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Isolation, Identification and Characterisation of Mangrove Rhizosphere Soil Fungi.

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ABSTRACT

Marine fungi were isolated from mangrove rhizosphere soil sediment from Pichavaram mangrove forest. It is located near Chidambaram in Cuddalore District, Tamil Nadu, in South India., by the Bay of Bengal. It is the world's second largest mangrove and it is located between two prominent estuaries, the Vellar estuary in the north and Coleroon estuary in the south. The Vellar - Coleroon estuarine complex forms the Killai backwater and Pichavaram mangroves. In this, *Avicennia marina* and *Rhizophora mucronata* are the dominant mangrove rare species only limited to Pichavaram when compare to other wetlands. In the study of mangrove rhizosphere soil, 19 morphologically different fungi were isolated by serial dilution agar plating methods. The fungi were identified and characterized based upon their microscopic examination and cultural characteristics on media. Findings of this study evidenced that Pichavaram mangrove forest is the potential source for antagonistic fungal populations.

Keywords: Marine fungi, Pichavaram mangrove forest, *Avicennia marina* and *Rhizophora mucronata*

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INTRODUCTION

Mangroves are coastal wetland forests located at the intertidal zones of estuaries, backwaters, deltas, creeks, lagoons, marshes and mudflats of tropical and sub- tropical latitudes. In 112 countries nearly one fourth of the world's coastline is surrounded by mangroves and among marine fungi mangrove fungi are the second largest group (1). The mangrove environment has some special physiochemical character due to its salinity, tidal currents, winds, high temperature and muddy anaerobic soil probably with highly developed adaptation to extreme conditions none other than other plant except mangrove plant (2)

Recently evolved marine mycology as a specialized branch of science comprising 1500 species, Recently there are 444 higher marine fungi have been reported and are grow on diverse substrate including wood, sediments, seaweeds, dead corals, calcareous tubes of mollusks and intertidal grasses it is mangrove substrata viz. decaying leaves, seedlings, prop roots and pneumatophores (3). There are 70,000 fungi in marine environment is described worldwide it is estimated as high as 1.5 million species. Marine fungi comprise saprobic forms present in the open ocean waters (pelagic) and in bottom (benthic) zones. Study revealed that marine fungi are responsible to forms various types of submerged materials in waters and sediments nearest to land, the neritic and littoral zones (4). Fungi are play a vital role in ecological process of mangroves saprophytic fungus in the mangrove environment are well studied whereas the diversity, mutualistic and pathogenic fungi in the mangrove environment are poorly studied (5).

MATERIALS AND METHODS

Sample collection:

Soil samples were collected from Pichavaram mangrove forest during three seasons namely Summer - April to June, Pre monsoon - July to September and Monsoon - October to December.

Isolation of fungi from Rhizosphere Soil –Serial Dilution Agar Plating Method:

In serial dilution agar plate method, a known amount of material is suspended in a known volume of sterile sea water blank to make a microbial suspension. Serial dilutions 10^{-2}, 10^{-3} to 10^{-7} are made by pipetting measured volumes (usually 1ml or 10 ml) into additional dilution blanks. Finally 1ml aliquot of various dilutions are added to sterile Petri dishes (triplicate for each dilutions) to which are added 15ml of the sterile, PDA medium, supplemented with chloromphenicol,10µg/ml. The dilutions of 10^{-2} to 10^{-5} are selected for enumeration of fungi. Upon solidification, the plates are incubated in an inverted position for 3 -7 days at 25°C. Fungal outgrowths from the soil sample were sub-cultured on fresh antibiotic- free medium for identification based on morphological examination and conidial characters (6).Colonization Frequency (CF) was calculated as described by (7).

Analyzing of relative frequency

$$\text{Colonization frequency of endophyte} = \frac{\text{Number of segments colonization}}{\text{Total number of fungal segment analyzed}} \times 100$$

Rhizosphere soil Fungus

Rhizosphere soil samples were taken at 20cm of depth from the root environment of each mangrove species, and placed in sterilized glass jars and kept at 4◦C for further microbial or physio chemical analysis (8). Soil sampling consisted on a randomized collection of two samples of rhizosphere soil from each mangrove species in a month from *Avicennia marina* and *Rhizophora mucronata*. Soil samples were used for either analyzing soil texture or chemical properties for fungal populations.

Identification by Culture technique:

The fungal isolates were inoculated into fresh Potato Dextrose Agar medium and incubated at 25ºC for 3-7 days after which the fungal growth were identified by colony morphology.

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Identification by Lacto phenol Cotton Blue Mounting:

A loopful culture was picked up with the help of a sterile inoculation loop and semi-permanent slides were prepared using lacto phenol cotton blue. The slides were gently heated in a spirit lamp so as to release the air bubbles, if any present inside the cover glass. The excess stain was removed by using tissue paper and the cover glass was sealed with white nail polish. (9)

RESULTS AND DISCUSSION

Isolation of fungi from Mangrove Rhizosphere soil sediment:

In the present study, 36 soil samples used for the enumeration of fungi, 19 common species of rhizosphere soil fungus was isolated and identified in both mangrove plant as shown in the Table-1.The fungi were identified using microscopic examination with lactophenol cotton blue staining (Table-2) and characterized using culture technique in Potato Dextrose agar. The cultural characteristics are shown in (Table-3)

Table -1: **Fungi isolated from mangrove rhizosphere soil sediment**

		Mangrove plant	
S.NO	Name of the fungus	*Avicenia marina*	*Rhizophora Mucronata*
1	*Aspergillus flavus*	Present	Present
2	*Aspergillus niger*	Present	Present
3	*Aspergillus nidulans*	Present	Absent
4	*Aspergillus verisicolor*	Present	Present
5	*Cladosporium sps*	Present	Present
6	*Curvularia sps*	Present	Present
7	*Fusarium oxysporum*	Present	Present
8	*Fusarium sps*	Present	Present
9	*Trichoderma harzianum*	Present	Present
10	*Trichoderma inhamatum*	Present	Present
11	*Trichoderma viridae*	Present	Present
12	*Trichophtonepidermophyte*	Present	Absent
13	*Penicillium sps*	Present	Present
14	*Phoma sps*	Absent	Present
15	*Rhizophus oryzae*	Present	Present
16	*Mucor sps*	Present	Present
17	*Nigrospora sps*	Absent	Present
18	*Unidentified sps*	Absent	Present
19	*Unidentified sps*	Absent	Present

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Table -2: Identification of fungal isolates by microscopic examination

S.No	Fungus	Microscopic appearance
1	*Aspergillus flavus*	It has septate hyphae with long conidiophores and have a rough texture or even spiny, it just below the vesicle it are spherical to elongate. Metulae cover three quarters to the entire surface of the vesicle from which the phialides form. Conidia are globose to ellipsoidal with smooth to finely roughened walls.
2	*Aspergillus niger*	Has septate hyphae with long conidiophores.
3	*Aspergillus nidulans*	It has septate hyphae with a woolly colony texture and white mycelia.
4	*Aspergillus verisicolor*	It has septate hyphae that appear glassy and transparent. Conidiphores terminate in small vesicles that are biseriate. The first layers of cells are called metulae upon which phialides are borne. The vesicles are variable in shape and are often described as spoon-shaped.
5	*Cladosporium sps*	condiophores usually present, erect, brown, sympodial, with 1-4 celled blackish- brown conidial scars at each end, pale to medium or dark brown,smooth walled to verrucose,arising in branched chains which readily disarticulate, lower conidia are often septate ramoconidia.
6	*Curvalaria sps*	Condiophores erect, brown, multicellular, producing conidia in sympodial order, conidial scars dark, flat. Conidia ellipsoidal its often curved with 3-4 true septa.
7	*Fusarium sp*	Condiogenous cells formed on aerial hyphae or in short. Conidia of three types macroconidia falcate with several transverse septate, microconidia ellipsoidal,ovoidal with rounded base and blastoconidia produced singly on polyblastic cells with septation. Chlamydospores often present in thick walled hyaline or pale, intercalary or terminal.
10	*Trichoderma sps*	Repeatedly branched conidiophores, irregularly verticillate, bearing clusters of divergent, often irregularly bent, flask - shaped phialides.
11	*Penicillium sps*	It consisting of networks of hyphae and ropes of hyphae,. Conidiophores arising from creeping hyphae or ropes of hyphae, with all smooth penicillus a single one-sided verticil of metulae with occasionally one branch from a lower node.
12	*Phoma sps*	The fungus produced semi immersed pycnidia in medium which were globose, thinwalled and brown in colour. Sometimes the pycnidia were erumpent and unilocular. Pycnidia were separate or aggregated and occasionally confluent on medium.

Table – 3: Identification of fungal isolates by culture

S.No	Fungus	Macroscopic appearance
1	*Aspergillus flavus*	Very rapid rate of growth, maturing in about three days. Surface is greenish-yellow to olive and may have a white border
2	*Aspergillus niger*	Rapidly growing on Saboraud-Dextrose Agar starting with a white to yellowish felt-like mat of mycelia, quickly turning black as conidia develop the pigment aspergillin during maturation. Reverse remains white to pale in colour
3	*Aspergillus nidulans*	The green color of wild-type colonies in Saboraud-Dextrose Agar
4	*Aspergillus verisicolor*	Colonies are yellow, orange and often green in colour
5	*Cladosporium sps*	Colonies spreading, powdery to wooly, grayish-green to lilaceous green.

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6	*Curvalaria sps*	Colonies are black, hairy, expanding.
7	*Fusarium sp*	Colonies are expanding, often colored in pink, yellow, red or purple shades.
10	*Trichoderma sps*	Colonies are white to greenish in colour
11	*Penicillium sps*	Colonies on wort gelatin bluish-green, gray-green, or bright green
12	*Phoma sps*	Phoma sps produced brown coloured colonies with regular margin on PDA medium. Reverse side of the colonies were brown.
13	*Rhizopus oryzae*	Colonies are grayish black, powdery in appearance
14	*Mucor sps*	Colonies are typically coloured white to beige or grey and are fast-growing. Colonies on culture medium may grow to several centimetres in height. Older colonies become grey to brown in colour
15	*Nigrospora sps*	Colonies expanding rapidly, cottony at first, later brown to black.

Colonization frequency of rhizosphere fungi from Avicenia marina

Of the total of 19 common fungal isolates, 15 fungal species were isolated and identified from A.marina. The colonization frequencies of the *A.marina* shown that the dominant rhizosphere fungus *Aspergillus* sps (27%) followed by *Trichoderma* sps (20%) and *Penicillim* sps (13%) almost all the other fungus present only in (7%) which was shown in FIG1

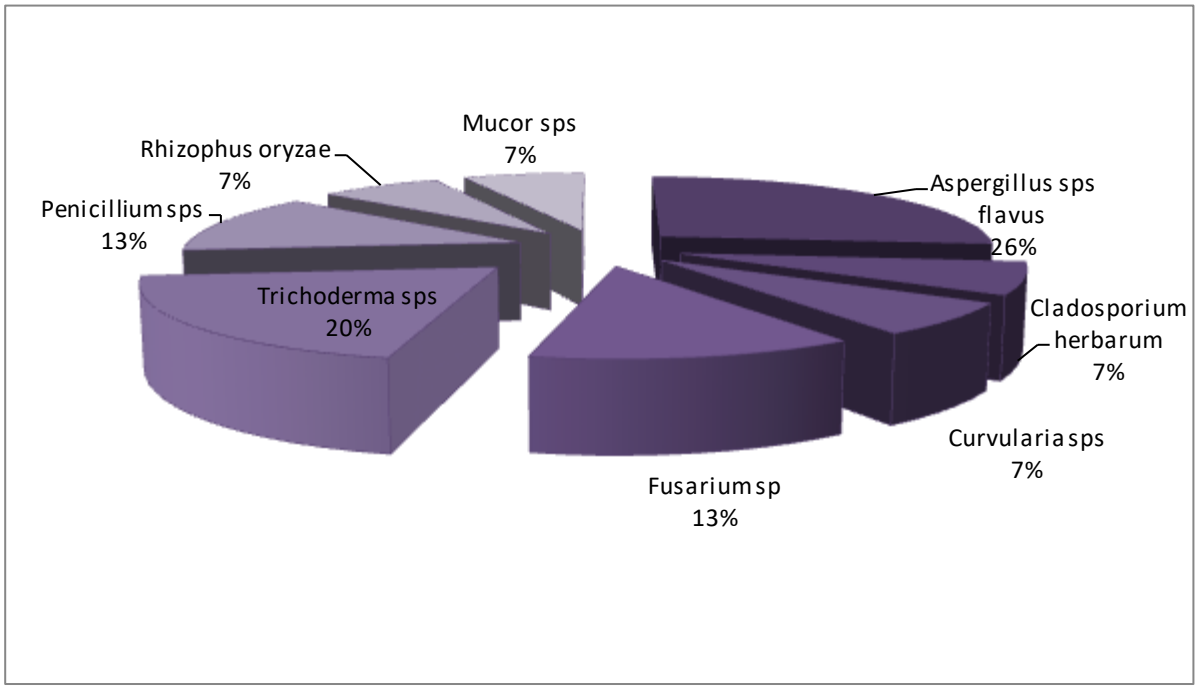

Figure 1: Colonization frequency of rhizosphere fungi from Avicenia marina

Colonization frequency of rhizosphere fungi from Rhizophora mucronata

Of the total 19 common fungal isolates, 17 fungal species were isolated and identified from *Rhizophora mucronata*.The colonization frequencies of the *Rhizophora mucronata* shown that the dominant

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rhizosphere fungus was *Trichoderma* sps (18%) and *Aspergillus* sps (18%) and followed by *Fusarium* sps (12%) almost all the other fungus present only in (6%) which was shown in FIG 2

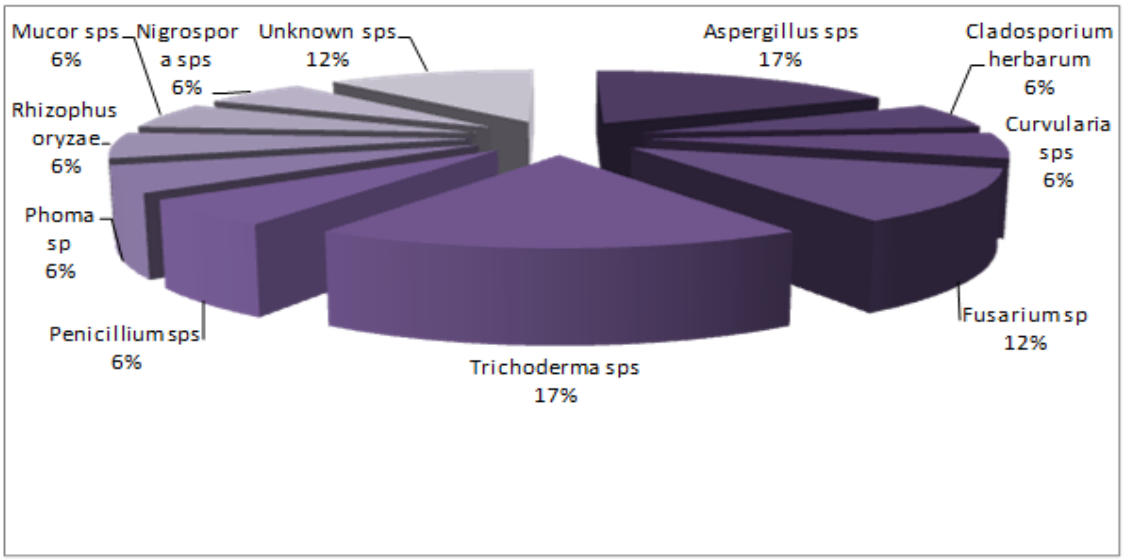

Figure 2: Colonization frequency of rhizosphere fungi from Rhizophora mucronata

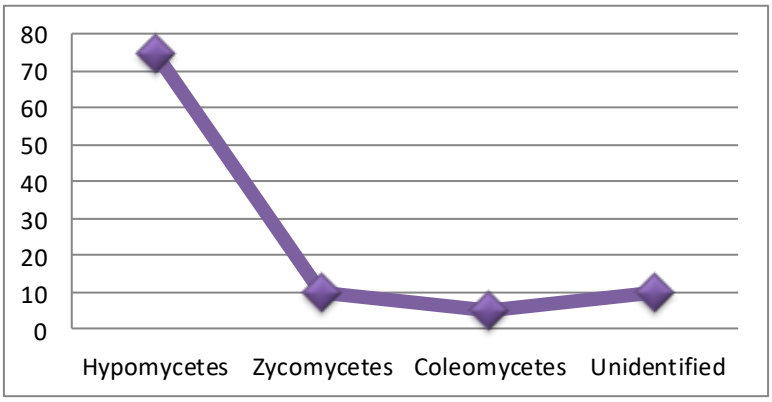

Fig.3.Colonization frequency of mangrove rhizosphere fungi

Among the fungal isolates, 14 species belongs to family Hypomycetes (75%), 2 species belongs to family zycomycetes (10%), 2 species belongs to Unidentified species (10%) and 1 species belongs to Coleomycetes (5%).Shown in FIG.3.Most of the species were isolated in monoculture.

Based On this study, the nutritional features and persistent nature of woody litter of *Avicennia* and *Rhizophora* in mangrove habitats might be responsible for yielding rich mycoflora (10).

Avicennia marina rhizosphere soil maximum number of fungi isolated from summer season, minimum number of fungi isolated from monsoon season. *Aspergillus sp* and *Penicillium sp* were most frequently isolated (11).In this study, the maximum number of fungus was isolated in summer season.

The present study assessed the rhizosphere soil fungus from *Avicenia marina* and *Rhizophora mucronata* as influenced by physio-chemical parameters in the mangrove ecosystem and there are very high fungal abundance, particularly pronounced for the rhizosphere samples. At the phylum level fungal communities were clearly dominated by Ascomycota (76%–85%) (12) In our studies most of the fungal communities were belongs to Ascomycota maximum

In this study, the colonization frequencies of the *Rhizophora mucronata* shown that the dominant rhizosphere fungus was *Trichoderma* sps (18%) and *A.marina* the colonization frequencies shown that the second dominant rhizosphere fungus was *Trichoderma* sps (20%).Trichoderma are predominant rhizosphere soil fungi of this genus frequently live in association with plant roots hence effective biocontrol agents to confer increased growth and systemic resistance to plants (13).

Covering a quarter of the world's tropical coastlines and being one of the most threatened ecosystems, mangroves are among the major sources of terrestrial organic matter to oceans and harbor a wide microbial diversity. In order to protect, restore, and better understand these ecosystems lack of knowledge is even more pronounced for specific fungal populations, such as the ones associated with the rhizosphere soil of the mangrove remain poorly characterized, and understanding of their fungal communities (14)

CONCLUSION

To conclude, the study revealed the presence of rich fungal communities in the rhizosphere soil of mangroves of *Avicennia marina* and *Rhizophora mucronata.* As these different fungi are unique in their characteristics, they would be potential sources of novel compounds which need to be explored.

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