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ORIGINAL ARTICLE

Fungi associated with ornamental plants in some Nurseries in Al–Qurayyat, Jouf region, Saudi Arabia

Mohamed Salah Massoud[1,2], Doaa Montaser Ahmed Khalil[2,3*]

[1]Department of Chemistry, Science and Arts College, Jouf University, Al-Jouf, Kingdom of Saudi, Arabia
[2]Department of Botany, Faculty of Sciences, Aswan University, Aswan, Egypt
[3]Department of Biological Science, Unit of Environmental Studies and Development, Aswan University, Aswan, Egypt

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ABSTRACT

Background and Objectives: Fungi communities are important soil components as decomposers and plant symbionts, and they play an important part in natural ecological and biogeochemical processes. In this study, isolation and identification of terrestrial and zoosporic fungi were detected.

Materials and Methods: Sixty–seven fungal species from thirty–four genera were isolated from 45 soil samples obtained randomly from nurseries in Al–Qurayyat, Jouf reagon, Saudi Arabia using the soil dilution technique on glucose–Czapek's agar medium, cellulose–Czapek's agar, and Potato dextrose agar medium.Authentic fungus manuals were then used to identify and characterise the mycoflora.

Results: A total of 46 fungal species belonging to 22 terrestrial fungal genera were recovered on glucose–Czapek's agar, 38 species belonging to 20 terrestrial fungal genera were recovered on cellulos–Czapek's agar and 27 fungal species belonging to 15 terrestrial fungal genera were recovered on PDA medium while 12 species belonging to 7 genera zoosporic fungal genera were discovered.

Conclusion: The most common terrestrial fungal genera were *Aspergillus, Penicillium, Fusarium, Trichoderma, Acremonium,* and *Cladosopium* while in zoosporic fungus. *Allomyces* was the most prevalent, followed by *Achlya* and *Pythium*.

Keywords: Isolation; Fungi; Soil; Plants; *Allomyces*

INTRODUCTION

Soil provides a rich environment for the growth of microorganisms more than any other microbial habitats which represent the main source of fungi (1). Fungi play important role in growth of the plant through mutualism, pathogenicity, making the availability of nutrient and recycling the different organic materials (2). Fungi are found in soil, air, roots, surfaces of plants, and skin of human and animals. It is responsible for many diseases that affect plants, as well as causing spoilage of preserved foods, grains, seeds, fruits and products rich in organic matter (3). Fungi are found in a wide range of ecosystems, including soils, decaying plants, waters, and fish samples, and have been seen by numerous researchers

*Corresponding author: Doaa Montaser Ahmed Khalil, Ph.D, Department of Botany, Faculty of Sciences, Aswan University, Aswan, Egypt; Department of Biological Science, Unit of Environmental Studies and Development, Aswan University, Aswan, Egypt. Tel: +2001100824842 Fax: +20973480450 Email: doaa.khalil30@sci.aswu.edu.eg

around the world including in the Arab world (4). The diversity and activity of fungi in soil are regulated by various biotic (plants and other organisms) and abiotic (soil pH, moisture, salinity, structure, and temperature) (5). Microbial communities in plant roots and shoots, as well as in soil, play a significant role in plant growth and health, as well as in ecosystem activities. As a result, it is important to know what factors influence their diversity (6). Some of the fungi are important pathogens of plants and animals (7). Kim et al. concentrated on influence of culture media and environmental factors on mycelial growth of isolated fungi and explaining their morphological characteristics based on their ability to grow in various suitable enrichment media (8).

The present study aimed to isolate and identify the soil and zoosporic fungi associated with the soil samples from Al–Jouf reagon.

MATERIALS AND METHODS

Soil samples collection. 45 soil samples were randomly collected from different nurseries in Al–Qurayyat, Jouf Region, Saudi Arabia that is located in the northwest of the Kingdom of Saudi Arabia at 31º 20' 43.5300 N, 37o 20' 1.0104 E. this region is generally dominated by a dry desert climate associated with extreme daytime temperatures, an abrupt drop in temperatures, and very low annual rainfall. The kingdom is generally hit by dry winds, and almost all the areas are characterized by drought. The temperatures and humidity are highly differentiated as a result of the impact of the tropical and subtropical high–pressure systems, where there is a significant difference between the coastal and intra–tropical areas (9). Soil samples were collected and transported to the lab in sterilized plastic bags.

Isolation of terrestrial fungi. The dilution–plate method that was used to determine the terrestrial fungal flora of samples by 25 gram of each sample was suspended in 90 ml of sterile water into 250 ml Erlenmeyer flasks. The flasks were mechanically shaken for 30 min. 10 ml suspension arc transferred immediately through successive 90 ml sterile water blanks until the desired final dilution is reached. One ml desired dilution is transferred aseptically in each Petri dishes contain medium, cooled to just above the solidifying temperature are added to each dish (10, 11).

For fungal isolation, three types of media were used: glucose–Czapek's agar medium g/L (Sodium nitrate, 3g; dipotassium hydrogen phosphate, 1g; magnesium sulphate $7H_2O$, 0.5g; potassium chloride, 0.5g; ferrous sulphate $7H_2O$,0.01g; glucose, 10 g and agar, 20g). The second medium was cellulose– Czapek's agar, but instead of glucose, CMC was used. Last medium: potato–dextrose agar (Sigma–Aldrich PDA, 39 gm/L). Three duplicates were set up at each study site, one meter apart and 20-35 cm below the surface. For each sample, nine plates were used: three with glucose – Czapek's agar, three with cellulose – Czapek's agar, and three with PDA media. Chloramphenicol (0.5 mg/m) and Rose bengal (1/15000) were added as bacteriostatic agents (12). The plates were incubated for 7-10 days at 28ºC, and the growing fungus were counted, identified, and thereafter calculated as colony forming units (CFU) per g of dry soil in each sample.

Isolation of zoosporic fungi. The zoosporic fungi were recovered from soil using the Sesame seed baiting method (13). On glucose–peptone (GP) agar medium, isolated zoosporic fungi were purified (14). The fungal slants were kept at 8-12ºC and sub–cultured every 2-3 months.

Identification of fungi. Standard identification manuals were used to identify all isolated fungal colonies based on microscopic observations of mycelia, asexual/sexual spores and colony morphology, cultural characteristics (colour, texture, and pigmentation), and spores and spore–bearing structure. The fungi were identified using relevant keys and taxonomic notes from various standard manuals (15-20).

RESULTS

From 45 soil samples obtained randomly from soil in several nurseries in Al–Qurayyat, Jouf reagon, KSA, 67 species belonging to 34 terrestrial and zoosporic fungus genera were isolated as showen in Tables 1 and 2.

On three mediums, glucose–Czapek's agar, cellulose agar, and PDA, fifty–five species from 27 terrestrial fungal genera were isolated (Table 1 and Fig. 1). on glucose–Czapek's agar, 46 species belonging to 22 terrestrial fungal genera were isolated from 45 soil samples. *Aspergillus*, *Penicillium* and *Fusarium* were recorded as high incidance respectively comprising 38.36%, 11.19% and 10.47% respectively of total soil samples.

Table 1. Number of cases of isolation (NCI), percentage number of cases of isolation (% NCI) and occurrence remark (OR) of terrestrial fungal genera which isolated from 45 soil Samples collected from nurseris in Al–Qurayyat, Jouf area on Glucose, Cellulose–Czapek's agar and Potato Dextrose Agar media at 28°C.

Fungal Genera	Glucose – Agar			Cellulose – Agar			PDA		
	NCI	% NCI	OR	NCI	% NCI	OR	NCI	% NCI	OR
Acremonium	18	6.5	M	11	3.77	M	13	5.8	M
Alternaria	1	0.36	L	0	0	0	0	0	0
Aspergillus	107	38.63	H	122	41.78	H	116	51.79	H
Botryotrichum	9	3.25	L	3	1.03	L	0	0	0
Chaetomium	0	0	0	0	0	0	5	2.23	L
Circinella	0	0	0	0	0	0	2	0.89	L
Cladosopium	15	5.42	M	7	2.4	L	5	2.23	L
Cochliobolus	2	0.72	L	8	2.74	L	0	0	0
Curvularia	9	3.25	L	8	2.74	L	10	4.46	L
Emericella	4	1.44	L	7	2.4	L	8	3.57	L
Epicoccum	3	1.08	L	0	0	0	0	0	0
Fennellia	6	2.17	L	2	0.68	L	8	3.57	L
Fusarium	29	10.47	M	27	9.25	H	21	9.38	M
Gibbrella	2	0.72	L	0	0	0	0	0	0
Humicola	1	0.36	L	4	1.37	L	3	1.34	L
Mucor	2	0.72	L	6	2.05	L	6	2.68	L
Mycosphaerella	2	0.72	L	6	2.05	L	0	0	0
Myrothecium	11	3.97	M	8	2.74	L	0	0	0
Penicillium	31	11.19	H	27	9.25	H	19	8.48	M
Pestalotia	0	0	0	3	1.03	L	0	0	0
Phoma	4	1.44	L	6	2.05	L	0	0	0
Rhizopus	5	1.81	L	0	0	0	4	1.79	L
Scopulariopsis	1	0.36	L	0	0	0	0	0	0
Stachybotrys	0	0	0	8	2.74	L	0	0	0
Torula	2	0.72	L	5	1.71	L	0	0	0
Trichoderma	13	4.69	M	17	5.82	M	11	4.91	M
Ulocladium	0	0	0	7	2.4	L	0	0	0

Occurrence Remarks (OR): High occurrence (H) = 30-125 total count; Moderate occurrence (M) = 11-30 total count; Low occurrence (L) = 1-10 total count

Acremonium, *Cladosporium*, and *Trichoderma* were recorded in moderate occurrence, while 16 remaining fungal genera were observed as low incidence.

Genus *Aspergillus* represented by 11 species and isolated from all samples. The most common species were *Aspergillus niger, A. flavus* and *A. terreus* were classified as high and moderate incidence, respectively while remaining 8 species were listed as low incidence. *Fusarium* and *Penicillium* were represented by 5 species for each and recovered from 26 and 28 soil samples (out of 45 soil samples), respectively. *Cladosporium* (3 species), *Acremonium* (2 species), and

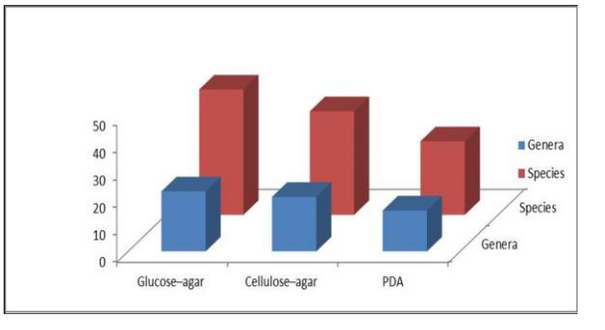

Fig. 1. Number of fungal genera and species in diffrent studies media

Table 2. Number of cases of isolation (NCI), percentage number of cases of isolation (%NCI) and occurrence remarks of zoosporic fungi of 45 soil samples collected from nurseris in Al–Qurayyat, Jouf area.

Zoosporic fungal genera	Total		
	NCI	% NCI	OR
Achlya:	12	26.6	M
Allomyces	45	100	H
Aphanomyces	2	4.4	L
Dictyuchus	4	8.8	L
Phytophthora	12	26.6	M
Pythium	15	33.3	M
Saprolegnia	10	22.2	L

Occurrence Remarks (OR): High occurrence (H) = 16-43 total count; Moderate occurrence (M) = 11-15 total count ; Low occurrence (L) = 1-10.

Trichoderma (one species) were recorded in moderate occurrence during this study and were isolated from 13, 19 and 13 soil samples out of 45 samples, respectively. The remaining 16 fungal genera in this study were representing by 2 or 1 species and recoreded in low occurrence and these genera were recovered from 1 to 12 soil samples and contributing.

Thirty–eight species belonging to twenty genera were encountered on cellulose – Czapek's agar at 28ºC as showen in Table 1. *Aspergillus, Penicillium* and *Fusarium* genera were recorded as high occurrence which comprising 41.78%, 9.25% and 9.25% of total samples, respectively and represented by 10, 5 and 4 species, respectively. *Aspergillus niger* and *A. flavus* were classified as high incidence while *A. terreus* and *Aspergillus fumigatus* were recorded in moderate occurrence and the remaining 6 species were listed as low incidence. The genus *Trichoderma* and *Acremonium* were represenred by one species and were recorded in moderate incidence which comprising 5.82% and 3.77% of total fungal species, respectively. Low incidence was found for the other fungal species, which were represented by three or one species.

Fivteen genera and 27 species were recovered on PDA medium during this study. *Aspergillus* was observed as high incidence which recovered from 41 soil samples and contributing 51.79% of total samples. Six species of the *Aspergillus* genus were recorded. *Aspergillus niger, Aspergillus terreus* and *A. flavus* were the most frequent and recorded as high occurence while *Aspergillus fumigatus* was recorded in moderate occurrence and the remaining species were listed as low incidencee as showen in Table 1. The genus *Fusarium* and *Penicillium* were represented by 4 species followed by *Acremonium* (2 species) and *Trichoderma* (one species) were recorded in moderate occurrence comprising 9.38% and 8.48%, 5.8% and 4.91% respectively. Nine genera namely:, *Chaetomium, Circinella, Cladosporium, Curvularia, Emericella, Fennellia, Humicola, Mucor* and *Rhizopus* were recovered in low occurrence. In this study, *Alternaria alternate, Epicoccum nigrum, Gibbrella inticans,* and *Scopulariopsis brevicaulis* were found only on Glucose–agar medium and not on any other investigated media, while *Pestalotia* species, *Stachybotrys atra,* and *Ulocladium atrum* were found only on Cellulose– Czapek's agar medium and were not found on Glucose–Czapek's agar or PDA media. Only two terrestrial species, *Chaetomium* species and *Circinella* species, were found on PDA and were completely absent from all other medium. Images of some fungi genera isolated during the present study were showen in Fig. 2.

As shown in Table 2, 45 soil samples revealed 12 zoosporic fungus species belonging to 7 genera. The species *Allomyces* was found to be the most dominant genus in soil samples and isolated from all samples in high occurrence comprising 100% of total fungal isolates. It was only represented by *A. macrogynus* which was the only species identified. The genus *Pythium, Achlya* and *Phytophthora* were isolated in moderate occurrence (15, 12 and 12 samples, comprising 33.3%, 26.6, and 26.6% of total soil samples, respectively). The *Pythium* genus was represented by *P. pulchrum, P. vexans* and an unknown species followed by *Achlya* genus that represented by *A. dubia*

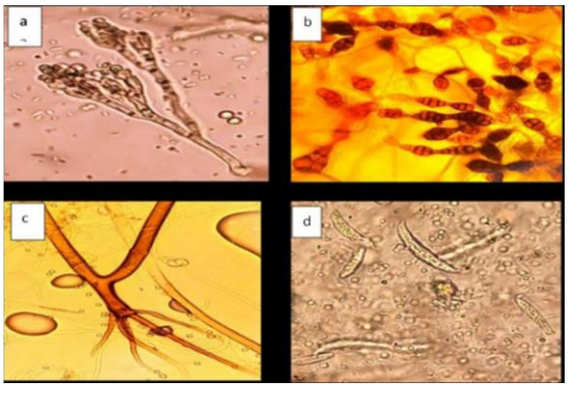

Fig. 2. Some of fungi genera which isolated during the present study

Image of a *Penicillium,* b *Alternaria,* c *Rhizopus,* d *Fusarium*

and unidentified species, which failed to form sex organs and *Phytophthora* which represented by only an unidentified species. *Saprolegnia* was found in ten soil samples (corresponding to 22.2%), and it was classed as a low occurrence species with three species (*S. ferax* and *S. parasitica* and an unknown species). *Aphanomyces arbuscula* and *Dictyuchus sterilis* were the remaining fungal genera isolated in low numbers.

DISCUSSION

Sixty–seven species from thirty–four fungal genera were isolated from 45 soil samples collected randomly from soil in several nurseries in Al–Qurayyat, Jouf reagon, KSA. On three type of mycological media, glucose– Czapek's agar, cellulose– Czapek's agar and PDA there were fifty–five species representing 27 terrestrial fungal genera were isolated. On glucose–Czapek's agar media, 22 genera and 46 species were discovered, with three genera, *Aspergillus, Fusarium,* and *Penicillium*, being identified as having a high occurrence. In this study, members of *Aspergillus* genus had the highest incidence and represented by with 11 species followed by *Fusarium* and *Penicillium* genera. It is known that the soil serves as a reservoir for many microorganisms which plays major role in soil ecosystem (21). Six identified species belonging to *Acremonium, Cladosporium,* and *Trichoderma* were recorderd as moderate incidence, as that the remaining fungal genera were observed as low incidence. El–Said and Saleem (22) were previously isolated all of these genera and species from various locations.

On cellulose–Czapek's agar media, twenty genera were isolated and identified. The genera *Aspergillus, Penicillium* and *Fusarium* genera have high incidence, respectively. The genus *Trichoderma* and *Acremonium* were recorded in moderate occurence, respectively. The remaining fungal species were recorded as low incidence. From 77 samples obtained from the Red Sea's coastal zones. Gómez et al. (23) isolated *Aspergillus, Penicillium, Cladosporium* and *Alternaria.* Helal (24) was previously isolated all of these genera and species from various locations.

Fifteen genera and 27 species were recovered on PDA medium during this study. *Aspergillus, Fusarium* and *Penicillium* were the most frequent and recorded as high occurrence. The genus *Acremonium* and *Trichoderma* were recorded in moderate occurrence while the remining nine genera namely:, *Chaetomium, Circinella, Cladosporium, Curvularia, Emericella, Fennellia, Humicola, Mucor* and *Rhizopus* were recovered in low occurrence. From soil samples collected from different sites in Saudi Arabian deserts, *Aspergillus, Penicillium, Alternaria, Fusariam, Ulocladium, Trichoderma, Chaetomium, Curvularia, Stemphylium, Talaromyces* and *Cladophialophora* genera were isolated (25). Many investigators have previously recorded all of the above genera and species at various levels on a variety of media using soil samples collected from various locations in the Saudi Arabian desert (26-30).

From 45 soil samples, 12 species of zoosporic fungi belonging to 7 genera were identified. *Allomyces macrogynus* was recovered from all tested samples as high occurrence, followed by *Achlya* (*A. dubia* and *Achlya* species), *Phytophthora* species and *Pythium* (*P. pulchrum, P. vexans* and *Pythium* species) while, the remaining genera *Saprolegnia* (*S. ferax* and *S. parasitica* and *Saprolegnia* species), *Aphanomyces arbuscula* and *Dictyuchus sterilis* were found in low incidences. These discoveries were consistent with previous results from investigations on water, soil, and plant samples conducted around the world (14, 31-34).

CONCLUSION

Fungi of many genera and species live in the soil of the research area. From 45 soil samples obtained randomly from soil in several nurseries in Al–Qurayyat, Jouf reagon, KSA, 67 species belonging to 34 terrestrial and zoosporic fungus genera were isolated. During this investigation, 22 genera and 46 species were revealed on Czapek's agar medium, twenty genera on cellulose–agar Czapek's media, and fifteen genera and 27 species on PDA medium. The genera *Aspergillus, Penicillium,* and *Fusarium* were found in high abundance in all studied samples, while Allomyces was the most prevalent genus in zoosporic fungi, followed by *Achlya* and *Pythium.* According to our results, four fungal species of terrestrial fungi were only discovered on Glucose–agar medium and were not isolated on any other studied media , whereas three species were only identified on Cellulose–Czapek's agar medium and were not found on Glucose–agar or PDA media. Only two terrestrial species were found on PDA, and none were found on any other medium.

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