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Diversity of *Aspergillus* and *Penicillium* of Farmland in Egypt

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Abstract: *The biodiversity of terrestrial Aspergillus and Penicillium of farmland in Egypt until now have been ignored. The aim of this work was to identify these distinguished fungal species which isolated from farmland at Al Sharqia Governorate. Different soil samples from patches free of roots were collected from different farmland at Al Sharqia Governorate. The isolated fungi were identified using the different microscopic and morphological feature. A. flavus, A. terrus, A. niger, A. versicolor, E.nidulans , P. chrysogenum and P. funiculosum were represented as the most common species isolated with high frequencies of occurrence while A. ficuum, A. sclerotiorum and P. janthinellum have low frequencies of occurrence. According to available data, it is new record of fungal biodiversity in farmland at sharqia governorate but the results of this study showed that the fungal biodiversity in farmland is similar to the fungal biodiversity in different localities of Egypt with differentiation in number and frequency of occurrence. According to available data, it is first estimation to terrestrial Aspergillus and Penicillium in farmland at Egypt.*

Keywords: *Aspergillus; Penicillium; terrestrial; farmland; biodiversity.*

1. INTRODUCTION

Fungi form a kingdom diverse from plants and animals gradually became accepted. Hawksworth has estimated that approximately 1.5 million fungal species are present on Earth (1, 2). Out of this number it is proposed that about 100 000 valid species have been described implying that only about 7% of the world's fungi have been described today. Records of the Egyptian fungi may be dated back to 4500 B.C., when ancient Egyptians created a number of hieroglyphic illustration of plants (many of which are psychedelic) on walls and within texts throughout Egypt. Temples with uncounted poles are shaped like huge mushrooms with tall stems, umbrella caps, and mushroom inscriptions distributed all over the country. No researches were executed on the terrestrial fungi until the 1930s, yet it was to be foreseeable that, in Egypt with rich agricultural traditions, knowledge of these fungi should have catch considerable interest. The number of fungi recorded in Egypt is 2 281 species and about 105 taxa have been described from Egypt as new to science (3). In Egypt, different researches have been made on terrestrial fungi. They found that the most common fungi in different kind of Egyptian soils were members of *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, and some dematiaceous Hyphomycetes (4, 5). The Nile Delta is a fruitful riverine triangle wedged in the middle of one of the driest deserts in the world. Located in northern Egypt, the Delta prolongs about 175 km from its top at Cairo to the Mediterranean Sea, and is about 260 km wide along the coast (6). Al Sharqia Governorate Located in the northern part of the country. *Aspergillus* is a diverse genus with high economic and social impact. Species occur worldwide in different habitats and they are known to spoil food, produce mycotoxins and are frequently reported as human and animal pathogens. So, many species are used in biotechnology for the industrial output of different bioactive compounds. The classification and identification of *Aspergillus* has been based on phenotypic characters. *Penicillium* is one of the most common fungi occurring in a prolong range of habitats, from outdoor environments such as soil, plants and air, indoor environments and different food and feed stuffs. *Penicillium* has wide range distribution and a high economic importance on human life. The major function of *Penicillium* in nature is the biodegradation of organic materials, where species have destructive mildew as pre- and postharvest pathogens on food crops (7, 8), as well as producing a prolong range of mycotoxins (9). Some species also have important role in the food industry. Some *Penicillium* species have an important role in food industry in the production of specialty cheeses, such as Camembert or Roquefort (10, 11).

2. MATERIAL AND METHOD

2.1 Collection of Samples

Different isolates of filamentous fungi were collected. These isolates were isolated from soil samples collected from different farmland at Al Sharqia Governorate.

Soil samples were collected from patches free from roots according to the method described by Johnson *et al.,* 1959 (12) and could be summarized as the following:

1- A sample tube is used which be washed thoroughly before starting the sampling. Samples are taken to a depth of 5 inches and the soil is shaken directly into clean and sterilized plastic bags (at least 5 samples are taken at random from each replication).

2- The five or more samples from each replication are brought together into composite sample, which is mixed thoroughly.

2.2 Isolation of Fungi

The dilution-plate method was used to determine soil fungi as described by Johnson *et al.* 1959 (12) and can be summarized as follows:

1- Ten gram of soil particles were placed in a graduated cylinder; sterilized distilled water was added to the soil so that a total volume of 100 ml was reached. The suspension was stirred and poured into 1000 ml Erlenmeyer flask. The flask containing the suspension was shaken on mechanical shaker for 30 minutes.

2- Ten milliliter of the suspension were immediately drawn (while in motion) using sterile Menzies'dipper and transferred immediately through a known volume of sterile distilled water blank until the desired final dilution was reached. Each suspension was shaken by hand for few minutes, and was in motion while being drawn by the dipper.

3- One milliliter of the desired dilution was transferred directly into each of sterilized 12 Cm Petri-dishes then 12-15 ml of a Czapek's agar medium cooled to just above solidifying temperature were added to each dish. The dishes were rotated by hand in a broad swirling motion, so that, the dilution soil was dispersed in the agar.

4- Five plates were used for each sample and incubated at 28°C for 7 days. The developing colonies were isolated and grown again for three times until purification; the purified colonies were identified and counted. The average number of colonies per plate was multiplied by the dilution factor to obtain the number per gram in the original soil samples.

5- Czapek's agar was used throughout the present investigation for and identification of fungi. This was supplemented with Rose-bengal and chloramphenicol as bacteriostatic agents (13).

2.3 Identification of fungi

Identification of the isolated fungi during our investigation was carried out using the morphological characteristics as colony diameter, the color of condia, extracellular exudates, pigmentation and the color of reverse mycelium and microscopic features were examined also as conidial heads, fruiting bodies, degree of sporulation and the homogeneity characters of conidiogenous cells by optical light microscope (10×90) Olympus CH40 according to the following references: Ainsworth, 1971(14), Klich and Pitt 1992 (15) for *Aspergillus* species, Ramirez 1982 (16) and Pitt 1979 and 1985(17, 18), for *Penicillium* species. Fungal isolates were grown onto Malt extract-agar (MA) medium at 28°C for several days (7-10). The cultures were then kept in 4°C.

3. RESULT

Ninety five isolates of filamentous fungi belonging to two genera (*Aspergillus* and *Penicillium*) and thirty-six species plus to two species varieties were collected. These isolates were isolated from different areas of farmland at at **Al Sharqia Governorate**. Scientific names of fungal isolates were pointed in **Tables (1 and 2)**. *Aspergillus* was represented by 11 sections in this study according to previous references as the following: Candidi (one species), Circumdati (two species), Flavi (three species and one species variety), Nigri (four species), Wentii (one species), Fumigati (one species and one species variety), Cervini (two species), Terii (one species), Usti (one species), Versicolores (two species) and Oranti (one species). Three *Aspergillus* related genera (based on anamorph/teleomorph): *Emericella* (*E. nidulans*), *Eurotium* (*E. amstelodami*) and *Fennellia* (*F. nivea* ,*F. flavipes*) were also collected as in **Table (1).**

Genus *Penicillium* was represented by four subgenus: Aspergilloides (Three species), Biverticillium (three species), Furcatum (three species) and Penicillium (8 fungal Species) were isolated and tested in this study **Table (2).**

Table 1: *Classification and name of the different tested species and varieties of Aspergillu*

Genus	Subgenus	Section	Species and Varieties
Aspergillus Link	*Circumdati*	*Candidi*	*A .candidus* Link
		Circumdati	*A. ochraceus* Wihelm
			A. sclerotiorum Huber
		Flavi	*A. flavus* Link

			A. flavus var. *columnaris* Raper and Fennell
			A. oryzae (Ahlburg) Cohn
			A. parasiticus Speare
		Nigri	*A. aculeatus* Iizuka
			A. ficuum
			A. niger Van Tieghem
			A. phoenicis (Cda.) Thom
		Wentii	*A. wentii* Wehmer
	Fumigati	*Fumigati*	*A. fumigatus* Fresenius
			A. fumigatus var. *ellipticus* Raper and Fennell
Aspergillus Link	*Fumigati*	*Cervini*	*A. cervinus* Massee
			A. versicolor (Vuillemin)Tiraposchi
	Nidulantes	*Terri*	*A. terreus* Thom
		Usti	*A. ustus* (Bain.) Thom and Church
		Versicolores	*A. sydowii* (Bain. And Sart.) Thom and Church
			A. versicolor (Vuill.) Tiraboschi
	Ornati	*Oranti*	*A.ornatus* Raper, Fennell and Tresner
Emericella Berekeley and Broome			*E.nidulans* (Eidam) Vuillemin
Eurotium Link			*E. amstelodami* Mangin
Fennellia Wiley and Simmons			*F.nivea* (Wiley and Simmons) Simmons
			F.flavipes Wiley and Simmons

Table 2*: Classification and name of the different tested species and. varieties of Penicillium*

Subgenus	Species and varieties
Aspergilloides Pitt	*P. capsulatum* Raper and Fennell
	P. lividum Westling
	P. spinulosum Thom
Biverticillium Diercks	*P. funiculosum* Thom
	P. purpurogenum Stoll
	P. rugulosum Thom
Furcatum Pitt	*P. citrinum* Thom
	P. herquer Bainier and Sartory
	P. janthinellum Biourge
Penicillium Pitt	*P. albidum* Sopp
	P. aurantiogriseum Dierckx
	P. camemberti Thom
	P. chrysogenum Thom
	P. digitatum (Persoon Ex Fr.) Saccardo
	P. multicolor Grigorieva-Manoilova and Poradielova
	P. nigricans (Bainier) Thom
	P. stoloniferum Thom

4. DISCUSION

Nile delta in the north (Lower Egypt) composes a riparian oasis that frames the densely inhabited farmlands of Egypt. At the generic level, some genera show high species plenty as *Aspergillus* (100 spp.) and *Penicillium* (83 spp.) (3) and this agreement with our results in which *Aspergillus* genus represent by 60 isolates while *Penicillium* genus represent only by 35 isolates. The isolates of *Aspergillus* genus were belonging to 19 species plus two species varieties and four related species. *A. flavus*, *A. terrus, A. niger, A. versicolor* and *E.nidulans* were represented as the most common species isolated with high frequencies of occurrence while *A. ficuum* and *A. sclerotiorum* have low frequencies of occurrence and the other *Aspergillus* species were represented by normal and moderate frequencies. *Penicillium* genus was represented by 35 isolates belonging to 17 species. *P. chrysogenum* and *P. funiculosum* ranked the most common species and in the other hand, *P. janthinellum* was considered a rare frequency of occurrence. The remaining species were isolated in moderate and normal rank. The above results showed that Most of the tested species were also prevalent in soils formerly examined and collected from different localities in Egypt but only may be differ in the numbers and frequency of occurrence (4, 5, 19 and 20*)*.

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