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Biodiversity of Phylloplane and Endophytic Fungi from Different Aged Leaves of Medicinal Mangrove Plant Species, *Avicennia marina*

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Abstract

Aim:

Variety of phylloplane and endophytic fungi is used to imitate as one of the rich source of novel compounds of biological accomplishments and have a high level of organizational diversity on the leaf surfaces. Bioactive composites produced by these phylloplane and endophytes have shown inspiring potentiality towards therapeutic medicine, for which it is necessary to appreciate and employ this important microbial resource and make it more valuable for the wellbeing of mankind. During the present study, isolation and enumeration of phylloplane (ectophytic) and endophytic fungal species was carried out from one medicinal plant, *Avicennia marina* collected from marine mangrove environments of Puducherry coast.

Methods:

During the study, two methodologies viz., moist chamber and direct agar plate methods were used for the isolation and enumeration of phylloplane and endophytic fungi.

Results & Discussion:

Altogether, 25 fungal species of 13 genera were isolated from the mangrove plant, *Avicennia marina*. Among the isolated fungi, *Aspergillus niger, Cochilobolus victoria, Colletotrichum* sp., *Curvularia lunata, Drechslera* sp., *Glomerella sp. Fusarium oxysporum, Penicillium chrysogenum, P. oxalicum, Phoma* sp., *Sordaria* sp. White sterile mycelia were found common in both phylloplane and endophytes. Moist chamber method was found suitable to record more number of fungi than agar plate method. The host relative preference and tissue specification evidence was found between the phylloplane and endophytes based on the fungal community distribution and composition.

Key words: Biodiversity, Phylloplane and Endophytic fungi, Medicinal mangrove plant *Avicennia marina*

INTRODUCTION

The endophytic fungi are the microbes which occupy the tissues of plants during their life cycle without causing disease indications and many are significant in biotechnological processes. In general, vascular plants harbor phylloplane and endophytic organisms [1]. The fungi who reside in the internal part of plant tissues called endophytes, which constitute a group of plant symbionts and are a component of microbial diversity and those who reside over the leaf surfaces are recognized as phylloplane fungi [1, 2]. Endophytic fungi that are existing in living tissue of diverse plant parts viz., root, fruit, stem, seed, leaf etc. establishing a reciprocal relationship without making apparently any dysfunction or maladies in the host. Endophytic fungi are capable of living in host plant without causing any types of symptoms [3, 4]. Endophytic fungi are ubiquitous in nature and found in the plants, residing intercellular or intracellular at least for a portion of their life without causing apparent symptoms of infection [5,6]. Fungi are group of organisms having a great biodiversity and they are the largest group of microbes of tropical ecosystems throughout the world. They are present in most plant parts, especially the leaves where the tissue is apparently healthy [4, 5, 6]. It is beneficial for us to study the relations between the phylloplane and endophytes with their host plants and to develop a interchangeable approach for competently producing these unusual and valuable bioactive compounds [6, 10]. The endophytes produce a number of bioactive compounds for helping the host plants to resist external biotic and abiotic stresses and benefiting for the host growth in return [3, 11]. Limited endophytic fungi have developed the ability to produce the same or similar bioactive substances as those originated from the host plants. During the present study, isolation and identification of phylloplane and endophytic fungi were carried out from one medicinal plant of mangrove origin, *Avicennia marina* collected from dense stand at the mouth region of estuary of Veerampattinam, in the vicinity of coastal area of Puducherry, India.

MATERIALS AND METHODS

Collection of leaf samples

Leaves of different age group viz., Young, Mature, Yellow and Litter of *Avicennia marina* were collected from dense stand at the mouth region of estuary of Veerampattinam, in the vicinity of coastal area of Puducherry, India. Young, Mature, Yellow and Litter leaves were carefully segregated and brought to the Microbiology Laboratory, Department of Botany with utmost care and kept in room temperature for further experiments.

Description of the plant

Binomial name **:** *Avicennia marina*
Family : Acanthaceae
Common name : Grey Mangrove or White mangrove
Vernacular name : Kanna

Avicennia marina is a species of mangrove also known as Indian mangrove. It is an evergreen shrub or small tree about 1-10m high, trunk equivalent to 40 cm in diameter. Numerous upright pneumatophores are of 10-15 cm high and 6 mm in diameter. Trunk often with masses of small air roots but no prop or stilt roots. Bark whitish to grayish or yellow-green, smooth, often powdery with raised dots, scaly, exposing greenish inner bark. Leaves opposite, ovate, lanceolate to elliptical, 3.5-12 cm long, 1.5-5cm wide, mostly acute at both ends, entire, thick leathery, shiny green and hairless upper surface, pale on long stalks at ends and sides of twigs. Flowers are few to many, sessile, 4 mm long, 5mm across. Calyx is 5-lobedm green, hairy, persistent; corolla tubular, white, white turning yellow or orange with 4 nearly equal, short lobes. They are generally distributed in East and South Africa, southern Asia, Australia, and Oceania. From Egypt and Arabia along shore of Red Sea and Western Indian Ocean, eastward along shore of Arabian sea, Bay of Bengal, Southeastern and Eastern Indian ocean, South china sea north to Hong Kong and Taiwan, and island from Philippines Sea , Coral sea, South Pacific to Western Australia and New Zealand etc.

Surface sterilization of leaves

In order to isolate the endophytic fungi, the collected healthy leaves were thoroughly washed in running tap water. Then 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. Without washing the segments, they were placed on the PDA and moist chamber plates equidistantly.

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, 13]. 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

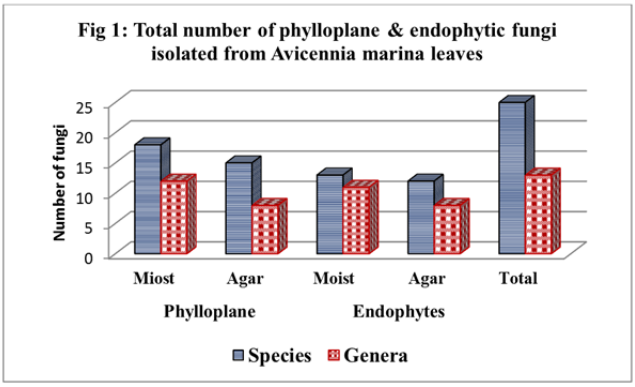
In our present study, altogether 25 phylloplane and endophytic fungal species under 13 genera were isolated and identified from different aged leaf samples viz., young, mature, yellow and litter of the mangrove plant, *Avicennia marina* by agar plate and moist chamber methods. The phylloplane fungi were recorded under 22 genera and 12 species and endophytic fungi were of 18 genera and 12 species by both the methods (Fig 1), of which moist chamber method was found to be good enough to identify fungi was confirmed by our previous work [3]. Occurrence of phylloplane and endophytic fungi isolated by agar plate and moist chamber methods from the medicinal plant, *Avicennia marina* is given in Table 1. Total number of isolated phylloplane and endophytic fungal species is given in (Fig 1). Moist chamber method was found suitable to isolate most of the fungal species from the leaf samples of the mangrove plant. It was also observed that moist chamber was not expensive to prepare and to inoculate the materials like agar plate method [3,4]. Moreover it was

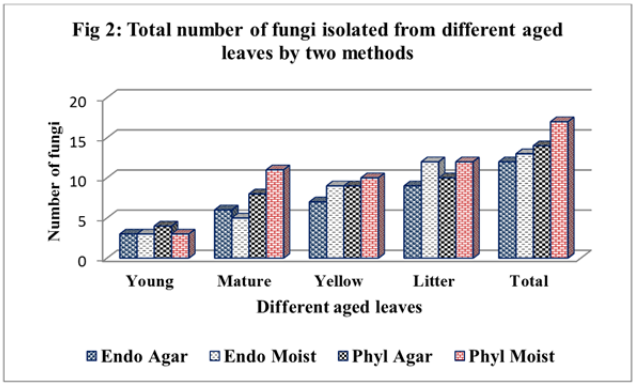
observed that the growth of endophytic and phylloplane fungi was very slow in the moist chamber than the agar plate method. All obligate parasitic or restricted fungi were found to grow in the moist chamber in better way than agar plates since they are likely to grow in their own host in the humidity 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), which may be related to the saprophytic nature of the fungi in order to grow in littered materials [14, 15]. Fungi like *Aspergillus niger, Cochilobolus victoria, Colletotrichum* sp., *Curvularia lunata, Drechslera* sp., *Glomerella sp. Fusarium oxysporum, Penicillium chrysogenum, P. oxalicum, Phoma* sp., *Sordaria* sp. White sterile mycelia were found common in both phylloplane and endophytes. Most of the endophytes were also included with phylloplane fungi and few were like *Phoma* sp., *Sordaria* sp. and *Glomerella* sp. were found as exclusively endophytes, Our present result is found similar to Bharathidasan and Panneerselvam [10], who recorded a total 10 fungal species viz. *Aspergillus flavus, A. niger, Aspergillus sp., Penicillium sublateritium, Phoma chrysanthemicola, P. hedericola, Phoma sp.* and *Candida albicans* from *Avicennia marina*. But they [10] isolated very few fungi in comparison to us and they were used moist chamber method and not used different aged leaves from the plant.

Phylloplane and endophytic organisms 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, Zygomycetes and Ascomycetes [3]. The isolated fungi of the endophytic and phylloplane origin may lead to the production of special compound within the host, mangrove plant, *Avicennia marina* [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 marina* 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 23 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]. Endophytes, are now considered as an outstanding source of bioactive natural products, because they occupy unique biological niches as they grow in so many unusual environments [10,11]. 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 23 different species with high frequency were isolated from *Avicennia marina* which is slightly more than the above cited study by Suryanarayanan [8].

Table 1: Phylloplane and endophytic fungi isolated from the medicinal mangrove plant, *Avicennia marina* from Puducherry coastal area by two methods.

Sl. No.	Fungal species	Phylloplane		Endophytes	
		Moist chamber	Agar plate	Moist chamber	Agar plate
1	*Alternaria alternata*	+	+	-	-
2	*Aspergillus awamori*	+	+	-	
3	*Aspergillus flavus*	+	+	-	+
4	*Aspergillus nidulans*	-	+	-	-
5	*Aspergillus niger*	+	+	+	+
6	*Aspergillus ochraceus*	+	-	+	-
7	*Aspergillus terreus*	-	+	-	+
8	*Aspergillus versicolar*	-	+	-	-
9	*Aspergillus* sp.	-	-	-	+
10	*Cochilobolus victoriae*	+	-	+	-
11	*Colletotrichum* sp.	+	+	+	-
12	*Curvularia lunata*	+	-	+	+
13	*Drechslera* sp.	+	+	+	
14	*Fusarium oxysporum*	+	+	+	+
15	*Fusarium* sp.	+	+		
16	*Glomerella sp.*	+	-	+	+
17	*Penicillium citrinum*	-	-	-	+
18	*Penicillium chrysogenum*	+	-	+	-
19	*Penicillium digitatum*	+	+	-	-
20	*Penicillium fellutanum*	-	+	-	-
21	*Penicillium oxalicum*	+	+	+	+
22	*Phoma* sp.	+	-	+	+
23	*Sordaria* sp.	+	+	+	-
24	Grey sterile mycelia	-	-	-	+
25	White sterile mycelia	+	+	+	+
25/13gen		18/12	15/8	13/11	12/8





CONCLUSION

From the early twentieth century, fungi are used as medicinal agents with natural products once serving as the basic antimicrobial source of most of the infections. *Avicennia marina* is mangrove plants which have broad spectrum medicinal properties. In the present study, a total of 23 phylloplane and endophytic fungal species under 13 genera were recorded by moist chamber and agar plate methods. The most of the fungi isolated belonged to the class deuteromycetes and ascomycetes. Among the phylloplane and endophytic fungal population, *Aspergillus niger*, *Cochilobolus victoria*, *Colletotrichum* sp., *Curvularia lunata*, *Drechslera* sp., *Glomerella sp. Fusarium oxysporum*, *Penicillium chrysogenum*, *P. oxalicum*, *Phoma* sp., *Sordaria* sp. White sterile mycelia were found common in both agar plate and moist chamber methods. This one was confirmed from the current 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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