

Isolation of Micro-Fungi from Some Macro-Fungi Soils

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Abstract: *Micro-fungi were isolated and diagnosed from the soil of some large fungi growing in some areas of Salah Al-Din Governorate, as 6 types of micro-fungi were isolated and classified (Alternaria alternate † Aspergillus terreus † Fusarium ventricosum † Fusarium ventricosum † Penicillium corylophilum † Rhizopus stolonifer † Trichoderma koningii) Growing in the soil of large fungi, (Coprinellus micaceus † Coprinellus radians † Lactocollybia variicystis † Coprinellus flocculosus † Coprinellus disseminatus † Psathyrella spadiceogrisea) Based on the phenotypic and microscopic characteristics, and the reason for the presence of these species in some areas of Salah al-Din Governorate is due to the diversity of their botanical presence (trees, shrubs, herbs, cultivated plants and wild plants) and the diversity of their geographical characteristics (river banks, desert and hills) and that this fungus has important roles in recycling elements (Cracking of permanent polymers) Analytical dye such as malformed cellulose and in the medical, pharmaceutical and industrial fields, as well as in the agricultural fields and the treatment of pollutants. The frequency of microfungi on soil Coprinellus micaceus 2 types of micro fungi and Coprinellus radians 6 types of micro fungi was the highest compared to the frequency of micro fungi on the rest of the soil of large fungi.*

Keywords: *Macro-fungi, micro-fungi, mycorrhizal fungi.*

1. INTRODUCTION

Fungi are industrially based on size are divided into microfungi (Fungi that form fruiting bodies doesn't see with the naked eye) and Macrofungi Fungi that form fruiting bodies that are seen with the naked eye, that is, without the use of a microscope (Redhead, 1997). In general the definition of macrofungi (Mushroom) They are those fungi that produce fruiting bodies that are visible to the naked eye (≤ 1 cm), above the soil Epigenous or under the soil Hypogenous. The majority of them belong to the two phylum Basidian fungi Basidiomycota and sac fungi Ascomycota And a few of them belong to his division zygote fungi Zycomycota (Kinge, et al., 2017; Al-Abbasi, S. H. A., et al., 2021). These fungi are divided ecologically into Saprotrophic fungi and mutualistic (Mycorrhizal), and a few of them are plant pathogens (Al-khesraji, et al., 2017).

From the estimated total number of fungal species on Earth (1.5 million fungal species), there are about 14,000-16,000 diagnosed species of large fungi, which constitutes 1% of the total number of expected fungal species on Earth, about 15% of the total number of diagnosed

fungal species (110,000 species), and about 11% of the estimated number of large fungal species on Earth, which is 150,000 (Badaylan and Rapior, 2020 ; AL-Samarraie¹, M. Q., et al.,2014) . Macrofungi are widespread in natural ecosystems agro and systems (Kinge, et al.,2017).

Aim of this study:

The current study was aimed to study the isolation and diagnosis of microfungi found in the soils of some large fungi.

2. MATERIAL AND METHODS:

Samples collection:

The current study included the isolation and diagnosis of 6 genera of microfungi in Salah Al-Din Governorate, where samples were collected after conducting several field trips for the period between March - July 2022, and large and fine fungi were diagnosed depending on the culture traits, microscopic examinations, as well as taxonomic keys.

Isolation of microfungi from macro fungus soils:

10 gram of soil was taken on a dry weight basis and placed in a 200 ml cylinder and then sterile distilled water was added to complete the volume to 100 ml. The cylinder was shaken well for the purpose of homogenization with the Shaker electric shaker, 1 ml of homogeneous soil suspension was taken and 9 ml of sterile distilled water was added in a 10 ml cylinder and thus the first dilution was obtained 10^{-1} The process was repeated to obtain the best concentration 10^{-4} Add 1 ml of suspended to each dish sterilized by a sterile pipette and then add 20 ml of PDA plus 1 ml of chloramphenicol antibiotic suspension I worked three Duplicates to mitigate above. Then the dishes were incubated under a temperature of 25 ± 1 ° C. The first count of developing colonies was carried out after five days of incubation and the examination followed for four weeks to record the growing genus and species. The fungus was diagnosed according to their taxonomic keys (Watanabe, 2002) .

Diagnosis of isolated microfungi:

Diagnosis by culture characteristics:

Planted dishes were examined after 7 days of the appearance of fungal growth, which is one of the most important means of identifying fungi and included several characteristics, including the incubation period, the shape of the colonies (depression, elevation), their color and texture (powder, cotton, fluffy) where the examination is carried out from the opposite side and the diameter of the colony is measured after growth stops.

Microscopic examination of colonies by wet mounting method:

This examination was done by placing a drop of cotton blue dye in lactophenol on a clean glass slide and by sterile needle, part of the fungal spreads were transferred from the edge of the colony to a glass slide and mixed with the dye and then placed the slide cover and pressed gently for the purpose of brushing the sample, and the sample was examined under a light microscope to observe the fungal filaments, their shapes, branches, dimensions and different shapes and sizes of spores.

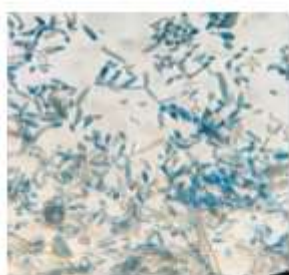
Microscopic examination by adhesive tape method:

This examination was done by using 2 cm transparent adhesive tape by touching and pressing the adhesive front of the tape on the surface of the colony and under sterile conditions and the tape is lifted and pasted on the glass slide that contains a drop of cotton blue dye and examining the slide under light microscopy to examine the characteristics of the fungus (Baron et. Al., 1994). Microfungi were diagnosed by taxonomic key (Watanabe, 2002; Abdullah, S. K., et al., 2015).

3. RESULTS AND DISCUSSION:

Six genera of microfungi isolated from the soils of large fungi have been diagnosed, and these genera are

(*Alternaria alternate*, *Aspergillus terreus*, *Fusarium ventricosum*, *enicillium corylophilum*, *Rhizopus stolonifer*, *Trichoderma koningii*)



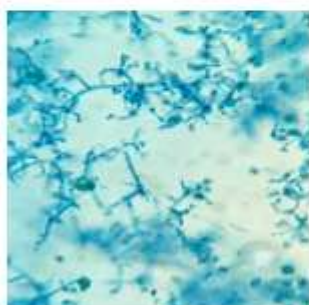
Fusarium ventricosum



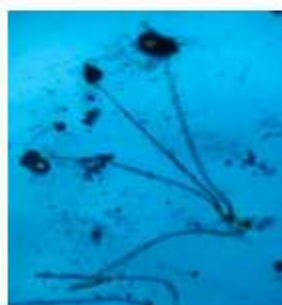
Aspergillus terreus



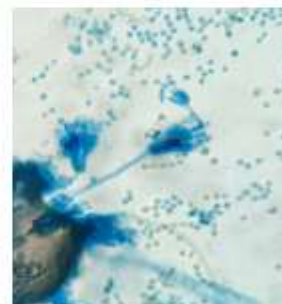
Alternaria alternate



Trichoderma koningii



Rhizopus stolonifer



Penicillium corylophilum

Panel (1) Microfungi isolated from the soils of large fungi in Salah al-Din Governorate. As for the macro fungi that have been isolated from the microfungi from their soil, they are shown in the figures below:



Coprinellus flocculosus



Coprinellus micaceus



Lactocollybia variicystis



Coprinellus disseminatus



Psathyrella spadiceogrisea



Coprinellus radians

Plate (2) Large fungi from which microfungi have been isolated

The descriptions of macro fungi:

1- *Coprinellus micaceus* :

External features

- Cap: 1.5-3 cm long, grainy, bell-shaped or convex, light brown or yellowish-brown and with a brown center.
- Gills: attached to the stem, crowded, sandy color changes to dark brown or black at maturity.
- Stalk: length 2-6 cm, diameter 6-2 mm, white, cylindrical, hollow, hallow granular granulate.

2- *Coprinellus flocculosus*

External features

- Cap: growing's are oval, conical to flat and decomposed at maturity, width 2-3.5 cm, height 1.5-2.5 cm, straight, sandy, pale yellow to brown in its central disc, covered with burqa residues.
- Gills: crowded, free, black color.
- Stalk: length 2-8 cm, width 4-8 mm, cylindrical, hollow, white, without volva and ring.

3- *Coprinellus disseminates*

External features

- Cap: width 0.5-1 cm, height 0.4-0.8 cm, oval, bell-shaped, yellow color, white and becomes gray at maturity, with a brown or blackish central disc and straight lines extending from the edge of the disc to the edge of the cap.
- Gills: eggs are young to black at maturity, attached to the leg attached and do not decompose at the maturity.
- Neck: length 1.3 – 4.3 cm, thickness 0.1 – 2.5 cm, white, cylindrical, hollow, smooth, central, no volva and ring.

4- *Lactocollybia variicystis*

External features

- Cap : 0.3 – 1 cm diameter, brown in color and then become convex to flat white or cream color at maturity, center (disc) concave, edges brown flat at maturity.
- Gills: eggs, somewhat spaced, disconnected.
- Stalk: length 2-6 cm, 2-4 mm thickness, cylindrical, central to lateral, white or creamy white, smooth, hollow.

5 - *Coprinellus radians*:

- Cap: oval becomes expanded, decomposed at maturity, 1.3-2.5 cm high 2-5 cm wide, straighten from the central disc, containing creamy-colored granules, discolored with increased humidity hygrophanous, edges curved upwards.
- Gills: free, crowded, black or pontifical at maturity.
- stalk: 1.5 – 8.5 cm length, 0.3 – 0.7 cm width, cylindrical, narrow at the top, central, hollow, white, no volva and ring.

6- *Psathyrella spadiceogrisea*

External features

- Cap : width up to 7 cm, conical, bell-shaped, flat at maturity, light brown, smooth.
- Gills : connected to the leg massive, eggs become black brown color at maturity.
- Stalk : length up to 5 cm, thickness 1 cm, cylindrical, white, smooth, hollow.

Table (1). Microfungi accompanied with the studied macrofungi

<i>Psathyrella spadiceogrisea</i>	<i>Coprinellus disseminatus</i>	<i>Coprinellus flocculosus</i>	<i>Lactocollybia variicystis</i>	<i>Coprinellus radians</i>	<i>Coprinellus micaceus</i>	Macrofungi Microfungi
+	-	-	+	+	+	<i>Alternaria alternate</i>
-	+	+	+	+	+	<i>Aspergillus terreus</i>
+	+	+	-	+	-	<i>Fusarium ventricosum</i>
-	+	+	+	+	-	<i>Penicillium corylophilum</i>

+	-	+	-	+	-	Rhizopus stolonifer
+	-	-	-	+	-	Trichoderma koningii

+ Exist, - absent

In Table 1, it is noted that the most frequent microfungus that appears with the accompaniment of the large fungus is the type *Aspergillus terreus* And that the least frequent species to appear accompanied by large fungi in some of the samples studied, as the species recorded *Alternaria alternata* Appeared in four large species included *Psathyrella spadiceogrisea*, *Coprinellus micaceus*, *Coprinellus*, *radians* *Lactocollybia*. While there is no appearance of the two species *Coprinellus flocculosus* , *Coprinellus disseminates*, the microfungus *Aspergillus terreus*, it has been recorded in all species except the genre *Psathyrella spadiceogrisea*. as well as the species *Fusarium ventricosum* It appeared in most of the samples studied, while no appearance was recorded in the two species *Lactocollybia variicystis*, *Coprinellus micaceus*. The microfungus *Penicillium corylophilum* It appeared in most species and no appearance was recorded in the two species *Psathyrella spadiceogrisea* and *Coprinellus micaceus* but the fungus *Rhizopus stolonifer*, It has recorded an appearance in three species and disappeared in three others. While mushrooms were *Trichoderma koningii*, it was the minimum appearance species in both two types *Psathyrella spadiceogrisea* and *Coprinellus radians* This indicates the difference of organic matter secreted from the macrofungi in the rhizospheric region and therefore the microbial diversity.

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