http://www.pjbs.org



ISSN 1028-8880

Pakistan Journal of Biological Sciences



Species Diversity and Seasonal *Occurrence* of Fungi on Seedlings of *Avicennia marina* (Forsk.) Vierh

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Abstract: Thirty nine different species of fungi were isolated from seedlings of black mangrove *Avicennia marina* (Forsk.) Vierh growing at Clifton area of Karachi and their habitat, eleven exclusively from seedlings, fifteen from habitat and thirteen common to both sources. *Aspergillus* was the most diverse genus with six species followed by *Penicillium* with four species. *Deuteromycotina* was also the most common and dominant group. The frequency of fungal colonization and species diversity (number of species) both were highest during the south west monsoon season (summer) and negligible during the north east monsoon season (winter), indicating preference for high values of temperature and precipitation by these organisms.

Key words: Species diversity, Avicennia marina, Fungi

Introduction

Mangrove community of Clifton area, Karachi, is composed of only one species *Avicennia marina* (Forsk.) Vierh, which grows in anaerobic muddy substrate. The species adapts to the anaerobic conditions by producing numerous aerial roots, the pneumatophores, which emerge above the mud from the subterranean cable roots. These peg like projections, the seedlings and all other wet parts of the tree provide ideal sites for fungal growth. The fungi along with other microbes play an important role in the energy turnover of the ecosystem (Hyde and Jones, 1988). In fact they are more effective as decomposers than bacteria, as they penetrate the tissue of the host. The fallen leaves, floral parts, fruits, twigs, seedlings, bark of the trunk, all provide favourite substrate for growth of fungi.

Although significant studies have been done on manglicolous fungi in different parts of the world, that in Pakistan is very scanty. Recently Mehdi and Saifullah (1992a,b) described the fungi associated with mature trees and their parts from Clifton and also neighbouring area of Korangi Creek. The present study focuses on those fungi that grow on seedlings and their habitats. The seedlings colonized on shores during the germinating season. It is also the first ever study on the monthly and seasonal variations in incidence of such fungi from Pakistan.

Materials and Methods

The sampling site chosen for the present study is located on rocky banks of water ways along 5 km long coast line of Clifton area. This site was colonized by mangrove seedlings during the winter-summer monsoon season of 1994 (Fig. 1). Regular monthly collections of water, mud and the mangrove seedlings (Approx. 16 cm in height) were made at random from the sampling station. Approx (marey 04 seeorithgs ailing wreei water and mud samples were collected each month.

Mangrove seedlings along with roots and 7-8 leaves were washed thoroughly in sterile sea water containing streptomycin and penicillin at the rate of 2000 Units/L. Each part of the seedling was separated off and placed on the sea water agar plates. The surface of the plates was flooded with sterile sea water. The inoculated cultures were incubated at 20°C over a period of 24 hours. The plates were examined microscopically, and any developing colonies were aseptically removed and inoculated on the fresh water agar plates. The initial leaf fungal population was determined by punching out 5 mm discs of leaf tissue with a sterilized cork borer. These discs were washed in five changes of 15 ml sterile sea water and then

placed aseptically on the surface of sea water agar plates at the rate of one disc per dish. The total number of discs taken was 25. The cultures were incubated at 20°C in the dark and examined microscopically at two to three days intervals over a period of four weeks. Any fungi appearing were sub-cultured on the plates of PDA, CDA and CMA to encourage growth and sporulation and thus allowing identification to be made.

Results and Discussion

In all 36 different species of micro-fungi were isolated from the water, mud and mangrove seedlings (Fig. 1,2, Table 1,2). They were all secondary marine fungi (Kohlmeyer, 1986) basically terrestrial forms. The species composition was similar to that found in other estuarine waters (Raghu-Kumar, 1988a,b). Like the Indian manglicolous fungi, Deuteromycotina sp. were also the most diverse group in the area (Venkatesan and Natarajan, 1985a,b). However, it was different in Hawaii where Kohlmeyer and Volkmann-Kohlmeyer (1989) found Ascomycetes to be the most dominant group. In the present study Ascomycotina were recorded most of the year while Zygomycotina were the most easily grown group in culture with the exception of Pilobolus sp. which is selective in isolation. In general the species composition of the area was limited in number as compared to other areas (Hyde and Jones, 1988) and this discrepancy may be due to limited range of methodology used and sampling in the present study, Mangrove fruits started arriving in the area from isolated mangrove stands in March and continued until May 1993. A large proportion of fruits perished due to human activity, animal consumption and decomposition. The remaining small fraction survived inside the narrow crevices and sheltered places which eventually emerged as small seedings.

The fungi colonizing the fruits were *Acremonium* sp., *Alternaria maritime, Aspergillus flavus, A. fumigatus, A.nidulans, Cladosporium herbarum, Chaetomium* sp., *Fusarium* spp., *Peniciftium* spp., *Pilobolus* sp., *Rhizopus* sp. and *Trichoderma* sp. Apart *Acremonium* sp. and *Pilobolus* sp., the rest of the mycoflora was broadly similar to the mycoflora of Clifton and Korangi Creek (Mehdi and Saifullah, 1992a). Highest number of fungi were isolated in July and August (Fig. 1) and the lowest number in November and December.

Out of twenty four species recorded earlier from mature trees of *A. marina* in Clifton and Korangi Creek areas (Mehdi and Saifullah, 1992b) seventeen were also isolated in the present study. In all, therefore, forty-five species have been isolated from mangroves of

| Fungi | Water | Mud |
|-------------------------|-------|-----|
| Achlya americana | + | + |
| Alternaria maritima | + | + |
| Aspergillus flavus | + | + |
| A. niger | + | + |
| A. terreus | + | + |
| A. sulphurus | + | - |
| Aureobasidium pullulans | + | + |
| Botrytis cinerea | + | + |
| Cladosporiurn herbarum | - | + |
| Curvularia sp. | - | + |
| Fusarium culmorum | + | + |
| F. solani | + | + |
| Penicillium sp. | - | + |
| P. chrysogenum | + | - |
| P. digitatum | + | - |
| Phoma sp. | - | + |
| <i>Pythium</i> sp. | - | + |
| P. proliferum | + | + |
| Rhizopus nigricans | + | + |
| Schizochytrium sp. | + | - |
| Sigmoidea marina | + | + |
| Saprolegnia o'iclina | + | - |
| S. ferax | + | - |
| Syncephalostrum sp. | - | + |
| Trichoderma sp. | + | + |
| Tricladium sp. | - | + |
| Trichocladium sp. | - | + |
| Varico sporina sp. | + | - |

Table 1: Microfungi isolated from water and mud samples of sea view (clifton) (+) indicates presence; (-) indicates absence



Fig. 1: Fungal texa recorded each month each month from magrove seedling.

| Table 2: Percentage frequency of fun al colonization in Man rove s | seedling (fruit, root, stem, leaf) During January-December, 1993 |
|--|--|
|--|--|

| Fungal species | | Janı | Jary | | | Febr | uary | | | Ma | rch | | April | | | | |
|-----------------------|-------|-------|-------|-------|-------|-------|------|-------|-------|-------|-----|-------|-------|---|---|---|--|
| | F | R | S | L | F | R | s | L | F | R | S | L | F | R | S | L | |
| Acremonium sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Alternaria maritima | - | - | - | - | - | - | - | - | 3 | - | - | 4 | - | - | - | - | |
| Aspergillus flavus | - | - | - | - | - | - | - | - | 4 | | - | 4 | - | - | - | - | |
| A. fumigatus | - | - | - | - | - | - | - | - | 8 | - | - | 4 | - | - | - | - | |
| A. nidulans | - | - | - | - | - | - | - | - | - | - | - | 4 | - | - | - | - | |
| Cladosporium herbarum | - | - | - | - | - | - | - | - | - | - | - | 6 | - | - | - | - | |
| Curvularia sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Chatomium sp. | - | - | - | - | - | - | - | - | - | - | - | 5 | - | - | - | - | |
| Fusarium sp. | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - | |
| Fusarium culmorum | - | - | - | - | - | - | - | - | 2 | - | - | 3 | - | - | - | - | |
| F. solani | - | - | - | - | - | - | - | - | 6 | - | - | 6 | - | - | - | - | |
| Penicfilium sp. | - | - | - | - | - | - | - | - | 10 | - | - | 12 | - | - | - | - | |
| P. chrysogenium | - | - | - | - | - | - | - | - | 10 | - | - | 10 | - | - | - | - | |
| P. brefeldianum | - | - | - | - | - | - | - | - | - | - | - | 4 | - | - | - | - | |
| <i>Phoma</i> sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Pyrhium sp. | - | - | - | - | - | - | - | - | 10 | - | - | - | - | - | - | - | |
| P. profiferum | - | - | - | - | - | - | - | - | 3 | - | - | - | - | - | - | - | |
| Pilobolus sp. | - | - | - | - | - | - | - | - | 6 | - | - | - | - | - | - | - | |
| Rhizopus sp. | - | - | - | - | - | - | - | - | - | - | - | 9 | - | - | - | - | |
| Syncephalastrum sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Trichoderma lignosum | - | - | - | - | - | - | - | - | - | - | - | 4 | - | - | - | - | |
| Trichocladium sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Tricladium sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Ascomycete-1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Total Species = 24 | - | - | - | - | - | - | - | - | 11 | - | - | 13 | - | - | - | - | |
| Isolated | | | | | | | | | | 11 | | | 13 | | | | |

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Table 2: (contd..)

| Fungal Taxa | | Мау | | June | | | | | | July | July | | | August | | | | |
|-----------------------|-------|-------|----|-------|-------|----|----|-------|-------|------|------|-------|-------|--------|----|----|--|--|
| | F | R | S | L | F | R | S | L | F | R | S | L | F | R | S | L | | |
| Acremonium sp. | - | - | - | - | - | - | - | - | - | 7 | - | - | - | 10 | - | - | | |
| Afternaria maritima | - | - | - | - | - | 6 | - | - | - | - | 10 | 10 | - | - | 10 | 12 | | |
| Aspergillus flavus | 4 | - | - | 6 | 5 | - | - | 10 | - | - | 15 | 10 | - | - | 20 | 10 | | |
| A. fumigatus | 5 | - | - | - | 10 | - | - | 15 | - | - | 15 | 20 | - | - | 10 | - | | |
| A. nidulans | - | - | - | 10 | - | - | 20 | 16 | - | - | 10 | - | - | - | 6 | 8 | | |
| Cladosporium herbarum | - | - | - | 8 | - | - | 10 | 15 | - | - | 15 | 20 | - | - | 15 | 25 | | |
| Curvularia sp. | - | - | 11 | 10 | - | - | - | 16 | - | - | 20 | 18 | - | - | 10 | 10 | | |
| Chatomium sp. | - | - | 4 | - | - | - | 4 | 6 | - | - | 2 | 3 | - | - | 2 | - | | |
| Fusarium sp. | 10 | 15 | - | 10 | - | - | 15 | 13 | 10 | 12 | 2 | 5 | 8 | 6 | 2 | 3 | | |
| Fusarium culmorum | - | - | 10 | 15 | - | - | 8 | 10 | 10 | 10 | 15 | 10 | 8 | 10 | 10 | 15 | | |
| Fsolani | - | - | 5 | - | - | - | 5 | - | 10 | 8 | 6 | - | 10 | 15 | 10 | 17 | | |
| Penicillium sp. | 15 | - | 10 | - | 6 | - | 5 | - | 5 | 6 | 3 | 8 | - | 5 | 10 | 10 | | |
| P.chrysogenium | - | - | - | 10 | - | - | - | 10 | 5 | 10 | - | 10 | 8 | 10 | - | 10 | | |
| P. brefeldianum | - | - | - | - | 6 | 5 | - | - | - | - | 5 | 4 | 3 | 2 | - | 2 | | |
| Phoma sp. | - | - | - | - | - | - | - | - | - | - | 6 | 10 | - | - | 4 | - | | |
| Pythium sp. | 10 | 10 | - | - | - | 15 | - | - | - | 10 | 10 | - | - | 15 | 10 | - | | |
| P. proliferum | - | 6 | - | - | - | - | 16 | - | - | - | - | - | - | - | - | - | | |
| Pilobolus sp. | - | 4 | - | - | - | 3 | - | - | - | 2 | - | - | - | 2 | 3 | - | | |
| Rhizopus sp. | 2 | 3 | - | - | - | - | 5 | 6 | - | - | 6 | 4 | 2 | 2 | - | 4 | | |
| Syncephalastrum sp. | 2 | - | 3 | 3 | - | 2 | - | - | - | 4 | 6 | - | - | 3 | 2 | - | | |
| Trichoderma lignosum | - | - | 10 | 5 | - | - | 8 | 10 | - | - | 6 | - | - | - | 6 | - | | |
| Trichocladium sp. | - | - | - | 5 | - | - | - | 6 | - | - | 8 | 10 | - | - | 6 | 9 | | |
| Tricladium sp. | - | - | - | 6 | - | - | - | 3 | - | - | 6 | 9 | - | - | 3 | 8 | | |
| Ascomycete-1 | - | - | - | - | - | - | - | - | - | - | 20 | - | - | - | 25 | - | | |
| Total Taxa = 24 | 7 | 5 | 7 | 11 | 4 | 5 | 10 | 13 | 5 | 8 | 20 | 15 | 6 | 10 | 19 | 14 | | |
| Isolated | | 19 | | | 21 | | | 23 | | | | 23 | | | | | | |

Table 2: (contd.)

| Fungal Taxa | September | | | | Octo | ber | | | Nov | vember | December | | | | | |
|---------------------------|-----------|----|----|-------|-------|-----|----|-------|-------|--------|----------|-------|-------|---|---|----|
| | F | R | S | L | F | R | S | L | F | R | S | L | F | R | S | |
| Acremonium sp. | - | | | - | - | 5 | - | - | 2 | - | - | 8 | - | - | - | - |
| Aternaria maritima | - | 10 | - | 5 | - | - | - | 10 | - | - | - | - | - | - | - | 8 |
| Aspergillus flavus | - | - | - | 10 | - | - | 20 | 18 | - | - | - | - | - | - | - | 20 |
| A. furnigatus | - | - | 10 | 20 | - | - | 30 | 40 | - | - | - | - | - | - | - | - |
| A. nidulans | - | - | 20 | 30 | - | - | 30 | 40 | - | - | - | 10 | - | - | - | 5 |
| Cladosporium herbarum | - | - | 30 | 40 | - | - | 40 | 60 | - | - | - | - | - | - | - | - |
| Curvularia sp. | - | | - | 10 | - | - | - | 30 | - | - | - | - | - | - | - | - |
| Chatornium sp. | - | - | - | 12 | - | - | 10 | - | - | - | - | - | - | - | - | 2 |
| Fusarium sp. | - | 10 | - | 15 | - | 15 | 20 | 10 | - | - | - | - | - | - | - | - |
| Fusarium culmorum | - | - | - | 20 | - | - | - | 15 | - | - | - | 8 | - | - | - | - |
| F. solani | - | 10 | - | 20 | - | - | 10 | 20 | - | - | - | 6 | - | - | - | - |
| Penicilliurn sp. | - | - | - | 10 | - | - | 6 | 10 | - | - | - | 6 | - | - | - | - |
| P. Chrysogenium | - | - | - | - | - | - | - | 16 | - | - | - | - | - | - | - | - |
| P. brefeldianum | - | - | 5 | 10 | - | - | - | 10 | - | - | - | - | - | - | - | - |
| Phams sp. | - | - | 4 | - | - | - | 8 | - | - | - | - | - | - | - | - | - |
| Pythium sp. | 10 | - | 10 | - | - | 5 | 10 | - | - | 4 | - | - | - | - | - | - |
| P. proliferum | | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Pllobolus sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Rhizopus</i> sp. | - | - | - | 10 | - | - | - | 15 | - | - | - | - | - | - | - | - |
| <i>Syncephalastrum</i> sp | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Trichoderma lignosum | - | - | - | 30 | - | - | - | 40 | - | - | - | - | - | - | - | - |
| Trichocladium sp. | - | - | 10 | - | - | - | - | 10 | - | - | - | - | - | - | - | - |
| <i>Tricladium</i> sp. | - | - | - | - | - | - | - | 10 | - | - | - | - | - | - | - | - |
| Ascornycete-1 | - | - | 20 | - | - | - | 40 | - | - | - | 20 | - | - | - | - | - |
| Total Taxa = 24 | 1 | 4 | 8 | 14 | - | 3 | 11 | 16 | | 1 | 1 | 5 | | | | |
| 5olated | | 19 | | | 20 | | | 7 | | | 5 | | | | | |

Mehdi and Saifullah:



Fig. 2: Number of texa colonizing different parts of mangrove seedings F = Fruit, R = Root L = Leaf

Karachi area so far. Twenty two species are new records from the area, that is, they were not found earlier by Mehdi and Saifullah, (1992a,b). This indicates that manglicolous fungi prefer seedlings over mature trees. It is also obvious from the results that non-living substrate (mud and water) is more favourable than living substrate (seedling) for their growth. Thus in all nine species were isolated from the seedlings but fourteen species from mud and water. Mehdi and Saifullah (1992a,b) also reported the same situation with mature trees habitat. *Aspergillus* was the most diverse genus with six species and next in rank was *Penicillium* sp. with four species. Overall, *Deuteromycotina* was the most dominant group with largest number of species.

The percentage frequency of occurrence of manglicolous fungi on parts of the seedlings during the course of one complete year is presented in Table 1 and Fig. 1-2. It is quite evident that maximum number of species were isolated during the south-west monsoon season (May-September) and almost none during the north-east monsoon season (December-February). This indicates that manglicolous fungi prefer high values of temperature and precipitation for their growth, which is characteristic of the former season.

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