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Aquatic Pycnidial and Hyphomycetes Fungi from Macrophytes and Riparian Plants in the River Nile

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Abstract: This study extends our knowledge of aquatic pycnidial and hyphomycetes fungi that isolated from aquatic macrophytes plants in Qanatir city. Thirteen fungal texa have been recorded from eleven submerged plant substrates, ten aquatic pycnidial fungi, two Hyphomycetes and one Ascomycetes. *Clyeopycnis aeruginascens* was recorded on most plants. *Phragmites* and *Cyperus* were the most substrates which had most species, while *Rorripa palustris* and *Persicaria salicifolia* were the least substrates which had species. Aquatic pycnidial and Hyphomycetes fungi were screened for their ability to produce extracellular degradative enzymes on solid media. Most of species were positive for cellulase and pectinase. Twigs powders of *Rorripa palustris* were inhibited growth of most species.

Key words: Aquatic pycnidial fungi, Hyphomycetes, extracellular enzymes

INTRODUCTION

Aquatic pycnidial fungi, have been reported from submerged shoots of aquatic macrophytes including *Phragmites*, *Carex* and *Schoenoplectus* (Webster and Descals, 1981; Soars and Barneto, 2006; Kobayashi *et al*., 2005; Collade *et al*., 2006).

Macrophytes have several intrinsic properties that make them an indispensable component of constructed wetlands. The most important functions of the macrophytes in relation to the treatment of wastewater are the physical effects brought about by the presence of the plants. The macrophytes stabilize the surface of the beds, provide good conditions for physical filtrations, prevent vertical flow systems from clanging, insulate against frost during winter and provide a huge surface aero for attached microbial growth (Brix, 1994).

Anthony (1999) examined the occurrence of macro fungi and the decay of roofs thatched with water reed, *Phragmites australis*. Sampling from 20 north- and 20 south-facing roof sides showed that several ascomycetes usually associated with reed *in situ* are common on thatch. The only basidiomycetes recorded were Mycena species.

Aquatic Hyphomycetes occur commonly on a wide variety of decaying submerged plant substrates in fresh water (Barlocher and Kendrick, 1981; Chauvet, 1992; Gessner and Chauvet, 1994; Descals *et al*., 1995; Faber, 1998a, b). Many of these spore found in terrestrial habitat (Bandoni, 1981; Fisher *et al*., 1991; Shearer, 1993, 2001). With the exception of studies by Yuen *et al*. (1998) and Abdel-Raheem and Shearer (2002) little is known about the enzymatic capabilities and decomposition activities of these fungi. These fungi produce a wide range of plant cell wall degrading enzymes (Chamier, 1985; Abdel Rheem and Shearer, 2002).

In order to decompose plant litter, saprotrophic microbes produce extracellular enzymes. The most relevant enzymes from this aspect involve those that break down the plant fibers (cellulases, hemicellulases, pectinases, phenoloxidases, chitinases) as well as enzymes important for microbial acquisition of nitrogen and phosphorous (peptidases, ureases and phosphatases) (Sinsabaugh *et al*., 2002). Fungi in general have a more forceful enzymatic capacity than other microbes.

Several of the fungal species generally considered to be terrestrial can also be found in aquatic systems, but also truly aquatic species have been shown to produce a wide range of enzymes (Zemek *et al*., 1985; Abdel-Raheem and Shearer, 2002).

Decomposition of *Phragmites* has been found to relate to activity of cellulolytic and xylanolytic enzymes, which can be produced by fungi and other microbes (Tanaka, 1991, 1993; Boschker *et al*., 1995).

Present study was carried out to inveswtigate Aquatic pycnidial and Hyphomycates fungi occurring on root of plant species near to fresh water of River Nile at Qanatir city and determined the kinds of enzymes produced by pycnidial and Hyphomycetes fungi that could play a role in plant decomposition.

MATERIALS AND METHODS

Description of Study Sites

Twigs and branches of aquatic macrophytes and aquatic different plants that grown on wet slopes and water edges were collected at El-Qanatir city front of the bridge about 1 km at sandy area flooded with water frequently. The vegetation in the mire in which the river rises is dominated by *Cyperus papyrus*, *Phragmites australis*, *Persicaria senegalensis*, *Persicaria lapathifolia*, *Eclipta prostrata*, *Rorripa palustris*, *Rumex dentatus*, *Cyperus articulatus*, *Cyperus alopecroides* and *Pseudognaphalium luteo-album* and *Persicaria salicifolia*.

Trapped twigs and branches were collected in clean blastic bags and returned to the laboratory for examination and incubation. The twigs were examined under a dissecting microscope at a magnification of x 50. Spores that were found on the wood were removed by a dissecting needle and fungal species were recorded immediately. After that, the twigs were put in a plastic lunch box lined with moist tissue paper. They were examined within 1 week of incubation and at regular intervals for up to 3 months at 27°C. Aquatic pycnidia present were recorded. Isolations were made for some of the fungi on malt and patato, dextrose agar media. Identification of pycnidial fungi were carried out according to Sutton (1980).

The physico-chemical characteristics of water samples during the investigation were as follows: the temperature ranged from 18°C (winter) to 34°C (summer), the pH from 9.5-10.4 and the dissolved oxygen from 3.3-11.4 (ppm).

Culture Media

Malt Extract Agar Medium

This medium was used for isolation of aquatic pycmidia and it has the following composition (g L^{-1}): malt extract, 1; agar, 15; distilled water, 1000 mL. For control of bacterial growth, 0.1% crystamycin was added to the warm agar medium (Descals *et al*., 1977). Crystamycin is mixture of penicillin and streptomycin. When required, 1 mL of the antibiotic was pipetted into the bottom of the petri-dish before the warm medium was poured. Single conidia were located and cultures were prepared by transferring single germinated spores to 2% Malt extract agar media.

Production of extracellular enzymes was determinel by incorporation of test substrates into a basic medium, inoculating the medium with discs of fungal hyphae, allowing the fungi to grow out on the medium and adding reagents to the plates to detect the test substrate remaining. Colony radial growth rates and substrate clearing zones were measured for each fungal species on each substrate. Three replicates of each treatment were assayed and non-inoculated plates with substrates served as negative controls. Inoculated plates were checked at 5-7 days depending on the growth rates of the individual species.

Amylase

Amylase activity was assayed by growing the fungi on starch medium (starch, 2 g; peptone, 1 g; agar, 20 g; distilled water, 1 L). After 5-10 days, the plates were flooded with a 1% aqueous IKI solution. A yellow one around the colony in an otherwise blue medium was considered a positive test for starch hydrolysis (Gessner, 1980).

Proteolytic Enzyme

The medium used to detect proteolytic enzyme activity contained gelatin as the protein substrate (Hankin and Anagnostakis, 1975).

Pectolytic Enzyme

To detect pectolytic activity, we used the medium described by Liao and Wells (1987).

Cellulase

The basal media were supplemented with carboxymethyl cellulose, were proved to be best sources for the production of cellulase, according to Bland and Douglas (1977) and Datta *et al.* (1989).

Effect of Ground Twigs of *Rorripa palustris* and *Persicaria salicifolia* on Growth

Samples were dried separately at 50°C for 2 days, after which time they were finely milled. The mill (2g L^{-1}) was added to 2% MEA medium. These samples were thoroughly mixed and poured into Petri-dishes aseptically. Cultures were cut into 1 cm disks using a flamed cork borer and transferred to the petri-dishes that contained the twigs powders and the plates were incubated at 25°C for 10 days. Three plates were prepared for each organism and the increase in colony diameter was measured as usual.

RESULTS AND DISCUSSION

Results shown in Table 1 showed 10 aquatic pycnidial fungi, 2 Hyphomycetes and one Ascomycetes isolated from submerged plant substrates were selected for study: five species have been reported only from *Phragmites australis* (*Dinemasporium cytosporoides*, *Sordaria* sp. *Cylindrocarpon* sp. *Camarosporula* sp. and *Cheilaria agrostis*. Two species have been reported from *Cyperus articulatus* (*Cystotricha striola* and *Leptomelanconium australiense*. Four species have been recorded on *Cyperus papyrus* (*Cylidrocarpon* sp. *Clypeopycnis aeruginascens*, *Fusarium* sp. and

Table 1: Fungal strains and substrate isolated from which they were isolated

Species/substrate	1	2	3	4	5	6	7	8	9	10	11	12
Camarosporula sp.	-	-	+	-	-	-	-	-	-	-		4.5
Cheilaria agrostis	-	-	+	-	-	-	-	-	-	-		4.5
Cylindrocarpon sp.	-	+	+	-	-	-	+	-	+	+	+	50.8
Cystotricha striola	+	+	-	-	-	-	-	-	-	-		4.5
Clypeopycnis aeruginascers	-	-	-	+	+	+	+	+	+	-	+	59.9
Dinemasporium cytosporoides	-	-	+	-	-	-	-	-	-	-		4.5
Fusamen amenamentorum	-	-	-	+	-	-	-	-	-	-		4.5
Fusarium sp.	-	+	-	-	+	-	+	-	+	+	+	50.0
Leptomelanconium australiense	+	-	-	-	-	-	-	-	-	-		4.5
Libertella faginea	-	-	-	-	-	+	-	-	-	-		4.5
Sordaria sp.	-	-	+	-	-	-	-	-	-	-		4.5
Stilbospera pistaciae	-	+	-	-	-	-	-	-	-	-		4.5
Pleurothyrium sp.	-	-	-	-	-	+	-	-	+	-		13.6
Total	2	4	5	2	2	3	3	1	4	2	3	100.0

1 = *Cyperus articulatus*, 2 = *Cyperus alopecroides*, 3 = *Phragmitesaustralis*, 4 = *Persicaria salicifolia*, 5 = *Pseudognaphalium luteo-album*, 6 = *Persicaria lapathifolia*, 7 = *Rumex dentatus*, 8 = *Rorripa palustris*, 9 = *Cyperus papyrus*, 10 = *Eclipta prostrate*, 11 = *Persicaria senegalensis*, 12 = % = Frequency of occurrence of fungus, + = Fungus recorded, - = Fungus not recorded

Pleurothyrium sp.). *Clyeopycnis aeruginascens* was found on most plants and it was only found on *Rorripa palustris*, although other species were not faund on it. *Sordaria* sp. and *Cammarosporula* sp. were only found on *Phragmites australis*.

Koriniak and Belomesytseva (2005) were identified 45 fungus species occurring in confierous forests of the Minsk elevation 8 genera living on 26 plant species from 18 families. Two new fungal were recorded from aquatic weeds native to Barzil and were described *Passalora barretoana* stat-et comb-nov. and *Paraphaeosphaeria michotii*. The latter was described in association with its anamorph, which belong to the genus *Microsphaeropsis* (Soares and Barreto, 2006). Two new pycnidial members of the Atractiollapes: *Basidiopycnis hyallina* and *Proceropycnis pinicola* (Oberwinkler *et al*., 2006).

Among plant-inhabiting fungi, Kobayashi *et al*. (2005) were described four fungi that found among plant-inhabiting collected in June 2001 and in September 2002 on Hachijo Island, Tokgo They consist of two new species, namaly *Stagonospora hachijoensis* on *Miscanthus sinensis* var. Condensatus and *Ascochyta ixorae* on *Ixora Chinensis* and two fungi newly added to the Japanese mycoflora, namely *Discosiella cylindrospora* on *Callistemon speciosum* and *Robillarda sessilis* on *Parthenocissus tricuspidatus*. Van Ryckegen and Verbeken (2000) were discovered a new *Rosellinia* species on dead culms of *Phragmites australis*.

We have summarized the occurrence and frequency values for the pycnidial fungi and Hyphomycetes for Nile River at El Qanatir as colonizers of submerged of riparian plant roots (Table 1). The maximum occurance was on *Cyperus alopecroides*, *Phragmites australis* and *Cyperus papyrus*. *Clypeopycnis aeruginascens* showed maximum frequency of occurance (60%) followed by *Cylindrocarpon* sp. and *Fusarium* sp. (50%) *Pleurothyrium* sp. had 14% frequency of occurrence. The minimum frequency of occurrence was recorded for the remaining species (4.5%). *Rorripa palustris* (Brassicaceae) and *Persicaria salicifolia* were not colonized by pycnidial fungi and Hyphomycetes only one or two species were found after long incubation time at 25°C. This results might be due to the presence of some substance (allelopathic compound) inhibited the growth of these fungi. McCarthy and Hanson, (1998) recorded that the production of potentially allelopathic compounds in members of the Brassicaceae did not have to mean that allelopathy was involved in the success of members of this family invading woodlands as exotic weeds.

Anthony (1999) were recorded eleven species (Ascomycetes and Basidiomycetes) in total on *Phragmites australis* and the average of 2.4 species per roof did not increase with the age of thatch or degree of decay.

Generally, species from macrophytes substrates and woody riparian substrates had the fastest growth rates on PCA and most of the test substrates (Table 2). The least growth generally occurred on 2% MEA which added to it ground Rorripa twigs and 2% MEA which added to it ground Persicaria twigs media. Otherwise, all species grew to some degree on most test substrates.

Table 2: Colony diam (cm) after growth for seven days on the different test media

	Media					
Species	Pr	AM	PEC	CL	MR	MP
Camarosporula sp.	2.1	3.0	5.3	5.6	1.5	2.5
Cheilaria agrostis	2.0	2.0	3.2	2.2	1.1	2.3
Clypeopycnis aeruginascens	3.5	4.5	4.7	4.8	2.0	2.0
Cylindrocarpon sp.	4.1	4.6	4.5	3.2	2.0	2.5
Cystotricha striola	4.1	4.5	3.5	3.0	2.0	2.5
Dinemasporium cytosporoides	2.1	2.0	2.5	2.0	2.0	2.0
Fusamen amenamentorum	2.3	3.5	2.5	2.2	1.9	2.0
Fusarium sp.	3.0	2.5	3.5	3.0	1.7	2.0
Leptomelanconium australiense	3.0	2.1	2.6	2.0	1.7	2.1
Libertella faginea	2.0	1.8	1.6	2.0	1.7	2.0
Pleurothyrium sp.	3.7	3.6	2.5	3.2	2.0	2.0
Polystigmina sp.	3.6	3.8	4.2	4.2	2.0	2.8
Sordaria sp.	3.7	4.6	3.5	3.2	2.0	2.0
Stilbospera pistaciae	3.6	3.8	4.2	4.2	2.0	2.5

MR = Malt extract agar media + ground Rorripa palustris twigs, MP = Malt extract agar media + ground Persicaria salicifolia twigs, AM = Amylase; Pr = Proteolytic; PEC = Pectinase; CL = Cellulase

Table 3: Production of extracellular enzymes by Aquatic pycnidial and Hyphomycetes fungi

Species	AM	Pr	PEC	CL
Camarosporula sp.	-	-	+	+
Cheilaria agrostis	-	-	+	+
Clypeopycnis aeruginascens	-	++	++	++
Cylindrocarpon sp.	-	-	++	++
Cystotricha striola	++	+	++	++
Dinemasporium cytosporoides	-	-	+	+
Fusamen amenamentorum	-	+	++	++
Fusarium sp.	-	+	+	+
Leptomelanconium australiense	-	-	++	+
Libertella faginea	-	+	+	+
Pleurothyrium sp.	-	-	+	++
Polystigmina sp.	-	++	-	-
Sordaria sp.	-	+	++	+
Stilbospera pistaciae	-	-	+	+

++ = Strong reaction; + = Weak reaction; - = No reaction; AM = Amylase; Pr = Proteolytic enzyme; PEC = Pectinase; CL = Cellulase

Table 4: Production of extracellular enzymes by pycnidial and Hyphomycetes fungi; as measured by width of clearing zone (cm)

Species	AM	Pr	PEC	CL
Camarosporula sp.	-	-	-	-
Cheilaria agrostis	-	-	-	-
Clypeopycnis aeruginascens	-	2.0	2.5	3.0
Cylindrocarpon sp.	2	4.0	2.0	4.5
Cystotricha striola	3	2.0	2.0	2.0
Dinemasporium cytosporoides	-	-	-	-
Fusamen amenamentorum	-	0.9	2.0	1.6
Fusarium sp.	-	-	2.6	1.8
Leptomelanconium australiense	-	-	-	-
Libertella faginea	-	-	-	-
Pleurothyrium sp	-	-	-	-
Polystigmina sp.	-	-	-	-
Sordaria sp.	1	1.2	0.8	0.6
Stilbospera pistaciae	-	-	-	-

AM = Amylase; Pr = Protease; PEC = Pectinase; CL = Cellulase, - = No reaction

Most of species were positive for cellulase and pectinase (Table 3). *Cystotricha striola* was able to degrade all substrates tested. *Libertella faginea* and *Fusarium* sp. and *Sordaria* sp. *Fusamen amenamentorum* were postive for all enzymes except amylase. One species (*Cystotricha striola*) was positive for amylase. Six species were positive for proteolytic enzyme while 12 species were positive for pectinase and cellulase. Danninger *et al.* (1979) were investigated the ability of five aquatic Hyphomycetes to produce amylase, pectinase and cellulose. They found that all the tested strains were week productions of amylase and good producers of pectinase, whereas degradation of cellulose was only found with two strains. Boschker *et al.* (1995) suggested that enzymatic hydrolysis of polysaccharides in common reed litter (*Phargmites australis*) which contains cellulose and arabinose as its main polysaccharides, was the main source of glucose, xylose, arabinose and galactose accumulation which was probably caused by lyses of the microbial population in toluene-treated reed litter.

Cylindrocarpon sp., *Cystotricha striola*, *Sordaria* sp. were able to degrade to some degree, starch, protein, pectin and cellulose (Table 4). *Fusamen amenamentorum* and *Clypeopycnis aeruginascens* were only decomposed protein, pectin and cellulose, but Fusarium sp. degraded only pectin and cellulose. Although all species grow on test media but not all produce enzymes (Table 3 and 4). Positive growth but negative enzymes results could be due to the ability of the fungus to use other materials in the medium rather than the test substrate. These results were similar to those recorded for tropical freshwater fungi (Yuen *et al.*, 1998).

Pettersen (1984) found that, the most abundant polymer in wood, cellulose, may account for about 40-50% of dry weight of temperate woods. Native cellulose requires three hydrolytic enzymes acting synergistically for its complete degradation (Kirk and Cowling, 1984). All species in this study tested positive for cellulose. Rohrmann and Molitoris (1992) recorded cellulose activity on acid-swollen avicel for marine Ascomycetes and Raghukumar *et al.* (1994) recorded about 80% of the marine species they tested were positive for cellulotic enzymes.

Au *et al.* (1992) reported that tested aquatic hyphomycetes showed higher cellulolytic activity in the winter than summer leaf litter. On the other hand, Parado and Forchiassim (1999) found that temperature between 50-55°C was the optimal temperature for cellulose system in *Nectria catalinensis*.

This study demonstrates that aquatic pycnidial and Hyphomycetes which found on submerged substrates of macrophytes are able to produce many extracellular enzymes in the decomposition of these substrates and species specificities in types of substrates decomposed.

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