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Research Article
A Study on distribution of fungi in Muthupettai mangrove along the East Coast of Tamil Nadu, India

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Abstract

Diversity of fungi in Muthupettai mangrove along the East coast in Tamil Nadu was studied in relation to mangrove vegetation in Koraiyar (S1), Korimunai (S2), Manakkattu (S3), Lagoon (S4) and Kadalmunai (S5) estuarine system during April to August (2003).Highest diversity (60 species) was recorded in samples collected from Manakkattu (S3) and least (51 species) at Koraiyar (S1). Among the 160 species isolated, 156 were facultative and remaining 4, obligate marine forms, *Varicosporina ramulosa, Halosphaeria maritima, Didymosphaeria maritima* and *Pleospora aquatica*. A total of 102 species were isolated from sediment samples, 95 from water, 64 from sea foam and 47 from natural substratum. A total of 137 fungal species were enumerated during pre-monsoon than 88 during summer. Baiting technique alone allowed recovery of 21 species. *Aspergillus* was the common genus represented with 28 species followed by *Curvularia* (13 species), *Alternaria* (12 species) and *Penicillium* (7 species).

Introduction

Mangroves are one of the richest and most productive habitats and litter from mangrove trees forms the base of food chain in tropical estuarine environments. They are open systems with respect to energy and matter and thus couple upland terrestrial and coastal estuarine eco-systems (Lugo and Snedakar, 2001). Furthermore, mangroves are storehouses of flora and fauna, which are dependent on products of microbial degradation at mangrove floor. The concentration of mangroves is particularly important in clean tropical waters where nutrient levels are usually low (Kathiresan and Bingham, 2001). These organic resources ultimately enrich the coastal eco-system and in turn the fisheries. However, mangrove eco-system provides hostile habitat for a number of organisms, including fungi. In spite of their ecological significance little is known about the microbial ecology of mangrove swamps. During the past several years, considerable work has however been done on the taxonomy and ecology of mangrove swamp fungi of India(Padhye *et al.*, 1967; Pawer *et al*., 1963& 1965; Rai and Tewari,1963) . Mangrove fungi are almost exclusively saprobes and belong to the family Ascomycetes, Deuteromycetes and Basidiomycetes. The majority of magnicolous marine fungi are omnivorus and occur mostly on dead cellulosic substrates around the tropics (Kohlmeyer and Kohlmeyer, 1979). The present study was undertaken to assess the distribution of fungi and their ecological behavior within the mangrove vegetation in Muthupettai mangrove along the South East coast of Tamil Nadu, India.

Materials and Methods

A total of five sampling stations were selected based on the richness of the mangrove vegetation. These were, Koraiyar (S1), Korimunai (S2), Manakkattu (S3), Lagoon (S4) and Kadalmunai (S5). In order to follow the sampling procedures for water, sediment, sea foams and natural substratum of mangrove plants were collected. All the samples were transported to the laboratory by keeping them in iceboxes and stored in the refrigerator condition for further analyses.

All the collected samples were plated on Potato Dextrose Agar, Corn Meal Agar, Rose Bengal Agar, Low Nutrient Growth Medium, and Sabouarud's Dextrose Agar with addition of mixture antibiotics (Booth, 1970). After incubation at room temperature (28°C), the fungal colonies were identified (Kohlmeyer and Kohlmeyer,1979; Subramanian,1971; Ellis,1971& 1976; Ellis and Ellis,1985; Gilamn,1957 & 1998) . Yeast flora was also isolated by employing Membrane Filtration technique using Yeast Extract Glucose Agar (YEGA).

The natural substrates such as leaf litter, wood, root samples were placed aseptically on the surface of agar media and incubated at 28-30° C for 4-5 days (Vrijmoed, 2000). The baits samples were regularly observed under aseptic conditions using Stereoscopic Dissection microscope under 2X and 4X magnification.

The water and sediment sample were collected separately and according to Venugopalan and Paulpandian (1989), Jayaraman (1996); Plummer (2003); Geetha Swaminathan and Mary George (2002) .

At the end of four months, the per cent frequency of occurrence of fungi was determined based on the number of fungi isolates in particular station and total fungal isolation in all the stations.

$$\text{Frequency of occurrenc (\%)} = \frac{\text{Number of fungi isolates in particular stations}}{\text{Total number of fungi in all the stations}} \times 100$$

The diversity of fungi in the mangrove samples of five sampling stations were assessed on the diversity indices,

$$\text{Simpson index } D' = \frac{1}{\sum (pi)^2}$$

and

$$\text{Shannon index, } H' = -\sum (p_i \ln p_i),$$

Where *pi* is the proportion of individuals that species *i* contributes to the total(Magurran,1988).

The Shannon Evenness, J′, was expressed by,

$$J' = \frac{H'}{H'_{max}}$$

Where *H′* mark is the maximum value of diversity for the number of species present (Pielou, 1975).

To compare the fungal species richness among the samples of each sampling stations with unequal number of samples and isolation, rarefaction indices were calculated (Ludwig, 1988).

The expected number of fungal species, *E(s)*, is a random samples of *n* isolation taken from a total population of *N* isolation was estimated using the formula,

$$E(s) = \sum_{n=i}^{s} \{-(({}^{N-ni})/ (N))\}$$

where *n′* is the number of isolation of the *i* th species.

Results and Discussion

Distribution of fungi in relation to physico-chemical status

The physico-chemical parameters of water and sediment in all stations, salinity and Nitrogen were 53.9 and 42.56 respectively. Altogether 88 fungi belonging to 4 Mastigomycotina, 7 Zygomycotina, 7 Ascomycotina and 70 Deuteromycotina were recovered in summer (Table.1).

The parameters of water and sediments in all five stations, salinity, N and K were 50.4, 42.4 and 380.5 respectively in pre-monsoon. A total of 137 fungi were recovered, of which 4 Mastigomycotina, 9 Zygomycotina, 12 Ascomycotina and 112 Deuteromycotina (Table 2). Total number of fungi per stations were 36.0(Range: 1-160). Altogether 160 fungi (including 13 unidentified) belonging to 60 genera comprising 117 Deuteromycotina, 18 Ascomycotina, 10 Zygomycotina and 5 Mastigomycotina (Figure.1).

Species diversity of fungi in the estuarine system

A total of 160 fungal species were isolated and enumerated. Among these, 51 species were represented in Koraiyar (S1), 55 in Korimunai (S2), 60 in Manakkattu (S3), 58 in Lagoon (S4) and 56 in Kadalmunai (S5). Thirteen of these species were common to all the five stations. Maximum fungal species diversity was observed in Manakkattu (S3) represented by 60 species and minimum of 51 at Koraiyar (S1) was recorded (Figure 1).

Maximum number of species diversity belonged to Hyphomycetes (37 genus; 115 species) followed by Zygomycetes (9 genus; 10 species and Hemiascomycetes (1 genus;5species). Among the Hyphomycetes, *Aspergillus* was the common genus represented by 28 species followed by

Table 1: Details of physico-chemical parameters of water and sediment in five stations of the East Coast of India.

Parameters	Mean ±S.D (n=5)
Water samples Temperature(° C)	34±1.4
pH	7.82±0.26
Dissolved oxygen(mg/l)	4.8±1.6
Biological oxygen demand(mg/l)	2.32±0.3
Chemical oxygen demand(mg/l)	0.06±0.03
Salinity (%)	53.9±3.4
Alkalinity(mg/l)	37.6±4.8
Dissolved carbohydrate(mg/l)	12.82±4.0
Particulate organic carbon(mg/l)	15.17±4.05
Total dissolved solids	2.93±0.46
Sediment samples pH	7.94±0.98
Salt (Nacl)	7.22±1.98
Nitrogen	42.56±16.03
Phosphorus	5.44±0.71
Potassium	414±142.77
Mastigomycotina	0.8±0.40
Zygomycotina	0.7±0.45
Ascomycotina	0.45±0.41
Deuteromycotina	0.54±0.5

Sampling period: summer, April and May, 2003.

Table 2: Details of physico-chemical parameters of water and sediment in five stations of the East Coast of India.

Parameters	Mean ±S.D (n=5)
Water samples Temperature(° C)	31.5±0.5
pH	7.7±0.15
Dissolved oxygen(mg/l)	6.4±1.96
Biological oxygen demand(mg/l)	2.5±0.5
Chemical oxygen demand(mg/l)	0.2±0.07
Salinity(%)	50.4±4.63
Alkalinity(mg/l)	32±2.53
Dissolved carbohydrate(mg/l)	13.78±3.8
Particulate organic carbon(mg/l)	11.99±2.22
Total dissolved solids	3.15±0.39
Sediment samples pH	8.5±0.06
Salt (Nacl)	8.68±2.67
Nitrogen	42.4±14.4
Phosphorus	7.56±0.56
Potassium	380.5±114.68
Mastigomycotina	0.8±0.4
Zygomycotina	0.9±0.3
Ascomycotina	0.70±0.45
Deuteromycotina	0.87±0.33

Sampling period: pre-monsoon, July and August, 2003.

Table 3: Frequency of occurrence of fungi on Muthupettai Mangrove in five sampling stations of East coast on India.

Name of the fungi	Mangrove locations	Frequency of occurrence (%)
Mastigomycotina		
Allomyces arbuscules Butler.	3	0.6
Achlya ambisexualis John Raper	1	0.6
Zygomycotina		
Absidia sp.Micheli	1	0.6
Mucor sp.Van Tiegham	2,3	1.2
Rhizopus oryzae Went & Gerlings	1-3,5	2.5
R.nigricans Ehernberg	1-5	3.1
Syncephalastrum recemosum Coln Exd Schroet	2	0.6
Ascomycotina		
Saccharomyces sp. Fell	1-5	3.1
Emericella nidulans Eidam ex Daturvuill	5	3.1
Halosphaeria martima (Linder) Kohlm	1	0.6
Didmosphaeria maritima Saccardo	1	0.6
Pleospora aquatica Griffths	3	0.6
Deuteromycotina		
Aspergillus clavatus Desmazieres	1-5	3.1
A.conicus Blochwitz	1,2	1.2
A.flavus Link	1-5	3.1
A.fumigatus Fresenius	1-5	3.1
A..humicola Chudhuri and Sachar	1	0.6
A.koningi Oudemans	1	0.6
A.luchensis Inui	1-5	3.1
A.oryzae Cohn	1,3-5	2.5
A.sacchari Thom & Church	3-5	2.5
A.terricola Marchal	1,2	1.2
Penicillium funiculosum Thom	2,3	1.2
P.janthinellum Biourge	2,3,4	1.8
P.rubrum Stoll	3,4	1.2
Trichoderma sp. (Persoon) Harz	3,5	1.2
Varicosporina ramulosa Meyers et Kohlm	3	0.6
Alternaria citri Ellis &Pierce	3-5	1.8
A.dennissii Ellis	5	0.6
A.solani Sorauer	3	0.6
A.sonchi Sorauer	1	0.6
A.tenuis Auct	1	0.6
Cladosporium britanicum Ellis	1,3	1.2
C.uredinicola Spey	3,5	1.2
Curvularia indica Subram	4	0.6
C.palmaram Subram	1,3,4	1.8
C.lunata (Walkar) Boedijn	1,2,4,5	2.5
Drechlera avenacea Shoemaker	3,5	1.2
Helminthosporium oryzae Shoemaker	5	0.6
Periconia laminella Manson & Ellis	3	0.6
Scoleobasidium gyrocarpii Ellis	1-4	2.5
Tetraploa aristata Berk&Br (Ellis)	2	0.6
Fusarium oxysporum Schlectennndhal	4,5	1.2
F.semitectum Berkeley&Revenel	2,4	1.2
Ascochyta vulgarius Kabat & Bubak	2-4	2.5
Mycelia sterilia (2)	2,3	1.2
Unidentified fungi (8)	1-5	3.1

Sampling stations - Koraiyar, 2- Korimunai, 3-Manakkattu,4-Lagoon and 5-Kadalmunai.

Table: 4. Species richness, diversity and evenness of fungi recovered from five stations of Muthupettai mangrove along the East coast of India.

Sampling Stations	Species richness		Diversity indices		
	Species Recovered	E(S) (160)*	Simpson (D′)	Shannon (H′)	Shannon Evenness (J′)
Koraiyar	51	46	0.974	3.782	0.962
Korimunai	55	50	0.972	3.803	0.949
Mannakkattu	60	55	0.982	4.225	0.951
Lagoon	58	53	0.970	3.794	0.942
Kadalmunai	56	51	0.971	3.802	0.948
Mean ± S.D	56 ±3.0351 ± 3.03		0.973 ± 0.004	3.881 ± 0.172	0.950±0.006

***** Expected number of species: E(s) (160) out of 160 random fungal isolations based on rarefaction indexes.

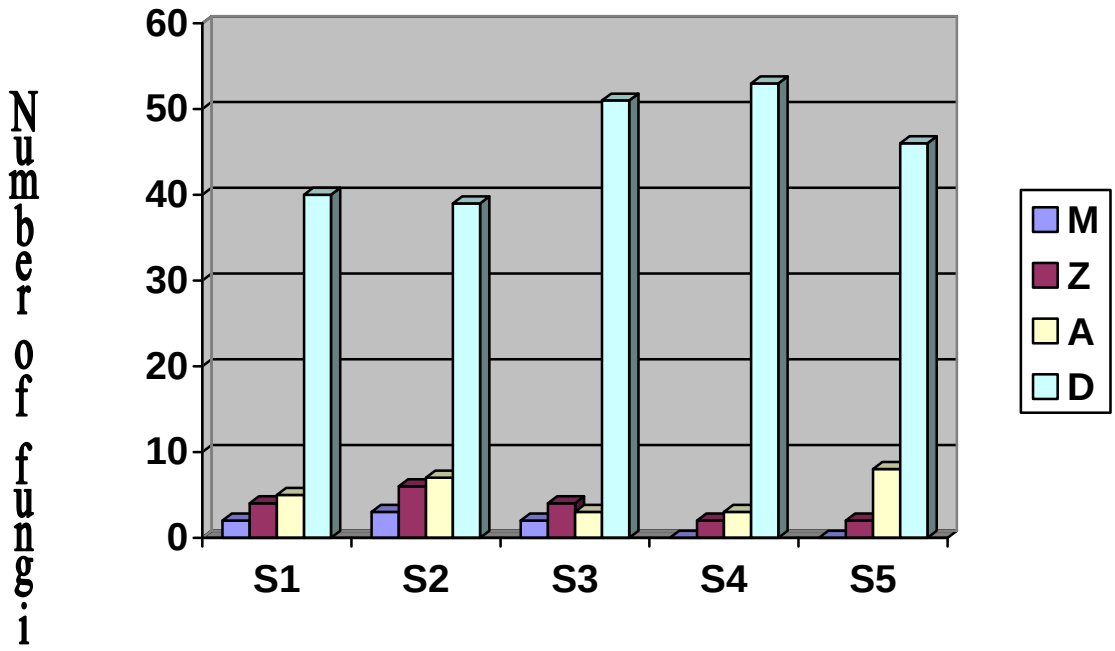

Figure: 1. Number of fungi in five stations along the east coast of India (Stations 1, Koraiyar; 2, Korimunai; 3, Manakkattu; 4, Lagoon and 5, Kadalmunai. M- Mastigomycotina, Z-Zygomycotina, A-Ascomycotina and D-Deuteromycotina

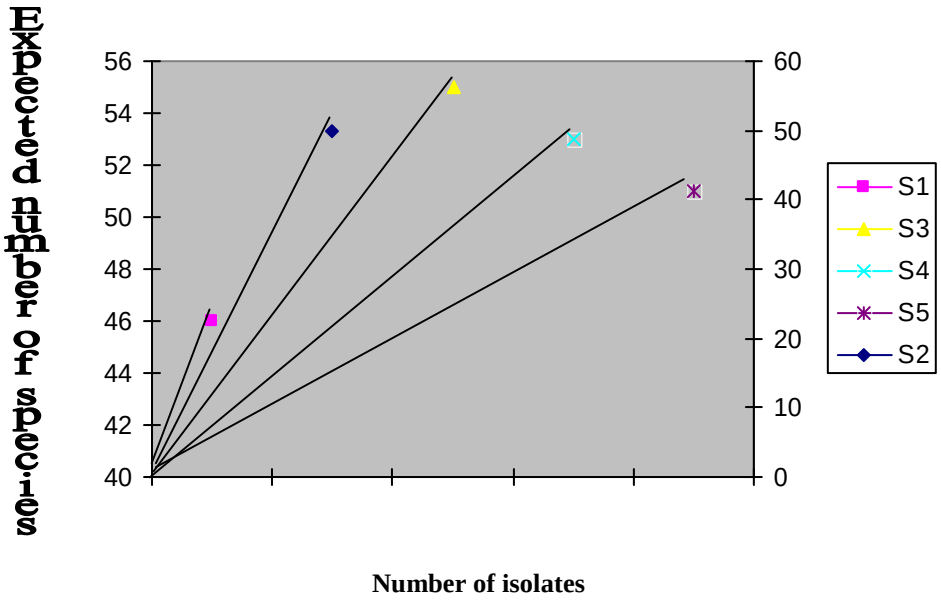

Figure 2: Rarefaction curves of expected number of species of fungi E(s) from random isolations. S1-Koraiyar,S2-Korimunai,S3-Manakkatu,S4-Lagoon and S5-Kadalnmunai.

Curvularia (13 sp), *Alternaria* (12 sp) and *Penicillium* (7 sp). In this study, 102 species of fungi were recovered from sediment samples whereas water samples yielded 95 species and natural substrates with 47 species This is to conformity with the findings of Garg(1983); Rai and Chowdhery (1978) ; Raper and Fennel (1965) and Rooth *et al*., (1964).

From the sea foams, a total of 64 fungal species were isolated. Of these, 48 species were recovered by direct and dilution plating technique whereas 29 species were observed directly in the centrifuged sedimental layer of the seafoam. Four obligate marine fungi represented by *Varicosporina ramulosa*, *Halosphaeria maritima*, *Didymosphaeria maritima* and *Pleospora aquatica* were recovered from seafoams while 156 species were facultative. Such obligate marine fungi of seafoams have been reported by Kohlmeyer (1966,1968 & 1969) ; Kohlmeyer and Kohlmeyer(1971) . Besides, 10 different types of yeasts were isolated in YEGM.

Seasonal variations of fungi

At the species level, 60 species were common to both summer and pre-monsoon seasons while observed the seasonal occurrence of fungi during the study period, maximum of 137 species were recovered in pre-monsoon and than 88 in summer. Fungal species diversity behaviour observed during the pre-monsoon is in conformity with the ecological studies of Dayal and Tandon (1962); Manoharachary (1974).

Frequency of occurrence of fungi

The number of species recovered was highest at Manakkattu (37.5%) and lowest in Koraiyar (31.8%). In the present study *R. nigricans*, *Emericella nidulans*, *Aspergillus luchensis*, *A. flavus*, *A. fumigatus*, *A. clavatus* was 3.1% of frequency of occurrence (Table 3). It is well accepted with previous findings by Sarma and Vittal (2001); Allem (1980), Leong et al., (1991) and Hyde (1990 &1991).

Species richness, diversity and evenness of fungi in five stations

Both Simpson and Shannon indices were highest at two mangrove stations i.e Manakkattu and Korimunai (0.982 and 0.974 respectively). The highest diversity coincided with the observed species richness (60) as well as expected number of species (46 to 55) in five stations. The Shannon evenness was least 0.942 at Lagoon while it was 0.962 at Koraiyar. Lagoon showed least Simpson indices (0.970) and Shannon indices at Koraiyar (3.782). So also observed least species richness 51 at Koraiyar (Table. 4). Species richness and diversity of fungi in all the five stations during two seasons is in conformity with the diversity studies of Maria and Sridhar (2002). A figure 2 shows the rarefaction curves for five sampling stations. It clearly shows that the sampling station Koraiyar consists of the least expected number of species than other stations (46 vs 50-55) out of 160 fungal isolations.

Distribution of fungi in relation to mangrove vegetation and their substrates

47 species of fungi were enumerated from natural substrates attempted with direct plating technique whereas, 21 species of fungi were isolated by baiting technique. The fungal diversity of prop roots, seedlings and wood of *R. apiculata* and wood and pneumatophores of *Avicennia* sp. were investigated by Sarma and Vittal (2001).

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