FUNGI ASSOCIATED WITH SOME AQUATIC PLANTS COLLECTED FROM FRESHWATER AREAS AT ASSIUT (UPPER EGYPT)

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SUMMARY: Sixty three species in addition to one variety belonging 25 fungal genera were recovered during this investigation. Three species of aquatic Hyphomycetes are new records to Egypt. The composition of water-borne fungi was basically similar to those of the washwater (phyllosphere) and plant part samples (phylloplane). No qualitative variations but quantitative differences were observed. Of the 17 identified zoosporic species, Dictyuchus sterilis, Pythium thalassium and P. intermedium were the commonest species. On glucose Czapek's agar medium the most frequent species in three substrates were members of Fusarium, Aspergillus and Penicillium. Some fungal species were isolated only from water samples and not from phyllosphere or phylloplane and vice versa. Aspergillus niger and A. flavus were the commonest species in water samples and phyllosphere whereas F. moniliforme was the prevalent species in phylloplane.

Key Words: Aspergillus niger, A. flavus, dictyuchus sterilis.

INTRODUCTION

Fungi are universally present in all types of natural waters and form one of the most important components of an ecosystem as decomposers. The adjacent terrestrial vegetation supplies the water with substantial amount of dead organic material mostly in the form of leaves, branches and twigs. Although an increasing number of investigations concerned with the study of the occurrence and distribution of zoosporic fungi (18, 20, 21, 40, 56) and terrestrial fungi (2, 22, 23, 37, 38, 42, 47, 48) in various water areas, a few informations are available concerning the association between fungi and plant leaves and other parts collected from water areas (8,33,41). In Egypt, no efforts were performed dealt with association between fungi (zoosporic and terrestrial) and aquatic plants. Thus, the present investigation aimed to study the occurrence and frequency of fungal population on some aquatic plants collected from Nile system of Assiut (Upper Egypt).

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MATERIALS AND METHODS

Thirteen aquatic plant samples related to eight species namely, *Eicchornia crossipes* (4 samples), *Ceratophyllum demersum* (2 samples), *Polygonum salicifolium* (1 sample), *Potamogeton nodosus* (1 sample), *Lactuca serriola* (1 sample), *Typha domingensis* (2 sample), *Myriophyllum spicattum* (1 sample) and Potamogeton pectinatus (1 sample) were randomly collected from eight water sites at Assiut and brought to the laboratory in clean plastic bags. For comparison, a water sample was also taken from each of these water sites and brought to the laboratory in sterile conical flasks (1 liter capacity each) and used for further experimentation.

Water Samples

For the recovery of zoosporic fungi, a baiting technique was used (21). The seeded plates (5 plates for each sample) were incubated at 22°C for two weeks during which the growing colonies were examined and identified. For the determination of fungal population (density), the zoosporic fungal species appearing on one plate was counted as one colony.

For the recovery of terrestrial fungi, 1 ml using sterile Menzies's (39) dipper of each of water sample was transferred to

10 cm sterile Petri dish containing about 20 ml of glucose-Czapek's agar medium (5 plates for each sample). Rose bengal was added as a bacteriostatic agent (54). The plates were incubated at 28°C for 2 weeks during which the growing colonies were examined, identified and counted.

The plant samples

Freshwater plant samples were collected and brought to the laboratory in clean plastic bags. In order to have the wash water samples, a part of shoot system (about 10 gm) of each plant sample was placed in a sterile flask (500 ml) which contained 300 ml sterilized distilled water. Flasks were shaken gently in rotating motion for 10 minutes.

Isolation of fungi from wash water (phyllosphere)

For the recovery of zoosporic fungi, the baiting technique using sesame and hemp seeds was used (21). Five replicates (each contained 20 ml of wash water and sterilized seeds as baits) were used to each sample. The seeded plates were incubated at 22°C for 2 weeks during which the growing hayphae were examined and identified.

For the recovery of terrestrial fungi, aliquots of wash water (1 ml each) was transferred into sterilized petri dishes which were poured with about 20 ml glucose-Czapek's agar medium (5 plates to each sample). Rose bengal was added as bacteriostatic agent (54). The plates were incubated at 28°C for 2 weeks during which the growing colonies were examined, identified and counted.

Isolation of fungi from plant part samples (phylloplane)

The plant parts after being thoroughly shaken in a series of sterile distilled water were removed and dried between sterilized filter papers.

For the recovery of zoosporic fungi, two pieces of each dried plant parts were put in petri dish containing about 20 ml of sterile distilled water and sesame and hemp seeds as baits and incubated at 22°C for 2 weeks during which the growing heyphae were examined, identified and counted.

For the recovery of terrestrial fungi two pieces of a dried plant parts were inserted on the surface of agar medium in each plant (5 plates to each plant sample). Plates were incubated at 28°C for two weeks during which the growing colonies were examined, identified and counted.

Identification of fungal genera and species

The following references were used for the identification of isolated fungi genera and species:

a - Zoosporic fungi

- Coker (13), for Saprolegniaceae and other water moulds.
- Johnson (34), for *Achlya* species.
- Sparrow (55), for aquatic phycomycetes in general.
- Waterhouse (59, 60), for *Pythium* species.
- Seymour (52), for *Saprolegnia* species.

b - Terrestrial fungi

- Barnett (9), for the genera of imperfect fungi.
- Barron (10), for the genera of Hyphomycetes.
- Booth (11), for *Fusarium* species.
- De Vries (14), for *Cladosporium* species.
- Domsch and Gams (16), for different fungal species.
- Raper and Fennell (51), for *Aspergillus* species.
- Raper and Thom (50), for *Penicillium* species and related genera.

RESULTS AND DISCUSSION

Zoosporic fungi

Seventeen species which belong to six zoosporic fungal genera were recovered from 8 water samples, 13 wash water samples (phyllosphere) and 13 plant part samples (phylloplane) during this investigation. In addition 150 colonies (48.70% of total zoosporic fungi) of three genera namely, *Pythium* (93 colonies), *Saprolegnia* (47 colonies) and *Achlya* (10 colonies) did not form the sexual organs and were identified as genera only (Table 1).

The water samples (8) contributed 10 identified species in addition to 3 unidentified species (6 genera) constituting 33.77% of total zoosporic fungi. The wash water samples (phyllosphere) contributed also 10 identified species in addition to 2 unidentified species (4 genera) representing 49.68% of total zoosporic fungi. On the other side, plant part samples (phylloplane) contributed 5 identified species in addition to 3 unidentified species (4 genera) constituting 16.56% of total zoosporic fungi.

Three genera namely, *Pythium* (8 species), *Dictyuchus* (3 species) and *Saprolegnia* (5 species) were the most prevalent genera and were represented in 79.42%, 73.53% and 55.88% of total samples respectively.

Three species namely, *Dictyuchus sterilis Pythium thalassium* and *P. intermedium* were the commonest identified species and were represented in 67.65%, 32.35% and 20.59% of total samples respectively. The remaining species were of moderate, low or rare occurrence (Table 1).

Six species namely, *Pythium helicoides, Dictyuchus pseudoachlyoides, Saprolegnia anisospora, S. eccentrica. Olpidiopsis pythii* and *Aphanomyces leavis* were recovered from the water samples only and were not represented in neither phyllosphere nor phylloplane of test plants. On the other side, five species namely, *Pythium irregular, Dictyuchus monosporus, Saprolegnia*

Table 1: Total conts (TC), number of cases of isolation (NCI) and occurrence remarks (OR) of zoosporic fungi recovered from water samples, phyllosphere and phylloplane of collected plant samples at 22°C using baiting techniques.

Fungal genera and species	Water			Phyllosphere			Phylloplane		
	TC	NCI	OR	TC	NCI	OR	TC	NCI	OR
Total	104	8		153	13		51	13	
Pythium	40	7	H	65	12	H	28	8	H
P. butleri Subram	1	1	R	2	2	L	-	-	-
P. debaryanum Heese	-	-	-	3	2	L	5	4	M
P. thalassium Atkins	6	5	M	6	4	M	2	2	L
P. ultivuum Trow	-	-	-	-	-	-	1	1	R
P. intermedium de Bary	5	3	M	4	3	M	1	1	R
P. irregulare Bouisman	-	-	-	3	3	M	-	-	-
P. helicoides	1	1	R	-	-	-	-	-	-
Pythium species	27	7	H	47	10	H	19	8	H
Dictyuchus	32	7	H	51	11	H	11	7	H
D. sterilis Coker	31	7	H	39	9	H	11	7	H
D. pseudoachlyoides Beneke	1	1	R	-	-	-	-	-	-
D. monosporus Leitgeb	-	-	-	12	6	M	-	-	-
Saprolegnia	23	6	M	38	8	H	6	5	M
S. anisospona de Barry	6	2	L	-	-	-	-	-	-
S. eccentrica (Coker) Seymour	2	2	L	-	-	-	-	-	-
S. diclina Humhrey	-	-	-	9	6	H	-	-	-
S. hypogyna (Pringsheim) de Bary	-	-	-	3	2	L	-	-	-
Saprolegnia species	15	6	M	26	7	H	6	5	M
Olpidiopsis pythii (Bntler) Korling	2	1	R	-	-	-	-	-	-
Aphanomyces laevis de Barry	3	2	L	-	-	-	-	-	-
Achlya	4	2	L	5	3	M	6	4	M
A. racemosa Hildebrand	-	-	-	5	3	M	-	-	-
Achlya sp.	4	2	L	-	-	-	6	4	M

Occurrence remarks:

High (H): More than 46% of samples

Moderate (M): Between 23% and 45%

Low (L): Between 13% and 22%

Rare (R): Less than 13%

dicline, S. hypogyna and *Achlya recemosa* were recovered from the samples of wash water (phyllosphere) and were not represented in neither phyhoplane nor water samples. Moreover, one species namely, *Pythium ultimum* was recovered from phylloplane and was not emerged from neither phyllosphere nor water samples.

Concerning the fungi of phyllosphere, it was observed that *Pythium butleri* was recovered from the phyllosphere of two species of *Potamogeton (P. pectinatus* and *P. nodosus)* only, *Pythium intermedium* from phyllosphere of *Eicchornia crassipes* (2 samples) only and *Saprolegnia hypogyna* emerged from the phyllosphere of *Eicchornia crassipes* and *Ceratophyllum demersum* only. *Achlya recemosa* was encountered from phyllosphere of *Ceratophyllum demersum* and *Eicchornia carssipes*. Unidentified species of *Phytium* were emerged from the phyllosphere of all plant species except those of *Ceratophyllum demersum* and *Typha domingensis*.

Concerning the phylloplane samples, it was found that *Pythium thalassium* was recovered from the phylloplane of *Polygonum salicibolium* and *Eicchornia crassipes* only and *P. intermedium* emerged from the phylloplane of *Lactuce serriola* only.

It is worthy to mention that, within the collected water samples, the richest samples with zoosporic fungi was that collected from the water site covered by *Eicchornia crassipes*.

Unfortunately, no available literature concerning the zoosporic fungi associated with aquatic plants. However, these recovered fungal genera and species were previously isolated in our laboratory from various Egyptian water areas (18, 20, 21, 23, 24, 35, 36) as well as from submerged mud and soil (1, 19, 35).

Terrestrial fungi

Forty-three species and one variety which belong to 16 terrestrial fungal genera were isolated in his investigation (Table 2). *Fusarium* was the leading genus (79.41% of total samples; 28.71% of gross total count). *Aspergillus* came behind and was represented in 76.47% of total samples yielding 46.85% of gross total count. The broadest spectrum of species was produced by *Aspergillus* (8 species and one variety), *Penicillium* (7 species) and *Fusarium* (6 species). The most common species were *Aspergillus niger* (58.82% of total fungal count) and *Fusarium moniliforme* (29.41% of total samples and 3.82% of total fungal count).

In comparison, it was found that, five species *(Aspergillus ustus, Penicillium viridicatum, Trichodenna hamatum, Scopulariopsis brevicaulis* and *Myrothecium verrucaria)* were isolated from water samples only and disappeared completely in phyllosphere and phylloplane of plant samples. On the other side, eight species *(Fusarium graminae, Aspergillus gluacus, A. fischerii, Penicillium chrysogenum, P. corylophilum, Drechslera specifera, Alternaria chlamydosporum, Paecilomyces terricola* and *Cladosporium cladosporioides)* were isolated from washwater (phyllosphere) samples and were not emerged from neither water samples nor phylloplane. Moreover, two species namely, *Botryotrichum atrogriseum* and *Humicola grisea* were encountered in phylloplane only and were not represented neither in phyllosphere nor in water samples.

Aspergillus niger, A. flavus, A. terreus, A. nidulans, Fusarium solani and *F. oxysporum* were the commonest species in water samples. *Aspergillus niger, A. flavus* and *Trichoderma viride* were the most prevalent species in phyllosphere whereas *Fusarium moniliforme* was the prevalent species in phylloplane samples.

The fungal population in water samples was mainly *Fusarium, Aspergillus* and *Penicillium* which were represented in 100%, 100%, and 50% of total water samples respectively. This is in accordance with the statement of Barron (10) that *Aspergillus* is biologically one of the most successful of all fungi and is expected to occur on all sorts of organic debris. Nasar and Munchi (45) reported that the fungal population in freshwater pond of Bhagalpur (India), is mainly composed of the *Aspergillus, Penicillium, Fusarium, Cladosporium, Epicoccum* and *Mucor*. Most of isolated species (Table 2) were previously isolated from Egyptian water areas (7, 22, 23, 42) and from other water areas in the world (28, 48). Park (47) mentioned that the more obvious types of origin of these terrestrial fungi in fresh water would be animal or plant, the whole or part, living or dead and soil letter having been in contact with soil. Indeed no available literature concerning the fungi isolated from phyllosphere or phylloplane of aquatic plants. However, most of isolated terrestrial fungi were previously isolated from phyllosphere of cultivated plants (2-4, 15, 29, 43, 44, 49) and from the phylloplane of some plants (5, 6, 12, 25, 57).

It was interesting to recover three fungal genera of aquatic Hyphomycetes when leaves of collected plants were placed in Petri dishes containing sterile distilled water. Each of these three genera was represented by one species only. These are *Flagellospora curvula, Triscleophorus monosporus* and *Actinospora megalospora* (New records to Egypt). These species were previously isolated from submerged decaying leaves (27, 30, 31). Ingold (32) reported that *Flagellospora curvula*

Table 2: Total count (TC), number of cases of isolation (NCI) and occurrence remarks (OR) of terrestrial fungi isolated from 8 water samples, 13 phyllosphere samples and 13 phylloplane samples of collected plants samples on glucose-Czapek's agar at 28°C.

	Water			Phyllosphere			Phylloplane			Total %	
Fungal genera and species	TC	NCI	OR	TC	NCI	OR	TC	NCI	OR	Count	NCI
Total	195.81	8		152.00	13		40	13		100	100
Fusarium	60.66	8	H	42.67	11	H	8	8	H	28.71	79.41
F. equiseti (Corda) Sacc.	14.00	4	H	23.17	4	M	-	-	-	9.58	23.53
F. solani (Mart.) Sacc.	30.00	4	M	6.08	5	M	-	-	-	9.30	26.47
F. oxysporum Schlecht. ex Fr.	10.33	4	H	4.92	4	M	1	1	R	4.19	26.47
F. moniliforme Sheldon	3.33	2	M	7.50	4	M	4	4	M	3.82	29.41
F. semitectum Berk. and Rav.	3.00	1	R	-	-	-	3	3	M	1.55	11.76
F. graminearum Schwabe	-	-	-	1.00	1	R	-	-	-	0.26	2.94
Aspergillus	103.50	8	H	64.17	12	H	14	6	H	46.85	76.47
A. niger Van Tieghem	14.00	8	H	35.33	10	H	5	2	L	14.01	58.82
A. flavus Link	4.17	6	H	10.67	10	H	1	1	$	4.08	50.00
A. terreus Thom	38.00	4	H	15.00	3	M	2	2	L	14.18	26.47
A. fumigatus Fresenius	38.00	3	M	1.34	4	M	2	2	L	10.66	26.47
A. nidulans (Eidam) Wint.	9.00	4	H	0.67	2	L	2	1	R	3.01	20.59
A. amstelodami (Margin) Thom and Chucrh	-	-	-	0.50	1	R	-	-	-	0.13	2.94
A. flavus var. *columnaris* Raper and Fennelll	-	-	-	0.50	2	L	2	1	R	0.64	8.82
A. ustus (Bain.) Thom and Church	0.33	1	R	-	-	-	-	-	-	0.09	2.94
A. fischeri Wehmer	-	-	-	0.16	1	R	-	-	-	0.04	2.94
Penicillium	20.50	4	H	21.34	7	H	4	3	M	11.82	41.18
P. oxalicum Currie and Thom	2.00	1	R	2.00	2	L	2	1	R	1.55	11.76
P. funiculosum Thom	16.50	2	L	1.00	2	L	-	-	-	4.51	11.76
P. steckii Zaleski	-	-	-	15.00	1	R	1	1	R	4.13	5.88
P. chrysogenum Thom	-	-	-	2.67	3	M	-	-	-	0.69	8.82
P. viridicatum Westling	2.00	1	R	-	-	-	-	-	-	0.52	2.94
P. martensii Biourge	-	-	-	0.17	1	R	1	1	R	0.30	5.88
P. corylophilum Dierckx	-	-	-	0.50	1	R	-	-	-	0.13	2.94
Trichoderma	5.33	4	H	4.17	7	H	-	-	-	2.45	32.35
T. viride Pers. ex S.F. Gray	5.00	3	M	4.17	7	H	-	-	-	2.36	29.41
T. hamatum (Bon.) Bain.	0.33	1	R	-	-	-	-	-	-	0.09	2.94
Mucor	2.00	1	R	8.66	5	M	2	2	L	3.26	32.53
M. racemosus Fresenius	2.00	1	R	8.33	5	M	1	1	R	2.92	17.65
M. circinelloides van Tiegh.	-	-	-	0.33	1	R	1	1	R	0.34	5.88
Drechslera	-	-	-	1.50	4	M	3	3	M	1.16	20.59
D. miyakei (Nisikado) Subram and Jain	-	-	-	-	-	-	1	1	R	0.26	2.94
D. spicifera (Bain.) von Arx	-	-	-	1.17	3	M	-	-	-	0.30	8.82
D. pallescens Boedijn	-	-	-	-	-	-	1	1	R	0.26	2.94
D. rostrata (Drechsler) Richardson and Fraser	-	-	-	0.33	1	R	1	1	R	0.34	5.88
Alternaria	-	-	-	2.00	4	M	2	2	L	1.03	17.64
A. alternata (Fries) Keissler	-	-	-	1.00	3	M	2	2	L	0.77	14.71
A. chlamydospora Mauchacca	-	-	-	1.00	1	R	-	-	-	0.26	2.94
Curvularia	0.50	1	R	1.33	1	R	1	1	R	0.73	8.82
C. Lunata (Wokker) Boedijn	0.50	1	R	1.33	1	R	1	1	R	0.73	8.82
Rhlzopus stoloni Ser Ehrenb. ex Fr. Lindt	0.33	1	R	-	-	-	2	2	L	0.73	8.82
Scopulariopsis	0.33	1	R	2.00	1	R	-	-	-	0.73	5.88
S. brevicaulis (Sacc.) Bainier	-	-	-	2.00	1	R	-	-	-	0.52	2.94
S. brumptii Salvanet-Duval	0.33	1	R	-	-	-	-	-	-	0.09	2.94
Paecilomyces	2.00	1	R	0.33	1	R	1	1	R	0.86	8.82
P. variotii Bainier	2.00	1	R	-	-	-	1	1	R	0.77	5.88
P. terricola Miller	-	-	-	0.33	1	R	-	-	-	0.09	2.94
Cladosporium	-	-	-	3.50	1	R	2	1	R	1.42	5.88
C. cladosporioides (Freseninus) de Vries	-	-	-	1.00	1	R	-	-	-	0.26	0.94
C. herbarum (Pers.) Link ex Fr.	-	-	-	2.50	1	R	2	1	R	1.16	5.88
Acremonium strictum W. Gams	0.33	1	R	0.33	1	R	-	-	-	0.17	5.88
Botryotrichum atrogriseum Van Beyma	-	-	-	-	-	-	1	1	R	0.26	0.94
Humicola grisea Traaen	-	-	-	-	-	-	1	1	R	0.26	0.94
Myrothecium verrucaria (Albertini and Schweinitz) Ditmar	0.33	1	R	-	-	-	-	-	-	0.09	0.94

was one of the commonest of all aquatic Hypholycetes and was found all over the world. Nilsson (46) and Singh and Musa (53) mentioned that aquatic Hyphomycetes have a world-wide distribution and have been repeatedly observed on decaying leaf litter in temperate and tropical streams. A major role of these fungi in decomposition of leaf materials in water has been suggested by various investigations.

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