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# 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*, followed by *Ascomycota*, which 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.
**Article type:** Research Article.

## 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-Khazraji 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, aspargine, grginine) and organic acids (acetic).

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-Khazraji 2012).

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## METERIALS 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 121ºC 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

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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 containes nine taxonomic orders: *Aspergillus parasiticus, A. ocraceus, A. niger, A. flavus, Aspergillus* sp., *Penicillium expansum, P. corylophilum, P. resticulosum,* 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 Curmycota containing three taxa, Zyporycota and Curmycota fungi. Its taxa are *Mucor* Sp 1, *Mucor* Sp 2 and *Rhizopus oryzae,* followed by Oomycota, 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).

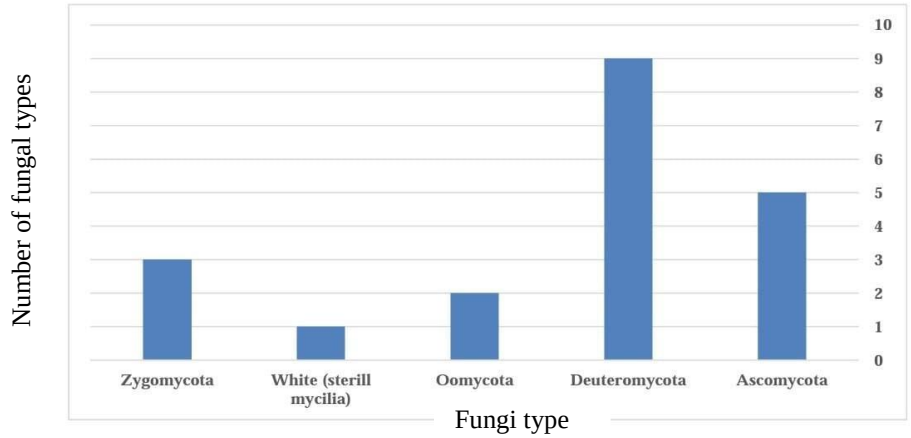


**Fig. 1.** Order of fungal centers by number of types.

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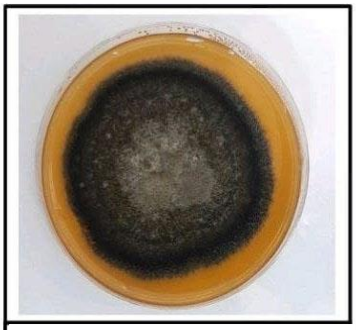
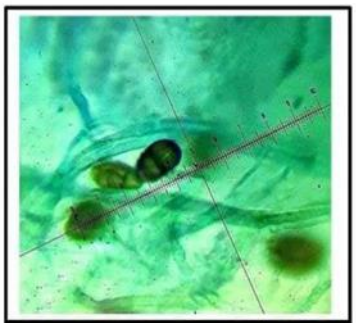
*A. alternate* (400X)

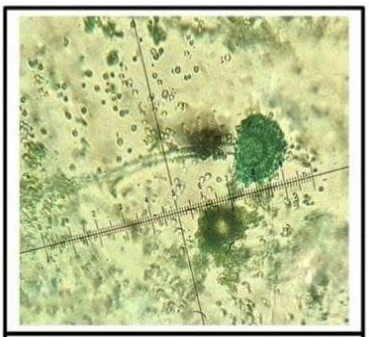


*Alternaria alternate* colonies



*Alternaria* sp. (400X)

*Alternaria* sp. colonies



*A. flavus* (400X)

*Aspergillus flavus* colonies

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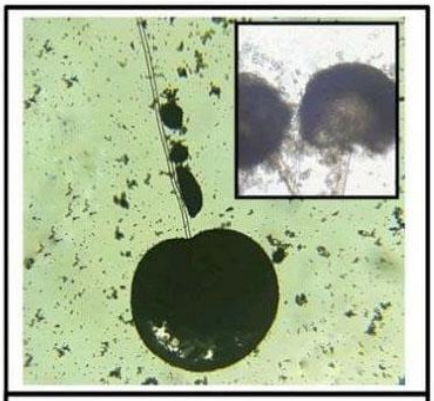
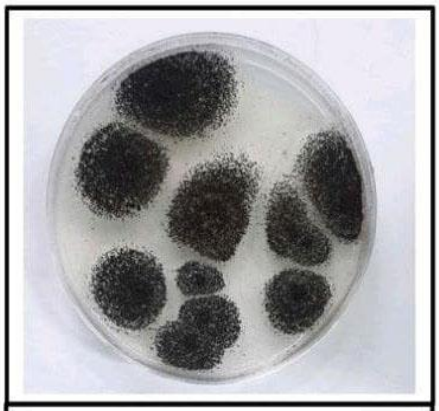
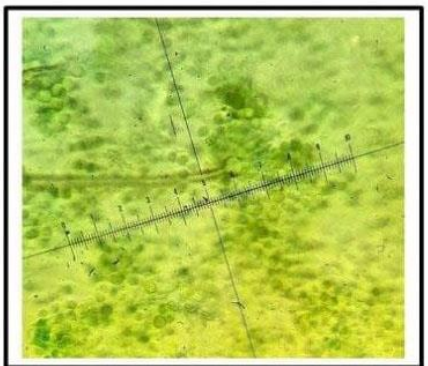


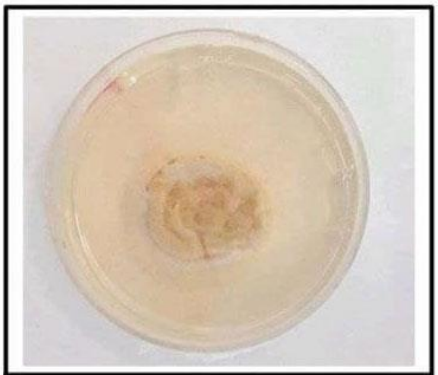
*A. niger* (400X)



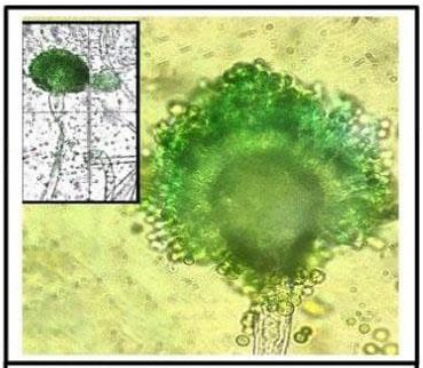
*As. niger* colonies



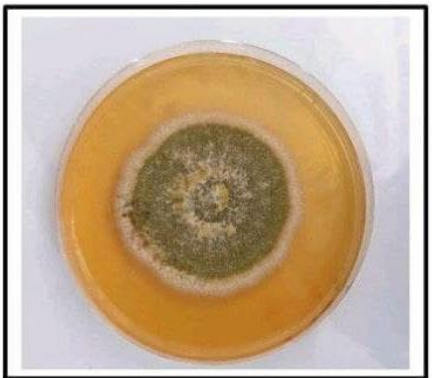
*A. ocraceous* (400X)



*Aspergillus ocraceous* colonies



*A. parasiticus* (400X)



*Aspergillus parasiticus* colonies

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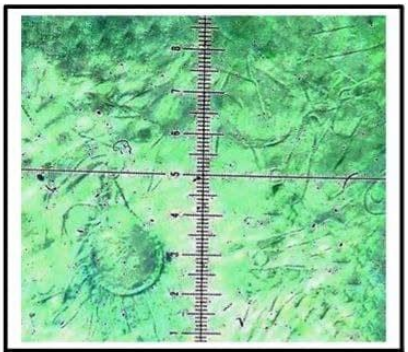
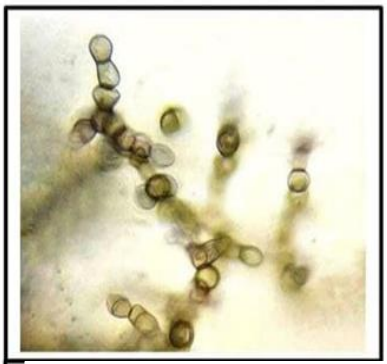
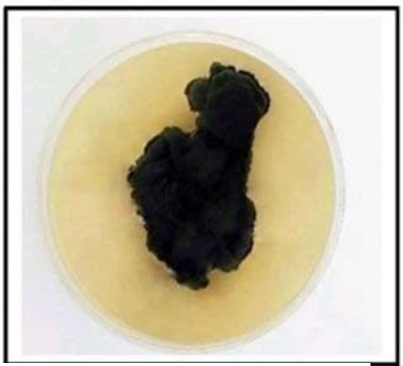


*Aspergillus* sp. (400X)

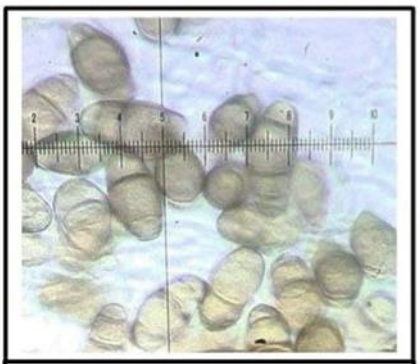
*Aspergillus* sp. colonies



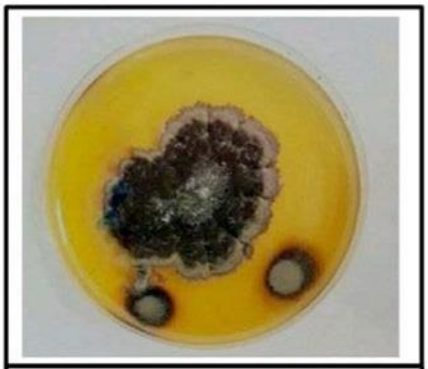
*Cl. Cladosporioides* (400X)



*Cladosporium cladosporioides* colonies



*C. lunata* (400X)



*Curvularia lunata* colonies

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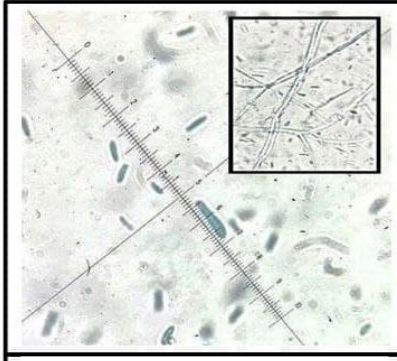
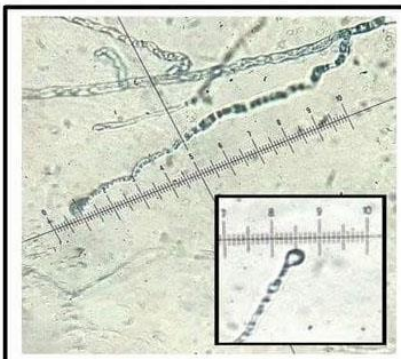
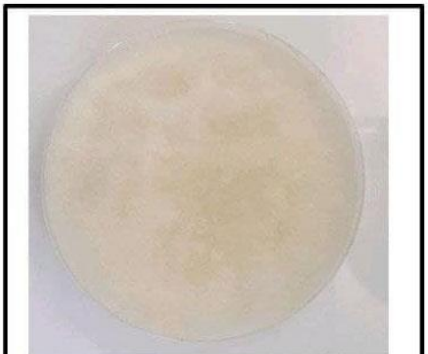


*F. oxisporium* (400X)

*Fusarium oxisporium* colonies

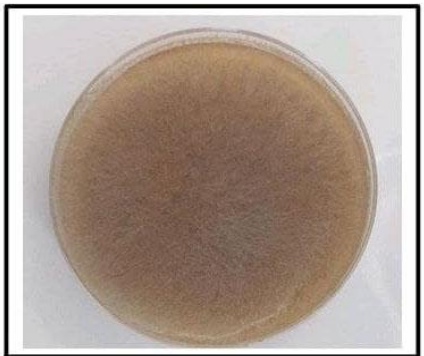


*Mucor* sp. 1 (400X)



*Mucor* sp. 1 colonies

*Mucor* sp. 2 (400X)



*Mucor* sp. 2 colonies

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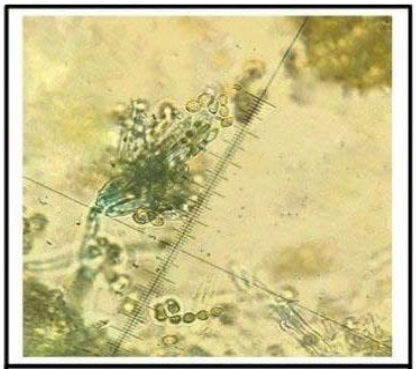
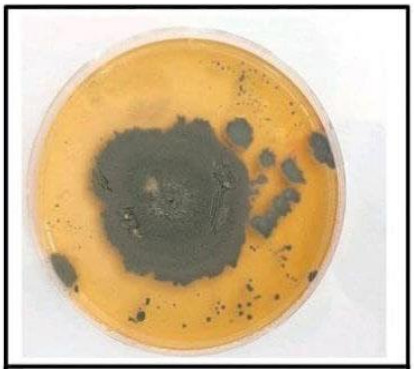
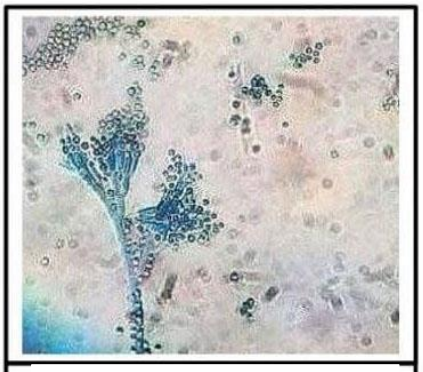


*P. corylophilum* (400X)

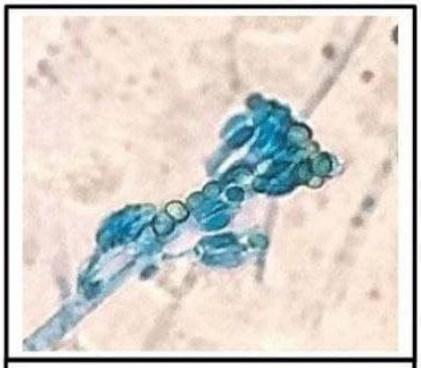


*Penicillium corylophilum* colonies

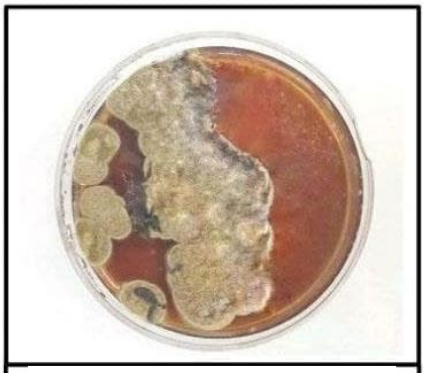


*P. expansum* (400X)

*P. expansum* colonies



*P. resticulosum* (400X)



*P. resticulosum* colonies

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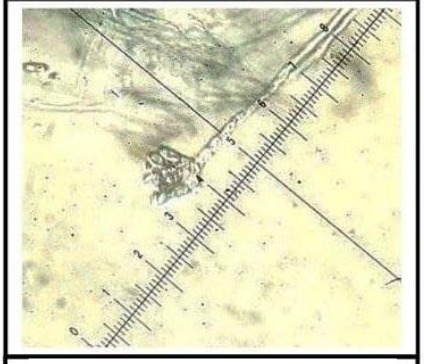
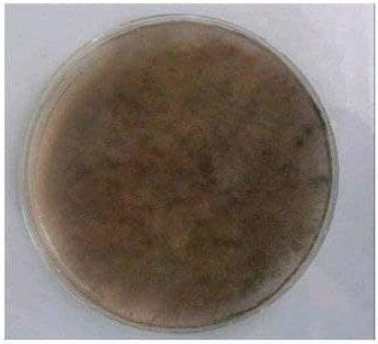
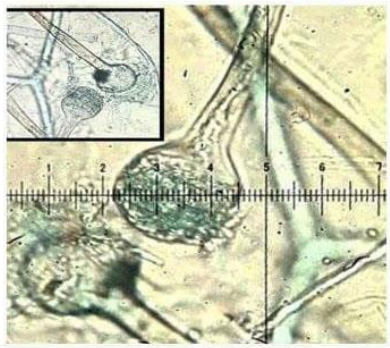


*P. janthinellum* (400X)

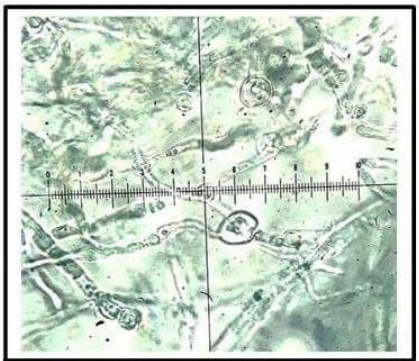


*P. janthinellum* colonies



*Pithium* sp. 1 (400X)

*Pithium* sp. 1 colonies



*Pithium* sp. 2 (400X)

*Pithium* sp. 2 colonies

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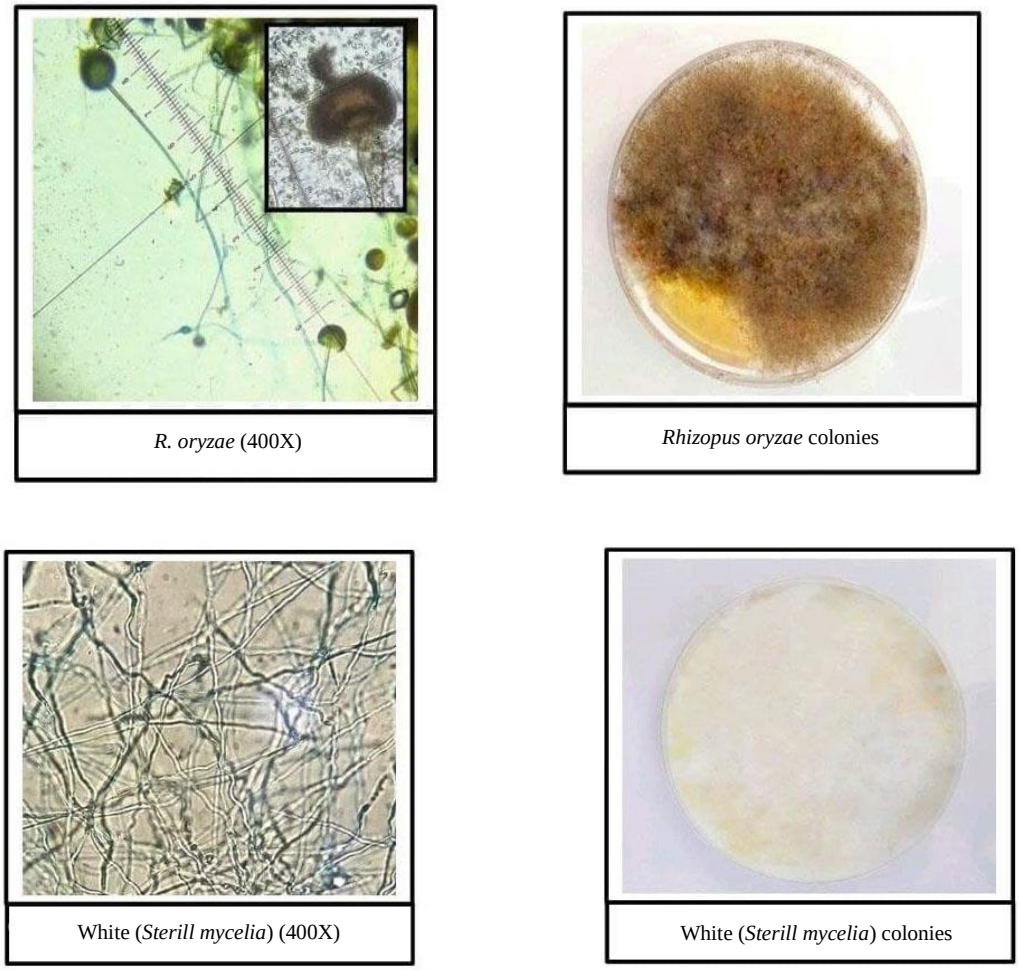


**Fig. 2.** The appearance of taxonomic types of diagnosed for fungi.

## CONCLUSION

Rhizosphere soil is rich of the different fungi species of various kinds. Expected the denseout duometercota and the asceomicot 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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