Mycoflora Associated with Aquatic Plants in Ponds and Lakes in Central West of Florida, USA

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ABSTRACT

Twenty species belonging to fifteen freshwater fungal genera were recovered from 9 aquatic plants species that were collected from different ponds and lakes in Hillsborough County in central west of Florida. Five genera and eight species of zoosporic fungi were isolated in this study. *Saprolegnia* was the most common genus among all recovered genera during this study. *Alternanthera philoxeroides* was recorded as the highest aquatic plant yield fungal species while, *Salvinia molesta* and *Phragmites australis* produced the lowest number of species. Twelve species related to ten terrestrial fungal genera were isolated on corn meal agar (CMA) and potato dextrose agar (PDA); *Trichoderma* was the most frequent genus recovered on two types of tested media. *Phragmites australis* and *Hydrocotyl* spp were recorded as the highest plant yielded terrestrial fungi on the two investigated media, while *Alternanthera philoxeroides* contributed the lowest number of species on PDA medium. Some fungal species were isolated on only one tested medium either CMA or PDA and vice versa.

Keywords: Aquatic plants, Terrestrial fungi, *Trichoderma, Saprolegnia, Zoosporic* fungi

INTRODUCTION

Fungi found in all kinds of aquatic habitats (freshwater and marine). They live as saprophytes or parasites of plants and animals. Saprophytic fungi grow on decaying plant and animal remains, submerged branches, twinges, fruits and leaves (Dube, 1983). Aquatic fungi mainly belong to Mastigomycotina (the zoosporic fungi) and the Deuteromycotina. However, some Asco-, and Basidiomycotina are also known to have aquatic forms. The saprophytic activity of microorganisms on the leaf surface of plants has, in recent years, been described by several investigators (Leben, 1972), (Sharma, 1974), (Garge and Sharma, 1983), (Mishra and Dickinson, 1984), (Charudattan, 1990) and (Gaur *et al,* 1992). In Egypt there have been a few studies on the association between fungi and aquatic plants (Khallil 1990), (El-Hissy *et al.* 1990), (Nassar, 1991), (Abdel-Hafez *et al,* 1990), (Khallil *et al,* 1991) and (Bagy *et al,* 1992). So, this investigation aims to study the association of freshwater (terrestrial and zoosporic) fungi with aquatic plants that were collected from different ponds and lakes in Hillsborough County in central west of Florida, USA.

MATERIALS AND METHODS

Collection of aquatic plants:

45 aquatic plant samples related to nine species were collected randomly from different sites in the ponds and lakes of Hillsborough County central – west of Florida. These plants namely: *Spirodela polyrhiza, Salvinia molesta, Phragmites australis, Hydrocotyl spp, Potamogeton nodosus, Alternonthera philoxeroides, Potamogeton crispus, Hydrochloa caroliniensis* and *Potamogeton diversifolius* (5 samples of each plant) each aquatic plant sample was collected in dry clean plastic bags and brought to the laboratory immediately for further experimentation.

a) Recovery of zoosporic fungi:

Plant samples were collected and brought to the laboratory in clean plastic bags. A part of the shoot system (about 10 gm) of each plant sample was placed in a sterile flask (500 ml) which contained 250 ml sterilized distilled water. The flasks were shaken gently in a rotating motion for 30 minutes. For the recovery of zoosporic fungi; the wash water of plants was poured into Petri-dishes (12 cm diameter). These plates (four plates for each sample) were left overnight at room temperature to allow colonization of seeds by aquatic fungi (El-Hissy and Khallil, 1989).

After 24 hours the colonized sesame seeds were transferred into other sterile Petri-dishes which contained sterilized distilled water and crystalline penicillin to inhibit bacterial growth (Roberts 1963);and these were incubated at 22 ± 2^{o}C + and these dishes were incubated at for three weeks. The colonized seeds were examined and identified for zoosporic fungi.

b) Recovery of terrestrial fungi

For isolation of fungi which associated with aquatic plant species, the dilution-plate method as described by (Johnson and Curl, 1972) was used. Ten grams of each sample were put in a 250 ml Eyrlenmeyer flask under aseptic condition. A sufficient quantity of sterile distilled water was added to obtain the desired dilution.

The flask was shaken for 15 minutes. One ml of water suspension of each plant samples was transferred into a 12 cm sterile Petri-dish, which was then mixed with about 20 ml of Corn Meal Agar (CMA) and Potato Dextrose agar medium (PDA). Ten plates were used for each water sample (5 plates of CMA and 5 plates of PDA). These plates were then incubated at 28^{o}C for 7 – 10 days and the growing colonies were examined; identified and counted.

Identification of fungal genera and species

The following references were used for the identification of isolated fungal genera and species during this investigation:

a) *Zoosporic fungi:* (Coker, 1923), (Johnson, 1956), (Sparrow, 1960), (Waterhouse, 1967), (Seymour, 1970), (Rattan *et al*, 1978), and (Van Der Plaats Niterink, 1981).

b) *Terrestrial fungi:* (Raper and Thom, 1949), (De Vries, 1952), (Raper and Fennell, 1965), (Barron, 1968), (Ames, 1969), (Ellis, 1971) and (Booth, 1977).

RESULTS AND DISCUSSION

Twenty identified species belonging to 15 fungal genera in addition to 2 unidentified species of *Achlya* and *Saprolegnia* were recovered from 45 aquatic plant samples during this investigation. From these fungi there were 7 zoosporic species belonging to 5 aquatic fungal genera in addition to 13 species belonging to 10 terrestrial fungal genera (Table 1).

Among all investigated aquatic plants, *Alternanthera philoxeroides* was the richest in fungal species (12 species), whereas *Salvinia molesta* and *Potamogeton nodosus* were lowest in fungal species (5 identified species for each). Concerning the occurrence of the common aquatic fungal genera it was found that, *Saprolegnia* was the most frequent genera, these genera was represented by two identified species which namely (*S. hypogyna* and *S. parasitica*) in addition to an unidentified one, it was recovered from 6 plants among of all tested plants. *Pythium catenulatum* occupied the second position after *Saprolegnia* in the investigated aquatic plants samples and it was recovered from 5 plants.

Fig 1: Relation between plants and number of species

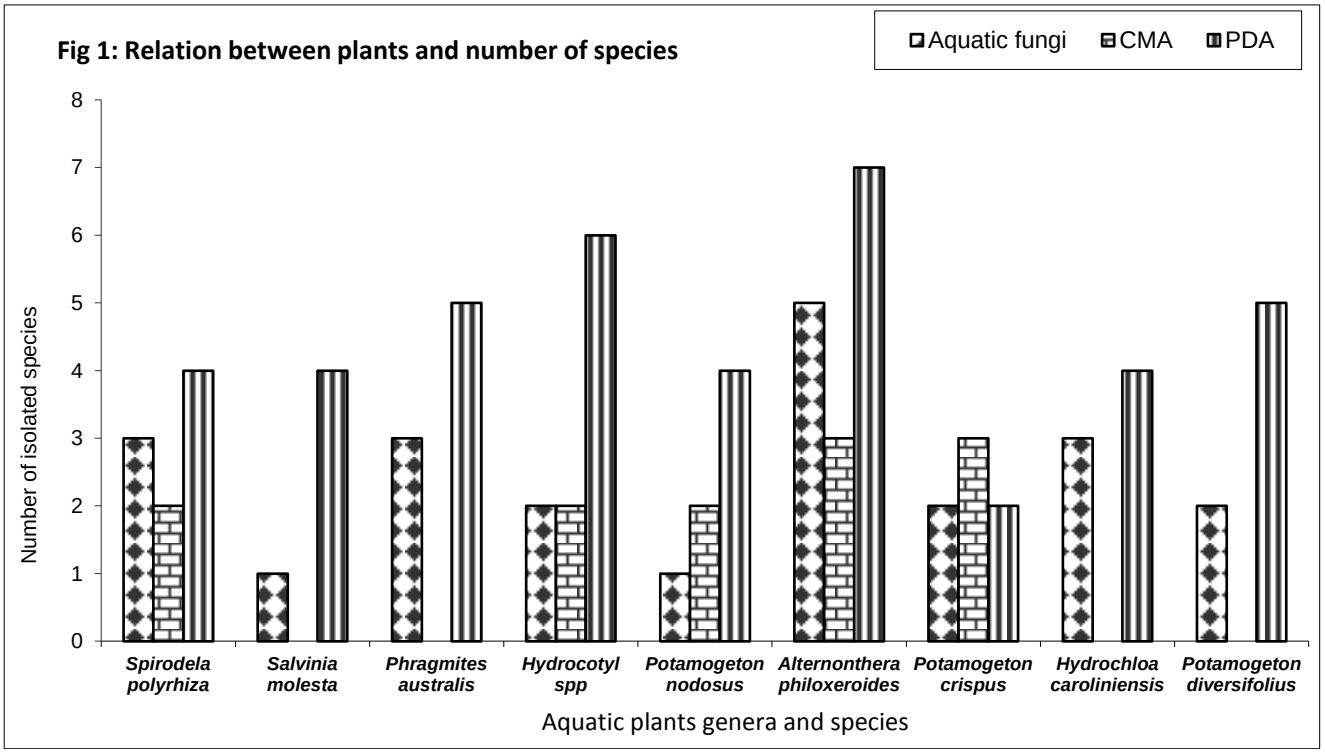

Table (1): Fungal genera and species which were recovered from 9 aquatic plants which collected from water ponds and lakes from different ponds and lakes of Hillsborough County, central – west of Florida.

Fungal genera & species	1			2			3			4			5			6			7			8			9		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Achlya:	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-		-	-	-	-	-
A. dubia	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
A. hypogyna	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Achlya species	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alternaria alternata	-	+	+	-	-	-		-	+	-	-	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	+
Aphanomyces levis	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus:	-	-	+	-	-	+	-	-	-	-	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	+
A. fumigatus	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A. niger	-	-	+	-	-	+	-	-	-	-	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	+
A. terreus	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium sp	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Dictyuchus steriles	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Epicoccum nigrum	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium:	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
F. javanicum	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. solani	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
Mucor stolenefer	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	-	+	-	-	+
Pecilomyces	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
Penicillium chrysogenium	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Pythium catenulatum	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Saprolegnia:	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-
S. hypogyna	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
S. parasitica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-
S. species	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-
Trichoderma viroide	-	-	+	-	-	+	-	-	+	-	+	+	-	+	+	-	+	+	-	-	+	-	-	+	-	-	+
Verticillium album	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Total no. species	3	2	4	1	-	4	3	-	5	2	2	6	1	2	4	5	3	7	2	3	2	3	-	4	2	-	5

1- Spirodela polyrhiza 2- Salvinia molesta 3- Phragmites australis 4- Hydrocotyl spp 5- Potamogeton nodosus 6- Alternonthera philoxeroides 7- Potamogeton crispus 8- Hydrochloa caroliniensis 9- Potamogeton diversifolius

A: Aquatic fungi B: CMA medium C: PDA medium

Achlya which represented by (*A. dubia, A. hypogyna* and *Achlya* species) was came after *Saprolegnia* and *Pythium* and isolated from three aquatic plants; *Spirodela polyrhiza, Potamogeton diversifolius* and *Hydrochloa caroliniensis.*

The remaining two genera *Aphanomyces* and *Dictyuchus* were represented by only one identified species namely *A. levis* and *D. sterilies*, respectively and isolated from one plant only.

Most of the aquatic fungi which were recovered and identified during this investigation were previously isolated from aquatic plants, water resources, soil and fishes in different parts of the world by: (El-Hissy *et al*, 1990), (Khallil, 1990), (Khallil *et al*. 1991) and (Bagy *et al*, 1992) and different water sources in Egypt and all different parts of the world; (El-Nagdy, 1981), (El-Hissy *et al*, 1982), (El-Hissy *et al*., 2000), (El-Hissy, 1994), (El-Hissy and Oberwinkler 1999), (El-Hissy *et al*. 1982 and 2000), (El-Nagdy and Abdel-Hafez, 1990), (Khallil *et al.* 1991), (Czeczuga and Snarska, 2001), (Paxton and Willoughby 2000), (Nassar *et al*., 2002), (Prasad *et al,* 2009), (Kiziewicz, 2005) and **(**Paliwal and Sati, 2009).

Thirteen species belonging to 10 terrestrial fungal genera and species were recovered from 45 aquatic plant samples on Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA) media during this investigation (Table 1).

The broadest spectra of terrestrial fungal species recovered from aquatic plants were yielded by *Aspergillus* (3 species). This genus was recovered from eight on PDA, while it recovered from tow on CMA. *Aspergillus niger* was the most prevalent species and it was isolated from six investigated aquatic plants on PDA medium while it was recovered from two aquatic plants on CMA medium. *Aspergillus fumigatus* and *A. terreus* were completely similar they recovered from only one tested plant on PDA medium where they were missed on CMA media.

Aspergillus was isolated previously as the most frequent genus on leaf surface of some Egyptian plants by (Khallil, 1990), (El-Hissy *et al*. 1990), (Bagy *et al*, 1992) and (Nassar *et al*, 2002). *Fusarium* was came in the second position after *Aspergillus,* it isolated from five examined plants on PDA medium while, it was completely absent on CMA medium. *Fusarium solani* was recovered from three tested plants in order to *Fusarium javanicum* was isolated from two examined plants on PDA medium.

Trichoderma (*T. species*) and *Alternaria* (*A. alternate*) were the most frequent genera on both tested media, they isolated from nine and eight examined plants on PDA while, they recovered from three and two examined aquatic plants on CMA, respectively. *Mucor* (*M. hiemalis*) came after *Trichoderma* and *Alternaria* it was recovered from five and two examined plants on PDA and CMA media, respectively.

Another all remaining genera; *Cladosporium cladosporioides*, *Epicoccum nigrum, Paecilomyces varioti, Penicillium chrysogenum* and *Verticillium album* were recovered from two to one examined aquatic plants on two tested media.

All of these genera and species were previously recovered from different parts around the world as: (Khallil, 1990), (El-Hissy *et al,* 1990), (Bagy *et al,* 1992), (Khallil *et al,* 1991), (Galal, 2010), (Leticia *et al,* 2007) and (Abdulmoneim, 2011). As a general outlook on the recovered fungal genera and species during this investigation (Fig. 1) it can be seen that the highest number of aquatic fungal species (5 species) were recovered from *Alternonthera philoxeroides* and the lowest number isolated from *Salvinia molesta* and *Potamogeton nodosus* (one species) for each. *Alternonthera philoxeroides* and *Potamogeton crispus* were yielded highest number of terrestrial fungal species on CMA (3 species for each) followed by *Salvinia molesta, Hydrocotyl spp* and *Potamogeton nodosus* (two species) each, while it not recovered on other plants. On PDA (7 species) were recorded as highest number of terrestrial fungi isolated from *Alternonthera philoxeroides* and *Potamogeton crispus* was yielded lowest number of species (2 species).

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