Study of soil mycobiota diversity in some new reclaimed areas, Egypt

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The objective of this survey was to study the diversity of soil fungi, as they play an important role in knowing the soil quality and increase plant productivity. In this work, the chemical composition of soil samples in newly reclaimed localities at Assiut Governorate was studied; mycobiota of those areas were studied reporting some medically important substances produced by three fungal species, namely *Aspergillus terreus*, *Emericella nidulans*, and *Penicillium chrysogenum*, which recorded the highest occurrence all over the year in different studied areas. Soil samples were collected monthly for a whole year from the four selected reclaimed regions at Assiut Governorate, namely Protectorate of Assiut (PR), El-Ghorayeb (GH), El-Wady El-Assiuty (WA), and Petroleum's Farm (PF), and samples were identified using the morphological and microscopic features according to many references and confirmed by Assiut University Mycological Center (AUMC) followed by physiochemical analysis of soil, including measuring total soluble salts and determining the organic matter content, maximum and minimum temperatures, relative humidity, and soil texture. The highest numbers of fungal genera and species were recorded in PR followed by WA and PF, whereas the lowest numbers were recorded in GH. *E. nidulans* var. *acristata* and *Penicillium funiculosum* were isolated with moderate frequency from GH; *Aspergillus aegyptiacus* and *Aspergillus ustus* were isolated with moderate frequency from WA; *A. aegyptiacus*, *Eurotium amstelodami*, and *Fusarium solani* were isolated with moderate frequency from PF; *Eurotium repens* was isolated only from GH; *Arthrinium sacchari*, *Cochliobolus sativus*, and *Fusarium xylarioides* were isolated only from WA; *Aspergillus deflectus*, *Penicillium expansum*, *and Rhizopus arrhizus* were isolated only from PF; *Aspergillus niger*, *Fusarilla indica*, *Fusarium semitectum*, and *Trimmatostroma eriodictyonis* were isolated only from PR. There are no adequate mycological studies carried out to describe the fungal flora of these areas. Hence, any information on the endemic mycobiota is of great significance.

Keywords:
fungal isolates, identification, isolation, reclaimed soil

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Introduction

Many investigations have been carried out on the occurrence of soils fungal isolates in many parts all over the world [1–8]. In Egypt, numerous investigations have been made on soil fungi from Upper Egypt, Delta area and Sinai Peninsula as well as from some Wadies at eastern desert of Egypt [9–15]. They found that members of *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, and some dematiaceous Hyphomycetes were the most common in various types of Egyptian soils. Mazen and Shaban [16] studied the fluctuation of soil fungi in wheat fields and found that the most common fungi isolated were *Aspergillus* (*Aspergillus niger*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus versicolor*), *Humicola grisea*, *Fusarium solani*, *Fusarium oxysporum*, *Trichoderma viride*, and *Stachybotrys chartarum*. Moubasher *et al*. [17] isolated 167 species and 10 varieties belonging to 45 genera from 100 soil samples collected from Wadi Bir-El-Ain, and the most common species were *A. fumigatus*, *A. niger*, *A. terreus*, and *Aspergillus glaucus* group (represented by *Aspergillus amstelodami*, *Aspergillus chevalieri*, *Aspergillus ruber*) and *Penicillium chrysogenum*. Abdel-Hafez *et al*. [14] isolated 122 species and nine varieties belonging to 36 genera from soil samples collected from Sinai Peninsula on glucose-Czapek's agar at 28°C, and the most common species were *A. flavus*, *A. flavus* var. *columnaris*, *A. fumigatus*, *Aspergillus nidulans*, *A. niger*, *A. terreus*, *Botryotrichum atrogriseum*, *Chaetomium globosum*, *F. solani*, *P. chrysogenum*, and *S. chartarum*. Mazen *et al*. [18] mentioned that the occurrence of *Fusarium* spp. in Egyptian soils was influenced by the soil type and by locality; they also noticed that the most common species were *F. oxysporum*, *F. solani*, *Fusiform equiseti*, *Fusiform acuminatum*, and *Fusarium semitectum*. Abdel-Hafez *et al*. [15] isolated 118 species in addition to seven varieties belonging to 51 genera from cultivated and desert soils. The results obtained from the three soil type were basically similar, and the most common fungi were *A. flavus*, *A. flavus* var. *columnaris*, *A. fumigatus*, *A. niger*, *Aspergillus sydowii*,

A. terreus, *B. atrogriseum*, *Cladosporium cladosporioides*, *Emericella quadrilineata*, *F. oxysporum*, *Gibberella fujikuroi*, *H. grisea*, *Nectria haematococca*, *P. chrysogenum*, *Penicillium citrinum*, and *Penicillium oxalicum.* Most investigations have been made on the fungus flora of agricultural or forest soils. Very few investigations have been carried out on the mycobiota of the newly reclaimed localities at Assiut Governorate.

Protectorate of Assiut (PR), El-Ghorayeb (GH), El-Wady El-Assiuty (WA), and Petroleum's Farm (PF) represent the largest newly reclaimed areas at Assiut Governorate, cultivated with different important crops. There are no adequate mycological studies carried out to describe the fungal flora of these areas. Hence, any information of these areas on the endemic mycobiota is of great significance.

Selected areas

Four areas representing the largest and distinctive regions of newly reclaimed soil at Assiut Governorate were selected. These were PR that lies at 25 km southeast Assiut, GH that lies at 20 km southeast Assiut, WA that lies at 10 km east Assiut, and PF that lies at 10 km northwest Assiut.

Collection of samples

Soil samples

Soil samples were collected monthly for a whole year from the four selected reclaimed regions at Assiut Governorate. Soil samples were collected from patches free from roots according to the method described by Johnson *et al.* [19] and could be summarized as the following:

(1) A sample tube is used, which should be washed thoroughly before starting the sampling. Samples are taken to a depth of 5 inches and the soil is taken directly into clean and sterilized plastic bags (at least five samples are taken at random from each replication).
(2) The five or more samples from each replication are brought together into one composite sample, which is mixed thoroughly.
(3) Finally, 10 g (on an oven-dry basis) of the mixed composite samples are used for determining fungal counts by the dilution-plate method as recommended by Johnson *et al*. [19] and used in this laboratory by Moubasher *et al.* [5].

Chemical analysis of soil

Total soluble salts

The specific electrical conductance in the prepared soil extract was measured by means of conductance meter (YS1 Model 35); the total soluble salts (TSS) in the soil was estimated using the following equation

$$\%\text{TSS in the dry soil} = 0.064 \times \text{EC(m/cm)} \times \text{Extrack ratio.}$$

The conversion factor to percentage salts (0.064) was fairly applied for solutions extracted from alkaline and saline soils [20,21].

Organic matter content

The organic matter content of soil samples was determined by Jackson [20]. A certain amount of soil was digested by chromic acid (for oxidation of organic matter to CO_2) and the excess chromic acid was back titrated against standard ferrous sulfate solution in the presence of diphenylamine as an indicator.

Ca^{2+} and Mg^{2+}

The Versene (disodium dihydrogen ethylenediaminetetraacetic acid) titration method as recommended by Schwarzenbach and Biedermann [21] was used for Ca^{2+} and Ca^{2+}+Mg^{2+} determination.

Na^{+} and K^{+}

Flame photometer method [22] using Carl Zeiss flame photometer was used for determination of cations such as Na^{+} and K^{+}.

pH value

A pH meter (WTW pH 90) was used for the determination of soil pH. The electrode was immersed in the soil suspension in a ratio of 1 : 5 (w/v) to avoid the error through higher dilution [21].

Maximum and minimum temperatures and relative humidity

The values of maximum and minimum temperatures and relative humidity all over the year were obtained from Assiut Forecasting Authority.

Determination of soil texture

The soil type of each locality was determined by the hydrometer method as described by Piper [23] and used in this laboratory by Moubasher *et al.* [5]. Soil type was determined from the textural triangular as recommended by Alexander [24].

Isolation and identification of fungi

Soil fungi

The dilution-plate method was used to determine soil fungi as described by Johnson *et al*. [19] and used in this laboratory by Moubasher *et al.* [5] and can be summarized as follows:

(1) Soil to be diluted was sifted through a 9-mesh sieve. Three aliquots, each of 5–10 g soil, were placed in previously weighed metal container and dried overnight in an oven at 105°C. The aliquots were then reweighed and the moisture content of the soil sample was calculated.
(2) Ten grams of soil sample (based on an oven-dry weighed soil) was placed in a graduated cylinder, and 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 min.
(3) Ten milliliters of the suspension was immediately drawn (while in motion) using sterile Menzies' dipper [25] and transferred immediately to a known volume of sterile distilled water blank until the desired final dilution was reached. Each suspension was shaken by hand for few minutes.
(4) One milliliter of the desired dilution was transferred directly into each of the sterilized Petri dishes followed by 12–15 ml of glucose-Czapek's agar medium cooled to just above solidifying temperature. The dishes were rotated by hand in a broad swirling motion so that the diluted soil was dispersed in the agar.
(5) Five plates were used for each sample and incubated at 28 ± 1°C for 7 days during which the developing colonies were identified and counted [expressed as colony forming unit (cfu)/g dry soil].
(6) Glucose-Czapek's agar [26] used in this laboratory by Moubasher *et al.* [5] was used throughout the present investigation for isolation and identification of fungi. This media was supplemented with bacteriostatic agents. The composition of this medium is as follows: agar, 15.0 g; $NaNO_3$, 3.0 g; KH_2PO_4, 1.0 g; $MgSO_4 \times 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 \times 7H_2O$, 0.01 g; glucose, 10.0 g; and 1000 ml distilled water.
(7) For further investigation, growing fungal species were obtained on slants of glucose-Czapek's agar medium and kept in refrigerator at 4°C.

Identification of fungal genera and species

Identification of the isolated fungi during this investigation was carried out using the morphological and microscopic features according to the following reported books:

(1) Ainsworth [27] as a dictionary of the fungi.
(2) Barron [28] for the genera of Hyphomycetes.
(3) Booth [29,30] for *Fusarium* spp.
(4) Onions and Barron [31] for *Paecilomyces* spp.
(5) Domsch *et al.* [32] for soil fungi in general.
(6) Ellis [33,34] for dematiaceous Hyphomycetes.
(7) Kendrick [35] for the genera of imperfect fungi.
(8) Moubasher [36] for fungi in general.
(9) Pitt [37] for *Penicillium* spp.
(10) Raper and Fennell [38] for *Aspergillus* spp.
(11) Rifai [39] for *Trichoderma* spp.
(12) Simmons [40] for *Alternaria* and *Ulocladium* spp.
(13) Zycha [41] for Mucorales.

In addition, identification of the isolated fungi was reviewed and compared with the same species deposited in Assiut University Mycological Center (AUMC).

Results and discussion

Mycobiota of protectorate of assiut soil

Thirty-three species and three species varieties belonging to 18 genera in addition to the fungi with sterile mycelia were isolated and identified during this study. *Aspergillus* was the most common genus, with 10 species in addition to one species variety. Results showed that A. *flavus* and *Aspergillus japonicus* were the most common species. *A. terreus* and *Aspergillus aegyptiacus* were also isolated in high frequencies, whereas *A. sydowii* and *A. versicolor* were isolated in moderate frequencies. *Chaetomium* was the second common genus and *C. globosum* was the commonest species. *Emericella* was the third commonest genus. The most common species and variety were *Emericella nidulans* and *E. nidulans* var. *acristata. Penicillium* and *Stachybotrys* were also isolated in high frequency, and the most common species were *P. chrysogenum* and *S. chartarum*. The remaining genera and species were isolated either in moderate, low, or rare frequency of occurrence as shown in Table 1.

Mycobiota of El-Ghorayeb area soil

Twenty-eight species and two species varieties belonging to 17 genera in addition to fungi with sterile mycelia were isolated and identified during this study. *Aspergillus* was the most frequent genus. Eight species were isolated and identified. *A. japonicus* ranked the first commonest species. *A. flavus* and *A. terreus* were also isolated with high (H) frequencies. The remaining species were isolated in moderate (M), low (L), or rare (R) frequencies of occurrence as in Table 1. *Emericella* came behind *Aspergillus* and the isolated species were as following: *E. nidulans*, *E. nidulans* var. *acristata*, and *E. nidulans* var. *lata*. *Penicillium* ranked the third genus, with two species, *P. chrysogenum* and *Penicillium funiculosum*, recorded in moderate frequencies. *C. globosum* in addition to the fungi with white sterile mycelia were recovered in moderate frequencies. The remaining genera and species were isolated either in low or rare frequencies of occurrence.

Table 1 Occurrence remark of fungi isolated from the different selected areas

Genera and species	El-Ghorayeb soil	El-Wady El-Assiuty soil	Petroleum's Farm soil	Protectorate of Assiut soil	Genera and species	El-Ghorayeb soil	El-Wady El-Assiuty soil	Petroleum's Farm soil	Protectorate of Assiut soil
Acremonium strictum	R	R	L	M	*F. dimerum*	—	—	—	R
Alternaria alternate	—	L	—	L	*F. graminearum*	—	R	R	—
Aspergillus aegyptiacus	R	M	M	H	*F. m.* var. *subglutinanas*	—	—	R	—
A. carbonarius	—	—	—	R	*Fusarium oxysporum*	R	R	M	—
A. carneus	—	R	—	—	*Fusarium solani*	—	L	M	—
Aspergillus deflectus	—	—	R	—	*F. sporotrichioids*	—	—	R	—
A. flavipes	—	—	—	R	*Fusarium xylarioides*	—	R	—	—
Aspergillus flavus	H	M	H	H	*Gliocladium roseum*	—	—	R	—
Aspergillus flavus var. *columnaris*	—	—	—	R	*Humicola fuscoatra*	—	—	R	—
Aspergillus japonicus	H	M	H	H	*Humicola grisea*	—	—	R	—
A. melleus	R	—	L	—	*Hypomyces chrysospermus*	R	—	—	—
Aspergillus niger	—	—	—	R	*Macrophomina phaseolina*	—	—	L	—
A. ochraceus	—	L	R	—	*Memnoniella echinata*	—	—	—	R
Aspergillus sydowii	M	H	L	M	*Mucor fuscus*	L	R	—	R
A. tamari	R	—	R	R	*M. circinelloids*	—	—	R	—
Aspergillus terreus	H	H	H	H	*Myrothecium roridum*	—	—	R	—
Aspergillus ustus	R	M	H	R	*M. verrucaria*	R	L	L	R
Aspergillus versicolor	L	R	M	M	*Papulaspora immerse*	—	R	L	—
Botryotrichum piluliferum	R	H	M	—	*Penicillium brevicompactum*	R	L	M	L
Beltrania querna	—	—	R	—	*Penicillium chrysogenum*	M	M	M	M
Chaetomium globosum	M	H	M	H	*Penicillium citrinum*	—	—	—	L
C. spirale	R	—	R	L	*P. corylophilum*	R	R	—	R
Cladosporium cladosporioides	—	R	L	L	*P. frequentans*	—	—	R	—
C. herbarum	—	—	R	—	*Penicillium funiculosum*	M	R	—	—
C. sphaerospermum	—	R	—	—	*P. purpurogenum*	—	—	R	R
C. hawaiiensis	—	—	—	R	*Pestalotia* spp.	—	—	R	—
Cochliobolus spicifer	L	L	M	H	*Phoma glomerata*	—	—	L	—
Cunninghamilla echinulata	—	R	R	—	*P. herbarum*	—	—	L	—
C. elegans	R	L	—	—	*P. leveillei*	—	—	R	—
Doratomyces microsporus	—	L	L	M	*Rhizopus arrhizus*	—	—	R	—
D. stemonitis	—	—	—	R	*Rhizopus stolonifer*	L	L	M	M
Emericella nidulans	H	M	H	H	*Scopulariopsis brevicaulis*	—	L	R	—
E. nidulans var. *acristata*	M	L	L	M	*Stachybotrys chartarum*	L	L	M	H
E. nidulans var. *dentate*	—	—	L	—	*Thermoascus aurantiacus*	L	R	R	—
E. nidulans var. *Lata*	L	—	—	R	*T. harzianum*	—	R	R	—
Emericella quadrilineata	—	—	L	—	*Trichothecium roseum*	—	—	R	—
E. violacea	—	—	R	—	*U. botrytis*	R	—	—	—
Epicoccum nigrum	—	—	R	L	*U. chartarum*	—	—	L	R
Eurotium amstelodami	L	L	M	R	*U. chlamydosporum*	—	—	R	—
F. culmorum	—	R	L	–					

H, high occurrence (more than 5 months along the year); L, low occurrence (2 months); R, rare occurrence (1 month); M, moderate occurrence (between 3 and 5 months).

Mycobiota of El-Wady El-Assiuty soil

Thirty-seven species and one species variety belonging to 21 genera were isolated and identified. *Aspergillus* was the leader and *A. japonicus* was the most dominant among its eight isolated species followed by *A. sydowii* and *A. terreus*, whereas *A. aegyptiacus*, *A. flavus*, and *Aspergillus ustus* were isolated in moderate frequencies. The remaining species were detected in either low or rare frequencies of occurrence. *Botryotrichum*, *Chaetomium*, and *Penicillium* were also isolated in high frequencies, and *Botryotrichum piluliferum*, *C. globosum*, and *P. chrysogenum* were the most common species as can be seen in Table 1. *E. nidulans* and *E. nidulans* var. *acristata* were recorded in moderate frequencies. The remaining genera and species in addition to the fungi with dark sterile mycelia were isolated either in low or rare frequencies of occurrence.

Mycobiota of Petroleum's Farm soil

Fifty-six species and three species varieties belonging to 29 genera in addition to the fungi with sterile mycelia were isolated and identified during this study. The most common genus was *Aspergillus* and 11 species were isolated and identified. *A. japonicus* was the common species and *A. terreus* was the second common species isolated. *A. flavus* and *A. ustus* were also isolated in high frequencies, whereas the remaining *Aspergillus* spp. were isolated either in moderate, low, or rare frequencies. *Emericella* (three species and two varieties) and *Fusarium* (five species and one variety) in addition to the fungi with dark sterile mycelia were also isolated in high frequencies, and the most common species were *E. nidulans*, *F. oxysporum*, and *F. solani*. *Penicillium* (five species) was isolated in high frequency and the most common species was *P. chrysogenum*. *B. piluliferum*, *Rhizopus stolonifer*, *C. globosum*, *Cochliobolus spicifer*, *Eurotium amstelodami*, and *S. chartarum* in addition to the fungi with white sterile mycelia were isolated in moderate frequencies. The remaining genera and species were isolated in either low or rare frequencies of occurrence as shown in Table 1.

Few investigations have been carried out on the soil mycobiota of the newly reclaimed areas at Assiut Governorate especially in four reclaimed areas, namely GH, WA, PF, and PR. Analysis of some biotic factors such as TSS, organic matter, and pH of the studied soil samples demonstrated that amount of TSS was varied depending on the area and fluctuated between 1.58 and 2.68%. This finding was supported by many other researchers [11,17,19]. The soil samples collected from the different areas were generally poor in their organic matter content compared with other cultivated areas, which have clay or silt nature, and there was no big difference between the areas. This might be attributed to the sandy nature of these areas. Moisture content of the samples was also relatively low (0.78–5.2%). This is in agreement with desert soil characteristics. pH values of all samples were alkaline (9.5–10.5). In this respect, Egyptian soil was mentioned in most areas as alkaline [17,19]. The amount of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} in tested soil samples was wide ranged. The amount of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} contents in dry soil varied between 20 μg/g, 3–10 μg/g, 5.8–10.8 mg/g, and 0.26–0.78 mg/g, respectively, in all samples tested of the four localities. This is almost in agreement with the results previously obtained from Egyptian soils [11,17]. Several investigations mentioned that fungal population in a certain soil is more influenced by several factors such as its salt content, organic matter content [5], water content, pH value, and the type of soil. Seventy-two species and six species varieties belonging to 32 genera of fungi were collected from GH (17 genera and 28 species+2 varieties), from WA (21 genera and 37 species+1 variety), from PF (29 genera and 56 species+3 varieties), and from PR (18 genera and 33 species+3 varieties) on glucose-Czapek's agar at 28±1°C. The results obtained from the four localities were similar, and the most common genera were *Aspergillus*, *Emericella*, *Penicillium*, and *Chaetomium*. They emerged from 100, 33.3–75, 50–66.7, and 33.3–75% of the samples comprising 42.6–57.4, 1.1–32.2, 2.05–5.13, and 1.03–8.74% of total fungi, respectively. The most common species was *A. terreus* and its natural products of medical importance were reported by many other researchers [42,43], followed by *E. nidulans* that also produces some bioactive metabolites [44] and then *P. chrysogenum*, which was reported to have some important bioactive metabolites [45]. In contrast, some species were common only in one or more of the four localities tested, such as *A. flavus* in GH, PF, and PR; *B. piluliferum* in WA and PF; *A. ustus*, *F. oxysporum*, *F. solani*, and *R. stolonifer* in PF; *S. chartarum* in PR; and *A. sydowii* in WA. Most of the above species were also common in soils previously tested and gathered from different localities in Egypt [9,12,16,19].

Conclusion

This survey discussed the basic similarities between fungi associated with the different localities (PR, GH, WA, and PF). There are no specific fungal flora characteristics for each locality. In addition, no considerable differences between the mycobiota recovered from one locality to another were detected. Comparative study of the genera and species for the different localities showed that they could differ in the numbers and frequency of occurrence.

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Conflicts of interest

There are no conflicts of interest.

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