Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2012, 2 (4):452-459

ISSN : 2249-7412
CODEN (USA): AJPSKY

Isolation, identification of fungi from *Avicinnia marina* Muthupet mangroves Thiruvarur Dt.

[*]S. Madavasamy and [**]A.Panneerselvam

[*]Prist University, Vallam, Thanjavur (Dt), Tamilnadu, India
***A.V.V.M. Sri Pushpam College, Poondi, Thanjavur (Dt), Tamilnadu, India*

ABSTRACT

In the present study the leaf surface mycoflora of green, senescent and brown leaves of Avicinnia marina was studied by plating of leaf surface washings and washed leaf bits. Twenty two species of fungi were isolated during the course of four samplings. They include Absidia glauca, Acrphialophora fusispora, Alternaria alternate, Aspergillus candidus, A. flavus, A. luchueusis, A.niger, A.sydowi, A.fumigatus, A. sulphureus, Cladosporium, Cunninghamella sp., Curvularia sp., *Gliocladium* sp., *Fusarium* sp., *Melanospora sp., Myrothecium* sp., *Nigrospora sphaerica, Pestalotia, Penicillium* sp., *Rhizopus* sp., *Trichoderma viride. The population of the fungi was comparatively more on the surface of the senescent leaves than the green and brown fallen leaves. The Cladosporium sp. was more during the months of December and January, and Aspergillus sp., during the months of March. The secretion of cellulase by Curvalaria sp., Gliocladium sp., Penicillium sp., and Trichoderma sp., was studied in vitro by viscometric method. It was more in Trichoderma than other species of fungi tested. Thus the present study will give a clue to know about the degradation of litter and alive woody species of mangrove ecosystem. This study also helps to know about the cellulolytic activity of some of the phylloplane Mycoflora which indirectly enrich the nutrient status of the mangrove soils and maintenance of equilibrium status. This study will pave the way for further projects in various angle.*

Key words: Mycoflora, fungal strains and *Avicinnia marina*

INTRODUCTION

Mangroves are the unique forests, representing intermediate vegetation between Land and Sea that grow in oxygen deficient water loged soils. To survive in such harsh conditions mangrove have evolved a number of physiological and structural adaptations like Vivipary, Pneumatophores, Prop roots, Salt secretion, Ultra filtration etc. All mangrove species have mechanism to provide air to their root system from the atmosphere. Hence they can tolerate anaerobic conditions to some extent.

Mangroves perform more vital for sustenance of both man and animal. However human dependency on mangrove resources has claimed heavily on its area and function (Krishnamurthy, 1984). As mangroves occur mostly in tropical regions where majority of the world population reside they have been gradually cleared through the years to meet the needs of the burgeoning population of late conversion in to shrimp farms aquaculture is emerging as a major threat to the future mangroves also. The mangrove ecosystem is at serious threats owing to anthropogenic pressure (Mohamed, 1996).

Fungi are one of the important microbial components of the soil. Since 1860's, research have been carried out on the fungi of different soil types, such as soils of forest,[4,2,12] driftwood, grasslands (Ray and Dwivedi, 1962) polar region, desert, marine and mangrove habitats and coastal sand belt 24 from various parts of the world. All these studies revealed that the fungi might reside permanently, temporarily for a period in the soil. Their number and species composition in the soil habitat differs from place to place depending upon the physical, chemical and biological factors of the particular habitat (Ainsworth *et al.*, 1973).

Mangroves are widespread in tropical and sub tropical regions, growing in the saline intertidal zones of sheltered coast lines. Pakistan has a coastline of about 1,000 km. The Indus River delta extends over 250 km from Sir Creek at the Indian Border and Karachi in the west with about 250,000 ha of mangroves (Khan, 1966; Mirza *et al*., 1983). Fungi make a very important part of the ecosystem along with other microbes of the biomass (Hyde, 1990, 1992; Harrison *et al.*, 1994; but unfortunately they have revealed very little information. Fungi growing on mangroves from different parts of the world have been reported. Mehdi and Saifullah, (2000) reported the species diversity and seasonal occurrence of fungi on seedlings of *A. marina.*

MATERIALS AND METHODS

Sample Collection

Mangrove leaves (*Avicinnia marina*) sample collected from the Muthupettai, Thiruvarur District, Tamilnadu.

Sterilization of Plant Materials

The disease free and fresh leaves were selected for this investigation. About 20gms of fresh and healthy leaves were taken for each solvent extract including aqueous. Then surface sterilized with 0.1% mercuric chloride or alcohol for few seconds. Again the plant materials were washed thoroughly with distilled water (Three times).

Processing of plant leaves

20 leaves were taken and plated on potato dextrose agar plate and plate was incubated for 36 hours. After incubation, the colonies were grown. The isolated fungi were sub cultured in a potato dextrose agar medium. The pure cultures were used for further studies.

Preparation of PDA Medium

The potato tubers were peeled and weighed for about 200g. The tubers were chopped into small piece with the help of sterile knife. The chopped potatoes were transferred into a conical flask containing about 100ml of distilled water. The content was boiled for 20 minutes. The supernatant was decanted and filtered by muslin cloth and the filtrate was collected. Dextrose and Agar were transferred into the extract and shaken to dissolve the ingredients. The medium was made up to 1 litre using distilled water. The pH of the medium was checked and checked and adjusted to 5.6. Finally, the medium was cotton plugged and autoclaved at 121^{o}C for 15 minutes of 15 lbs.

Identification of Fungi (Mounting Process Using Lactophenol Cotton Blue Stain)

Lacto phenol cotton blue is a strain commonly used for making semi permanent microscopic preparations of fungi. It strains the fungal cytoplasm and provides a blue background against with the walls of hyphae can readily been seen.

Reagents

Lactic acid	-	200 ml
Phenol crystal	-	20g
Glycerol	-	40ml
Distilled water	-	20ml
Cotton blue (1% aqueous)	-	2ml

Lactic acid and glycerol were added to the distilled water and mixed thoroughly. Phenol crystals were added and heated gently in hot water with frequent agitation until the crystals completely dissolved. Then the dye was added and mixed thoroughly.

Procedure
Place a drop of Lacto phenol cotton blue on a clean slide. Transfer a small tuft of the fungus with the sterilized inoculation needle with spores and spore bearing structures. in to the drop. Gentle tease the sample using the two mounted needles. Mix gently the strain with the mold structure place a cover glass over the preparation and taking care to avoid trapping air bubbler in the stain. The slides were observed under bright field microscope with oil immersion objective.

Identification and Photo Micrography
Morphological features of fungi were photographed using Nikon microscope. All the fungi were identified with referred the standard manual of Gillmans, 1957.

Studies on the cellulolytic activity of some phylloplane fungi
The phyloplane fungi namely *Fusarium*, *Trichoderma*, *Penicillium Gliocladium* and *Curvularia* were inoculated individually in conical flasks containing 100 ml czapeks medium with Carboxy methyl cellulose as carbon source. The flasks were incubated for 7 to 10 days. Then the filtrate was taken and filtered through Whatman No.1 filter paper. Then centrifuged at 200g for 30 minutes and supernatant was diarized in cellophane tubing against distilled water at 40ºC for 24 hour. The water was changed at 8 hour interval.

The enzyme was assayed by physical methods based upon loss in viscosity of the substrate. To measure the loss in viscosity the viscometer was used. The loss in the viscosity was calculated by using following formula.

$$V = \frac{To - T}{To - TH_2O} \times 100$$

Where
V = Percent loss in viscosity
To = flow time in seconds at zero time
T = flow time of the reaction mixture at time T. and
TH_2O = flow time of distilled water

RESULTS

Characters of Fungi
***Absidia* sp.**
Mycelium formed as in the genus Rhizopus byu frequently branched stolons; more or less incurved into arches and producing at the point of contact with the substratum more or less richly branched rhizoids. Sporangiophores straight, rarely single, more often in groups of two to five, occurring at the curve of the stolon (internodal) and not at the point of origin of the rhizoid (nodes). A cross-wall is placed at a definite distance below the sporangium. Spores small, 5-6µ, round or oval (not angular) with a smooth wall, rarely echinulate, colorless or bluish-black. Zygospores formed on the stolons. They are surrounded by circinate filaments, cutinized, which are borne in a whorl from one or both of the suspensors.

Alternaria alternata
Sterile hyphae creeping, septate, conidiophores single or in groups erect, septate, mostly unbranced, short, conidia inverted club shaped mostly elongate at the tip muriform in the lower portion dark colored lighter at the points – usually simple chains.

Aspergillus candidus
Surface growth usually stalks and heads with scanty sterile mycelium or anastomosing ropes of hypae bearing short fertile stalks. Conidiophores very with the strain. Walls thick, smooth Heads white globose radiate. Conidia colorless, globose, smooth sclerotic occasionally produced.

Aspergillus flavus
Colonies widely spreading conidial areas ranging in color from sea-foam yellow through chartreuse-yellow. Heads in every colony vary from small with a few chains of conidia to large columnar masses or both mixed in the same area, small heads with small doom like vesicles conidia pyriform to almost globose colorless to yellow green.

A. fumigatus
Colonies green to dark green conidiophores short, usually densely crowded submerged hyphae or as branches from aerial hyphae septate or non septate, gradually enlarged conidia dark green in mass, globose.

A. luchuensis
Colonies black-brown single series of phialides 7-9 to 5 with conidia 4 – 45/u and finely roughned. Conidiophores smooth; vesicles 30-40/u in diameter, showing pores (or) marking where phialides face off.

A.niger
Colonies blackish brown abundant submerged mycelium aerial hyphae usually scantily produced; conidiophores mostly arise directly from the substratum smooth. Septate or nonseptate varying greatly in length and diameter. Vesicles globose phialides typically in two series, thickly covering the vesicle.

A. sulphureus
Colonies sulphur yellow in colour conidiophore arise from aerial hyphae, stalk walls smooth. Heads loose columns of conidial chains rarely radiate phialides in two series; conidia globose, thick walled.

A. sydowi
Colonies blue green velvety with some aerial interlacing and trailing hyphae. Conidiophores mostly arise from submerged hyphae colorless, smooth thick walled heads radiate or globose, phialides radiate in two series.

A. terreus
Colonies brown shades in age conidiophores more or less flexuous with walls smooth septate or non septate with apex enlarged to form a vesicle; phialides usually in two series upon its dome-like upperface.

***Cladosporium* sp.**
Hyphae creeping, septate on the surface, conidiophores almost erect branced, and floccose, often forming a turf, olive coloured conidia globose and ovate usually greenish terminal and then passed to the side.

Curvularia lunata
Colony spreading sub floccose, dark olive gray, reverse bluish black hyphae septate and much branched olive conidiophores erect unbranced, three septate curved brown.

***Cunninghamella* sp.**
Mycellium white floccose,slightly thickened continuous when young later becoming septate. Septa disposed here and there without order conidiophores straight branched. The main axis as well as side brabceus. Little or non septate conidia spherical or oval. The external membrane spiny with needle chlamydospores globose intercalary in the mycelium.

***Fusarium* sp.**
Conidial layer cushion shaped or some what extended without a spindle or sickle shaped, many celled with indistinct cross walls stroma brownish, white to violet sclerotial hard bodies. Medium, high aerial mycelium but are lacking in the typical fruiting layers of the macroconidia. Chlamydospores terminal and intercalary globose.

***Gliocladium* sp.**
Conidiophores erect simple or branched septate producing at the apex a fructification composed of successive vertical of primary branches conidia in chains.

***Humicola* sp.**
Sterile hyphae creeping, branched, septate, usually plain. Conidiophores erect, straight, septate, unbranched, rather long, brown. Conidia single, apical., globose or subglobose, brown, one-celled.

Sporangia less than 100µ in diameter, Young turf white to golden brown; No growth at 37°C. or above sporangia very variable, columellae usually globose, sporangia of uniform size, columellae elongate. Growth at 37°C, limited, spores 5-7µ long, spores 4-5µ long. Young turf gray to gray-brown; sporangia brown, sporangia black, sporangia more than 100µ in diameter.

Helminthosporium
Colonies consist of conidiosphores, loose or dense, regularly or irregularly velvety, brown to black, with strict or spreading margin. Conidiophores usually arise in groups, erect and straight, sometimes reclining, usually unbranched, only seldom with small side branches, septate, geniculate at points below the conidia, brown, green-brown to black, transparent or nontransparent. Conidia terminal or lateral on the geniculations, elongate, cylindrical, clavate or obclavate, smooth, mostly rounded at both ends, or sometimes pointed at the base or at both ends, straight or bent, with more than four cross-walls, dark brown, green-brown to black, often with the end-cells lighter colored.

***Melanospora* sp.**
Perithecia light colored superficial without stroma globose pyrifom with a long neck usually with a fringe of hairs about ostiole ascospores one celled, brown or brownish black.

***Myrothecium* sp.**
Conidial layer, shield or cusion shaped black surrounded at the edge by fine hyaline hairs condiophores short rod-shaped, conidia very small ovate or cylindric.

***Nigrospora* sp.**
Hyphae creeping, at first hyaline, later dark, ultimate branchlets bearing jar-shaped conidiophores either laterally or terminally. Conidia solitary, subglobose, smooth. This gtenus approaches the genus Pachybasium among the Botrytideae and Rhinocladium among the Trichosporieae. A single species treated.

***Penicillium* sp.**
Vegetative hyphae creeping septate branched, conidiophores erect, usually unbranched septate, the apex with a vertical of erect primary branches conidia borne in chains which typically form a brush-like head, conidia globose, ovate or elliptical.

***Rhizopus* sp.**
Mycelium of two kinds one submerged and the other aerial. The sporangia white at first become bluish black at maturity spores round or oval, angular colorless or colored bluish or brown.

Table 1. Population of fungi isolated from the brown leaf of *Avicennia marina* by plating the leaf washings

Fungi	Sampling			
	I (Dec)	II (Jan)	III (Feb)	IV (Mar)
Absidia glauca	1	-	2	-
Alternaria alternata	1	-	1	1
Aspergillus fumigatus	-	1	-	-
A. flavus	-	-	-	2
A. lucheunsis	-	1	3	3
A. niger	1	-	-	2
A. sydowi	-	-	-	2
A.sulphureus	-	-	-	1
Cladosporium sp.	4	1	-	-
Cunninghamella sp.	3	-	-	2
Curvularia lunata	1	-	1	1
Fusarium sp.	-	-	-	2
Glicocladium sp.	-	-	1	1
Melanospora sp.	3	1	-	-
Pestalotia sp.	-	-	-	-
Rhizopus sp.	-	-	3	-
Trichoderma viride	1	1	1	1

Trichoderma viride
Vegetative hyphae usually forming a thin inconspicuous growth on media with little organic food but usually fluffy in rich media. Chains of spores produced conidia arising by the extrusion of protoplasm through the neck of the phialide conidia globoid pale bright green under the microscope. *Spores globose, Spores not globose:* Turf delicate, at first white, later gray or borwn; sporangiosphores richly branched, sporangial walls fragile or slowly diffluent. Gemmae in sporangiophores numerous, spores short oval. Gemmae in sporangiophores scarce or lacking, spores more than twice as long as broad. Turf remaining white, yellow, or gray, spore walls diffluent. Turf usually less than 20mm high, sporangia less than 100µ in diameter. Larger species: Primary sporangiosphores more or less

sympodially branched. Primary sporangiosphores, not sympodially branched, sporangia large, 100-300µ, nogemmae.

***Stachybotrys* sp.**

Mycelium creeping, spreading over the substratum, septate, branched, hyaline, or slightly colored. Conidiophores arise as branches of the mycelium, erect, variously branched, septate, dark colored or almost hyaline, bearing at the apex of the main stalk and branches small sterigma-like cells (phialides), which are nonseptate, hyaline or slightly dark colored, and either borne in whorls or arise irregularly below the point of the branch, appearing singly or more or less grouped. Conidia borne singly on the points of the phialides, round or elongate, black, smooth or echinulate.

Table 2. Population of fungi isolated from the brown leaf of *Avicennia marina* by plating the washed leaf bits

Fungi	Sampling			
	I (Dec)	II (Jan)	III (Feb)	IV (Mar)
Alternaria alternata	+	-	-	+
Aspergillus candidus	+	-	-	-
A. terreus	-	+	-	-
Cladosporium sp.	-	+	-	+
Curvularia sp.	-	+	-	-
Fusarium sp.	+	-	-	+
Pestalotia sp.	+	-	-	-
Pencillium sp.	-	+	-	-

Table 3. Percent occurrence fungi on the brown leaf of *Avicennia marina* by plating the leaf washings

Fungi	Sampling			
	I (Dec)	II (Jan)	III (Feb)	IV (Mar)
Absidia glauca	6.6	-	16.6	-
Alternaria alternata	6.6	-	8.3	5.5
Aspergillus fumigatus	-	20	-	-
A. flavus	-	-	-	11
A. lucheunsis	-	20	25	6.6
A. niger	6.6	-	-	11
A. sydowi	-	-	-	11
A.sulphureus	-	-	-	5.5
Cladosporium sp.	26.6	20	-	-
Cunninghamella sp.	20	-	-	11
Curvularia lunata	6.6	-	8.3	5.5
Fusarium sp.	-	-	-	11
Glicocladium sp.	-	-	8.3	5.5
Melanospora sp.	20	20	-	-
Pestalotia sp.	-	-	-	-
Rhizopus sp.	-	-	25	-
Trichoderma viride	6.6	20	8.3	5.5

Table 4. Percentage of cellulase secreted by different groups of fungi

Name of Fungi	% loss in Viscosity	% cellulose utilized
Trichoderma sp.	32.81	32.81
Gliocladium sp.	6.83	6.83
Fusarium sp.	11.84	11.84
Penicillium sp.	16.50	16.50

DISCUSSION

The Endophytic fungi are one of the most unexplored and diverse group of organisms that make symbiotic associations with higher life forms and may produce beneficial substances for host (Weber, 1981: Shiomi *et al*., 2006) Fungi have been widely investigated as a source of bio active compounds. An excellent example of this is the anticancer drug, taxol, which had been previously supposed to occur only in the plants (Strobel & Daisy, 2003).

Endophytic organisms have received considerable attention after they were found to protect their host against insect pests, pathogens and even domestic herbivorous (Weber, 1981).

A study of endophyte biodiversity of the two dry and moisture of mangrove forest in Ramanathapuram District, Karankadu in Tamilnadu, India was conducted by the Suryanarayana *et al*., (2003). They have reported diversity of fungal species ranging from 10 to 26 in the host. Among the one plant species the lowest number of fungal diversity was 10 in *Gmelina arborea* Roxb. In the present study 17 different species with 8.86% colonization frequency were isolated from *Avicennia marina.*

However only a few plants have been studied for their endophyte biodiversity and their potential to produce bioactive compounds. Recently studies have been carried out about the endophytic bio diversity, taxonomy, reproduction, host ecology and their effort on host (Arnold *et al.,* 2001; Clay & Schardl, 2002).

It is well known that cellulose is the major constituent of the plant, material and it makes up about one third of biomass of annual plants, plants and about half that of perennials. Cellulose biodegradation is thought to be brought about by the confined and synergestic activities of atleast three different groups of hydrolytic enzymes (Burns 1983), and microorganism differ widely in which they attack cellulosic substrates. It was found that the cellulolytic activity was comparatively more in *Trichoderma* than *Gliocladium*, *Fusarium* sp. and *Penicillium* sp.

Totally 17 species of fungi were isolated during the course of the investigation, *Abisidia*, *Cunninghamella* and *Rhizopus* belonged to phycomycetes, *Melanospora* belonged to ascomycetes and all other species belonged to fungi imperfect. The basidiomycetous fungi were not represented. The fungi occur on the leaf surfaces could very well documented by using different methods and media. Among the different groups of fungi isolated, *Cladosporium* and species of *Aspergillus* showed variations in their distribution during the course of samplings. The population of *Cladosporium* was more during the months of December and January and *Aspergillus* was more during the month of March onwards. Such seasonal variations in the distribution of *Cladosporium cladosporides*, during winter and *Aspergillus* during summer have been reported on the leaves of *Psidium guajava* (Pandey and Drivedi, 1984). Another interesting observation was that the population of *Aspergilli* was more than any other species recorded in the investigation which may be due to the face that they are heavy spores.

It is well known that cellulose is the major constituent of the plant, material and it makes up about one third of biomass of annual plants, plants and about half that of perennials. Cellulose biodegradation is thought to be brought about by the confined and synergestic activities of atleast three different groups of hydrolytic enzymes (Burns 1983), and microorganism differ widely in which they attack cellulosic substrates. It was found that the cellulolytic activity was comparatively more in *Trichoderma* than *Gliocladium*, *Fusarium* sp. and *Penicillium* sp.

Acknowledgement
The authors are grateful to the the Managing Director, Sri Gowri Biotech Research Academy, Thanjavur (Dt), Tamilnadu for their permission to utilized the laboratory facility.

REFERENCES

[1] Ainsworth, GC, Sparrow, FK and Sussman AS, *The fungi – an advanced treatise: A taxonomic review with keys. Ascomycetes and fungi imperfecti*, (Academic Press, New York) **1973**, 621.
[2] Arnold, AE, Maynard, Z and Gilbert, GS, **2001**. *Mycological Research*, 105: 1502-1507.
[3] Clay, K. and C. Schardl. **2002**. *American Naturalist*, 160: 99-127.
[4] Harrison, PJ. Snedaker, SC, Ahmed, SI and Azam, F, **1994**, *Tropical Ecology*, 35: 155-184.
[5] Hyde, K D, *Asian Marine Biology*, **1990,** 7: 93-107.
[6] Khan, SA, **1966,** Working plan of the Coastal Zone Afforestation Division from 1963-64 to 1982- 1983. Government of West Pakistan. Agriculture Dept., Lahore.
[7] Krishnamurthy, KMJ, Prince Jeyaseelan, **1984,** Human impacts on the Pichavaram mangrove Ecosystem a case study from South India.
[8] Mehdi, FS and Saifullah, SM, **2000,** *Pak. J. Biol. Sci*, 3(2): 265-268.
[9] Mirza, MI, Hassan, MZ, Akhtar S, and Ali J, **1983**, *Pak. Agri. Council, Islamabad*, 19-21.
[10] Mohamed, AD, **1996,** Mangrove Forests. Valuable resources under the threat of development Pressure, Ocean years. 12: 247 – 267.
[11] Pandey, RR, and Dwivedi, RS, **1984**, *Botanica Indica*, 12: 1-8.
[12] Ray, RY. and Dwivedi, RS, **1962,** *Proc. Natn. Acad. Sci. India.*, 32: 421-428.
[13] Shiomi, HF, Silva, HAS, De Melo, IS, Nunes, FV and Bettiol, W, **2006**, *Sci. Agric.*, 63(1): 32-39.

[14] Strobel, G. and Daisy, B, **2003**, *Microbiology and Molecular Biology Review*, 67: 491-502.
[15] Suryanarayanan, TS, Venkatesan, G. and Murali, TS, **2003,** *Current Science*, 85(4): 489-492.
[16] Weber, J, **1981,** *Nature*, London, 292: 449-451.