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e-ISSN 0976-7614

Volume 3, Issue 4, October 2012

Research Article

# Isolation, Identification and Seasonal Distribution of Soilborne Fungi in Different Areas of Erbil Governorate

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**Abstract:** A survey study was undertaken for the isolation and identification of soilborne fungi inhabiting different areas in Erbil, Iraq. A total of 15 soil samples collected seasonally during August 2008-July 2009.

Fungi were isolated from soil during this study's period, by soil dilution plate method on selective medium: Potato Dextrose Agar (PDA), eighty-one different genera of fungi were isolated during the four seasons; (30, 33, 70, and 53) genera isolated in (summer, autumn, winter and spring) respectively, the most frequently isolated fungi during four seasons were *Aspergillus* sp. (539x10<sup>3</sup>) colony forming units /g.soil, followed by *Penicillium* sp. (215x10<sup>3</sup>) cfu/g.soil, *Rhizopus* spp. (115x10<sup>3</sup>) cfu/g.soil, *Emericella* spp. (109x10<sup>3</sup>) cfu/g.soil, *Fusarium* spp. (47x10<sup>3</sup>) cfu/g.soil, and *Ulocladium botrytis* (47x10<sup>3</sup>) cfu/g.soil, while the least frequently isolated fungal genera were *Blakeslea tuningtam*, *Clasterosporium cyperi*, *Idriella* sp., *Naranus cryptomeriae* and *Torula alternata*, (1x10<sup>3</sup>) cfu/g.soil, for each one.

Fungi isolated from soil by washing method counted for ninety three species belonging to fifty six genera, among them: Aspergillus sp., Circinella sp., Cunninghamella sp., Mucor spp., Mycelia sterilia, Rhizopus sp.

**Keyword:** Soil fungi, *Aspergillus* sp., Washing method.

#### 1. Introduction

Soil is defined as a mantle of weathered rock which, in addition to organic matter contains minerals and nutrients (Sylvia *et al.*, 2005).

The soil is a rich habitat for the growth of microorganisms more than any other microbial habitats. Among these microorganisms, fungi are one of the dominant group present in soil, which represent the main reservoir of fungi (Ali-Shtayeh and Jamous, 2000; Rane and Gandhe, 2006).

Fungi belong to kingdom Myceteae. The distinguishing characteristics of this group as a whole are that they are eukaryotic, nonphotosynthetic, lack tissue differentiation, have a cell wall of chitin or other polysaccharides and propagate by spores (sexual and /or asexual) (Benson, 2002).

Soil fungi can be grouped into three general functional grouped 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 (Tugel *et al.*, 2000; Brady and Weil, 2002). 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 (Ingham, 2007).

#### 2. Material and Methods

## 2.1 Media used for isolation and identification of fungi

A. Potato Dextrose Agar (PDA) [Onuorah, 1982].
Chemical compound Quantity
Dextrose: 20g.
Potato: 200g.
Agar: 20g.
Distill water (D.W.): 1 L.

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#### B. Czapek Dox Agar (Johnston and Booth, 1983).

Chemical compound	Quantity
NaNO <sub>3</sub> :	2g.
KH <sub>2</sub> PO <sub>4</sub> :	1g.
$MgSO_4.7H_2O:$	0.5g.
KCl:	0.5g.
FeSO <sub>4</sub> .7H <sub>2</sub> O:	0.01g.
Sucrose:	30g.
Agar:	20g.
D.W:	1L.

#### 2.2 Soil Samples Collection

In the collected samples, first a soil profile was extracted and then the surface of the profile was cleaned. Fifteen soil samples were taken from Erbil city at different locations from (Glkand, Agricultural Research Center in Ankawa, field on Salahaddin summer resort Road, Agricultural College Research in Grdarash, Mosul Road, field behind technical institute, Erbil citadel (Qalat), Sami-Abdulrahman (Erbil) park, garden in Rizgari Hospital, garden in Erbil Teaching Hospital, Tavrawa (Mostofi) neighborhood, Mnara park, garden in Salahaddin neighborhood, Hawari hawri school, and College of Science/University of Salahaddin). Soil samples were collected from all these sites vertically at the depth of (0-20) cm during all the four seasons summer, autumn, winter and spring. Three soil samples taken from each study site with disinfected spatula were mixed thoroughly into one composite sample. The samples were placed in sterilized packets then stored in a cool box until they reached the laboratory.

In the laboratory, the samples were milled and sieved twice to remove large stones and debris to obtain soil samples with small particles. Physical and Chemical analysis of soil samples were measured (Saadoun *et al.*, 2008; Scholtz, 2006). The samples then were processed in an isolated process of fungi using the soil dilution plate and soil washing methods.

#### 2.3 Isolation techniques

#### 2.3.1 Standard dilution plate

Serial dilution was prepared by adding one gram of each soil sample to 9ml of sterile distilled water (SDW) in a sterilized test tube, shaken well, a serial dilution up to (10<sup>-3</sup>) were made in the same method. One ml of (10<sup>-3</sup>) dilution was poured in each Petri dish (9cm) in diameter, containing prepared medium (PDA + Chloramphenicol) by sterile pipette each sample made by three replication plates and incubated at 25°C for 10 days.

#### 2.3.2 Soil washing technique

Twenty gram of fresh soil was placed in a glass funnel lined with cloth. The soil samples were first washed with 2 Liters of tap water and the outflow was collected in a funnel. The procedure was then repeated using 2 Liters of sterile water. After this treatment, the

cloth and its contents were transferred into a sterile Petri dish. Organic particles and the washed soil particles were picked up with forceps and transferred onto plate of Czapek Dox Agar with streptomycin (in order to suppress bacterial growth, 30mg/L of streptomycin was added). The plates were incubated at 25°C for 10 days (Gams *et al.*, 1987).

#### 2.4 Diagnosis

#### 2.4.1 Identification of the fungal genera

The fungal isolates were transferred to sterilize plates for purification and identification. The grown fungi were mounted on a slide, Stained with lactophenol cotton blue to detect fungal structures (Basu, 1980), covered with a coverslip, examined under microscope and identified on the basis of their colony morphology and spore characteristics (Ronhede *et al.*, 2005 and Rajankar *et al.*, 2007).

### 2.4.2 Keys used for identification of the fungal genera

The texts (books) used for identification of soil fungi, depending on their taxonomic keys are as follows; Barnett, 1962; Gilman, 1966; Bessy, 1968; Ellis, 1971; Barnett and Hunter, 1972; Ainsworth *et al.*, 1973; Frey *et al.*, 1979; Samson *et al.*, 1981; Beneke *et al.*, 1984; Moubasher, 1993; Larone, 1995; Pitt and Hocking, 1997; Guarro *et al.*, 1999; Howard, 2002; Watanabe, 2002; Ulhan *et al.*, 2006; Uztan *et al.*, 2006; Chaturvedi and Ren, 2007; McClenny, 2007 and Pornsuriya *et al.*, 2008).

#### 3. Results and Discussion

## 3.1 Fungi isolated from soil by dilution plate method (The identity of fungi on PDA medium)

The result presented in Table (3.1) shows the identity and the total colony forming units (CFU) of fungi during the four seasons on PDA medium. Eighty one different genera of fungi were isolated during the four seasons; (30) genera were isolated in summer, 33 genera in autumn, 70 in winter and 53 genera in spring respectively. The total number of isolated fungi during different seasons, from the fifteen selected sites, was (1968×10<sup>3</sup>) cfu/g.soil.

The result showed that the total colony forming units for isolated fungi at summer, autumn, winter and spring was  $(307\times10^3, 327\times10^3, 868\times10^3, \text{ and } 466\times10^3)$  cfu/g.soil respectively.

Maximum diversity of fungi was observed during the winter while the minimum number of fungi was isolated during the summer, and the number of fungi in spring was higher than in autumn.

Table (3.1): Identification of soil F	unș	gi	isol	ate	ed	fro	m fi	ftee	n lo	oca	tion	by	dih	ıtio	n p	late	e m	eth	od																																	
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Metarhizium anisopliae	Τ.												١.		١.									1									- 3																				3		3
Microascus desmosporus	١.	1									1		t.		١.												П																				П			2			2		2
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Geotrichum candidum	-	-	-   -		-	_		-		-	_ 4	-	-	-		-	-		_	-	-		+	_		-		-	-		-		-	-		-	-			-	-			-	+	-	3
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Verticillium sphaerosporum	-		-			-							-		-	-		-			+				-		-		-		-	_		-		-			-		-	-	-	1
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The most frequently isolated fungi during four seasons were *Aspergillus* sp.  $(539\times10^3)$  cfu/g.soil, followed by *Penicillium* sp.  $(215\times10^3)$  cfu/g.soil, *Rhizopus* sp.  $(115\times10^3)$  cfu/g.soil, *Emericella* sp.  $(109\times10^3)$  cfu/g.soil, *Fusarium* sp.  $(47\times10^3)$  cfu/g.soil, and *Ulocladium botrytis*  $(47\times10^3)$  cfu/g.soil. While, the least frequently isolated fungal genera were *Blakeslea tuningtam*, *Clasterosporium cyperi*, *Idriella* sp., *Naranus cryptomeriae* and *Torula alternata*  $(1\times10^3)$  cfu/g.soil, for each one.

The highest frequency of isolated fungi was found during winter season within location (14),  $(92\times10^3)$  cfu/g.soil, followed by location (4),  $(89\times10^3)$  cfu/g.soil, and location (7),  $(79\times10^3)$  cfu/g.soil, while the lowest isolated found during summer season in the location (3), (5) and in the autumn season in the location (7),  $(4\times10^3)$  cfu/g.soil, for each one.

Hashem (1991) who isolated twenty nine fungal species belonging to eleven genera from soil samples of eight different places in Saudi Arabia. viz Afif, Ashafa, Dawadmi, Moayh, Radwan, Toraba, Wahat and Wehat. The most frequent genera isolated were *Aspergillus* (7 sp.) and *Penicillium* (5 sp.) followed by *Mucor* and *Ulocladium* (3 sp. each). *Mycelia sterilia* was also found to be among the dominating fungi.

Azaz and Pekel, (2002)isolated cosmopolitan genera found that Aspergillus, Penicillium and Alternaria were found in greater densities while Trichoderma, Cladosporium and Chaetomium were obtained in lesser densities in normal forest soil in Alanya (Turkey). El-Amin and Saadabi (2007) used soil-dilution plate method for isolating the fungi, specified (23) different species in sixteen genera and six sterile fungal taxa. The richest taxa in abundance were Aspergillus sp., Penicillium sp., Alternaria sp., Rhizopus sp., Curvularia sp. and these were new records for the area and thirteen of which were new records for the flora of Sudan.

### 3.2 Fungi isolated from soil by washing method

The results presented in Table (3.2), show 93 species belonging to 56 genera been isolated from soil. Arif and Hashem (1998), who collected soil samples from five localities in Jizan city, Saudi Arabia, to analyze for metals and fungal flora. Soil type is sandy and alkaline; it contains a low percentage of total organic matter and high percentage of total soluble salts. Seventeen species belonging to eight genera of fungi were isolated *Aspergillus* (4 sp.), *Fusarium* and *Penicillium* (3 sp.), *Alternaria* and *Cladosporium* (2 sp.), *Curvularia*, *Mucor* and *Nigrospora* (1 sp.).

Azaz (2003) who isolated microfungi from 105 soil samples of field soils irrigated by southeastern Anatolia project in Harran plain, using the soil dilution plate and the soil washing method, identified the following fungi: Absidia, Circinella, Cunninghamella echinulata, Mucor sp., Acremonium, Aspergillus sp., Humicola grisea,

Penicillium sp., Trichoderma harzianum, Ulocladium sp. and Verticillium lecanii.

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