Isolation and identification of some fungi from rhizospheric soils of some wild plants at Samarra University, Iraq

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ABSTRACT
In this study, 20 taxonomic ranks were diagnosed, and Deuteromycota outperformed all the isolates, as it recorded nine taxonomic ranks, including five of them belonging to the genus Aspergillus and four of them to the genus Penicillium. Ascomycota displayed five taxonomic ranks, two ranks belong to the genus Alternaria and three to the genus Alternaria. Species belonged to different races, while the vaccinated fungi, Zygomycota exhibited three taxonomic ranks, two of them belong to the genus Mucor and one to the genus Rhizopus, while the oval fungi, Oomycota displayed two species belonging to the genus Pythium, while the sterile fungi, Sterill mycilia revealed the lowest numerical level among the rest of the studied fungi.

Keywords: Rhizosphere, Fungi, Plant.

INTRODUCTION
There is a microecological zone surrounding the plant root and affected by the biological, chemical and physical properties of the soil called the rhizosphere. Rhizospheric (RS) soil is highly effective in which many chemical and biological reactions take place. The number and activity of microorganisms may depend on the soil content of the organic and amino acids secreted by the roots. These compounds have an effect on the movement of nutrients in the soil. This area was described for the first time by the German scientist Hiltner in 1904. It was defined as an area of soil or soil affected by root infiltrates that surrounds the roots and is affected by plant growth and is effective with microorganisms (Al-Dabbagh 2011; Al-Khazraj 2012; Abdullah et al. 2015). Fan et al. (1997) and Uren (2000) found that plant roots secrete compounds of low molecular weight, including sugars: arabinose, maltose, mannose and amino acids (aspartic, cystine, asparagine, grginine) and organic acids (acet). Benzoicimalic and high molecular weight compounds such as enzymes, carbohydrates, fatty acids, growth regulators, nucleotides, vitamins and stimulants encourage the growth of fungi in huge numbers in that region. Morgan et al. (2005) added that root growth through the soil and the secretion of organic and amino acids, sugars, vitamins and growth regulators will lead to an increase in the activity and numbers of microorganisms in RS soil due to the availability of carbon and energy sources in this region, referred to as the effect of the rhizosphere.

The rhizospheric fungi are represented by saprophytes, pathogenic fungi and symbionts, and the rate of preparation ranges between 105 and 106 per gram of rhizospheric soil. Among the fungi that are endemic to the rhizosphere are zygotes and imperfect fungi (Al-Khazraj 2012).
MATERIALS AND METHODS

The media used in the study
The culture media listed below were prepared according to the instructions of the producing company installed on each package, and were sterilized by oxidizer at a temperature of 121°C and 1 atmospheric pressure for 20 minutes.

Preparation of culture media

Potato Dextrose Agar (PDA)
It is prepared by dissolving 39 g of potato dextrose agar (PDA) powder (HIMEDIA Company) in 1000 mL distilled water, then stirring it well with heating by a hotplate until it boils, then its sterilized by autoclave under 121°C and 1 atmosphere for 20 minutes. After cooling, 1 mL of the suspension of the antibiotic chloramphenicol (100 μg mL⁻¹) produced by the Indonesian Company, Bekasi, was then distributed in sterile Petri dishes. This medium was used to isolate and diagnose the fungi.

Medium sweet potato dextrose
Flasks of 500 mL-capacity were taken and 250 mL of broth PD liquid medium (HIMEDIA Company) was poured into them, then sterilized by autoclave and after cooling the flasks, the antibiotic (chloramphenicol) was added. One capsule containing 250 mg of chloramphenicol was dissolved in 2.5 mL of sterile distilled water. One mL of the solution was taken and added to one liter of the nutritional medium to be a diluted to 100 μg mL⁻¹.

Isolation of Fungi

Isolation of fungi from rhizosphere soil
The rhizospheric soil was separated by shaking it with a mixer and washing with sterile distilled water. The Volume Displacement Technique was used to isolate the fungi from the rhizosphere (Reyes & Mitchell 1962) where 2 cm pieces of roots and the adherent soil were placed in beakers containing 90 mL of sterile distilled water. These flasks were shaken for 15 min, the roots were lifted, the process was repeated and other roots were added until the final volume of soil with water was 100 mL (Al-Dabbagh, 2011). The 10⁻³ dilution was prepared and these dilutions were placed in Petri dishes, then it was incubated in a shaking incubator at 25 ± 1°C.

Purification and preservation of isolated fungi
Fungi colonies were purified after planting the dishes with 1 mL of water collected as mentioned above. Different colonies were obtained, purified by taking a small part from the tip of each colony using a loop and placed in new dishes containing PDA medium and kept in the refrigerator at a temperature of 4 °C. until use, taking into account its renewal whenever the need arises.

Diagnosis of isolated fungi

Diagnosis according to the characteristics of the plant
The culture dishes were examined 7 days after the appearance of the fungal growth, which is one of the most important means of identifying the fungi. Cultivation characteristics include several things, including: the incubation period, the shape of the colony (sunken, prominent), its color and texture (powdery, cottony, fluffy), and the examination is re-examined from the opposite side, and the diameter of the colony is measured after growth stops.

Microscopic examination of colonies by wet loading method
This test was carried out by applying a drop of cotton blue dye (prepared by adding 10 g phenol crystals to 10 mL glycerin and mixing it with 10 mL distilled water and also 10 mL lactic acid). The mixture was well mixed and then 0.2 g of cotton blue was added (Ellis 1994) on a clean glass slide and by means of a sterilized needle. Thereafter, a part of the fungal hyphae was transferred from the edge of the colony to the glass slide and mixed with the dye, then the slide cover was placed on it and gently pressed for the
purpose of spreading the sample (Forbes et al. 1998). Then, the sample was examined under a light microscope using the 100 X, followed by 400 X, and higher magnifications, to observe the fungal hyphae, their shapes, branches, dimensions, and the conidia of different shapes and sizes such as micro and macro conidia. They were used for the purpose of measuring the dimensions of the conidia, the ocular micrometer, after calibration. Microscopic imaging was done using Sony digital camera.

**Microscopic examination using tape**
This examination was carried out using a transparent adhesive tape with a length of 2 cm by touching and pressing the adhesive side of the tape to the surface of the colony and under sterile conditions. The slide was examined under the microscope in 100 X at first, then 400 X and higher magnifications, to observe the distinctive characteristics of the small and large conidia in terms of shape, arrangement and size (Baron et al. 1994).

**RESULTS AND DISCUSSION**
In the present study, 20 fungal taxa were isolated and identified as shown in Figs. 1 - 2. from rhizospheric soil obtained from some plant species taken from Samarra University, Iraq, where the vast majority of those taxonomic orders belonged to Deuteromycota. This order contains nine taxonomic orders: Aspergillus parasiticus, A. ocraceus, A. niger, A. flavus, Aspergillus sp., Penicillium expansum, P. corylophilum, P. resticulosus, and P. janthinellum, followed by the cystic fungi, i.e., Ascomycota, containing five taxonomic orders: Alternaria sp., Alternarianata, and Cladosporium cladosporiodes, Cl. luna as well as Currycota containing three taxa, Zygomycota and Curmycota fungi. Its taxa are Macor Sp 1, Macor Sp 2 and Rhizopus oryzae, followed by Oomyxota, which contained two taxonomic orders, Pythium Sp1 and Pythium Sp 2. The sterile fungi, Sterill mycilia achieved the lowest order by containing one taxonomic order, which is White (Sterill mycilia).

Our study has shown that the imperfect fungi are dominant, and this is consistent with what was found by Rizek (2013), who isolated 36 fungal species during three seasons of the year, and recorded the deficient fungi in 94.4% of the root circumference of three plants including sedge, reed and rush, similar to the other studies (Hamoudi 1999; Mashhad 2010 & Al-Abbasi 2014, AL-Samarraie et al. 2014).

![Fig. 1. Order of fungal centers by number of types.](image)
*alternate* 

*Alternaria* 

*Alternaria* sp. 

*Alternaria* sp. colonies 

*Aspergillus* 

*A. flavus* (400X) 

*Aspergillus flavus* colonies 

*Aspergillus flavus* colonies
Aspergillus sp. (400X)

Aspergillus sp. colonies

Cl. Cladosporioides (400X)

Cladosporium cladosporioides colonies

C. lunata (400X)

Curvularia lunata colonies
Fusarium oxysporium (400X)

Mucor sp. 1 (400X)

Mucor sp. 2 (400X)

Mucor sp. 1 colonies

Mucor sp. 2 colonies
**Penicillium corylophilum colonies**

**P. corylophilum (400X)**

**Penicillium expansum colonies**

**P. expansum (400X)**

**Penicillium resticulosum colonies**

**P. resticulosum (400X)**
P. janthinellum (400X)

P. janthinellum colonies

Pithium sp. 1 (400X)

Pithium sp. 1 colonies

Pithium sp. 2 (400X)

Pithium sp. 2 colonies
CONCLUSION
Rhizosphere soil is rich of the different fungi species of various kinds. Expected the denseout duometercota and the ascomiicot crossings fungal on all the types of fungus are in this study. The sterile fungals of Sterill mycilia have been recorded by the lowest frequency between the entire fungal types.

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