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Isolation and Identification of Microfungi from Soil Samples Collected at Different Location in Erbil Governorate

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By

Mahmood A. Anwar & Rahima A. Darwesh

Supervised by: MSc. Shna Ibrahim Ismail

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Mahmood A. Anwar **Rahima A. Darwesh**

MSc. Shna Ibrahim Ismail

Department of Biology/College of Science/University of Salahaddin-Erbil, Iraq

Abstract

Soil is defined as a mantle of weathered rock which, in addition to organic matter contains minerals and nutrients. Soil fungi are the important part of the terrestrial ecosystem and it plays a major role in nutrient cycle as decomposer. The diversity of soil fungi indicates the good or defective condition of the soil health. This work was designed to assess the prevalence and species diversity of microfungi inhabiting from the soil of different areas in Erbil governorate. About thirteen species belonging to nine genera of fungi, including *Aspergillus, Alternaria, Candida, Emericella, Eurotium, Fusarium, Mucor, Penicillium* and *Rhizopus* were isolated from soil samples of five different locations, by using two methods; Soil plate method and Soil dilution method. Among them *Aspergillus* (32.3%) and *Rhizopus* (23.5%) were the most predominant isolated genera, followed by *Candida* (11.8%), *Mucor* and *Fusarium* (8.9%). While, *Eurotium* (5.9%), *Alternaria, Emericella* and *Penicellium* (2.9%) were the least frequently isolated soil borne fungi.

Key Word: Soil Fungi, *Aspergillus*, *Rhizopus.*

I. INTRODUCTION

The soil is the junction of minerals, organic matter, air and water that allows biological activity in the environment. Compared to other terrestrial habitats, "the soil in their nature complex and heterogeneous dynamics, allows organisms with different metabolisms live side by side, interacting in a state of dynamic equilibrium, providing ideal conditions for an extremely high biodiversity" (Moreira Garcia et al., 2015). The biodiversity refers to the variability of life on the earth, including all of the living species of animals, plants and microorganisms (Hussain, 2015). Actually, soil is dynamic structure, inhabiting various diverse groups of microorganisms. Most important amongst them are bacteria, actinomycetes, algae, fungi and protozoans (Pandey et al., 2017). The relationship between biodiversity of soil fungi and ecosystem function is an issue of paramount importance, particularly in the face of global climate change and human alteration of ecosystem processes. Fungi are an important component of the soil micro biota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. The saprobic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in the decomposition of plant structural polymers, such as, cellulose, hemicelluloses and lignin, thus contributing to the maintenance of global carbon cycle (Saravanakumar and Kaviyarasan, 2010).

Fungi are one of the most diverse kingdoms of eukaryotes. Most of them are spore-producing, heterotrophic organisms because they do not contain plastids. Fungi are like other organisms, especially animals, in that they need organic compounds as a source of food. The diversity in fungal nutrition methods whether symbiosis, saprophytism or parasitism, has given them great importance for influencing all terrestrial ecosystems, including plant communities. Fungi are classified according to the structure, where the fungal body may be either unicellular or more commonly filamentous, in addition to life cycles, taking into account the mode of sexual and asexual reproduction. Glucans, chitin and glycoproteins are characteristic compounds of fungal cell wall structure. This makes it an excellent target for antifungal therapy, since fungal cell wall components are not present in humans (Jwieli et al., 2021). They are one of the most widespread living organisms in nature, as the number of diagnosed species reached (100,000) species. It exists in humans, animals and plants. It can spread in the soil, air and waters (MAHMUT et al., 2023). Many of fungi occur as saprophytes in the environment and are scattered throughout the world. Although these fungi had previously been considered to be nonpathogenic, are now being encountered as causes of humans and animals' infection especially in hosts with impaired immune systems. In recent years, opportunistic fungal infections have increased significantly, and the species of the genus *Aspergillus, Mucor, Penicillium, Rhizopus, Fusarium, Alternaria*, etc. are emerging as the cause of a variety of infections in human. Many of potentially pathogenic fungi such as *Histoplasma capsulatum*, *Sporothrix schenckii*, *Coccidioides immitis*, *Blastomyces dermatitidis*, etc. also inhabit freely in the soil and can cause different degrees of allergy or serious fungal diseases. Pathogenic fungi can cause harm to humans, animals and plants (Nosratabadi et al., 2017).

Soil fungi can be grouped into three general functional groups based on how they get their energy, Decomposers, mutualists and pathogens, the decomposers are responsible for the breakdown of dead organic material. Mutualist fungi will colonize plant roots and provide nutrients to the plant in exchange for carbon (an energy source). The pathogens cause disease or death when they colonize and feed on living organisms like plant roots or nematodes. Fungal hyphae physically bind soil particles together, creating stable aggregates that help increase water infiltration and soil water holding capacity. Some soil fungi are potential pathogen to both humans and animals. Soils that are rich in keratinous material are the most conductive for the growth and occurrence of keratinophilic fungi (Toma and Abdulla, 2012). Genera and species of fungi in soil are related to diverse chemical and physical factors, for instance, oxygen, pH, moisture and organic compounds (Jwieli et al., 2021). The diversity of soil fungi indicates the good or defective condition of the soil health (Chandini and Rajeshwari, 2017).

Aim and objectives

The present study is an attempt to assess the prevalence and species diversity of microfungi inhabiting from the soil of different areas in Erbil governorate.

II. MATERIALS AND METHODS

Collection of Soil Samples

Samples for this research work were collected from five different zones of Erbil including; Kurdistan quarter, Sami-Abdulrahman Park, College of Science Garden, Galyawa and Koya. Approximately 500gm of soil was removed with a sterile trowel from depth of 2-8 cm from site after first scraping away the upper 0-2 cm of surface soil. Soil samples were stored in sterile polythene bags labelled and transferred to the laboratory for mycological analysis. In the laboratory the samples were milled and sieved twice to remove large stones and debris for obtaining soil samples with small particles.

Media preparation:

Potato dextrose agar used in this study was prepared and sterilized according to the manufacturer's guideline. Bacterial contamination was inhibited by adding 500mg of chloramphenicol into 500mls of the agar solution prior to autoclaving and pouring into petri dishes. Media was placed in the autoclave to allow for homogenization and sterilization. Reliable sterilization with moist heat requires temperatures above that of boiling water. These high temperatures are most commonly achieved by steam under pressure in an autoclave. Steam at a pressure about 15 psi, attaining temperature 121°C will kill all organisms and their endospores in about 15 minutes. A basic principle of chemistry is that when the pressure of a gas increases, the temperature of the gas increase proportionally (Thliza *et al*. 2020).

Isolation of fungi:

Two methods of isolation have been used, soil plate method and soil dilution method, by using potato dextrose agar media.

Soil plate method: Approximately 0.005 gram of each soil sample have been scattered on the bottom of sterile petri dishes, then a molten cooled (40-45°C) media of potato dextrose agar has been added, rotated gently to disperse the soil particles in the medium. They let to solidify, and incubated at 26-28 °C for 7-10 days (Hussain, 2015).

Soil dilution method: One gram from each soil samples have been suspended in 9ml sterile distilled water to prepare the soil suspension. Six dilutions until 10^{-6} have been prepared from each soil suspension. 1ml from dilution 10^{-1}, 10^{-3} and 10^{-6} was added to the sterile petri dishes containing prepared medium (PDA + Chloramphenicol) and incubated at 26-28 °C for 7-10 days (Hussain, 2015).

Purification of the Fungal Isolates:

Pure isolates were generated by sub-culturing on Potato Dextrose for both visual and microscopic examinations of cultural (color and growth pattern) and morphological characteristics respectively for further differentiation (Umar et al., 2016). Occasionally identification of fungal species was done from the culture of the original petri dish (Abdel-Gawad and Zohri, 1993).

Identification of fungal isolates:

The isolated fungi were identified to the genus level and to the species when possible on the basis of macro morphological (The colonies were examined for slow or for rapid growth, topography (flat, heaped, regularly or irregularly folded), texture (yeast like, powdery, granular, velvety or cottony), surface pigmentation and reverse pigmentation) and micro morphological (Hyphae, macro conidia, micro conidia, chlamydospores and other special fungal structure) characteristics using suitable media, slide cultures and the most updated keys for identifications. The identified fungi confirmed with microbial expert (Raja et al., 2017).

Macroscopic characteristics:

The macroscopic characteristics (color of the surface and reverse, topography, and texture) of fungal colonies observed after incubation were thoroughly studied and observations were recorded.

Microscopic characteristics:

Microscopic identification was done according to (Fawole and Oso, 2001). A drop of lactophenol cotton blue stain was placed on a clean slide and with the aid of a mounted needle, a small portion of the mycelium from the fungal cultures was removed and placed in the drop of the stain. The mycelium was spread very well on the slide with the aid of the two mounted needles and a cover slip was gently lowered on it. The slide was then examined under the microscope. The observation was done at high power objective 40X of the microscope. Morphological characteristics of the fungi such as type of hyphae and asexual reproductive structure were observed (Muhammad et al., 2018). Each filamentous fungal colony growing from the inoculum pieces was identified at least to genus level.

III. RESULTS AND DISCUSSION

Fungi are important components in soil microbiota. Saprotrophic fungi constituted the largest proportion of fungal species in soil, played a major role in the decomposition of plant structural polymers such as cellulose, hemicellulose and lignin, thus contributing to the maintenance of the global carbon cycle. Monitoring fungal diversity is essential to detect fungi hazardous to humans, animals and plants.

In this investigation nine fungal genera were isolated by two methods; Soil plate method and Soil dilution method. From the fungal isolates the most of the species belonging to the genera *Aspergillus* and *Rhizopus.*

The identified soil fungi (Table 1) namely *Aspergillus flavus, Aspergillus fumigatus Aspergillus niger, Aspergillus ochraceous, Aspergillus terrus, Alternaria, Emericella, Eurotium, Fusarium, Mucor, Penicillium, Rhizopus* and Candida.

Table 2 show the identity and Total CFU (Colony forming Unite) of fungi isolated from five selected site of soil samples. The most frequently isolated fungal genera were Aspergilli

11X10^3 CFU/gm of soil, followed by *Rhizopus* 8X10^3 CFU/gm, *Candida* 4X10^3 CFU/gm, *Mucor* and *Fusarium* (3X10^3 CFU/gm). While, the least frequently isolated fungal genera were *Eurotium* 2X10^3 CFU/gm, then *Alternaria, Emericella* and *Penicillium* (1X10^3 CFU/gm).

The results are in conformity with many of previous works. (Toma and Abdulla, 2012) In their survey for identification of seasonal soil fungi in Erbil city, they discovered that *Aspergillus* had the highest incidence in soil, followed by *Penicillium, Rhizopus, Emericella, Fusarium* and *Clasterosporium*. Furthermore, in Erbil the investigation conducted by (Ramadan and Ismael, 2011) They discovered several distinct fungal genera, including dermatophyte and non-dermatophyte fungi, among non-dermatophytes they highlighted that *Aspergillus niger* was the most frequently isolated fungi. In Duhok, the paper which published by (Abdullah and Mohammed, 2011) They observed that black aspergilli were the most often isolated species in soil in vineyards in Duhok. In Kirkuk (MAHMUT et al., 2023) showed that the (*Aspergillus flavus* 20.83%) was the most abundant fungus, while the (*Penicillium citrinum* 8.30%) was the less prevalent one in all resources and locations. In Baghdad (Hussain, 2015) isolated 89 fungal genera from the soil and showed that the highest percentage of fungus isolates was *penicillium* followed by *Mucor* and *Alternaria*.

In Iran (Shahbazy et al., 2015) who investigated the seasonal distribution of fungi in soil found in two hospitals in Bandar Abbas, reported *Aspergillus* was the dominant fungus in both hospitals. Additionally in Iran, (Nosratabadi et al., 2017) isolated 30 fungal genera from the soil including *Aspergillus* spp. (22.99%), *Mycelia sterilia* (16.15%), *Penicillium* spp. (8.9%), *Chrysosporium* spp. (6.83%), *Cladosporium* spp. (5.6%), *Fusarium* spp. (4.97%), *Alternaria* spp. (4.76%), *Acremonium* spp. (3.73%) and other fungi (26.07%). (Karaoglu and Ulker, 2006) isolated soil borne fungi from different altitudes of Iyidere-Ikizdere vicinity in Rize, Turkey, reflected that majority of *Penicillium, Aspergillus, Trichoderma* and *Fusarium* were the most abundant genera. (Jwieli et al., 2021) isolated and identified nine genera of fungi (*Phyllactinia, Fusarium, Penicillium, Alternaria, Mucor, Aspergillus, Chaetomium, Phytophthora and Rhizoctonia*) from nine regions in Ghemins, which located on the eastern coast of Libya, in the south of Benghazi. (Nazri et al., 2020), a total of 25 isolates were identified into ten genera based on internal transcribed spacer region (ITS) sequence analysis, namely *Aspergillus, Clonostachys, Colletotrichum, Curvularia, Gliocladiopsis, Metarhizium, Myrmecridium, Penicillium, Scedosporium* and *Trichoderma* consisting 18 fungi species. *Aspergillus* and *Penicillium* species were claimed as predominant microfungi inhabiting the soil in Serdang, Selangor, Malaysia. (Afzal et al., 2013) indicated that *Aspergillus fumigatus* and *Aspergillus flavus* were dominating species isolated from all areas of Larkana in Pakistan. (Gaddeyya et al., 2012) isolated15 species belonging to 6 genera of fungi from agricultural fields at Salur Mandal, India (*Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp., *Fusarium* spp., *Curvularia* spp. and *Rhizopus* sp) by using soil dilution technique and soil plate technique. Fungal species belonging to the genera *Aspergillus, Penicillium, Colletotrichum, Mucor, Rhizopus, Cunninghamella, Scopulariopsis* and *Cladophialophora* were isolated from soil samples collected from 20 zones in Loyola college campus, Chennai, and the method that was used is soil dilution method beside staining technique by using Lactophenol and cotton blue (Raja et al., 2017). Also, in a study of determination of the soil Microflora of in Nigeria (Oyeyiola et al., 2013), they isolated *Rhizopus oryzae, Aspergillus*

niger, Aspergillus flavus, Mucor hiemalis, Penicillium chrysogenum, Fusarium oxysporium, and *Trichophyton rubrum*. And these results were almost similar to our results of soil borne fungal isolation.

There is relationship between soil fungi and the soil environment properties like physical and chemical properties. also, some soil fungi related positively with the P, Ca and Mg content in the soil. the maximum species and numbers of soil fungi were found in soil collected in the rainy season (Kosol et al., 1999).

Another research showed that the effect of microbial diversity on microbial functions in the soil depends on the function measured. Some functions increased (substrate induced respiration, SIR) with decreasing microbial diversity in soil, others were not affected (thymidine and leucine incorporation, NO3 accumulation, respiratory growth response), and some declined when microbial diversity was small (short-term respiration from added grass, potential nitrification rates (Griffiths et al., 2001).

No relationship exists between microbial diversity and decomposition of organic matter, and a reduction in any group of species has little effect on overall soil process because the surviving microorganisms can carry out the decomposition of organic matter (Nannipieri et al., 2003).

The fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic contents, and moisture. And there was no much of a variance in the three types of soil (agricultural fields, garden and barren land soil), the diversity was found to be higher in the unattended barren land as compared to the agricultural fields and garden soils (Nilima Wahegaonkar et al., 2011).

Because of the importance of the soil fungi category, it should receive much more attention, and more researches should be done to find any promising species that could be use in the industry, medicine and agriculture, also to preserve any important species that might be disappeared.

Table 2: The percentage of fungal genera isolated from soil samples.

No.	**Fungal Genera**	**Frequency (n) x 10^3 CFU/gm soil**	**Prevalence (%)**
1	*Aspergillus*	11	32.3
2	*Rhizopus*	8	23.5
3	*Candida*	4	11.8
4	*Mucor*	3	8.9
5	*Fusarium*	3	8.9
6	*Eurotium*	2	5.9
7	*Alternaria*	1	2.9
8	*Emericella*	1	2.9
9	*Penicillium*	1	2.9
Total		**34X10^3**	**100**

Table 1: Isolated fungal genera from soil samples by Soil plate method & Soil dilution method

No.	Locations	Soil plate method	Soil dilution method		
			Dilution 10^{-1}	Dilution 10^{-3}	Dilution 10^{-6}
1	**Kurdistan quarter**	*A. fumigatus* *Rhizopus*	*A. fumigatus*	*Eurotium*	*Eurotium* *Candida*
2	**Sami-Abdulrahman Park**	*A. flavus* *A. niger*	*A. ochraceous* *A. niger*	*Fusarium*	*Penicillium* *Candida*
3	**College of Science Garden**	*Rhizopus* *A. niger*	*Rhizopus*	*Rhizopus*	*A. fumigatus* *Candida*
4	**Galyawa**	*Mucor* *Emericella*	*Mucor*	*Mucor* *Alternaria*	*A. terrus* *A. flavus*
5	**Koya**	*Rhizopus*	*Rhizopus*	*Rhizopus* *A. flavus* *Fusarium*	*Rhizopus* *Fusarium* *Candida*

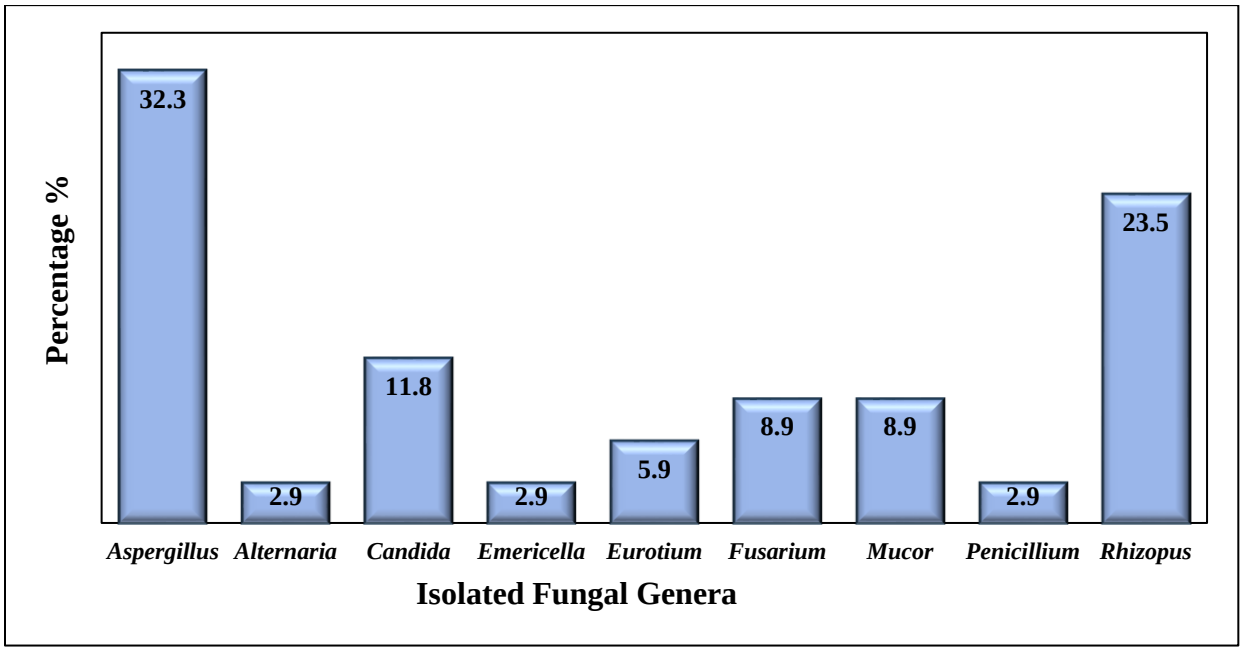
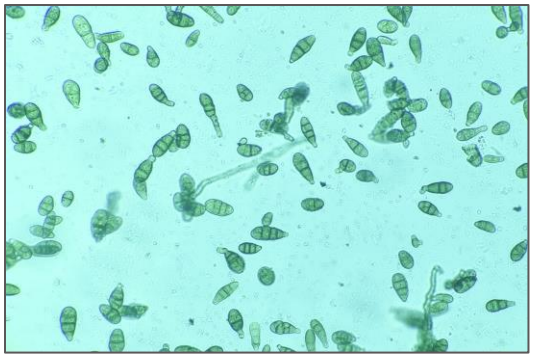
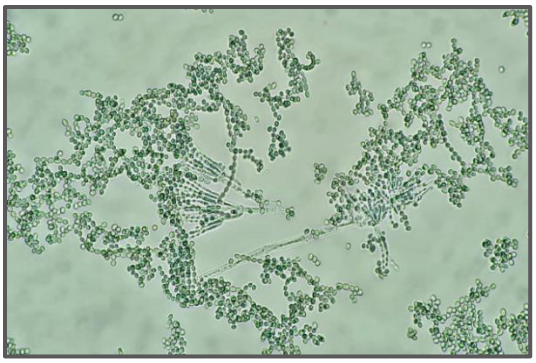


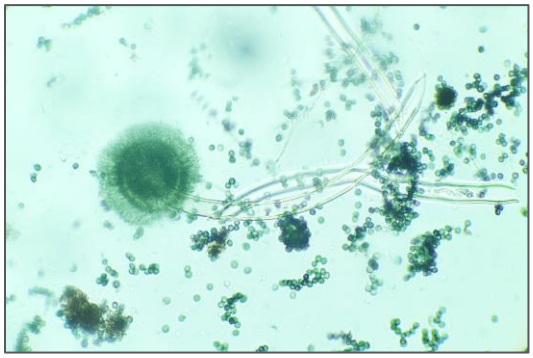
Figure 1: Percentage of Isolated Fungal Genera From Soil Samples.



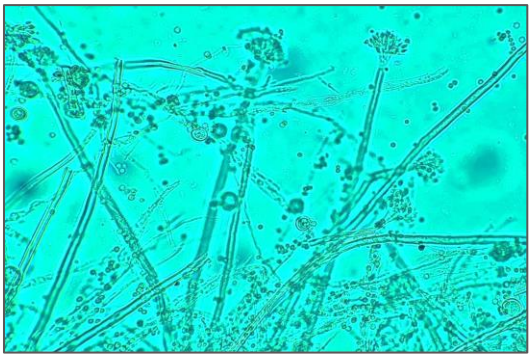
Alternaria



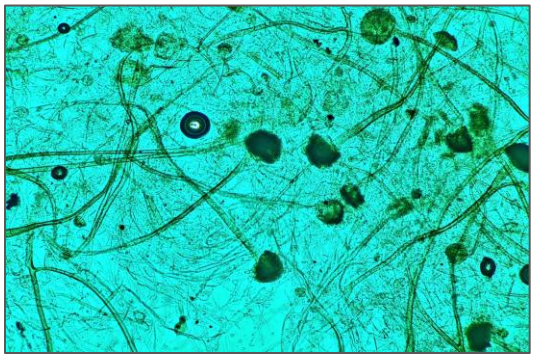
Penicillium



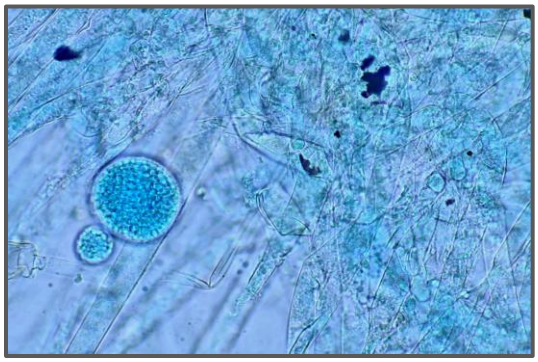
Aspergillus ochraceous



Aspergillus terrus



Rhizopus



Mucor

Figure 2: Microscopic morphology of different fungal genera isolated from tested soil samples.

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