International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 466-473, 2017

Studies on biodiversity of phylloplane and endophytic fungi from different aged leaves of mangrove plant, Avicennia officinalis

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Abstract : Assortment of phylloplane and endophytic fungi is used to consider as one of the rich source of innovative compounds of biological activities and have a high level of structural diversity on the leaf surfaces. Bioactive composites produced by these phylloplane and endophytes have shown promising potentiality towards good health for human beings, for which, it is necessary to recognize and manipulate this important microbial resource and make it more beneficial for the welfare of mankind. In the present study, isolation and enumeration of ectophytic (phylloplane) and endophytic fungal species was carried out from one mangrove plant, Avicennia officinalis with the host relationship based on two methodologies, agar plate and moist chamber. Altogether, 27phylloplane and endophytic fungal species of 18 genera were isolated from the mangrove plant. Among the phylloplane and endophytic fungal population, *Absidia spinosa, Aspergillus niger, A. flavus, Alternaria alternata, Curvularialunata, Drechslera, Chaetomium, Helminthosporium*sp., *Humicola* sp., *Phoma* sp., *Fusarium oxysporum, Neurospora* sp., *Penicillium* sp., *Penicillium digitatum, Penicillium oxalicum, Rhizopus stolonifer, Ulocladium* sp., white sterile mycelia were the most dominant fungal species in both agar plate and moist chamber methods. Fungi isolated from both the surface and sub-surface of the leaf samples was more or less similar to each other. The host relative favorite and tissue description indication was found between the phylloplane and endophytes based on the fungal community distribution and composition and thus the fungi isolated are dependent on the used methodologies.

Key words : Phylloplane and Endophytic fungi, Biodiversity, Mangrove plant, Ectophytic, *Avicennia officinalis.*

Introduction

Phylloplane fungi grow on the surfaces of leaves [1]. There are two groups of phylloplane fungi, residents and casuals [1, 2]. Residents can multiply on the surface of healthy leaves without noticeably affecting the host whereas casuals land on the leaf surface but cannot grow [3]. Phylloplane fungi have been poorly studied as compared to endophytes, saprobes and pathogenic fungi[1, 2].Endophytic fungi are well-definedasthe fungi which spend the whole or part of their lifecycle inhabiting inter or intracellular way inside the healthy tissues of the plants, typically causing no outward pathogenic indications. These are significant components of plant micro-ecosystems [2-3]. Endophytic fungi are found in eachplants examined and it is predicted that there are over one million fungal endophytes were in the nature [4]. Endophytic fungi are laredy documented as an important and novel source of natural bioactive products with potential application in agriculture, medicine and food industry [5-7].

Asone of the bioactive compound paclitaxel (taxol) revealed from the endophytic fungus, *Taxomyces andreanae* in 1993 [8].Many of the scientists are mounting their interests in studying the fungal endophytes as potential producers of novel and biologically dynamic compounds. Within two decades, many valuable bioactive compounds with different activities are successfully discovered from the endophytic fungi. These bioactive compounds are generally classified as alkaloids, terpenoids, steroids, quinones, lignans, phenols and lactones secreted from the plant parts[2,9]. There is an evolutionary friendly relationship is being set up between phylloplane and endophytic fungi wit htheir host plant [2]. The host plant supply the required nutrients and easeful habitation for the survival of its phylloplanes and endophytes. Henceforth, the endophytes produce a number of bioactive composites for helping the host plants to resist external biotic and abiotic stresses and benefiting for the host growth in return [3, 10]. Little endophytic fungi have established the ability to produce similar bioactive materials as those originated from the host plants. It would be beneficial for us to record the relations between the phylloplanes and ectophytes with their host plants and to develop a interchangeable tactic for capably generating these rare and valuable bioactive compounds [6, 11].During the present study, isolation and identification of phylloplane and endophytic fungi were carried out from one mangrove plant species viz., *Avicennia officinalis* collected from Murugampakkam, Ariyankuppam and Nonankuppam regions of Pondicherry, India employing two methods. The potential relationships of the phylloplanes and endophytes with their host plants were also discussed.

Materials and Methods

Collection of sample

Leaves of different age group viz., Young, Mature, Yellow and Litter of *Avicennia officinalis* were collected in fresh condition from the mangrove vegetation of Murugampakkam, Ariyankuppam and Nonankuppam regions of Puducherry, India. Leaf sampleswere carefully brought to the Microbiology Laboratory, Department of Botany with utmost care and kept in room temperature for the experimental processes on the same day of collection without delay.

Description of the plant

Binomial name**,** *Avicennia officinalis*
Family , Acanthaceae
Common name , Indian Mangrove, white mangrove
Vernacular name , Venkantal

Avicennia officinalis

Avicennia officinalis is a mangrove species found in most of the brackish water blocks on the vicinity of estuaries and marine shorelines. The young tree customs a columnar tree up to 15 m and may rise up to 30 m. The plants have glossy green leaves, 10 cm long by 5 cm wide, have rounded apexes and golden-brown beneath leaf and grown in opposites. The flower is orange yellow to lemon yellow in color and the largest amid the *Avicennia* species and has a diameter of 6 to 10mm. The bark of the plant is smooth, dirty green to dark gray in colour. It is also slightly fissured and does not flake. The fruit looks green or brown, heart shaped abruptly narrowed to a short beak is 2.5 cm long or more. *Avicennia officinalis* is found occasionally on the banks of rivers and rarely found near the sea. The plant prefers clay soil and usually found in inland areas. The plant is

found in mostly south Asia viz., Bangladesh, Brunei, Cambodia, India, Indonesia, Malaysia, Myanmar, Papua New Guinea, Philippines, Singapore, Sri Lanka, Thailand, and Vietnam.

Surface sterilization of leaves

The collected healthy leaves were thoroughly washed in running tap waterin order to isolate the endophytic fungi. The leaves were cut into small segments (about 1cm2) including midrib portion. The leaf samples were surface sterilized by 0.1 % mercuric chloride for 60 seconds and then rinsed in sterile distilled water for 10 seconds (three times). For phylloplane mycoflora study, the leaf segments were not surface sterilized since phylloplane fungi grown on the surface of the leaves. They were placed on the PDA and moist chamber plates equidistantly without washing the segments.

Culture of leaf samples on agar plates

After sterilization, the excess water was blotted out by sterile filter paper from the leaf segments and kept separately. Then the surface sterilized segments were placed in a petridishes containing PDA supplemented with streptomycin as well as in moist chamber. Five (5) leaf such as Young, Mature, Yellow and Litter segments of a centimeter square, both sterile and unsterile were placed separately on the PDA media plates equidistantly by the help of sterile forceps and pressed later on followed by incubation for 3 to 7 days.

Culture of leaf sample on moist chamber

The moist chamber plates don't need any type of medium for the growth of endophytic as well as phylloplane fungi. Same like agar plates, five (5) leaf Young, Mature, Yellow and Litter segments of centimeter square, both sterile an unsterile were placed separately on the moist chamber petriplates equidistantly by the help of sterile forceps and pressed later on followed by incubation for 7 to 21 days. The fungi on moist chamber were enumerated later on based on their growth on the leaf segments.In this method, the fungi grow on its own on the host, getting the moister produced from the wet condition prevailing inside the petriplates.

Incubation for the growth of fungi

All the plates were incubated at 25±3ºC temperature in the incubation chamber. Incubation time was maintained differently since, 7-8 days is meant for the fungal growth of fungi in agar plate method, but in moist chamber method, 1 to 3 weeks are required for the growth of fungi. Every day watch of the petriplates and check the growth of fungi was almost necessary in our present study after 3rd day of incubation.

Identification of fungi

After three days of incubation, the fungal colonies were counted for individual species and the total number was enumerated. Microscopic slides stained with lacto phenol cotton blue were prepared from each colony of the fungus and observed microscopically under the trinacular digital photography microscope to identify up to species level. The colony which was not be identified directly from plates was sub cultured in SDA/PDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal CFUs up to species level [12-16]. The presence and absence based on the occurrence of individual fungus in the phylloplane and endophytic were determined and plotted in the form of tables and figures.

Results & Discussion

During the present study, altogether 27 fungal species under 18 genera were isolated and identified from different aged leaf samples viz., young, mature, yellow and litter of the mangrove plant, *Avicennia officinalis* by agar plate and moist chamber methods. Thephylloplane fungi were recorded under 26 genera and 18speciesandendophyticfungiwere of 22 genera and 17 species by both the methods, of which moist chamber method was found to be good enough to sporulate fungi was confirmed by our previous works[3]. Occurrence of phylloplane and endophytic fungi isolated by agar plate and moist chamber methods from the mangrove plant, *Avicennia officinalis* is given in Table 1-4.Complete number of isolated phylloplane fungi and endophytes are given in Fig 1 &2, likewise the total number of fungi recorded separately by the two methods are given in Fig 2 & 3. Moist chamber method was found suitable to isolate most of the fungal species from the leaf samples of the mango plant. It was also observed that moist chamber was not expensive to prepare and to inoculate the materials like agar plate method. Moreover it was seen that the growth of endophytic and phylloplane fungi was very slow in the moist chamber than the agar plate method. All obligate parasitic or restricted fungi were found

to grow in the moist chamber in better way than agar plates since they are likely to grow in their own host in the humidity condition than the agar plates where no humidity is prevailed[3,17].Littered leaves was found to harbor the maximum number of fungal species in both endophytic and phylloplane followed by yellow and mature leaves (Fig 2 & 3), which may be related to the saprophytic nature of the fungi in order to grow in littered materials. Fungi like *Absidia spinosa*, *Aspergillu sniger*, *A.flavus*, *Alternaria alternata*, *Curvularia lunata*, *Drechslera*, *Chaetomium, Helminthosporium* s p., *Humicola sp.*, *Phoma* sp., *Fusarium oxysporum*, *Neurospora* sp., *Penicilliums*p, *Penicillium digitatum*, *Penicillium oxalicum*, *Rhizopusstolonifer,Ulocladium* sp.,White sterile mycelia and Grey sterile mycelia species were identified as endophytes. Most of the endophytes were also included with phylloplane fungi and few were like *Absidia spinosa, Alternaria alternata, Aspergillus awamori,A. fumigatus, A.nidulans, A. terreus, Chaetomium*sp., *Cladosporium herbarum,Curvulari alunata, Fusarium*sp., *Helminthosporium* sp., *Neurospora*sp., *Penicilliu mfellutanum, Phoma*sp,.*Rhizopus stolonifer, Ulocladium*sp., white and grey sterile mycelia (Table 1-4). Bharathidasan and Panneerselvam[10] recorded a total 10 fungal species viz. *Aspergillusflavus, A. niger, Aspergillus sp., Penicillium sublateritium, Phomachrysanthemicola, P. hedericola, Phoma sp.* and *Candida albicans* from *Avicennia marina*. Among the endophytic flora, *Phoma* was the most prominent genus. Interestingly no endophytes were isolated from 110 leaves samples and overall colonization frequency from surface in their work.

Table 1, Occurrence of phylloplane fungi isolated by agar plate method from the mangrove plant, *Avicennia officinalis*.

Sl. No.	Fungal species	Different aged leaves			
		Young	Mature	Yellow	Litter
1	*Absidia spinosa*	-	-	+	+
2	*Aspergillus awamori*	-	+	+	+
3	*Aspergillus flavus*	+	+	-	-
4	*Aspergillus niger*	-	+	+	+
5	*Cladosporium herbarum*	-	-	-	+
6	*Colletotrichum* sp.	-	+	+	+
7	*Drechslera indica*	-	-	+	+
8	*Fusarium oxysporum*	+	+	+	+
9	*Fusarium* sp.	-	-	+	+
10	*Penicillium digitatum*	-	-	+	+
11	*Penicillium oxalicum*	+	+	-	-
12	*Phoma* sp.	-	+	+	+
13	White sterile mycelia	+	+	+	+
14	Grey sterile mycelia	-	+	+	+

Table 2, Occurrence of endophytic fungi isolated by agar plate method from the mangrove plant, *Avicennia officinalis*.

Sl. No.	Fungal species	Different aged leaves			
		Young	Mature	Yellow	Litter
1	*Absidia spinosa*	-	-	-	+
2	*Alternaria alternata*	-	-	+	+
3	*Aspergillus awamori*	-	-	+	+
4	*Aspergillus niger*	+	+	+	+
5	*Cladosporium herbarum*	-	+	+	+
6	*Drechslera indica*	-	-	+	+
7	*Fusarium oxysporum*	-	-	+	+
8	*Penicillium digitatum*	-	-	-	+
9	*Penicillium oxalicum*	+	-	+	-
10	*Phoma* sp.	-	+	+	+
11	White sterile mycelia	+	-	+	+
12	Grey sterile mycelia	-	+	-	+

Table 3, Occurrence of phylloplane fungi isolated by moist chamber methods from the mangrove plant, *Avicennia officinalis.*

Sl. No.	Fungal species	Different aged leaves			
		Young	Mature	Yellow	Litter
1	*Absidia spinosa*	-	-	-	+
2	*Alternaria alternata*	-	-	+	+
3	*Aspergillus awamori*	+	-	-	+
4	*Aspergillus flavus*	+	+	-	-
5	*Aspergillus fumigatus*	+	-	+	-
5	*Aspergillus nidulans*	-	-	+	+
6	*Aspergillu sniger*	-	+	+	+
7	*Aspergillu sterreus*	-	+	+	-
8	*Chaetomium* sp.	-	+	+	+
9	*Cladosporium herbarum*	-	-	-	+
10	*Curvularia lunata*	+	-	-	+
11	*Fusarium oxysporum*	-	+	-	+
12	*Fusarium* sp.	+	-	+	+
13	*Helminthosporium*s p.	+	-	-	+
14	*Humicola* sp.	-	+	-	+
15	*Neurospora* sp.	-	-	+	+
16	*Penicillium fellutanum*	-	-	+	+
17	*Phoma* sp.	-	-	+	+
18	*Rhizopus stolonifer*	-	-	+	+
19	*Ulocladium* sp.	-	+	-	+
20	White sterile mycelia	+	+	+	+

Table 4, Occurrence of endophytic fungi isolated by moist chamber methods from the mangrove plant, *Avicennia officinalis.*

Sl. No.	Fungal species	Different aged leaves			
		Young	Mature	Yellow	Litter
1	*Absidia spinosa*	-	-	-	+
2	*Alternaria alternata*	-	-	+	+
3	*Aspergillus awamori*	+	-	-	-
4	*Aspergillu sflavus*	+	+	-	-
5	*Aspergillus nidulans*	-	-	+	+
6	*Aspergillus niger*	-	+	+	+
7	*Chaetomium* sp.	-	+	+	+
8	*Curvularia lunata*	+	-	-	+
9	*Fusarium oxysporum*	-	+	-	+
10	*Fusarium* sp.	+	-	+	+
11	*Helminthosporium* sp.	+	-	-	+
12	*Humicola* sp.	-	+	-	-
13	*Neurospora* sp.	-	-	+	+
14	*Phoma*sp.	-	-	+	+
15	*Rhizopus stolonifer*	-	-	+	+
16	*Ulocladium* sp.	-	+	-	+
17	White sterile mycelia	+	+	+	+

Fig 1, Total number of fungi isolated from different aged leaves by moist chamber method

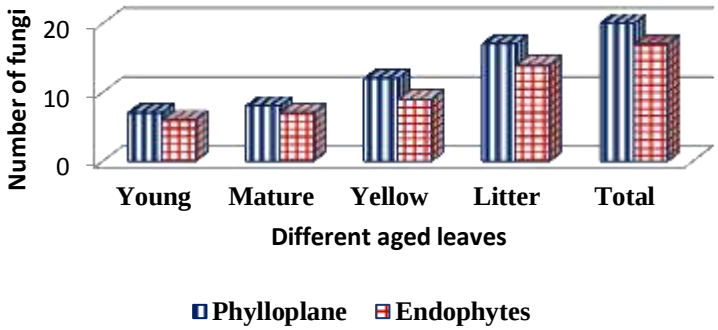


Fig 2, Total number of fungi isolated from different aged leaves by agar plate method

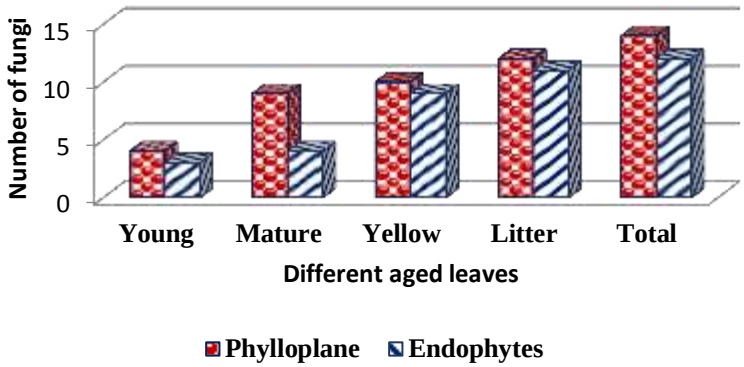
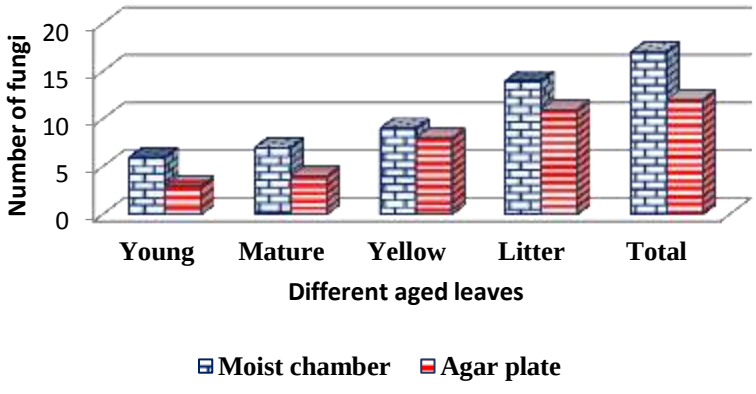
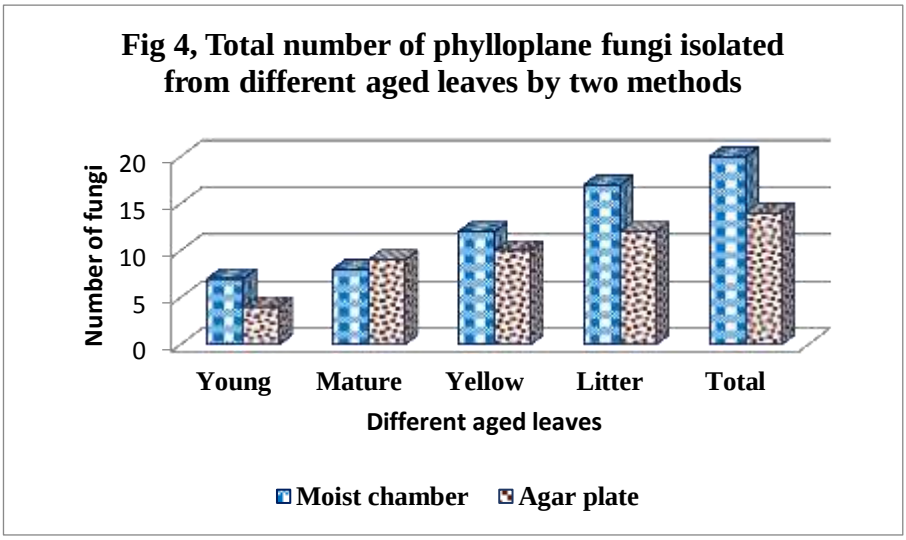


Fig 3, Total number of endophytic fungi isolated from different aged leaves by two method





Phylloplane and endophytic microbes have received considerable attention as they are found to protect their host against pest pathogens and even domestic herbivorous[1]. Most of the isolated fungi belonged to anamorphic fungi in particular to Deuteromycetes and Zygomycetes[3]. The isolated fungi of the endophytic and phylloplane origin may lead to the production of special compound within the host, mangrove plant, *Avicennia officinalis*[7, 9]. Fungi have been widely known as a source of bioactive compounds, an excellent example for the anti-cancer drug taxol, which was previously supported to occur only in the plant[9]. *Avicennia officinalis* is a plant having a broad spectrum of medicinal properties. Every part of the plant is used in one or the other types of medicine. Isolation of only 27 taxa of phylloplane and endophytic fungi showed that the medicinal property of the plant has some role to play in the colonization of fungi[11].

Recently studies have been carried out about the endophytic biodiversity, taxonomy, reproduction, host ecology and their effort on host [1,2,11,18,19]. Endophytes, are now considered as an outstanding source of bioactive natural products, because they occupy uniquebiological niches as they grow in so many unusual environments [10,11, 19]. 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 and his coworkers[8]. They have reported diversity of fungal species vanging 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 27 different species with high frequency were isolated from *Avicennia officinalis* which is slightly more than the above cited study by Suryanarayanan[8].

Conclusion

Fungi are used as medicinal means with natural products once serving as the basic source of most of the illnesses. *Avicennia officinalis* is a mangrove plant which has broad spectrum medicinal properties. In the present study, a total of 27phylloplane and endophytic fungal species under 18 genera were recorded by moist chamber and agar plate methods. The most of the fungi isolated belonged to the class Deuteromycete sand Zygomycetes. Among the phylloplane and endophytic fungal population, *Absidia spinosa, Aspergillus niger, A.flavus, Alternaria alternata, Curvularia lunata, Drechslera, Chaetomium, Helminthosporium* sp., *Humicola sp., Phoma* sp., *Fusarium oxysporum, Neurospora* sp., *Penicilliums*p.,*Penicillium digitatum, Penicillium oxalicum, Rhizopusstolonifer,Ulocladium*sp.,white sterile mycelia were the most dominant fungal species in both agar plate and moist chamber method. It was confirmed from the present study that, moist chamber method was superior to the agar plate method in order to isolate phylloplane and endophytic fungi from plant materials.

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