaluation in seedling test based on the highest ITs value were made (Roelfs et al., 1992). The reaction scores were evaluated in three groups as resistance (R, 0-3), moderate resistance (MR, 4-6) and susceptible (S, 7-9).

**Results**

The reactions of all durum wheat varieties to *Pst* race at the seedling stage were given in Table 2. In the present study, seedling resistance was found in Sarı çanak 98, Altın toprak 98, Eyyubi Şahinbey, Gediz 75, Altıntaş 95, Şölen 2002, Tüten 2002 Turabi, Sham 1, Pınar-2001, Fırat 93

durum wheat varieties (ITs 1-3) while Çeşit 1252, Mirzabey 2000, Dumlupınar, Fata Sel, Meram-2002, Tunca 79, Gökgöl 79, Zühre, Sarı Başak and Gündaş durum wheat varieties showed susceptible reactions (ITs 7-9) to *Pst* race. The remaining other durum wheat varieties showed moderate resistance reaction (ITs 4-6).

**Table 2**. Infection types (ITs) scores of durum wheat varieties to *Pst* race at seedling stage

| Varieties | ITs | Varieties | ITs | Varieties | ITs |
|---|---|---|---|---|---|
| Akbaşak 073/144 | 6 | Selçuklu-97 | 6 | Şölen 2002 | 2 |
| Kunduru 414/44 | 5 | Meram-2002 | 7 | Tüten 2002 | 2 |
| Berkmen 469 | 6 | Tunca 79 | 7 | GAP | 6 |
| Çakmak 79 | 6 | Gökgöl 79 | 7 | Turabi | 2 |
| Kızıltan 91 | 6 | Diyarbakır-81 | 6 | Sham-1 | 2 |
| Altın 40/98 | 6 | Ceylan 95 | 6 | Amanos-97 | 6 |
| Yılmaz 98 | 6 | Sarı çanak 98 | 1 | Fuatbey 2000 | 6 |
| Ankara 98 | 6 | Altın toprak 98 | 2 | Sarı Başak | 7 |
| Çeşit-1252 | 7 | Aydın-93 | 5 | Akçakale-2000 | 5 |
| Mirzabey 2000 | 7 | Fırat-93 | 3 | Gündaş | 7 |
| Eminbey | 5 | Artuklu | 6 | Özberk | 5 |
| İmren | 6 | Eyyubi | 1 | Pınar-2001 | 2 |
| Kunduru 1149 | 6 | Şahinbey | 1 | Zenit | 6 |
| Altıntaş 95 | 2 | Zühre | 7 | Svevo | 5 |
| Kümbet 2000 | 4 | Güney Yıldızı | 5 | Levante | 5 |
| Yelken 2000 | 5 | Gediz-75 | 1 | Saragolla | 5 |
| Dumlupınar | 7 | Ege 88 | 6 | Maestrale | 6 |
| Fata Sel | 7 | Salihli 92 | 6 | Bisante | 4 |

**Discussion**

Two major types of rust resistance, seedling and adult plant resistance, are mostly used for rust disease in wheat. It is mainly preferred that seedling resistance, also known as all-stage resistance (ASR) and race-specific resistance, has been used in wheat varieties. Therefore, it is important that growing wheat varieties are tested against *Pst* race/races at the seedling stage. From this context, in a study conducted by Li et al. (2016), 115 wheat varieties are evaluated *Pst* race *CYR32* at seedling stage and they found that 81 varieties (80.4%) showed intermediate resistance. Likewise, Tahir et al. (2020) stated that 46 of 80 wheat landraces obtained from Pakistan showed resistance reactions against to *Pst* race 574232 at the seedling stage. A similar study conducted by Hu et al. (2022) reported that 305 wheat cultivar/landrace were evaluated for seedling reactions to *Pst* race *CYR32* and *CYR34*. Their results showed that 58 were resistant to both *Pst* races, 72 were resistant to *CYR32* but susceptible to *CYR34*, 28 were resistant to *CYR34* but susceptible to *CYR32*, and 147 were susceptible to both *Pst* races. In the present study, based on the infection types (ITs) values, among the 54 durum wheat varieties, it was determined that 12 were resistant (ITs 0-3), 32 were moderate resistance (ITs 4-6) and 10 were susceptible reactions (ITs 7-9) accounting for .22.2%, 59.2% and 18.5% respectively. These findings obtained from the current study was compatible with the previous research (Yang et al., 2017; Tahir et al., 2020; Tekin et al., 2022). Accordingly, wheat varieties should be tested against *Pst* races both seedling

and adult plant stage. In addition to this, such studies related to resistance reactions of wheat varieties against more *Pst* races should be routinely carried out to reduce the severity of the disease.

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# EVALUATION OF TOXICOKINETIC PROPERTIES OF MAJOR MUSHROOM TOXINS THROUGH IN SILICO ANALYSIS

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## ABSTRACT

Mushrooms have an important place as a food source, and its consumption is common worldwide. Data on the toxicokinetic parameters of nine major mushroom toxins (α-amanitin, orellanine, gyromitrin, coprin, muscarine, psilocybin, psilocin, ibotenic acid and muscimol) are limited in the literature. The reason for this is that the signs of intoxication that may be mushroom are noticed late, the victims go to the hospital long after the mushroom exposure, and therefore, the toxic substance in body fluids cannot be adequately monitored. However, these limitations can be overcome with *in silico-ADMET (*absorption, distribution, metabolism, excretion, and toxicity) models using algorithms technologies. This study aimed to contribute to the literature by evaluating the toxicokinetic parameters of nine toxins by using the *in silico-ADMET* models. Toxicokinetic analyzes were performed by using the free web tools SwissADME, and pkCSM. In our study, the absorption rank of the toxins from the intestines from high to low has found muscarine, gyromitrin, psilocin, psilocybin, muscimol, orellanine, ibotenic acid, coprin, and α-amanitin, respectively. The distributions of toxins were examined, it has been seen that the toxins orellanine, psilocybin, and psilocin have a high distribution volume as specified, while the others have a low distribution volume. It was shown that psilocin could pass the Blood Brain Barrier (BBB). Other molecules didn't have Central Nervous System (CNS) distribution. The potential of toxins to affect the activities of cytochrome P450 enzymes has not been identified. The Maximum Tolerable Dose (MTD) level is low for orellanine, muscimol, psilocybin, and psilocin. Hepatotoxic toxins are α-amanitin and orellanine. In conclusion, the toxicokinetic analyses of nine major mushroom toxins were successfully performed using multiple *in silico* analysis web tools in this study. It is recommended that *in silico* analysis methods be used prior to studies to provide a preliminary idea for comprehensive and multi-molecular *in vivo* toxicokinetic analyses.

***KEYWORDS*****:** Mushroom, Toxins, In silico, Toxicokinetic

## In Silico Analiz Yolu ile Başlıca Mantar Toksinlerinin Toksikokinetik Özelliklerinin Değerlendirilmesi

## ÖZET

Mantarlar besin kaynağı olarak önemli bir yere sahiptir ve tüketimi dünya çapında yaygındır. Literatürde dokuz ana mantar toksininin (α-amanitin, orellanin, gyromitrin, koprin, muskarin, psilosibin, psilosin, ibotenik asit ve muskimol) toksikokinetik parametrelerine ilişkin veriler sınırlıdır. Bunun nedeni mantar zehirlenme belirtilerinin geç fark edilmesi, mağdurların mantar maruziyetinden çok sonra hastaneye gitmesi ve bu nedenle vücut sıvılarındaki toksik maddenin yeterince izlenememesidir. Bununla birlikte, algoritma teknolojileri kullanılan In silico-ADMET

(absorpsiyon, dağılım, metabolizma, atılım ve toksisite) modellerinde bu sınırlamaların üstesinden gelinebilir. Bu çalışma, In silico-ADMET modellerini kullanarak dokuz toksinin toksikokinetik parametrelerini değerlendirerek literatüre katkıda bulunmayı amaçlamıştır. Toksikokinetik analizler SwissADME ve pkCSM ücretsiz web araçları kullanılarak yapıldı. Çalışmamızda toksinlerin bağırsak emilimi yüksekten düşüğe doğru sırasıyla muskarin, gyromitrin, psilosin, psilosibin, muskimol, orellanin, ibotenik asit, koprin ve a-amanitin bulunmuştur. Toksinlerin dağılımları incelendiğinde, orellanin, psilosibin ve psilosin toksinlerinin yüksek dağılım hacmine sahip olduğu, diğerlerinin ise düşük dağılım hacmine sahip olduğu görülmüştür. Psilosinin Kan Beyin Bariyerini (BBB) geçebileceği görülmüştür. Diğer moleküllerin Merkezi Sinir Sistemine (MSS) dağılımı zayıf bulunmuştur. Toksinlerin Sitokrom P450 enzimlerinin aktivitelerini etkileme potansiyeli bulunmamaktadır. Orellanin, muscimol, psilosibin ve psilosin için Maksimum Tolere Edilebilir Doz (MTD) seviyesi düşüktür. Hepatotoksik toksinler a-amanitin ve orellanindir. Sonuç olarak, bu çalışmada dokuz ana mantar toksininin toksikokinetik analizleri, çoklu In Silico analiz ağ araçları kullanılarak başarıyla gerçekleştirilmiştir. Kapsamlı ve çok moleküllü in vivo toksikokinetik analizlere ön fikir sağlamak amacıyla çalışmalardan önce In Silico analiz yöntemlerinin kullanılması önerilmektedir.

***ANAHTAR KELIMELER*:** Mantar, Toksinler, In silico, Toksikokinetik

## Introduction

Mushrooms, which are commonly found in nature, are heterotrophic, spore-forming, chlorophyll-free, parasitic, and sporadically-living eukaryotic creatures. Chang and Miles (1992) defined mushrooms as "macro fungi with a unique hat that can be easily seen with the naked eye and collected by hand, growing on or under the soil" (Coşkun ve Kaya 2020). Mushrooms have an important place as a food source, and its consumption is common worldwide and in Türkiye. While the term mushroom is mainly used for edible species of macrofungi, macrofungi containing some toxic substances for human health are known as "poisonous mushrooms." Intoxications due to mushroom consumption are quite common (Toka et al., 2016). Mushroom intoxications vary according to the toxidromes of the main toxins in the mushroom content. Among the main toxins, alpha-amanitin (α-amanitin) is in the "cyclopeptide" group, orellanine is in the "orellanine" group, gyromitrin is in the "monomethyl hydrazines" group, coprin is in the "disulfiram-like reaction" group, muscarine is in the "central nervous system (CNS)" influencing group, psilocybin and psilocin are in the "hallucinatory indoles" group, and ibotenic acid and muscimol are in the "isoxazoles" group (Diaz, 2005). α-Amanitin containing mushroom species are *Amanita phalloides (A. phalloides), Amanita verna (A. verna), Amanita virosa (A. virosa), Galerina unicolor, Lepiota helveola (L. helveola), and Lepiota brunneoincarnata (L. brunneoincarnata).* α-Amanitin is cytotoxic (White et al., 2019). It causes necrosis in the liver and kidneys and induces the Phalloides Syndrome. The median lethal dose ($LD_{50}$) of α-amanitin in humans is 0.1 mg/kg (Flament et al., 2020). The mushroom species containing orellanine are *Cortinarius orellanus (C. orellanus)* and *Cortinarius orellanoides (C. orellanoides).* It reduces glomerular filtration by acting on the kidneys and causes the Orellanus Syndrome. The $LD_{50}$ in mice is between 30-90 mg/kg. The lethal dose of orellanine in humans is 100-200 g/kg (Karlson-Stiber and Persson, 2003). The mushroom species containing gyromitrin are *Gyromitra esculenta (G. esculenta), Gyromitra gigas (G. gigas), Gyromitra ambiua (G. ambigua), Helvella crispa (H.crispa), Helvella lacunose (H. lacunose), Paxina leucomelas,* and *Sarcosphaera crassa.* Gyromitrin causes the Gyromitra Syndrome. Hydrazines released by the biotransformation of gyromitrin and its metabolites cause necrosis in the liver. The lethal dose

of gyromitrin is 10-30 mg/kg in children and 20-50 mg/kg in adults (Puschner, 2018). Coprin is a colorless, crystallized compound formed by the condensation of glutamine and cyclopropanone. The mushroom species containing coprin is *Coprinus atramentarius.* It causes the Coprinus Syndrome. It inhibits the destruction of alcohol in the body and stops it at the acetaldehyde stage. The mushroom species containing muscarine *are Inocybe*, *Clitocybe* and *Mycena* (Govorushko et al., 2019). Muscarine is effective on the peripheral nervous system and causes the Muscarinic Syndrome. The lethal dose of muscarine in humans is around 180-300 mg (Puschner, 2018). The mushroom species containing psilocybin are *Psilocybe, Panaeolus, Stropharia,* and *Conocybe.* They cause the Psilocybin Syndrome. They are effective in CNS, causing hallucinations. Psilocybin taken orally is rapidly hydrolyzed to psilocin by the action of alkaline phosphatase in the body. Psilocybin is effective at very low doses. An amount of 4 mg causes mild poisoning, and 6-20 mg causes significant psychotropic changes. The mushroom species containing ibotenic acid and muscimol are *Amanita muscaria (A. muscaria)* and *Amanita pantherine (A. pantherine).* It causes the Pantherina Syndrome. Both compounds are effective in CNS. Ibotenic acid in CNS also stimulates glutamate receptors and causes excitotoxicity. The LD50 in rats is 129 mg/kg (Flament et al., 2020). Muscimol is five times more active than ibotenic acid. Since its chemical structure is similar to GABA (gamma-aminobutyric acid), it can stimulate GABA receptors in CNS cells, and thus has a GABA-like effect. The LD50 in rats is 129 mg/kg (Flament et al., 2020).

Data on the toxicokinetic parameters of these nine major toxins that cause mushroom intoxication in humans are limited in the literature. The reason for this is that the signs of intoxication that may be mushroom are noticed late, the victims go to the hospital long after the mushroom exposure, and therefore, the toxic substance in body fluids cannot be adequately monitored. In the toxicokinetic studies in the literature, it is seen that the parameters can be examined to a limited extent due to the large cost and extensive time required by toxicokinetic studies, and comparative toxicokinetic studies cannot be conducted at a sufficient level. These reasons limit the ability of researchers to conduct desired toxicokinetic studies. However, these limitations can be overcome with *in silico* research models using today's technologies. *In silico* research models enable to estimate the absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters of molecules using algorithms. In today's drug development studies, *in silico* models are used before preclinical and clinical analyses to determine the correct molecule in a short period and to eliminate the cost and time loss experienced in the traditional drug research process. In the literature and scientific research, it is accepted that web-based analysis techniques are an alternative approach for ADMET studies. This study aimed to contribute to the literature by evaluating the toxicokinetic parameters of α-aminitin, orellanine, gyromitrin, coprin, muscarine, psilocybin, psilocin, ibotenic acid, and muscimol molecules, which are among the main toxins causing mushroom intoxication, by using the *in silico* ADMET analysis methods.

**Material and Method**

Physicochemical and toxicokinetic analyses of nine major toxins (α-aminitin, orellanine, gyromitrin, coprin, muscarin, psilocybin, psilocin, ibotenic acid, and muscimol) were performed *in silico* by using the free web tool SwissADME (http://www.swissadme.ch/), and pkCSM (http://biosig.unimelb.edu.au/pkcsm/) web tools, which can analyze the physicochemical, pharmacokinetic, and drug similarity properties of molecules with fast and robust predictive models, were utilized to predict the small-molecule pharmacokinetic properties using graph-based signatures. First of all, the molecular SMILES (Simplified Molecular Input Line Entry System) structures of each toxin were downloaded from the "pubchem" (https://pubchem.ncbi.nlm.nih.gov/) page. The downloaded SMILES structures were uploaded

to the SwissADME and pkCSM programs. The physicochemical and toxicokinetic properties of each toxin were evaluated with the parameters in the program.

**Results**

The SwissADME database analyzes the data by considering five different evaluation criteria (Ro5) known as Lipinski, Ghose, Veber, Egan, and Muegge rules to determine the oral absorption of molecules (Lipinsky et al., 1997; Ghosa et al., 1999; Veber et al., 2002). The general properties of the molecules with high oral absorption in the program are determined by the molecular weight (MW) ≤ 500 g/mol, H-donor ≤ 5, H-acceptor ≤ 10, Rotatable bonds 10, MLogP ≤ 5, topoplogical polar surface area (TPSA) <140 Å² criterion. The results of the physicochemical properties of α-amanitin, orellanine, gyromitrin, coprin, muscarine, psilocybin, psilocin, ibotenic acid, and muscimol toxins obtained from SwissADME databases are given in Table 1. When the molecular weights, H-donor, H-acceptor, TPSA, rotatable bond, and MLogP values of the toxins in Table 1 were evaluated according to the criteria determined by the program, it was seen that α-amanitin did not meet the criteria. The physicochemical properties of the other toxins were included in the Ro5 rule. The toxins were individually analyzed in both the SwissADME and pkCSM data tools to determine the toxicokinetic properties of α-amanitin, orellanine, gyromitrin, coprin, muscarine, psilocybin, psilocin, ibotenic acid, and muscimol toxins. Table 1 shows the gastrointestinal absorption data obtained from SwissADME, the transition status of the Blood Brain Barrier (BBB), and the status of being a P-glycoprotein substrate.

**Table 1.** Result of physicochemical properties of major mushroom toxins by SwissADME

| Mushroom | Toxin | Physicochemical Properties Molecular formula | MW g/mol | TPSA Å² | H donor | H acceptor | Rotb | Log $P_{O/W}$ (MLOGP) | Log S (ESOL) | Assessment of GIS, BBB and pGP subs. status |
|---|---|---|---|---|---|---|---|---|---|---|
| *A. phalloides* | α-Amanitin | $C_{39}H_{54}N_{10}O_{14}S$ | 918.97 | 400.09 | 13 | 14 | 7 | -5.74 | -2.40 | GIS: low BBB: no Pgp subs: yes |
| *Cortinaceae* | Orellanine | $C_{10}H_8N_2O_6$ | 252.18 | 124.92 | 4 | 6 | 1 | -1.51 | -1.21 | GIS: high BBB: no Pgp subs: no |
| *Gyromitra* | Gyromitrin | $C_4H_8N_2O$ | 100.12 | 32.67 | 0 | 2 | 2 | 0.31 | -0.15 | GIS: high BBB: no Pgp subs: no |
| *Clitocybe, Mycena, Inocybe* | Muscarine | $C_9H_{20}NO_2^+$ | 174.26 | 29.46 | 1 | 2 | 2 | -3.35 | 1.67 | GIS: high BBB: no Pgp subs: no |
| *A.pantherine, A.muscaria* | Muscimol; | $C_4H_6N_2O_2$ | 114.10 | 72.02 | 2 | 3 | 1 | -1.08 | 0.56 | GIS: high BBB: no Pgp subs: no |
| | Ibotenic acid | $C_5H_6N_2O_4$ | 158.11 | 109.32 | 3 | 5 | 2 | -3.80 | 1.43 | GIS: high BBB: no Pgp subs: no |
| *Plocybe, Panaeolus, Gymnopillus, Conocybe* | Psilocybin ; | $C_{12}H_{17}N_2O_4P$ | 284.25 | 95.60 | 3 | 5 | 5 | 0.27 | -6.02 | GIS: high BBB: no Pgp subs: no |
| | Psilocin | $C_{12}H_{16}N_2O$ | 204.27 | 39.26 | 2 | 2 | 3 | 1.22 | -2.68 | GIS: high BBB: yes Pgp subs: no |
| *C.atramentarius* | Coprin | $C_8H_{14}N_2O_4$ | 202.21 | 112.65 | 4 | 5 | 6 | -3.72 | 1.46 | GIS: high BBB: no Pgp subs: no |

*A. phalloides; Amanita phalloides, A. pantherine; Amanita pantherine, A.muscaria; Amanita muscaria, C. atramentarius; Coprinus atramentarius,* MW; moleküler weight (g/mol), TPSA;

Topological polar surface area ( Å²), $H_{donor}$: Hydrogen donor, $H_{acceptor}$: Hydrogen acceptor, Rotb; Rotatable bond, GIS; Gastro Intestinal System, BBB; Blood Brain Barrier, pGP subs.; P-glycoprotein substrate.

Table 2 demonstrates the parameters that may affect the absorption of the molecules in the gastrointestinal tract using the pkCSM data tool. The interaction of the molecules with P-glycoprotein, which is one of the carrier proteins and affects absorption, Caco2 permeability, and skin permeability, which affects absorption from the intestines, is presented in Table 2.

**Table 2**. Result of absorption properties of major mushroom toxins by pkCSM

| Mushroom | Toxin | ABSORPTION | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Water solubility<br><br>Log mol/L | Caco2 permeability (log $P_{APP}$ in $10^{-6}$ cm/s) | Intestinal absorption (human; % Absorbed) | Skin Permeability<br><br>Log Kp | pGP subs.<br><br>Yes/No | pGP I inhibitor<br><br>Yes/No | pGP II inhibitor<br><br>Yes/No |
| *A. phalloides* | α-Amanitin | -2.85 | -0.54 | 0 | -2.74 | Yes | No | No |
| *Cortinaceae* | Orellanine | -2.90 | 0.05 | 71.53 | -2.74 | No | No | No |
| *Gyromitra* | Gyromitrin | -0.06 | 1.48 | 100 | -2.97 | Yes | No | No |
| *Clitocybe, Mycena, Inocybe* | Muscarine | -0.18 | 1.58 | 100 | -3.51 | No | No | No |
| *A.pantherine, A.muscaria* | Muscimol; | -0.45 | 0.63 | 87.04 | -3.02 | No | No | No |
| | Ibotenic acid | -2.89 | -0.32 | 48.33 | -2.74 | Yes | No | No |
| *Plocybe, Panaeolus, Gymnopillus, Conocybe* | Psilocybin; | -3.13 | 0.94 | 91.78 | -2.77 | Yes | No | No |
| | Psilocin | -2.90 | 1.54 | 92.40 | -2.83 | Yes | No | No |
| *C.atramentarius* | Coprin | -2.89 | -0.37 | 33.07 | -2.74 | No | No | No |

*A. phalloides; Amanita phalloides, A. pantherine; Amanita pantherine, A.muscaria; Amanita muscaria, C. atramentarius; Coprinus atramentarius*, pGP subs.; P-glycoprotein substrate, pGP I inhibitor; P-glycoprotein I inhibitor, pGP II inhibitor; P-glycoprotein II inhibitör.

The evaluation of intestinal absorption of oral molecules is performed with Caco2 permeability (log PAPP in $^{10-6}$ cm/s) and intestinal absorption (human; % Absorbed) values in pkCSM web scanning. Since Caco2 permeability is associated with small intestinal absorption, Caco2 permeability is considered high when log $P_{APP}$ is > 0.90, which gives information about the intestinal absorption of orally administered drugs. When the intestinal absorption (human; % Absorbed) value is < 30% in the program output, the intestinal absorption of the molecule is considered weak. As demonstrated in Table 2, the absorption rank of the toxins from the intestines from high to low is muscarine, gyromitrin, psilocin, psilocybin, muscimol, orellanine, ibotenic acid, coprin, and α-amanitin, respectively. α-Amanitin, gyromitrin, ibotenic acid,

psilocybin, psilocin, and coprin are substrates of p-glycoprotien, a carrier protein. Regarding the skin permeability in the program, low skin permeability occurs when log Kp > -2.5. The log Kp values of these toxins ranged from -2.74 to -3.51.

The distribution and metabolism analysis results of the toxins performed through the pkCSM web tool are given in Table 3. The program includes the distribution volume (VDss (human); Log L/kg), molecular level not dependent on plasma proteins and BBB and CNS transition that affect the distribution of molecules. For metabolism analysis, the most effective Cytochrome P450 (CYP) enzymes in our body are evaluated.

**Table 3.** Result of distribution and metabolism properties of major mushroom toxins by pkCSM

| Mushrom | Toxin | Distribution | | | | Metabolism | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VDss | Fu | BBB perm. | CNS perm. | CYP 2D6 subs. | CYP 3A4 subs. | CYP 1A2 inhibitor | CYP2C19 inhibitor | CYP 2C9 inhibitor | CYP2D6 inhibitor | CYP 3A4 inhibitor |
| | | Log L/kg | | Log BB | Log PS | Yes/No | Yes/No | Yes/No | Yes/No | Yes/No | Yes/No | Yes/No |
| *A. phalloides* | α-Amanitin | -0.92 | 0.34 | -2.07 | -6.07 | No | No | No | No | No | No | No |
| *Cortinaceae* | Orellanine | 1.24 | 0.69 | -1.24 | -3.58 | No | No | No | No | No | No | No |
| *Gyromitra* | Gyromitrin | -0.20 | 0.76 | -0.22 | -2.75 | No | No | No | No | No | No | No |
| *Clitocybe, Mycena, Inocybe* | Muscarine | 0.23 | 0.78 | 0.09 | -3.27 | No | No | No | No | No | No | No |
| *A.pantherine, A.muscaria* | Muscimol; | -0.19 | 0.98 | -0.05 | -3.38 | No | No | No | No | No | No | No |
| | Ibotenic acid | -0.36 | 0.55 | -0.74 | -3.53 | No | No | No | No | No | No | No |
| *Plocybe, Panaeolus,* | Psilocybin; | 0.46 | 0.48 | -1.09 | -2.84 | No | No | No | No | No | No | No |
| *Gymnopillus, Conocybe* | Psilocin | 0.98 | 0.45 | 0.24 | -1.58 | Yes | No | No | No | No | No | No |
| *C.atramentarius* | Coprin | -0.46 | 0.51 | -0.91 | -3.63 | No | No | No | No | No | No | No |

*A. phalloides; Amanita phalloides, A. pantherine; Amanita pantherine, A.muscaria; Amanita muscaria, C. atramentarius; Coprinus atramentarius*, VDss; Human distribution volume (log

L/kg), Fu; Fraction unbound (human), BBB perm.; Blood Brain Barrier permeability (log BB), CNS perm.; Cental Nervous System permeability (log PS), CYP2D6 subs.; cytochrome P450 2D6 substrate, CYP3A4 subs.; cytochrome P450 3A4 substrate, CYP1A2 inhibitor; cytochrome P450 1A2 inhibitor, CYP2C19 inhibitor; cytochrome P450 2C19 inhibitor, CYP2C9 inhibitor; cytochrome P450 2C9 inhibitor, CYP2D6 inhibitor; cytochrome P450 2D6 inhibitor, CYP3A4 inhibitor; cytochrome P450 3A4 inhibitor.

As shown in Table 3, the toxins investigated have different distribution volumes. The Log VDss value is considered when evaluating the distribution volume of pkCSM web tool molecules. The distribution volume of molecules with a Log VDss value of < -0.15 is accepted to be low by the program. In Table 3, α-amanitin, gyromitrin, muscimol, ibotenic acid, and coprin toxins are among the molecules with low distribution volume since their Log VDss value is < -0.15. BBB and CNS transitions of molecules are evaluated with Log BB and Log PS values in the pkCSM web tool, respectively. The distribution of molecules with a Log BB value of < -1 to the brain is considered low. As Table 3 illustrates, the Log BB values of α-amanitin, orellanine, and psilocybin toxins are < -1. Coprin has a relatively low BBB passage. When the CNS transitions of the toxins are examined, it is shown by the program that the CNS permeability of those with log PS < -3 is low. The distribution of α-amanitin and orellanine to both the BBB and CNS is low. Although the transition of muscarine to the BBB is weak, the CNS distribution is borderline. While ibotenic acid and muscimol can pass the BBB, CNS distribution is low. The coprin BBB transition is relatively low, whereas its distribution to CNS is borderline.
The excretion and toxicity analysis results of the toxins performed through the pkCSM web application are given in Table 4. When Table 4 is examined, the Maximum Tolerable Dose (MTD) is low for orellanine, muscimol, psilocybin, and psilocin. The dose level at which the lowest side effect appears is low in muscimol, gyromitrin, and ibotenic acid. According to the results, hepatotoxic toxins are α-amanitin and orellanine. On the other hand, skin sensitivity is caused by gyromitrin, muscarine, and psilocin.

**Table 4.** Result of excretion and toxicity properties of major mushroom toxins by pkCSM

| Mushroom | Toxin | Excretion | | Toxicity | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CL tot. Log ml/min/kg | OCT2 subs. Yes/No | AMES Yes/No | MTD Log mg/kg/day | hERG I inhibitor Yes/No | hERG II inhibitor Yes/No | LD50 mol/kg | LOAEL Log mg/kg bw/day | Hepatotoxicity Yes/No | Skin Sensitisation Yes/No | *T.Pyriformis* toxicity Log ug/L | Minnow toxicity Log mM |
| *A. phalloides* | α-Amanitin | 0.09 | No | No | 0.50 | No | No | 2.45 | 4.81 | Yes | No | 0.29 | 18.24 |
| *Cortinaceae* | Orellanine | 0.51 | No | No | 0.39 | No | No | 1.97 | 2.46 | Yes | No | 0.29 | 4.38 |
| *Gyromitra* | Gyromitrin | 0.90 | No | No | 0.94 | No | No | 2.34 | 1.11 | No | Yes | -0.16 | 2.58 |
| *Clitocybe, Mycena, Inocybe* | Muscarine | 0.99 | No | No | 0.77 | No | No | 2.31 | 1.88 | No | Yes | -0.33 | 2.78 |
| *A.pantherine, A.muscaria* | Muscimol; | 0.76 | No | No | 0.02 | No | No | 2.36 | 0.89 | No | No | -0.38 | 3.34 |
| | Ibotenic acid | 0.36 | No | No | 0.99 | No | No | 2.17 | 1.43 | No | No | 0.29 | 3.70 |
| *Plocybe, Panaeolus, Gymnopillus, Conocybe* | Psilocybin; | 0.60 | No | No | 0.29 | No | No | 1.7 | 2.07 | No | No | 0.35 | 1.00 |
| | Psilocin | 1.03 | Yes | No | -0.36 | No | No | 2.47 | 2.08 | No | Yes | 0.46 | 1.01 |
| *C.atramentarius* | Coprin | 0.92 | No | No | 1.23 | No | No | 2.06 | 2.47 | No | No | 0.28 | 3.52 |

*A. phalloides; Amanita phalloides, A. pantherine; Amanita pantherine, A.muscaria; Amanita muscaria, C. atramentarius; Coprinus atramentarius*, CLtot; Total Clearance (Log ml/min/kg), OCT2 subs.; Renal OCT2 substrate, AMES; AMES toxicity, MTD; Maximum Tolerated Dose (human; log mg/kg/day), Oral Rat Acute Toxicity- median Lethal Dose (LD50; mol/kg), Oral Rat Chronic Toxicity- the Lowest Observed Adverse Effect Level (LOAEL; log mg/kg bw/day).

## Discussions

The SwissADME web tool evaluates the suitability of compounds for oral administration according to five different assessment scales known as Lipinski, Ghose, Veber, Egan and Muegge rules (Lipinsky et al., 1997; Ghosa et al., 1999; Veber et al., 2002). For the evaluation of pharmacokinetic parameters, five rules (Ro5) published by Lipinski et al. (1997) as a result of the investigation of the physicochemical properties of 2245 drugs in the World Drug Index (WDI) database are theoretically taken as a reference (Lipinsky et al., 1997). This rule, which is used to estimate the oral bioavailability of drugs that may be absorbed by passive diffusion, is

based on the physicochemical properties of the compounds tested. Common features of molecules that are easy to absorb by passive diffusion include MlogP ≤ 5, MW ≤ 500 g / mol, Number of hydrogen bond recipients (HBA) (total N and O atoms) ≤ 10, Number of hydrogen bond donors (HBD) (total OH and NH groups) ≤ 5, Number of rotatable bonds (nRotb) ≤ 10, and the Topological polar surface area (TPSA) Å² < 140 Å² (Ghosa et al., 1999; Yamashita and Hashida, 2004). When the physicochemical properties (Table 1) of α-amanitin, orellanine, gyromitrin, coprin, muscarine, psilocybin, psilocin, ibotenic acid, and muscimol toxins obtained from SwissADME in our study are examined, it is seen that α-amanitin is not included in the Ro5 rule, and its gastrointestinal absorption is low. The evaluation of intestinal absorption of oral molecules is performed with Caco2 permeability (Log $P_{APP}$ in $^{10-6}$ cm/s) and intestinal absorption (human; % Absorbed) values in the pkCSM web tool (Pires et al., 2015). Since Caco2 permeability is associated with small intestinal absorption, Caco2 permeability is considered high when Log $P_{APP}$ is > 0.90. This situation gives information about the intestinal absorption of oral drugs. When the intestinal absorption (human; % Absorbed) value is < 30% in the program output, the intestinal absorption of that molecule is considered weak. Since the Log $P_{APP}$ and Intestinal absorption level of α-amanitin is found to be low in Table 2, it is seen that the absorption of α-amanitin from the small intestine is lower than other toxins. α-Amanitin is included as a p-glycoprotein substrate. When the physicochemical properties of muscarin, gyromitrin, psilocybin, and psilocin toxins specified in Table 1 are examined, it is seen that these toxins meet the Ro5 rule, and their gastrointestinal absorption is high. In addition, the Log $P_{APP}$ and intestinal absorption levels of these molecules are also found to be high as seen in Table 2. Therefore, it is predicted by both web tools that the absorption of the toxins muscarine, gyromitrin, psilocybin, and psilocin from the small intestines is high. Gyromitrin, psilocybin and psilocin are included as p-glycoprotein substrates. When the physicochemical properties of muscimol, orellanine, ibotenoic acid, and coprin in Table 1 are examined, it is seen that these molecules also meet the Ro5 rule and have high gastrointestinal absorption. In addition, there are differences in the Log $P_{APP}$ and intestinal absorption levels of these molecules (Table 2). Although the Log $P_{APP}$ levels are below >0.9, they can be absorbed from the small intestine since the intestinal absorption (human; % Absorbed) value is > 30%. According to the program, low skin permeability occurs when Log Kp > -2.5. The Log Kp values of the toxins studied range from -2.74 to -3.51. When the distributions of α-amanitin, orellanine, gyromitrin, coprin, muscarine, psilocybin, psilocin, ibotenic acid, and muscimol toxins in the body are examined, it is seen that the toxins orellanine, psilocybin, and psilocin have a high distribution volume as specified in Table 3, while the others have a low distribution volume. Besides, the distribution of α-amanitin and orellanine in both BBB and CNS is weak. Other molecules without CNS distribution include muscarine, muscimol, ibotenoic acid, and coprin. However, since both web tools evaluate BBB transitions through molecules that can perform passive diffusion, there may be those that can perform BBB transitions between these molecules with the active transport system. In both programs, it is observed that there is a limitation in the evaluation of BBB transitions according to the active transport system. In the web tool evaluation, the effect of psilocybin and psilocin on CNS appears. CYP enzymes play an important role in the biotransformation of drugs to be excreted from the body. Their inhibition leads to delayed excretion of drugs from the organism. Five basic enzymes of this system (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) play an important role in neutralizing therapeutic molecules. According to the study conducted as *in silico*, the potential of molecules to affect these enzyme activities is presented in Table 3. The potential of toxins to affect the activities of CYP enzymes has not been identified. When the toxicokinetic data obtained in animals and during human poisoning are examined, it is stated that after intravenous administration of α-amanitin in dogs, the half-life of α-amanitin in plasma is between 26.7 and 49.6 minutes, and its detection in blood

is not possible after 4 to 6 hours (Park et al., 2021). It has been reported that the majority of α-amanitin is excreted unchanged by urine 72 hours after exposure (Le Dare et al., 2021). In an animal study, Sun et al. 2018 reported that alanine transaminase, aspartate transaminase, total bilirubin, and alkaline phosphatase levels in plasma peaked 36 hours after exposure to α-amanitin (Sun et al., 2018). Hepatotoxicity is a hallmark of this toxin and leads to hepatocellular failure within three days of exposure. Toxic mechanisms of action mainly include RNA polymerase II inhibition and oxidative stress formation, which leads to hepatic cell apoptosis or necrosis depending on the doses ingested. In our study, α-amanitin was found to be hepatotoxic (Table 4). *In silico* analysis method in our study is similar to the study data performed by means of the *in vivo* method.

There is very limited information about gyromitrin in the literature. Gyromitrin is converted to its toxic metabolites N-methyl-N-formylhydrazine and monomethylhydrazine after exposure. When hydrazines reach the liver, they are metabolized to reactive intermediates such as methyl cations and free methyl radicals. In our study, it was shown by *in silico* methods that gyromitrin doesn't cause hepatotoxicity (Table 4). It has been reported in the literature that hepatotoxicity is caused by its metabolite, not gyromitrin. Considering these data, it is seen that the results of our study are compatible with the literature.

Studies indicate that the distribution of muscarine, a quaternary ammonium compound, in the body is within two hours. In the present study, the volume of the distribution of muscarine was moderate. In our study, it was shown by *in silico* analyses that muscarine could not pass BBB. In the studies conducted, it is stated that muscarine cannot pass BBB due to its quaternary configuration, and its cholinergic effect is completely peripheral (Puschner, 2018). The toxicokinetic data analysis performed through *in silico* analysis method in our study is similar to the study data performed by means of the *in vivo* method. In the literature reviews, it is seen that some of the muscarine was eliminated unchanged in the urine, but detailed toxicokinetic studies could not be performed.

Precise data on the toxicokinetics of ibotenic acid and muscimol have not been determined. Based on the rapid onset of clinical signs after oral exposure, rapid absorption of toxins is suspected. In our study, the *in silico* analysis methods revealed that the gastrointestinal absorption of ibotenic acid and muscimol was high. According to the findings of the study, *in silico* methods demonstrated that ibotenic acid and muscimol could not pass the BBB. In the literature, this is explained by the fact that ibotenic acid and muscimol pass the BBB with an active transport system (Moss and Hendrickson, 2019). Since the toxicokinetic evaluations used in the *in silico* analysis programs are made by considering the Ro5 rule, the BBB transitions of molecules distributed by active transport cannot be reflected in the program. This is among the limitations of the software. Studies have reported that ibotenic acid is decarboxylated in the stomach, liver, and brain and converted to muscimol. These toxins can be detected in the urine one hour after exposure. Psilocybin is a prodrug and is rapidly dephosphorylated to psilocin (Diaz, 2005). The general assumption is that the conversion of psilocybin to psilocin occurs before it enters the systemic circulation. In humans, the absolute bioavailability of psilocin is estimated to be 52.7-62.0%. The absorption is rapid, and maximum concentrations are achieved within 1.5 hours. In plasma, psilocin is also metabolized to 4-hydroxytryptophol (4HT) and 4-hydroxyindol-3-acetic acid (4HIAA). Psilocin passes the BBB and concentrates in the brain tissue. It is excreted unchanged or in the urine as psilocin glucuronide and to some extent unchanged via the bile. In our study, it was shown by *in silico* analysis methods that the absorption of psilocin was rapid and that it was a toxin that could pass the BBB. The data obtained from our study are consistent with the literature. The MTD level is low for orellanine, muscimol, psilocybin, and psilocin. The dose level with the lowest side effect is low in muscimol, gyromitrin, and ibotenic acid. Hepatotoxic toxins are α-amanitin and orellanine (Table 4).

Similarly, it is reported in the literature that α-amanitin is a hepatotoxic toxin and is also responsible for kidney necrosis (Sun et al., 2018). Other molecules that cause hepatotoxicity and renal toxicity include orellanine and gyromitrin. The toxins affecting the CNS include muscimol, ibotenic acid, psilocybin, and psilocin (Diaz, 2015).

In conclusion, the toxicokinetic analyses of α-amanitin, orellanine, gyromitrin, coprin, muscarine, psilocybin, psilocin, ibotenic acid, and muscimol, which are the main mushroom toxins, were successfully performed using multiple *in silico* analysis web tools in this study. It is seen that the toxicokinetic properties of these molecules can be evaluated in a wide range through *in silico* analysis. The predicted *in silico* analysis results are highly consistent with the analysis results in the literature. It is recommended that *in silico* analysis methods be used prior to studies to provide a preliminary idea for comprehensive and multi-molecular *in vivo* toxicokinetic analyses.

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## ÖZET

Bu çalışmada, Karaman ilinin en küçük ilçesi olan Başyayla'nın miksomisetlerinin tespit edilmesi ve Türkiye miksobiotasına katkı amaçlanmıştır. 2016 yılında Başyayla ilçesinin ormanlık alanlarına arazi çalışması düzenlenmiştir. Araştırma bölgesinden orman döküntü katı ve canlı ağaç parçaları gibi materyaller toplanmıştır. Materyallere nem odası tekniği uygulanmış ve laboratuvar çalışmaları sonucunda 19 miksomiset taksonu tespit edilmiştir.

***ANAHTAR KELİMELER*:** Başyayla, Biyoçeşitlilik, Myxomycetes, nem odası tekniği.

**Myxomycetes of Başyayla (Karaman) District**

## ABSTRACT

In this study, it was aimed to determine the myxomycetes of Başyayla, the smallest town of Karaman province, and to contribute to the myxobiota of Türkiye. In 2016, field work was carried out in the forested areas of Başyayla district. Materials such as forest zone and live wood pieces were collected from the research area. The moist chamber technique was applied to the materials and 19 myxomycete taxa were determined as a result of laboratory studies.

***KEY WORDS:*** Başyayla, Biodiversity, Myxomycetes, moist chamber technique.

## Giriş

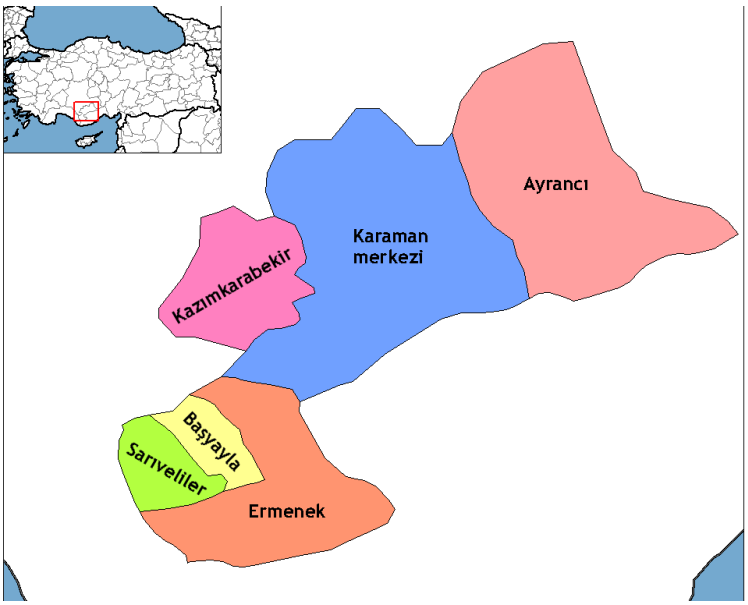
Miksomisetler amoeboid ökaryotik organizmaların büyüleyici bir grubudur. Uzun yıllar boyunca mantarlarla ilişkilendirilmiş, ancak yapılan son çalışmalarda protistlere dahil edilmiştir. Miksomisetlerin yaşam tarzlarına bakıldığında ne ayrıştırıcı ne de patojen olarak değil, amoeboid evreleri sırasında bakteri, maya ve mantar sporları ile beslendikleri için daha çok mikrobiyal predatörler olarak değerlendirilmektedir. Karmaşık yaşam döngülerinde bir hücreli amoeboid safha veya çift kamçılı oğul hücre ve çok nukleuslu plasmodium veya diploid eşeyli safha ile karakterize edilirler (Dadamac vd., 2015).

Türkiye'deki miksomiset çalışmalarının geçmişi yakın tarihe dayanmaktadır (Sesli vd., 2020). Türkiye'den şimdiye kadar 305 miksomiset taksonu bildirilmiştir (Eroğlu, 2021; Baysal ve Eroğlu, 2022). Bu çalışma ile Türkiye'nin alan literatürüne katkı sağlanmıştır.

## Materyal ve Metot

Karaman ili Başyayla ilçesinin İl merkezine uzaklığı 180 km'dir. İlçenin deniz seviyesinden ortalama yüksekliği 1.400 metredir. İlçenin, kuzeyinde Konya ili Taşkent ilçesi, güneyinde Ermenek, batısında Sarıveliler ve doğusunda yine Ermenek ilçesi bulunmaktadır (Şekil 1). İlçenin yüzölçümü 228,99 km²'dir. Başyayla ilçesi 22.899,6 ha'lık alan ile Karaman'ın %2,60'ını, Türkiye'nin ise %0,03'ünü kapsamaktadır. İlçenin ormanlık alanı toplam alanın %20,57'sini oluşturmakta ve bu alan Karaman toplam ormanlık alanın %2,93'ünü

kaplamaktadır. Alan Taşeli platosunda yer almakta ve oldukça dağlık ve sarp kayalıklar bulunmaktadır. Orman alanında ise meşe, ladin, karaçam, sedir, köknar ve ardıç ağaçları (URL 1), dere kenarında söğüt, kavak ve çınar ağaçları vardır. Ayrıca elma, ceviz, armut, nar ve incir ağaçları da vardır.




**Şekil 1.** Çalışma alanı (URL 2)

Bu çalışmadaki materyal 2016 yılında yapılan arazi çalışmalarında toplanmıştır. Çalışmalarda orman içindeki çürümüş ağaç kabukları ve odunları, canlı ağaçların kabukları, yaprak, ibre ve kozalak döküntüleri gibi orman döküntü katından örnekler toplanmıştır. Üzerinde spor veya plasmodium olma ihtimali olan bu materyallerin her birine arazi numarası verilerek kilitli poşet veya küçük ebatlardaki karton kutulara konulmuştur. Toplama numaraları arazi bilgi formuna işlenerek materyallerin hangi substratlardan toplandığı not edilmiştir. Materyallere Nem Odası Tekniği uygulanmıştır (Gilbert ve Martin, 1933). Materyaller plastik kaplara yerleştirilmiş ve kaplar distile su ile doldurulmuştur. Her bir kutuya materyallerin toplama numarası yazılmıştır. 24-48 saat bekletildikten sonra kaplardaki fazla su boşaltılmıştır. Nem odası olarak iklimlendirme odası kullanılmıştır. (Sıcaklık 24-25˚C, su püskürtme ile nem %80-90 oranında ve ışık 12 saat yanacak şekilde otomatik olarak ayarlanmıştır.) Her gün veya gün aşırı olarak stereomikroskopta incelemeler yapılmış ve sporokarp gelişimleri kaydedilmiştir.

Elde edilen örnekler substratlarıyla beraber mukavvalara yapıştırılıp, aynı boydaki kutulara yerleştirilmiştir. Böylece fungarium örneği haline getirilmiştir. Mikroskobik incelemeler için geçici preparat ortamları hazırlanırken alkol, saf su veya %3'lük KOH kullanılmıştır. Geçici daimî preparat hazırlanırken Amann'ın laktofenol ortamı kullanılmıştır. Laktofenol ortamıyla hazırlanmış preparat kuruduktan birkaç gün sonra lamelin etrafına şeffaf oje sürülerek geçici daimî preparat haline getirilmiştir (Kaşık vd., 2005). Teşhis için başlıca eserler kullanılmıştır (Martin ve Alexopoulos, 1969, Stephenson ve Stempen, 2000, Neubert vd., 1993; 1995; 2000, Nannenga-Bremekamp, 1991, Poulain vd., 2011). Taksonların isimleri, yazar isimleri ve sinonimleri online nomenklatür bilgi sisteminden kontrol edilmiştir (Lado, 2022). Bu çalışmada, Puolain vd. (2011) tarafından kullanılan sistematik takip edilmiştir. Bunlara ilave olarak miksomisetlerin ek bilgileri www.mycobank.org web sitesinden kontrol edilmiştir (URL 3).

Karaman ili Başyayla ilçesi miksomisetlerine ait fungarium örnekleri, S. Ü. Mantarcılık Uygulama ve Araştırma Merkezi Fungariumu'nda saklanmaktadır.

**Sonuçlar**

Başyayla ilçesinin miksomisetlerini tespit amacıyla yapılan arazi ve laboratuvar çalışmaları sonucunda toplam 19 takson tespit edilmiştir. Tespit edilen taksonlar takım seviyesinde alfabetik sırayla verilmiştir. Ayrıca taksonların lokalitesi, substrat ve toplama numaraları da verilmiştir. Çalışma bölgesinin koordinatı metin içinde tekrarlanmaması için Tablo 1'de verilmiştir.

**Tablo 1.** Başyayla ilçesinin koordinatı

| Lokalite | Lokalite kısaltması | Koordinat | Yükseklik | Toplama tarihi |
|---|---|---|---|---|
| Karaman, Başyayla | B1 | 36°45'40.02"K-32°40'09.52"D | 1594m | 15.10.2016 |

***Protozoa*** R. Owen
***Myxomycota*** Whittaker
***Myxomycetes*** G. Winter

***Clastodermatales*** Leontyev, Schnittler, S.L. Stephenson, Novozh. & Shchepin
***Clastodermataceae*** Alexop. & T.E. Brooks
**1. *Clastoderma debaryanum*** A. Blytt
**Lokalite:** B1, bilinmeyen kütük odunu, GE400.

***Echinosteliales*** G.W. Martin
***Echinosteliaceae*** Rostaf. ex Cooke
**2. *Echinostelium minutum*** de Bary
**Lokalite:** B1, canlı kavak ağacı kabuğu, GE390.

***Liceales*** E. Jahn
***Cribrariaceae*** Corda
**3. *Cribraria lepida*** Meyl.
**Lokalite:** B1, kesik ceviz kütüğü kabuğu, GE403.
**4. *Cribraria violacea*** Rex
**Lokalite:** B1, canlı ceviz ağacı kabuğu, GE392.

***Liceaceae*** Chevall.
**5. *Licea castanea*** G. Lister
**Lokalite:** B1, kesik kütük kabuk, GE464.
**6. *L. denudescens*** H.W. Keller & T.E. Brooks
**Lokalite:** B1, canlı ceviz ağacı kabuğu, GE405; GE429; canlı söğüt ağacı kabuğu, GE434.

***Physarales*** T. Macbr.
***Didymiaceae*** Rostaf. ex Cooke
**7. *Didymium annulisporum*** H.W. Keller & Schokn.
**Lokalite:** B1, su kenarı, canlı yaşlı söğüt ağacı kabuğu, GE444.
**8. *D. difforme*** (Pers.) Gray
**Lokalite:** B1, elma bahçesi, canlı elma ağacı kabuğu, GE424.

**9. *D. squamulosum*** (Alb. & Schwein.) Fr. & Palmquist
**Lokalite:** B1, dere kenarı döküntü yaprak, GE375.

***Physaraceae*** Chevall.
**10. *Physarum cinereum*** (Batsch) Pers.
**Lokalite:** B1, canlı kavak ağacı kabuğu, GE433.
**11. *P. didermoides*** (Ach. ex Pers.) Rostaf.
**Lokalite:** B1, canlı meşe kabuk, GE383; meşe döküntü kabuk, GE387; canlı ceviz kabuğu, GE405; döküntü ceviz dal, GE406; canlı söğüt kabuk, GE413; canlı ceviz kabuk, GE441; canlı çınar kabuk, GE447; karaçam canlı ağaç kabuk, GE466.

***Stemonitidales*** T. Macbr.
***Stemonitidaceae*** Fr.
**12. *Collaria lurida*** (G. Lister) Nann.-Bremek.
**Lokalite:** B1, döküntü çam odunu, GE471.
**13. *Comatricha laxa*** Rostaf.
**Lokalite:** B1, karaçam canlı ağaç kabuğu, GE461.

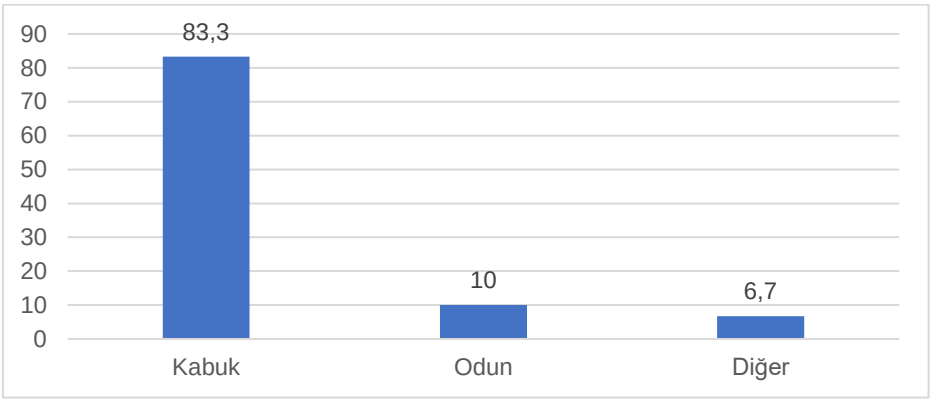
**14. *Macbrideola cornea*** (G. Lister & Cran) Alexop.
**Lokalite:** B1, canlı ardıç kabuğu, GE362.
**15. *Stemonitopsis typhina*** (F.H. Wigg.) Nann.-Bremek.
**Lokalite:** B1, döküntü kavak kabuğu, GE359.

***Trichiales*** T. Macbr.
***Arcyriaceae*** Rostaf. ex Cooke
**16. *Arcyria denudata*** (L.) Wettst.
**Lokalite:** B1, dere kenarı, canlı yaşlı söğüt ağacı kabuğu, GE444.

***Trichiaceae*** Chevall.
**17. *Hemitrichia clavata*** (Pers.) Rostaf.
**Lokalite:** B1, kesik meşe kütük odunu, GE380.
**18. *Perichanea depressa*** Lib.
**Lokalite:** B1, döküntü dal, GE402; canlı söğüt kabuk, GE413; canlı ardıç kabuk, GE416.
**19. *P. vermicularis*** (Schwein.) Rostaf.
**Lokalite:** B1, canlı söğüt kabuğu, GE427.

**Tartışma**
Karaman iline bağlı Başyayla ilçesine yapılan arazi çalışmalarında miksomisetlerin gelişimine uygun yaklaşık 115 civarında organik materyal toplanmıştır. Toplanan bu materyallere nem odası tekniği uygulanmış ve laboratuvar çalışmaları sonucunda toplam 19 miksomiset taksonu tespit edilmiştir. Arazi çalışmaları sırasında olgun sporokarp bulunamamış ve bütün örnekler nem odasıyla gelişmiştir. Laboratuvar çalışmaları sonucunda tespit edilen türlerin cinslere göre dağılımı ise; *Arcyria*'dan 1, *Clastoderma*'dan 1, *Collaria*'dan 1, *Comatricha*'dan 1, *Echinostelium*'dan 1, *Hemitrichia*'dan 1, *Macbrideola*'dan 1, *Stemonitopsis*'den 1, *Cribraria*'dan 2, *Licea*'dan 2, *Perichaena*'dan 2, *Physarum*'dan 2, *Didymium*'dan 3 takson tespit edilmiştir. Çalışma bölgesinde ormanlık alanların darlığı, tarım ve meyve bahçesi alanlarının çok olması sebebiyle çok fazla materyal toplanamamıştır. Bu olumsuzluklara rağmen tür sayısı iyi olarak değerlendirilebilir.

Çalışmada örneklerin %83.3'ü kabuk üzerinde tespit edilmiş kortikol miksomisetlerdir (Şekil 2). Ağaçların dalları ve gövdelerinin kabukları (Ing 1994) miksomisetler için uygun substratlardır (Policina and dela Cruz 2020). Kabuğun su tutma kapasitesi, ağacın genel şekli, ağaç kabuğunun yüzey dokusu ile alg, yosun, ciğerotu ve likenlerin epitik örtüsü (Everhart and Keller 2008, Stephenson and Stephen 2000) daha fazla tür tarafından kolonizasyonu kolaylaştırır (Keller and Braun 1999). Bu da çalışmadaki sonuçla uygunluk göstermektedir.




**Şekil 2.** Taksonların substratlara göre dağılımı

Çalışmada *P. didermoides* 8 farklı substrat üzerinde tespit edilmiş olup %26.6'lık bir oranla en fazla bulunan taksondur. Sırasıyla *L. denudescens* 3 (%10) ve *P. depressa* 2 (%6.6) farklı substratta tespit edilmiştir. Diğer taksonlar ise 1'er farklı substratta tespit edilmiştir. Ayrıca toplama numaraları farklı olsa da *C. violacea*, *L. denudescens* ve *P. didermoides* canlı ağaç kabuğunda, *M. cornea* ve *P. depressa* canlı ardıç kabuğunda, *E. minutum* ve *P. cinereum* canlı kavak ağacı kabuğunda, *P. didermoides*, *L. denudescens*, *P. vermicularis*, *P. depressa*, *A. denudata* ve *D. annulisporum* canlı söğüt ağacı kabuğunda tespit edilmiştir. Takahashi ve Fukasawa (2022) yaptıkları çalışmada canlı ağaç kabuklarıyla miksomisetlerin ilişkisini değerlendirmişlerdir. Bundan dolayı kortikolöz miksomisetlerin kabuk yüzeyindeki bakteri ve mantar sporlarını temizlemesiyle ekolojik biri işleve sahip olduğu şeklinde değerlendirmişlerdir. Başyayla ilçesinin miksomisetleri Türkiye miksobiotasının zenginliği açısından önemli olup fungaryum materyali haline getirilen miksomiset örnekler, S.Ü. Mantarcılık Uygulama ve Araştırma Merkezi Fungaryumu'nda saklanmaktadır.

**Teşekkür**
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URL 3 www.mycobank.org

# EXPRESSION OF SAP GENES 1 10 AND BIOFILM FORMATION IN *CANDIDA* SPP COMPARISON OF HEALTHCARE ASSOCIATED INFECTION ISOLATES FROM BLOODSTREAM WITH ISOLATES FROM OTHER SOURCES

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## ABSTRACT

*Candida* species are yeast fungi that are common in nature and can cause both superficial and deep infections. According to recent data, *Candida* species are considered to be the main causative agents of healthcare-associated infections. In this study, it was aimed to determine *Candida* species, Sap genes and biofilm formation isolated from blood cultures, which are health care-associated infectious agents, and to evaluate them together with antifungal susceptibility tests. *Candida* spp. were identified by the Vitek 2 Compact method and then the results were verified by multiplex PCR and DNA sequence analysis. The biofilm formation of the species was investigated by microplate method and presence of SAP (1-10) genes were investigated by PCR. In species identification with VITEK-2, 41 (47.1%) strains were *C.albicans*, 14 (16%) *C.glabrata*, 10 (11.5%) *C.parapsilosis*, 9 (10.3%) *C.tropicalis*, 6 (6.9%) *C.krusei*, 3 (3.4%) *C. guilliermondii*, 2(2.4%) *C.lusitaniae*. 1 (1.2%) *C. dubliniensis*, 1 (1.2%) was defined as *C. kefyr*. After verification by multiplex PCR 10 strains were found to be incorrectly identified by VITEK-2 Compact System. All multiplex PCR results proved to be 100% agreement with DNA sequence analysis. While SAP1, SAP2, SAP3, SAP4, SAP6, SAP7, SAP8, SAP9 and SAP10 gene positivity were detected in all 31 *C.albicans* strains (100%), the presence of the SAP5 gene was detected in 30 (98.8%) *C. albicans* strains. to the results of the study, it was determined that *C.albicans* strains isolated from patient care-associated infections had intense biofilm-forming properties and carried almost all Sap genes. Therefore, it has been concluded that studies on biofilm formation and Sap genes will be important in the face of increasing antifungal resistance.

***KEYWORDS*:** Antifungal resistance, Biofilm, Candida species, SAP.

## Introduction

*Candida* spp. are an important pathogen responsible for fungal infections and are detected as causative agents in most invasive fungal infections. Many important virulence factors such as morphogenesis, adhesion, biofilm formation, phenotypic change, production of hydrolytic enzymes are effective in occupying various anatomical regions. The production and release of secreted aspartyl proteinases (SAP), plus biofilm formation, are believed to be the key determinants of virulence for *Candida* species ( Hamid et al.,2018).

Biofilms are microbial communities of cells that adhere to abiotic or biotic surfaces, and these adherent cells are often embedded in a polymeric extracellular matrix. *C. albicans* has a tendency to form biofilms on the surfaces of almost all medical devices such as catheters, prostheses, implants, stents, shunts, endotracheal tubes. *C. albicans* cells in biofilms exhibit severe resistance

to a wide variety of clinical antifungal agents, including amphotericin B and fluconazole, contributing to therapeutic failure and increased mortality (Cowen et al.,2014).
Secretory aspartyl proteinases (SAPs) have the ability to degrade proteins such as immunoglobulins, complement and cytokines, and various host cell proteins such as collagen, keratin, mucin, and are encoded by a large family of genes. The SAP gene family consists of 10 genes and was first described in *C. albicans*. The contribution of SAPs to mucosal and systemic infections and their role in adhesion, tissue damage, and escape from the host immune response has been demonstrated by SAP-deficient mutants and protease inhibitors (Naglik et al.,2003).
In this study, it was aimed to compare the VITEK 2 Compact system and Multiplex Polymerase Chain Reaction (PCR) methods for the identification of *Candida* strains and to determine their antifungal susceptibility. In addition, it was aimed to determine the biofilm formation and SAP 1-10 genes of *C. albicans* strains determined as agents in healthcare-associated infections.

**Materials and Methods**
In the study, 87 *Candida* strains isolated from patients with healthcare-associated infections and hospitalized in various clinics in Selçuk University Medical Faculty Hospital between February 2019 and February 2020 were included. *C.albicans* ATCC 10231 was used as the standard strain. VITEK-2 (BioMérieux, France) ID YST cards were used to identify isolated yeasts and AST-YS01 cards were used to detect their antifungal susceptibility. For the molecular typing of *Candida* spp, firstly, genomic DNA was obtained from the isolates by the modified phenol-chloroform-isoamyl alcohol method. *Candida* species were identified by Multiplex PCR using DNA extractions.
The presence of SAP (1-10) genes in *C. albicans* strains was investigated by PCR. DNA sequencing analysis (ITS 1 and ITS 2 gene region) was performed to confirm species identification on samples with discordant VITEK and Multiplex PCR results. All PCR products made in the study were run on a 1.5% agarose gel in 1X Tris-Borate-EDTA (TBE) buffer and visualized using the Fusion FXV (Vilber, France) gel documentation system. Biofilm formation in *C. albicans* strains was carried out in flat-bottomed 96-well microplates. Various methods (IBM SPSS® 21.0, Krippendorff's alpha coefficient and Fleiss Kappa value) were used for statistical analysis of the data.

**Results**
Of the 87 included strains, 52 (59.8%) were isolated from male patients and 35 (40.2%) from female patients. The mean age of the patients was 52.87 ± 26.08 years (median=62). *Candida* strains included in the study were most commonly isolated from samples belonging to the Anesthesia Reanimation Intensive Care Unit (20.7%) and 16.1% of the strains were from Chest Diseases Intensive Care Unit. While strong biofilm activity was detected in 29 (93.5%) of 31 C. albicans strains included in the study, moderate biofilm formation was detected in 2 strains. It was isolated from the sputum of 2 strains with moderate biofilm activity.

**Table 1.** Distribution of *Candida* strains according to clinical samples from which they were isolated

| Samples | n (%) |
|---|---|
| Blood | 44 (50.6 %) |
| Tracheal Aspirate | 6 (6.9 %) |
| Sputum | 13 (14.9 %) |
| Bronchoalveolar lavage | 5 (5.7 %) |
| Cerebrospinal fluid | 2 (2.3 %) |
| Drainage | 3 (3.4 %) |
| Catheter | 2 (2.3 %) |
| Peritoneum | 4 (4.6 %) |
| Pleural fluid | 2 (2.3 %) |
| Wound | 4 (4.6 %) |
| Vaginal Swab | 2 (2.3 % ) |
| **Total** | **87 (%100)** |

**Table 2.** Comparison of VITEK 2 and Multiplex PCR Method Results

| **VITEK 2** | | **Multiplex PCR** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Type** | **Number of Strains** | ***C. albicans*** | ***C. dublinie nsis*** | ***C. glabrata*** | ***C. Guilliermo ndii*** | ***C. kefyr*** | ***C. krusei*** | ***C. lusitaniae*** | ***C. parapsilosis*** | ***C. tropicalis*** | **VITEK Misidentification (%)** |
| ***C. albicans*** | 41 | **31** | 0 | 0 | **1** | **2** | 0 | 0 | **3** | **4** | 24.4 (10/41) |
| ***C. dubliniensis*** | 1 | 0 | **1** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 (0/1) |
| ***C. glabrata*** | 14 | 0 | 0 | **11** | 0 | 0 | 0 | 0 | **2** | **1** | 21.5 (3/14) |
| ***C. guilliermondii*** | 3 | 0 | 0 | 0 | **2** | **1** | 0 | 0 | 0 | 0 | 33.3 (1/3) |
| ***C. kefyr*** | 1 | 0 | 0 | 0 | 0 | **1** | 0 | 0 | 0 | 0 | 0.0 (0/1) |
| ***C. krusei*** | 6 | 0 | 0 | 0 | 0 | 0 | **6** | 0 | 0 | 0 | 0.0 (0/6) |
| ***C. lusitaniae*** | 2 | 0 | 0 | 0 | 0 | 0 | 0 | **2** | 0 | 0 | 0.0 (0/2) |
| ***C. parapsilosis*** | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **10** | 0 | 0.0 (0/10) |
| ***C. tropicalis*** | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **9** | 0.0 (0/9) |
| **Total** | 87 | 31 | 1 | 11 | 3 | 4 | 6 | 2 | 15 | 14 | 16.1 (14/87) |

**Table 3.** Antifungal susceptibility profiles of *Candida* species according to VITEK-2 Compact System

| **Antifungal** | ***C. albicans*** **(n=31)** | ***C. dubliniensis*** **(n=1)** | ***C. glabrata*** **(n=11)** | ***C. guilliermondii*** **(n=3)** | ***C. kefyr*** **(n=4)** | ***C. krusei*** **(n=6)** | ***C. lusitaniae*** **(n=2)** | ***C. parapsilosis*** **(n=15)** | ***C. tropicalis*** **(n=14)** |
|---|---|---|---|---|---|---|---|---|---|
| **Amfoterisin B (µg/ml)** | | | | | | | | | |
| Sensitive (≤ 0.25) | 27 (%87,09) | 1 (%100) | 10 (%90,90) | 3 (%100) | 4 (%100) | 4 (%66,66) | 2 (%100) | 13 (%86,66) | 14 (%100) |
| Moderately Sensitive (0.25-16) | 3 (%9,67) | 0 (%0) | 1 (%9,09) | 0 (%0) | 0 (%0) | 1 (%16,66) | 0 (%0) | 1 (%6,66) | 0 (%0) |
| Resistant (≥ 16) | 1 (%3,22) | 0 | 0 (%0) | 0 (%0) | 0 (%0) | 1 (%16,66) | 0 (%0) | 1 (%6,66) | 0 (%0) |
| **Flukonazol (µg/ml)** | | | | | | | | | |
| Sensitive (≤ 1) | 26 (%83,87) | 1 (%100) | 11 (%100) | 2 (%66,66) | 3 (%75) | 0 (%0.0) | 2 (%100) | 15 (%100) | 13 (%92,85) |
| Moderately Sensitive (1-64) | 2 (%6,45) | 0 (%0) | 0 (%0) | 1 (%33,33) | 1 (%25) | 0 (%0.0) | 0 (%0) | 0 (%0) | 1 (%7,14) |
| Resistant (≥ 64) | 3 (%9,67) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 6 (%100) | 0 (%0) | 0 (%0) | 0 (%0) |
| **Vorikonazol (µg/ml)** | | | | | | | | | |
| Sensitive (≥ 0.12) | 23 (%74,19) | 1 (%100) | 10 (%90,90) | 3 (%100) | 4 (%100) | 6 (%100) | 2 (%100) | 14 (%93,33) | 12 (%85,71) |
| Moderately Sensitive (0.12-8) | 6 (%19,35) | 0 (%0) | 1 (%9,09) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 1 (%6,66) | 1 (%7,14) |
| Resistant (≥ 8) | 2 (%6,45) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 1 (%7,14) |
| **Flusitozin (µg/ml)** | | | | | | | | | |
| Sensitive (≤ 1) | 24 (%77,41) | 1 (%100) | 11 (%100) | 3 (%100) | 4 (%100) | 6 (%100) | 1 (%50) | 15 (%100) | 14 (%100) |
| Moderately Sensitive (1-64) | 3 (%9,67) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 1 (%50) | 0 (%0) | 0 (%0) |
| Resistant (≥ 64) | 4 (%12,90) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) |
| **Kaspofungin (µg/ml)** | | | | | | | | | |
| Sensitive (≤ 0.25) | 23 (%74,19) | 1 (%100) | 9 (%81,81) | 3 (%100) | 3 (%75) | 5 (%83,33) | 2 (%100) | 14 (%93,33) | 11 (%78,57) |
| Moderately Sensitive (0.25-4) | 3 (%9,67) | 0 (%0) | 2 (%18,18) | 0 (%0) | 1 (%25) | 0 (%0) | 0 (%0) | 0 (%0) | 3 (%21,42) |
| Resistant (≥ 4) | 5 (%16,12) | 0 (%0) | 0 (%0) | 0 (%0) | 0 (%0) | 1 (%16,66) | 0 (%0) | 1 (%6,66) | 0 (%0) |

**Table 4.** PCR results with SAP 1-10 primers specific to C. albicans

| Shush no | Sample | SAP 1 | SAP 2 | SAP 3 | SAP 4 | SAP 5 | SAP 6 | SAP 7 | SAP 8 | SAP 9 | SAP 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **2** | Blood | + | + | + | + | + | + | + | + | + | + |
| **3** | Blood | − | − | − | − | − | − | − | − | − | − |
| **4** | Blood | + | + | + | + | + | + | + | + | + | + |
| **6** | Blood | + | + | + | + | + | + | + | + | + | + |
| **7** | Blood | + | + | + | + | − | + | + | + | + | + |
| **9** | Blood | + | + | + | + | + | + | + | + | + | + |
| **10** | Blood | + | + | + | + | + | + | + | + | + | + |
| **11** | Blood | + | + | + | + | + | + | + | + | + | + |
| **13** | Blood | + | + | + | + | + | + | + | + | + | + |
| **14** | Blood | + | + | + | + | + | + | + | + | + | + |
| **15** | Blood | + | + | + | + | + | + | + | + | + | + |
| **17** | Blood | + | + | + | + | + | + | + | + | + | + |
| **18** | Blood | + | + | + | + | + | + | + | + | + | + |
| **19** | Blood | + | + | + | + | + | + | + | + | + | + |
| **21** | Blood | + | + | + | + | + | + | + | + | + | + |
| **24** | Blood | − | − | − | − | − | − | − | − | − | − |
| **26** | Blood | + | + | + | + | + | + | + | + | + | + |
| **27** | Blood | + | + | + | + | + | + | + | + | + | + |
| **28** | Blood | + | + | + | + | + | + | + | + | + | + |
| **29** | Blood | + | + | + | + | + | + | + | + | + | + |
| **31** | Blood | + | + | + | + | + | + | + | + | + | + |
| **102** | Aspirate | + | + | + | + | + | + | + | + | + | + |
| **75** | BAL | + | + | + | + | + | + | + | + | + | + |
| **76** | CF | + | + | + | + | + | + | + | + | + | + |
| **1** | Sputum | + | + | + | + | + | + | + | + | + | + |
| **4** | Sputum | + | + | + | + | + | + | + | + | + | + |
| **66** | Sputum | + | + | + | + | + | + | + | + | + | + |
| **67** | Sputum | + | + | + | + | + | + | + | + | + | + |
| **88** | Sputum | + | + | + | − | + | + | + | + | + | + |
| **94** | Sputum | + | + | + | + | + | + | + | + | + | + |
| **99** | Sputum | + | + | + | + | + | + | + | + | + | + |
| **28** | Drainage | + | + | + | + | + | + | + | + | + | + |
| **80** | Pleura | + | + | + | − | + | + | + | + | + | + |

**Discussion**

In similar studies conducted around the world, eight common pathogenic species (C. albicans, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* , *C. guilliemondii* , *C. dubliniensis* and *C. lusitanae*) of *Candida* spp. are generally detected ( Pfaller et al.,2014). In 2016, Turk Dagi et al., (2016) conducted a study where 47.5% *C. albicans* 18% *C.glabrata*, 14% *C. parapsilosis*, 12% *C. tropicalis*, 5% *C. kefyr* and 3.5% *C. lusitanae* were detected in blood cultures. In a study conducted by Kord et al (2020) in Iran using the Vitek 2 Compact system, it was determined

that 120 out of 132 samples were defined correctly and 12 incorrectly. In addition, according to the results of the study, almost 92% of the *C. albicans* species were correctly identified in the Vitek 2 Compact system. However, almost all of the misidentified strains were non-*C.albicans* (*C. parapsilosis* and *C. glabrata* and *C. tropicalis*) species.

One of the major virulence traits of *C. albicans* consists of aspartic proteases (SAP) secreted by the 10 SAP gene families (Schaller et al., 2005). In a study conducted in Poland, the presence of the SAP 1-3 gene was found in all 13 *C.albicans* strains isolated from patients. In addition, the presence of the SAP 4 gene in 11 strains, the SAP 5 gene in 6 strains and the SAP 6 gene in 11 strains were detected (Sikora et al.,2011). In the study by Kadry et al (2018) it was reported that there is a correlation between the prevalence of the SAP9 and SAP10 genes and the strong biofilm production of *C. albicans* isolates. Because 66.7% of these isolates had both SAP9 and SAP10, while 25% had SAP9 or SAP10.

In recent years, many studies have been conducted on increasing antifungal resistance. In a study by Kaur et al (2016) amphotercin B had 87.8% susceptibility and 12.2% resistance, caspofungin 92.7% susceptibility and 7.3% resistance, fluconazole 87.8% susceptibility and 12.2% resistance, voriconazole 95%, susceptibility and 4.5% resistance, flucytosine 95.1% susceptibility and 4.5% resistance, micafungin 87.8% susceptibility and 12.2% resistance. In a similar study conducted in Brazil, amphotericin B was found to be 6.25% resistant and 93.75% susceptible (Siqueira RA. et al.,2016). According to the results of the study conducted by ElFeky et al (2015) Amphotericin B was found to be effective antifungal agent (98.4%), and was followed by ketoconazole (85.7%).

**Conclusion**

As a result, it was seen that the agreement between VITEK and PCR was at an acceptable level when compared statistically. In addition, it was determined that biofilm formation was very strong in *C.albicans* strains isolated from healthcare-associated infections and carried almost all of the SAP genes. Furthermore, at least one antifungal resistance was found in 41% of *Candida* species. For this reason, the relationship between virulence factors and antifungal resistance is thought to further increase the severity of invasive *Candida* infections. Finally, it is thought that studies on biofilm formation and SAP genes will be important in the face of increasing antifungal resistance.

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# DETERMINATION OF MEDICINAL EFFECTS OF *GANODERMA ADSPERSUM* SCHULZER DONK

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## ABSTRACT

Species in the genus *Ganoderma*, whose therapeutic effects have been known for thousands of years, are the most popular in Asia, particularly in Traditional Chinese Medicine. These species have recently been used as an alternative to the treatment of many diseases in our country. *Ganoderma* species are used to treat chronic diseases such as hypertension, nephritis, diabetes, insomnia, asthma and arthritis and have anticancer, antihepatotoxic, antioxidant, antimicrobial and immunomodulatory effects. In this study, it was aimed to determine the antimicrobial and antioxidant effects of methanol and ethanol extracts of *Ganoderma adspersum* (Schulzer) Donk. Antimicrobial effect of extracts was determined by disk diffusion method using pathogenic microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus megaterium*, *Staphylococcus aureus* and *Candida albicans*. Total antioxidant level, total oxidant level and 2.2-diphenyl-1-picrilhydrazyl (DPPH) radical scavenging capacity were detected for the antioxidant activity of the mushroom. In the results obtained, it was determined that the inhibition zones of methanol and ethanol extracts of *G. adspersum* at a concentration 1000 µg against the microorganisms used were in the range of 14-27 mm. Total antioxidant values of methanol and ethanol extracts of the mushroom were determined as 0.65 mmol and 0.05 mmol, respectively. Total oxidant values were determined as 12.89 and 10.42 µmol, respectively. It has been observed that the DPPH radical scavenging effect of *G. adspersum* increased depending on increasing concentrations, but showed a lower antioxidant effect compared to the control. As a result of the study, it can be said that methanol and ethanol extracts of *G. adspersum* can be good antimicrobial agents.

***KEYWORDS*:** Inedible mushroom, *Ganoderma adspersum*, antimicrobial effect, antioxidant effect

### *Ganoderma adspersum* (Schulzer) Donk'un Tıbbi Etkilerinin Belirlenmesi

## ÖZET

Tedavi edici etkileri binlerce yıldır bilinen *Ganoderma* cinsindeki türler, Asya'da, özellikle de Geleneksel Çin Tıbbında en popüler olanlarıdır. Bu türler ülkemizde son zamanlarda birçok hastalığın tedavisinde alternatif olarak kullanılmaktadır. *Ganoderma* türleri, hipertansiyon, nefrit, diyabet, uykusuzluk, astım ve artrit gibi kronik hastalıkların tedavisinde kullanılır ve antikanser, antihepatotoksik, antioksidan, antimikrobiyal ve immünomodülatör etkilere sahiptir. Bu çalışmada *Ganoderma adspersum* (Schulzer) Donk'un metanol ve etanol ekstraktlarının antimikrobiyal ve antioksidan etkilerinin belirlenmesi amaçlanmıştır. Ekstraktların antimikrobiyal etkisi, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus megaterium*, *Staphylococcus aureus* ve *Candida albicans* gibi patojen mikroorganizmalar kullanılarak disk difüzyon yöntemiyle belirlendi. Mantarın antioksidan aktivitesi için toplam antioksidan seviyesi,

toplam oksidan seviyesi ve 2.2-difenil-1-pikrilhidrazil (DPPH) radikal süpürme kapasitesi tespit edildi. Elde edilen sonuçlarda 1000 µg konsantrasyonda *G. adspersum* metanol ve etanol ekstraktlarının kullanılan mikroorganizmalara karşı inhibisyon zonlarının 14-27 mm aralığında olduğu belirlendi. Mantarın metanol ve etanol ekstraktlarının toplam antioksidan değerleri sırasıyla 0.65 mmol ve 0.05 mmol olarak belirlendi. Toplam oksidan değerleri sırasıyla 12.89 ve 10.42 µmol olarak belirlendi. *G. adspersum*'un DPPH radikal süpürücü etkisinin artan konsantrasyonlara bağlı olarak arttığı ancak kontrole göre daha düşük bir antioksidan etki gösterdiği gözlemlendi. Çalışma sonucunda *G. adspersum*'un metanol ve etanol ekstraktlarının iyi bir antimikrobiyal ajan olabileceği söylenebilir.

***ANAHTAR KELİMELER*:** Yenmeyen mantar, *Ganoderma adspersum*, antimikrobiyal etki, antioksidan etki,

**Introduction**

Mankind has always benefited from nature in the treatment of diseases and benefited from many natural products, including mushrooms, in the treatment (İnci et al., 2022). Mushrooms have been used in the treatment of diseases since ancient times, especially in Asia (Venturella et al., 2021). Today, medicinal mushrooms are recognized as functional foods and are available as over-the-counter supplements used in complementary and alternative medicine (Antmen and Ögenler, 2021). Numerous medicinal effects have been reported for extracts and compounds obtained from mushrooms, such as various immunomodulatory, antidiabetic, cytotoxic, antioxidant, hepatoprotective, anticancer, antioxidant, antiallergic, antihyperlipidemic and prebiotic properties (Gunde-Cimerman, 1999; Goswami et al., 2021; Venturella vd., 2021). In addition, fungi play a central role in the synthesis of pharmaceutical products. Bioactive compounds produced by species ranging from micro-sizes such as molds and yeasts to macro-scales such as capped mushrooms are also very diverse. The development of resistance to currently used antibiotics has increased the search for new antibiotics and mushrooms have been used in research with the increasing orientation to natural antioxidant sources (Atabay and Gül, 2021). They are natural sources of antibiotics, especially thanks to the components they contain such as triterpenes, schizophyllan, lovastatin, pleuran, steroids, glycopeptides, terpenes, saponins, alkaloids, purine, purimidine, quinone, phenylpropanoid, calvacin, volvotoxin, flammutoxin and poricin (Çınar Belyurt, 2014).

Reactive oxygen species (ROS) are products of the normal cellular metabolism of living organisms. To counter these ROS, living cells have a natural antioxidant production system that helps keep the body's redox state below the threshold level. However, in case of imbalance such as high ROS production or loss of natural endogenous antioxidant production, "oxidative stress" can occur. Since oxidative stress is associated with various diseases, including neurodegenerative ones, a significant number of natural compounds with antioxidant activity have been investigated in recent years (Hameed ve ark., 2017; Atabay and Gül, 2021).

*Ganoderma* species have been used for centuries for medicinal purposes, especially in Far East countries: China, Japan and Korea (Baby et al., 2015). These species are also traditionally used in some West African countries such as Nigeria for the treatment of skin diseases, hypertension and digestive disorders (Basnet et al., 2017; Wang et al., 2020) The popularity of these species has increased in our country in recent years. Especially teas are consumed against cancer. Recent studies have revealed the therapeutic potential of *Ganoderma* species as antitumor, immunomodulatory, antioxidant, anti-inflammatory, antiallergic, neuroprotective, hepatoprotective, hypoglycemic, hypotensive, antimicrobial (Li et al., 2019; Sułkowska-Ziaja et al., 2022). In addition, these species are used as functional foods to prevent and treat many diseases such as anorexia, arthritis, asthma, cardiovascular problems, diabetes, gastritis,

hepatitis, hypercholesterolemia, hypertension, insomnia, migraine, nephritis, obesity and tumor formation (Bishop et al., 2015; Hapuarachchi et al., 2017).
In this study, it was aimed to determine the antimicrobial and antioxidant effects of methanol and ethanol extracts of *Ganoderma adspersum* (Schulzer) Donk.

## Materials and Methods

### Material

*Ganoderma adspersum* was collected in Kahramanmaraş. The species was identified by performing macroscopic and microscopic studies. The mushroom identification was made by Prof. Dr. Sevda KIRBAĞ from Fırat University. After the mushroom was ground, 0.5 g was weighed. 100 mL of 96% methanol (MetOH) and ethanol (EtOH) were added to the samples and left in an orbital shaker at 100 rpm for 72 h. Then, samples were filtered with blank disc.

### Antimicrobial Effect

#### Microorganisms

In this study; *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Bacillus megaterium* DSM32, *Staphylococcus aureus* COWAN1 and *Candida albicans* FMC17 were used. Microorganism were obtained from Firat-University, Department of Biology.

#### Preparation of Microorganism Cultures and Antimicrobial Testing

The antimicrobial activity was determined by the disc diffusion method (Collins and Lyne, 1987). Mueller Hinton Agar and Yeast Malt Extract Agar were sterilized separately in an erlen, then cooled to 45-50°C and inoculated with culture of bacteria and yeast ($10^6$ cells / mL of bacteria, $10^4$ cells / mL yeast as per Mc Farland standard). After shaking well, they were poured into sterile petri dishes and homogeneously dispersed. Discs (6 mm diameter) impregnated with 100 µL (1000 µg) of extracts were placed on microorganism-inoculated plates. Then the plates were kept at $4^0$C for 2 h. The inoculated petri dishes were incubated at 37 ± $0.1^0$C at 24 h for bacteria and at 25 ± $0.1^0$C at 72 h for yeasts. Standard discs were used for bacteria (Streptomycin sulphate 10 µg / disc) and yeasts (Nystatin 30 µg / disc). The antimicrobial effect was detected by measuring the inhibition zone in mm.

### Antioxidant Effect

#### Total Antioxidant Activity (TAS) and Total Oxidant Activity (TOS)

TAS and TOS levels of MetOH and EtOH extracts of sample were determined with Rel Assay kits (Rel Assay Kit Diagnostics). TAS value was given as mmol Trolox equiv./L and Trolox was used as the calibrator (Erel, 2004). The TOS value was expressed as µmol $H_2O_2$ equiv./L and hydrogen peroxide was used as the calibrator (Erel, 2005).

#### 2.2-diphenyl-1-picrilhydrazyl Radical Scavenging Capacity (DPPH)

The antioxidant activity of different concentrations of MetOH and EtOH extracts of *G. adspersum* was detected according to the DPPH radical scavenging capacity method (Cuendet et al., 1997). The solution was prepared in methanol and ethanol at a concentration of 25 mg/mL of the obtained extract and diluted 4 times to obtain the calibration curve of DPPH. 40 µL of the solution was taken and 160 µL of DPPH solution was added. After mixing well, the plate left in the dark for 30 min. Methanol and ethanol were used as controls. At the end of the period, absorbances of each mixture were read at 570 nm in the spectrophotometer. percent inhibition values were calculated;

% DPPH inhibition = [(AbsControl - AbsSample) / AbsControl] x 100

#### Statistical Analysis

The statistical analysis was performed according to the Kruskal Wallis test.

## Results

### Antimicrobial effect

Antimicrobial activities of *G. adspersum* extracts obtained from MetOH and EtOH against, used microorganisms are given in Table 1. According to the results obtained from, methanol and ethanol extracts of *G. adspersum*, it inhibited the development of *E. coli* the most.

**Table 1.** Inhibition zones of extracts of *G. adspersum* against some microorganisms (mm)

| | *G. adspersum*-MetOH | *G. adspersum*-EtOH | Standard antibiotics |
|---|---|---|---|
| *E.coli* | $22 \pm 0.35^{ab}$ | $27 \pm 0.33^{b}$ | 20.21 ± 0.57* |
| *K. pneumoniae.* | $16 \pm 0.33^{ab}$ | $14 \pm 0.46^{ab}$ | 19.13 ± 0.43* |
| *B. megaterium* | $15 \pm 0.33^{b}$ | $16 \pm 0.33^{b}$ | 20.25 ± 0.57* |
| *S. aureus* | $14 \pm 0.58^{ab}$ | $18 \pm 0.33^{b}$ | 20.23 ± 0.33* |
| *C. albicans* | $21 \pm 0.46^{ab}$ | $15 \pm 0.48^{ab}$ | 21.32 ± 0.42** |

(Streptomycin sulphate *10 µg/disc for bacteria, Nystatin** 30 µg/disc for yeast and dermatophyta), Means in the same column with the different superscript are significantly different ($p<0.05$)

### Antioxidant Effect

The TAS and TOS values of the MetOH extract of mushroom was found to be 0.65 mmol and 12.89 µmol, respectively and the EtOH extract of mushroom was 0.05 mmol and 10.42 µmol, respectively (Table 2).

**Table 2.** TAS and TOS values of *G. adspersum*

| | **TAS(mmol Trolox equiv./L)** | **TOS (µmol $H_2O_2$ equiv./L)** |
|---|---|---|
| ***G. adspersum*-MetOH** | 0.65 ± 0.241 | 12.89 ± 0.167 |
| ***G. adspersum*-EtOH** | 0.05 ± 0.255 | 10.42 ± 0.141 |

Values are means ±S.D.n:3, $p<0.05$ importantly dissimilar

The percent inhibition of the DPPH of *G. adspersum* is seen in Table 3. It was detected that the antioxidant effects of MetOH and EtOH extracts increased with increasing concentration.

**Table 3.** Percent inhibition of the DPPH radical of *G. adspersum*

| | ***G. adspersum*-MetOH** | ***G. adspersum*-EtOH** |
|---|---|---|
| **1000 µg/mL** | 35.36 ± 0.321 | 49.11 ± 0.524 |
| **500 µg/mL** | 15.24 ± 0.274 | 43.24± 0.465 |
| **250 µg/mL** | 11.58 ± 0.440 | 40.00 ± 0.322 |
| **125 µg/mL** | 6.70 ± 0.365 | 38.00 ± 0.321 |

Values are means ±S.D.n:3, $p<0.05$ importantly dissimilar with Kruskal Wallis's test

## Discussion

The antimicrobial effects of the methanol extract of *G. adspersum* against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus megaterium*, *Staphylococcus aureus* and *Candida albicans* were determined as 22mm, 16mm, 15mm, 14mm and 21mm, respectively (Table 1). Inhibition zones of ethanol extracts against the same microorganisms were in the range of 14-27mm (Table

1). In previous studies, antimicrobial effects of *G. adspersum* against *S. aureus*, *E. coli* and *P. aeruginosa* were reported to be 10mm, 10mm and 8mm, respectively (Shomali et al., 2019). It was reported that some compounds isolated from *G. adspersum* showed weak antimicrobial activity against *Streptococcus pneumonia* (7.7 mm), *Streptococcus pyogenes* (9.7 mm) and *Cryptococcus neoformans* (8.0 mm) (Mayaka et al., 2019). It was reported that the ethanol extract of *Ganoderma lucidum* at 50 mg/ml concentration formed a 13-29mm inhibition zone against *E. coli*, *S. aureus*, *S. thypi*, *P. aeruginosa* and *Klebsiella* sp. (Tamilselvan and Rajesh, 2019). *G. tuberculosum*, *G. tornatum*, and *G. weberianum* showed antibacterial activity against *Clavibacter michiganensis* in the concentration range of 31.5 to 1000 µg/mL (Espinosa-García et al., 2021). It was determined that the extracts of *Ganoderma lucidium* obtained from ethanol and methanol solvents have antifungal effects (6.6-10.3 mm) against *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus sydowii*, *Fusarium* sp. (Radhika and Rajan, 2021). When the results obtained are compared with the results of previous studies, it is seen that some are higher than some (Shomali et al., 2019; Mayaka et al., 2019; Radhika and Rajan, 2021) and similarities with others (Tamilselvan and Rajesh, 2019; Espinosa-García et al., 2021). Antimicrobial effects in mushroom extracts vary depending on the bioactive components they contain, the type of fungus, the microorganism used and the concentrations (Shomali et al., 2019; Mayaka et al., 2019; Radhika and Rajan, 2021; Tamilselvan and Rajesh, 2019; Espinosa-García et al., 2021).
The IC50 value of the antioxidant effect of the ethanol extracts of *G. adspersum* was determined as 48.002 µg/mL (Shomali et al., 2019). In a different study, the scavenging effect of the same species on DPPH radical was reported to be 5.61 mg TE/g (Sułkowska-Ziaja et al., 2022). It has been reported that the DPPH radical scavenging effects of *G. adspersum* extracts obtained from different solvents at 200 µg/ml concentration range from 71.19% to 78.24% (Tel-Çayan et al., 2015). The IC50 value of the antioxidant effect of the polysaccharides of *G. adspersum* was determined as >200 µg/ml (Deveci et al., 2019). It has been reported that galactomannans obtained from *Ganoderma adspersum* have weak antioxidant effects (Tel-Çayan et al., 2020). In a different study, the IC50 value of the antioxidant effect of *G. adspersum* was determined as 1.70±1.13 µg/ml (Raks et al., 2018). When the results obtained are compared with other studies in the literatüre (Shomali et al., 2019; Ziaja et al., 2022; Tel-Çayan et al., 2015; Deveci et al., 2019; Tel-Çayan et al., 2020; Raks et al., 2018), it is seen that the antioxidant effects of *G. adspersum* show differences. The difference in the results of the study varies according to the place where the species was collected, the concentration used, the bioactive components it contains, the solvent and the part where the species is used.

## Conclusion

In this study, antimicrobial and antioxidant effects of ethanol and methanol extracts of *G. adspersum* were determined. The ethanol extract of *G. adspersum* showed the highest antimicrobial effect against *E. coli*. It was determined that *G. adspersum* had low TAS values and high oxidant values. It was determined that the scavenging effects of DPPH radical increased depending on the concentration, but the inhibition percentage was below 50%. The results obtained may be pharmacologically important and fungal extracts can be used as antimicrobial agents.

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# DETERMINATION OF ANTIMICROBIAL AND ANTIOXIDANT EFFECT OF *SUILLUS LUTEUS* L ROUSSEL

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## ABSTRACT

Edible mushrooms have been collected from nature for centuries and used for both food and therapeutic purposes. One of these mushrooms, *Suillus luteus* (L.) Roussel, is widely consumed in our country and it is known that it is rarely consumed as food in Elazığ. *Suillus luteus* is known to have antimicrobial, antioxidant, anticancer and hypoglycemic activity. In this study, it was aimed to determine the antimicrobial and antioxidant effects of methanol and ethanol extracts of *S. luteus*. Its antimicrobial effect was determined by disk diffusion method using pathogenic microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus megaterium*, *Candida albicans* and *Candida glabrata*. Total antioxidant level (TAS), total oxidant level (TOS) and 2.2-diphenyl-1-picrilhydrazyl (DPPH) radical scavenging capacity were detected for the antioxidant activity of the mushroom. In the results obtained, the ethanol extract of *S. luteus* showed the highest antimicrobial effect against *C. glabrata* (27±0.43 mm), while the ethanol extract showed the lowest antimicrobial effect against *E. coli* (9±0.30 mm). The TAS values of the methanol and ethanol extracts of the mushroom were determined as 0.61±0.26 mmol and 0.40±0.20 mmol, respectively. TOS values were determined as 18.83±0.36 and 14.27±0.30 µmol, respectively. It has been observed that the effect of removing DPPH radical of *S. luteus* increased depending on increasing concentrations, but showed a lower antioxidant effect compared to the control. As a result of the study, it can be said that methanol and ethanol extracts of *S. luteus* can be good antimicrobial agents.

***KEYWORDS***: Edible mushroom, *Suillus luteus*, antimicrobial effect, antioxidant effect

## *Suillus luteus* (L.) Roussel'in Antimikrobiyal ve Antioksidan Etkisinin Belirlenmesi

## ÖZET

Yenilebilir mantarlar yüzyıllardır doğadan toplanmış ve hem yemek hem de tedavi amaçlı kullanılmıştır. Bu mantarlardan biri olan *Suillus luteus* (L.) Roussel ülkemizde yaygın olarak tüketilmekte olup, Elâzığ'da yemek olarak nadiren tüketildiği bilinmektedir. *S. luteus*'un antimikrobiyal, antioksidan, antikanser ve hipoglisemik aktiviteye sahip olduğu bilinmektedir. Bu çalışmada, *S. luteus'*un metanol ve etanol ekstraktlarının antimikrobiyal ve antioksidan etkilerinin belirlenmesi amaçlanmıştır. Antimikrobiyal etkisi, *Escherichia coli*, *Klebsiella pneumoniae*, Staphylococcus aureus, *Bacillus megaterium*, *Candida albicans* ve *Candida glabrata* gibi patojen mikroorganizmalar kullanılarak disk difüzyon yöntemi ile belirlendi. Mantarın antioksidan aktivitesi için toplam antioksidan seviyesi (TAS), toplam oksidan seviyesi (TOS) ve 2.2-difenil-1-pikrilhidrazil (DPPH) radikal süpürme kapasitesi tespit edilmiştir. Elde edilen sonuçlarda, *S. luteus*'un etanol özütü *C. glabrata*'ya karşı en yüksek antimikrobiyal etkiyi (27±0.43 mm) gösterirken, *E. coli'*ye karşı en düşük antimikrobiyal etkiyi etanol özütü (9±0.30 mm) gösterdi. Mantarın metanol ve etanol ekstraktlarının TAS değerleri sırasıyla 0.61±0.26 mmol ve 0.40±0.20 mmol olarak belirlendi. TOS değerleri sırasıyla 18,83±0,36 ve 14,27±0,30

µmol olarak belirlendi. *S. luteus*'un DPPH radikalini uzaklaştırma etkisinin artan konsantrasyonlara bağlı olarak arttığı ancak kontrole göre daha düşük bir antioksidan etki gösterdiği görülmüştür. Çalışma sonucunda *S. luteus*'un metanol ve etanol ekstraktlarının iyi bir antimikrobiyal ajan olabileceği söylenebilir.

***ANAHTAR KELİMELER*:** Yenilebilir mantar, Suillus luteus, antimikrobiyal etki, antioksidan etki

## Introduction

Today, the use of natural products for the prevention and treatment of diseases is becoming more and more widespread. Since these products are easily accessible and cheap and at the same time, the amount of use is increasing among the people because of the thought that they are not harmful from nature (Vardar et al., 2018). In many studies conducted in recent years, medicinal uses of mushrooms have been stated (İnci et al., 2022; Akyüz et al., 2021).

Mushrooms have been used both as food and medicine throughout history. It is one of the sought-after foods of gourmet meals, thanks to its unique taste and aroma contents, and its protein, carbohydrates, vitamins, dietary fiber and minerals (Pinky et al., 2019; Mwangi et al., 2022). In recent years, mushrooms have become functional foods due to their positive effects on human health (Sharma et al., 2021). Many studies conducted today have reported that mushrooms have antioxidant, antimicrobial, antiproliferative, anticancer, DNA protective, anti-inflammatory, immunomodulatory and antihypertensive activities (İnci et al., 2018; Akyüz et al., 2021). Extracts are prepared from macrofungi and actinomycetes used in the treatment of many diseases (Sevim et al., 2021).

*Suillus luteus (L.) Roussel,* an edible fungus native to temperate and subtropical regions of China, is a species that can form ectomycorrhizae with *Pinus elliottii* and *P. tabulaeformis* (Li and Sun 2002; Feng et al., 2019; Liu et al. 2018). *S. luteus* is a member of the Boletales within the Agaricomycetes (Aytar et al., 2020). *Suillus luteus is an edible mushroom commonly called "slippery jack" because its cap cuticles are sometimes sticky: Also known as "Kallampa" and used in traditional medicine to treat gastrointestinal ailments and headaches* (Andrade et al., 2022).

In this study, it was aimed to determine the antimicrobial and antioxidant effects of methanol and ethanol extracts of *S. luteus*.

## Materials and Methods

### Material

*Suillus luteus* was collected in Elazığ. The species was identified by performing macroscopic and microscopic studies. The mushroom identification was made by Prof. Dr. Sevda KIRBAĞ from Fırat University. After the mushroom was ground, 0.5 g was weighed. 100 mL of 96% methanol (MetOH) and ethanol (EtOH) were added to the samples and left in an orbital shaker at 100 rpm for 72 h. Then, samples were filtered with blank disc.

### Microorganisms

In this study; *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Bacillus megaterium* DSM32, *Staphylococcus aureus* COWAN1, *Candida albicans* FMC17 and *Candida glabrata* were used. Microorganism were obtained from Firat-University, Department of Biology.

### Preparation of microorganism cultures and antimicrobial testing

The antimicrobial activity was determined by the disc diffusion method (Collins and Lyne, 1987). Mueller Hinton Agar, Yeast Malt Extract Agar and Sabouraud Dextrose Agar were sterilized separately in an erlen, then cooled to 45-50°C and inoculated with culture of bacteria,

yeast and dermatophyte ($10^6$ cells / mL of bacteria, $10^4$ cells / mL yeast as per Mc Farland standard). After shaking well, they were poured into sterile petri dishes and homogeneously dispersed. Discs (6 mm diameter) impregnated with 100 µL (1000 µg) of extracts were placed on microorganism-inoculated plates. Then the plates were kept at $4^0$C for 2 h. The inoculated petri dishes were incubated at $37 \pm 0.1^0$C at 24 h for bacteria and at $25 \pm 0.1^0$C at 72 h for yeasts. Standard discs were used for bacteria (Streptomycin sulphate 10 µg / disc) and yeasts (Nystatin 30 µg / disc). The antimicrobial effect was detected by measuring the inhibition zone in mm.

**Antioxidant effect**

**Total Antioxidant Activity (TAS) and Total Oxidant Activity (TOS)**

TAS and TOS levels of MetOH and EtOH extracts of sample were determined with Rel Assay kits (Rel Assay Kit Diagnostics). TAS value was given as mmol Trolox equiv./L and Trolox was used as the calibrator (Erel, 2004). The TOS value was expressed as µmol $H_2O_2$ equiv./L and hydrogen peroxide was used as the calibrator (Erel, 2005).

**2.2-diphenyl-1-picrilhydrazyl Radical Scavenging Capacity (DPPH)**

The antioxidant activity of different concentrations of MetOH and EtOH extracts of *S. luteus* was detected according to the DPPH radical scavenging capacity method [13]. The solution was prepared in methanol and ethanol at a concentration of 25 mg/mL of the obtained extract and diluted 4 times to obtain the calibration curve of DPPH. 40 µL of the solution was taken and 160 µL of DPPH solution was added. After mixing well, the plate left in the dark for 30 min. Methanol and ethanol were used as controls. At the end of the period, absorbances of each mixture were read at 570 nm in the spectrophotometer. percent inhibition values were calculated;
% DPPH inhibition = [(AbsControl - AbsSample) / AbsControl] x 100

**Statistical analysis**

The statistical analysis was performed according to the Kruskal Wallis test.

## Results

**Antimicrobial Effect**

Antimicrobial activities of *S. luteus* extracts obtained from MetOH and EtOH against, used microorganisms are given in Table 1. According to the results obtained from, methanol (25 mm) and ethanol extracts (27 mm) of *S. luteus*, it inhibited the development of *C. glabrata* the most (Table 1).

**Table 1.** Inhibition zones of extracts of *S. luteus* against some microorganisms (mm)

| | *S. luteus*-MetOH | *S. luteus*-EtOH | Standard antibiotics |
|---|---|---|---|
| *E.coli* | $10 \pm 0.45^{ab}$ | $9 \pm 0.30^{b}$ | $20.21 \pm 0.57$* |
| *K. pneumoniae.* | $20 \pm 0.43^{ab}$ | $15 \pm 0.56^{ab}$ | $19.13 \pm 0.43$* |
| *B. megaterium* | $15 \pm 0.33^{b}$ | $15 \pm 0.23^{b}$ | $20.25 \pm 0.57$* |
| *S. aureus* | $17 \pm 0.58^{ab}$ | $14 \pm 0.23^{b}$ | $20.23 \pm 0.33$* |
| *C. albicans* | $17 \pm 0.66^{ab}$ | $14 \pm 0.48^{ab}$ | $21.32 \pm 0.42$** |
| *C. glabrata* | $25 \pm 0.53^{ab}$ | $27 \pm 0.43^{ab}$ | $22.45 \pm 0.51$** |

(Streptomycin sulphate *10 µg/disc for bacteria, Nystatin** 30 µg/disc for yeast and dermatophyta), Means in the same column with the different superscript are significantly different ($p<0.05$)

**Antioxidant Effect**

The TAS and TOS values of the MetOH extract of mushroom was found to be 0.61 mmol and 18.83 µmol, respectively and the EtOH extract of mushroom was 0.40 mmol and 14.27 µmol, respectively (Table 2).

**Table 2.** TAS and TOS values of *S. luteus*

| | TAS(mmol Trolox equiv./L) | TOS (µmol $H_2O_2$ equiv./L) |
|---|---|---|
| ***S. luteus*-MetOH** | 0.61±0.26 | 18.83±0.36 |
| ***S. luteus*-EtOH** | 0.40±0.20 | 14.27±0.30 |

Values are means ±S.D.n:3, $p<0.05$ importantly dissimilar

The percent inhibition of the DPPH of *S. luteus* is seen in Table 3. It was detected that the antioxidant effects of MetOH and EtOH extracts increased with increasing concentration.

**Table 3.** Percent inhibition of the DPPH radical of *S. luteus*

| | ***S. luteus*-MetOH** | ***S. luteus*-EtOH** |
|---|---|---|
| **1000 µg/mL** | 51.82 ± 0.421 | 50.10 ± 0.635 |
| **500 µg/mL** | 45.73 ± 0.671 | 33.33 ± 0.582 |
| **250 µg/mL** | 27.43 ± 0.643 | 18.33 ± 0.542 |
| **125 µg/mL** | 18.29 ± 0.580 | 10.90 ± 0.367 |

Values are means ±S.D.n:3, $p<0.05$ importantly dissimilar with Kruskal Wallis's test

**Discussion**

It was determined that methanol extracts of *S. luteus* formed a 9mm inhibition zone against *E. faecalis*, *B. subtilis* and *K. pneumaniae*. In the same study, ethanol extract created a 9 mm inhibition zone against *E. faecalis*, *S. aureus* and *S. typhimurium*, while it created a 10 mm inhibition zone against *P. aeruginosa* (Aytar et al., 2020). It was reported that ethanol extracts prepared from *S. luteus* showed low antimicrobial activity (9mm) against *S. mutans* (Duman et al., 2003). It was determined that *S. luteus* did not have antimicrobial effects against *E. coli*, *S. aureus*, *C. albicans*, *M. smegmatis* and *S. schenkii* (Patricia et al., 2010). Ethanol extracts of *S. luteus* were reported to form inhibition zones against *K. pneumaniae* (18mm), *P. aeruginosa* (10mm) and *S. typhimurium* (11mm) at 500 µg (Park et al., 1995). According to the antimicrobial activity results, it was found that *S. granulatus* extract inhibited *E. coli* and *S. aureus* at 64 µg/mL concentration, while concentrations higher than 16 µg/mL inhibited the growth of *L. monocytogenes*, *S. typhimurium* and *P. florescens* (Soyuçok et al., 2022). When the results obtained are compared with other studies in the literature, the results show variability. Because the results of the study vary depending on the bioactive components contained in the mushroom, the type of fungus, the microorganism used and the concentrations.

In previous studies, the IC50 values of the DPPH radical scavenging effects of methanol and ethanol extracts of *S. luteus* were reported as 63.72 and 80.72 µg/mL, respectively (Aytar et al., 2020). The IC50 values of the antioxidant effect of some compounds isolated from *S. luteus* were determined in the range of 1.55 to 19.89 µM. (Yao et al., ) In a different study, the antioxidant effect of the same species was reported as 2.86 mg/mL (Riberio et al., 2014). TAS and TOS values of *S. collinitus* were determined as 2.467 mmol/L and 17.845 µmol/L, respectively. (Bal, 2021). TAS value of *S. granulatus* was determined as 3.143 mmol/L and TOS value was determined as 18.933 µmol/L (Mustaq et al., 2020). The DPPH radical scavenging activity of *S. granulatus* was 175. 64 µmol TE/g dry weight. (Soyuçok et al., 2022). When the results of the study were compared with the results of other studies in the literature, they showed variability

depending on the type of mushroom used, the concentration, the study method, the place where the species was collected, and the components it contained.

**Conclusion**

In this study, antimicrobial and antioxidant effects of ethanol and methanol extracts of *S. luteus* were determined. In the study, methanol and ethanol extracts of *S. luteus* showed the highest antimicrobial effect against *C. glabrata*. It was determined that *S. luteus* had low TAS values and high oxidant values. It was determined that the scavenging effects of DPPH radical increased depending on the concentration, but the inhibition percentage increased above 50% at 1000 µg/mL concentration. Although it is a known fungus species, we think that the results of antimicrobial and antioxidant studies of S. luteus will be brought to the literature and may be antifungal agents in pharmacological studies.

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# MOLECULAR DIVERSITY ON THE RACES OF *FUSARIUM OXYSPORUM* F. SP. *CICERIS* CAUSING WILT OF CHICKPEA IN KURDISTAN PROVINCE OF IRAN

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## ABSTRACT

*Fusarium* wilt by *Fusarium oxysporum* f. sp. ciceris (Padwick) Matuo & K. Sato, (FOC) is the most important soil-borne disease of chickpea in Kurdistan province of Iran. The distribution of pathogenic races of FOC in Kurdistan province was determined during this research. Plant samples with disease symptoms were collected from 45 infected farms in the chickpea production area along with Kurdistan province. First, the isolation purification and morphological identification of the FOC have been done from each sample, and the DNA extraction, the molecular identification of species, and the races of isolates are performed using FOC-specific linked primers. Among the isolate collected, the 38 isolates were identified as *Fusarium oxysporum* f. sp. *ciceris*. Molecular identification of races using SCAR-linked markers for detection of races (B/C1, 0, 2,3,4,5, 6, and 1A) revealed that 28 out of 38 isolates of FOC belonged to race 0, and other isolates belonged to the race B/C1. We could not find any relation between the area of race prevalence and the geographical distribution of the disease in the Kurdistan province. Identification of the races is crucial for the chickpea landrace evaluation for resistance and development of new commercial cultivars. It is a fundamental strategy for the integrated management of the disease for durable crop production.

***KEYWORDS***: Races, diversity, *Fusarium oxysporum* f.sp. *ciceris*

## Introduction

Chickpea (*Cicer arietinum* L.) is the most cultivated legume crop after dry bean (*Phaseolus vulgaris* L.) and dry pea (*Pisum sativum* L.) in the word. Fusarium wilt caused by the asexual soil-born fungus *Fusarium oxysporum* Schelchtend.: Fr. F. sp. *ciceris* (Padwick) Mantuo & K. Sato (FOC), is one of the most destructive chickpea diseases worldwide (Jalali and chand, 1992). This pathogen can cause complete crop loss (Halila and S trange, 1996; Haware and Nene, 1980), with average annual yield losses of 10-15% (Jalali and Chand, 1992; Tropero-Casas and Jimenez-Diaz, 1985). The most applied and cost-efficient method for the management of this disease is through the use of resistant cultivars (Jalali and Chand, 1992). However, pathogenic variability in FOC limits the effectiveness of this strategy (Haware and Nene, 1982; Jimenez-Diaz *et al*., 1993; Trapero-Casas and Jimenez-Diaz, 1985). Therefore, the identification of pathogenic races of FOC is important for disease management, and the efficient use of available resistant cultivars. Races and genetically diverse FOC populations have been reported in India, Türkiye, Tunisia, Spain, Sudan, Iran, and Ethiopia (Hware and Nene, 1982; Jimenez-Diaz *et al*.,

1989., Zamani *et al*., 2004; Bayraktar *et al*., 2008; Shehaboo *et al*., 2008; Gurjar *et al*., 2009). Eight races (0, 1A, 1B/C, 2,3,4,5, and 6) of FOC have been identified using chickpea differential lines and molecular markers. Based on aboveground symptoms, two groups reported; one those causing yellowing and other causing wilting (Trapero-Casas and Jimenez-Diaz 1985). Races 1A, 2,3,4,5, and 6 induce the wilt syndrome (severe chlorosis and flaccidity combined with vascular discoloration followed by plant death). Races 0 and 1B/C induce the yellowing syndrome (Trapero-Casas and Jimenez-Diaz 1985, Jimenez-Diaz *et al*., 1993). Races 1A, 2,3, and 4 were first identified in India (Haware and Nene, 1982). Races 2 and 3 have been reported in Ethiopia, India, and Türkiye. Whereas race 4 has only been reported in Ethiopia and India (Haware and Nene, 1982; Jalali and Chand, 1992; Shehabu *et al*., 2008). Races 0, 1A, 1B/C, 5, and 6 have been found in California (USA) and Spain; races 0 and 1A, 1B/C in Syria, Tunisia, and Türkiye; races 0, 1A, and 6 in Palestine; races 1A and 6 in Morocco; and race 0 in Lebanon ( Jimenez-Diaz *et al*., 1993; Hlila and Strange 1996). Races 0, 2 and 3 identified in Türkiye (Bayraktar and Dolar 2012) and races 0 and 2 in Sudan (Mohamed *et al*., 2015). Knowledge of the genetic diversity of the pathogen is very important for better targeting key races and populations of FOC in its breeding program. Therefore, this study aimed to estimate the genetic diversity of FOC isolates collected from infected farmers' fields of Kurdistan province of Iran.

## Material and methods

### Infected plant sampling

Samples of infected plants were collected in July 2017 from Kurdistan province, Iran. Infected plants with symptoms of fusarium rot such as yellowing and wilting selected from infected farms in 42 area of chickpea cultivation. Latitude and longitude information of each sampling location were recorded. The samples were transferred to the laboratory in numbered bags and stored at 4 C° in a refrigerator.

### Isolation and purification of the disease agent

To isolate the disease agent transverse sections of the infected roots were cut from the areas with signs of infection in the root and crown area of collected plants. The samples were first washed with sterilized distilled water and then disinfected with sodium hypochlorite (containing 1% of active substance) for one minute and then washed again with sterilized distilled water and dried on sterilized filter paper. Then they were transferred to a PDA culture medium and kept in the incubator for 5-7 days at 25-30 C°. Then each fungi isolate was purified separately by the standard single spore method. To store the isolates, 1.5 cc vials filled to half with a mixture of 10 parts washed sand, one part perlites, and two parts straw, and then 0.5 cc distilled water was added to it and sterilized using an autoclave. After the growth of the single spores, a piece of culture medium containing the grown isolate transferred for long-term storage and subsequent identification.

### Morphological identification of isolates

To evaluate macroscopic characteristics, on the PDA medium at 25 C° and 8/16-hour fluorescent lamp light frequency after 14 days the fungus phenotypic characters measured. The characters colony growth rate, the colony color, and color diffusion in agar were recorded. Microscopic characteristics such as macroconidia, microconidia, and chlamydospores shape and size, sporodochium color, phialide shape, the shape of branched conidiophore, and shape of conidia on conidiophore after growth on CLA medium were evaluated after three weeks using the Ogawa model microscope with a magnification of 40X and 100X. Characteristics of sporodochium and macroconidia; include presence and absence, color, size, shape and terminal and primary cell type, production method, and the number of transverse bands recorded. Although characteristic of microconidia include presence or absence of microconidia, microconidia, production method and type of arrangement (single, false head and chain), Number of transverse bands, phialide

type (mono or poly phialide), characteristics of chlamydospores including presence or absence, place of production, wall type, arrangement (single, batch, double and chain), Presence and absence of false chlamydospores and presence or absence of swollen cells in filaments were measured. For each sample, 50 dimensions were executed and their average was considered. The *Fusarium* species were identified based on measured characteristics using Leslie & Summerell (2006) and Nelson *et al.* (1983) key of identification.

**Pathogenicity tests**

The experimental setup given in Nene and Haware (1980) has been reproduced here to examine the pathogenicity of *Fusarium* isolates. Kaka susceptible cultivar seeds were disinfested with 0.25% sodium hypochlorite (NaOCl 0.25%) for 5 min. Then they were sown in 15 cm pots filled with sterilized soil for 2 weeks at 25 ± 1°C with a 12/12 h photoperiod. When the seedlings became three to four-leafed, inoculated with each *Fusarium oxysporum* f.sp *ciceris* isolates, the topsoil was removed and 15 ml of PDB liquid culture medium with a concentration of $5\times 10^6$ spores was poured at the base of each seedling and the soil was replaced. Pathogenicity was measured according to Dubai *et al*., (2010). The percentage of wilting and yellowing was scored at 15 days after sowing of chickpea till four weeks, based on wilt incidence, the isolates were grouped into three categories as weakly pathogenic (up to 30% wilt), moderately pathogenic (31 to 50% wilt) and highly pathogenic (50% wilt). Based on the percentage of seedling wilting up to 30% weak pathogenicity, between 31 to 50% moderate pathogenicity and above 50% high pathogenicity were considered for each isolate. The experiment was performed in a completely randomized design with three replications. PDB culture medium without contamination was used as control treatment.

**Molecular identification of isolates**

Total genomic DNA of *Fusarium* species was extracted using the method described by Zhong and Steffenson (2001). Approximately 100 mg mycelium of each isolate were finely grounded and suspended in 400 µL of extraction buffer (50 mM Tris-HCl (pH 8.0), 150 mM EDTA, 1% sarkosyl, and 300 µg proteinase K per mL). The contents were transferred into a 1.5 mL microtube, mixed by vortex for 10 second and incubated for 30 minutes at 65°C. After incubation, 400 µL of chloroform: isoamyl alcohol (24:1) was added, mixed for 10 min, and then centrifuged at 10,000 rpm for 10 min at 4°C. The aqueous clear phase was mixed with an equal amount of isopropanol and stored overnight for precipitation of DNA at -20°C. DNA was recovered by centrifugation at 10,000 rpm for 3 min and precipitated with 70% ethanol. The DNA pellet was then rinsed twice with 1 ml of 10 mM Tris-HCl and 0.1 mM EDTA or sterile distilled water buffer. The purity and quantity of extracted DNA were determined using NanoDrop 2000c Spectrophotometer (Thermo Scientific).

**Polymerase chain reaction**

A standard polymerase chain reaction (PCR) assay was performed using the primer pair FOC-f (5′- GGCGTTTCGCAGCCTTACAATGAAG-3′) and FOC-r (5′- GACTCCTTTTTCCCGAGGTAGGTCAGAT-3′) that was used to amplify a DNA fragment of 1500 bp for the detection of FOC molecules by PCR (Arvaio-Ortiz *et al.*, 2011). The reaction mixture of PCR was prepared in a final volume of 25 µL containing 12.5 µl 2X PCR Master Mix, 8.5 µl of injected distilled water, 1 µl (10 pmol / ml) specific F primer, 1 µl (10 pmol / ml) specific R primer , 20 ng of fungal DNA; using the following conditions: initial denaturation at 94°C for 10 min; 36 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 58 °C, initial extension for 1 min at 72°C, and final extension of 5 min at 72°C. PCR products were detected through gel electrophoresis using 0.8% agarose gel.

**Genetic diversity analyses**

Eight molecular markers were used to determine of the genetic diversity of the FOC populations (Table 1). The PCR amplifications were performed in a 25µl reaction volume. The mixture

contained (12.5 µl 2X Master Mix PCR, 8.5 µl distilled water, 1 µl (10pmol/ml) specific primer F of each race, 1 µl (10pmol/ml) specific primer R of each race and 20ng of fungus DNA). The thermocycling program for each primer was designed with their appropriate annealing temperatures (Table 1). The PCR cycles of SCAR were conducted as follows: one cycle of initial denaturation at 94 ◦C for 10 min, followed by 36 cycles at 94 ◦C for 1 min, 1 min of annealing temperature and 1 min at 72 ◦C, and finally at 72 ◦C for 5 min (Shokri *et al*., 2020).

**Table 1.** Primers used for molecular identification of pathogenic races of *Fusarium oxysporum* f. sp *ciceris*

| Primers | Primer sequence (5′ to 3′) | Melting point (◦C) | Race | Amplicon size (Kbp) |
|---|---|---|---|---|
| FOC- 0f | GGAGAGCAGGACAGCAAAGACTA | 65 | R0 | 0.9 |
| FOC- 0r | GGAGAGCAGCTACCCTAGATACACC | 69 | | |
| FOC-B/C1f | GAGAGCAGGGTCAGCGTAGATAG | 66 | RB/C1 | 0.5 |
| FOC-B/C1r | GCAGCAGAAGAGGAAGAAAATGTA | 62 | | |
| FOC- 5f | GGAAGCTTGGCATGACATAC | 58 | R5 | 0.9 |
| FOC- 5r | AAGCTTGGGCACCCTCTT | 56 | | |
| FOC- 6f | GAGCAGTCAATGGCAATGG | 57 | R6 | 1.0 |
| FOC- 6r | AGAGCAGGGTCAGCGTAGATA | 61 | | |
| OPF-12 | ACGGTACCA | 32 | RB/C1-R0-R2 | 1.1-0.39-0.9 |
| OPF-06 | GGGAATTCGG | 32 | RB/C1-R3 | 1.9-2 |
| OPF-10 | GGAAGCTTGG | 32 | RB/C1-R4- R5 | 0.51- 0.95-0.9 |
| OPL-01 | ACCTGGACAC | 32 | RA1-R0-RB/C1 | 1- 1.2- 2.7 |

**Result**

Based on the results obtained from sampling and isolation of fungi on selected samples from 42 infected farms in Kurdistan province, among *Fusarium* species, 37 FOC isolates were identified (Figure 1).

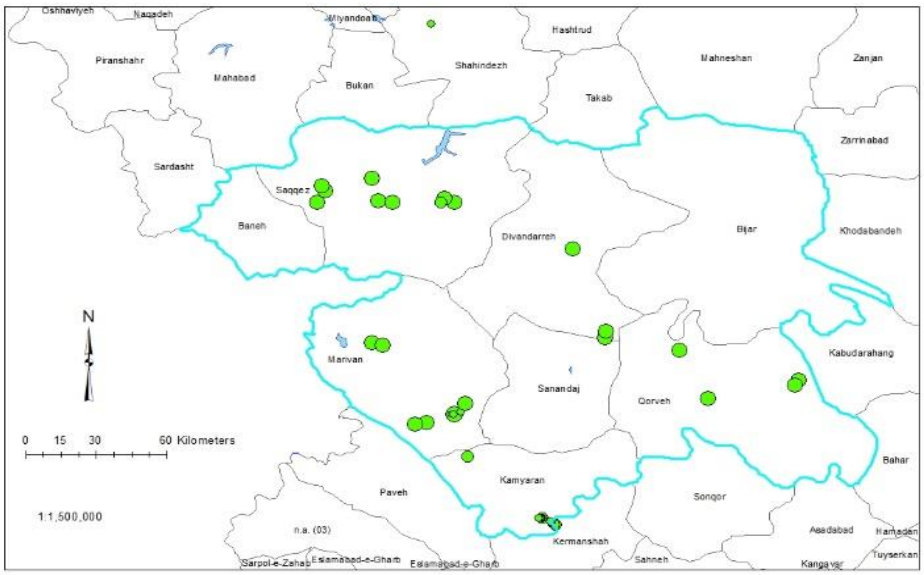


**Figure 1.** Distribution map of FOC isolates indicated by green circles

**Morphological characteristics**

Different species were identified among *Fusarium* isolates. The highest frequency is connected to two species complexes of *F. Solani* and *F. oxysporum*.

The *F. oxysporum* is a global complex species and has been reported as a source of vascular wilt disease in many plant species from all over the world. In addition, this species is known as a common soil saprophyte. On PDA medium the colony morphology is very diverse. The hyphae are low or very high cotton shape and their color varies from white to purple. In some isolates, large numbers of orange or purple macroconidia form on the filament mass observed in the center of the colony. On CLA medium, macroconidia were usually produced in large numbers on orange sporodochia and some isolates had no or few sporodochia. Macroconidia were short to medium in length and ranged from crescent to smooth, thin-walled, often with three transverse walls in shape. The primary cell was short and, in some isolates, slightly hooked or curved. The basal cell is foot-shaped and sometimes terminates. Macroconidia were formed mono-phialide on conidiophores branching in the sporodochia and in extremely small numbers as monofilaments on hyphae. The microconidia were often without transverse walls and in elliptical, elongated, and sometimes fragmentary shapes. Microconidias were produced in large numbers in the form of false heads on short monofilaments.

Chlamydospores formed in large numbers within hyphae on the surface or in agar. Their rate of formation was often as low as 2-4 weeks. A number of isolates were sometimes observed as complexes and chains inside or at the ends of the strings. The walls of chlamydospores were smooth or raised (Figure 2).

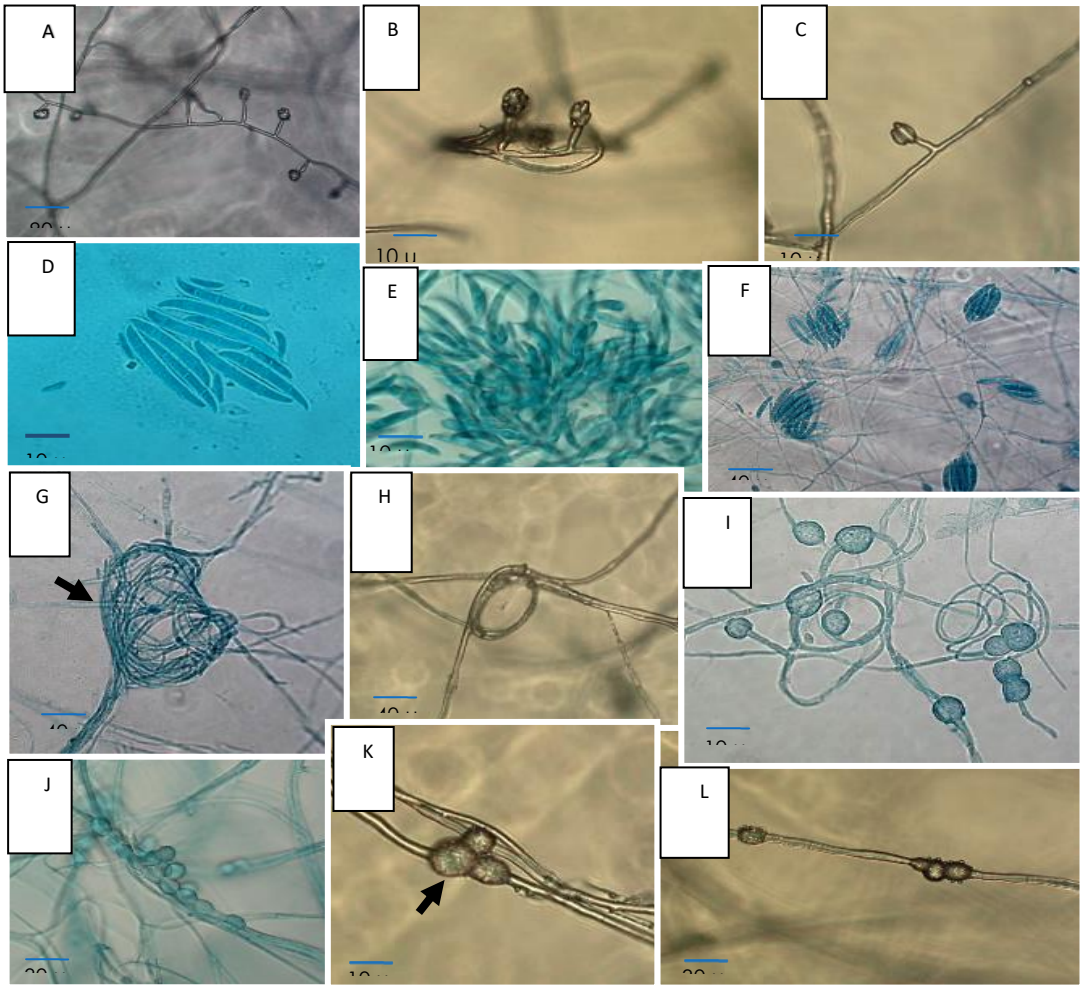


Figure 2. Microscopic properties of *Fusarium oxysporum* : A,B,C: Microconidia and Phialid, D, E, F: Macroconidia, G,H: Coil hyphae: I,J,K,L: Types of Chlamydospore on CLA medium

**Pathogenicity test results of isolates**

Disease index was determined according to the scoring system of Dobby *et al*. (2010). The pathogenicity of each isolate was determined separately based on the percentage of yellowing and wilting of each seedling. The disease development of each isolates were significantly different ($P \leq 0.01$) (Tables 1 and 2). Isolates related to Kamyaran region showed the highest (64.88%) and isolates related to Saqez region showed the lowest (18.44%) rate of disease development. Due to the fact that the disease occurs in the seedling stage until flowering and seed closure there is a difference in the severity of pathogenicity in the two stages of maturity and seedling.

**Table 1.** Results of variance Analysis of Disease severity (DS) of wilting, root and crown rot of chickpea on Kaka susceptible cultivar

| | Means of Square | |
|---|---|---|
| **Source of variation** | **Df** | **Disease incidence** |
| Isolates | 37 | 5.3 |
| Residual | 216 | |
| Total | 323 | |

**Table 2.** Comparing of the mean percentage of Disease incidence and Disease severity by *Fusarium oxysporum* f. sp *ciceris* isolates on Kaka cultivar

| Percentage of Wilting | Isolates | Percentage of Wilting | Isolates |
|---|---|---|---|
| 35.55egjfih | 61.1.2 | 64.88a | 62.2.1 |
| 31.55kgjfih | 38.2.3 | 64.77ab | 28.2.6 |
| 31.22kgjfih | 55.2.7 | 62.44ab | 51.2.2 |
| 30.11kgjih | 51.2.7 | 62.22ab | 34.1.7 |
| 29.66kgjih | 53.2.6 | 62.22ab | 34.2.6 |
| 27.22kjlih | 60.1.2 | 55.55abc | 40.1.2 |
| 27.22kjlih | 63.2.4 | 52.88abcd | 40.1.3 |
| 24.55kjli | 52.1.3 | 50.33abcde | 62.1.3 |
| 22.66kjl | 51.2.4 | 48.55bcde | 63.2.1 |
| 18.44kml | 61.1 | 46.22edfc | 33.1.2 |
| 18.33kml | 63.2.2 | 45.22egdfc | 37.1.2 |
| 18.11kml | 34.1.5 | 45egdfc | 35.1.5 |
| 18.44kml | 59.2.3 | 45egdfc | 34.11 |
| 16.55kml | 44.2.6 | 44.44egdfc | 38.2.2 |
| 13.55mln | 63.2.5 | 41.22egdfch | 43.2.4 |
| 13.22mln | 38.2.5 | 40.33egdfch | 58.1.6 |
| 5.11mn | 64.1.2 | 39.22egdfih | 64.2.2 |
| 1.55n | 43.2.3 | 37.11egjfih | 64.2.4 |
| | | 36.44egjfih | 52.1.1 |

Treatments with the same letters not significantly different according to LSD test in level 5%

**Molecular identification of *Fusarium oxysporum* f.sp *ciceris***

All isolates that had the morphological characteristics of FOC were selected for molecular studies with a pair of FOC-r and FOC-f primers. Those were *Fusarium oxysporum* f.sp *ciceris* amplified a DNA bands of 1500 bp on agarose gel 0/8% (Figure 3).

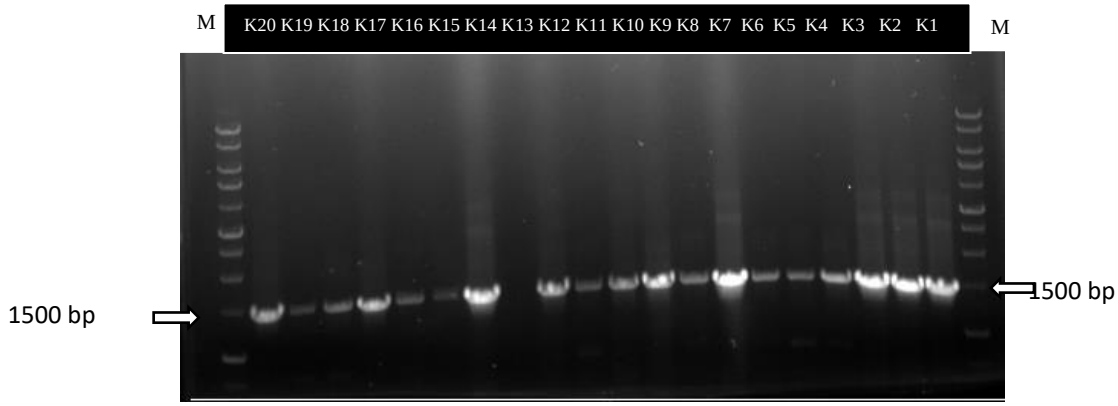


**Figure 3.** DNA bands of 1500 bp in agarose gel 0.8% amplified using FOC-f and FOC-r primer pairs

**Identification of FOC races**

Races of 37 isolates of FOC was determined by molecular markers. Pathogenic races 0 and 1B/C were identified among the isolates. The 28 isolates belongs to race 0 that by using primer R0, amplified a band which was a 900 bp long (Figure 4), and 9 isolates belongs to races 1B/C that were identified by using the 1B/C primer, produced a band of 500 bp long (Figure 5).

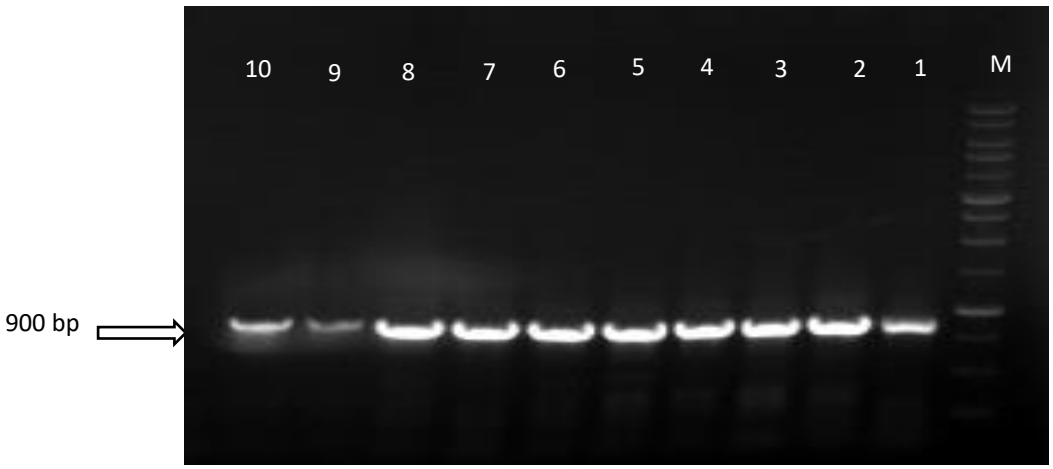


**Figure 4.** DNA bands of 900 bp on agarose gel 0.8% for detection of the races 0 of *Fusarium oxysporum* f.sp *ciceris* isolates

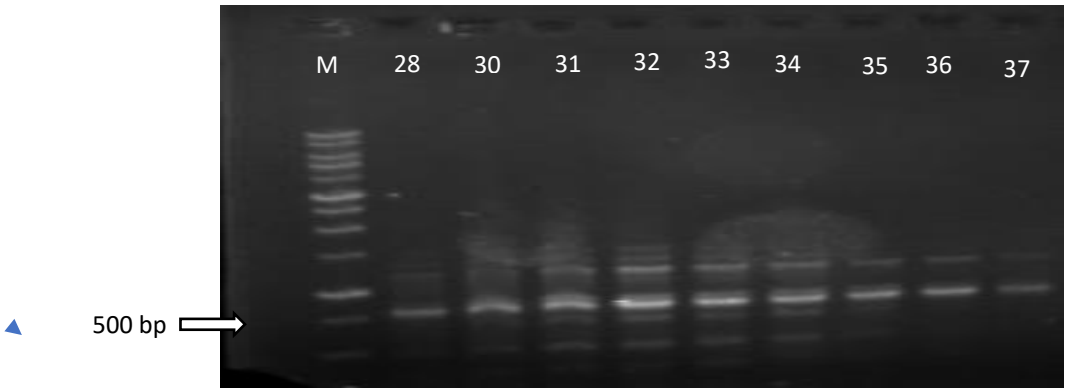


**Figure 5.** DNA bands of 500 bp on agarose gel 0.8% for detection of the races 1B/C of *Fusarium oxysporum* f.sp *ciceris* isolates.

**Discussion**

During this research Foc was isolated from plants that had signs of vascular wilt disease. The morphological characteristics of the isolates were consistent with the characteristics mentioned in the valid identification keys (Leslie & Summerell 2006 and Nelson *et al*., 1983). Among isolates under study, the races 0 and 1B/C were identified. It has already reported that two types of pathotypes generating yellowing and wilting could be collected. In addition to two pathotypes for FOC, eight pathogenic races (A1, 1B/C, 0, 2, 3, 4, 5, and 6) have been reported that have a wide geographical distribution in the world (Nene *et al*., 1988). Races 0, A1, 1B/C, 5, and 6 have been reported from the Mediterranean and California (Halila & Strange, 1996; Jimenez-Diaz *et al*., 1993; Jimenez-Gasco *et al*., 2001), races A1, 2, 3, and 4 from the Indian (Haware & Nene 1982), races 2 and 3 from Ethiopia (Shehaboo *et al*., 2008) and Türkiye (Bayraktar & Dolar, 2012) and race 4 from the Ethiopia (Shehabu *et al*., 2008). Yellowing including isolates were placed in two distinguished clusters which correspond to race 1B/C and 0. During our study the isolates of Kurdistan province that morphologically presented yellowing were found to be races 0 and 1B/C too. Wilting symptom presenting FOC isolates in another cluster grouping races i.e. A1, 2, 3, 4, 5, and 6 were reported from six different regions of Sudan. Based on the similarity coefficient, 14 isolates from Lebanon and Syria were investigated using SCAR, SSR and RPD markers. The isolates were grouped into two major clusters with seven groups. Races of 0 and 2 from Sudan isolates were placed in the first cluster and 1B/C and 6 races from Syrian and Lebanese isolates were placed in the second cluster. Race 0 was identified in Sudan (Elmahi Mohamed *et al*., 2015). Race 0 was identified by using race specific primers for FOC (Tekeoglu *et al*., 2017). Races 0, 1B/C, 5 and 6 was determined using SSR primer in Mexico (Arvaio-Ortiz *et al*., 2011). In the western and northwestern provinces of Iran, races 0, 1B/C, 1A, 6 and 5 were identified using SCAR markers (Shokri *et al*., 2020) and in this study two races 0 and 1B/C were identified using SCAR markers which confirms the distribution and importance of the presence of these two races in the west of Iran.

The result of this study on Kurdistan province chickpea cultivation only confirms the presence of the races 0 and 1B/C that could use to explain the diversity of resistant cultivars performance in Kurdistan province compare to elsewhere. The quick and appropriate identification of races in FOC is significant for the development and effective expansion of resistant chickpea cultivars. Our research achieved by markers similar to that of (Alloosh *et al*., 2019 and Shokri *et al*., 2020) prove the presence of 0 and 1B/C races in Kurdistan province and the ability of used SCAR markers for race Identification. The presence of these two races in Kurdistan province shows the importance of growing cultivars with the resistant gene for races 0 and 1B/C. There is a report

of other races of FOC elsewhere in Iran (Shokri *et al*., 2020). So during seed exchange, monitoring for other races should be considered.

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# BIODIVERSITY AND ECOLOGY OF MYXOMYCETES OF AYAZMA KAZ MOUNTAINS BAYRAMIC ÇANAKKALE

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## ABSTRACT

Many myxomycetes are cosmopolitan, free-living, multicellular, or plasmodial phagotrophic eukaryotes. The substrate, humidity, and temperature are the main factors that ensure myxomycete presence in an area. They are widespread on rotting logs, dead leaves, live bark, forest floor litter, animal manure, and some other organic matter, usually in moist forests in temperate regions. This research was carried out on samples such as tree bark, leaves, and rotten wood materials collected from field studies conducted in Kaz Mountains-Ayazma Recreation Area (Bayramiç-Çanakkale) between 2018-2019. "Moisture Chamber Technique" was applied to the collected materials and myxomycete sporophores were trying to be developed. In addition, samples of myxomycetes that develop and grow in their natural habitats were collected. As a result of field and laboratory studies, ten myxomycete taxa belonging to six families and eight genera were identified. The species/genus Ratio, which will show the richness of myxomycete biodiversity in our study area, was calculated as 1.43. When evaluated in terms of species diversity, this number is not very low (H'= 2.1972). However, the same is not the case when myxomycete record is evaluated.

***KEYWORDS***: Myxobiota, Ayazma, Çanakkale, biological diversity, ecology

**Kaz Dağları – Ayazma Mesire Yeri (Bayramiç-Çanakkale) Miksomisetlerinin Biyoçeşitliliği ve Ekolojisi**

## ÖZET

Serbest yaşayan, çok hücreli ya da plazmodial fagotrofik ökaryot olan miksomisetlerin birçok türü kozmopolit olup substrat, nem ve sıcaklık bir bölgede miksomiset bulunurluğunu sağlayan ana faktörlerdir. Genellikle ılıman bölgelerdeki rutubetli ormanlarda; çürüyen kütükler, ölü yapraklar, canlı ağaç kabukları, orman tabanındaki ölü örtü tabaka, hayvan gübreleri ve diğer bazı organik maddeler üzerinde çok yaygın bulunurlar.
Bu araştırma, 2018-2019 yılları arasında, Kaz Dağları-Ayazma Mesire Yeri (Bayramiç-Çanakkale)'ne yapılan arazi çalışmaları sonucunda toplanan ağaç kabukları, yapraklar ve çürümüş ağaç materyalleri gibi örnekler üzerinde yapılmıştır. Toplanan materyallere "Nem Odası Tekniği" uygulanmış ve miksomiset sporoforları geliştirilmeye çalışılmıştır. Ayrıca doğal ortamında bulunan ve gelişim gösteren miksomiset örnekleri de toplanmıştır.
Arazi ve laboratuvar çalışmaları sonucu 6 familya ve 8 cinse ait 10 miksomiset taksonu tespit edilmiştir. Çalışma alanımızda miksomiset biyoçeşitliliğinin zenginliğini gösterecek tür/cins oranı 1,43 olarak hesaplanmıştır. Tür çeşitliliği açısından değerlendirildiğinde bu sayının çok

düşük olmadığı görülmektedir (H'= 2,1972). Ancak miksomiset kaydı bakımından değerlendirildiğinde aynı durum söz konusu değildir.

***ANAHTAR KELİMELER:*** Miksobiyota, Ayazma, Çanakkale, Biyolojik çeşitlilik, Ekoloji

**Giriş**

Gerçek cıvık funguslar ya da plazmodial cıvık funguslar adıyla da bilinen miksomisetler "plazmodyum" denen özel bir yapı ile karakterize edilirler ki, bu yapı hücre çeperi olmayan, çok nukleuslu, protoplazma yığını halindeki, yapışkan bir kın ile çevrelenmiş, bir veya daha fazla sporofor meydana getirebilen bir özelliktedir. Plazmodyum, gelişiminin ileriki safhalarında bir veya daha fazla sporofor meydana getiren dev bir amibe benzetilebilir. Serbest yaşayan, çok hücreli ya da plazmodial fagotrofik beslenme özelliğindeki ökaryotik olan miksomisetlerin birçok türü kozmopolit olup substrat, nem ve sıcaklık bir bölgede miksomiset varlığını etkileyen başlıca faktörlerdir. Miksomisetlerin büyük çoğunluğu çok küçük organizmalar olup büyüklükleri 1-2 mm'den fazla değildir (Stephenson ve Stempen, 2000).

Miksomisetler önceleri hayvanlar ve bitkiler âlemi içerisinde sınıflandırılmıştır. Daha sonra flagella içermeleri ve diğer bazı özellikleri nedeniyle amoeboid protozoa (*Mycetozoa*) ve üreme yapıları ile sporları bakımından da bir grup tarafından Fungi âlemi içerisinde (*Myxomycetes*) tanımlanmıştır (Ing, 1999; Stephenson ve Stempen, 2000). Bu organizma grubunun farklı gruplar içerisinde gösterilmesinin nedeni, taksonomistlerin miksomisetlerin hayat devrelerinin farklı bölümlerini incelemelerinden kaynaklanmaktadır. Son zamanlarda r-RNA analizlerine göre yapılan filogenetik sınıflandırmada Protista âlemi içinde olduğu üzerinde durulmaktadır (Rammeloo ve Bogaerts, 2002). Fizyolojisi, morfolojisi, yaşam tarihi ve genetik analiziyle diğer ökaryotik mikroorganizmalar ile Protoctista âlemi içinde yer almıştır (Everhart ve Keller, 2008). Ancak yapılan bu araştırmalara rağmen sporla çoğalmaları, funguslarla benzer habitatlarda yaşamaları ve benzer karakteristik özelliklere sahip olmaları nedeniyle miksomisetler halen mikologlar tarafından Fungi âlemi içerisinde incelenmeye devam etmektedir. Alexopoulos ve ark. (1996) yaptıkları fungal sınıflandırmaya göre miksomisetler *Myxomycota* altında incelenmektedir.

Miksomisetlerin birçoğu kozmopolit olup genellikle ılıman bölgelerdeki rutubetli ormanlarda; çürüyen kütükler, odun, ölü yapraklar, canlı ağaç kabukları, orman tabanındaki ölü örtü, hayvan gübreleri ve diğer bazı organik maddeler üzerinde çok yaygın bulunurlar ve mikroorganizmalarla, canlı ve çürümekte olan bitki parçaları ya da ölü yapraklarla beslenirler (Lado 2001). Orman döküntü katındaki bu gibi substratlar üzerinde plasmodium, sporofor olarak veya sadece sporları bulunabilmektedir (Stephenson ve Stempen, 2000; Ocak, 2015). Üzerinde plazmodyum veya spor bulundurma ihtimali olan materyallerde laboratuvar ortamında miksomiset gelişimini sağlamak için kullanılan en önemli metot "nem odası tekniği" dir. Doğada sporofor olarak bulunamayan miksomisetlerin nem odası tekniğiyle gelişimi için uygun ortam şartları sağlanarak üç ay veya daha kısa bir sürede gelişimleri sağlanır (Ing, 1999).

Miksomiset çeşitliliği dünyada yaygın olarak çalışılmakta ve ekolojik önemleri üzerinde detaylı olarak durulmaktadır (Lado ve ark., 2002). Ancak ülkemizde yapılan miksobiyota çalışmaları daha çok sistematik ve taksonomik çalışmalar üzerine yoğunlaşmış olup ekolojik özellikleri bakımından sınırlı sayıda çalışma mevcuttur (Ocak, 2015). 1957-2019 yılları arasında yapılan çalışmalarda Türkiye'den sadece 282 miksomiset taksonu bildirilmiştir (Sesli ve ark., 2020). Son kayıtlara göre bu sayının dünya üzerinde 994 takson olduğu belirtilmiştir (Lado 2005-2021). Miksomisetler kozmopolit canlılar olmakla birlikte bazı örnekler dünyanın tropik, subtropik ve bazıları da sadece sıcak, karasal iklimsel bölgelerinde yaşamaktadır (Martin ve ark., 1983). Genellikle sıcak orman bölgelerinde daha çok görülürler. Çölde bile yaşayan türleri tespit edilmiştir (Stephenson ve Stempen, 2000).

Miksomisetler içinde yaşadıkları ekosistemde önemli ekolojik rollere sahiptirler (Rollins ve Stephenson, 2011). Miksomisetlerin ekolojik sınıflandırılması yapılırken, fruktifikasyon yapısının nerede şekillendiği ve plazmodial beslenmenin nerede olup olmadığı ile ilişkilendirmiştir. Araştırmacılar özellikle yaşadıkları substrata göre miksomisetleri lignikoloz, kortikoloz, litter-inhabiting (çöpte yaşayan) ve koprofiloz miksomisetler olarak bilinen dört büyük ekolojik grup altında toplamakla birlikte çeşitli küçük grupların da bulunduğunu ifade etmişlerdir (Stephenson ve Stempen, 2000; Novozhilov ve ark., 2000). Kortikoloz miksomisetlerin plazmodyum ve fruktifikasyon yapısının bulunduğu yer ağaç kabuklarıdır. Bu grubun üyeleri gerek plazmodyum yapıları gerekse çok küçük olan fruktifikasyon yapıları itibarıyla arazi çalışmalarında çıplak gözle fark etmek oldukça zordur (Ing 1999). Büyük bir olasılıkla da nem odası tekniği keşfedilene kadar da pek keşfedilmemişlerdir. Lignikoloz miksomisetler ölü odunların içinde gelişip fruktifikasyon oluştururlar. Çöpte yaşayan miksomisetler ormanda yere düşen küçük dal, yaprak, kabuk, meyve ve çiçek gibi çeşitli bitki parçacıkları üzerinde gelişirken, koprofiloz miksomisetler de herbivorların gübreleri üzerinde yaşarlar. Bununla birlikte ayrıca toprakta, henüz dökülmemiş yapraklarda, küçük dal parçalarında, bryofitler üzerinde ve erimiş kar sınırları üzerinde yaşayan olmak üzere yaşadıkları özel habitatlara göre de adlandırılırlar (Rollins ve Stephenson, 2011).

Miksomiset ekolojisi ile ilgili olarak bazı çevresel parametrelerin türlerin dağılımı üzerindeki etkilerine dair yapılmış çalışmalar bulunmaktadır. Sıcaklık, yağış ve nem, pH ve ışık bunlardan en yaygın olanlarıdır. Buna göre, miksomisetler kendi iç sıcaklıklarını düzenleyemedikleri için, çevresel sıcaklıklardaki günlük ve mevsimsel dalgalanmalar, yaşam döngüleri ve metabolizmalarının tüm yönleri üzerinde hemen ve doğrudan etkilidir. Sıcaklık, plazmodyum ve miksoamip üzerinde fizyolojik sınırları etkiler ve diğer taraftan sporulasyon, büyüme ve spor çimlenme oranlarını kontrol eder (Gray ve Alexopoulos, 1968). Sıcaklık tropik, subtropik, Akdeniz ve alpin türlerde sınırlayıcı faktör olarak önemli bir role sahiptir, fakat ılıman türlerde bu söz konusu değildir (Ing, 1994). Mevcut subsratların pH'ı bu organizmaların dağılımını etkileyen bir faktör olarak temsil edilmektedir (Härkönen 1977; Stephenson 1989). Birçok miksomiset nispeten geniş bir pH toleransına sahip gibi görünmektedir, ancak tüm türler için durum böyle değildir. Kullanılabilir su miksomisetlerin oluşması ya da ortaya çıkması için birinci derece öneme sahiptir ve su tutan substratlar önemlidir. Ormanlardaki nem miksomiset dağılımını etkiler. Ancak yağış, tür çeşitliliği ve zenginliği ile ilişkili değildir (Stephenson 1988, Heilmann 2001).

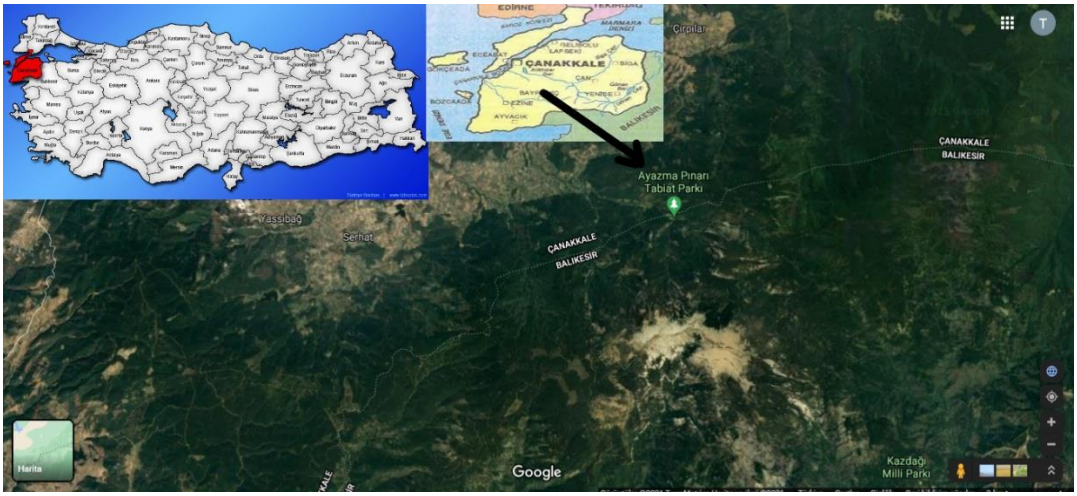
Çalışma alanı olan Kaz Dağları-Ayazma Bölgesi Biga Yarımadası'nda yer alan önemli doğal alanlardan biridir. Miksomisetlerin gelişimi için bu alan vejetasyon ve iklim özellikleri bakımından da oldukça uygun bir arazidir. Ayazma-Mesire Yeri özellikle iç turizm ve kısmen de dış turizme açık bir alan olduğundan antropojenik etkilere açıktır yani doğal ortama insanların etkisi kaçınılmazdır. Bu bölgede bulunan baskın ağaç türleri (Gemici ve ark., 1998) bölgenin önemli bir orman vejetasyonuna sahip olduğunu göstermektedir. Dolayısıyla vejetasyonun zengin olması miksomiset tür çeşitliliğinin de fazla olabileceğini düşündürmektedir. Dünya genelinde miksomisetler üzerine yapılan morfolojik, fizyolojik ve moleküler çalışmalara bakıldığında ülkemizde bu konu üzerine yapılan çalışmaların aslında ne kadar yetersiz ve sistematik çalışmalarla sınırlı olduğu anlaşılmaktadır (Oran ve Ergül 2004; Ocak ve Konuk 2018).

Bu çalışmanın ana amacı; Ayazma Mesire Alanı'nın miksomiset tür çeşitliliğini belirlemek ve kaydedilen ekolojik parametrelerle türlerin ekolojik özelliklerine ait bilgiler ortaya koymaktır. Ülkemizdeki miksobiyota çeşitliliğinin tüm dünya miksobiyota çeşitliliğinin 1/5'i kadar olduğu bilinmektedir (Sesli ve ark., 2016). Bu çalışmada ayrıca, Kaz Dağları-Ayazma Mesire Yeri (Bayramiç-Çanakkale)'nin miksobiotasını ortaya koymak, varsa yeni kayıtlar ile Türkiye miksobiotasına önemli bir katkı sağlamayı da hedeflemektedir.

## Materyal ve Metot

### Araştırma Alanının Özellikleri

Çalışma alanı olarak seçilen Kaz Dağları- Ayazma Pınarı Tabiat Parkı (Bayramiç-Çanakkale) Orman ve Su İşleri Bakanlığı II. Bölge Müdürlüğü Çanakkale İl Şube Müdürlüğü'ne bağlı Çanakkale İli, Bayramiç İlçesi, Evciler Köyü sınırları içerisinde yer almakta olup Evciler Köyü'ne yaklaşık 6 km mesafede ve 490 m rakımda, 39° 44' 44,8" N, 26° 50' 33,53" E koordinatları üzerindedir. Kazdağları'nın kuzey yamacında yer alır ve insanlar tarafından piknik alanı olarak da kullanılan bir bölgeyi teşkil ettiğinden antropojenik etkiler altında olan bir bölge olarak tarif edilebilir. İçerisinden alanı ikiye ayıran Ayazma deresi geçmektedir (http://ayazmapinari.tabiat.gov.tr/).




**Şekil 1.** Araştırma Alanı (https://cografya.sitesi.web.tr, https://www.google.com.tr/maps).

Ayazma Pınarı Tabiat Parkı florası ile ilgili yapılmış özel bir araştırma bulunmamakla birlikte alanın güneyinde yer alan Kazdağı floristik yönden iyi bilinen, yaklaşık 800 taksonun kayıtlı olduğu önemli bitki alanlarından biridir. Kazdağı florasında 23'ü yalnız bu dağa özgü olmak üzere ülke çapında bilinen en az 68 nadir bitki bulunmaktadır. Tabiat Parkı içerisinde ise; karaçam ormanı ve dere vejetasyonu (kestane, gürgen, meşe, ıhlamur kayın, menengiç, akçakesme) hakimdir (http://ayazmapinari.tabiat.gov.tr/). Derenin kenarlarında özellikle yüksek boylu iyi gelişmiş *Platanus orientalis* (çınar), *Alnus glutinosa* subsp. *glutinosa* (kızılağaç) ağaç türleri yayılış göstermektedir. Tepeler yüksek kalker kayalıklardan oluşmaktadır. Bunların üzerinde kalker seven *Pinus nigra* subsp. *pallasiana* (karaçam) ve az miktarda *Juniperus excelsa* (boz ardıç) yayılış göstermektedir. Bunların aralarında özellikle nemli alanlarda *Tilia rubra* subsp. *rubra* (kafkas ıhlamuru), *Tilia argentea* (gümüşi ıhlamur), *Castanea sativa* (kestane), *Corylus avellana* (fındık) ağaçları yayılış göstermektedir. Derenin kenarlarında vadi yamaçlarında elma ve kiraz bahçeleri yer almaktadır (Gemici ve ark., 1998). Çalı türlerinden orman altında *Arbutus unedo* (kocayemiş), *Cistus creticus* (laden), *Cistus salviifolius* (kartli), *Prunus spinosa* (çakal eriği), *Pistacia terebinthus* var. *terebinthus* (menengiç), *Anthyllis hermanniae* (akıllı geven), *Crataegus monogyna* (yemişen), *Rhus coriaria* (sumak) ve *Hedera helix* (duvar sarmaşığı) bulunmaktadır. Eğreltiler nemli yerlerde yaşamaları ile karakterize olmuş bir bitki grubu olup alanda nem oranının yüksek olmasından dolayı oldukça çok sayıda tür ile temsil edilmektedirler. Bunlar; *Dryopteris pallida* (solucan eğreltisi), *Asplenium trichomanes*

(saçakotu), *Asplenium scolopendrium* (geyikdili), *Polystichum setiferum* (kızıl pilunç), karaçam orman altlarında *Pteridium aquilinum* (eğrelti) yaygın olarak bulunmaktadır (Özel, 1999).

Bölge'nin Kaz Dağı gibi dağlık yöreler dışında kalan geniş bir bölümünde, coğrafi olarak Akdeniz ile Karadeniz iklimleri arasında bir geçiş özelliği taşıyan, geleneksel sınıflandırmaya göre 4 makro iklim tipinden biri olan, Akdeniz İklim Tipi'nin, yarı nemli Marmara geçiş iklimi görülür (Türkeş, 2006). Sıcak ve ılıman bir iklim hakimdir. Yazları sıcak ve kurak, kışları yağışlıdır ve Akdeniz iklimine göre soğuk geçer. Kış aylarında yaz aylarından çok daha fazla yağış düşmektedir (https://tr.climate-data.org/location/30695/).

Çanakkale meteoroloji istasyonunun verilerine göre en fazla esen rüzgârlar; Kuzey- Doğu ve Kuzey- Kuzeydoğu yönlüdür. Diğer yönlerden esen rüzgârlar ise yok denecek kadar azdır. Ortalama fırtınalı gün sayısı 25,5 ve ortalama kuvvetli rüzgârlı gün sayısı 114,4 tür. Bu da yörenin son derece rüzgârlı olduğunu göstermektedir. Kullanım periyodunu oluşturan Mayıs-Eylül ayları arasında ortalama sıcaklık 17,3 derece ile 24,6 derece arasında iken, yıllık ortalama sıcaklık Bayramiç'te 14,0ºC'dir. En sıcak ve en soğuk aylar, sırasıyla Temmuz ve Ocak'tır. Ancak yörede Akdeniz ikliminin etkisiyle özellikle kışın bir su fazlalığı, buna karşın yaz mevsiminde önemli bir su açığı yaşanır. Aylık olarak değerlendirildiğinde, yağışın en yüksek ve en düşük olduğu aylar sırasıyla Aralık (118,2 mm) ve Ağustos (10,6mm)' tur (Türkeş, 2006). Ortalama nispi nem %62 ile %72 arasında değişmektedir. Yağışlar Haziran-Temmuz-Ağustos ve Eylül aylarında ortalamanın çok altına düşmektedir. Bu yüzden yağış rejimi ya da mevsimselliği açısından yöre yazları kurak, kışları yağışlı Akdeniz yağış rejimi bölgesine girer (Türkeş, 1998).

**Örneklerin Toplanması ve Nem Odası Tekniği Uygulaması**

Araştırma materyali 2018-2019 yılları arasında, yağışlı ve ılıman günleri takip eden zaman dilimlerinde, Ayazma Pınarı Tabiat Parkı (Çanakkale-Bayramiç)'ndan belirlenen pilot alanlardan (yaklaşık 10 m x 100 m) özellikle ilkbahar ve sonbahar aylarında yapılan arazi çalışmalarında toplanmıştır. Orman içindeki çürümüş ağaç kabukları ve odunları, canlı ağaçların dış kabukları, yaprak, ibre ve kozalak döküntüleri vb. örnekler araştırma materyalini oluşturmuştur. Üzerinde spor veya plasmodium olma ihtimali olan materyallerin her biri arazi numarası verilerek uygun ebatlı kese kâğıtlarına konulmuştur. Toplama numaraları arazi bilgi formuna işlenerek materyallerin hangi substratlardan toplandığı not edilmiştir.

Araziden döndükten sonra kese kâğıtları içindeki materyaller hemen boşaltılmış ve toplanan materyallere Nem Odası Tekniği uygulanmıştır. Nemli ortamların hazırlanmasında kültür ortamı olarak genellikle petri kabı veya cam kaplar kullanılmaktadır. Uygulama kolaylığı ve kolay taşınabilirliği açısından nem odası tekniği uygulamalarında zaman zaman 12 x 12 cm'lik şeffaf plastik kaplar da kullanılmıştır. Kullanılan kaplara uygun filtre kâğıtları kesilerek kapların içine yerleştirilmiştir. Toplanan materyaller üst üste gelmeyecek şekilde kaplara konulmuştur. Kapların üzerine arazi toplama numaraları da yazılmıştır. Daha sonra kapların içindeki materyallerin üzerine distile su ilave edilerek, 24-48 saat suyu iyice almaları için bekletilmiştir. Bu işlemden sonra fazla su boşaltılıp kültürler miksomiset gelişimi için oda sıcaklığı tabir edilen sıcaklıkta (23ºC-25ºC) bırakılmıştır.

Nem odası tekniğinin uygulanması ile elde edilen örnekler 8,5 x 8,2 cm ebatlarındaki kartonların orta kısmına substratlarıyla birlikte yapıştırılarak uygun ebattaki kutulara yerleştirilmiş, ağzı kapatılmış ve etiketlenmiştir. Böylece örnekler muhafazalı şekilde kutulara alınmış ve koleksiyon örneği halinde hazırlanmıştır. Nem odası tekniği ile geliştirilen sporoforların teşhislerinin yapılması ve daha sonraki araştırmalarda kullanmak amacıyla geçici ve daimi preparatlar hazırlanmıştır. Geçici preparat ortamları hazırlanırken alkol, saf su veya KOH kullanılmıştır. Alkol, preparat hazırlanırken hava kabarcığının oluşmasını engellerken su veya KOH, kapillitial ipliklerin ve sporların şişmesini ve normal boyutlarında kalmasını sağlamıştır. Daimi preparat hazırlanırken Hoyer ortamı kullanılmış ve hazırlanan preparat kuruduktan birkaç

gün sonra lamelin etrafına şeffaf oje sürülerek daimi preparat haline getirilmiştir. Daha sonra cama yazar kalemle kayıt numaraları verilen örnekler teşhisleri yapılmak üzere preparat kutularına kaldırılmıştır.

Stereomikroskop yardımıyla sporofor tipi, rengi, boyutları, saplı-sapsız olması, sap uzunlukları, peridiumun kireçli yapılar ihtiva edip etmemesi, peridiumun açılma tipi, sporların yığın halindeki renk özellikleri, ışık mikroskobunda sporların boyutları, mikroskop ışığındaki renkleri, çeper süsleri, kapillitial ipliklerin süsleri, boyutları, kolumellanın, kalikulusun olup olmaması, sap yapısı, peridium özellikleri, kapillitial sistemin kireçli olup olmaması gibi mikroskobik özellikleri tespit edilen örneklerin teşhisi Nannenga-Bremekamp (1991), Martin ve Alexopoulos (1969), Martin ve ark. (1983), Stephenson (2003), Lado ve Pando (1997) Stephenson ve Stempen (2000), Farr (1976), Thind (1977), Ing (1999) gibi başlıca sistematik eserler ve Ergül (1993), Bican Süerdem (2010), Demirel (2010), Ocak (2001)'in çalışmalarından yararlanılarak yapılmıştır. Taksonların isimleri, otörleri ve sinonimleri Juan Carlos Hernández-Crespo'nun hazırladığı online nomenklatür bilgi sisteminden kontrol edilmiştir (Lado 2005-2021

Koleksiyon örneklerinin saklandığı kutuların üzerine türün adı, familyası, örnek numarası, toplayanın adı, toplama tarihi, substrat ve rakım bilgilerinin yer aldığı etiketler yapıştırılmıştır. Ayrıca, preparatlar da preparat saklama kabına yerleştirilerek korunmaktadır. Bayramiç-Ayazma Mesire Yeri miksomisetlerine ait örnekler ilk araştırmacının özel kültür koleksiyonunda saklanmaktadır.

**Ekolojik Verilerin Toplanması**

Seçilen pilot alanlardan toplanan örneklerde pH, yerden yükseklik, güneş alma ve çürüme durumu olmak üzere 4 parametre değeri kaydedilmiştir. Bu parametre değerlerinden pH, yerden yükseklik ve güneş alma durumu 1 ile 3, çürüme 1 ile 5 arasında azdan çoğa doğru numaralandırılmıştır. Alınan her numune için bu sayı aralığında değerler belirlenerek kaydedilmiştir. Örneklerin toplandığı ortamda hava sıcaklığı ve nem higrotermometre ile ölçülmüştür.

**Verilerin Analizi**

Çeşitli veri kümeleri için çeşitlilik indeksleri, Shannon'ın formülü (Shannon ve Weaver, 1963) kullanılarak çeşitlilik (H') = -∑ (Pi ln Pi) olarak hesaplanmıştır. Pi, ağırlıklı bolluk cinsinden temsil edilen tüm tür kayıtların oranıdır. (Ocak, 2015)

Cins başına ortalama tür sayısı (tür/cins), çeşitli çalışmalarda miksomiset topluluklarını karşılaştırmak için kullanılmıştır (Stephenson ve ark., 1993, Novozhilov ve ark., 1999; Ocak, 2015).

**Bulgular**

**Taksonların Deskripsiyonları**

Taksonların listesi verilirken takson adı, yazarları, lokalite, habitatları sırasıyla verilmiştir. Lokalitenin verilişinde toplama yeri, rakım, substrat, toplama tarihi ve toplama numarası takip edilmiştir.

En son sınıflandırma sistemine göre bu canlılar Protista âleminin Amoebozoa şubesinde olup *Protostelea* ile *Myxogastrea* diye iki sınıftan ve *Protosteliida, Liceida, Echinosteliida, Trichiida, Stemonitida, Physarida* takımlarından oluşur.

***MYCETOZOA***

***PROTOSTELIDA***

***Ceratiomyxaceae***

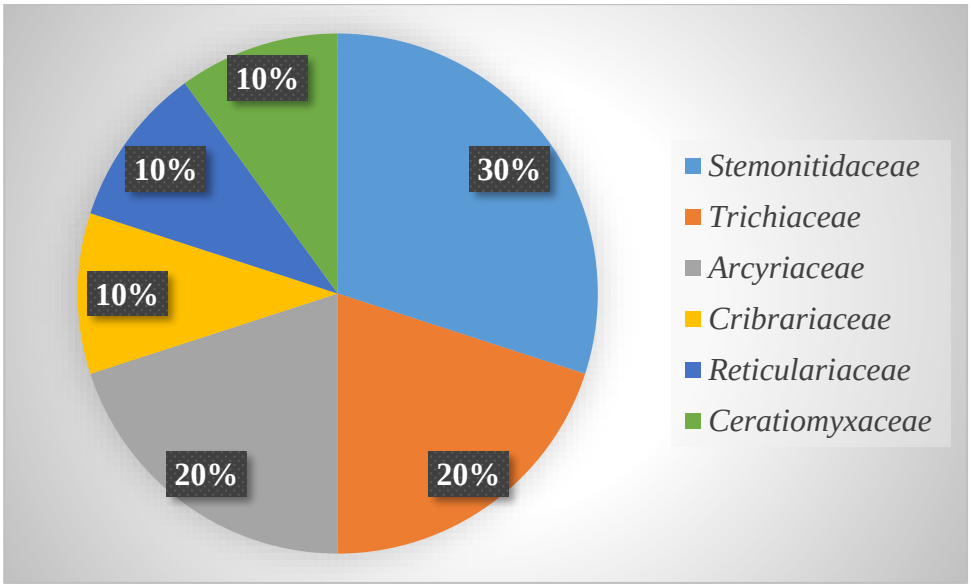
**3.1.1. *Ceratiomyxa fruticulosa*** (O.F. Müll.) T. Macbr.

**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 480 m, döküntü *Quercus* sp. dalı, 09.07.2018, TBS67.

***MYXOGASTRIA***
***LICEIDA***
***Reticulariaceae***
**3.1.2. *Lycogala flavofuscum*** (Ehrenb.) Rostaf.
**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 480 m, dere kenarı, *Platanus orientalis* üzerinden, 09.07.2018, TBS66.
***Cribrariaceae***
**3.1.3. *Cribraria cancellata*** (Batsch) Nann.-Bremek
**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 490 m, *Pinus brutia* ormanı, 09.07.2018, TBS68.
***PHYSARIDA***
***Arcyriaceae***
**3.1.4. *Arcyria incarnata*** (Pers. ex J.F. Gmel.) Pers.
**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 490 m, ağaç kabuğu üzerinden, 09.07.2018, TBS69.
**3.1.5. *Arcyria cinereae*** (Bull.) Pers.
**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 490 m, kara çam, meşe ormanı, *Pinus nigra* üzerinden, 09.07.2018, TBS70.
***Trichiaceae***
**3.1.6. *Trichia botrytis* (J.F. Gmel.) Pers.**
**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 475 m, kara çam, meşe ormanı, döküntü *Pinus nigra*, 09.07.2018, TBS73.
**3.1.7. *Trichia affinis* de Bary, in Fuckel**
**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 480 m, kara çam, meşe ormanı, döküntü *Pinus nigra*, 09.07.2018, TBS75.
***STEMONITOMYCETIDAE***
***Stemonitidaceae***
**3.1.8. *Stemonitopsis typhina*** (F.H.Wigg.) Nann.-Bremek.
**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 485 m, çürümüş ağaç kabuğu, 09.07.2018, TBS 72.
**3.1.9. *Enerthenema papillatum* (Pers.) Rostaf.**
**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 475 m, döküntü *Pinus nigra*, 09.07.2018, TBS74.
**3.1.10. *Stemonitis fusca* Roth.**
**Lokalite:** Bayramiç: Kazdağı, Ayazma mesire yeri, 350m, kesik kütük odun, 09.07.2018, TBS71.

**Taksonların Familyalara Göre Dağılımları**

Araştırma alanında tespit edilen taksonların familyalara göre dağılımı şu şekildedir. Tespit edilen toplam taksonlar içinde *Stemonitidaceae* 3 taksonla, *Trichiaceae* 2 taksonla, *Arcyriaceae* 2 taksonla, *Cribrariaceae* 1 taksonla, *Reticulariaceae* 1 taksonla, *Ceratiomyxaceae* 1 taksonla temsil edilmektedir. *Stemonitidaceae* familyası en fazla takson içeren familyadır. Bunu takip eden diğer iki familya ise *Trichiaceae* ve Arcyriaceae familyalarıdır.




**Şekil 2**. Taksonların % cinsinden familya dağılımları.

**Ekolojik Koşullar ve Türlerin Dağılımı**

Örneklerin toplandığı ekolojik koşullara ait veriler Tablo 1 ve Tablo 2'de verilmiştir. Toplanan arazi materyallerinden nem durumu, yerden yükseklik, güneş alma durumu ve çürüme durumu olmak üzere 4 parametre değeri kaydedilmiştir. Bu parametre değerlerinden nem durumu, yerden yükseklik ve güneş alma durumu 1 ile 3, çürüme1 ile 5 arasında azdan çoğa doğru numaralandırılmıştır. Alınan her numune için bu sayı aralığında değerler belirlenerek kaydedilmiştir.

**Nem durumu:** 1 (kuru), 2 (yarı nemli),3 (tamamen nemli)

**Yerden yükseklik:** 1 (zeminle teması var), 2 (zemine yakın ancak temas yok-1m. altı), 3 (zeminden 1 m üzeri)

**Güneş alma:**1 (güneş almıyor), 2 (az güneş alıyor), 3 (güneş alıyor-orta)

**Çürüme:** 1 (çürüme yok), 2 (az çürüme), 3 (orta çürüme), 4 (yarıdan fazla çürüme), 5 (tamamen çürüme)

**Tablo 1.** 2018 yılı örneklerine ait olan ekolojik veriler

| GRUP NO | NEM DURUMU | YERDEN YÜKSEKLİK | GÜNEŞ ALMA DURUMU | ÇÜRÜME DURUMU | MATERYAL ÇEŞİDİ |
|---|---|---|---|---|---|
| **1** | 1 | 2 | 2 | 3 | Kuru döküntü, dal parçası |
| **2** | 2 | 2 | 2 | 3 | Kuru döküntü, dal parçası |
| **3** | 1 | 2 | 2 | 1 | Kuru yaprak |
| **4** | 3 | 1 | 2 | 1 | Nemli yaprak örneği |
| **5** | 1 | 2 | 2 | 2 | Kuru dal ve yaprak |
| **6** | 3 | 1 | 2 | 5 | Odun parçası |
| **7** | 1 | 3 | 2 | 1 | Dal parçası-aerial-çınar ağacı |
| **8** | 2 | 3 | 2 | 1 | Aerial bark-çam ağ.-canlı odun parçası |
| **9** | 1 | 2 | 2 | 1 | Çam ağacı-canlı kabuk |
| **10** | 1 | 2 | 2 | 3 | Çınar ağacı-canlı ağaç kab. |
| **11** | 1 | 2 | 2 | 4 | Çınar ağacı-canlı ağaç kab. |
| **12** | 1 | 3 | 3 | 2 | Döküntü materyal |
| **13** | 1 | 2 | 3 | 1 | Kesik kütük-kabuk |
| **14** | 19 | 2 | 2 | 1 | Meşe-canlı kabuk örneği |
| **15** | 1 | 2 | 3 | 3 | Meşe-canlı kütük örneği |
| **16** | 1 | 2 | 3 | 1 | Çam canlı kabuk |
| **17** | 1 | 2 | 3 | 1 | Karayosunu canlı örnek |
| **18** | 1 | 2 | 2 | 1 | Meşe-canlı kabuk |
| **19** | 1 | 3 | 3 | 1 | Çam ağacı canlı kabuk |
| **20** | 1 | 3 | 3 | 1 | Çınar ağacı canlı kabuk |
| **21** | 1 | 3 | 2 | 1 | Aerial asılı yaprak örneği |
| **22** | 1 | 3 | 2 | 1 | Aerial asılı yaprak örneği |
| **23** | 1 | 1 | 2 | 1 | Kuru yaprak |
| **24** | 3 | 1 | 2 | 4 | Dipten yaprak, kabuk |
| **25** | 1 | 2 | 3 | 2 | Kütükten kabuk |
| **26** | 1 | 2 | 3 | 2 | Kesilmiş kütük |
| **27** | 3 | 2 | 3 | 1 | Çınar ağacı gövde-canlı kabuk |
| **28** | 1 | 3 | 3 | 1 | Ağaç yaprağı-canlı ağaç gövdesinden |
| **29** | 1 | 1 | 3 | 3 | Yerden döküntü yaprak |
| **30** | 1 | 2 | 2 | 3 | Yaprak örneği |
| **31** | 1 | 2 | 2 | 3 | Kütük parçası-odun |
| **32** | 1 | 2 | 2 | 2 | Yerde kütük parçası-gövde kabuk örn. |
| **33** | 1 | 2 | 2 | 2 | Kesilmiş kütük parçası |

**Tablo 2.** 2020 yılı örneklerine ait olan ekolojik veriler

| GRUP NO | NEM DURUMU | YERDEN YÜKSEKLİK | GÜNEŞ ALMA DURUMU | ÇÜRÜME DURUMU | MATERYAL ÇEŞİDİ |
|---|---|---|---|---|---|
| **1** | 1 | 1 | 1 | 1 | Yaprak örnekleri |
| **2** | 2 | 1 | 1 | 1 | Yaprak ve küçük dallar |
| **3** | 1 | 2 | 2 | 1 | Canlı çınar ağacı gövde kabuğu |
| **4** | 2 | 2 | 1 | 2 | Dere içi nemli yaprak |
| **5** | 1 | 1 | 1 | 1 | Dere kenarı dal yaprak |
| **6** | 1 | 3 | 1 | 1 | Canlı çınar ağacı gövde kabuğu |
| **7** | 1 | 1 | 1 | 1 | Dere içi kütük parçası |
| **8** | 3 | 2 | 1 | 1 | Nemli-dere içi canlı kütük gövde parçası |

Tanımlanan izolatların ekolojik verilerle birlikte değerlendirilmesi ise Tablo 3'te verilmiştir.

**Tablo 3**. Tanımlanan izolatlar ve ekolojik verileri

| TÜR ADI/KAYIT NO | NEM DURUMU | YERDEN YÜKSEKLİK | GÜNEŞ ALMA DURUMU | ÇÜRÜME DURUMU | MATERYAL ÇEŞİDİ |
|---|---|---|---|---|---|
| ***Ceratiomyxa fruticulosa* (TBS67)** | 1 | 3 | 2 | 2 | Döküntü materyal |
| ***Lycogala flavofuscum* (TBS66)** | 2 | 3 | 3 | 2 | Canlı çınar ağacı gövde kabuğu |
| ***Cribraria cancellata* (TBS68)** | 1 | 3 | 2 | 2 | Yerde kütük parçası-gövde kabuk örneği |
| ***Arcyria incarnata* (TBS69)** | 1 | 3 | 1 | 2 | Kesilmiş kütük parçası |
| ***Arcyria cinereae* (TBS70)** | 1 | 2 | 2 | 2 | Ağaç kabuğu parçası |
| ***Trichia botrytis* (TBS73)** | 1 | 2 | 2 | 2 | Kütük parçası-odun |
| ***Trichia affinis* (TBS75)** | 1 | 2 | 1 | 2 | Dal parçası |
| ***Stemonitopsis typhina* (TBS72)** | 1 | 2 | 2 | 2 | Kesilmiş kütük parçası |
| ***Enerthenema papillatum* (TBS74)** | 1 | 2 | 2 | 1 | Küçük dal parçası |
| ***Stemonitis fusca* (TBS71)** | 1 | 2 | 1 | 2 | Çınar ağacı büyük kütük parçası |

**Tür çeşitliliği**

Araştırmada tür çeşitliliği açısından bakıldığında sadece arazi koleksiyonu olarak 10 tür toplanmıştır. Tür çeşitliliği açısından değerlendirildiğinde bu sayının çok düşük olmadığı görülmektedir (H'= 2,1972). Ancak miksomiset kaydı bakımından değerlendirildiğinde aynı durum söz konusu değildir. İki kez arazi gezisi yapılmış ve nemli oda kültürü için miksomiset örnekleri, odun örnekleri belirlenen pilot bölgelerden herbir substrat için toplanmıştır. Ancak bu örnekler nemli oda kültürüne alınsalar da normal bir gelişim gösterememişlerdir ve plazmodyum aşamasında kalmışlardır. İkinci araştırma gezisinde farklı lokalitelerden arazi koleksiyonu olarak miksomiset örnekleri toplanmış ekolojik özelliklerini tespit etmeye yönelik döküntü, canlı ağaç kabuğu, havai kurumuş ve teşhis edilmesi sonucunda yukarıdaki toblolada yer alan türler tespit edilmiştir. Çalışma alanımızda miksomiset biyoçeşitlilik zenginliğini gösterecek Tür/Cins Oranı 1,43 olarak hesaplanmıştır.

**Türlerin cinslere göre dağılımı**

Araştırma alanında tespit edilen taksonların cinslere göre dağılımı şu şekildedir. Tespit edilen toplam taksonlar içinde *Ceratiomyxa* 1 taksonla, *Lycogala* 1 taksonla, *Cribraria* 1 taksonla, *Trichia* 2 taksonla, *Arcyria* 2 taksonla, *Trichia* 2 taksonla, *Stemonitopsis* 1 taksonla, *Enerthenema* 1 taksonla, *Stemonitis* 1 taksonla temsil edilmektedir. *Arcyria* ve *Trichia* diğerlerine göre daha fazla takson içeren cinslerdir.

**Yoğunluk**

Türlerin nispi bolluğu (RA) sayılmıştır ve tür bolluğu ölçeği Stephenson ve ark. (1993)'nın yöntemine göre sınıflandırılmıştır. Buna göre yüzdelik içerisindeki oranları A: bol (%>3); C: yaygın (%1,5–3); O: ara sıra (%0,5–1,5); R: nadiren (%<0,5) olarak bulunmuştur.

**Tür- substrat ilişkisi**

Tespit edilen türlerin subsrat tercihleri değerlendirildiğinde bu türlerden *Ceratiomyxa fruticulosa* döküntü *Quercus* sp. dalı üzerinde döküntüden tespit edilirken, *Lycogala flavofuscum* angiosperm kabuğu üzerinde tespit edilmiştir (kortikoloz miksomiset). *Cribraria cancellata* ve *Arcyria incarnata* döküntü kabuk üzerinden, *Arcyria cinereae* kütük üzerindeki kabuk üzerinden, *Stemonitopsis typhina* kütük üzerindeki kabuktan izole edilmişlerdir. *Trichia botrytis* döküntü odun üzerinden, *Stemonitis fusca* kesilmiş kütük çınar ağacı üzerindeki odundan izole edilen diğer türlerdir (lignikoloz miksomisetler). *Trichia affinis* ve *Enerthenema papillatum* döküntü *Pinus nigra* dalı üzerinde tespit edilmiştir.

**Tartışma ve Sonuç**

2018-2019 yılları arasında Çanakkale-Bayramiç Ayazma Mesire Yeri'ne doğal örneklerin ve miksomiset sporu taşıyan örneklerin toplanması amacıyla arazi çalışmaları yapılmıştır. Arazi çalışmaları sonucunda toplanan materyaller gruplandırılmış toplamda 33 grup elde edilmiştir. Toplanan materyallere nem odası tekniği uygulanmış ve bunun sonucunda elde edilen 10 örnekte miksomiset gelişimi olmuştur. Gelişen bu örneklerin laboratuvar çalışmalarında incelenmesi sonucunda ise 6 familya 8 cinse ait toplam 10 miksomiset taksonu tespit edilmiştir. Çalışmalar sonucunda yeni kayıt tespit edilememiştir.

Araştırma alanında tespit edilen taksonların familyalara göre dağılımı şu şekildedir. Tespit edilen toplam taksonlar içinde *Stemonitidaceae* 3 taksonla, *Trichiaceae* 2 taksonla, *Arcyriaceae* 2 taksonla, *Cribrariaceae* 1 taksonla, *Reticulariaceae* 1 taksonla, *Ceratiomyxaceae* 1 taksonla temsil edilmektedir. *Stemonitidaceae* familyası en fazla takson içeren familyadır. Bunu takip eden diğer iki familya ise *Trichiaceae* ve *Arcyriaceae* familyalarıdır.

Türkiye'nin özellikle orman formasyonunun zengin olması tür çeşitliliğinin de fazla olmasını doğrudan etkilemektedir. Şimdiye kadar tespit edilen çoğu türün ağaç kabuğu üzerinde yaşayan türler olduğu görülmektedir. Miksomisetlerin diğer yetişme yeri özellikleri tespit edildiğinde ve bu konu üzerine yoğunlaşıldığında dünya çapındaki tür zenginliğine önemli derecede katkı

sağlayacağı düşünülmektedir. Çanakkale-Bayramiç Ayazma Mesire Yeri'nde yapılan bu çalışmada 10 takson elde edilmiştir. Tür çeşitliliği fazla olmamakla birlikte yine de bölgenin tür biyoçeşitliliğini arttırdığı ve miksobiotasına önemli katkılar sağladığı düşünülmektedir.

Literatürde Türkiye'deki miksomisetlerin ekolojisi ile ilgili çalışmalar oldukça sınırlı olup, genellikle taksonomik çalışmalar bulunmaktadır. Yağız ve Afyon (2008), 1957-2006 arasında Türkiye'deki 18 floristik alandan 260 lokaliteden 39 genus ve 12 familyaya ait 216 takson ile çalışmışlardır. Toplam 37 örneği iğne yapraklı ağaçlar (6 tane), geniş yapraklı ağaçlar (30 tane) ve tanımlanamayan oldukça çürümüş bir substrat içeren materyalden toplamışlardır. Sonuç olarak, *Arcyria cinerea* türünün 11 floristik alanda 13 farklı substrattan tanımlanan en yaygın miksomiset türü olduğunu bildirmişlerdir. Ayrıca *A. cinerea, A. incarnata, Fuligo septica* ve *Lycogala epidendrum* türlerine ise çalışma alanının tüm yükseltilerinde rastlanmıştır. Ocak (2015) Afyonkarahisar İli Köroğlubeli ormanında dört farklı mevsim boyunca doğal şartlar altında yetişmiş olan miksomisetlerin mevsimsel bulunuşu ve dağılımını incelediği araştırmasında 17 cinse ait iki tanesi (*Fuligo cinerea, Diacheopsis minuta*) Türkiye için yeni kayıt olan 33 tür toplamıştır. Aynı zamanda *Diacheopsis* Türkiye için yeni bir cins kaydı olarak rapor edilmiştir. İlkbahar ve geç sonbahar mevsimlerinin en yüksek topluluk katsayısına (CC) sahip olduğu (0,53) ve çeşitliliğin düzgünlük bileşeninin yaz ile diğer mevsimler arasında değiştiği bulunmuştur. Çalışmamızda aynı cinse ait türler elde edilmiş olup, özellikle *Lycogala* cinsi için tür bazında farklılık bulunmaktadır. Çalışmalar karşılaştırıldığında; farklı türlerin görülmesi olağandır. Bunun nedeni ise bölgesel farklılık, dolayısıyla ikilm koşullarının ve bitki örtüsünün değişmesi faktörlerinin dikkate alınması gerekmektedir.

Baba (2012) Antakya-Hatay Bölgesi miksomiset çeşitliliği ve ekolojisi üzerine yaptığı bir çalışmada 11 familya ve 18 genusa ait 44 takson elde etmiştir. Miksomiset toplamak için en elverişli ayların yağış ve sıcaklığın uygun olduğu Ekim ve Kasım ayları olduğunu belirtmiştir. Çalışmamızda ise örneklerin toplanması aşaması Temmuz 2018 yılında gerçekleştirilmiştir. Bu nedenle yaz döneminde örneklerin toplanması çalışmamızda tür çeşitliliğini etkileyebilen bir faktör olarak karşımıza çıkmaktadır.

Baba ve ark. (2016a), Hatay ve çevresinde 4 farklı lokaitede miksomisetlerin biyocoğrafik dağılımlarını karşılaştırmalı olarak incelediği çalışmasında ise, 12 familya 28 genusa ait 102 takson elde etmiştir. En yüksek örnek sayısı sırasıyla *Physarales, Trichiales* ve *Stemonitales* ordolarına aittir. Dört farklı habitatın üçünde lignikoloz miksomisetler (%65-85) ve bir habitatta da kortikoloz miksomisetler (%65) dominant durumda bulunmuştur. Çalışmada miksomisetlerin biyocoğrafik dağılımının yanında ayrıca morfolojik, korolojik ve ekolojik özellikleri de tartışılmıştır. Çalışmamızda da, özellikle kortikoloz miksomisetler daha fazla görülmüştür.

Baba ve ark. (2016b), Adana'nın kuzey bölgesi miksobioatasını belirlemek için yaptığı bir çalışmada 10 familya ve 20 genusa ait 54 takson kaydı gerçekleştirmiştir ve bunların 3 tanesi Türkiye için yeni kayıttır. En yaygın dört familya toplam 38 takson ve %70 bulunurlukla *Stemonitidaceae, Cribrariaceae, Arcyriaceae* ve *Physaraceae* olup 45 tanesi doğal ortamlarından toplanmıştır. Yağmur ve nispi nemin optimum olması, sıcaklığın ılıman seyretmesi nedeniyle bölgede en iyi miksomiset gelişimi sonbahar, kış ve ilkbahar aylarında olmuştur. Daha çok çürümüş ve ölü odun üzerinde gelişen lignikoloz miksomisetler %70 oranında bulunurken, kortikoloz miksomisetler %11 olarak bulunmuştur. Çalışmamızda görülen en fazla örnek elde edilen familya önceki çalışmada bahsedildiği gibi *Stemonitidaceae* olup, bunu *Arcyriaceae* takip ederken, üçüncü en yaygın familyası ise diğer çalışmadan farklı olarak *Trichiaceae* olmuştur.

Sonuç olarak çalışmamızda *Stemonitidaceae*, *Trichiaceae*, *Arcyriaceae*, *Cribrariaceae*, *Reticulariaceae*, *Ceratiomyxaceae* familyalarından toplam 10 miksomiset taksonu elde edilmiş olup, yeni tür kaydı bulunmamaktadır. Dolayısıyla bu konu ile ilgili daha fazla ayrıntılı çalışmaların ve örneklemenin yapılması gerekmektedir. Burada özellikle sistematik çalışmaların

dışında yurtdışında olduğu gibi ekolojik faktörlerin ve dağılımların Çanakkale bölgesi için detaylandırılması, daha fazla örnek alanı içeren çalışmaların yapılması önem arz etmektedir. Bölgenin daha önce çalışılmış olması yeni tür kayıtların çıkmayacağı anlamına gelmemelidir. Bunun nedeni ise, küresel ısınma ve iklim değişikliğine bağlı olarak değişen ekolojik koşullar ve bitki örtüsü sebebiyle biyoçeşitliliğinde değişkenlik göstermesidir.

Tür/cins oranı Stephenson ve ark., (1993) tarafından ilk kez hesaplanmış ve Güneydoğu Hindistan için 2,24, Kuzey Amerika için 4,13 olarak bulunmuştur. Bu oran ne kadar düşük olursa (1'e yaklaşırsa) biyoçeşitliliğin o kadar zengin olduğunu gösterir. Baba ve Arslan (2017) Kuzey Amanos Dağları (Hatay-Türkiye) miksomisetleri biyoçeşitliliğinin tür/cins oranını 2,42 belirlemişlerdir. Çalışmamızda ise 1,43 belirlenmiş olup, biyoçeşitliliğin fazla olduğu görülmektedir ancak bu oranın yüksek çıkmasının nedeni az örnekte miksomiset gelişiminin sağlanmış olmasıdır.

Miksomisetlerin fruktifikasyon yapılarının en bol göründüğü dünyanın ılıman bölgelerinde, bu organizmaların bir dizi farklı mikrohabitatlarla ilişkili olduğu görülmüştür. Bu mikrohabitatlar, kaba odunsu döküntüleri, canlı ağaçların kabuk yüzeyini, yerdeki döküntü çöpleri ve ölü fakat hala ayakta duran otsu bitkilerin havadaki kısımlarını içerir ve bu ortamların her biri, farklı bir tür topluluğu ile karakterize olma eğilimindedir (Stephenson 1988, 1989; Stephenson ve Stempen 1994).

Dünya çapında karışık angiosperm ormanlarında çürümemiş odunun pH'ı ~4.9'dur ve çürümüş odun ve ağaç kabuğu için tropik enlemlerde ~6.5 ve 35 enlemde ~5.5'tir, bu nedenle çürümede pH'daki değişim enlemle değişir. Çürümüş kereste, ağaç kabuğu ve çeşitli orman altı türleri için ortalama pH değerlerinin tümü büyük bir dağılıma sahiptir. Çürüyen substratların ortalama pH değerleri birbirinden önemli ölçüde farklı değildir ve tropik enlemlerde ortalama 6,5 ve 35 enlemde ortalama 5,6'dır. Miksomiset türlerinin tercih ettiği pH, fruktifikasyon veren gövdelerde kirecin mevcudiyeti ile ilişkili değildir, fakat genellikle cins içinde sabittir. (Wellman, 2021). Bazı araştırmalar, asidik kabuklu kozalaklı ağaçların üzerinde, örneğin *Paradiacheopsis* spp. Alaska'daki *Picea glauca* türünde (Schnittler ve ark., 2016) ve *Leocarpus fragilis* Vietnam tropik dağ ormanlarında *Pinus kesiya* türünde tespit edilmiştir (Novozhilov ve ark., 2020). Stephenson ve ark. (2020), Amerika Birleşik Devletleri'ndeki dört bölgeden toplanan *Pinus* kabuğu ile nem odası uygulamalarını yürütmüş ve 11'i *Liceales* takımından olmak üzere 34 miksomiset türü elde etmiştir. Bu sonuç, bazı *Liceales, Trichiales* ve *Stemonitidales* türlerinin iğne yapraklı ormanları tercih ettiğini ve daha düşük pH'lı kabuklara en iyi adapte olduklarını göstermiştir. Bu çalışmada, CCA (Canonical correspondence analysis) ayrıca pH'ın kabukta yaşayan miksomiset topluluğu üzerindeki önemli etkisini de ortaya çıkarmıştır ve önceki çalışmalarla da tutarlılık göstermektedir (Ndiritu ve ark., 2009; Schnittler ve ark., 2016). Blackwell ve Gilbertson (1980), Stephenson (1989) ve Novozhilov ve ark. (2006) ile benzer olarak, *Perichaena* ve *Physarales* türlerinin geniş yapraklı orman olan Yangjia Nehri ve çevresinde daha bol olduğunu göstermiştir. *Perichaena*'nın yüksek pH'lı substrat üzerinde büyüme eğiliminde olan *Trichiales* takımının bir istisnası olabileceğini öne sürmüşlerdir. Ayrıca iğne yapraklı ormanlardaki *Physarales* çeşitliliğinin de genel olarak düşük olduğu bulunmuştur.

Mikro habitat belirli miksomiset türünün tercihini belirleyen substratı sağlayan spesifik bitki türü değil, bitkinin pH, doku ve su tutma gibi fiziksel ve kimyasal özellikleridir. (Dagamac ve ark., 2021; Li ve ark., 2021). Mikro habitatta tipik olarak bulunan odunsu (ağaçta yaşayan) türler, arazide çıplak gözle tespit edilebilecek yeterli büyüklükte karakteristik olarak fruktifikasyon veren gövdeler arasında olma eğiliminde olduğundan, kaba odunsu artıklarla ilişkili miksomisetler en iyi bilinenlerdir (Martin ve Alexopoulos 1969). Çeşitli *Arcyria*, *Lycogala*, *Stemonitis* ve *Trichia* türleri dahil olmak üzere daha yaygın ve yaygın olarak bilinen miksomiset taksonlarının çoğu, ağırlıklı olarak odunsudur. Kaba odunsu döküntüler üzerinde bulunan miksomiset toplulukları, ayrışma aşaması ile değişir (Takahashi ve Hada 2009). Örneğin,

*Badhamia gibi* bazı taksonlar büyük ölçüde kabuğun hala mevcut olduğu erken aşamalarla sınırlıdır. Büyük ve bileşik fruktifikasyona sahip türlerin çoğu dahil olmak üzere birkaç yüz miksomiset türü, ağırlıklı olarak veya tamamen odunsudur, bu nedenle, odunsu döküntüler miksomisetler için en çeşitli mikro habitatlardan biridir.

Araziden ve/veya nemli oda kültüründeki ağaç kabuklarından 200'den fazla "kortikoloz" (kabukla ilişkili) miksomiset türü rapor edilmiştir (Mitchell 1980; Snell ve Keller 2003). Bu türlerin birçoğunun diğer mikro habitatlarda da gelişim gösterdiği bilinmektedir, ancak en azından bazı türlerin gelişim ortamlarının canlı ağaçların kabuğuyla sınırlı olduğu görülmektedir. Öne çıkan örnekler, küçük fruktifikasyona sahip çeşitli *Echinostelium, Licea* ve *Macbrideola* türlerini içermektedir (Alexopoulos 1964; Mitchell 1980). Arazideki taban örtüsünde, *Physarales*'in kültüre edilebilen birçok üyesi de dahil olmak üzere, bu mikro habitattan yaklaşık 400 tür rapor edilmiş olup, son derece çeşitli bir miksomiset topluluğunu destekler. Üst döküntü katmanlarında fruktifikasyon oluşturan birçok miksomisetin aslında amip gibi toprak-döküntü ara yüzünde yaşaması muhtemel görünmektedir (Stephenson ve ark., 2011). Bir dizi özel mikrohabitat, görünüşte özelleşmiş türlerin bulunduğu nadir miksomiset topluluklarını destekler. Tropik bölgelerde, canlı yapraklarda (Schnittler, 2001) ve büyük tropik otsu bitkilerin çiçek salkımlarının çürüyen kısımlarında, özellikle de oldukça bazik bir pH sağlayan *Zingiberales* takımının üyelerinde gelişen epifillöz (yapraksı) ciğer otlarından miksomisetler rapor edilmiştir (Schnittler ve Stephenson, 2002). İlaveten ılıman bölgelerde yaklaşık iki düzine briyofilik (karayosunu üzerinde gelişen) miksomiset türünü destekleyen mikrohabitatlar da vardır ki, bunlar nemli serin vadilerde genellikle kumtaşı olmak üzere kayaların yüzeyini kaplayan yosunlarla ilişkili olarak bulunurlar (Schnittler ve ark., 2010). Benzer şekilde, bazıları özel olarak kalın duvarlı sporlarla uyumlu, yaklaşık 25 türün gübre üzerinde geliştiği (koprofilöz miksomiset) bilinmektedir (Eliasson ve Keller, 1999). Çöllerde, sukulent bitkilerin çürüyen kısımları, yaklaşık 50 "sukulentöz miksomiset" türünün rapor edildiği başka bir özel mikrohabitatı temsil eder (Lado ve ark., 1999). Bu miksomisetlerin amipsi hücreleri muhtemelen mayaları besler ve sporlarının meyve sinekleri (*Drosophila spp.*) tarafından dağılması muhtemeldir (Stephenson, 2010).

Li ve ark. (2021) yaptıkları çalışmada kortikoloz miksomiset topluluklarının, orman tipi ve yükseklik gibi mekansal değişkenlerden daha fazla etkilendiğini bildirmişlerdir. Miksomiset gelişiminde zeminin, yalnızca yükseklik ve eğim gradyanından değil, aynı zamanda sıcaklık, nem ve yağıştan da etkilendiği bulunmuştur, çünkü Kirschbaum, (2006)'a göre bu son faktörler, yerdeki zemin malzemesinin durumunu ve bozunma hızını etkileyebilir. Kochergina ve Markina (2021) substrat olan bitki türleri ile kortikoloz miksomisetlerin spesifik kompleksler oluşturduğunu belirtmişlerdir. Dolayısıyla, farklılıklar, tür kompozisyonunu, bazı türlerin bolluğunu gibi çok faktörlü parametrelerin karşılaştırılmasını içermektedir. CCA'dan elde edilen sonuçlar doğrultusunda miksomiset türlerinin sıcaklık, nem ve rüzgâr hızından etkilendiği belirlenmiştir.

Son zamanlarda miksomisetlere olan ilgi artmış ve pek çok mikolog tarafından daha fazla ilgi gösterilmeye başlanmıştır. Hatta mikoloji biliminin dışında da genetik, sitoloji ve farmakoloji gibi birçok alanda bu organizmalardan etkili bir şekilde yararlanılmaktadır. Ancak yine de hala bu organizmaların değerinin tam olarak anlaşıldığı söylenemez. Özellikle de elde edilen örneklerin tanımlanmasında geleneksel yöntemler önemini korumakla birlikte, özellikle yeni tür kayıtlarının olşuturulması ve hızlı tanımlamaya olanak vermesinden dolayı günümüzde altın standart olan moleküler identifikasyonların yapılması gerekliliği gündeme gelmektedir.

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# BRONKOALVEOLER LAVAJ ÖRNEKLERİNDEN İZOLE EDİLEN *ASPERGILLUS* TÜRLERİNİN ARAŞTIRILMASI

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## ÖZET

*Aspergillus* türleri, doğada yaygın olarak bulunan küf mantarlarıdır. Özellikle immünsüpresif hastalarda hayatı tehdit eden ciddi enfeksiyonlara neden olmakla birlikte konağın immün durumuna bağlı olarak noninvaziv veya invaziv enfeksiyonlara, aşırı duyarlılık reaksiyonları gibi farklı klinik tablolara neden olabilmektedir. İnsanlardaki enfeksiyonlara en sık neden olan tür Aspergillus fumigatus'tur. Bu çalışmada Nisan 2017 – Mart 2020 tarihleri arasında Fırat Üniversitesi Tıp Fakültesi Mikrobiyoloji laboratuvarına gönderilen bronkoalveolar lavaj (BAL) örneklerinde saptanan *Aspergillus* türlerinin dağılımı retrospektif olarak araştırılmıştır. Laboratuvara gönderilen BAL örnekleri beyin kalp infüzyonlu kanlı agar ve Sabouraud dekstroz agara ekimleri yapılarak inkübatörde 25°C ve 35°C'de inkübe edilmiştir. Ayrıca BAL örneklerinden direkt preparatları hazırlanarak Gram ve Giemsa boyama ile lökositler, epitel ve mantar elemanlarının varlığı araştırılmıştır. Küf mantarı üreyen kültürdeki kolonilerin görünüşleri, laktofenollü pamuk mavisi ile hazırlanan preparatlardaki hif ve spor yapılarının mikroskobik görünümleri, 25°C, 35°C ve 44°C'de üreme hızlarına göre ve matris destekli lazer desorpsiyon/iyonizasyon uçuş süresi kütle spektrometresi (MALDI-TOF MS) tekniği ile tanımlanması sonrasında tür düzeyinde isimlendirmeleri yapıldı. Bulgular: Aspergillus izole edilen 21 BAL örneğinden 10 (%47,6)'unda *Aspergillus fumigatus*, 9 (%42,9)'unda *Aspergillus niger*, 1 (%4,8)'er örnek olmak üzere *Aspergillus terreus* ve *Aspergillus flavus* olduğu saptanmıştır. *Aspergillus* türleri bulaşma yoluyla ilişkili olarak sıklıkla solunum yollarında enfeksiyona neden olmaktadır. Günümüzde mantar enfeksiyonlarının sıklığı giderek artmakta ve bununla birlikte ampirik antifungal kullanımı da yaygınlaşmaktadır. Aspergillus'un tür düzeyinde identifikasyonu, türlerin gösterdiği farklı direnç paternleri nedeniyle klinik olarak önem taşımaktadır.

***ANAHTAR KELİMELER***: Bronkoalveolar lavaj, *Aspergillus* spp., *Aspergillus fumigatus*, MALDI-TOF MS

## Investigation of Aspergillus Species Isolated from Bronchoalveolar Lavage Samples

## ABSTRACT

*Aspergillus* species are mold fungi commonly found in nature. Although it causes life-threatening serious infections especially in immunosuppressive patients, it can cause different clinical pictures such as noninvasive or invasive infections and hypersensitivity reactions depending on the immune status of the host. The most common species that causes infections in humans is

*Aspergillus fumigatus*. In this study, the distribution of *Aspergillus* species detected in bronchoalveolar lavage (BAL) samples sent to the Microbiology laboratory of Fırat University Faculty of Medicine between April 2017 and March 2020 was investigated retrospectively. BAL samples sent to the laboratory were inoculated on blood agar with brain heart infusion and Sabouraud dextrose agar and incubated at 25°C and 35°C in the incubator. In addition, direct preparations of BAL samples were prepared and the presence of leukocytes, epithelial and fungal elements were investigated by Gram and Giemsa staining. The appearance of the colonies in the mold growing culture, the microscopic appearance of the hyphae and spore structures in the preparations prepared with lactophenol cotton blue, according to the growth rates at 25°C, 35°C and 44°C and matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI- After identification with the TOF MS) technique, they were named at the species level. Of 21 BAL samples from which *Aspergillus* was isolated, 10 (47.6%) were found to be *Aspergillus fumigatus*, 9 (42.9%) *Aspergillus niger*, 1 (4.8%) as *Aspergillus terreus* and *Aspergillus flavus*. *Aspergillus* species often cause respiratory tract infections in association with transmission. Today, the frequency of fungal infections is increasing, and the empirical use of antifungals is also becoming widespread. Identification of *Aspergillus* at the species level is clinically important because of the different resistance patterns exhibited by the species.

***KEYWORDS*:** Bronchoalveolar lavage, *Aspergillus* spp., *Aspergillus fumigatus,* MALDI-TOF MS

**Giriş**

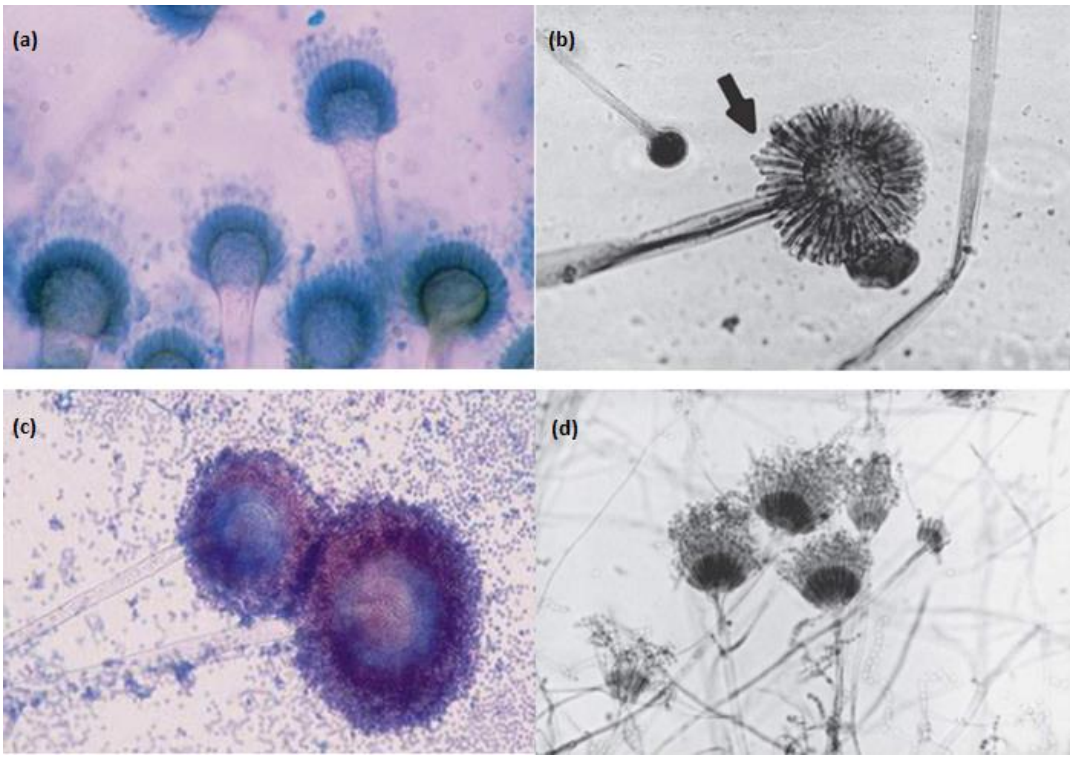
*Aspergillus* cinsi, hastane ve ev ortamlarında havalandırma ekipmanları gibi nemli yüzeylerde, doğada toprak, su ve organik atıklar gibi birçok yerde yaygın olarak bulunan saprofitik, küf mantarlarıdır (Chadeganipour & Mohammadi, 2020; Khambati et al., 2022). *Aspergillus*'un vücuda temel giriş ve kolonizasyon yeri solunum yolu olmakla birlikte daha nadir olarak cilt, böbrek, kemik, göz gibi vücudun çeşitli bölgelerinde enfeksiyonlara neden olabilir (Khambati et al., 2022). Konağın immünolojik durumuna bağlı olarak noninvaziv enfeksiyondan hayatı tehdit eden invaziv veya sistemik enfeksiyonlara kadar değişen farklı klinik tablolara neden olabilir (Chadeganipour & Mohammadi, 2020). Alerjik astım, pnömoni, alerjik bronkopulmoner aspergilloz gibi aşırı duyarlılık reaksiyonları, aspergilloma, invaziv pulmoner aspergilloz sporlarının solunmasıyla ortaya çıkabilecek klinik durumlardır (Mousavi et al., 2016). Doku invazyonu nadirdir ve en yaygın olarak immünosupresif tedavi alan hematolojik maligniteli veya solid organ transplantasyonu yapılan hastalarda görülür (*Aspergillosis: from Allergic Bronchopulmonary Aspergillosis to Invasive Aspergillosa*, 2021). Uzun süreli steroid kullanımı, kronik obstrüktif akciğer hastalığı (KOAH), siroz, kronik böbrek hastalığı gibi diğer immünsupresyon durumları da invazif pulmoner aspergilloz gelişimi için risk faktörleridir (Timurkaynak, 2011). *Aspergillus* cinsi 200'den fazla türe sahiptir ve bunlardan yaklaşık 20 tanesi insan patojenleri olarak sınıflandırılır. İnsanlardaki enfeksiyonlara en sık neden olan tür *Aspergillus fumigatus* olup, diğer sık görülen türler *Aspergillus flavus*, *Aspergillus niger* ve *Aspergillus terreus*'tur (Hernández-Solí et al., 2022).

Bu çalışmada, Fırat Üniversitesi Tıp Fakültesi Mikrobiyoloji Laboratuvarına gönderilen bronkoalveolar lavaj (BAL) örneklerinde saptanan *Aspergillus* tür dağılımının retrospektif olarak değerlendirilmesi amaçlanmıştır.

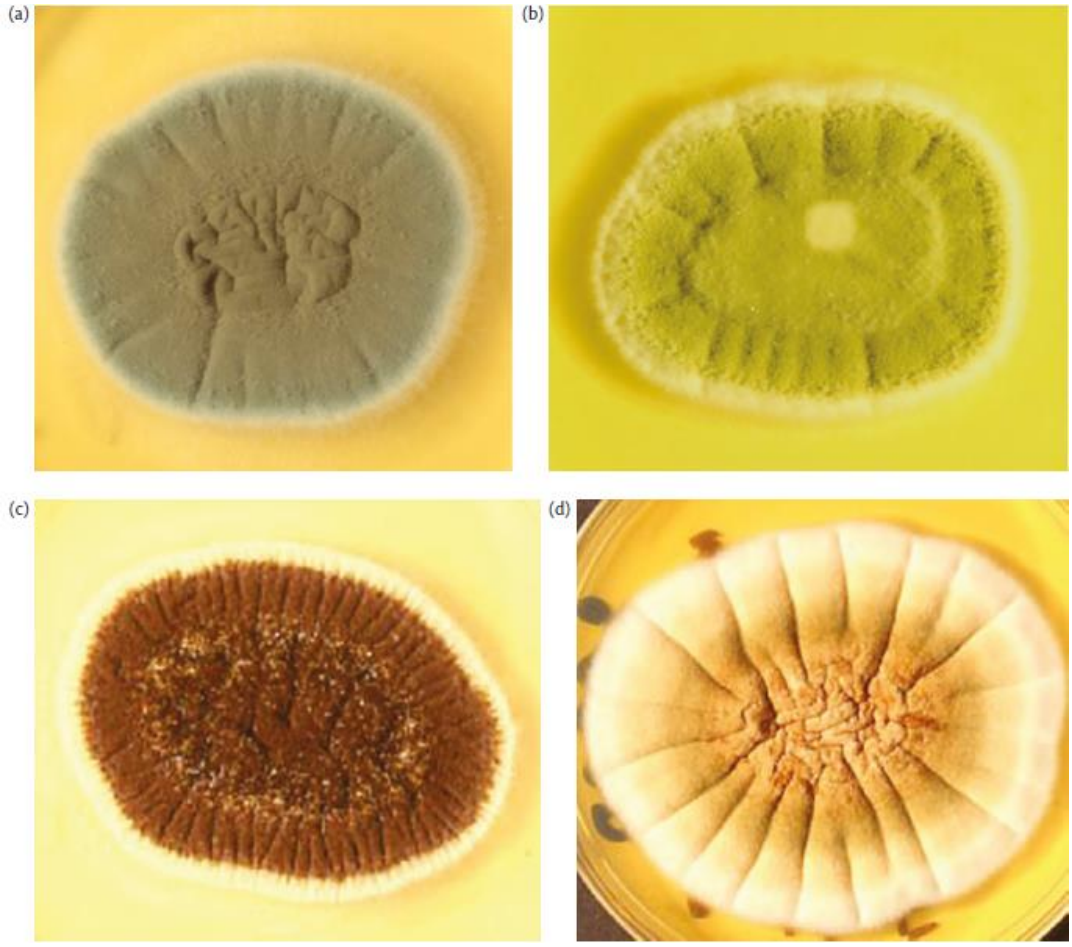
**Gereç ve Yöntem**

Nisan 2017 – Mart 2020 tarihleri arasında Fırat Üniversitesi Tıp Fakültesi Mikrobiyoloji Laboratuvarına çeşitli kliniklerden gönderilen BAL örneklerinde saptanan *Aspergillus* türleri retrospektif olarak araştırılmıştır. Laboratuvara gönderilen BAL örneklerinin beyin kalp

infüzyonlu kanlı agar ve Sabouraud dekstroz agara ekimleri yapılarak 25°C, 35°C ve 44°C etüvde inkübe edilmiş ve ardından 21 güne kadar mantar gelişimi açısından kontrol edilmiştir. Ayrıca BAL örneklerinden direkt preparatları hazırlanarak %10-30 KOH, Gram ve Giemsa boyama ile lökositler, epitel ve mantar elemanlarının varlığı araştırılmıştır. Küf mantarı üreyen kültürdeki kolonilerin morfolojisi, üreme hızları, laktofenollü pamuk mavisi ile hazırlanan selofan bant preparatlardaki hif ve spor yapılarının mikroskobik görünümleri dikkate alınarak, matris destekli lazer desorpsiyon/iyonizasyon uçuş süresi kütle spektrometresi (MALDI-TOF MS) yöntemi ile tür düzeyinde tanımlaması yapılmıştır. İzolatların kolonizan/etken ayrımı mikroskopik inceleme, kültür, histopatoloji sonuçları, radyoloji bulguları ve klinisyen görüşüne göre karar verilmiştir. Her hastaya ait tek bir örnek çalışmaya dahil edilmiştir.




**Şekil 1.** *Aspergillus* türlerinin tipik mikroskopik görünümleri. a. *Aspergillus fumigatus*, b. *Aspergillus flavus*, c. *Aspergillus niger*, d. *Aspergillus terreus* (Procop et al., 2017).




**Şekil 2.** *Aspergillus* türlerinin tipik koloni görünümleri. a. *Aspergillus fumigatus*, b. *Aspergillus flavus*, c. *Aspergillus niger*, d. *Aspergillus terreus* (Kibbler et al., 2018).

**Bulgular**

Etken olarak kabul edilerek *Aspergillus spp.* izole edilen 21 BAL örneğinin 11 (%)'i erkek, 10 (%)'u kadın hastaya aitti. Hastaların yaşı 19 ile 83 arasında değişmekte olup yaş ortalaması 55,3'tü. Örneklerin kliniklere göre dağılımına bakıldığında 10 (%47,6)'unun Göğüs Hastalıkları kliniği, 3 (%)'ünün Hematoloji kliniği, 2 (%)'sinin Romatoloji kliniği ve 1 (%)'er örneğin Dahili Yoğun Bakım, Nefroloji Yoğun Bakım, Enfeksiyon, Göz, İç hastalıkları ve Onkoloji kliniklerinden geldiği belirlenmiştir. *Aspergillus i*zole edilen 21 BAL örneğinden 10 (%47,6)'unda *Aspergillus fumigatus*, 9 (%42,9)'unda *Aspergillus niger*, 1 (%4,8)er örnekte *Aspergillus terreus* ve *Aspergillus flavus* olduğu saptanmıştır.

**Tartışma**

Kronik hastalıklar, maligniteler, uzun süreli kortikosteroid kullanımı, AIDS, transplantasyon, yetersiz beslenme, diyabetes mellitus gibi immün sistemi baskılayan çeşitli durumların artışıyla birlikte pulmoner mikozlar da küresel olarak artmaktadır (Hernández-Solí et al., 2022).

*Aspergillus* türleri, toprak, çürüyen bitki örtüsü, organik maddeler olmak üzere doğada her yerde mevcuttur ve klinik laboratuvarlarda mikotik hastalıkların etkeni olarak sıklıkla izole

edilmektedir. Etrafa yayılan sporlarının inhalasyonuyla gelişen sinüzit veya bronkopulmoner hastalık, insanlarda en sık görülen enfeksiyon şeklidir. Ciddi enfeksiyonların büyük kısmında etken olan tür *Aspergillus fumigatus*'tur (Procop et al., 2017).

Birçok epidemiyolojik çalışmada da enfeksiyonlardan sıklıkla izole edilen türlerin laboratuvarımızdaki verilerle uyumlu olarak *A. fumigatus*, *A. flavus*, *A. niger* ve *A. terreus* olduğu bildirilmiştir (Baddley et al., 2009; Balajee et al., 2009; Pinto et al., 2018; Steinbach et al., 2012). Laboratuvarımızda *Aspergillus* spp. izole edilen BAL örnekleri incelendiğinde, bu örnekler çoğunlukla Göğüs Hastalıkları Kliniğinden gelmiş olup daha önceki raporlarda olduğu gibi en çok izole edilen tür *Aspergillus fumigatus* (%47,6)'tur (Baddley et al., 2009; Mortensen et al., 2011; Pinto et al., 2018; Xu et al., 2020). Ayrıca yayınlanmış bazı çalışmalarla uyumlu şekilde, en yaygın ikinci kompleksin *Aspergillus niger* (%42,9) olduğu gözlemlenmiştir (Baddley et al., 2009; Lortholary et al., 2011).

**Sonuç**

*Aspergillus* türlerinin neden olduğu klinik hastalıklar çeşitlidir ve genellikle konakçının bağışıklık durumuna bağlıdır. Ancak bulaşma yoluyla ilişkili olarak sıklıkla solunum yollarında infeksiyona neden olmaktadır. Doğru tanımlama karakterize edilen türlerin birçoğunun farklı antifungal duyarlılık modellerine sahip olması nedeniyle klinik olarak önemlidir.

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# BRONKOALVEOLER LAVAJ ÖRNEKLERİNDE *PNEUMOCYSTIS JIROVECII*'NİN GERÇEK ZAMANLI PZR YÖNTEMİYLE ARAŞTIRILMASI

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## ÖZET

*Pneumocystis jirovecii* immün sistemi baskılanmış hastalarda ciddi enfeksiyonlara neden olan bir mantardır. Özellikle *Pneumocystis jirovecii* pnömonisi (PJP) günümüzde yüksek mortalite hızıyla seyreder. *P. jirovecii* kültürde üreyemediği için tanıda kültür dışındaki yöntemlerden yararlanılmaktadır. Son yıllarda rutin boyama ve direkt floresan antikor yöntemlerinin yanında, duyarlılığı yüksek olan polimeraz zincir reaksiyonu (PZR) yöntemi kullanılmaya başlamıştır. Bu çalışmada moleküler laboratuvarımıza çeşitli kliniklerden gönderilen bronkoalveoler lavaj (BAL) örneklerinde moleküler yöntemle tanımlanan *P. jirovecii*'nin retrospektif olarak araştırılması amaçlanmıştır. Temmuz 2017-Temmuz 2022 tarihleri arasında Fırat Üniversitesi Tıp Fakültesi Mikrobiyoloji Laboratuvarı Moleküler Bölümüne gönderilen 465 hastaya ait BAL örneği çalışmaya alınmıştır. Gönderilen BAL örneklerinden *P. jirovecii*, gerçek zamanlı PZR yöntemiyle Fast Track Diagnostics, Respiratory Pathogens 33 (FTD®, GER.) kiti kullanılarak araştırılmıştır. Retrospektif olarak da hastaların demografik özellikleri, klinik ve laboratuvar verileri kaydedilmiştir. Bu çalışmada toplam 465 hastaya ait BAL örneğinin 25 (%5,4)'inde P. jirovecii yönünden pozitif olarak saptanmıştır. Bunların 18 (%72)'i erkek 7 (%28)'si kadın hastaydı. 25 pozitif örneğin 6 (%24)'sı 0-18 yaş aralığında olup, bunların hepsi 1 yaş altı bebekleri içeriyordu. 12 (%48) hasta 18-65, 7 (%28) hasta ise 65 yaş üstü hasta grubunu oluşturuyordu. Hastaların altta yatan hastalıkları irdelendiğinde en sık malignite olduğu saptandı. Günümüzde gelişen tetkik ve tedavi olanaklarına rağmen PJP yüksek mortalite ile seyreden bir hastalıktır. Klinik verilerin dikkatle değerlendirilmesi ve hastaların immün durumlarının göz önünde bulundurulması önemlidir. PJP'nin erken tanısında multidisipliner yaklaşıma ihtiyaç bulunmaktadır.

***ANAHTAR KELİMELER:*** *Pneumocystis jirovecii*, pnömoni, bronkoalveoler lavaj, gerçek zamanlı PZR

# Investigation of *Pneumocystıs jirovecii* in Bronchoalveolar Lavage Samples by Real-Time PCR Method

## ABSTRACT

*Pneumocystis jirovecii (P. jirovecii)* is a fungus that causes serious infections in immunocompromised patients. Especially *Pneumocystis jirovecii* pneumonia (PJP) has a high mortality rate today. Since *P. jirovecii* cannot grow in culture, methods other than culture are

used for diagnosis. In recent years, besides routine staining and direct fluorescent antibody methods, polymerase chain reaction (PCR) method with high sensitivity has started to be used. In this study, it was aimed to retrospectively investigate *P. jirovecii* identified by molecular method in bronchoalveolar lavage (BAL) samples sent to our molecular laboratory from various clinics. BAL samples of 465 patients sent to Fırat University Faculty of Medicine Microbiology Laboratory Molecular Department between July 2017 and July 2022 were included in the study. In BAL samples, *P. jirovecii* was investigated by real-time PCR using the Fast Track Diagnostics, Respiratory Pathogens 33 (FTD®, GER.) kit. Demographic characteristics, clinical and laboratory data of the patients were recorded retrospectively. In this study, *P. jirovecii* was found to be positive in 25 (5.4%) of the BAL samples of 465 patients. Of these, 18 (72%) were male and 7 (28%) were female. Of the 25 positive samples, 6 (24%) were in the 0-18 age range, and all of them were under 1 year old. Twelve (48%) patients were 18-65 years old, and 7 (28%) patients were over 65 years old. When the underlying diseases of the patients were examined, it was found that the most common malignancy was. the developing examination and treatment possibilities, PJP is a disease with high mortality. It is important to carefully evaluate the clinical data and to consider the immune status of the patients. A multidisciplinary approach is needed in the early diagnosis of PJP.

***KEYWORDS*:** *Pneumocystis jirovecii*, pneumonia, bronchoalveolar lavage, real time PCR

**Giriş**

*Pneumocystis jirovecii* morfolojik yapısı, mantar besiyerinde üreyememesi, antifungal ilaçların etkisizliği, antiprotozoal ilaçlara duyarlılığı ile protozoonlara benzerlik gösterdiğinden keşfedildiği 1909 tarihinden 1980'lerin sonlarına kadar protozoon olarak sınıflandırılmış ancak daha sonra rRNA ve diğer gen sekans analizlerine göre mikroorganizmanın protozoondan çok mantarlara benzerlik gösterdiği saptanmıştır (Stringer et al., 2002). *P. jirovecii* bağışıklığı baskılanmış popülasyonda sıklıkla pnömoni etkeni olan fırsatçı bir patojendir. Ateş, öksürük, nefes darlığı en sık görülen semptomlardır. Ağır vakalarda solunum yetmezliği ortaya çıkabilir. Tipik radyolojik bulgusu bilateral diffüz interstisyel akciğer lezyonlarıdır (Truong & Ashurst, 2022). HIV'li hastalar, organ transplantasyonu yapılan hastalar, malignite, otoimmün hastalık veya enflamatuar hastalığı olan hastalar başlıca risk gurubunu oluşturmaktadır. PJP, hayatı tehdit eden ciddi bir enfeksiyon olup özellikle immünsüpresif bireylerde önemli bir morbidite ve mortalite nedenidir (Zhang et al., 2022). PJP, HIV hastalarında en yaygın fırsatçı enfeksiyonlardan biridir (Salzer et al., 2018). Son yıllarda geliştirilen etkili antiretroviral tedavi ve kemoprofilaksilerle HIV hastalarında PJP insidansında azalma olduğu gözlenirken, kemik iliği veya organ transplantasyonları, maligniteler, otoimmün inflamatuar hastalıkların artması ve immunsupresif tedavilerin kullanımının yaygınlaşmasıyla birlikte, HIV olmayan hastalarda PJP enfeksiyonlarında artışlar görülmektedir (Wang et al., 2021).

*Pneumocystis jirovecii*, fungal kültür ortamında üretilemediğinden PJP tanısı, klinik ve radyografik bulgularla birlikte respiratuar örneklerde organizmanın kist ve trofozoit formlarının Giemsa, metanamin gümüş, toluidin mavisi, kalkoflour beyazı, papanicolaou gibi geleneksel boyalar, immünfloresan boyama yöntemi ile mikroskobik olarak gösterilmesi veya moleküler yöntemlerle *P. jirovecii* DNA'sının saptanması ile konmaktadır. *P. jirovecii* laboratuvar tanısında altın standart yöntem, kist veya trofozoit formlarının mikroskobik olarak gösterilmesidir. Ancak HIV negatif veya PJP kemoprofilaksisi alan hastalar gibi mikroorganizma yoğunluğunun düşük olduğu hastalarda mikroskobik tanının duyarlılığı oldukça azalmaktadır. Bu nedenle duyarlılığı yüksek olan moleküler testler *P. jirovecii* tespitinde giderek daha önemli hale gelmektedir. PZR testi günümüzde etkenin tespitinde en değerli tanı yöntemidir (Bateman et al., 2020; Franconi et al., 2022).

Bu çalışmada moleküler laboratuvarımıza çeşitli kliniklerden gönderilen bronkoalveolar lavaj (BAL) örneklerinde moleküler yöntemle tanımlanan *P. jirovecii*'nin retrospektif olarak araştırılması amaçlanmıştır.

**Gereç ve Yöntem**

Temmuz 2017-Temmuz 2022 tarihleri arasında Fırat Üniversitesi Tıp Fakültesi Mikrobiyoloji Laboratuvarı Moleküler Bölümüne gönderilen atipik pnömoni şüpheli 465 hastaya ait BAL örneği çalışmaya alınmıştır. Gönderilen BAL örneklerinden *P. jirovecii* saptanması, Fast Track Diagnostics (FTD®), Respiratory Pathogens 33 kiti ve Rotor-Gene Q (Qiagen, Almanya) cihazı kullanılarak, multipleks gerçek zamanlı PZR ile çalışılmıştır. Retrospektif olarak da hastaların demografik özellikleri, klinik ve laboratuvar verileri (lenfosit sayısı, nötrofil sayısı, C-reaktif protein (CRP), laktat dehidrogenaz (LDH), albümin, prokalsitonin) kaydedilmiştir. Bu kit *P. jirovecii* dışında İnfluenza A, İnfluenza A (H1N1), İnfluenza B, İnfluenza C, Parainfluenza 1, 2, 3 ve 4, Coronavirus NL63, 229E, OC43 ve HKU1, Human metapneumovirus A ve B, Rhinovirus, Respiratory syncytial virus A ve B, Adenovirus, Enterovirus, Parechovirus, Bocavirus, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus influenzae* tip B, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Bordetella* spp. (*Bordetella parapertussis* hariç), *Klebsiella pneumoniae*, *Legionella pneumophila*/ *Legionella longbeachae*, *Haemophilus influenzae*, *Salmonella* spp. gibi solunum yolunda birçok enfeksiyon etkeni virüs, bakteri ve mantarların saptanması için kullanılmaktadır.

**İstatistiksel Yöntem**

İstatistiksel analiz, SPSS programı 21. versiyon kullanılarak yapıldı. Değişkenlerin normal dağılıma uygunluğu görsel (histogram ve Q-Q grafiği) ve analitik yöntemlerle (Shapiro Wilk testi) kullanılarak incelendi. Sonuçlar, sürekli değişkenler için median (minimum – maksimum) olarak sunuldu. Kategorik değişkenler için sonuçlar yüzde ve frekans olarak verildi. Normal dağılmayan sürekli değişkenler arası karşılaştırmalar için Mann-Whitney U testi kullanıldı. Kategorik değişkenler arası karşılaştırmalar için Fisher exact test kullanıldı. İstatistiksel anlamlılık için $p < 0,05$ kabul edildi.

**Bulgular**

Bu çalışmada toplam 465 hastaya ait BAL örneğinin 25 (%5,4)'inde *P. jirovecii* yönünden pozitif olarak saptanmıştır. Bunların 18 (%72)'i erkek 7 (%28)'si kadın hastaydı. Hastaların yaşı 0 ile 89 yaş arasında değişmekte olup yaş ortalaması 44,2 olarak bulundu. 25 pozitif örneğin 6 (%24)'sı 0-18 yaş aralığında olup, bunların hepsi 1 yaş altındaydı. 12 (%48) hasta 18-65, 7 (%28) hasta ise 65 yaş üstü hasta grubundaydı. Örneklerin 20 (%80)'si servis, 4 (%16)'ü yoğun bakım ünitesi ve 1 (%4)'i poliklinik hastasına aitti.

*Pneumocystis jirovecii* pozitif hastaların altta yatan hastalıkları irdelendiğinde 9 (%36) hastada hematolojik malignite, 4 (%16) hastada solid organ tümörü, 3 (%12) hastada HIV pozitifliği, 2 (%8) hastada kronik obstrüktif akciğer hastalığı (KOAH) ve 1 (%4) hastada renal transplantasyon öyküsü olduğu saptanmıştır. 6 (%24) hastada altta yatan herhangi bir risk faktörü tespit edilmemiştir. PJP olan hastaların 4 (% 16)'ünde mortalite gelişmiştir.

**Tablo 1.** *Pneumocystis jirovecii* pozitif hastaların demografik özellikleri, altta yatan hastalık ve laboratuvar sonuçları

| **Değişkenler** | **Sayı** | **Yüzde** |
|---|---|---|
| **Cinsiyet** | | |
| Kadın | 7 | 28 |
| Erkek | 18 | 72 |
| **Altta yatan hastalık** | | |
| Hematolojik malignite | 9 | 36 |
| Solid organ tümörü | 4 | 16 |
| HIV pozitifliği | 3 | 12 |
| KOAH | 2 | 8 |
| Renal transplantasyon | 1 | 4 |
| **Laboratuvar testleri** | | |
| Lenfopeni | 17 | 68 |
| Nötropeni | 6 | 24 |
| CRP ortanca değeri (mg/L) (en büyük-en küçük) | 40,1 (201-3,02) | |
| Procalsitonin ortanca değeri (mg/L) (en büyük-en küçük) | 0,15 (46-<0,12) | |
| Albümin ortanca değeri (g/dL) (en büyük-en küçük) | 3,2 (5,2-2,3) | |
| LDH ortanca değeri (u/L) (en büyük-en küçük) | 308 (1043-181) | |
| **Mortalite** | | |
| Yok | 21 | 84 |
| Var | 4 | 16 |

CRP; C reaktif protein, LDH; Laktat dehidrogenaz

**Tablo 2.** *Pneumocystis jirovecii* pozitif hastalardan ölümle sonuçlanan ve sonuçlanmayan olguların özelliklerinin karşılaştırması

| **Değişkenler** | **Ölüm yok** | **Ölüm Var** | **p** |
|---|---|---|---|
| **Cinsiyet** | | | |
| Kadın n(%) | 6 (%28,6) | 1 (%25) | 1[1] |
| Erkek n(%) | 15 (%71,4) | 3 (%75) | |
| **Yaş** ortanca değeri (en büyük-en küçük) | 49(0-80) | 76,5(28-89) | 0,68[2] |
| **Altta yatan hastalık** | | | |
| Var n(%) | 15 (%71,4) | 3 (%75) | 1[1] |
| Yok n(%) | 6 (%28,6) | 1 (%25) | |
| **Laboratuvar testleri** | | | |
| **Lenfopeni** (<800) | | | |
| Var n(%) | 13 (%65) | 4 (%100) | 0,283[1] |
| Yok n(%) | 7 (%35) | 0 (%0) | |
| **Nötropeni** (<500 µl) | | | |
| Var n(%) | 6 (%30) | 0 (%0) | 0,539[1] |
| Yok n(%) | 14 (%70) | 4 (%100) | |
| **CRP** ortanca değeri (mg/L) (en büyük-en küçük) | 35,9 (3-124) | 133 (41,5-202) | **0,019[2]** |
| **Procalsitonin** ortanca değeri (mg/L) (en büyük-en küçük) | 0,13 (0,1-1,1) | 0,36 (0,1-46) | 0,290[2] |
| **Albümin** ortanca değeri (g/dL) (en büyük-en küçük) | 3,2 (2,3-3,7) | 2,8 (2,6-3,5) | 0,183[2] |
| **LDH** ortanca değeri (u/L) (en büyük-en küçük) | 333(207-1043) | 519 (181-891) | 0,892[2] |

Sütun yüzdeleri verildi.
[1]Fisher exact test, [2]Mann Whitney U test

**Tartışma**

*Pneumocystis jirovecii* pnömonisi, günümüzde immünsüpresif bireylerde önemli morbidite ve mortalite sebebidir. Bu nedenle hızlı ve doğru tanı, diğer pnömoni etkenlerinden ayrım ve tedavinin yönlendirilmesi açısından önem taşımaktadır (Bozzi et al., 2022). Solunum yolu enfeksiyon etkenlerin teşhisinde moleküler testler, geleneksel yöntemlere kıyasla daha iyi bir etkinliğe, duyarlılığa ve hıza sahiptir. Multipleks PZR testlerin avantajı ise hem çok sayıda patojeninin hızlı tespitine olanak tanıması hem de patojenlerin tanısı için ayrı ayrı alınması gereken numune hacmini ve kontaminasyon riskini azaltmasıdır (Kenmoe et al., 2019).

*Pneumocystis jirovecii* pnömonisi sıklığını bildiren yayınlanmış çalışmalar incelendiğinde farklı oranlar göze çarpmaktadır. Ülkemizde PJP şüpheli 255 solunum yolu örneğinde direkt floresans antikor (DFA) yöntemiyle *P. jirovecii'*nin araştırıldığı bir çalışmada %54 pozitif sonuç saptanmıştır (Yanık et al., 2012). Şengel ve arkadaşları dört yıllık süre içinde PZR ile çalıştıkları toplam 133 BAL örneğinin 17 (% 13)'sinde *P. jirovecii* pozitifliği elde etmişlerdir (Ertürk Şengel et al., 2020). Bir başka çalışmada *P. jirovecii* pozitifliği %35'tir (Alshahrani et al., 2020). Hindistan'da HIV'li hastalarda yapılan bir araştırmada bu oran %27.2 bulunmuştur (Kaur et al., 2015). Çalışmamızda, 465 hastaya ait BAL örneğinin 25 (%5,4)'inde *P. jirovecii* pozitifliği tespit edilmiştir. Bu oranın, ülkemizden bildirilen oranlardan düşük olmasının sebebi, yöntem olarak multipleks PZR testinin kullanılması ve gelen atipik pnömoni şüpheli tüm örneklerin çalışmaya dahil edilmiş olmasıdır.

*Pneumocystis jirovecii*'nin görülme sıklığı, immünsüpresif kişilerde immünkompetan bireylere göre daha yüksektir. Daha önce yapılan çalışmalarda özellikle hematolojik malignite, organ transplantasyonu, inflamatuvar/otoimmün hastalık, solid organ tümörü olan hastalarda yüksek *P. jirovecii* pozitiflikleri bildirilmiştir (Özkoç et al., 2015; Yang et al., 2020; Yanık et al., 2012). Bu çalışmada da en yüksek PZR pozitifliği sırasıyla; hematolojik malignite (%36), solid organ tümörü (%16) ve HIV pozitifliği (%12) olan hastalarda gözlenmiştir.

HIV ile enfekte hastalar PJP için önemli risk grubunu oluşturmaktadır. Ancak son yıllarda PJP insidansının, uygulanmaya başlanan yüksek aktiviteli antiretroviral tedavi (HAART) ve kemoprofilaksilerle birlikte HIV pozitif hastalarda azalırken, immünosupresif tedaviler ve kemoterapötik ajanların sıklıkla kullanılmasıyla HIV-negatif immünosuprese hastalarda arttığı bildirilmektedir (Hamada et al., 2020). Bir çalışmada *P. jirovecii* PZR pozitif hastaların sadece %9.9'u HIV ile enfekte olduğu bildirilmiştir (Yang et al., 2020). Ülkemizdeki bir çalışmada *P. jirovecii* saptanan hastalarında % 41 HIV pozitifliği görülmüştür (Ertürk Şengel et al., 2020). Literatür verileriyle uyumlu olarak çalışmamızdaki pozitif hastaların büyük kısmı (%88) HIV negatiftir.

Yapılan çalışmalarda PJP hastalarında ileri yaş, kadın cinsiyet, LDH yüksekliği, albümin düşüklüğü, nötropeni, lenfopeninin kötü prognozla ilişkili olduğu belirlenmiştir (İnci et al., 2021; Liu et al., 2017). Bu çalışmada hayatını kaybeden ve hayatta kalan hastalar arasında yaş ve cinsiyet açısından anlamlı fark saptanmamıştır (p=0,68, p=1). Laboratuvar testlerinden CRP değerinin ölen hastalarda anlamlı derecede yüksek olduğu tespit edilirken (p=0,019), diğer laboratuvar testlerinde gruplar arasında anlamlı fark bulunamamıştır.

*Pneumocystis jirovecii* pnömonisi, genel durumu bozuk, bağışıklığı baskılanmış hastalarda mortalitesinin yüksek olması nedeniyle önemlidir. Özkoç ve arkadaşları PZR ile *P. jirovecii* saptadıkları 31 hastadan 5 (%16,1)'inin hayatını kaybettiğini bildirmişlerdir (Özkoç et al., 2015). Başka bir çalışmada PZR ile *P. jirovecii* pozitif saptanan hastalarda %12 ölüm bildirilmiştir (Ertürk Şengel et al., 2020). Çalışmamızda *P. jirovecii* pozitif olan hastaların 4 (% 16)'ü kaybedilmiştir.

*Pneumocystis jirovecii* pnömonisi semptomlarının, HIV ile enfekte olmayan bağışıklığı baskılanmış hastalarda HIV ile enfekte bireylere göre daha hızlı başladığı ve daha hızlı ilerlediği

gösterilmiştir. Bu nedenle, bu hasta grubunda HIV ile enfekte hastalara kıyasla daha yüksek ölüm oranları bildirilmektedir (Chung et al., 2022; Sokulska et al., 2015; Tadros et al., 2017). 13 çalışmanın dahil edildiği bir meta analizde, PJP'li HIV negatif hastalarda mortalite %30,6 olarak belirlenmiştir (18). İtalya'da yapılan bir çalışmada PJP teşhisi konan HIV-negatif 20 hastada mortalite %20 olarak bulunmuştur (Bozzi et al., 2022). Bu çalışmada hayatını kaybeden 4 hasta da HIV negatiftir ve HIV olmayan grupta mortalite oranı % 18,2'dir. Elde ettiğimiz veriler, PJP'li HIV pozitif ve HIV negatif hastaların farklı prognoza sahip olduklarına dair daha önceki çalışma sonuçlarını desteklemektedir.

Sonuç olarak, PJP yüksek mortalite ile seyreden bir hastalıktır. PJP HIV-negatif immünosüprese hastalarda giderek artmaktadır. Özellikle bağışıklığı baskınlanmış pnömonili hastalarda *P. jirovecii*'nin oldukça sık görüldüğü göz ardı edilmeden hastalar dikkatle değerlendirilmelidir. Şüphelenilen hastalarda erken dönemde tanı konması, uygun tedavi sürecini sağlayarak hayat kurtarıcı olacaktır.

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# ORAL PRESENTATIONS

# Dermatophyte Fungi

# A CASE WITH FUNGAL KERATIT DEVELOPED TO CORNEAL MELTING

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## ABSTRACT

Fungal keratitis is a serious corneal infection. It causes decreased visual acuity and blindness. In this article, the clinical findings and treatment results of a case who developed keratitis due to *Candida albicans* are presented. The most common risk factors for fungal keratitis are ocular trauma, contact lens use, ocular surgery, and topical corticosteroid use. An 83-year-old female patient without any systemic disease was admitted to the ophthalmology clinic with complaints of pain, redness, watering, and decreased vision 1 week after cataract surgery on her left eye. In the ophthalmological examination of the patient, the vision was 0.05 in the left eye. Hyperemia in the conjunctiva, corneal ulcer near the limbus at 3 and 9 o'clock in the cornea, 2 positive cells in the anterior camera and intraocular lens were observed. Scraping material was taken from the cornea for microscopic examination and culture. Without waiting for the laboratory results, empirical topical fortified vancomycin (1%) and ceftazidime drops were started. As a result of microbiological evaluation, candida albicans was detected and the treatment protocol was changed to intravenous voriconazole (400 mg/day) and topical enhanced voriconazole (1%) drops. Due to the continued thinning of the cornea and the risk of perforation, amniotic membrane transplantation was performed after voriconazole injection into the anterior chamber and stroma. Two months after the operation, therapeutic penetrating keratoplasty was performed as the infection did not heal in the cornea. The corneal lesion caused by the fungus is raised, dry, grayish-white or yellowish-white in color. Satellite lesions, hypopyon and immune ring can be observed. Clinical diagnosis alone is not sufficient for fungal keratitis. Culture and microscopic examination are important in diagnosis. Confocal microscopy, anterior segment optical coherence tomography, polymerase chain reaction are other fast and reliable diagnostic methods used today. In conclusion, fungal keratitis is a damaging eye infection that causes permanent vision loss. Detailed anamnesis, early diagnosis and effective treatment are very important in preventing complications.

***KEYWORDS***: Fungal keratit, *Candida albicans*, terapötik keratoplasti, fungal keratitis, therapeutic keratoplasty

# SYNERGISTIC ANTIFUNGAL ACTIVITIES OF P GRISEOFULVUM E7P METABOLITES WITH SILVER NANOPARTICLES

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## ABSTRACT

Many fungal species are part of the natural microbiota, which has an important role in human health. However, when the immune system is compromised, commensal fungal species can transform into opportunistic pathogens that systematically migrate to develop invasive fungal infections that affect multiple systems. Natural bioactive molecules that can be used against these pathogens are often synthesized by filamentous microfungi. Silver nanoparticles (AgNP) with unique properties such as shape, size and increased surface area are known to indicate antimicrobial properties. Combining biogenic AgNP with other bioactive metabolites is a promising new strategy to control resistant infections. In this study, ethyl acetate extract was obtained from *P. griseofulvum* E7P, which was isolated and identified from İzmir Çamaltı Saltern, and the fractions of this extract purified by column chromatography were used. The antifungal activities of the purified fractions (A-F) and AgNP were determined by microdilution method. The checkerboard method was used to determine the synergistic activities of fungal extracts and AgNP on yeast and dermatophytes. The Fractional Inhibition Concentration value was calculated for each well that did not grow at the end of the incubation period. This value was used to determine the effect type. The MIC value of Fraction D against *C. albicans* and *C. krusei* was found to be 200 µg/ml, and Fraction F against dermatophytes as 15.6 µg/ml. AgNP MIC values were in the range of 25-1.56µg/ml against both test microorganism groups. The synergy and partial synergy effects obtained from the combinations examined in synergistic activity studies give hope in the fight against resistant pathogens.

***KEYWORDS***: Fungal metabolite, biogenic AgNP, dermatophyte, synergetic activity

# THE ROLE OF NATIVE PREPARATION IN THE DIAGNOSIS OF SUPERFICIAL DERMATOPHYTE INFECTIONS

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## ABSTRACT

Superficial dermatophyte infections are classified according to the anatomical location and are named tinea. The factors of dermatophyte infections may vary depending on socioeconomic status, geographical region, climate, and lifestyles. The diagnosis of dermatophytosis is made by clinical findings and some laboratory tests. In our study, native preparation results of patients who were admitted to our dermatology clinic with a pre-diagnosis of dermatomycoses were evaluated retrospectively according to gender, age, and localization. This study included 592 patients with dermatomycoses who were admitted to our clinic between the 1st of August 2018 and the 1st of December 2021. Swabs were taken from each of 592 patients. Swabs were placed in a drop of 10% potassium hydroxide (KOH) solution on a microscopic slide and covered with a coverslip. While nail and hair samples were kept on the slide for one hour, skin samples were kept on the slide for half an hour. The findings were evaluated retrospectively. In our study, 265 (48.71%) of 544 patients who applied to the dermatology clinic of our hospital with a preliminary diagnosis of dermatomycosis were female and 279 (51.28%) of them were male. As a result of direct microscopic evaluation with a native preparation, 272 (50%) patients were positive. While Tinea corporis was the most common reason for patients to apply to the hospital, the highest positive rate was determined as Tinea cruris with 58.97%. In the samples, the distribution of the highest positivity according to body sites was determined as 60% tinea pedis in children, and 60.60% tinea cruris in adults. As a result, fungal infections have increased dramatically in recent years. The correct and early diagnosis together with early treatment initiation is extremely important in fungal infections. In addition, early diagnosis is very important in terms of minimizing the harmful effects of antifungal drugs that are prescribed unnecessarily. It has been emphasized that the native preparation method in superficial dermatophyte infections is cheap and practical, and it can quickly guide the treatment.

***KEYWORDS***: Dermatophytosis, direct microscopic examination, superficial fungal infections

# Environmental Mycology

# STUDIES ON BASIDIOMYCETES FROM THE FIELD TO THE LABORATORY

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## ABSTRACT

Molecular taxonomy is developing day by day. However, classical morphological methods have certainly not lost their importance and are even being developed further. Although many molecular works have been carried out until today, less than five percent of described species have been analyzed for DNA. It is very difficult or even impossible to obtain a proper sequence in some groups. Additionally, some mycologists do not fully apply collection techniques, so some problems arise in DNA analysis. Sometimes samples are mixed during the studies, or similar problems may exist. My advice to mycologists is that they should never publish new species without detecting strong morphological evidence, even if they find the DNA results interesting. Another problem is that existence of subspecies is not respected by some mycologists. I believe that, the members of the same species distributed in different geographical regions or habitats may not be exactly the same and the problem of varieties, subspecies and similar taxa has not been solved yet. Mushrooms of small size close to each other may belong to different species. This should be taken into account during collection. We carried out some studies on the fruiting bodies of basidiomycetes from the Eastern Black Sea Region of Türkiye between 1990-2022. Some data belonging to these collections have been previously published. After these publications we have collected some of basidiomata again and studied microscopically. Most macro- and micro figures belonging to these collections are newly presented here. We used Axio Imager Stemi 2000-C to get thin sections from basidiomata. New razor blades, congo red, potassium hydroxide solution and some other laboratory equipments were used to prepare the material for microscopic examination. Axio Imager A2 research microscope was used to illustrate of hyphae, cystidia, basidia and other structures. Some of the photos belong to new records or newly described species during the previous studies. The purpose of this presentation is focusing on some interesting structures and experiences from the field and laboratory.

***KEYWORDS***: Agaric, fungal diversity, microscopy, new species, Trabzon

# BIOLOGICAL CONTROL OF *TRICHODERMA* SPP AGAINST *FUSARIUM OXYSPORUM* AND *RHIZOCTONIA SOLANI* IN MINT MENTHA MULTIMENTHA L

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## ABSTRACT

In this study, the effects of *Trichoderma harzianum* and *Trichoderma virens* biological control agents against *Fusarium oxysporum* and *Rhizoctonia solani*, which are fungal pathogens that cause important diseases in mint cultivation, were investigated. In the study, *F. oxysporum* and *R. solani* isolates were obtained and identified as a result of the isolations made from the roots of mint plants showing wilting symptoms. The effects of *T. harzianum* and *T. virens* bio-agents on growth parameters and pathogen applications of peppermint (*Mentha multimentha* L.) in vivo were investigated. As a result of the experiment, the fresh and dry weights of the plants were taken, their heights were measured and disease scale values were calculated. It was determined that *T. harzianum*, which showed the most activity among them, suppressed the growth of R. solani by 44% and the growth of *F. oxysporum* by 57%. It was determined that *T. harzianum* application was the highest and *R. solani* was the lowest in plant height, fresh and dry weight parameters. It was determined that *T. harzianum* and T. virens reduced pathogen growth in both *F. oxysporum* and *R. solani* scale values.

***KEYWORDS***: *Trichoderma harzianum*, *Trichoderma virens*, *Fusarium oxysporum*, *Rhizoctonia solani*, mint (*Mentha multimentha* L.).

# MINERAL COMPOSITIONS AND MORPHOLOGICAL CHARACTERIZATIONS OF SOME EDIBLE NATURAL BASIDIOMYCOTA SPECIES

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## ABSTRACT

Fungi, together with bacteria, are important decomposers that ensure the cyclical operation of the ecosystem and the circulation of nutrients. Soil and above-substrate tissues of edible mushrooms are rich in nutrients and are used by humans for various purposes. Most fungi are saprophytic creatures. They feed by breaking down plant residues (tree trunk, branch pieces, leaves, pine needles, herbs, fruits, cones, etc.) that they use as food sources. They can absorb and accumulate macro and micro elements in the substrates on which they live. One of the aims of this study is to determine how the accumulated minerals will affect human health. The specimens (*Auricularia auricula-judae* (Bull.) Quél., *Lentinus arcularius* (Batsch) Zmitr., *Lentinus tigrinus* (Boğa) Fr., *Schizophyllum commune* Fr., *Trametes versicolor* (L.) Lloyd, *Tremella mesenterica* (Schaeff.) Pers.) that were the subject of our study are the specimens belonging to the basidiomycota, which produces white rot, collected from their natural habitats. Specimens were collected from the field and morphological character studies were carried out in the laboratory for species identification. Heavy metal (Al, As, Cd, Co, Cu, Cr, Fe, Mg, Mn, Ni, Pb, Se and Zn) ion contents of these mushroom samples were determined using ICP-OES spectrometry. Another aim of this study which mushrooms can be used especially as environmental cleaners in order to perform one of the important tasks of fungi in nature? is to answer the question. Thanks to the data obtained from this study, mycoremediation methods can be developed. Fungi can be used as an inexpensive, effective and environmentally sound method to decompose a wide variety of pollutants from contaminated environments or wastewaters. As a result, minimum and maximum heavy metal ion concentrations were determined for the mushroom samples used. Arsenic (As) was not determined at all in the samples examined. Magnesium (772.1-2739 mg/kg), Iron (49.91-1583 mg/kg) and Aluminum (8.009-1298 mg/kg) minerals were the heavy metal ions detected at the highest rates in all samples used in the research.

***KEYWORDS***: Minerals, heavy metals, macrofungi, Çorum

# MICROFUNGI BIODIVERSITY OF PINK KALPLI LAKE IN THE ÇANAKKALE EZINE DALYAN REGION

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## ABSTRACT

The fungi are widely distributed worldwide and can thrive in various habitats, from deserts or areas of high salt concentration to the deep-sea bottoms, even in environments with extreme environmental conditions such as ionizing radiation. Fungi have vital roles in the ecosystem. Essentially, fungi which is closely associated with bacteria, plants and animals are decomposers, symbiotics, and pathogens. Despite their functional importance, fungal diversity, distribution, and ecology are much less studied than bacteria. Interest in the study of microorganisms living in extreme conditions is increasing day by day. There are more studies on bacterial and archaeal biodiversity among microorganisms living in extreme environmental conditions, but studies on fungal biodiversity are limited in literature. Moreover, studies involving enzyme production, secondary metabolite production, and industrial evaluation of fungi adapted to extreme environmental conditions are also minimal. Therefore, further studies are needed to identify further characteristics of these microorganisms and to study and investigate their biodiversity, distribution and ecology. This study aimed to reveal the microfungi biodiversity of Kalpli Lake in the Çanakkale-Ezine-Dalyan Region, known as the pink lake, which has only 8 in the world and only one in Türkiye, by traditional methods. For this purpose, water samples were taken from Kalpli Göl for the first time on March 25, 2022. The samples were inoculated into DRBC and DRBC media prepared with lake water by the membrane filtration method. After 30 days of incubation at 25°C, plates were counted. Microfungus isolates were taken from counting plates and were identified by traditional methods. As a result, the microfungi load of Kalpli Göl, which is a unique ecosystem, was determined as 0-30 CFU/100 ml in DRBC medium and 5-35 CFU/100 ml in DRBC medium containing lake water. A total of 78 microfungi were isolated from the counting plate. Isolates were defined as *Cladosporium*, *Aspergillus*, *Penicillium*, *Epicoccum*, *Scophuloriopsis*, *Cunninghamella*, *Alternaria*, *Ulocladium*, *Curvularia*, and sterile hyphae (unidentified genera without spores). The molecular identification and industrial importance of the microfungi species identified in this study will also be evaluated with high-level studies.

***KEYWORDS***: Kalpli Lake, Pink Lake, microfungi, biodiversity

# İKLİM DEĞİŞİKLİĞİNİN FUNGUSLAR ÜZERİNDEKİ ETKİSİ

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## ABSTRACT

Devam eden küresel ısınma, organizmaların Dünya üzerindeki coğrafi dağılımını değiştiriyor, fizyolojileri ve mevsimsel aktiviteleri de iklim değişikliği ile çarpıcı biçimde değişiyor. Öte yandan iklim değişikliğinin etkisi besin zincirinde önemli ekolojik rolleri olan funguslar için oldukça yıkıcıyken patojen türlerin ortaya çıkmasına veya yeniden patojen özellik kazanmasına neden olabilir. Mantar türlerinin büyük çoğunluğu için, yüksek sıcaklıklarda büyüme kapasitesi, memelilerde enfeksiyon ve yerleşme yeteneklerini sınırlar. Bununla birlikte, mantarlar yüksek sıcaklıklara uyum geliştirebilir ve iklim değişikliğinin neden olduğu artan sıcaklığa kademeli olarak uyum sağlamaları, hastalığa neden olabilecek organizmaların artmasına neden olur. İklim değişikliğinin yıllık ortalama sıcaklıktaki artış gibi etkilerle yararlı mikroorganizmalar üzerine olumsuz etkileri, patojen mikroorganizmalar üzerinde artırıcı etkileri yapılan araştırmalarla kanıtlanmıştır. Ancak kommensal yaşam içerisindeki mikroorganizmaların stres koşullarına adaptasyon sağlaya bilme yetenekleri birlikte yaşadıkları diğer organizmalara da adaptasyon yetenekleri kazandırabilir. Örneğin; Uzun vadeli evrim sırasında bitkiler, endofitik mantar kolonizasyonuna yanıt olarak sayısız faydalı stratejiler edinmiştir. Endofitik mantarların bitkilerle etkileşimi hem biyotik (mikrobiyal, virülans vb.) hem de abiyotik (sıcaklık, nem, pH, yağış vb.) stresler arasındaki ilişkiyi düzenler. Bu tür simbiyotik etkileşimler iklim değişikliği ile oluşan stres koşullarında hayatta kalmak için oldukça önemli. Bu derleme ile patojen mantarların ortaya çıkışı veya yeniden patojenite kazanmaları ile oluşabilecek potansiyel sağlık problemleri için acil ve uzun vadeli alınması gereken önlemlerden bahsetmekteyiz. Ayrıca iklim değişikliğinin etkisi ile azalan tarım arazileri ve besin tedariki için kommensal bitki-mantar ilişkileri sayesinde değişen iklim koşullarına adaptasyon ile sürdürülebilirliği sağlayabilmek için deliller öneriyoruz.

***KEYWORDS***: İklim değişikliği, funguslar, fungusların iklim değişikliğine adaptasyonu, iklim değişikliğinin mantar biyoçeşitliliğine etkisi

# ISOLATION AND IDENTIFICATION OF MICROFUNGI WITH POTENTIAL DEGRADATION FACTORS DETECTED ON HISTORICAL RUINS IN IZMIR ARCHAEOLOGICAL MUSEUM

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## ABSTRACT

Historical artifacts are the most important remains that tell the past of a country as well as all people. We can say that these works, which have come from the past to these days and should remain from these days to the future, are the treasures of civilization and bridges connecting the past and the present. Many historical buildings are destroyed as a result of physical, chemical and biological degradation and erosion. The most important of these factors is biodegradation because irreversible losses are experienced. Biodegradation; It can be defined as the development and damage of unwanted microorganisms on an object. Biodegradation is often considered part of the natural aging process of stone. However, biological growth is destructive and it is very important to develop a control mechanism in order to prevent or control this destruction. These cultural heritages will be preserved for a longer period of time by cleaning, repairing, consolidating and maintaining the stone works at regular intervals after the protection stages. Fungi need organic matter for their growth. Existing microbial communities on the stone surface utilize metabolic intermediates and polysaccharides from storage materials. Fungi are also important decomposers in nature and play a role in the degradation of natural stones by forming a mutualist, symbiotic and pathogenic life style with other living groups. In this study, 100 microfungi were isolated from stones with historical value exhibited and stored in the Izmir Archeology Museum. Phenotypic determination was made for each of the 100 microfungi and for 20 samples known to play an important role in the destruction of stones. For genotypic determination, PCR method using ITS1 and ITS4 primers, which are specific universal primers for the ITS gene region, which is the conserved gene region in fungi, was used. 15 of the obtained PCR products were sent for sequence analysis. Sequence results from sequence analysis were evaluated using Finch TV (Blast) and APE program. Species determination was made by comparing nucleotide sequences and examining similarity on GenBank's website https://blast.ncbi.nlm.nih.gov/Blast.cgi (NCBI).

***KEYWORDS***: Microfungus, historical artifact, biodegradation, archeology

# Lichenology

# A NEW SPECIES OF GENUS *BACIDINA* (RAMALINACEAE: ASCOMYCOTA) FROM PAKISTAN

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## ABSTRACT

*Bacidina pakistanica* from the Himalayan moist temperate forest in Pakistan is described as new to science. nrITS sequences confirm its position within the genus *Bacidina* and, together with its morphology and chemistry, suggest that it is distinct from other species of this genus. The taxon is characterized by olive green to pale green thallus, purplish black to almost black apothecia, 0.7–2 mm wide, a hymenium 65–80 µm high, 3–4 septate large ascospores, 28–55 × 1.9–3 µm and absence of pycnidia.

***KEYWORDS***: Polyphyletic, taxonomic diversity, tropical latitudes

# KESTEL ORMANI (KADINHANI: KONYA) EPİFİTİK LİKENLERİ

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## ABSTRACT

Bu çalışmada, Konya Kadınhanı İlçesi'ndeki Kestel Ormanı'nda bulunan epifitik liken türlerinin tespiti amaçlanmıştır. 2022 yılında yapılan arazi çalışmalarında tüm bölge taranmıştır. Çalışma alanında liken türlerinin gözlemlendiği 3 farklı istasyon belirlenmiştir. Belirlenen bu istasyonlardaki vejetasyon *Pinus nigra* J.F.Arnold, *Quercus pubescens* Willd, *Juniperus oxycedrus* L. gibi ağaçları içermektedir. Substrat olarak bu ağaçların üzerinde gelişen likenler, ağaçların gövde, dal, kabuk kısımlarından toplanmıştır. Arazi çalışmaları tamamlandıktan sonra laboratuvara getirilen örnekler üzerinde makroskobik, mikroskobik ve kimyasal incelemeler yapılmıştır. Tür teşhisleri için çeşitli teşhis anahtarlarından yararlanılmıştır. Laboratuvar çalışmaları sonucunda 21 cinse ait 28 farklı tür teşhis edilmiştir. Teşhisi yapılan türler Selçuk Üniversitesi Mantarcılık Uygulama ve Araştırma Merkezi Fungaryumu'nda saklanmaktadır. Alanda en yaygın bulunan ilk beş tür; *Pseudevernia furfuracea* (L.) Zopf var. *furfuracea*, *Evernia prunastri* (L.) Ach., *Phaeophyscia nigricans* (Flörke) Moberg, *Parmelia sulcata* Taylor, *Hypogymnia tubulosa* (Schaer.) Hav.'dır. Teşhis edilen 28 türün deskripsiyonları oluşturulmuştur. Epifitik likenlerin ağaç türleri ile istasyonlar arasındaki dağılımı ve karşılaştırılması yapılmıştır. Sonuç olarak, Kestel Ormanı'ndan ilk kez yapılan bu çalışma ile Türkiye liken biyoçeşitliliğine katkı sağlanmıştır.

***KEYWORDS***: Biyoçeşitlilik, epifitik, Konya, liken

# Ethnomycology

# MAKROFUNGUSLARA YÖNELİK ÖLÇEK GELİŞTİRME VE ETNOMİKOLOJİK ÖZELLİKLERİNİN ARAŞTIRILMASI KONYA MODELİ

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## ABSTRACT

Konya'da özellikle orman açısından zengin bölgelerde araziden toplanan mantarlar besin olarak değerlendirildiği görülmüştür. Bazı türler ise ticari olarak değerlendirilmek için taze veya kurutulmuş olarak pazarlarda satışa sunulur. Semt pazarlarında özellikle bahar mevsimlerinde belli mantar türlerine sıkça rastlandığını görmek mümkündür. Çoğunlukla da *Terfezia*, *Morchella*, *Clitocybe*, *Boletus*, *Lactarius*, *Tricholoma*, *Lepista*, *Volvariella*, *Macrolepiota*, *Amanita*, *Agaricus*, *Pleurotus*, *Cantharellus*, *Craterellus*, *Hydnum*, *Lentinula*, *Ganoderma*, *Tuber* cinsine ait türlerdir. Ülkemizdeki mantar çeşitliliğinin ortaya çıkarılması, yok olma tehlikesine karşı gelecek nesiller için muhafazaya alınması, yerel halka bu mantarların tanıtılması ve onların hangi mantarları gıda ve ticari gelir elde etmek amacı ile kullandıklarını tespiti için yapılan etnomikolojik çalışmalar büyük önem taşımaktadır. Bu çalışma ile de Konya ili ve ilçelerinde yaşayan halkın geleneksel olarak faydalanmakta olduğu doğal olarak bulunan mantarları belirlemek ve bu mantarların kullanım çeşitliliğini saptayarak etnomikolojik açıdan önemini ortaya koymak amaçlanmıştır. Bu amaçla hazırlanan tutum ölçeği Konya ili ve ilçelerinde toplam 515 kişiye uygulanmıştır. Ayrıca bireylerin mantara yönelik görüşlerini ortaya koymak amacıyla hazırlanan açık uçlu sorular yardımı ile halkın mantarla ilgili düşünceleri, uygulama alanları ve ekonomik olarak nasıl değerlendirdikleri araştırılması hedeflenmiştir. Ölçeğe yönelik toplanan verilere sırasıyla açımlayıcı, doğrulayıcı ve uyum analizleri yapılmıştır. Bu analizler sonucunda ölçeğin 3 boyutlu olduğu belirlenmiştir. Ölçeğin boyutları ve toplamında betimsel ve çıkarımsal istatiksel analizler yapılarak değerlendirmeler yapılmıştır. Sonuç olarak Türkiye genelinde olduğu gibi Konya ilinde de yerel halkın mantarlara olan ilgisi ve bilgisinin kısıtlı olduğu görülmüş, yerel halkın mantarlar hakkındaki bilgileri geleneksel bilgilerin ötesine geçmediği ve bu nedenle bölgelerinde sadece büyüklerinden gördükleri veya duydukları kadarı ile mantarları topladıkları tespit edilmiştir. Ayrıca belli bölgelerde mantara olan ilginin yüksek olduğu, bu doğal ürünlerden ticari kazanç elde etmek istekleri olduğu da görülmüştür.

***KEYWORDS***: Makrofungus, etnomikoloji, tutum ölçeği, Konya

# ETHNOMYCOLOGICAL STUDIES IN ANATOLIA FROM PAST TO PRESENT

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## ABSTRACT

Edible and medicinal wild mushrooms (WM) have been valuable natural sources of ethnofood and ethnomedicine since the ancient times. WM growing in Anatolia has seasonally been collected from nature for traditional recipes and traditional treatments by indigenous people for hundreds of years. In addition, economically important WM such as *Amanita caesarea*, *Boletus* spp., *Cantharellus cibarius*, *Craterellus cornucopioides*, *Hydnum repandum*, *Lactarius* spp., and *Morchella* spp. have been sold in local markets and exported abroad as a source of income by the people living in rural areas since the end of the 20th century. The traditional knowledge, including collection times mainly after rainy periods of spring and autumn, and locations regarding the forests, and meadows of various districts and villages in different altitudes, aspects, and slopes of WM have been passed on from generation to generation in Anatolia until today. Moreover, utilization aims for nutrition and/or traditional medicine, traditional recipes such as soup, dible, rice, and menemen, consumption patterns including roasting, frying, baking, grilling, and preservation methods regarding freezing, fermentation, canning, drying, and salting of WM have previously been informed by the indigenous people. This review has aimed to keep and publish comprehensive traditional knowledge about the collection and consumption of WM by compiling the studies on ethnomycology in Anatolia.

***KEYWORDS***: Wild mushrooms, indigenous beliefs, ethnofood, ethnomedicine, traditional knowledge

# Fungal Physiology

# NANOELICITOR CONSTRUCTED FROM FUNGAL METABOLITES FOR PLANT IMMUNITY

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## ABSTRACT

Research findings revealed that the bioactive compounds from metabolites of each active strains of *Chaetomium* spp. produced various new compounds e.g. *Ch. globosum*, *Ch. cupreum*, *Ch. elatum*, *Ch. cochliodes*, *Ch. brasiliense*, *Ch. lucknowense*, *Ch. longirostre*, *Ch. siamense*, *Emericella* sp and *Trichoderma harzianum* PC01. These natural products of new compounds produce from those investigated species are not only inhibited human pathogens (antimalaria, anti-tuberculosis, anti-cancer cell lines and anti-*Candida albicans* etc) but also plant pathogens. These active natural products from different effective strains are further developed to be nanoparticles as a new discovery of scientific investigation which used to induce plant immunity, namely microbial nano-elicitors for inducing immunity in plants. The nano-elicitors are developed to induce plant immunity through phytoalexin production in plants e.g. inducing tomato to produce alpha-tomaline against *Fusarium* wilt of tomato, capsidiol against *Chilli* anthracnose, sakuranitin and oryzalexin B against rice blast, Scopletin and anthrocyaidin against *Phytophthora* or *Pythium* rot, scoparone against *Phytophthora* or *Pythium* rot of citrus, flavonoid against *Papaya anthracnose* etc. Chaetomium biofungicide is registered in Laos, Vietnam, Cambodia. Microbial nano-elicitors are registered in Cambodia which developed from active metabolites from *Chaetomium* and *Trichoderma* species are the new unique science for plant immunity which have been contributed in Thailand, Finland, Russia, Indonesia, Laos, Cambodia, Myanmar, Vietnam and China. Our experiences of research in this area of specialization will be continued to find more biological products for sustainable development goals (SDGs).

***KEYWORDS***: Nanoelcitor, nanoparticles, Thailand

# Medicinal Mushrooms

# MANTARDAN İLACA *GANODERMA APPLANATUM* EKSTRAKTININ KOLON KANSERİNDEKİ TERAPOTİK ETKİSİ

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## ABSTRACT

*Ganoderma applanatum* (Pers.) Pat. yapısındaki sekonder metabolitlerin zenginliğinden dolayı oldukça etkin biyolojik aktiviteye sahip bir mantar türüdür. Dünya genelinde kanser nedenli ölüm nedenleri arasında üçüncü sırada yer alan kolorektal kansere (iKRK) karşı yeni ve alternatif tedavi yöntemleri arayışı hala devam etmektedir. Bu çalışmada *G. applanatum*'un metanol ekstraktının iKRK'deki terapotik etkisinin moleküler mekanizmasının aydınlatılması amaçlanmıştır. Bu amaç doğrultusunda liyofilize edilen *G. applanatum* methanol Ekstraktında (GAME) HPLC analizi sonrasında 14 fenolik bileşiğin miktarı belirlenmiştir. GAME'nin antioksidan aktivitesi, antioksidan kapasitesi, toplam fenolik ve flavanoid miktarları spektrofotometrik yöntemlerle belirlenmiştir. GAME' nin iKRK hücre hatlarından DLD-1 ve HT-29 ile sağlıklı kolon epitel hücre hattı olan CCD-18Co üzerindeki antiproliferatif etkisini Alamar mavisi yöntemi ile belirlenerek IC50 değerleri sırasıyla 20.79 µg/mL, 140 µg/mL ve 180 µg/mL olarak hesaplanmıştır. Konfokal mikroskop çalışmaları neticesinde GAME'nin hücrelerde yoğunlukla sitoplazma etrafında akümüle olduğu tespit edilmiştir. In vitro hücre göçü çalışmaları neticesinde kontrol grubuna kıyasla GAME muamele grubunda hücre göçü miktarının %90 inhibe edildiği belirlenmiştir ($p<0.0001$). Hücre ölüm mekanizmasının belirlenmesi amacıyla gerçekleştirilen Akış sitometresi analizleri sonucunda DLD-1 hücrelerinde kontrol grubuna oranla GAME grubunda erken ve geç apoptozun 2-kat arttığı, HT-29 hücresinde ise erken apoptozun 6-kat oranında arttığı tespit edilmiştir. Apoptozun moleküler temelini belirlemek amacıyla DLD-1 ve HT-29 hücrelerinde GAME muamelesi sonrasında tümör baskılayıcı TP53 geninin 3-kat ve 2.5-kat arttığı ve antiapoptotik genlerden BcL-2 geninin ekspresyonunun ise %80 ve %75 azaldığı qRT-PCR çalışmalarıyla gösterilmiştir. Western Blot çalışmaları sonucunda protein ekspresyonlarındaki değişimin mRNA ekspresyonları ile korelasyon gösterdiği belirlenmiştir. Elde edilen bulgular doğrultusunda *G. applanatum*'un iKRK' ne karşı umut verici bir terapotik ajan adayı olabileceği belirlenmiştir. Bu çalışmalar preklinik çalışmalara ışık tutarak GAME'nin tedavi stratejisinin oluşturulmasına kılavuzluk yapacaktır.

***KEYWORDS:*** *Ganoderma applanatum*, kolorektal kanser, sitotoksisite, apoptoz

# YOĞUN BAKIM HASTALARININ ENDOTRAKEAL ASPIRAT ÖRNEKLERİNDE ÜREYEN CANDİDAL ETKENLER VE ANTİFUNGAL DUYARLILIKLARI

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## ABSTRACT

Yoğun bakım üniteleri, çoklu organ fonksiyon bozukluğu nedeniyle yoğun ilaç tedavisi alan, birçok invazif girişim yapılması gereken ve mekanik ventilasyon uygulanan hastaların takip edildiği, dirençli patojenlerin üremesiyle geniş spektrumlu antibiyotik kullanımının yoğun bir şekilde uygulandığı, nazokomiyal enfeksiyonların yaygın olarak görüldüğü birimlerdir.
Yoğun bakım ünitelerinde Candida albicans ve diğer Candidalara (C.) bağlı gelişen fungal infeksiyonlar önemli mortalite ve morbidite sebeplerindendir.
Candidalara bağlı enfeksiyonlarda türlerin tanımlanması ve antifungal duyarlılıkların tespiti tedavilerden sonuç almak için çok önemlidir.
Çalışmamızda, bir yıllık bir dönem içerisinde hastanemiz yoğun bakım ünitelerinde yatan hastaların endotrakeal aspirat örneklerinden izole edilen Candida türlerinin ve antifungal duyarlılıklarının belirlenmesi amaçlandı.

***KEYWORDS***: Dermatophytosis, superficial fungal infections

# DISTRIBUTION OF *CANDIDA* SPECIES ISOLATED FROM BLOOD CULTURES DURING THE PANDEMIC PERIOD AND EVALUATION OF ANTIFUNGAL SUSCEPTIBILITY

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## ABSTRACT

*Candida*, which is usually seen in immunosuppressive patients, is being reported at increasing rates in different patient groups. Although *Candida albicans* is the most common cause of candidemia detected in blood cultures, the frequency of non- albicans *Candida*'s is increasing day by day. As with antibiotics, the widespread use of antifungal drugs has changed the susceptibility patterns of *Candida* species to antifungal agents. Therefore, it is of great importance to apply species-level identification and antifungal susceptibility tests in determining the antifungal treatment. In this study, it was aimed to evaluate the distribution and antifungal susceptibility of *Candida* species isolated from blood culture samples sent to our laboratory from various clinical units during the pandemic period. Blood culture results sent to Selçuk University Medical Faculty Hospital Medical Microbiology Laboratory from various clinical units between January 2020 and July 2022 were retrospectively examined. Repetitive isolates isolated from the same patient were not included in the study. Blood samples sent in appropriate BACTEC culture bottle were incubated in the BACTEC automatic blood culture device (Becton Dickinson, USA). Gram staining was performed from the bottles giving reproductive signal and it was planted in EMB and 5% sheep blood agar medias. Reproduce yeast isolates were identified conventional methods and/or using yeast identification cards (YST) with the VITEK® 2 Compact System (bioMérieux, France) and their antifungal susceptibility was investigated using antifungal susceptibility cards (AST-YST07). A total of 84 *Candida* species were isolated from 82 patients in the blood cultures studied. Of these patients were observed 42 (51.22%) were female and 40 (48.78%) were male. It was determined that 52 (62.65%) of the samples were sent from patients treated in intensive care units, 28 (33.73%) in services and 3 (3.62%) in polyclinics. Of the 84 isolates were typified as 36 (42.85%) *C. albicans*, 24 (28.57%) *C. parapsilosis*, 13 (15.47%) *C. glabrata*, 5 (5.95%) *C. tropicalis*, 4 (4.76%) *C. krusei*, 1 (1.20%) *C. guilliermondii* and 1 (1.20%) *C. sphaerica*. The resistance rates obtained from 54 isolates were determined as 5.76% in caspofungin, 3.84% in flucytosine, 3.84% in voriconazole, 1.92% in amphotericin B, 1.92% in fluconazole, 1.92% in mikafungin. In our study, it was found that candidemias were most frequently observed in patients (62.65%) treated in intensive care units of our hospital. It was observed that the most frequently isolated factor in blood culture samples was *C. albicans* (42.85%), followed by *C. parapsilosis* (28.57%). The isolated *Candida* species were found to be resistant to some antifungals. As a result, it has been suggested that the typing of *Candida* species and the reporting of their antifungal susceptibility, especially in newborns, elderly patients and ICU patients receiving treatment, are important for guiding treatment.

***KEYWORDS***: Antifungal sensitivity, blood culture, *Candida* species, pandemic

# THE ASSESSMENT OF ANTIOXIDANT ANTIMICROBIAL ANTIDIABETIC ACTIVITIES AND ANATOMICAL PROPERTIES OF *LEPISTA NUDA* BULL COOKE TRICHOLOMATACEAE

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## ABSTRACT

*Lepista nuda* (Bull. ex Fr). Cooke (Tricholomataceae) is utilized in public medicine. The sporophore of *L. nuda* includes Vit B. Infusion1 of this macrofungus is utilized to prevent beriberi. Moreover, decoctions are utilized for the therapy of abscesses and wounds. It possesses significant nutritional and medicinal importance, and particularly has high antioxidant, antitumor and antiviral effects. It is an edible fungus and its cap is lavender, and its round size is (6.1–8) × (4.0–5) µm. This is an unusual, wild mushroom, which includes perfect food constituents. Its cap includes numerous nutrients, containing 56.39% protein, 45.0% total sugar, 3.87% fat, and 7.92% fiber, with anti-vascular sclerosis, anti-virus, anti-gallstone, and anti-diabetes effects. It has been reported that regulates glucose metabolism. Mushrooms have been known as a significant component of gourmet cuisine global with their perfect flavors, nutritious properties and aromas. Owing to their significant nutritional properties, fungi, that are approved as nutraceutical foods are enhancing their interest owing to their medicinal properties and economic significance. In this study, in vitro antidiabetic, antioxidant, antimicrobial activities and anatomical properties of *L. nuda* have been performed. The mushroom (50 g) was powdered and macerated with methanol at room temperature for 8 h/3 days with a mechanical mixer. The methanolic extract evaporated to dryness and was dispersed in a mixture of methanol: water (1:9), then fractionated with solvents of different polarities (n-hexane, dichloromethane, ethyl acetate, n-butanol), respectively, and the sub-extracts gained via evaporating with a rotatory evaporator were weighed. Detection of α-Amylase Enzyme Inhibition Detection of α-amylase enzyme inhibition effect Nampoothiri et al. (2011). Acarbose was used as a positive control. Detection of α-Glucosidase Enzyme Inhibition α-glucosidase enzyme inhibition test, Bachhawat et al. (2011) method was applied by making some modifications. Acarbose was used as a positive control. Detection of Total Phenolic Component The total phenolic compound amounts of the extracts gained from the mushroom were determined utilizing the method developed by Folin and Denis and modified by Singleton. Gallic acid was used as the standard phenolic compound. DPPH• radical-scavenging activity the DPPH• radical scavenging activities of extracts obtained from the mushroom were tested according to the Blois method. 1 mM DPPH• solution was used as a free radical, and α-tocopherol and trolox were used as standard antioxidants. ABTS•+ radical-scavenging activity ABTS•+ cation radical scavenging activities were determined according to the study by Re (Re et al. 1999). 2 mM ABTS•+ solution was used as free radical,

and α-tocopherol and trolox were used as standard antioxidants. Antimicrobial Activity *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231 antimicrobial activity was performed using microorganism strains. Extracts were prepared at a concentration of 1 mg/ml. Moxifloxacin, ampicillin, and fluconazole used as standard were prepared at 128 µg/ml microscopic analysis. The investigations were made in a Jeol JSM 6490LV scanning electron microscope at the Turkish Petroleum International Company (TPAO) Research Centre SEM laboratory, Ankara. Results and Discussion: Determination of α-Amylase Enzyme Inhibition The hexane extract showed the best α-amylase inhibition activity at 5000 µg/ml with 11.22% (38.65% for acarbose). It showed lower activity than acarbose. Determination of α-Glucosidase Enzyme Inhibition The hexane extract showed the best α-glucosidase inhibition activity at 5000 µg/ml with 10.26% (56.59% for acarbose). It showed lower activity than acarbose. Determination of Total Phenolic Component Butanol and Aquas and Ethyl Acetate extracts showed the best total phenolic component with 4.375 µg GAE/ mg, 0.125 µg GAE/ mg ,4.312 µg GAE/ mg extracts. Other extracts were found to be lower. DPPH• radical-scavenging activity Butanol extract showed the best DPPH• radical scavenging activity with 9.221% at 100 µg/ml. It was found to be very low compared to the standards (α-tocopherol 91.957%, trolox 93.317%). ABTS•+ radical-scavenging activity Butanol with 25.111% showed the best ABTS•+ radical scavenging activitiy at 60 µg/ml. It was found to be lower than the standards (α-tocopherol 90.127%, trolox 96.597%). Antimicrobial Activity Ethyl acetate extract showed the best antimicrobial activity against E. coli ATCC 8739, S. aureus ATCC 6538, and C. albicans ATCC 10231 with 250, 250, and 125 µg/ml MIC values, respectively. Microscopic analysis According to anatomy studies, the shape of spore of mushroom was found as spheroidal and size of spore was determined as 4.9-5.5 µm. Acknowledgment: BİLGE Aydın and Enes TEKMAN would like to acknowledge the scholarship during their postgraduate program provided by the Turkish Scientific and Technical Research Council (TUBITAK).

***KEYWORDS***: *Lepista nuda*, antioxidant, antimicrobial, antidiabetic, total phenolic, spore, microscopic

# THE EVALUATION OF ANTIOXIDANT ANTIMICROBIAL ANTIDIABETIC ACTIVITIES AND ANATOMICAL PROPERTIES OF *ARMILLARIA MELLEA* VAHL P KUMM PHYSALACIRIACEAE

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## ABSTRACT

*Armillaria mellea* (Vahl) P. Kumm. is a species of fungus belonging to the family Physalaciriaceae (*A. mellea* (Vahl) P. Kumm. | COL, n.d.). *Armillaria mellea*, also called a honey mushroom, is an edible wild mushroom. To be eaten, it must be thoroughly cooked. Honey fungus, a saprophytic, mycorrhizal, and pathogenic fungus, grows on living and dead trees and decaying foodstuffs. *Armillaria mellea* is used in traditional medicine to treat dizziness, headache, insomnia, and numb arms and legs. In modern medicine, it has been reported that it has been effective against vertigo, aging, and allergies due to its polysaccharides. The antioxidant and antimicrobial activities of *A. mellea* were evaluated as well as phenolic compounds such as β-carotene, and lycopene. In another study, ethanol extract proved to be more effective in α-glucosidase inhibition assays. *Armillaria mellea* aqueous extract was found to alleviate depression-like behaviors. In the literature research, it was seen that there are not many studies on mushrooms. Therefore, in this study, alpha-glucosidase, alpha-amylase, antioxidant, antimicrobial activities, and total phenolic compounds of *Armillaria mellea* were determined. Also, the anatomical properties of the mushroom spores were evaluated by electron microscope. The mushroom (50 g) was powdered and macerated with methanol at room temperature for 8 h/3 days with a mechanical mixer. The methanolic extract (18.78 g) evaporated to dryness and was dispersed in a mixture of methanol: water (1:9), then fractionated with solvents of different polarities (n-hexane, dichloromethane, ethyl acetate, n-butanol), respectively, and the sub-extracts obtained by evaporating with a rotatory evaporator were weighed. Determination of α-amylase Enzyme Inhibition Determination of α-amylase enzyme inhibition effect Nampoothiri et al. (2011). Acarbose was used as a positive control. Determination of α-glucosidase Enzyme Inhibition α-glucosidase enzyme inhibition assay, Bachhawat et al. (2011) method was applied by making some modifications. Acarbose was used as a positive control. Determination of Total Phenolic Component The total phenolic compound amounts of the extracts gained from the mushroom were determined utilizing the method developed by Folin and Denis and modified by Singleton (Folin ve Denis 1912, Slinkard ve Singleton 1977). Gallic acid was used as the standard phenolic compound. DPPH• radical-scavenging activity the DPPH• radical scavenging activities of extracts obtained from the mushroom were tested according to the Blois method (Blois, 1958). 1 mM DPPH• solution was used as a free radical, and α-tocopherol and trolox were used as standard antioxidants. ABTS•+ radical-scavenging activity ABTS•+ cation radical scavenging

activities were determined according to the study by Re (Re et al. 1999). 2 mM ABTS•+ solution was used as free radical, and α-tocopherol and trolox were used as standard antioxidants. Antimicrobial activity *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Candida albicans* ATCC 10231 antimicrobial activity was performed using microorganism strains (CLSI, 2006; CLSI, 2008). Extracts were prepared at a concentration of 1 mg/ml. Moxifloxacin, ampicillin, and fluconazole used as standard were prepared at 128 µg/ml microscopic analysis Morphological investigations were made in a Jeol JSM 6490LV scanning electron microscope at the Turkish Petroleum International Company (TPAO) Research Centre SEM laboratory, Ankara. Determination of α-Amylase Enzyme Inhibition The dichloromethane extract showed the best α-amylase inhibition activity at 5000 µg/ml with 19.04% (38.65% for acarbose). It showed lower activity than acarbose. Determination of α-Glucosidase Enzyme Inhibition Dichloromethane with 2996 µg/ml IC50 value at 5000 µg/ml and hexane extracts with 2879 µg/ml IC50 value showed the best α-glucosidase inhibition activities (IC50= 3299 µg/ml for acarbose). Other extracts showed lower activity than acarbose. Determination of Total Phenolic Component Butanol extract showed the best total phenolic component with 0.562 µg GAE/ mg extract. Other extracts were found to be lower. DPPH• radical-scavenging activity Butanol extract showed the best DPPH• radical scavenging activity with 1.898% at 100 µg/ml. It was found to be very low compared to the standards (α-tocopherol 91.957%, trolox 93.317%). ABTS•+ radical-scavenging activity Butanol with 16.717% and ethyl acetate extracts with 16.375% showed the best ABTS•+ radical scavenging activities at 60 µg/ml. It was found to be lower than the standards (α-tocopherol 90.127%, trolox 96.597%). Antimicrobial activity Hexane extract showed the best antimicrobial activity against E. coli ATCC 8739, S. aureus ATCC 6538, and *C. albicans* ATCC 10231 with 125, 250, and 125 µg/ml MIC values, respectively., Microscopic analysis *Armillaria mellea* the size of the spore was 4-6 micrometers and its shape was determined as spheroidal or elliptical. Acknowledgment: BİLGE Aydın and Enes TEKMAN would like to acknowledge the scholarship during their postgraduate program provided by the Turkish Scientific and Technical Research Council (TUBITAK).

***KEYWORDS***: *Armillaria mellea*, antioxidant, antimicrobial, antidiabetic, total phenolic

# EVALUATION OF IN VITRO ANTIDIABETIC ANTIOXIDANT ANTIMICROBIAL ACTIVITIES AND ANATOMICAL PROPERTIES OF AN EDIBLE MUSHROOM *MACROLEPIOTA PROCERA* SCOP SINGER

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## ABSTRACT

Mushrooms have been vital for a long time as a source of food and used as a medicinal product. In addition to having high nutritional values thanks to the amino acids, minerals, and carbohydrates they contain; they are also used for therapeutic purposes thanks to organic acids, alkaloids, terpenoids, steroids, and phenolic compounds. Some studies have reported antimicrobial, anticancer, and antidiabetic effects of some mushrooms owing to these secondary metabolites they contain. In addition, it was reported antioxidant, antibiotic, and antineoplastic activities of mushrooms before. *Macrolepiota procera* (Scop.) Singer belongs to the Agaricaceae family of Basidiomycota. It is populary known as parasol mushroom, an edible species, *M. procera* has a good taste and very high medicinal value. This species has an erect, high, hollow stem with a large ring. Its cap is quite broad and in adult mushrooms it is umbrella-shaped. It has characteristic brown protrusions on its cap. The cap color is whitish while the body is white-brown. The edible part of the wild mushroom species is its cap. Its stem is inedible. Many therapeutic activity studies such as enzyme inhibitory, antioxidant, antimicrobial, and anticancer of *M. procera* are available in the literature. In this research, in vitro antidiabetic, antioxidant, and antimicrobial activities of *M. procera* extracts in different solvents were evaluated and anatomical properties were determined. Materials and the methanolic extract (MPM) were prepared from *M. procera* by maceration, then the main extract was solved with distilled water (MPW) and fractionated with n-hexane (MPH, dichloromethane (MPD), ethyl acetate (MPEA), and n-butanol (MPB). α-Glucosidase and α-amylase enzyme inhibitory acitivities of mushroom were evaluated for antidiabetic activity. Antioxidant activity was determined using DPPH and ABTS radical scavenging assays. Additionally, total phenolic content was determined. The antimicrobial activity of the mushroom was determined by using microdilution methods against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Candida albicans* ATCC 10231 (CLSI, 2006; CLSI, 2008). Anatomical investigations were made in a Jeol JSM 6490LV scanning electron microscope at the Turkish Petroleum International Company (TPAO) Research Centre SEM laboratory, Ankara. As a result of antioxidant studies, the MPEA extract showed a radical scavenging effect of 21.11% against ABTS (90.13% for α-tocopherol, and 96.60% for trolox) at 60 µg/mL and 4.80% against DPPH (91.96% for α-tocopherol, and 93.32 % for trolox) at 100 µg/mL. The total phenolic content was found as 3.60 µg GAE/mg extract for MPM (3.60) and 0.23 µg GAE/mg extract for MPB. The total phenolic contents for other

extracts were not determined. In our study for antidiabetic activity, at 5000 µg/mL, MPD extract was found the most effective extract with a value of 20.49% in α-amylase inhibition assay (38.65% for acarbose) among six different extracts. In inhibition assay of α-glucosidase (56.59% for acarbose), the MPEA extract is the most effective extract with a value of 7.24% at 5000 µg/mL. In antimicrobial studies, MPD and MPH extracts showed activity against *C. albicans* with 125 µg/mL MIC value. However, its antimicrobial effect was rather weak compared to the standard (0.5 µg/mL for fluconazole). On the other hand, MPH and MPB extracts displayed efficacy against *E. coli and S. aureus* with 250 µg/mL MIC values. However, their efficacy is rather weak compared to the standards (>0.125 for ampicillin and >0.125 for moxifloxacin). This study is the first antidiabetic activity evaluation of *M. procera*. In some studies, methanol extract of *M. procera* was found to be antioxidant, and antimicrobial. According to anatomy studies, the shape of spore of mushroom was found as ellipse and size of spore long axis was determined as 9-12 µm and size of short axis was found as 7.5-9 µm.

***KEYWORDS***: *Macrolepiota procera*, Agaricaceae, antioxidant, antimicrobial, antidiabetic, total phenolic

# THE EVALUATION OF ANTIOXIDANT ANTIDIABETIC ANTIMICROBIAL ACTIVITIES AND ANATOMICAL PROPERTIES OF *LYOPHYLLUM DECASTES* FR SINGER LYOPHYLACEAE

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## ABSTRACT

Oxidants are produced from mitochondria as well as from many endogenous and exogenous sources. In a healthy organism, oxidants and antioxidants are in balance. However, in some cases, the increase in the oxidant level is of vital importance for living things. Because the increase in oxidants can cause many diseases such as cardiovascular diseases, gastrointestinal diseases, respiratory and excretory disorders, cancer, diabetes, aging, sperm dysfunction and infertility. Some mushrooms can be effective in maintaining this balance thanks to the effective compounds they contain. *Lyophyllum decastes* (Fr.) Singer, commonly known as chicken mushroom, is an edible mushroom of the family Lyophylaceae, which has a radish-like flavor and grows in clusters on rotting debris. The aim of our study is to evaluate and compare *L. decastes* extracts in terms of antioxidant, antidiabetic, antimicrobial effects and total phenolic compound. After being dried and powdered under suitable conditions, this mushroom weighing 50 grams was macerated with methanol at room temperature. The main methanol extract prepared by magnetic mixer was evaporated in the rotavapor and then fractionated by treating with solvents of different polarity (n-hexane, dichloromethane, ethyl acetate, n-butanol). Sub-fractions were also obtained after all fractions were evaporated. All extracts were evaluated for their antioxidant activities by ABTS• and DPPH• methods, and phenolic content using Folin-Ciocalteu's reagent (FCR) with slight modifications. In the antioxidant activity tests, α-tocopherol (TK) and trolox (TR) were used as standard. The total phenolic compound amounts of the extracts were expressed as gallic acid equivalents in µg/ml. Determination of α-Glucosidase and α-Amylase Enzyme Inhibition: In vitro α-glucosidase inhibition assays were carried out according to method of Tao et al. 2013, in vitro α-amylase inhibitory activity was determined by the method of Nampoothiri et al. 2011 with slight modification. All of this experiments acarbose was preferred as the standard. Antimicrobial activities were evaluated by microdilution method with some modifications

against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231. Moxifloxacin, ampicillin, and fluconazole used as standard were prepared at 128 µg/ml. Morphological examination of fungal spores was made in a Jeol JSM 6490LV scanning electron microscope at the Turkish Petroleum International Company (TPAO) Research Center SEM laboratory in Ankara. The extracts of *L. decastes*, aqueous extract (A) extract showed highest activity compared to methanol extract (M), n-hexane (H), dichloromethane (D), ethyl acetate (E) and n-butanol (B) extract. [(TR)96.5>(TK)90.1>(A)44.8>(E)30.3>(B)25.6>(M)7.1>(D)4.7>(H)2.3 %; at 60 µg/ml for ABTS)]. [(TR)93.3>(TK)91.9>(A)9.3>(E)9.1>(B)7.0>(M)4.5>(D)3.2>(H)1.1 %; at 100 µg/ml for DPPH)]. Total phenolic compound test results for *L. decastes* [(A)15.0>(E)10.1>(B)9.9>(M)8.2>(D)4.9 µg GAE/ mg extract]. n-Hexane extract did not show any effect. In the α-glucosidase and α- amylase inhibitory activity of *L. decastes* extracts, acarbose (A) was used as standard. The dichloromethane extract of *L. decastes* showed the highest α-glucosidase inhibitory effect [(A)56.6>(E)3.4; at 5000 µg/ml)]. The methanol extract of *L. decastes* showed the highest α-amylase inhibitory effect [(A)38.7>(M)10.8>(H)6.2; at 5000 µg/ml)]. No significant activity was observed in the other extracts. All of the extracts showed similar antimicrobial activity. n-hexane, dichloromethane, ethyl acetate and n-butanol extracts were found to be effective against *C. albicans* (MIC=125 µg/ml). The spore shape is sphenoid and its size is 0.5-1 µm. In a conclusion; our results should be useful in future studies about this species. Acknowledgement: BİLGE AYDIN and Enes TEKMAN would like to acknowledge the scholarship during their postgraduate program provided by the Turkish Scientific and Technical Research Council (TUBITAK).

***KEYWORDS***: *Lyophyllum decastes*, antioxidant, diabetes, antimicrobial, morphologic analysis

# THE EVALUATION OF ANTIOXIDANT ANTIDIABETIC ANTIMICROBIAL ACTIVITIES AND ANATOMICAL PROPERTIES OF *TRICHOLOMA TERREUM* SCHAEFF P KUMM TRICHOLOMATACEAE

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## ABSTRACT

Oxidative stress can cause many serious health problems in the body, including heart disease, neurological disorders, diabetes and leukemia. Today, some medicinal plants and mushrooms consumed as food may be effective in preventing these diseases. In this respect, it is important to determine the number of phenolic compounds they contain and their antioxidant effects. *Tricholoma terreum* (Schaeff.) P. Kumm. belongs to Tricholomataceae family, it has 4-7 cm wide cap and evenly covered in fine grey scales. Convex with a slight boss, it is broadly conical in shape. *Tricholoma terreum*, which is one of the edible mushroom species, is known as "karakız" mushroom in our country. The aim of our study is to evaluate and compare *Tricholoma terreum* extracts in terms of antioxidant, antidiabetic, antimicrobial effects and total phenolic compound. This species, which was dried under suitable conditions, was weighed 50 grams and powdered by a grinder and macerated with methanol at room temperature via a mechanical mixer at 200 rpm. The prepared main extract was filtered and evaporated in a rotavapor. The sub-fractions were prepared by using solvents of different polarities (n-hexane, dichloromethane, ethyl acetate, n-butanol) based on the main extract. These main extract and sub-fractions were evaluated for their antioxidant capacities by ABTS• and DPPH• methods, and phenolic content using Folin-Ciocalteu's reagent (FCR) with slight modifications. In the antioxidant capacity tests, α-tocopherol (TK) and trolox (TR) were used as standard. The total phenolic compound amounts of the extracts were expressed as gallic acid equivalents in µg/ml. In vitro α-glucosidase inhibition assays were carried out according to method of Tao et al. 2013, in vitro α-amylase inhibitory activity was determined by the method of Nampoothiri et al. 2011 with slight modification. The results of both experiments are expressed by comparison with the standard

acarbose. Antimicrobial activities were evaluated by microdilution method with some modifications against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231. Moxifloxacin, ampicillin, and fluconazole used as standard were prepared at 128 µg/ml. Morphological examination of fungal spores was made in a Jeol JSM 6490LV scanning electron microscope at the Turkish Petroleum International Company (TPAO) Research Center SEM laboratory in Ankara. The extracts of *T. terreum*, n-butanol (B) extract showed highest activity compared to methanol extract (M), n-hexane (H), dichloromethane (D), ethyl acetate (E) and aqueous (A) extract. [(TR)96.5>(TK)90.1>(B)15.8>(E)12.3>(A)10.9>(M)5.2 %; at 60 µg/ml for ABTS)]. [(TR)93.3>(TK)91.9>(B)4.2>(E)3.4>(A)1.4>(M)1.2>(D)0.4 %; at 100 µg/ml for DPPH)]. Total phenolic compound test results for *T. terreum* [(B)11.3>(E)9.3>(A)7.1>(M)5.5>(D) 1.1 µg GAE/ mg extract]. n-Hexane extract did not show any effect at the determined concentrations. The ethyl acetate extract of T. terreum showed the highest α-glucosidase inhibitory effect [(A)56.6>(E)0.2; at 5000 µg/ml)]. No significant activity was observed in the other extracts. The n-hexane extract of *T. terreum* showed the highest α-amylase inhibitory effect [(A)38.7>(H)21.3>(M)14.3>(D)14.1>(E)6.7>(B)5.9; at 5000 µg/ml)]. Ethyl acetate extract of *T. terreum* showed best antimicrobial activity against *C. albicans* with MIC=62.5 µg/ml. All of the extracts showed similar antimicrobial activity against *E. coli*, *S. aureus*. The spore shape is ellipse, with dimensions of 6-7 µm on the long axis and 4-5 µm on the short axis. In a conclusion; our results should be useful in future studies about this species. Acknowledgement: BİLGE AYDIN and Enes TEKMAN would like to acknowledge the scholarship during their postgraduate program provided by the Turkish Scientific and Technical Research Council (TUBITAK).

***KEYWORDS***: *Tricholoma terreum*, antioxidant, diabetes, antimicrobial, morphologic analysis

# THE EVALUATION OF ANTIOXIDANT ANTIDIABETIC ANTIMICROBIAL ACTIVITIES AND ANATOMICAL PROPERTIES OF *CLITOCYBE NEBULARIS* BATSCH P KUMM TRICHOLOMATACEAE

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## ABSTRACT

Free radicals are highly reactive, short-lived, and unstable molecules that occur as a product of normal metabolism in the body or are caused by environmental factors. They need to bind to other molecules in order to become more stable and survive longer. However, the molecules to which they are bonded also lose an electron and turn into free radicals. This cycle continues in a balance in the organism. When the balance between oxidant and antioxidant molecules is disturbed in favor of oxidants, various metabolic diseases occur. Many studies have also shown that there is a relationship between diabetes and free radicals. Again, studies have shown that antioxidant-effective compounds have antimicrobial effects on microorganisms. The aim of our study is to evaluate and compare *Clitocybe nebularis* (Batsch) P. Kumm. extracts in terms of antioxidant, antidiabetic, antimicrobial effects and total phenolic compound. The genus *Clitocybe* belongs to Tricholomataceae family. *Clitocybe nebularis* inhabits coniferous and mixed forests and forms its fruiting bodies during late summer and early autumn, usually in fairy rings, which known as "dumanlı mantar, kara balkadın" in our country. Although *C. nebularis* is known as an edible mushroom, it can cause stomach problems. Dried mushroom (50 g) was powdered and macerated with methanol at room temperature via a mechanical mixer at 200 rpm. The prepared main extract was filtered and evaporated in a rotavapor and sub-fractions were prepared by treating with solvents of different polarities (n-hexane, dichloromethane, ethyl acetate, n-butanol). These extracts were evaluated for their antioxidant capacities by ABTS• and DPPH• methods, and phenolic content using Folin-Ciocalteu's reagent (FCR) with slight modifications. In the ABTS•+ and DPPH• scavenging activity tests, α-tocopherol (TK) and trolox (TR) were used as standard. The total phenolic component amounts of the extracts were expressed in gallic acid equivalents. In vitro α-glucosidase inhibition assays were carried out according to method of Tao et al. 2013, in vitro α-amylase inhibitory activity was determined by the method of Nampoothiri et al. 2011 with slight modification. Antimicrobial activities were evaluated by microdilution method with some modifications against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC. Moxifloxacin, ampicillin,

and fluconazole used as standard were prepared at 128 µg/ml. Morphological examination of fungal spores was made in a Jeol JSM 6490LV scanning electron microscope at the Turkish Petroleum International Company (TPAO) Research Center SEM laboratory in Ankara. The extracts of *C. nebularis*, n-butanol (B) extract showed highest activity compared to methanol extract (M), n-hexane (H), dichloromethane (D), ethyl acetate (E) and aqueous (A) extract. [(TR)96.5>(TK)90.1>(B)34.8>(E)20.4>(A)19.1>(D)12.8>(M)5.4>(H)1.5%; at 60 µg/ml for ABTS)]. [(TR)93.3>(TK)91.9>(B)8.0>(E)6.3>(A)5.5>(D)3.8>(M)3.4>(H)2.7%; at 100 µg/ml for DPPH)]. Total phenolic compound test results for *C. nebularis* [(B)26.3>(E)16.3>(A)12.6>(D)12.1>(M)7.7>(H) 4.2 µg GAE/ mg extract]. In the α-glucosidase and α- amylase inhibitory activity of *C. nebularis* extracts, acarbose (A) was used as standard. The n-hexane extract of *C. nebularis* showed the highest α-glucosidase inhibitory effect [(A)56.59>(H) 22.88; at 5000 µg/ml)]. The dichloromethane extract of *C. nebularis* showed the highest α-amylase inhibitory effect [(A)38.65>(D)17.30; at 5000 µg/ml)]. No significant activity was observed in the other extracts. All of the extracts showed similar antimicrobial activity. n-Hexane, n-butanol and ethyl acetate extracts were found to be effective against *C. albicans* (MIC=125 µg/ml). The spore shape is pyriform or oblong, with dimensions of 6.5-8.5 µm on the long axis and 3.5-4.5 µm on the short axis. In a conclusion; our results should be useful in future studies about this species. Acknowledgement: BİLGE AYDIN and Enes TEKMAN would like to acknowledge the scholarship during their postgraduate program provided by the Turkish Scientific and Technical Research Council (TUBITAK).

***KEYWORDS***: *Clitocybe nebularis*, antioxidant, diabetes, antimicrobial, morphologic analysis

# ANTIOXIDANT ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF *GANODERMA* ISOLATES FROM TÜRKİYE

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## ABSTRACT

In this study, the antioxidant, antimicrobial and cytotoxic effects of 50 *Ganoderma* isolates obtained from different provinces of Türkiye and three positive controls including commercial strains were investigated. One isolate, *G. lucidum* OBCC 6069, was selected by a screening program based on DPPH radical scavenging activities. DPPH radical scavenging, reducing power, metal chelating and beta-carotene linoleic acid methods were applied to investigate its antioxidant activity. It was determined that *G. lucidum* OBCC 6069 extract had EC50 values of 1.934, 0.41 and 2.730 mg/mL for DPPH radical scavenging, reducing power and metal chelating activities, respectively. Antimicrobial activity was observed against *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) strains by disc diffusion method. Effective doses were obtained by applying minimal inhibitory concentration and minimal bactericidal concentration experiments. After determining the cytotoxic effect of isolate extracts with MTT assay, the presence of apoptotic effect of *Ganoderma* sp OBCC 3 and *G. lucidum* OBCC 5109 coded isolates was investigated.

***KEYWORDS***: *Ganoderma*, macrofungus, antioxidant activity, cytotoxic activity, antimicrobial activity

# THE ESTIMATION OF ANTIOXIDANT ANTIMICROBIAL ANTIDIABETIC ACTIVITIES AND ANATOMICAL PROPERTIES OF AN EDIBLE MUSHROOM *PLEUROTUS OSTREATUS* JACQ P KUMM PLEUROTACEAE

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## ABSTRACT

*Pleurotus ostreatus* (Jacq.) P. Kumm. (Pleurotaceae) is known as oyster mushroom and is a macrofungus. The mushroom is respected to be the third largest macrofungus cultivated for nutrient and industrial goals global. It is an eatable mushroom. This mushroom grows in shelf-like layers on logs or tree stumps and has excellent flavor. They have a very short lateral stalk and the mushroom caps is a like oyster shaped pileus. *Pleurotus ostreatus* has high protein contents. This mushroom has been found to contain vitamins B1 (thiamine), B2 (riboflavin), B5 (niacin), B6 (pyridoxine and B7 (biotin). Fungi of the *Pleurotus* genus have a significant place between the commercially utilized mushrooms because of their gastronomic, nutritional, and medicinal properties. In the human body, there is a equilibrate between the number of free radicals (hydroxyl radical, superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, etc.) produced and antioxidants. It is necessary to enrich the diet with foods that have antioxidant effects to help the organism reduce oxidative damage. *Pleurotus ostreatus* has shown a wide variety of pharmacological effects such as immunomodulatory, antimicrobial, antitumor and antioxidant. Significant reduction in blood sugar level in a study in alloxan-induced diabetic rats showed that P. ostreatus is an effective antidiabetic diet. In another study, it was concluded that total polysaccharides extracted from *P. ostreatus* have potential antidiabetic activity and can be used for the treatment of type 2. Levostatin, a cholesterol-lowering drug, and its analogues were obtained from *Pleurotus* species. These are reported to be the best therapeutic agents for correcting hypercholesterolemia. This chemical substance used in San Francisco to decrease "bad" cholesterol (LDL) in hypercholesterolmic patients. In this paper, in vitro antidiabetic, antioxidant, antimicrobial activities and anatomical properties of *P. ostreatus* have been estimated. *Pleurotus ostreatus* was collected from Bolu in 2022 and identified by Ilgaz AKATA. Dried *Pleurotus ostreatus* was crushed and macerated with methanol at room temperature by a mechanical mixer. The extract was filtered and evaporated by a rotary evaporator and then was dispersed with methanol:

distilled water: 1:9. This extract was fractionated with n-hexane, dichloromethane, and ethyl acetate, and butanol, respectively. Assignment of α-Amylase and α-Glucosidase Enzyme Inhibition The antidiabetic (α-amylase and α-glucosidase inhibitory activities) activity of these extracts was studied. Acarbose was used as a positive control. Determination of the inhibition effect of α-amylase enzyme Nampoothiri et al. (2011) and determination of the inhibition effect of α-glucosidase enzyme Bachhawat et al. (2011) methods were modified. The total phenolic compound amount of the mushroom was determined utilizing the method developed by Folin and Denis and modified by Singleton. Gallic acid was used as the standard compound. DPPH• radical-scavenging activity. The DPPH• radical scavenging activities of the mushroom were experimented accordingly the Blois method. 1 mM DPPH• solution was utilized as a free radical, and α-tocopherol and Trolox were utilized as reference antioxidants. ABTS•+ radical-scavenging activity ABTS•+ cation radical scavenging activities were determined accordingly the investigation through Re et al. 1999. 2 mM ABTS•+ solution was utilized as free radical, and α-tocopherol and Trolox were utilized as standard antioxidants. *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231 antimicrobial activity was applied using microorganism strains. Extracts were prepared at a concentration of 1 mg/ml and moxifloxacin, ampicillin, and fluconazole utilised as reference were prepared at 128 µg/ml. The researches were made in a Jeol JSM 6490LV scanning electron microscope at the Turkish Petroleum International Company (TPAO) Research Centre SEM laboratory, Ankara. According to antioxidant studies, ethyl acetate extract displayed the best radical scavenging activity with 31.207% against ABTS (90.13% for α-tocopherol, and 96.60% for trolox) at 60 µg/mL and 4.695% against DPPH (91.96% for α-tocopherol, and 93.32 % for trolox) at 100 µg/mL. The highest phenolic content was determined in ethyl acetate extract with a 23.0 µg GAE/mg extract value. *Pleurotus ostreatus* n-hexane extract indicated α-amylase inhibitory activity with an IC50 value of 14.17 µg/mL when compared with positive control acarbose (IC50 = 38.65 µg/mL). The extracts indicated no inhibition against α-glucosidase enzyme when compared with acarbose. The n-hexane and methanol extracts of *P. ostreatus* indicated the best antimicrobial activity against *C. albicans* ATCC 10231 with MIC =125 µg/mL. According to the anatomical study, spores were not observed, but abundant crystals were present. According to the studies in the literature, there are studies on antimicrobial, antioxidant and antidiabetic activities. As a result of the analysis, antioxidant activity, antimicrobial and alpha amylase inhibitory activity were observed, while alpha glucosidase enzyme inhibitory activity was not observed. Acknowledgements: BİLGE AYDIN and Enes TEKMAN would like to thank the scholarship along their postgraduate program provided by the Turkish Scientifc and Technical Research Council (TUBITAK).

***KEYWORDS***: Antidiabetic, antimicrobial, antioxidant, *Pleurotus ostreatus*

# SCREENING OF IN VITRO ANTIDIABETIC ANTIOXIDANT ANTIMICROBIAL ACTIVITIES AND ANATOMICAL PROPERTIES OF AN EDIBLE MUSHROOM *LYCOPERDON PYRIFORME* SCHAEFF

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## ABSTRACT

Mushrooms have long been used as an important food source and traditional medicine over the world. Health-promoting properties of mushrooms such as antioxidant, antimicrobial, anticancer, cholesterol-lowering, and immunostimulatory effects have been reported. Mushrooms are rich in bioactive metabolites with high therapeutic properties such as lectins, polysaccharides, phenolics and polyphenolics, terpenoids, ergosterol, and flexible organic compounds. *Lycoperdon pyriforme* Schaeff. (Agaricaceae) known as pear-shaped puffball or stump puffball, is a worldwide macro-mushroom. It is considered edible if it is not yet ripe and the flesh inside is white. It is 1.5–4.5 cm wide and 2- 4.5 cm high and grows mainly in areas covered with pine trees. According to previous studies, the mushroom has antimicrobial, antioxidant, esterase, and nematicidal activities. Additionally, ergosta-5,7-dien-3-ol, 7,22-ergostadienone, ergosterol, ergosta-7,22-dien-3β-ol, fungisterol, ergosterol peroxide, β-sitosterol, 4-methoxy-benzene-1-azoformamide, 4-methoxybenzene-1-ONN-azoxyformamide, 3,5-dichloro-4-methoxybenzene-1-ONN-azoxyformide were isolated from the mushroom before. In this study, in vitro antidiabetic, antioxidant, antimicrobial activities and anatomical properties of *L. pyriforme* have been evaluated. *Lycoperdon pyriforme* was collected in Bolu, Türkiye, on October 5, 2021. The mushroom was authenticated by Ilgaz AKATA. The methanolic extract (LPM) was prepared from *L. pyriforme* by maceration, then the main extract was solved with distilled water (LPW) and fractionated with n-hexane (LPH), dichloromethane (LPD), ethyl acetate (LPEA), and n-butanol (LPB). α-Glucosidase and α-amylase enzyme inhibitory activities of mushroom were evaluated for in vitro antidiabetic activity. Antioxidant activity was determined using DPPH and ABTS radical scavenging assays. Additionally, total phenolic content was determined. The antimicrobial activity of the mushroom was determined by using microdilution methods against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Candida albicans* ATCC 10231 (CLSI, 2006; CLSI, 2008). Anatomy studies on the spore of mushroom were performed by using electron microscopy. According to antioxidant studies, LPEA displayed the best radical scavenging activity with 78.59% against ABTS (90.13% for α-tocopherol, and 96.60% for trolox) at 60 µg/mL and 11.57% against DPPH (91.96% for α-tocopherol, and 93.32 % for trolox) at 100 µg/mL. The total phenolic content (µg GAE/mg extract) range was found as LPEA (20.50)

> LPM (17.38) > LPW (14.81) > LPB (13.13) > LPD (9.50) > LPH (7.56) as parallel to antioxidant assays. The extracts showed no inhibition against α-glucosidase and α-amylase enzymes when compared with acarbose. In antimicrobial activity assay, LPM, LPH, LPD, and LPB extracts displayed activity with 250 µg/mL MIC values against E. coli, the LPD extract showed the best activity with 125 µg/mL MIC value against *S. aureus*, and LPH, LPD, LPE, and LPB extracts displayed activity with 125 µg/mL MIC values against *C. albicans*. According to anatomy studies, the shape of the spore of the mushroom was found as spheroidal, and the size of the spore was determined as 2.5-3.5 µm. It is the first evaluation of the antidiabetic activity of this mushroom. In a study, the methanolic extract of the mushroom showed antibacterial and antioxidant activities as in our study. It is seen that the phenolic compounds are responsible for the biological effects.

***KEYWORDS***: *Lycoperdon pyriforme*, Agaricaceae, antioxidant, antimicrobial, antidiabetic, total phenolic

# ASSESSMENT OF IN VITRO ANTIDIABETIC ANTIOXIDANT ANTIMICROBIAL ACTIVITIES AND ANATOMICAL PROPERTIES OF *COPRINUS ATRAMENTARIUS* BULL FR

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## ABSTRACT

Mushrooms are products that have been consumed both as food and medicinally for many years. Fungi are of great economic importance and are directly and indirectly involved in maintaining the ecological balance. The enumeration, taxonomy, biology, chemistry, cultivation of mushrooms, and their conversion are of great relevance and fAŞÇInation. Wild mushrooms have become important in our diet not only owing to their nutritional value but also due to nutraceutical attributes that provide medical or health benefits to consumers. *Coprinus atramentarius* (Bull.) Fr. is a mushroom found in Europe and North America. The cap is bell-shaped when young, becoming widespread, flattening, and fragmenting as it ages. The diameter of the cap is 3-10 cm. The color of the cap is gray-brown from the center outwards, and it is covered with brown scales. They usually occur in small groups and clusters, sometimes singly, in tree stumps or in the buried wood of deciduous hardwoods. They are also frequently seen in urban habitats such as vacant lots and lawns, and parks, often just after rains in spring and autumn. In addition, the most studied feature of *C. atramentarius* mushroom is the "disulfiram-like" effect on the liver when the coprin substance in its content is taken with alcohol. This has been proven by in vivo and in vitro studies. Therefore, it can also be used for the treatment of alcohol dependence. In this research, in vitro antidiabetic, antioxidant, antimicrobial activities, and anatomical properties of *C. atramentarius* have been evaluated. *Coprinus atramentarius* was collected in Bolu, Türkiye, on October 5, 2021. The mushroom was authenticated by Ilgaz AKATA. The methanolic extract (CAM) was prepared from *C. atramentarius* by maceration, then the main extract was solved with distilled water (CAW) and fractionated with n-hexane (CAH, dichloromethane (CAD), ethyl acetate (CAEA), and n-butanol (CAB). α-Glucosidase and α-amylase enzyme inhibitory acitivities of mushroom were evaluated for antidiabetic activity. Antioxidant activity was determined using DPPH and ABTS radical scavenging assays. Additionally, total phenolic content was determined. The antimicrobial activity of the mushroom was determined by using microdilution methods against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Candida albicans* ATCC 10231 (CLSI, 2006; CLSI, 2008). Anatomical investigations were made in a Jeol JSM 6490LV scanning electron microscope at the Turkish Petroleum International Company (TPAO) Research Centre SEM

laboratory, Ankara. In antioxidant acitivity studies, CAEA extract displayed 16.02% ABTS radical scavenging effect (90.13% for α-tocopherol and 96.60% for trolox) at 60 ug/mL while 6.039% DPPH radical scavenging effect (91.96% for α-tocopherol and 93.32% for trolox) at 100 µg/mL. According to the total phenolic content (µg GAE/mg extract) was found as parallel with antioxidant studies, the total phenolic content of CAEA extract was determined as the highest one (16 µg GAE/mg extract). It was concluded that no significant inhibitory effect on α-glucosidase and α-amylase enzymes was observed when compared to acarbose. It is the first evaluation on antidiabetic activity of this mushroom. In antimicrobial activity study, the CAW extract was effective with 250 µg/mL MIC value against *E. coli*, and the CAB extract was effective with 125 and 62.5 µg/mL MIC values against *S. aureus*, and *C. albicans*, respectively. In a previous study, the β-glucan extract of prepared from *C. atramentarius* displayed antioxidant and antimicrobial activities. According to anatomy studies, the shape of spore of mushroom was found as ellipse and size of spore long axis was determined as 6-7.5 µm and size of short axis was found as 3-4 µm.

***KEYWORDS***: *Coprinus atramentarius*, antioxidant, antimicrobial, antidiabetic, total phenolic, spore

# Parasitic Mushrooms

# PATHOGENICITY OF ENTOMOPATHOGEN *BEAUVERIA BASSIANA* AGAINST TO THE LARVAE OF *CADRA CAUTELLA* (LEPIDOPTERA: PYRALIDAE) *AND EPHESTIA KUEHNIELLA* (ZELLER LEPIDOPTERA: PYRALIDAE)

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## ABSTRACT

*Ephestia kuehniella* (Zeller, Lepidoptera: Pyralidae) and *Cadra cautella* (Walker, Lepidoptera: Pyralidae) are the most important insect's pests that invade the stored grains in the world. Entomopathogenic fungi are considered as potential candidates to replace chemical insecticides. In our study, soil samples were collected from Dursunbey, Balıkesir. Entomopathogenic fungi were isolated from soil samples using the ''Galleria bait method''. Identification of fungal species were done according to classical (colonial and microscopic morphology) and molecular methods. Genomic DNA isolation was performed using the Plant DNeasy (Qiagen Inc.) kit, following the kit protocol. One of our fungal isolates, LK1, mentioned here, was identified a shared 100.00 % percent identity with *Beauveria bassiana* (gen bank ac. No: MT533246.1) and our NJ tree also proved the similarity. We prepared the six different fungal spore concentrations (103,104,105,106,107,108 conidia/ml) and investigated the insecticidal effect of the entomopathogenic fungus *B. bassiana* against topically to the insect. LD50, LD60, LD70, LD80 and LD90 values were calculated based on larval mortality data, by probit analyses using SPSS software. Mortality of the last instars of *E. kuehniella* and *C. cautella* were depend on dose-dependent, while there were no dead larvae in control groups. The injection assay of fungal doses gave a LD50 of 105.861 conidia/ml of *E. kuehniella* and LD50 of 108.448 conidia/ml for *C.cautella*. The death of *E. kuehniella* larvae was observed between 100 % and 43.3%, and the death of *C.cautella* larvae was observed between 86.6% and 33.3%. The results showed that the entomopathogenic fungi can be used as an alternative method to pest control in Insect Pest Management (IPM) programs.

***KEYWORDS***: *Ephestia Kuehniella*, biological control, entomopathogenic fungi

# BIOLOGICAL DEGRADATION OF HISTORICAL BUILDINGS A RESEARCH IN TEOS ANTIQUE CITY TÜRKİYE

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## ABSTRACT

Historical monuments are exposed to many biological, physical, and chemical decay. Microorganisms can cause serious biodegradation on historical buildings, such as fungi, bacteria, algae, lichens, and cyanobacteria. Fungi in particular play an important role in the deterioration of cultural heritage. The aim of this study was to reveal the fungal degradation in the historical monuments in the ancient city of Teos. The antique port city of Teos located in the Sığacık District (Seferihisar) of Izmir Province, is approximately 60 km southwest of Izmir (Aegean Region, Türkiye). In the antique city of Teos, which is estimated to exist since the protogeometric period, field studies have been carried out on monuments exposed to fungal deterioration. As a result of the field studies in Teos especially cauliflower-like black microcolonial fungi observed in historical artifacts. These fungi have been found to cause brown to black coloration, crater-shaped pits, blistering and fracture, and belong to the genera *Capnobotryella*, *Cladosporium*, *Coniosporium*, *Lophiostoma*, *Massarina*, *Monodyctis*, *Mycocalicium*, *Phoma*, *Phaeococcomyces*, *Rhinocladiella* and *Sarcinomyces*. The deterioration of historical artifacts is a threat in the ancient city of Teos, as in the whole world. It is necessary to increase biological control studies in this field.

***KEYWORDS***: Black microfungi, fungal deterioration, Teos ancient city

# Fungal Biodiversity

# STUDY ON GENETIC DIVERSITY OF *FUSARIUM OXYSPORUM* F SP DIANTHI BASED ON VEGETATIVE COMPATIBILITY GROUPS AND RACES IN FIVE PROVINCES OF IRAN

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## ABSTRACT

*Fusarium* wilt of carnation caused by *Fusarium oxysporum* f.sp. *dianthi* (Fod) W.C.Snyder & H.N. Hensen, is the most important fungal disease in all main carnation growing regions of Iran. In 2020, sampling of infected plants was randomly carried out from carnation greenhouses in five provinces located in the North (Gilan, Golestan, and Mazandaran) and Middle (Tehran and Markazi) of Iran. Eighty-eight fungal isolates were identified as Fod by morphological characters (based on Nelson's key identification), molecular method (PCR amplified of ITS region), and pathogenicity tests (according to the root dipping on sensitive cv. Rendezvous). In order to investigate on biodiversity of Iranian Fod isolates based on Vegetative Compatibility Groups (VCGs) and races, 70 isolates were randomly selected. These isolates were compared with eight Fod isolates from collection of Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali (Di.Va.P.R.A) Patologia vegetale, Italy; which were used as references for races and VCGs. For DNA amplifying, four primers IMP2, Ft3, R 1.8, and R 4.2 were employed. Amplified fragments of 295 bp and 564 bp were identified to race 1 (or 8; because these primers applied are not able to discriminate between the races), and race 2 (or 5 or 6), respectively. Furthermore, two VCG 0021 and VCG 0022 were identified amongst the Iranian Fod isolates. The relationship between the races and VCGs demonstrated that all race 1 (23 isolates) were categorized in VCG 0022, and race 2 (44 isolates) were grouped in VCG 0021. In addition, three isolates did not produce any amplification by using the mentioned primers.

***KEYWORDS***: Carnation, *Fusarium*, vegetative compatibility groups, races, Iran

# REVEALING SOIL FUNGAL BIODIVERSITY IN NATURAL AND AGRICULTURAL ECOSYSTEMS THE ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI AMF AND THEIR POTENTIAL AS BIOFERTILIZERS

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## ABSTRACT

Plant roots host a wide range of microorganisms -the root microbiota- that, together with the quality of soil and climatic conditions, represent the main factors that influence plant health, growth and development. Some microorganisms can put their host in the condition to capitalize soil fertility and optimize plant growth at a point that they can be referred as natural biofertilizers. The extraordinary genetic and functional diversity of fungi and their species richness in the soil make them a key component of the root microbiota with a great potential to impact several areas of agricultural biotechnology. Among these, a central role is offered by the mycorrhizal fungal symbionts. Mycorrhizae are symbiotic partnerships that are established between fungi that live in soil and the roots of most terrestrial plants. A key ecological role is played by endomycorrhizal symbioses, and with greater emphasis by the obligate mutualistic Arbuscular Mycorrhizal Fungi (AMF). AMF can bridge the host root apparatus to distant portions of undepleted soil, providing water and soil mineral nutrients to the plant. In exchange, photosynthetic products are transferred to the fungus. AMF can symbiotically interact with more than 80% of vascular plant species, including important crops and ornamental plants. Therefore, AMF are primary biotic soil components that, when missing or impoverished can lead to a less efficient ecosystem functioning. Human activities can have a detrimental impact on the microbiological features of soil. Agricultural fields, degraded lands and soilless cultures are all ecosystems in which the anthropogenic input has altered the ecological balances. Most of conventional agricultural practices can exert strong selective pressure on AMF, remodel community structure and reduce species diversity. This is a drawback for agriculture since loss of AMF diversity can result in fewer beneficial traits the host plant can benefit from. Consequently, a process that aims at the re-establishment of the natural level of AMF richness is pivotal towards the restoration of the soil microbial balances. Through the development of innovative '-omics' technologies such as high-throughput sequencing, study of root microbiota has advanced rapidly, allowing scientists to also describe AMF communities in different soils. This talk outlines as these fungi can offer novel potential applications for a sustainable agriculture, based fundamentally on the limitation and partial replacement of chemical fertilizers mediated by the respect of natural microbiological balances.

***KEYWORDS***: Arbuscular mycorrhizal fungi, root microbiota, sustainable agriculture

# ASIAN MYCOLOGY IN THE NEXT DECADE CHALLENGES SOLUTIONS AND FUTURE DIRECTIONS

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## ABSTRACT

DNA sequence-based species identification and phylogenetic analyses are standard practices in this era of mycology. Taxonomists regularly use DNA sequence data in taxonomy in addition to morphological characteristics; thus, the polyphasic approach in taxonomy is currently popular. Resolving species complexes, defining generic boundaries of polyphyletic genera, and linking different morphs of pleomorphic genera have become popular among taxonomists. It is shown that the taxonomy-related studies in Asian regions, especially China, India, and Thailand, have dramatically increased mainly due to the economic development of these countries, while rich biodiversity and human resources (experts/specialists) also play vital roles. However, the fungal taxonomic studies in other South Asian countries with higher biodiversity are not well-developed, e.g., Sri Lanka, Pakistan, Nepal, Bangladesh, and Bhutan. The British mycologists did most of the mycology work in Sri Lanka before 1948. Nevertheless, recent fungal diversity estimations mentioned that these biodiversity-rich regions are essential in revealing missing fungal species. Besides, epitypification and revisiting old species are also necessary as many taxa lack DNA sequences and decent recent works. It is essential to carry out systematic surveys in these mycologically least developed countries with the collaboration of foreign scientists and national-level experts. Establishing national fungaria and culture collections will be necessary for fungal taxonomy and application developments. It is essential to train new, budding mycologists to encourage these enormous tasks in the future.

***KEYWORDS***: Mycology, fungal diversity

# MOLECULAR IDENTIFICATION OF *ASPERGILLUS* SPECIES IN COVID 19 PATIENTS ASSOCIATED WITH FUNGAL RHINOSINUSITIS

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## ABSTRACT

Invasive aspergillosis (IA) caused by various *Aspergillus* species, can be a potentially lethal opportunistic fungal infections in patients with underlying immune deficiencies. The most susceptible individuals to this infection are those with diabetes mellitus, transplant recipients, individuals taking immunosuppressive drugs, and latterly, COVID-19 patients. The concurrence IA with other infections such as COVID-19 can disrupt the treatment and control of the infection. In this study, *Aspergillus rhinosinusitis* was detected using sinus biopsy specimens among COVID-19 patients. The sinus biopsy specimens of 68 patients with fungal rhinosinusitis affected COVID-19 were studied during eight months (April 2021 to November 2021). In a specific mycology laboratory, direct examination with 20% potassium hydroxide (KOH) and cultivation with sabouraud dextrose agar have been performed. Finally, molecular method of PCR sequencing using calmodulin and β-tubulin (benA) genes were used to identify *Aspergillus* species. Thirteen (19.11%) of the patients showed *Aspergillus rhinosinusitis*. Among patients with *Aspergillus rhinosinusitis*, four patients (30.77%) were male and nine patients (69.23%) female, with a mean age of 56 years (range: 33-68 years). Among the affected patients, eight (61.53%), three (23.07%) and one (7.69%) had diabetes mellitus, corticosteroid users and kidney transplant, receptively; that five (38.46%) them died. Nine (69.23%) of specimens showed septate, branched, hyaline hyphae in direct microscopic examination, and 10 specimens showed *Aspergillus* culture for seven *A. flavus* (70%), two *A. tubingensis* (20%), and one *A. welwitschiae* (10%). Our study revealed that *Aspergillus rhinosinusitis* had a high incidence among COVID-19 patients. Also, diabetes mellitus was an important predisposing factor in these patients. *Aspergillus flavus* was the most common fungal agent of *Aspergillus rhinosinusitis*.

***KEYWORDS***: Molecular identification, *Aspergillus rhinosinusitis*, covid-19, PCR sequencing

# CONTRIBUTIONS TO THE AGARICALES OF TÜRKİYE

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## ABSTRACT

In the present study, some macrofungi specimens were collected with field studies in Geçitli village of Hakkâri town of Türkiye between the years of 2021 and 2022. During field studies, the colorful photographs were taken, ecological features and the information on collected samples provided from local people were recorded. Macrofungi specimens were identified according to morphological and microscopic studies. In conclusion, it has been determined that the *Pterula subulata* Fr. (Pterulaceae, Agaricales) and *Hymenopellis megalospora* (Clem.) R.H. Petersen (Physalacriaceae, Agaricales) have been collected for the first time in our country by this research.

***KEYWORDS***: New record, *Pterula*, *Hymenopellis*, Geçitli, Hakkâri

# MICROFUNGUS DIVERSITY OF SALDA LAKE TÜRKİYE AN ALKALINE LAKE

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## ABSTRACT

Salda Lake is one of the rare alkaline lakes in the world. It is a magnesium-rich lake with an average pH of 9.1. In this study, it is aimed to illuminate the microfungi diversity of Salda Lake as an extreme environment. It was studied with one water sample and four soil samples from the Salda Lake basin. Membrane filtration method and soil dilution method were used for microfungi isolation from water and soil samples, respectively. DRBC agar plates prepared with salt addition and lake water were used for isolation. While microfungi varying between 20-400 CFU/g dry soil were counted in soil samples, an average of 18 CFU/100 ml microfungi were counted in water samples. After purification steps, totally 43 isolates obtained were identified by using conventional methods. As a result of the classical identification of the isolates based on morphology, it was determined that they belong to the genera *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Metarhizium*, *Penicillium* and *Stachybotrys*. In addition, the pH tolerance ranges of the isolates were determined. Most isolates were found to grow over a wide pH range. These alkalotolerant/alkalophilic isolates can be used as model organisms to elucidate life in extreme conditions and may even provide valuable information about extraterrestrial life.

***KEYWORDS***: Alkaliphilic, alkali tolerant, pH tolerance, fungal diversity

# FRESHWATER FUNGAL DIVERSITY IN SELECTED ANURADHAPURA LAKES OF SRI LANKA

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## ABSTRACT

Sri Lanka is an island with high biodiversity; nonetheless, the diversity of fungi in Sri Lanka is understudied and under documented. A recent study estimated that Sri Lanka is home to over 31,000 fungal species, however only ca. 3000 taxa are currently known, and this estimation was mainly based on the host-fungi relationship of terrestrial habitats. Particularly, Sri Lanka's freshwater fungi are poorly documented. Thus, this research's main objective was to study the diversity and taxonomy of freshwater fungi in selected lentic aquatic habitats in the district of Anuradhapura located in the city of Mihintale (North-Central Province). Submerged aquatic substrates (such as dead leaves and dead woody samples) were randomly collected from selected reservoirs (viz., Mihintale tank, Maha Kanadarawa tank and Iluppukanniya tank). The specimens were transferred to the laboratory and incubated in wet chambers at room temperature with ambient light for three days to ensure the best growth of aquatic fungi. After the appropriate incubation period, single spore isolation was performed using Water Agar (WA) and Potato Dextrose Agar (PDA) as culture synthetic media to isolate fungal freshwater strains/species. Germinated spores were then transferred to a new PDA. Different fungal colonies and spore morphological characters were used to differentiate the isolated taxa. On the basis of morphological evidence, fifty targeted species were preliminarily identified and all dry fungal cultures have been deposited at the Faculty of Technology, Rajarata University of Sri Lanka as the first culture collection of freshwater fungi in Sri Lanka. In this study, our results demonstrate that the Anuradhapura district of Sri Lanka provides a diversity rich in freshwater fungal taxa in lentic aquatic environments.

***KEYWORDS***: Aquatic fungi, Anuradhapura district, lentic habitats, single spore isolation, mycological biodiversity

# HYPOGEOUS SEQUESTRATE HIGHER BASIDIOMYCETES OF TÜRKİYE AND THEIR DISTRIBUTION

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## ABSTRACT

Basidiomycota is a phylum of kingdom Fungi having more than 31.500 known species belonging to 16 classes, 52 orders, 177 families and 1589 genera. Members of the phylum are characterized by the presence of basidium bearing basidiospores, clamp connections, dolipore septa and double layered wall. Basidiomycota members can be found in almost every terrestrial environment. Fruit-bodies of many higher Basidiomycetes can be seen on ground, but some members produce their fruit-bodies partially or completely embedded in soil or humus, and referred as hypogeous. This study makes a revision on the hypogeous Basidiomycetes of Türkiye, and aims to make a contribution to the mycobiota of Türkiye by organizing the accumulated data on hypogeous basidiomycetous macromycetes growing in Türkiye. The studies on Turkish higher fungi including the members of hypogeous sequestrate Basidiomycetes were traced. Then a list of the hypogeous taxa belonging to the phylum Basidiomycota were prepared together with their habitat and distribution localities. The list was also cross-checked with the current checklists and latest contributions. As a result, 58 hypogeous sequestrate members of the higher Basidiomycetes within 23 genera and 16 families have been compiled. The taxa are listed together with their distributions in Türkiye.

***KEYWORDS***: Basidiomycota, biodiversity, hypogeous fungi, Türkiye

# CONTRIBUTIONS TO THE TURKISH MYCOBIOTA NEW GENERA RECORDS

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## ABSTRACT

To contribute to mycobiota of Türkiye was aimed in this study. The microfungi samples were collected during periodic mycological excursion from the Kazdağı National Park between 2019 and 2022. They were transferred to the laboratory and microscopic investigations were carried out. The collections were examined in distilled water and for morphological photographs Olympus SZX16 with Olympus DP digi-CAM (Japan) stereo microscope, and microphotographs Laica DM3000 LED with Leica MC190 HD digi-Cam (Germany) research microscope was used. The specimens were identified with the help of different mycology monographs and papers. As results: *Agyrium rufum*, *Curreya pithyophila*, *Fujimyces oodes*, *Kalmusia sarothamni*, *Keissleriella pinicola*, *Lojkania platani*, *Neocucurbitaria cava*, *Ne. subcaespitosa*, *Patellariopsis atrovinosa*, *Pseudolachnea hispidula*, *Sclerencoelia fAŞÇIcularis*, and *Septopatella septata* were recorded as genera first time for Turkish mycobiota. Morphological and microscopic characteristics of the records were presented depend on collected samples, and supported by macro and microphotographs. The financial support of the TUBITAK (Project no: 119Z047) is gratefully acknowledged.

***KEYWORDS***: New Records, microfungi, Kazdağı national park

# ANTIFUNGAL ACTIVITY OF SIX NANOPARTICLES OF ZINC COPPER CERIUM OXIDE SILVER GOLD AND SELENIUM AGAINST CLINICAL ISOLATES OF *ASPERGILLUS* SPECIES

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## ABSTRACT

*Aspergillus* species are found as a saprophyte agent to cause a wide variety of infections in normal to immunocompromised hosts. Antifungal resistance in *Aspergillus* isolates is increasing on a global scale. Regarding the drug resistance against *Aspergillus* species, new aspects of using extracts and nanoparticles as antifungal agents were considerable in the past few years. The antifungal activity of zinc, copper, cerium oxide, silver, gold, and selenium nanoparticles were studied against a total of 11 clinical *Aspergillus* isolates, including *A. flavus* (n=4), *A. welwitschiae* (n= 4), and *A. fumigatus* (n=3) based on the M38-A2 guideline. It showed significant minimum inhibitory concentration (MIC) in nanoparticles of zinc, silver, gold, and selenium. Unfortunately, copper and cerium oxide didn't show an inhibitory effect against *Aspergillus* isolates. The inhibitory ranges of zinc, silver, gold, and selenium nanoparticles were 128-512, 26-53, 21-85, 6-26 µg⁄ml for *A. fumigatus*; and 512, 26-53, 85, 1-13 µg⁄ml for *A. welwitschiae*, respectively. In addition, the inhibitory ranges of silver and selenium nanoparticles were 26-53 and 106-425 µg⁄ml for *A. flavus*, respectively. The zinc and gold nanoparticles didn't affect *A. flavus*. All of the studied nanoparticles showed a significant effect against *Aspergillus* isolates except the copper and cerium oxide. Among the nanoparticles, zinc, silver, gold, and selenium exhibited outstanding antifungal activity against *Aspergillus* isolates. However, silver seemed to be the most effective nanoparticle against the *Aspergillus* species.

***KEYWORDS***: Antifungal, nanoparticles, *Aspergillus*, MIC

# EUROPEAN SPECIES OF THE GENUS *MELANOLEUCA* (BASIDIOMYCOTA: AGARICALES)

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## ABSTRACT

Genus *Melanoleuca* (Agaricales, Pluteaceae) is easily distinguishable at the generic level, but is one of the taxonomically most complicated genera of Agaricomycetes, due to overlapping macro- and micromorphological characters. This is characterized by collybioid to tricholomatoid basidiomata, a convex to slightly depressed pileus, often with a low central umbo, emarginate, adnate to shortly decurrent lamellae (with tooth), amyloid ornamented basidiospores and the absence of the clamp connections. The most important specific characters are the colour of basidiomata (in some species), the colour of the context in the stipe base, the stipe surface and its superficial structures, the presence or absence of cheilo- and pleurocystidia and their type, the presence or absence of a caulohymenium, and the size and shape of basidiospores (in some species). After our extensive taxonomic revision based on both revisions of the type material and studies of the recent collections 35 taxa are recognized in Europe divided in two subgenera: subgen. *Melanoleuca* (species with well-developed macrocystidioid cheilocystidia) includes 11 species and subgen. *Urticocystis* (species with either urticoid or absent cheilocystidia) includes 23 species and one variety. The *Melanoleuca* species are delimited by macro- and micromorphological characters and by multilocus molecular data based on ITS, rpb2 and tef1 markers. The survey and characteristics of the European *Melanoleuca* species is provided.

***KEYWORDS***: Basidiomycota, phylogeny, taxonomy

# NEW STRATEGIES FOR MOLECULAR IDENTIFICATION OF *ASPERGILLUS* SECTION *NIGRI* SPECIES

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## ABSTRACT

The species of *Aspergillus* section *Nigri* are economically significant to the production of enzymes, organic acids, secondary metabolites and some fermentation processes. Some of these *Aspergillus* species are also the most common food spoilage fungi contaminating various agricultural products such as nuts, cocoa, coffee, almond and onions. Identification/classification of the species of the *Aspergillus* section *Nigri* group is quite complex and difficult due to the slight differences between them. For this reason, the polyphasic approaches (morphological, chemical and molecular methods together) are currently used, which give accurate and reliable identification, but time consuming and expensive. The use of new strategies like DNA barcoding together with Polymerase Chain Reaction (PCR)-based molecular techniques in identifying Aspergillus species enables rapid and accurate differentiation of them. DNA barcoding allows the design of species-specific primers based on the polymorphic regions of *Aspergillus* species. Our study was aimed to obtain species-specific DNA barcodes by using molecular methods based on PCR. For this purpose, the PCR studies targeting two different gene regions (β-tubulin and calmodulin gene) of *Aspergillus* species (such as *A. tubingensis*, *A. brasiliensis*, *A. welwitschiae*), which we isolated in different studies were carried out. The sequence analyses of these amplified regions were performed. Following molecular identifications based on these two different gene regions, polymorphic regions in the nucleotide sequences of these genes were determined using bioinformatics tools. In the light of the data obtained, new species-specific primers were designed based on different base regions between *Aspergillus* species. Then, the species-specific band sizes will be determined by using PCR with these new primers. Thus, it will be possible to make rapid and low-cost molecular identification independent of sequence analysis by using primers unique to these species in the future.

***KEYWORDS***: *Aspergillus* section *Nigri*, polyphasic approach, molecular identification, polymerase chain reaction, DNA Barcoding

# *FUSARIUM* SPECIES ISOLATED FROM BOZCADA CAVUS AND KARALAHNA GRAPE

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## ABSTRACT

Bozcaada is the third largest island of Türkiye, connected to Çanakkale, and is located northeast of the Aegean Sea. It has a low and flat structure geographically. Since most of Bozcaada's vegetation consists of vineyards, viticulture is the most significant source of income. It has a Mediterranean climate and due to its geographical location at the exit of the Bosporus, north and south winds are very effective. For this reason, it allows the world's most delicious Çavuş grape and various grapes to be grown. The risk of contamination of grapes with mycotoxin-producing mold species such as *Aspergillus*, *Alternaria*, and *Penicillium* is well-known. Especially *Aspergillus* species and their mycotoxins have been studied in grapes and grape products. Recently, the presence of fumonisin B2 mycotoxin in grapes and their products has been determined. Still, there is minimal information about toxinogenic *Fusarium* species in grapes and their products. For this reason, our study identified 10 *Fusarium* isolates isolated from Karalahna and Çavuş grape varieties grown in Bozcaada between 2015-2016. Identification of the isolates was made according to the guidelines of Leslie and Summerell (2006), Pitt and Hocking (2009), Samson et al. (2010), and Walsh et al. (2018). As a result, it was determined that the isolates were probably *Fusarim equiseti*, *Fusarium fujikuroi*, *Fusarium oxysporum*, *Fusarium poae*, and *Fusarium verticillioides*.

***KEYWORDS***: *Fusarium*, Bozcaada, Çavuş grape, Karalahna grape, Iden

# PULMONARY CANDIDIASIS MOLECULAR IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS

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## ABSTRACT

*Candida* species are opportunistic yeasts that can responsible for life-threatening lung infections. Although *C. albicans* is considered a major causative agent of pulmonary candidiasis, due to the rapid development of resistance to antifungal drugs, non-albicans species have increased nowadays. The objective of this study was to identify and evaluate antifungal susceptibility patterns of *Candida* isolates obtained from bronchoalveolar lavage (BAL) specimens. In this study, 36 BAL specimens were collected from suspected patients. The genomic DNA of Candida colonies was amplified by Multiplex PCR, and *Candida* species were identified based on the electrophoresis pattern. Broth microdilution, according to the M27-A3 CLSI guideline, was employed for determining the antifungal susceptibility tests of itraconazole, fluconazole, clotrimazole, sertaconazole, and terbinafine. Then, the Minimum Inhibitory Concentration (MIC) of each antifungal agent was determined separately. Of the 36 patients, 24 (66.67%) patients were male and 12 (33.33%) patients were females, with a mean age 39.7 years (range: 3-76 years); that 3 (38.46%) of them died. Among the affected patients, COVID-19 (n = 7), diabetes mellitus (n = 5), trauma (n = 3), corticosteroid user (n=2), hematological malignancies (n = 1) were major predisposing factors. The distribution of species was as follows: *C. albicans* 27 (75%); *C. glabrata* 3 (8.33%); *C. parapsilosis* 2 (5.55%); *C. tropicalis* 2 (5.55%); *C. dubliniensis* 2 (5.55%). According to the results, the MIC ranges of sertaconazole, terbinafine, fluconazole, itraconazole, and clotrimazole were estimated at 0.125-128, 0.016-4, 0.064-1, 0.032-16, and 0.016-4 µg/ml, respectively, for *C. albicans* species. Also, the MIC ranges of non-albicans isolates were order as follow: sertaconazole: 0.25-128 µg/mL, terbinafine: 0.008-0.5 µg/mL, fluconazole: 0.25-32 µg/mL, itraconazole: 0.125-16 µg/mL, clotrimazole: 0.016-16 µg/mL. The results of the study showed that *C. albicans* was the most common *Candida* isolate in the BAL specimens. Resistance to clotrimazole was rarely found, occurring in only 2.77% of all isolates. Most of the isolates were resistant to terbinafine which occurred at 60% in *C. albicans* isolates and 33.3% in non-albicans isolates.

***KEYWORDS***: Molecular identification, antifungal susceptibility, Candidiasis, BAL

# TERFEZIA SPECIES NATURAL DISTRIBUTED IN TÜRKİYE AND THEIR ECOLOGIES

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## ABSTRACT

*Terfezia* species, known as Keme, truffles, soil mushrooms and some other names in Türkiye, are classified as desert truffles because they are generally widespread in arid and semi-arid regions. Türkiye, especially Central Anatolia, Southeastern Anatolia and Eastern Anatolia regions are the natural distribution areas of *Terfezia* species. These species, which attract attention with their superior flavors and high nutritional values as well as their medicinal properties, are in great demand all over the world. More than 50 species have been identified in the *Terfezia* genus and 7 of them have been recorded in different regions of Türkiye. The two most common *Terfezia* species in Türkiye are *Terfezia claveryi* and *Terfezia bourdieri*. On the other hand, *T. olbiensis*, *T. arenaria*, *T. leptoderma*, *T. albida* and *T. cistophila* are the other species distributed in Türkiye. In this review, some morphological and microscopic features of *Terfezia* species, their distribution, ecological demands and economic values were discussed based on the present literature.

***KEYWORDS***: Ascomycetes, keme, domalan, desert truffles, habitat

# HAKKARİ (TÜRKİYE) İLİNDEN YENİ BİR *VIBRISSEACEOUS* MANTAR KAYDI

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## ABSTRACT

Vibrisseaceae familyası 1990 yılında Korf tarafından *Vibrissea*, *Chlorovibrissea* ve *Leucovibrissea* cinslerini kapsayacak biçimde düzenlenmiştir. Familya üyeleri suda veya ıslak topraklarda yaşayan, apotestumları sapsız veya uzun saplı, disk şeklinde, beyazımsı, sarımsı, kahverengimsi, zeytinimsi veya siyahımsı yeşil renkte, ektal eksipulum textura globosa, textura angularis'ten textura prismatica'ya ve filiform askosporlar ile karakterize edilir. Üç cins, apothecia rengi, ektal eksipulum yapısı ve askal apeks aparatı ile ayırt edilebilir. Bu üç cinsin yanı sıra *Acephala* ve *Phialocephala* cinsleri de yapılan son çalışmalarda bu familya içerisine dahil edilmiştir. Günümüzde familya yaklaşık 80 tür içermekte, bunlardan 2'si Acephala'da, 6'sı Chlorovibrissea'da, 1'i Leucovibrissea'da, 33'ü Phialocephala'da ve 36'sı Vibrissea içerisinde sınıflandırılmaktadır. Daha önce Türkiye'de yalnızca 2 Vibrisseaceous mantar kaydı bulunmaktadır. Bu çalışmada morfolojik verilere dayalı olarak yeni bir Vibrisseaceous mantar kaydı olan *Vibrissea decolorans* Türkiye için yeni kayıt olarak verilmiştir.

***KEYWORDS***: Vibrisseaceae, yeni kayıt, *Vibrissea decolorans*, Türkiye

# CONTRIBUTIONS TO THE TURKISH MYCOBIOTA NEW SPECIES RECORDS

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## ABSTRACT

To contribute to mycobiota of Türkiye was aimed in this study. The microfungi samples were collected during periodic mycological excursion from the Kazdağı National Park between 2019 and 2022. They were transferred to the laboratory and microscopic investigations were carried out. The collections were examined in distilled water and for morphological photographs Olympus SZX16 with Olympus DP digi-CAM (Japan) stereo microscope, and microphotographs Laica DM3000 LED with Leica MC190 HD digi-Cam (Germany) research microscope was used. The specimens were identified with the help of different mycological monographs and papers. As results: *Amphisphaeria fibricola*, *Aposphaeria multiformis*, *Camarosporium arbuti*, *Ca. salicinum*, *Chalara fusidioides*, *Coniothyrium cytisellum*, *Cryptosporella corylina*, *Cucurbitaria arbuti*, *Cu. laurina*, *Cu. rubefaciens*, *Cytospora lauri*, *Dinemasporium lanatum*, *Diplodia cydoniae*, *D. laurina*, *D. meliae*, *D. nerii*, *D. preussii*, *Lophiostoma isomerum*, *L. vagans*, *Lophodermium conigenum*, *Microdiplodia harknessii*, *M. salicina*, *Monochaetia bicornis*, *Phoma nerii*, *Phomopsis radula*, *Ph. salicina*, *Pleospora cytisi*, *Stilbospora cistina*, and *Teichospora trabicola* were recorded as species first time for Turkish mycobiota. Morphological and microscopic characteristics of the records were presented depend on collected samples, and supported by macro and microphotographs. The financial support of the TUBITAK (Project no: 119Z047) is gratefully acknowledged.

***KEYWORDS***: New Records, microfungi, Kazdağı national park

# HAKKÂRI İLİNDEN TÜRKİYE MİKOTASI İÇİN İKİ YENİ *GALERINA* KAYDI

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## ABSTRACT

*Galerina*, dünya çapında 300'den fazla türü bulunan küçük kahverengi sporlu saprotrofik bir cins olarak tanımlanır. Bu grup en çok halüsinojenik *Psilocybe* türleri ile karıştırılmaktadır. Ülkemizde şimdiye kadar tespit edilen 14 türü vardır. 2021-2022 yılları arasında Hakkâri merkez ve Çukurca ilçesinden toplanan makromantar örnekleri, arazi ve laboratuvar çalışmalarından sonra tanımlanmış ve *Galerina calyptrata* P.D. Orton ve *Galerina tibiicystis* (G.F. Atk.) Kühner türlerinin Türkiye mikotası için yeni kayıt olduğu tespit edilmiştir. Bu yeni kayıtlar ile birlikte ülkemizdeki tür sayısı 16'ya ulaşacaktır. Yeni kayıtlar ile ilgili makroskobik ve mikroskobik veriler kısa bir tartışma sunulacaktır.

***KEYWORDS***: *Galerina*, Hakkâri, Türkiye, yeni kayıt

# TÜRKİYE MİKOBİYOTASI İÇİN BİR YENİ CİNS KAYDI

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## ABSTRACT

Hakkâri ili ve Çukurca ilçesi sınırları içerisinde 2020-2022 yılları arasında farklı zaman aralıklarında çeşitli arazi çalışmaları gerçekleştirilmiştir. Bu arazi çalışmaları sonucunda bazı makromantar örnekleri toplanmıştır. Toplanan bu örneklerin morfolojik, ekolojik ve entomikolojik özellikleri kayıt altına alınmış olup örneklerin kendi doğal alanında fotoğrafları çekilmiştir. Laboratuvara taşınan örneklere gerekli mikolojik teknikler uygulanarak örneklere ait mikroskobik veriler elde edilmiştir. Bütün bu aşamalar göz önünde bulundurularak arazi ve laboratuvar çalışmaları neticesinde elde edilen bilgilerin ışığında bu makromantar örneklerinin teşhisleri yapılmıştır. Sonuç olarak *Pleuroflammula ragazziana* (Bres.) E. Horak türünün cins seviyesinde ülkemizde ilk defa tespit edildiği ve Türkiye mikobiyotası için yeni bir cins kaydı olduğu belirlenmiştir. Teşhis edilen örnekler Van Yüzüncü Yıl Üniversitesi, Fen Fakültesi, Biyoloji Bölümü Fungaryumu'nda (VANF) muhafaza edilmektedir.

***KEYWORDS***: Çukurca, Hakkâri, makromantar, Türkiye, yeni cins kaydı

# Fungal Chemistry and Biochemistry

# FUNGAL PIGMENTS AND THEIR ADVANTAGES IN INDUSTRIAL BIOTECHNOLOGY

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## ABSTRACT

The interest in the use of natural colorants has increased due to the prohibition of synthetic dyes due to their potential carcinogenic effects. Studies are reporting that especially synthetic dyes cause behavioral disorders and hyperactivity in children. The non-toxicity of natural colorants and their easy industrial production make these colorants attractive. During the production of most natural food dyes, some problems arise due to changes in the supply of raw materials and pigment extraction. Microbial pigments are a promising alternative to other additives extracted from animals and plants. Fungi are one of the interesting natural sources of pigments. The reason for the use of fungi in industrial applications is that they have a high production capacity in pigment yield. In addition, the production of natural dyes by fungi enables the producer to minimize some external factors, seasonal raw material supply problems and variations between batches and they also allow production under controlled conditions in bioreactors. In recent years, it has been observed that the interest in these pigments has increased due to their positive effects on health such as antioxidant properties. Due to their easy development and manipulation, fungi are preferred by researchers in carotenoid production. For example, *Monascus* sp. has pigments ancaflavin and monAŞÇIn (yellow), rubropunctatin and monascorubrin (orange), rubropunctamine and monascorubramine (red). These pigments have shown antihyperlipidemic effects. Many patents are also issued in Japan, the United States, France and Germany regarding the food use of Monascus pigments. Application areas in food industries include coloring processed meat, seafood such as fish paste, surimi and ketchup production. The natural pigment production from different fungi such as *Fusarium* sp., *Aspergillus* sp. is currently becoming widespread due to these advantages.

***KEYWORDS***: Fungi, pigments, *Monascus* sp., production, biotechnology

# EXTRACTION CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF CHITOSAN FROM A FUNGAL MYCELIAL WASTE

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## ABSTRACT

In this study, firstly, the extraction process of fungal chitosan from waste *Rhizomucor miehei* biomass was optimized using response surface methodology. When the concentration of NaOH, the deproteinization time and temperature, the concentration of acetic acid, the deacetylation time and temperature were 3N, 20 min, 95 °C, 4%, 6 hrs and 85 °C, respectively, high chitosan yield (70.6 mg/g) was achieved. The evaluation of the quality of chitosan from fungal mycelial waste includes parameters for the degree of deacetylation, molecular weight, antibacterial and wound healing activities. The fungal chitosan obtained under the optimized conditions showed a deacetylation degree of %78 and molecular weight of 14kDa. The antimicrobial activity of the fungal chitosan showed a broad spectrum at low concentrations against five species of bacteria and fungi. As an in vitro model, the scratch assay was performed for wound healing activity of the fungal chitosan. At five days after scratching, HaCaT cells cultured in the presence of the fungal chitosan exhibited %57 migration rate. Furthermore, starch/gelatin-based films were prepared with the extracted fungal chitosan, and some of their physical, antimicrobial and antioxidant properties were characterized. Fungal mycelial wastes would be cost effective and eco-friendly source for chitosan production and obtaining antimicrobial and antioxidant films.

***KEYWORDS***: *Rhizomucor miehei*, mycelial waste, chitosan, biopolymer, statistical modelling

# OYSTER MUSHROOM *PLEUROTUS OSTREATUS* AS A HEALTHY INGREDIENT FOR SUSTAINABLE FUNCTIONAL FOOD PRODUCTION

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## ABSTRACT

Oyster mushroom (*Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm.) is one of the most popular edible mushrooms, both wild-growing in the forests and cultivated on various materials. *Pleurotus ostreatus* contains essential nutrients in terms of human nutrition and has medicinal benefits as a natural source of both traditional and modern medicine. Nowadays, *P. ostreatus* can be used in the production of dietary supplements, mycoceuticals, and nutraceuticals. Foods, which have health benefits besides their macro and micronutrients, are called functional foods. In recent years, *P. ostreatus* has also been used in vitro studies as a food additive in the development of value-added functional foods such as meat, bakery and dairy products, traditional foods, and various alcoholic and non-alcoholic beverages. Fresh and other various forms of *P. ostreatus* were used for food fortification in terms of important nutritional and medicinal properties for human health. Moreover, it was aimed to improve sensory quality and physicochemical properties and prolong the shelf life of functional foods by adding *P. ostreatus*. In general, this review has focused on using the potential of *P. ostreatus* as a food additive in sustainable functional food production and the effects of its utilization on food quality.

***KEYWORDS***: *Pleurotus ostreatus*, functional food, nutrient, biological activity, food quality

# THE BIOTECHNOLOGICAL POTENTIAL OF THE MACROFUNGI ISOLATES OBTAINED FROM BILECIK PROVINCE

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## ABSTRACT

A study on macrofungi diversity in Bilecik province has been initiated. In this study, the biotechnological potential of the macrofungi isolates obtained from Bilecik province was investigated in three different subjects. Totally 19 macrofungi isolates were screened for their biodegradation, biotechnologically important enzyme and plant growth regulator production capacities including *Fomitopsis pinicola*, *Gloeophyllum abietinum*, *G. trabeum*, *Macrolepiota procera*, *Trametes versicolor*, *T. hirsuta*, *Phaeoclavulina flaccida*, *Phellinus igniarius*, *Psathyrella tephrophylla*, *Tricholomopsis rutilans*, *Gymnopus* sp., and *Lentinellus* sp. To represent intraspecific diversity, different isolates of *G. abietinum*, *G. trabeum*, and *T. hirsuta* species were included the study. The activities of laccase, amylase, protease, tyrosinase, and urease as biotechnologically important enzymes were compared. *Gymnopus* sp. BY7, *Gloeophyllum abietinum* BG22, *Tricholomopsis rutilans* BP 10, *Macrolepiota procera* BP25, and *Psathyrella tephrophylla* BG57 isolates have best enzyme production capacities, respectively. In order to determine plant growth regulator (PGR) production capacity, gibberellic acid (GA) and indole acetic acid (IAA) amounts in the extracts of the growth media of the isolates were determined spectophotometrically. As a first record, *T. hirsuta* BG85 was the top producer, 430 and 824 mg/L, respectively. On the other hand, other *T. hirsuta* isolates BG106, BG86, and BP76, also produced GA and IAA in the range of 141-315 mg/L and 111-499 mg/L, respectively, representing intraspecific diversity. The commercial dye RBBR, malachite green and amaranth degradation capacity of *Lentinellus* sp.BG51, *Gymnopus* sp.BY7, and *Gymnopus* sp.BY8 were the highest according to degradation index.

***KEYWORDS***: Bilecik, degradation, enzyme, macrofungi, plant growth regulator

# TÜRKİYE'DEN ZEHİRLİ İKİ MANTAR TÜRÜ *RUSSULA EMETICA* SCHAEFF PERS VE *BOLETUS SATANAS* LENZ'IN ANTİMİKROBİYAL ANTİOKSİDAN AKTİVİTELERİ VE ENZİM İNHİBİSYONU

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## ABSTRACT

Ülkemizin farklı bölgelerinden toplanan, sırasıyla Russulaceae ve Boletaceae familyalarına ait iki zehirli mantar türü *Russula emetica* (Schaeff.) Pers. ve *Boletus satanas* Lenz'ın metanol ve su ekstreleri ultrasonik su banyosu ekstraksiyonu yöntemiyle elde edilip, bu çalışmada iki mantar türünün farklı ekstre tiplerinin antioksidan, antimikrobiyal aktiviteleri ve enzim ihnibisyonunu belirlemek amaçlanmıştır. Yapmış olduğumuz çalışmada elde ettiğimiz sonuçlara göre, en iyi veriler tirozinaz inhibisyon aktivitesi (REW IC50 = 9 ± 0,007 / KAE 58,39 ± 0,46 µg/mL), ABTS radikal süpürücü aktivitesi (BSW IC50 = 282,5 ± 0,72 / TE 84,83 ± 1,16 µg/mL) ve total flavonoid içeriği (BSW 519 ± 507 / RE 209 ± 0,42 µg/mL)'de tespit edilmiştir. Bu ekstrelerin antimikrobiyal aktivitelerini belirlemek için sıvı mikrodilüsyon metodu kullanılmıştır. *R. emetica*'nın metanol ekstresi *Pseudomonas aeruginosa*'ya 1,56 mg/ml dozunda etkiliyken, *Staphylococcus aureus* ve *Sarcina lutea*'ya ise 3,12 mg/ml dozunda zayıf bir antimikrobiyal etki göstermiştir. Çalışılan diğer mikroorganizmalara karşı ise herhangi bir aktivite göstermediği tespit edilmiştir. *B. satanas*'ın metanol ekstresinin *Escherichia coli*, *Pseudomonas aeruginosa* ve *Sarcina lutea*'ya 1,56 mg/ml dozunda, *Staphyloccus aureus*'a ise 3,12 mg/ml dozunda zayıf antimikrobiyal aktivite gösterdiği tespit edilirken çalışılan diğer mikroorganizmalara karşı herhangi bir aktivite saptanmamıştır. *R. emetica* ve *B. satanas*'ın su ekstrelerinin ise hiçbir mikroorganizmaya karşı antimikrobiyal aktivite göstermediği görülmüştür.

***KEYWORDS***: Antioksidan aktivite, enzim inhibisyonu, antimikrobiyal aktivite, *Boletus*, *Russula*

# FUNGAL BIOTECHNOLOGY OF TÜRKİYE

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## ABSTRACT

Fungi are ecological, morphological, metabolically and phylogenetic diverse and are a source of many bioactive molecules. Fungi can convert organic materials into various useful products by using their enzymes. Therefore, Fungi are a group of biotechnological valuable organisms that have not been adequately studied. Because fungi live in a wide variety of habitats and have to compete with various other fungi, bacteria and animals, they have developed numerous survival mechanisms. These properties hold great promise for applications in biotechnology and industry. In addition, the relatively easy growth of fungi allows for biotechnological appropriate scale production. The exploration of fungal biodiversity and the construction of a living mushroom collection will have incredible economic potential in finding organisms with new industrial uses that will lead to new crops. The purpose of this review is to review the potential uses of mushrooms in biotechnology and to emphasize the biotechnological importance of fungi by referring to the studies done in this field in the world and Türkiye. In addition, strategies against human and plant diseases, agriculture and forestry, food and beverages from mushrooms, mushrooms grown in compost, saving our planet, to contribute to the Turkish biotechnology industry by giving examples of commodities and future topics.

***KEYWORDS***: Biotechnology, Türkiye

# EVALUATION OF B GALACTOSIDASE ENZYMES PRODUCTION BY *ASPERGILLUS* SECTION *NIGRI* SPECIES ISOLATED FROM DRIED FIGS

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## ABSTRACT

Lactose intolerance is the most common carbohydrate absorption disorder in individuals. When the percentage distribution of lactose intolerance by countries is analyzed, it is seen that it is quite common in Türkiye (70-80%). Currently, the β-galactosidase enzyme is frequently used in the production of products that individuals with lactose intolerance can consume. The enzyme β-galactosidase breaks down lactose into glucose and galactose in general. In addition to preventing lactose intolerance, this enzyme also draws attention to its production of prebiotics (galactooligosaccharides, lactulose and lactosucrose). The facts that the enzymes obtained from *Aspergillus* species have extracellular character, high thermal tolerance and activity at low pH compared to microorganisms from other taxonomic groups are important in terms of being more applicable in the industry. This study aimed to determine the β-galactosidase enzyme production potential of *A. brasiliensis*, *A.tubingensis* and *A. welwitschiae* which were identified at the molecular level and did not produce mycotoxins isolated from dried figs. Little or no studies have been found in the literature on the production of β-galactosidase for these three species. In this study, a total of 48 strains for β-galactosidase activity were screened by the "rapid plate scanning method" using 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) as a substrate. As a result of the study, 29.16% (n=14) of the strains had a high level of the enzyme, 16.66% (n=8) had a moderate level, 16.66% (n=8) had a weak level, and it was determined that 14.58% (n=7) of them produced a very weak enzyme and 22.91% (n=11) of them did not produce the enzyme. The earliest enzyme activity was detected on the 4th day, while the latest activity was observed on the 8th day. Screening the potential of *Aspergillus* species to produce β-galactosidase medical enzyme and identifying new producers producing this enzyme is promising for lactose intolerance disease.

***KEYWORDS***: β-Galactosidase, lactose intolerance, *Aspergillus*

# POSSIBILITIES OF USING MYCONANOTECHNOLOGY IN COMBATING *VERTICILLIUM DAHLIAE* FUNGAL PATHOGEN

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## ABSTRACT

*Verticillium dahliae* is a soil-borne plant pathogen and its control is limited with chemical fungicides. The disease-causing fungus, which causes significant economic agricultural losses all over the world, has a very wide host range. The scarcity of successful methods in the control of the disease further increases the importance of the disease. In addition, the effort to develop alternative control methods is based on the inadequacy of environmentally friendly fungicides, the emergence of fungicide resistance in pathogens and the breaking of host resistance of pathogen populations. Today, the popularity of nanoparticle applications, which provide better efficiency in the control of diseases, is increasing day by day. The current research aims to synthesize a bionanomaterial (RDEK) with the wild mushroom *Russula delica* methanol extract (RDE) without using any hazardous substances, with a new, simple, cost-effective and environmentally friendly method, and to point out the use of this synthesized material in agricultural applications as an antifungal agent for the first time. aims. In the study, it was determined that RDE and RDEK applied in different concentrations showed antifungal activity against *Verticillium dahlia*. RDEK bionanomaterial, which shows the best activity at 0.02 mg/ml concentration, can be evaluated as a fungicide in agricultural applications.

***KEYWORDS***: *Verticillium dahlia*, *Russula delica*, bionanometrical, fungicide

# KESTEL BÖLGESİNDE (KADINHANI KONYA) YAYGIN OLARAK YETİŞEN BAZI MAKROMANTARLARIN MİNERAL KOMPOZİSYONU

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## ABSTRACT

Canlı organizmalar içinde ayrı bir alem olarak yer alan özellikle makromantarlar vitamin, mineral ve protein özellikleri bakımından sağlıklı bir diyet besini olarak beslenme zincirinde yer almaktadırlar. Bitkilerde olduğu gibi mantarlarda da elementlerin miktarı yetiştikleri substrata bağlı olarak değişiklik göstermektedir. Bu nedenle besin olarak tüketilen mantarların yetiştikleri alanların kirlenmemiş ve sağlık açısından temiz alanlar olmasına dikkat edilmesi gerekmektedir. Substratta bulunan ağır metaller ve makro/mikro elementler mantarların akümülatif özelliğinden dolayı mantar yapısına geçebilmektedir. Bu çalışmada Kestel Bölgesinde (Kadınhanı-Konya) yaygın olarak yetişen makromantarların ve yetiştikleri topraktaki mineral kompozisyonları ve aralarındaki korelasyonun incelenmesi amaçlanmıştır. Bu maksatla bölgede yapılan arazi çalışmaları sonucunda tespit edilen makrofunguslar "*Macrolepiota procera* (Scop.) Singer (Şemsiye Mantarı), *Suillus collinitus* (Fr.) Kurtze (Benekli Süngermantarı), *Boletus edulis* Bull. (Çörek Mantarı)" türleridir. Arazi çalışmalarında elde edilen mantar örneklerinin ve yetiştikleri substratlara ait toprak örnekleri ayrı ayrı polietilen torbalar içinde alınarak, laboratuvar getirilmiştir. Makromantarların ve toprak örneklerinin mineral madde analizinde ICP-MS cihazı kullanılarak 65 elementin varlığı PPB (Parts Per Billion=Milyarda bir) oranında araştırılmıştır. Araştırılan numunelerden üç farklı toprak örneğinde en yüksek seviyede sırasıyla Al, K, Ca, Mg, Na elementleri, en düşük seviyelerde ise Re (Renyum), Ru (Rutenyum), Pt (Platin), Ta (Tantal) ve Te (Tellür) elementleri tespit edilmiştir. Buna karşılık elde edilen mantar örneklerinde ise en yüksek oranda sırasıyla K, Mg, Na, Al, B elementleri, en az seviyede ise Tm (Tulyum), Lu (Lutesyum), Tb (Terbiyum), Ho (Holmiyum), U (Uranyum) elementleri tespit edilmiştir. Araştırılan elementlerden hem toprakta hem de mantar örneklerinde rastlanmayanlar ise Sc (Skandiyum) ve Ir (İridyum) elementleridir. Ti (Titanyum), V (Vanadyum), Ga (Galyum) Re (Renyum) elementleri ise toprakta tespit edilip mantarlarda rastlanmamıştır. Sonuç olarak; toprakta bulunan elementlerin yoğunlukları ile mantar örneklerindeki element yoğunlukları pozitif yönde korelasyon göstermektedir. Bundan dolayı ağır metal kirliliği veya endüstriyel kirlilik bulunan alanlarda yetişen makromantarların besin olarak tüketilmelerinin sağlık açısından uygun olmadığı yönünde değerlendirme yapılmıştır.

***KEYWORDS***: Kestel, Kadinhani, Konya, makromantar, mineral kompozisyonu, Icp-Ms

# Mushroom Cultivation

# EVALUATION OF WASTE MUSHROOM COMPOSTS AS PLANT GROWING MEDIA

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## ABSTRACT

After mushroom cultivation, the partially degraded paddy or wheat straw and other agricultural waste, have been defined as spent Mushroom Compost (SMC). These products are valuable by-products of edible mushroom cultivation. After the mushroom harvest, storage of this spent substrate may cause environmental contamination. Using waste mushroom compost as organic fertilizer is one of the best solutions to evaluate it beneficially. SMC has rich organic matter, moderate nutrient content, near-neutral pH and beneficial microbial population. Due to these properties, SMC can be turned into a quality fertilizer for crops. In addition, the use of SMC as organic amendments contributes to the use of residues in an environmentally friendly way and increases soil fertility and crop yields.

***KEYWORDS***: Mushroom compost, spent, evaluate, soil, crop

# USE OF BIOGAS WASTE FERMENTED CORN SILAGE AS A CASING MATERIAL IN THE PRODUCTION OF CULTIVATED MUSHROOMS *AGARICUS BISPORUS*

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## ABSTRACT

The expectations of the enterprises, which have kept energy resources and energy production on the agenda, especially in recent years, have directed many researchers to studies in this field. In recent years, energy production with renewable energy resources is getting popular in Türkiye. Of these, biogas, which can be obtained by the processing of organic wastes, is a clean, environmentally friendly and efficient energy resource. The wastes of biogas production can also be used as fertilizer in agriculture. In this research, effects of fermented corn silage, considered as an alternative or supplementary casing soil material to turf and obtained from biogas production, on yield and quality of mushroom quality was investigated by means of individual or mixed usage with turf. In the first season, five casing soil treatments prepared with fermented corn silage (BCS) and turf (T) as volumetric mixtures, namely 100%T (control treatment), 25%BCS + 75%T, 50%BCS + 50%T, 75%BCS + 25%T and 100%BCS was tested. The results of first season showed that, yield obtained from 25%BCS + 75%T and 50%BCS + 50%T mixtures were 52% and 8% higher than control treatment. Moreover, the same mixtures provided 51% and 27% increase in mushroom number, when compared to control treatment, also. In the second season, 10%BCS + 90%T, 20%BCS + 80%T, 30%BCS + 70%T, 40%BCS + 60%T, 50%BCS + 50%T mixtures together with 100%T (control treatment) were selected as treatments to be tested in the light of the first season's results. The highest yield was obtained from 20%BCS + 80%T mixture. On the other hand, 10%BCS + 90%T and 30%BCS + 70%T mixtures had higher yield than control treatment. Only the differences in cap height were statistically significant in the first season. Other quality parameters such as cap diameter, stalk diameter and stalk height were not significant in both seasons. By considering the rapid development of mushroom sector in our country, mixture of fermented corn silage with main casing soil turf can help to reduce the increasing turf demand and provide higher economic income by means of higher yields for mushroom producers.

***KEYWORDS***: *Agaricus bisporus*, biogas waste fermented corn silage, casing soil, yield, quality

# HASAT SONRASI KESTANE MANTARLARA *AGARICUS CAMPESTRIS* UYGULANAN MELATONİN VE JELATİNİN TOPLAM FENOLİK VE ANTİOKSİDAN KAPASİTESİ İÇERİĞİ ÜZERİNE ETKİSİ

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## ABSTRACT

Mantarlar tokoferoller, organik asitler, karotenoidler, fenolik bileşikler ve antioksidan bileşiklerin kaynağı olduğu bilinmektedir. Özellikle fenolik bileşikler ve antioksidanlar mantarların kalite özelliklerinin korunmasında belirleyici bir rol oynamakla beraber hasat sonrası depolama süresince kalite kontrolünde de önemli bir role sahiptir. Deneme için, ticari bir şirketten kompost satın alındı. Mantarlar, Van Yüzüncü Yıl Üniversitesi Mantar Araştırma Merkezi'ne ait yetiştirme koşullarının kontrol edilebildiği odalarda yetiştirildi. Mantarlar başarılı bir şekilde yetiştirilip hasat edildikten sonra, kararma belirtisi ve mekanik hasarı olmayan aynı boyuttaki mantarlar seçildi. Yenilebilir kaplama olan jelatin (%1), melatonin (0.5 mM) ve melatonin (0.5 mM) + Jelatin (%1) hazırlandıktan sonra mantarlar dört gruba ayrıldı. Birinci grup kontrol olarak ultra saf suya, ikinci grup mantarlar jelatin (%1) kaplama solüsyonuna, üçüncü grup mantarlar melatonin (0.5 mM) solüsyonuna ve dördüncü grup mantarlar melatonin (0.5 mM) + Jelatin (%1) daldırıldı. Uygulamalardan sonra tüm örnekler vantilatör yardımıyla soğuk hava deposunda kurutuldu. Daha sonra mantarlar köpük tabaklara (her paket 300 g) yerleştirilip üzeri streç filmle (sekiz mikron) kaplanarak 16 gün boyunca 4 °C sıcaklıkta ve %85-90 bağıl nemde depolandı. Mevcut çalışmanın amacı, yukarda bahsi geçene uygulamaların depolama süresince mantarlarda toplam fenolik içeriği ve antioksidan kapasitesi üzerine etkisini araştırmaktır. Çalışmadan elde edilen bulgulara göre, uygulama yapılan mantarlarda kontrol örneklerine kıyasla toplama fenolik içeriğinde ve antioksidan kapasitesinde artışa neden olduğu tespit edildi. Nitekim, en yüksek toplam fenolik içeriği ve antioksidan kapasitesi sırasıyla melatonin (0.5 mM) + Jelatin (%1), melatonin (0.5 mM) ve Jelatin (%1) uygulamalarından elde edildi. Çalışmada incelenen parametrelerde, kontrol ile uygulama yapılan örneklerde arasında istatistiki olarak önemli farklılıklar gözlemlendi ($p < 0.05$). Sonuç olarak, yukarda bahsi uygulamaların mantarlarda bulunan toplam fenolik içeriği ve antioksidan kapasitesi üzerine olumlu etki yarattığı söylenebilir.

***KEYWORDS***: Depolama, kestane mantarı, yenilebilir kaplama, melatonin, kalite

# A RESEARCH ON THE CULTIVATION OF *PLEUROTUS NEBRODENSIS* INZENGA QUEL

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## ABSTRACT

In this study, the cultural possibilities of *P. nebrodensis* collected from the vicinity of Tunceli-Ovacık (28.05.2019) and identified as molecular (GenBank: HM998799.1) were investigated. For the propagation of the main culture, 2.0% malt-extract agar was used whereas wheat grains were used for the propagation of spawn. For the formation of basidiocarp, bean pods (*Phaseolus vulgaris* L. (BP) residues were used as culture media. It was determined that the mycelium of *P. nebrodensis* completely covered the malt-extract agar medium in 18 days, wheat grains in 15 days, and bean pod waste (1 kg) in 17 days at 25ºC. After the substrate was fully covered with mycelia and for primordium stimulation, the incubation room conditions were set as the temperature at 14±1°C, humidity at 85–90%, light intensity at 1000 lux (12 h a day), and aeration at 2-3 h daily. The cultures were irrigated twice daily by spraying with water. The first primordium formation of *P. nebrodensis* was observed on the 42nd day, the second primordium on the 71st day, the first harvest on the 58th day, and the second harvest on the 87th day. The yield obtained at the end of the first harvest was 80 g, and the yield obtained at the end of the second harvest was determined as 40 g. In conclusion, basidiocarp of *P. nebrodensis*, which is not cultivated in Türkiye, was obtained and the growing conditions were determined.

***KEYWORDS***: *Pleurotus nebrodensis*, tissue culture, mushroom cultivation, kınkor-kırkor mushroom

# UTILIZATION OF AGRICULTURAL AND FORESTRY BY PRODUCTS IN *GANODERMA LUCIDUM* CURT FR P KARST PRODUCTION

***FUNDA ATİLA***

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## ABSTRACT

The aim of the study was to evaluate the use of some local agricultural and forestry by-products as *Ganoderma lucidum* growing subsrate. In the study, 8 different growing media prepared by mixing agricultural and forestry wastes such as oak sawdust (OS), poplar sawdust (PS), wheat straw (WS), wood chips (WCH), green almond hulls (AH) and green walnut hulls alone or in different proportions, were used for G. lucidum cultivation and the effects of these substrates on spawn run time (SRT), days to pinhead initiation (DPI), days to the first harvest (DFH), yield (g/kg) and biological efficiency (BE%) were investigated. The results demonstrated that spawn run time (SRT) of *G. lucidum* was shorter in PS and WS: GWH12.5 (19.7 days and 20.8 days) as compared to other substrates and mixtures (22.2-28.5 days). The pinheads emerged in substrates between 43th- 49th days and the fruiting bodies were collected between 80.3 -93.5 days after the spawning. Total fresh mushroom yield and BE (%) varied from 71.39 to 110.10 g/kg of substrate and from 21.0% to 31.46, respectively. Moreover, PS substrate produced fruitbody also earlier compared with the other investigated substrates. The study revealed that although higher total yields were obtained by sawdust substrates, considering the difficulty of finding sawdust in some areas, WS the mixture of WS and GHW (87.5:12.5%) could be also successfully used in of *G. lucidum* production.

***KEYWORDS***: Reishi, sawdust, agricultural wastes, forestry wastes, mushroom yield

# EFFECTS OF DIFFERENT PLANT WASTES ON SOME SOIL PROPERTIES

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## ABSTRACT

Many wastes during harvesting and after processing of agricultural products emerges. In many studies, it has been reported that organic matter residues in the surface soil greatly affect the physical and chemical properties of mineral soils. Plant litter is a primary nutrient source for saprophytic microbiota in soils. Quantity and properties of plant litter strongly influence the formation and humification of soil organic matter in terrestrial ecosystems. Peanut shell, grape pockets, hazelnut husk, tea waste, olive pellet, sugar beet pulp, rice husk, straw, sawdust, tree bark are listed among these plant wastes. The accelerated decomposition of plant wastes and organic matter by soil organisms improve soil fertility by releasing mineral elements in the forms that are available for uptake by plants Sustainable functioning of soils, which are the basic elements of agricultural production, can only be possible by preserving and even improving basic soil characteristics and genetic capacities. Organic matter plays an essential role in the sustainability of farming systems. The use of organic and green manures, compost and similar products, and animal and vegetable wastes added to the soil afterwards are beneficial for increasing the amount of both soil organic matter and soil microorganism's activities. Using of organic amendments has been found efficient for improving soil physical and chemical properties such as aggregation, structure, total porosity, bulk density, electrical conductivity, moisture holding capacity, permanent wilting point of soils, and increasing soil microbial activity and cation exchange capacity, and finally crop yields.

***KEYWORDS***: Pant wastes, physical, chemical, biological soil properties

# PROBLEMS DETECTED IN TRUFFLE CULTIVATION IN TÜRKİYE

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## ABSTRACT

Truffles, one of the edible mushrooms; It is a mushroom that has been known to people since ancient times owing to their taste, smell and unique aroma and has a privileged place for gourmets. Today, Truffle sp.; It has been one of the most attractive mushroom species due to its dependence on nature, its long-term cultivation and its high price. Appropriate techniques should be determined very well in cultivating and developing the production of truffles, which have an important contribution to the economy of our country and are important in terms of biodiversity. The aim of this study is to emphasize the importance of the problems observed in truffle cultivation, which is a new investment area for our country. Briefly, the most important of the problems determined in truffle cultivation determined in this research: Due to the fact that truffle cultivation is a newly developing agricultural practice in our country, the problems that will arise and the necessary precautions for their solutions cannot be taken in advance; Due to the fact that truffle cultivation is affected by the underground flora and the risk of contamination is high, it is not known enough that the grown seedlings must be certified; The fact that truffle-grafted seedlings are not widely certified and sold in our country; In our country, the right techniques are not known enough about truffle cultivation and there are not enough experienced experts to teach the right techniques for truffle collectors. As to be success in truffle cultivation is dependent on knowledge, experience, use of advanced technologies, and controls.

***KEYWORDS***: Truffle cultivation, *Tuber* sp., Türkiye

# Molecular Fungal Taxonomy

# MOLECULAR AND MORPHOLOGICAL IDENTIFICATION OF *LEUCOAGARICUS LIDENSIS* FROM TÜRKİYE

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## ABSTRACT

The genus *Leucoagaricus* (Agaricaceae) is a saprophytic macrofungus that grows in the temperate climatic zone of the world. *Leucoagaricus* species have a slender and fragile basidiomata, a smooth or scaly pileal surface, free lamellae, simple annulus, dextrinoid and metachromatic basidiospores, with cheilocystidia, and hyphae without clamp connections. In this study, *Leucoagaricus lidensis* is described a new record species from Türkiye. The fresh basiomata was collected from Hakkâri/Türkiye in 2018. Macromorphological characters were recorded in the field. Micromorphological features were examined and measured with the Leica Application Suite (version 3.4.0) programme. The nuclear ribosomal internal transcribed spacer (nrITS) region was used to infer the species relationships as molecularly. The best matched sequences were selected from GenBank and UNITE databases to construct phylogenetic tree. Phylogenetic reconstruction was performed on the ITS nucleotide matrix, by Bayesian MCMC and Maximum Likelihood methods. In the phylograms, *Leucoagaricus lidensis* grouped with its representatives in section *Rubrotincti* with strongly support values. It is recognized by its whitish cap with pink-lilac centre accompanied by a minute central umbo; whitish annulus; subamygdaloid, metachromatic, dextrinoid spores; cylindrical to claviform cheilocystidia. *Leucoagaricus lidensis* indicated close phylogenetic relations with *La. truncates* and *La. purpureolilacinus*. However, *La. purpureolilacinus* is separated having a purplish brown pileus and clavate to subfusiform cheilocystidia while *La. truncatus* is differentiated carrying a truncated pileus with orange–white to gray–orange squamules and larger basidiospores.

***KEYWORDS***: DNA, ITS, fungal taxonomy, molecular identification, Agaricales

# IDENTIFICATION OF *HELVELLA* SPP WITH MOLECULAR TOOLS COMPARISON OF BARCODE AND PROTEIN CODING REGIONS

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## ABSTRACT

The phylogenetic position of the ten *Helvella* species using a two locus of nuclear DNA was assessed. The phylogenetic analyses were performed with Maximum likelihood and Bayesian inference methods based on the data set of ITS1-5.8S-ITS2 (ITS), and translation elongation factor 1-alpha (TEF1-α), and the results show that TEF1-α region is more informative than ITS to delimit *Helvella* species. Alignment of the TEF1-α sequences was easier due to less frequent indels and consistent variation than those of ITS and TEF1-α provide well resolution in *Helvella* spp. This gene was also support morphological differences of the species and indicated to be a more suitable marker compared to ITS which show difficulties in amplification, sequencing and alignment.

***KEYWORDS***: *Helvella*, barcode, ITS, TEF-1α

# DETERMINATION OF *PLEUROTUS ERYNGII* COMPLEX SPECIES GROWN IN EASTERN REGION OF TÜRKİYE

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## ABSTRACT

This study, it was aimed to identify the *Pleurotus eryngii* species complex grown naturally in the Eastern Region of Türkiye (Van, Şırnak, Hakkâri, Muş, Bingöl, Elazığ, Tunceli, Kahramanmaraş, Erzincan, Erzurum, Bitlis), used as food sources and sold commercially by local people. In the studies conducted between 2007-2022, and molecular identification of 60 morphological different samples was made and the obtained DNA sequence information was subjected to BlastN analysis via GenBank. As a result, four taxa belonging to the *P. eryngii* species complex were identified. According to molecular data, these taxa are *Pleurotus eryngii* var. *eryngii* (DC ex Fr.) Quel., *P. eryngii* var. *ferulae* Lanzi, *P. nebrodensis* (Inzenga) Sacc. and *P. fossulatus* Cooke.

***KEYWORDS***: *Pleurotus eryngii* complex species, macrofungi, molecular analysis

# MORPHO-MOLECULAR EVIDENCES REVEAL TWO NOTEWORTHY *HEBELOMA* (BASIDIOMYCOTA) SPECIES FROM TÜRKİYE

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## ABSTRACT

*Hebeloma* (Agaricales) is a mostly poisonous and ectomycorrhizal genus growing in arctic and alpine habitats. Member of the genus is characterized by mostly two-colored plano-convex pileus, pale brown lamellae, the existence of a veil, and most evident cheilocystidia. In the present study, *Hebeloma vaccinum* is newly recorded and *Hebeloma nauseosum* is confirmed for the Turkish Mycota using morphological and molecular data. Fresh basidiomata were collected from Bingöl and Hakkâri provinces of Türkiye during rainy season of the year 2017-2018. Macromorphological characters were recorded in the field. Micromorphological structures were examined and measured with the Leica Application Suite (version 3.4.0) programme. The nuclear ribosomal internal transcribed spacer (nrITS) and translation elongation factor 1-alpha (TEF1-α) regions were used to infer the species relationships as molecularly. The best matched sequences were selected from GenBank and UNITE databases to construct phylogenetic tree. The tree inference was performed with Maximum Likelihood (ML) and Bayesian Inference (BI) methods. In the phylograms, *Hebeloma vaccinum* clustered with its representatives in section *Denudata* with high support values. The species has clavate to lageniform cheilocystidia and strongly dextrinoid spores typical for the section *Denudata*. The main characters of the species are brown to reddish brown pileus, cream stipe and distinctly finely verrucose, strongly dextrinoid spores. *Hebeloma nauseosum* is molecularly distinct and forms well supported clade with related species in section *Saccharіolentia*. The sweetish smell is one of the most remarkable features that places *H. nauseosum* in this section. Moreover, slightly to distinctly viscid and smooth pileus differentiate it from the other members of the section.

***KEYWORDS***: Hymenogastraceae, ITS, molecular taxonomy, TEF1-α, phylogeny

# *INFUNDIBULICYBE ALKALIVIOLASCENS* (BASIDIOMYCOTA: AGARICALES) FROM AEGEAN REGION TÜRKİYE

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## ABSTRACT

*Infundibulicybe alkaliviolascens* has been reported only from one location in Trabzon Province (Türkiye) before the present study. *Infundibulicybe* specimen was collected during the field studies between 2013 and 2016. The species is presented as a new locality record in Denizli Province based on the taxonomic and molecular phylogenetic data with the DNA sequences corresponding to internal transcribed spacer (ITS) region. Descriptions of the species, color photograph, drawings and habitat information are also provided. According to the results of the phylogenetic analysis, *Infundibulicybe alkaliviolascens* formed a well-supported monophyletic clade, sister to *I. kotanensis* and also closely related to *I. bresadolana* and *I. mediterranea*.

***KEYWORDS***: Agaricomycetes, ITS, biodiversity, distribution, phylogeny, taxonomy

# A NEW LOCALITY RECORD OF *SINGEROCYBE PHAEOPHTHALMA* IN TÜRKİYE EVIDENCE FROM MOLECULAR AND MORPHOLOGICAL DATA

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## ABSTRACT

*Singerocybe phaeophthalma* belongs to the *Singerocybe* genus. The species has only been recorded from Kocaeli Province in Türkiye so far. New collections of *Singerocybe phaeophthalma* specimens were collected and described from the second location Aegean Region of Türkiye. Macro and microscopic descriptions of the presented species, drawings of micro-structures, photograph and habitat information are given. In additon, the first ITS and LSU sequences of the species are presented from this country.

***KEYWORDS***: Molecular phylogeny, biodiversity, ITS, LSU, habitat, new locality

# MOLECULAR PHYLOGENY AND MORPHOLOGY OF *CORTINARIUS DENSECONNATUS* (BASIDIOMYCOTA) FROM TÜRKİYE

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## ABSTRACT

*Cortinarius* (Pers.) Gray is a taxonomically complex genus of Cortinariaceae showing a high degree of morphological variability. Therefore, combination of phenotype characters and DNA barcoding marker are needed for species delimitation, recognition and identification of this genus. In the present study, *Cortinarius denseconnatus* is newly recorded for the Turkish Mycota using morphological and molecular data. Fresh basidiomata were collected from Hakkâri province of Türkiye in 2013. Macroscopic descriptions were made from fresh basidiomata and microscopic features were examined from dried material using the Leica Application Suite (version 3.4.0) programme. A primary fungal DNA barcode, nuclear ribosomal internal transcribed spacer (nrITS), was used for molecular identification. The best matched sequences were selected from GenBank and UNITE databases to construct phylogenetic tree. The phylogenetic history was inferred using the Maximum Likelihood (ML) and Bayesian Inference (BI) methods. In the phylograms, *Cortinarius denseconnatus* clustered with its representatives in section *Saturninus* with high support values. The species is originially described from France and collected from frondose woodland. The species is characterized by orange, brown-orange color, smooth, convex, umbonate with rolled margin pileus, cream brown adnate lamelles, cylindrical-fusiform stipe, 9-10 × 5-6 µm spores, and grow on deciduous shrubs in clusters.

***KEYWORDS***: Agaricales, ITS, molecular taxonomy, phylogeny

# Mycorrhiza

# A NEW TECHNIQUE FOR ENUMERATION OF ARBUSCULAR MYCORRHIZAL FUNGUS AMF SPORES FROM SOIL ULTRASONICATION TECHNIQUE

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## ABSTRACT

The spore bank dynamics produced by arbuscular mycorrhizal fungi (AMF) have significant potential for sustainable ecosystem management and protection. For measuring the AMF spore bank dynamics, it is necessary to detect and determine AMF spore populations in the rhizosphere soil. Generally, the most common method for detection and isolation of AMF spores from the soil is wet sieving (WS) and centrifugation at sucrose solution method. However, this method has many disadvantages including takes a lot of time, loses a lot of spores, does not provide a clean environment for spore enumeration, uses sucrose solution and wastes a lot of water. To overcome these problems, we first modified this method and obtained good results in the number of AMF spores with these modified techniques (MT), but, in the next steps, we developed a new method to overcome some other challenges as the Ultrasonication method (UT). For comparison of these three techniques and validation of our modified method we used different host plants rhizosphere soils including wheat (*Triticum aestivum* L.), Bean (*Phaseolus vulgaris* L.), tomato (*Solanum lycopersicum* L.), pepper (*Piper nigrum* L.), parsley ((*Petroselinum crispum* (Mill.) Fuss)) and turfgrass (*Lolium perenne* L.) collected from different regions. In all three methods, 20 ml of AMF spore suspensions were obtained. The number of AMF spores was determined in 1ml of spore suspension under stereomicroscope and calculated using a mathematical formula on 1 soil sample. The results showed that the highest number of spores were observed bu using the modified technique (UT) rather than other two techniques (MT and WS) In this modified technique, sieves are not used and only the ultrasonic water bath is needed. Also, this technique is so fast than other methods, gives more and clean spores and not needed to more chemicals and water. By using of developed mathematical formula, the number of AMF spores in 1 g soil sample could be easily determined rather than other previously methods. This modified new technique is suggested for better and easily isolation and enumeration of AMF spore population assays in future researches.

***KEYWORDS***: Arbuscular mycorrhizal fungi (AMF), spore bank dynamics, spore numbers assay, wet sieving technique

# IDENTIFICATION OF SUBTILISIN GENES IN DERMATOPHYTES IN ARDABIL IRAN

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## ABSTRACT

Dermatophytes are the keratinophilic pathogenic fungus that infects both humans and animals. Keratinase enzymes are important virulence factors that play critical roles in the invasive stage of dermatophyte infection. In the present study, we used PCR techniques to evaluate the presence of genes encoding subtilisins (SUB) in four dermatophyte species including *T. mentagrophytes*, *T. rubrum*, *E. flocossum*, and *M. canis*. Clinical specimens obtained from patients suspected of dermatophytosis referred to mycology laboratory were subjected to direct examination and culture. PCR technique using panfungal primers (ITS1 and ITS4) targeting the ITS region of the rDNA gene was used to confirm the identity of dermatophyte isolates. Finally, specific primers were used to determine the presence of SUB gene families in clinical isolates were evaluated. Sixty-two dermatophyte isolates were isolated via direct examination and culture. The prevalence of dermatophytosis in suspected patients was 13.7%. Tinea corporis (37.1%) was the predominant type of dermatophytosis. Totally, the difference reflected the low distribution of SUB1 (50%) and SUB6 (50%). In *T. mentagrophytes*, all isolates have SUB2, SUB3, SUB4, SUB6, and SUB7. As well as 100% of E. floccosum strains have SUB2 and SUB7. The most frequent SUB gene in *M. canis* isolates was SUB3 (75%); SUB1 and SUB2 each were observed in only one (25%) isolate. In *T. rubrum* SUB1 and SUB6 were not detected in any of the isolates but SUB2, SUB5, and SUB7 were more prominent (73.3%, 80%, and 93.3%, respectively). The possession of SUB genes by different dermatophytes species armed the fungi with virulence factors needed to invade keratinized tissue and cause dermatophytosis. However, the low profile of some SUB genes in our clinical isolates indicates that not all SUB genes are involved in the pathogenesis of dermatophytosis. The characterization of proteins coded by SUB genes remains crucial to providing useful data that may disclose the contributory role of SUB genes in dermatophytes pathogenesis.

***KEYWORDS***: Dermatophytosis, tinea, virulence, subtilisin, polymerase chain reaction, Iran

# THE IMPORTANCE OF EARLY DIAGNOSIS AND EFFECTIVE TREATMENT IN RINO ORBITO CEREBRAL MUCORMYCOSIS

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## ABSTRACT

Mucormycosis is a rare disease in humans, but can result in 80% death if treatment is delayed. Since it is such a highly fatal disease and it progresses rapidly and has an aggressive course, it is very important to diagnose it, to start its treatment as early as possible, and to have the necessary information about this disease. Although its incidence varies according to countries, it is encountered at a rate of 1-2 per million on average. Mucorales class saprophytic aerobic fungi from the Zygomycetes family that cause this disease, also known as zygomycosis. Mucormycetes can cause rhino orbito cerebral mucormycosis by inhaling through the respiratory tract and spreading to the upper respiratory tract and from there to the sinuses, as they are found in nature, in the form of spores in manure and soil. Pulmonary type by reaching the lower respiratory tract, gastrointestinal type in the gastrointestinal tract, and cutaneous type mucormycosis cases can be seen by infecting through abrasions on the skin. A 79-year-old male patient presented to the emergency department with fever, headache, necrotic areas on the facial skin and oronasal mucosa, epistaxis, loss of vision, proptosis, chemosis, facial swelling, ophthalmoplegia, and cranial nerve involvement. The patient, who was engaged in animal husbandry and had a history of diabetes and chronic renal failure, had significant neutropenia as a result of the hemogram. After debriding the necrotic areas on the right side of the face, wound and tissue cultures were taken for examination. After debridement, mechanical irrigations were performed from the bone defect area formed in the anterior wall of the maxillary sinus. The patient, who was diagnosed with mucormycosis as a result of wound culture, was administered amphotericin B both systemically and by putting it in irrigation solution. The patient's condition was stabilized by correcting the predisposing factors with blood sugar regulation and hemodialysis. Since general anesthesia involves risks for the patient, the defect area was repaired with a flap under local anesthesia. Aggressive treatment is essential to prevent fatal outcomes in this disease. The survival of the patients depends on the diagnosis of mucormycosis as early as possible and the simultaneous surgical treatment along with medical treatment.

***KEYWORDS***: Mucormycosis, early diagnosis, antifungal treatment, surgical treatment

# POSTER PRESENTATIONS

# STUDY OF THE EFFECT OF SOME PLANT ESSENTIAL OILS ON *VERTICILLIUM DAHLIA* PATHOGEN BY MIXING WITH CULTURE MEDIUM

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## ABSTRACT

In this study, the effects of plant essential oils including asafetida (Ferula assa-foetida), thyme (*Thymus daenensis*), lavender (*Lavandula angustifolia*), fennel (*Foeniculum vulgare*), fennel (*Satureja hortensis*) and lemon (*Aloysia citrodora*), The reduction of Verticillium wilt disease of cucumber *Verticillium dahlia* in vitro was investigated by mixing with culture medium. The concentrations used by the mixing method were 0, 50, 100, 150 and 300 ppm, respectively. Essential oil extraction was performed using Clevenger apparatus. Based on the results of the effect of the mentioned essential oils on reducing the growth of the pathogen, by mixing with culture medium, the maximum antifungal activity were essential oils of lavender, thyme, fennel, savory, savory and lemon with 92, 81, 69 and 61, 56 and 49% showed inhibition at a concentration of 300 ppm compared to the control. At each of the concentrations of 50, 100, and 150 ppm, a significant percentage of control of this pathogen occurred. Increasing the controlling power of these essential oils was accompanied by increasing their concentration. The results clearly showed that with increasing concentration, an increase in the level of control over essential oils occurred in this test. Increasing the antifungal effect of these essential oils along with increasing the concentration can be related to the antifungal compounds in these essential oils. Essential oils are a mixture of volatile oily compounds that are made as a secondary metabolite in medicinal plants. Control of these essential oils, showed that the mentioned essential oils had fungicidal potency by contact method.

***KEYWORDS***: Plant essential oils, mixing with culture medium, *Verticillium dahlia*

# *PNEUMOCYSTIS JIROVECII* (PNEUMONIA) IN CHILDREN CLINICAL PRESENTATION UNDERLYING DISEASES AND ACCURATE LABORATORY DIAGNOSIS

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## ABSTRACT

Pneumocystosis, caused by *Pneumocystis jirovecii*, is one of the opportunistic fungal infections that may result in life-threatening pneumonia in children with underlying illnesses or compromised immune systems. Due to the very small size of *P. jirovecii*, it is difficult to identify by direct examination, and their clinical symptoms are similar to other pulmonary infections. Therefore, accurate and timely identification of this organism can play an important role in effective treatment and saving patients. Hence, according to the approval of the ethics committee of Mashhad University of Medical Sciences, bronchoalveolar lavage (BAL) specimens of pediatrics hospitalized were examined by real-time polymerase chain reaction (PCR) and Giemsa staining in tertiary Hospitals in Mashhad, northeastern Iran. In this study, 180 BAL specimens were collected from hospitalized children at risk with respiratory disorders (infants to 16 years of age). Several demographic parameters, including the history of blood disorders and the condition of the immune system, were evaluated. The BAL fluid was centrifuged and the pellet was vortex mixed to prepare for direct examination (Giemsa staining), and DNA extraction. For DNA extraction, the AmpliSens® kit protocol was also followed. *P. jirovecii* was identified by the AmpliSens *P. jirovecii* (carinii)-FRT real-time PCR method. To analyze the data, version 22 of the SPSS software was used. Among the 180 specimens examined, 34 were positive and 8 were suspect for the presence of *P. jirovecii* by using the real-time PCR method. This organism was only recognized by Giemsa staining in two of the Real-time PCR method-positive cases (5.88 %). It was also determined that there is no correlation between the positive findings of real-time PCR and the sex, the outcome of the illness, the underlying disease, the status of the patient's immune system, neutropenia, and hematological malignancies. According to our data, the incidence of pneumocystosis pneumonia in susceptible children along with pulmonary symptoms is significant. Among the risk factors in these patients, neutropenia is predominant. Real-time PCR has a high sensitivity detection of the *P. jirovecii* with a high predictive value. In addition, Giemsa staining showed high false negative results, which can lead to misdiagnosis.

***KEYWORDS***: *Pneumocystis jirovecii*, real-time PCR test, giemsa, pneumonia

# İSTANBUL'DA HAVA KİRLİLİĞİNİN LİKENLER ÜZERİNDEKİ ETKİLERİNİN İNCELENMESİ

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## ABSTRACT

İnsan aktiviteleri sonucunda atmosfere salınan kirleticiler ve artan sera gazı emisyonları küresel iklim değişikliğine sebep olarak tüm biyosferi etkilemektedir. Doğada ekosistemler içinde kirlilikten olumsuz etkilenen canlı türlerinin sayısındaki azalma besin zinciri yoluyla diğer canlıları da etkilemektedir. Büyük şehirlerde insanların sağlıklı yaşam sürmeleri de hava kalitesiyle yakından ilgilidir. Bu amaçla uygulanan biyolojik izleme yöntemlerinde hava kirliliği düzeylerinin periyodik olarak takip edilmesi için biyoindikatör liken türleri kullanılmaktadır. Bu çalışmada bu atmosferik kirlenme ve küresel iklim değişikliğinin likenler üzerindeki etkilerinin tespit edilmesi amaçlanmıştır. Likenler, belirli alg/siyanobakteri ve mantarların simbiyotik ilişkisi ile oluşmaktadırlar. Likenlerde stoma ve kütikula bulunmayışı daha çok atmosferik kirletici biriktirmelerine ve kirleticilerden daha çok zarar görmelerine sebep olmaktadır. Talluslarında bol miktarda ağır metal, kükürt dioksit ($SO_2$), partikül madde (PM) ve benzeri kirleticileri biriktiren likenlerde alg ya da mantar ortağının morfolojinde ve fizyolojisinde çeşitli hasarlar oluşabilmektedir. Bu çalışmada İstanbul metropolü içinde bulunan liken türlerinde klorofil degradasyonu, pigment ölümü, lipit peroksidasyonu ve fotosistem bozulmaları gibi fizyolojik parametrelerde meydana gelen değişiklikler, bunun yanı sıra liken fotobiyontu ve mikobiyontundaki morfolojik ve anatomik semptomlar üzerine odaklanılmıştır. İstanbul ilindeki koru ve orman alanlarından örneklenen biyoindikatör likenlerden *Xanthoria parietina* türünde oluşan hasarlar incelenmiştir. Morfolojik gözlemler sonucunda, referans materyal (temiz bölge likeni) ve diğer lokalitelerden toplanan örneklerin; tallus yüzeyi ve apostesyum renkleri hava kirliliğine bağlı bir değişim ile ilişkilendirilememiştir. Fotobiyont ve mikobiyont partnerlerinin her ikisinde de iboyut ve şekil bakımından lokaliterdeki örnekler arasında anatomik ve morfolojik herhangi bir farklılık görülmemiştir. Fizyolojik çalışmalarda ise yol kenarlarına yakın, mesire yerlerinin bol olduğu lokasyonlardaki liken örneklerinin fotosistemlerinde, klorofil ve lipit metabolizmalarındaki bozulmaların diğer bölgelere nazaran daha fazla olduğu tespit edilmiştir. Elde edilen sonuçlar, hava kirliliği düzeyini gösteren biyoindikatör liken türlerinin belirlenebilmesine ve kirlilikten etkilenen ekosistemde meydana gelebilecek potansiyel değişimler hakkında öngörüde bulunabilmeye imkan sunacaktır. Bu çalışma FYL-2022-10416 kodlu proje ile Marmara Üniversitesi BAP Birimi tarafından desteklenmektedir.

***KEYWORDS***: Liken, hava kirliliği, klorofil degredasyonu, fotosistem, fizyolojik hasar

# PREPARATIONS FOR A DIGITAL LICHEN FLORA OF IRAN DILFI

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## ABSTRACT

The Digital Lichen flora of Iran is a collaborative research project and effort between Iranian and German lichenologists that relies on the lichenological data in the Museum of Iranian lichens (MOIL) and recent lichen collections from different parts of Iran to create and maintain digital library collections on the lichen flora of Iran. The project focuses on developing web-accessible collections for lichenologists and lichenologically-interested nonspecialists. The webpage of MOIL will be the main portal to the collections of the Digital Lichens Flora of Iran (DiLFI). As a first step, DiLFI pages will allow examination of the lichen genus *Lecanora* through several types of interactive information visualizations: descriptions, a list of examined specimens, distribution maps and relevant photos from habitat and morphology. This DiLFI page presents the currently known species of *Lecanora* from Iran, the second largest country in the Middle East. Prepared based on a bibliographical review as well as recent observations, this *Lecanora* list should be considered as preliminary pages for the DiLFI project, and may still contain errors and incomplete information. A total number of 29 *Lecanora* species in Seaward et al. (2008) e.g., *Lecanora albellula* (Nyl.) Th. Fr., *L. albula* (Nyl.) Hue, *L. allophana* (Ach.) Nyl., *L. argentata* (Ach.) Degel., *L. argopholis* (Ach.) Ach, *L. campestris* (Schaer.) Hue, *L. carpinea* (L.) Vain., *L. chlarotera* Nyl., *L. concolor* Ramond, *L. conferta* (Fr.) Grognot, *L. dispersella* J. Steiner, *L. dispersoareolata* (Schaer.) Lamy, *L. horiza* (Ach.) Linds., *L. lisbonensis* Samp., *L. marginata* (Schaer.) Hertel & Rambold, *L. meridionalis* H. Magn., *L. reagens* Norman, *L. riparia* (Flot.) J. Steiner, *L. rupicola* (L.) Zahlbr., *L. subrugosa* Nyl., *L. symmicta* (Ach.) Ach, *L. thysanophora* R. C. Harris, *L. valesiaca* (Müll. Arg.) Stizenb., *L. populicola* (DC.) Duby, *L. sulphurata* (Ach.) Nyl. *L. cenisia* Ach., *L. jamesii* J.R. Laundon, *L. xanthostoma* Fröberg, *L. umbrina* (Ach.) A. Massal., are already listed on this website and we are still regularly updating the database. At present, the list is presented in plain text version and a more feature-rich layout presentation/web is currently being prepared.

***KEYWORDS***: Lichens, Iran, *Lecanora*, digital dlora

# FIRST USE OF *PISOLITHUS ARHIZUS* SCOP RAUSCHERT MUSHROOM IN COTTON YARN DYEING IN TÜRKİYE

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## ABSTRACT

Mushrooms, have been used as natural colorants in different parts of the world throughout the history. In this study, natural dyes were extracted from *Pisolithus arhizus* collected from Muğla province (southwest Aegean region) of Türkiye. *Pisolithus arhizus* is an ectomycorrhizal fungus that interacts with some of the most important tree genera from temperate forests. According to the dyeing results, different color tones of brown were obtained from *Pisolithus arhizus*. It is the first study obtained from *Pisolithus arhizus* in which without mordant was used to dye cotton yarns.

***KEYWORDS***: Natural dyeing, dyeball, mushroom, color measurement

# DETERMINATION OF FATTY ACID CONTENT OF *PLEUROTUS ERYNGII* DC QUEL GROWING IN THE AEGEAN REGION

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## ABSTRACT

In this study, it was aimed to determine the Fatty Acid content of *Pleurotus eryngii* (DC.) Quél. which grow naturally abundantly and consumed as functional food or dietary supplement by the rural in Muğla (Bodrum), Izmir ve Çanakkale. In this study *Pleurotus eryngii* was collected from in Muğla (Bodrum) and the collected mushroom samples of Fatty Acid content was determined GC/MS with the device. The results showed that total of eighteen fatty acids were identified and quantified by GC-MS. Major constituent of *Pleurotus eryngii* mushroom was found to be 48.125% Oleic acid; 14.094% Linoleic acid; 14.736% Palmitic acid. The other high-rate fatty acids component was Stearic acid 6.469%; 9-Octadecenal 5.278% were determined in *Pleurotus eryngii*. This is a wild edible mushroom, importance in the food and pharmaceutical industry as a natural healthy product source.

***KEYWORDS***: Fatty acid composition, GC-MS, *Pleurotus eryngii*, Bodrum

# EVALUATION OF THE EFFECT OF PLANT ESSENTIAL OILS ON *VERTICILLIUM DAHLIA* PATHOGEN BY PAPER DISK METHOD

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## ABSTRACT

In the present study, the effects of plant essential oils including angula (*Ferula assa-foetida*), thyme (*Thymus daenensis*), lavender (*Lavandula angustifolia*), fennel (*Foeniculum vulgare*), savory (*Satureja hortensis*) and lemon (*Aloysia citrodora*) were reduced. Growth of *Verticillium dahlia* wilt in vitro was investigated by disk test in culture medium. The concentrations used on paper discs were 0, 100, 200, 300 and 500 ppm, respectively. Essential oil extraction was performed using Clevenger apparatus. Results showed that the effect of these essential oils on reducing the growth of the pathogen, by paper disk test, maximum antifungal activity of essential oils of lavender, thyme, fennel, savory, savory and lemon with 85, 79, 61 and 56, 51 and 45, respectively. They showed a percentage of inhibition at a concentration of 500 ppm compared to the control. However, in each of the concentrations of 100, 200 and 300 ppm, a significant percentage of control occurred for this pathogen. Another important point was the increase in the controlling ability of these essential oils along with the increase in concentration. The results clearly showed that with increasing concentration, an increase in the level of control over essential oils occurred. Increasing the antifungal effect of these essential oils along with increasing the concentration can be related to the antifungal compounds in these essential oils. Essential oils are a mixture of volatile oily compounds that are made as a secondary metabolite in medicinal plants.

***KEYWORDS***: Plant essential oils, paper disc, *Verticillium dahlia*

# GREEN SYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACT AND COMPARISON THEIR EFFECT WITH ANTIFUNGAL DRUGS ON THE GROWTH OF STANDARD STRAINS OF *ASPERGILLUS* AND *CANDIDA*

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## ABSTRACT

The biosynthesis of nanoparticles stands as an uncomplicated, biocompatible, inexpensive, and secure approach for replacing the available chemical and physical methods. Nanoparticles with a size of 1 to 100 nm have unique chemical and biological quantum features. Recently, the induced difficulties of drug resistance are being constantly reported in different species of fungi. *Candida* and *Aspergillus* fungi function as opportunistic human pathogens in the Immune system defect of individuals. Silver nanoparticles are known as substances that contain antifungal properties. Silver nanoparticles (Ag-NPs) were biosynthesized by using Berberine plant extract. Included UV-Vis spectroscopy, FT-IR analysis, and FESEM/EDAX images in the list of exerted methods for confirming the presence of nanoparticles. In addition, they were utilizing the Minimum Inhibitory Concentration (MIC) method to perform a sensitivity test on fungal species' leachates through duplicate usage. The application biosynthesized Ag-NPs at concentrations of 1 µg/mL and below caused no growth of fungal agents. However, antifungal drugs at concentrations higher than 1 µg/mL and even up to 64 µg/mL inhibited the growth of these drug-resistant fungi. Comparison of the results showed that even these nanoparticles, in some cases, have a better effect than the group of antifungal drugs. Therefore, the usage of biosynthesized nanoparticles is more convenient and economical due to the increasing drug resistance of opportunistic and pathogenic microorganisms towards the exerted target drugs.

***KEYWORDS***: Biosynthesis, silver nanoparticles, *Aspergillus*, *Candida*, drug resistance, MIC

# BİYOGÖRÜNTÜLEME İÇİN FLORESAN PROB OLARAK *AGARICUS BISPORUS*'TAN TÜRETİLEN KARBON KUANTUM NOKTALARININ MİKRODALGA DESTEKLİ YEŞİL SENTEZİ

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## ABSTRACT

Karbon kuantum noktalar (CQDs) boyutları 1-20 nm arasında değişen düşük toksisite, yüksek floresans, stabilite ve biyouyumluluk gibi özelliklere sahip yarı iletken kristallerdir. Biyogörüntüleme, biyomedikal malzemeler, sensörler, baskı mürekkepleri, fotokataliz ve ışık yayan diyotlar başta olmak üzere birçok kullanım alanına sahip olan CQD'ların bitkiler, mantarlar ve algler gibi canlılardan sentezlenebilme potansiyeli bulunmaktadır. Çalışmamızda ise CQDs sentezi yenilebilir bir mantar türü olan Agaricus bisporus kullanılarak gerçekleştirilmiştir. Toz haline getirilmiş olan mantarlar mikrodalga sentez yöntemi ile CQDs formuna dönüştürülmüş ve kullanılan mantar miktarı, mikrodalga enerji seviyesi, mikrodalgada ışınlanma süresi ve çözücü türü kriterlerine göre optimizasyon çalışmaları gerçekleştirilmiştir. Maksimum floresans dalga boyu 1.0 g mantarın %15 $H_2O_2$ çözgeni içerisinde, 700Watt enerji düzeyinde 10 dakikalık bir reaksiyon sonucunda elde edildiği belirlenmiştir. Akabinde saflaştırılan ve toz haline getirilen CQDs'lar Geçirimli elektron mikroskobu (TEM), Floresans spektroskopisi, FT-IR Spektroskopisi ve XRD yardımıyla karakterize edilmiştir. Çalışmalar sonucunda sentezlenmiş olan CQDs'ların 16 nm olduğu, N-H bağı, C-H bağı, C=O ve C=C bağına sahip olduğu ve X-ışının kırınım modelinde ise yaklaşık 19°'deki tepe noktası kristal grafitik karbonun varlığına sahip olduğu belirlenmiştir. İnsan epitel hücrelerinde gerçekleştirilen biyogörüntüleme çalışmaları neticesinde konfokal mikroskop altında CQDS'ların hücrelerin sitoplazmasında yoğunlaştığı ve kısmende hücre çekirdeğinde lokalize olduğu belirlenmiştir. Kolay üretimi ve düşük maliyeti neticesinde mikrodalga yöntemi ile *Agaricus bisporus*'tan üretilen CQDs'ların çevre dostu olması, optik ve biyo-görüntüleme için uygun bir prob olarak kullanılabilmesi nedeniyle yeni bir boyar madde potansiyeline sahiptir.

***KEYWORDS***: Karbon kuantum dot, *Agaricus bisporus*, hücre görüntüleme, floresan boyama, yeşil sentez

# İMMUNSUPRESE HASTALARDAN SOYUTLANAN KANDİDA SUŞLARININ TİPLENDİRİLMESİ VE ANTİFUNGAL İLAÇLARA KARŞI DUYARLILIKLARININ E TEST YÖNTEMİ İLE ARAŞTIRILMASI

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## ABSTRACT

Kandidaların immün sistemi baskılanmış veya genel durumu kötü olan hastalarda invaziv ve yaygın-sistemik enfeksiyonlara yol açarak ciddi bir mortalite nedeni olduğu bilinmektedir. Bu çalışmada, hastanemizde yatarak tedavi gören immün sistemi baskılanmış veya genel durumu bozuk hastalardan soyutlanan kandidaların tür ayrımlarının yapılması ve antifungal duyarlılıklarının araştırılması amaçlanmıştır. Çalışmada 39 febril nötropenik ve 7 genel durumu bozuk yoğun bakım hastasından alınan idrar (6), balgam (6), kan (11), yara (4), endotrakeal aspirat (7), boğaz (3), kateter (7) ve dışkı (2) örneklerinden yapılan mikolojik kültürlerinde toplam 46 kandida suşu soyutlanmıştır. Soyutlanan kandidaların tür ayrımları API ID 32 C (Bio Mérieux/Fransa) kitleri ile yapılmıştır. E test (AB Biodisk, İsveç) ile antifungal duyarlılıkları belirlenmiştir. Çalışılan suşların 24'ü (%52.1) *Candida albicans*, 5'i (%10.8) *Candida krusei*, 7'si (%15.2) *Candida tropicalis*, 4'ü (%8.6) *Candida glabrata*, 3'ü (%6.5) *Candida parapsilosis* ve 3'ü (%6.5) *Candida guillermondii* olarak tanımlanmıştır. E test (AB Biodisk, İsveç) ile yapılan antifungal duyarlılık deneyinde, kandidaların %2.1' i amfoterisin B' ye, %21.7'si ketokonazol ve flukonazola, %17.3' ü vorikonazola, %23.9' u ıtrakonazola ve %6.5'i ise flusitozine dirençli bulunmuştur. En etkin antifungal olarak saptanan amfoterisin B'nin MİK50 ve MİK90 değerleri sırasıyla 0.019 ve 0.75 µg/ml; en düşük etkinlikli antifungal olarak saptanan ıtrakonazolün MİK50 ve MİK90 değerleri ise sırasıyla 0.032 ve 16 µg/ml olarak bulunmuştur. Tıptaki ilerlemelere paralel olarak fırsatçı patojen olan kandidaların hedefindeki hasta popülasyonu gittikçe artmakta ve zamanla invaziv kandida enfeksiyonlarının sıklığında da yükseliş olduğu görülmektedir. Bu nedenlerle, özellikle riskli hastalarda gelişen ciddi enfeksiyonların tedavisinin başarılı bir şekilde yapılabilmesi için kandidaların tür ayrımlarının rutin olarak uygulanması ve güvenilir bir yöntemle antifungal duyarlılıklarının saptanması gerekmektedir.

***KEYWORDS***: İmmünsüprese hasta, kandida türleri, antifungal duyarlılık

# THE ASPECTS OF HEMATOLOGICAL PARAMETERS AMONG COVID 19 PATIENTS AFFECTED TO YEAST INFECTIONS

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## ABSTRACT

Since the spread of COVID-19, a highly transmittable and pathogenic viral infection, it causes many hospitalizations and deaths, especially in immunocompromised patients. Yeast infections (mostly candidiasis) occur most commonly as a secondary infection in immunocompromised individuals. However, the diagnosis of fungal infections is controversial, particularly among COVID-19 patients. Hematologic parameters in COVID-19 patients affected by yeast infections can predict prognosis and may help patient treatment. The aim of this study is to investigate the hematological parameters in COVID-19 patients associated with yeast infections. During the study, 1531 COVID-19 patients were examined to have yeast infections, from March 2020 - May 2022. The collected data included serum specimens used for hematological parameters such as platelets, white blood cells (WBC), and D-dimer; blood culture and urine specimens evaluated for any yeast infections. All data were analyzed by IBM SPSS statistics 22 software, and for comparison between two groups, Student t-tests were used. The average age of patients was 60.37 years, of which (47.9%) 732 were female and (52.1%) 796 were male. Among the 1531 COVID-19 patients, 227 (14.8%) showed yeast infections with 21.8% of positive blood cultures and 20.4% positive urine cultures. The comparison was between two groups of COVID-19 patients and COVID-19 patients with yeast infections. Hematological factors of lymphocytes, platelets, WBC, and D-dimer showed a significant relationship between these two groups. Moreover, the INR factor has changed only in the control group (COVID-19 patients without candidiasis). According to the measured data in hospitalized COVID-19 patients, the measurement of hematological factors can give a valuable prognosis for the detection of yeast infections.

***KEYWORDS***: Yeast, *Candida*, covid-19, infection, hematological factor

# BIODIVERSITY OF FUNGI AND FUNGUS LIKE ORGANISM ON *SOLANUM* SPECIES IN UZBEKISTAN

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## ABSTRACT

*Solanum* is the largest genus in the family Solanaceae with about 2500 identified species worldwide distributed, except Antarctica. This genus is regarded as economically significant based on their multiple uses in food crops as well as ornamental and medicinal plants. In Uzbekistan, about 24 species of Solanaceae have been reported and of these, 7 species belong to the genus *Solanum*, some of which are wild while others are cultivated with their leaves, fruits, or both eaten as raw or cooked vegetables. or used as folk medicine. However, in recent decades, numerous fungal, fungus-like and bacterial pathogens cause diseases on the roots and collar roots, and on the foliage of solanaceous crops. Therefore, this study initially aims to introduce our ongoing survey, mostly focused on fungi and fungus-like organisms dwelling on cultivated, wild, medicinal and alien invasive plant species in Uzbekistan followed by summarizing of the results of previous studies in the region, with a special view on microbiota occurring in the members of Solanaceae. It has been noted that there were several studies on mycobiota of natural wild plants in Uzbekistan, and our current mycological survey is on solanaceous cultivated and wild plant species of the same indicated study area. Field trips to urban and natural landscapes were organized in 2021-2022. Thirty-nine fungi and fungus-like taxa (33 ascomycetous, 2 basidiomycetous, and 4 fungus-like) were reported from stems, leaves and fruits of *Solanum* species. Most of these taxa belong to Sordariomycetes (9 genera and 12 species), Dothideomycetes (6 genera and 9 species) and Eurotiomycetes (2 genera and 5 species). The most frequent genera observed were *Alternaria*, *Aspergillus*, *Fulvia*, *Fusarium*, *Leveillula*, *Penicillium*, *Phytophthora*, *Rhizoctonia*, and *Verticillium*. Fungi and fungus-like taxa were found on six cultivated, medicinal and invasive species of *Solanum*. The number of microfungal and oomycetes species is reported in descending order for the following host species as *Solanum lycopersicum* (25 species), *S. tuberosum* (19 species), *S. melongena* (8 species); *S. dulcamara*, *S. nigrum* and *S. villosum*, each has one fungal species. Furthermore, it has been observed that fungi and fungi-like oomycete pathogens cause serious diseases of major crops such as various cultivars of tomato, potato and eggplant in the same study area. An ongoing survey is needed here to obtain a more precise assessment of the fungi and fungus-like pathogens. This work was supported by the Ministry of Innovative Development of the Republic of Uzbekistan "Producing

of digital maps of invasive and quarantine plants foci in natural landscapes" and MUK-2021-46 "R & D of bioactive polysaccharide from medicinal basidiomycetes *Phellinus linteus* grown in Uzbekistan" and State scientific and technical program of Uzbekistan (Diversity, monitoring, and creation of a database of pathogenic fungi causing diseases of economically important plants: export fruits, vegetables, and melon crops (in the case of Namangan and Bukhara regions).

***KEYWORDS***: Crops, diversity, invasive alien, medicinal plants, plant diseases, Solanaceae

# FUNCTIONAL PROPERTIES OF IRANIAN AGARIC MUSHROOMS

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## ABSTRACT

Agarics are a significant group of macrofungi with diverse ecological roles, yet their diversity and functional properties are poorly studied in Iran. Here, we analyzed Iranian agaric species to disclose their resources of edible, poisonous, wood-inhabiting, saprotrophic, as well as parasitic species. A full survey of radical scavenging and antioxidant activity of the species was also performed and the phylogenetic relationships were reconstructed based on concatenated phylogenies. Our results demonstrate that 47% of Iranian agarics are categorized as edible mostly compared to the recognized guidelines in some mycophilous countries, while 31% of the species are poisonous. Wood-inhabiting and ectomycorrhizal agarics comprise about 140 and 240 species, respectively. Moreover, ca. 30% of Iranian agarics in the order Russulales possess antioxidant activity in various degrees. Phylogenetic diversity of functional species shows that Iranian agarics provide a valuable resource of applications with various beneficial aspects.

***KEYWORDS***: Agarics, Basidiomycota, macrofungi

# *PSEUDOHYDROPUS FLOCCIPES* A NEW RECORD FOR TURKISH MYCOTA

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## ABSTRACT

*Pseudohydropus floccipes* (Fr.) Vizzini & Consiglio is reported for the first time from Türkiye based on the collection made from Trabzon province. The species was first introduced as *Agaricus floccipes* by Fries in 1838. Later on, it was presented within the genera *Collybia*, *Hemimycena*, *Hydropus*, *Marasmiellus* and *Mycena* with the same epithet. Though IndexFungorum currently list this taxon as *Hydropus floccipes* (Fr.) Singer, it was transferred to a newly erected genus, *Pseudohydropus* Vizzini and Consiglio, in 2021 by Consiglio et al., especially due to the inamyloid basidiospores and the absence of pleurocystidia. The current Turkish fungal checklist and subsequent contributions revealed that *Pseudohydropus floccipes* has not been reported from Türkiye before. A brief description of the species is provided together with its distribution and photographs related to its macro and micromorphologies. The work aims to contribute to the mycobiota of Türkiye by adding a new record.

***KEYWORDS***: Biodiversity, Mycenaceae, new record, Türkiye

# DETERMINATION OF *ASPERGILLUS* BIODIVERSITY IN INDOOR AIR IN THE WINTER MONTHS JANUARY FEBRUARY MARCH 2022 OF CATTLE BARNS IN IHSANGAZI DISTRICT KASTAMONU WHICH MAY CREATE POTENTIAL INFECTION RISK

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## ABSTRACT

Microorganisms that exist in the air in free form or in the form of substances are also called bioaerosols. Considering that air is ubiquitous, microbial contamination from the air can also pose a risk to the cattle in the barns and the workers there. Depending on the types and density of these microorganisms, various infectious diseases may occur in animals and workers in the environment. Therefore, controlling the ambient air of animal holdings is very important. Air intake from outside to areas used as barns can be reduced in various ways and measures can be taken to ensure fresh air enters inside. Bacteria and fungi constitute the majority of airborne microbial contaminants. Especially fungal factors; i.e. yeasts and molds are important in terms of air contamination. Molds infect humans and animals, act as allergens, are toxigenic, cause inflammatory reactions. Species belonging to the genus *Aspergillus* cause infection in cattle. Aspergillosis is a fungal disease caused by *Aspergillus* species, usually localized in the respiratory system and sometimes showing a generalized (systemic) condition. Many species cause Aspergillosis. *Aspergillus fumigatus* is the leading cause of infection in animals. Apart from that, *Aspergillus niger*, *A. flavus*, *A.terreus*, and *A. nidulans* are the main species that cause infections in animals. In cattle, aspergillosis may be symptomatic or cause bronchopneumonia, mastitis, placentitis, or abortion. They can lead to spoilage of feed through enzymes and mycotoxins. When these toxic metabolites, known as mycotoxins, are taken in high doses by inhalation or mouth, as a result of acute toxic effects, disorders that can result in death in a short time may develop. Animal husbandry is important in İhsangazi and its environs. The people earn their living from animal husbandry. As a result, knowing the microbial flora elements in the indoor environment of environments such as barns quantitatively and qualitatively is extremely important in terms of carrying out studies to control and prevent the reproduction of microbial populations that may be harmful to animal health. The research material is 10 barns in İhsangazi District of Kastamonu Province. For 3 months (January, February, March) when the cattle are not taken out from the indoor air of the barns. Microbial sampling was done twice a month. The isolated microfungi samples were incubated in the laboratory at 25ºC for 7 days. *Aspergillus* grown in Petri plates were transferred to slanted media containing Potato Dextrose Agar and stock culture was prepared. In the diagnosis process, PDA, Czapek-Dox Agar, Malt Extract Agar media were used. *Aspergillus* species were identified using the MALDI-TOF MS (Matrix assisted laser desorption ionization time of flightmass spectrometry) system. 12 *Aspergillus* species were identified.

***KEYWORDS***: *Aspergillus*, MALDI-TOF MS, indoor, microfungus

# EVALUATION OF BIOCHEMICAL PARAMETERS IN CORONAVIRUS DISEASE 2019 PATIENTS ALONG WITH *CANDIDA* INFECTIONS

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## ABSTRACT

As the global COVID-19 pandemic spreads worldwide, new challenges arise in the clinical landscape. A significant number of patients hospitalized with COVID-19 developed mycoses, especially *Candida* infections, and that led to serious complications and even death. COVID-19 patients are at risk for opportunistic fungal infections. This co-infection will be due to changes in biochemical factors in these patients. Hence, the aim of this study is to evaluate the biochemistry parameters in COVID-19 patients associated with *Candida* infections. During a two-year period, 1528 hospitalized COVID-19 patients were admitted to ICUs, from February 2020 to May 2022. The collected data included serum specimens used for biochemical parameters such as liver enzymes (AST and ALT), glucose, LDH, and C reactive protein (CRP); blood culture and urine specimens evaluated for *Candida* infections. We studied COVID-19 patients along with candidiasis and without it. All data were analyzed by IBM SPSS statistics 22 software, and for comparison between two groups, Student t-tests were used. Among all patients 732 (47.9%) were female and 796 (52.1%) were male, with a mean age of 60.74 years. Of the patients, 227(14.8%) showed a candidiasis infection. In this study, COVID-19 patients with candidiasis and without it showed a significant rising in FBS and CRP in serum. Of the patients, 329 (21.8%) showed bacterial infection in blood culture and 359(20.4%) showed microbial infection in urine culture. The collected data demonstrated significant changes in FBS and CRP in both groups affected and non-affected by *Candida* infections. But, liver enzymes (AST and ALT) and LDH didn't show any significant differences between the two groups

***KEYWORDS***: Biochemical parameters, COVID-19, patients, infection, *Candida*

# DETERMINATION OF *PENICILLIUM* BIODIVERSITY IN WINTER MONTH JANUARY FEBRUARY MARCH 2022 OF BATTLE STABLES IN IHSANGAZI DISTRICT KASTAMONU

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## ABSTRACT

Air and water are the two most important factors required for living things on earth to continue their lives. While air contains some substances necessary for living things to breathe and survive, it also contains foreign substances and microorganisms in the form of large and small particles. These microorganisms, which are found in the air in free form or in the form of substances, are also called bioaerosols. Bioaerosols can come from doors, windows, wall openings, heating, ventilation and cooling systems, plumbing pipes, and can also be carried indoors with shoes or clothes. Considering that air is ubiquitous, airborne microbial contamination can also pose a risk to barns and workers. Depending on the types and density of these microorganisms, various infectious diseases may occur in animals and workers in the environment. Therefore, it is very important to control the ambient air of animal enterprises. Air intake from outside to areas used as barns can be reduced in various ways and/or measures can be taken to ensure fresh air enters inside. Bacteria and fungi constitute the majority of airborne microbial contaminants. Especially fungal factors; ie yeasts and molds are important in terms of air contamination. These factors can easily live and multiply indoors and outdoors, especially in humid environments. The range of pH and water activity required for their survival and reproduction is also quite wide. The temperature of 18-25°C, which is accepted as room temperature in general living areas, is quite suitable for the development of fungal agents. Molds in the indoor environment; May affect the respiratory and immune system, and may also have an effect on the skin. In addition, molds can affect various organs in terms of animal health and cause life-threatening systemic infections in animals. Penicillium species are ubiquitous soil fungi that are generally found wherever organic material is found, preferring cool, temperate climates. *Penicillium* species are among the main causes of food spoilage. Many species produce highly toxic mycotoxins. Penicilliums grow in seeds, other stored foods and animal feed. They thrive in low humidity. They spread rapidly through the air. Some species are known to be pathogenic to animals. *Penicillium* species are found in the air and dust of indoor environments. It can be easily transported outside. They can lead to spoilage of feed through enzymes and mycotoxins. When these toxic metabolites, known as mycotoxins, are taken in high doses by inhalation or mouth, as a result of acute toxic effects, discomforts that may result in death in a short time may develop. Animal husbandry is important in İhsangazi and its environs. The people earn their living from animal husbandry. As a result, knowing the microbial flora elements in the indoor environment of environments such as barns quantitatively and qualitatively is extremely important in terms of carrying out studies to control and prevent the reproduction of microbial populations that may be harmful to animal health. The research material is 10 barns in İhsangazi District of Kastamonu Province. For 3 months (January, February, March) when the cattle are not taken out from the indoor air of the barns. Microbial sampling was done twice a month. The isolated microfungi samples were incubated in the laboratory at 25ºC for 7 days. Penicilliums grown in Petri plates were transferred to slanted

media containing Potato Dextrose Agar and stock culture was prepared. In the diagnosis process, PDA, Czapek-Dox Agar, Malt Extract Agar media were used. *Penicillium* species were identified using the MALDI-TOF MS (Matrix assisted laser desorption ionization time of flightmass spectrometry) system. 12 *Penicillium* species have been identified. Identified species; *Penicillium camemberti*, *Penicillium verrucosum*, *Penicillium roqueforti*, *Penicillium nalgiovense*, *Penicillium aurantiogriseum*, *Penicillium chrysogenum*, *Penicillium fellutanum*, *Penicillium brevicompactum*, *Penicillium crustosum*, *Penicillium digitatum*, *Penicillium glabrum*, *Penicillium expansum*.

***KEYWORDS***: *Penicillium*, MALDİ-TOF MS, indoor, microfungi, barn

# IDENTIFICATION OF FUNGI ASSOCIATED WITH DIFFERENT *PIGEON PEA* SEED ACCESSIONS FROM IRAN

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## ABSTRACT

One species of Leguminosae family is *Cajanus cajan* (L.) Millsp. (Pigeon pea) is an herbaceous, annual, self-pollinated legume pulse crop. The pigeon pea is mainly cultivated in South Asia due to its nutritious quality and a good source of minerals and protein in many countries. Seeds are a common carrier of plant pathogens, which act as the primary source for the distribution of diseases. Contaminated seeds can often create defecated germination and poor seedling vigor. They lose viability much faster than non-contaminated seeds. A large number of mycoflora were reported to be associated with the pigeon pea seeds. To identify fungi associated with seed of pigeon pea accessions this experiment was conducted at the genetic department and national plant gene bank of Iran, seed, and plant improvement institute in 2021 to assess the seed health and quality of some pigeon pea accessions from Iran. In the experiment, a total of 52 accessions from the pigeon pea collection were used for laboratory tests. During this study different species of *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Rhizopus* spp. were found along with germinating seed and seedlings of contaminated pigeon pea. The most identified species on the contaminated pigeon pea seeds were *Rhizopus* spp., with 50%, and the least were *Alternaria* spp. and *Aspergillus* spp., with 7.2%. The other species *Penicillium* spp., and *Fusarium* spp., were measured on 21.4% and 14.2% of the contaminated seeds respectively. On contaminated pigeon pea seeds, a 20 to 100% reduction in seed germination has been measured. Paying attention to seed health is the fundamental strategy for the integrated management of the disease for durable crop production and seed conservation.

***KEYWORDS***: Mycoflora, seed contamination, *Pigeon pea*

# DETERMINATION OF ANTIFUNGAL ACTIVITY OF DIFFERENT PLANT EXTRACTS

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## ABSTRACT

In this study, the antifungal activity of extracts of different plants using various chemicals was investigated on 5 different fungal species. Olive seed, grape seed, avocado seed, cotton seed, cotton leaf and plantain plant (Plantago sp.) were extracted with methanol, acetone, chloroform and ethyl acetate. Fungi species used to determine the antifungal activities of these extracts are *Penicillium citrinum* HBF337, *Aspergillus niger* HBF338, *Aspergillus fumigatus* HBF339, *Aspergillus foetidus* HBF340, *Aspergillus flavus* HBF341. In the study, soxhalet device and evaporator were used to extract plant extracts, and well diffusion method was used to determine antifungal activity. Nystatin (100 Units) was used as a positive control. The results of the study showed that avocado seed and cotton leaf ethyl acetate extracts were effective on fungi. The results of the study were evaluated according to Eucast standards by measuring the zone diameters formed around the well.

***KEYWORDS***: Antifungal activity, fungus, plant extract

# DETECTION AND MEASUREMENT OF AFLATOXINS B1 USING CDSE ZNS CORE SHELL QUANTUM DOTS

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## ABSTRACT

Aflatoxins are a group of carcinogenic mycotoxins that are produced during fungal development as secondary metabolites mainly by members of the *Aspergillus* section *Flavi*. Contamination of food, feed and agricultural commodities by aflatoxins impose an enormous economic concern, as these chemicals are highly carcinogenic, they can directly influence the structure of DNA. They can lead to fetal mutagenesis and miscarriages, and are known to suppress immune systems. Routine detection of AFB1 by chromatographic (TLC and HPLC) and spectrometric (ELISA and EIA) are time-consuming, high cost and non-precise due to cross-reactivity of anti-AFB1 antibodies. In recent years, use of nano-biosensors in rapid and accurate diagnosis of AFB1 in food, feed and agricultural commodities has gathered special attention due to low costs, high feasibility and precision and high level of flexibility. In the present study, CdSe/ZnS core/shell quantum dots were successfully synthesized and used in conjugation with AFB1 by competitive Fluorescence Labeled Immunosorbent Assay (FLISA) technique to detect the toxin in an aflatoxigenic *Aspergillus parasiticus* submerged cultures on sucrose-yeast extract broth as a conductive medium.

***KEYWORDS***: Quantum dots, aflatoxin B1, *Aspergillus parasiticus*, agricultural commodities, CdSe/ZnS, FLISA

# MORFOLOGICAL AND MOLECULAR CONFIRMATION OF AN EDIBLE FUNGUS *AGARICUS ARVENSIS* SCHAEFF AND *AGARICUS LITORALIS* PILAT

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## ABSTRACT

*Agaricus* L. is the type genus of the Agaricaeae family and, contains abundant species distributed across all continents. The genus includes both edible and poisonous species with more than 400 species worldwide and 43 of them are found in Türkiye. In the current study, edible fungi *Agaricus arvensis* Schaeff. and *A. litoralis* (Wakef. & A. Pearson) Pilát were identified based on morphological and molecular features. Cap structure, dimensions, connection to stipe, annular zone shape and spore structure were used as morphological characters. For molecular studies, Internal Transcribed Spacer gene of nuclear ribosomal DNA (nrITS) region was used. Phylogenetic tree was constructed based on nucleotide variations in the DNA sequence via MEGA 7.0 analysis software. DNA sequences of representative species were also downloaded from NCBI database to increase diversity and see phylogenetic positions of the species within the genus. In the phylogenetic tree, *A. arvensis* and *A. litoralis* placed into sect. *Arvenses* and sect. *Spissicaules*, respectively with 99% and 66% bootstrap values.

***KEYWORDS***: Phylogeny, ITS, *Agaricus*, edible species

# PHENOTYPIC AND MOLECULAR IDENTIFICATION OF GREEN PEA POWDERY MILDEW PATHOGEN FROM ALBORZ IRAN

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## ABSTRACT

Green pea (*Pisum sativum*) is a worldwide consumable vegetable crop rich in protein, vitamins, and nutrition that is cultivated in many parts of Iran, particularly during the summer season, and recently under protected greenhouse. Powdery mildew is an important disease of green pea, especially during the pod fill and maturation stage decreases the photosynthetic area of leaves and causes crop loss (4). To identify fungi associated with powdery mildews on some accessions under regeneration of green pea collection this experiment was conducted at the genetic department and national plant gene bank of Iran, seed, and plant improvement institute in 2021. Powdery mildew became epidemic on the plants at the pod stage at the end of July toward Aguste. The formation of white, floury patches initially on the leaf progressing towards tendrils, pods, and stems observed that on susceptible accessions covered the most aerial part of the plants. On pea; *Erysiphe pisi*, *Erysiphe trifolii*, and *Erysiphe baeumleri* were reported causing this disease (3, 2). Samples of infected plants were collected and transferred to the lab for morphological studies, and then total DNA was isolated from fungal specimens and used for amplification and sequencing of the internal transcribed spacers (1). The fungus was identified based on morphological characteristics of its asexual and sexual morphs, and rDNA ITS sequence analyses as *Erysiphe pisi*. Due to the presence of accessions with high levels of resistance to this disease, further investigation is recommended to produce resistant cultivars in this province.

***KEYWORDS***: *Pisum sativum*, *Erysiphe pisi*, rDNA ITS, sequence analyses

# A CLOSE FRIENDSHIP IN SUBSOIL ARBUSCULAR MYCORRHIZA AND *CLONOSTACHYS ROSEA*

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## ABSTRACT

With a growing population and climate change, ensuring food and nutrition security is an increasingly challenging task. Climate smart and sustainable agriculture, i.e. thinking as resilient and resilient to a changing climate while keeping agriculture viable over the long term, is probably the best solution. The role of soil biota, especially fungi such as arbuscular mycorrhiza (AM) and *Clonostachys rosea* (Cr) is believed to be of great importance in this new farming. In this context, we thought that Cr could be a way to encourage subsoil AM communities. To test this hypothesis, we investigated the effect of Cr in alfalfa (*Medicago sativa* L.) on all symbiotic phases (Pre-, Early and mature symbiotic phases) of AM fungus *Rhizophagus irregularis* (Ri). As a matter of fact, we determined that Ri propagules (root; mycorrhizal frequency (%F), mycorrhizal density (%M), arbuscular density (%A), and vesicle density (%V), soil; g the number of spores that have formed and not formed hyphae in the soil) generally increased with the effect of Cr in all phases. We also found that Cr has a positive effect on mycorrhizal addiction. However, we found that exhausted fresh and dry biomass increased in all phases due to the friendship established by Cr and AM fusgus. As a result, we think that we have demonstrated that AMF and Cr are close friends in the subsoil biota. We also propose future research objectives that will maximize the benefits of this friendship.

***KEYWORDS***: Arbuscular mycorrhiza, symbiotic phases, *Clonostachys rosea*

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