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Comparative study of phylloplane fungi recorded from various aged leaves of two mangrove plant species collected from Puducherry coastal area.

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ABSTRACT

Phylloplane is the natural habitat of microbial community on leaf surface which comprises of both saprophytes and pathogens. The present study was designed to investigate fungal abundance on leaves of two mangrove plants viz., *Bruguiera cylindrica* and *Avicennia officinalis.* Direct inoculation with and without surface sterilization of leaf method were used in agar plate and moist chamber for the assessment of associated phylloplane fungal flora. A total of 33 phylloplane fungal species belonging to 19 genera were isolated from both the two plants. However, 25 fungal species of 17 genera were recorded especially from *Avicennia officinalis* and 22 fungal species of 14 genera were isolated from *Bruguiera cylindrica*. Fungi like *Alternaria alternata, Aspergillus awamori, Aspergillus fumigatus, Aspergillus niger, Cladosporium herbarum, Curvularia lunata, Drechslera* sp*,. Fusarium oxysporum, Helminthosporium* sp*., Penicillium fellutanum, Rhizopus stolonifer, Ulocladium* sp*.* Grey sterile mycelia, White sterile mycelia were common to both the plants. But *Absidia spinosa, Aspergillus flavus, Aspergillus nidulans, Aspergillus terreus, Chaetomium* sp*., Colletotrichum* sp*., Humicola* sp*.*, *Neurospora* sp*., Penicillium digitatum, Penicillium oxalicum* were exclusively recorded from *Avicennia officinalis* and *Aspergillus japonicus, Aspergillus ochraceus, Aspergillus versicolar, Aureobasidium pullulans, Cladosporium* sp*., Gliocladium* sp*., Penicillium digitatum and Penicillium oxalicum* were documented from *Bruguiera cylindrica* only. Litter leaves were found to harbor the maximum number of phylloplane fungi than other leaves viz., mature, yellow and young ones in both the mangrove plant studied herewith.

Keywords: Phylloplane fungi, Mangrove plants, Different aged leaves, *Avicennia officinalis, Bruguiera cylindrica.*

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INTRODUCTION

Phylloplane is a natural habitat of numerous microorganisms' viz., fungi, bacteria and actinomycetes on the surface of the leaf. Mostly fungi includes a variety of epiphytic and endophytic that colonizes the surface and internal tissues of the plants, respectively [1-4]. However, the quality and quantity of the microfungi differ with age of the plant on the leaf surface, leaf area, morphology and atmospheric factors such as temperature and humidity. Leaf surface topography and presence of nutrients on the leaf surface are generally recognized as important regulators of phyllosphere microbial communities. Very little research has been made on the whole plant community level [5-7]. Among different microbes, two groups of phylloplane fungi i.e. residents and casuals are generally present on leaves surface. Residents can multiply on the surface of healthy leaves devoid of noticeably affecting the host whereas casuals land on the leaf surface but cannot grow [8]. The phylloplane mycoflora is also reported to decompose plant material while act as allergic air borne spores [9]. Plants of medicinal value have been of age long remedies for human diseases because they contain components of therapeutic value [10,11]. Moreover mangrove plants have also medicinal properties in order to prevent and cure a number of diseases. *Avicennia officinalis* and *Bruguiera cylindrica* are the important mangrove medicinal plants that are traditionally used for treatment of several aliments. Both the mangrove plants have complex chemical composition. Some commonly recognized biologically active phytochemical constituents like eugenol, urosolic acid, alkaloids, flavonoids; tannins and carbohydrates have been reported. The present study was, therefore, planned to investigate the phylloplane fungi associated *Avicennia officinalis* and *Bruguiera cylindrica* using different methods viz., agar plate and moist chamber methods.

MATERIALS AND METHODS

Collection of leaf samples

Different aged leaves of two mangrove plants viz., *Bruguiera cylindrica* and *Avicennia officinalis* were collected from Murugampakkam, Ariyankuppam and Nonankuppam, Puducherry- 605008 in fresh condition. Young, Mature, Yellow and Litter leaves were brought separately to the Microbiology Laboratory, Department of Botany with sterile polythene cover, carefully segregated and kept in room temperature for further experiments.

Preparation of leaf segments

More than 100 leaves of the leaves were cut with sterile scissor into small segments (about $1cm^2$) including midrib portion. The leaf segments were kept in aseptic condition up to the completeion of experimental work.

Culture of leaf samples on agar plates

The leaf segments were placed in a petridishes containing PDA supplemented with streptomycin as well as in moist chamber. Five (5) leaf segments of Young, Mature, Yellow and Litter leaves of a centimeter square 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 phylloplane fungi. In this method, the fungi grow on its own on the host, getting the moister produced from the wet condition prevailing inside the petriplates. Same like agar plates, five (5) leaf segments of Young, Mature, Yellow and Litter leaves of centimeter square 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.

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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 4 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 of the said plants were determined and plotted in the form of tables and figures.

RESULTS & DISCUSSION

Altogether 33 phylloplane fungal species belonging to 19 genera were isolated from both the two plants. However, 25 fungal species of 17 genera were recorded especially from *Avicennia officinalis* and 22 fungal species of 14 genera were isolated from *Bruguiera cylindrica* (Fig 1). Fungi like *Alternaria alternata, Aspergillus awamori, Aspergillus fumigatus, Aspergillus niger, Cladosporium herbarum, Curvularia lunata, Drechslera* sp,. *Fusarium oxysporum, Helminthosporium* sp., *Penicillium fellutanum, Rhizopus stolonifer, Ulocladium* sp. Grey sterile mycelia, White sterile mycelia were common to both the plants. But *Absidia spinosa, Aspergillus flavus, Aspergillus nidulans, Aspergillus terreus, Chaetomium* sp., *Colletotrichum* sp., *Humicola* sp., *Neurospora* sp., *Penicillium digitatum, Penicillium oxalicum* were exclusively recorded from *Avicennia officinalis* and *Aspergillus japonicus, Aspergillus ochraceus, Aspergillus versicolar, Aureobasidium pullulans, Cladosporium* sp., *Gliocladium* sp., *Penicillium digitatum and Penicillium oxalicum* were documented from *Bruguiera cylindrica* only (Table 1). Litter leaves were found to harbor the maximum number of phylloplane fungi in compared to other leaves viz., mature, yellow and young (Fig 2 & 3). Moist chamber method was found suitable to record most of the fungal species from the leaf samples of the mangrove plants. 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 phylloplane fungi was very slow in the moist chamber than the agar plate method. All restricted parasitic or pathogenic 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 (Nayak). Phylloplane fungi isolated from two mangrove plants, *Avicennia officinalis* and *Bruguiera cylindrica* by two methods is given in Table 1, which showed their incidence and abundance in the leaf surfaces.

Table 1: Phylloplane fungi isolated from two mangrove plants, *Avicennia officinalis* and *Bruguiera cylindrica* by two methods.

Sl. No.	Phylloplane fungi	Moist chamber		Agar plate	
		Avicennia	*Bruguiera*	*Avicennia*	*Bruguiera*
1	*Absidia spinosa*	+		+	
2	*Alternaria alternata*	+	+		
3	*Aspergillus awamori*	+	+	+	+
4	*Aspergillus flavipes*		+		
5	*Aspergillus flavus*	+		+	
6	*Aspergillus fumigatus*	+	+		
7	*Aspergillus japonicus*				+
8	*Aspergillus nidulans*	+			
9	*Aspergillus niger*	+	+	+	
10	*Aspergillus ochraceus*				+
11	*Aspergillus terreus*	+			
12	*Aspergillus versicolar*				+

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13	*Aureobasidium pullulans*		+		+
14	*Chaetomium* sp.	+			
15	*Cladosporium herbarum*	+	+	+	
16	*Cladosporium* sp.		+		
17	*Colletotrichum* sp.			+	
18	*Curvularia lunata*	+	+		+
19	*Drechslera* sp.		+	+	+
20	*Fusarium oxysporum*	+	+	+	+
21	*Fusarium* sp.	+		+	
22	*Gliocladium* sp.		+		
23	*Helminthosporium* sp.	+	+		
24	*Humicola* sp.	+			
25	*Neurospora* sp.	+			
26	*Penicillium chrysogenum*				+
27	*Penicillium digitatum*			+	
28	*Penicillium fellutanum*	+	+		
29	*Penicillium oxalicum*			+	
30	*Rhizopus stolonifer*	+	+		+
31	*Ulocladium* sp.	+	+		
32	Grey sterile mycelia			+	+
33	White sterile mycelia	+	+	+	+
33/19 = 25/17 Av, 22/14 Br.		20/14	17/13	13/9	12/9

Fig 1: Total number of phylloplane fungi recorded from the mangrove plants by two methods

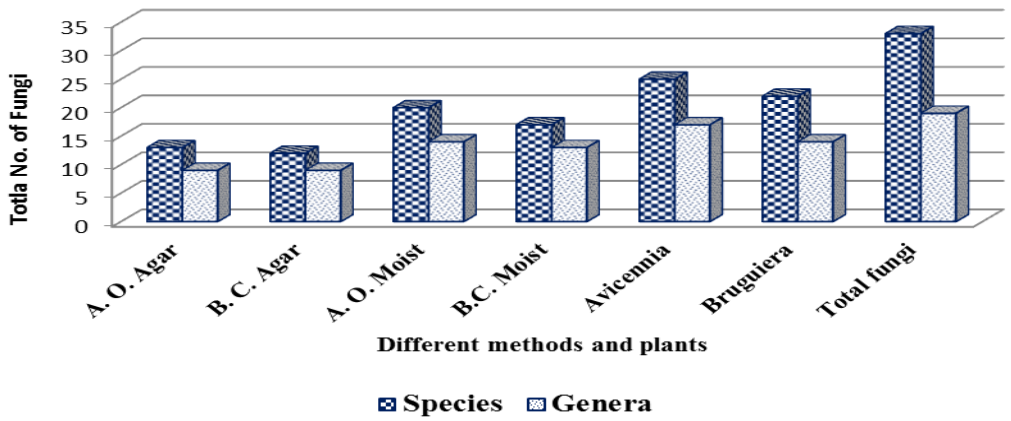
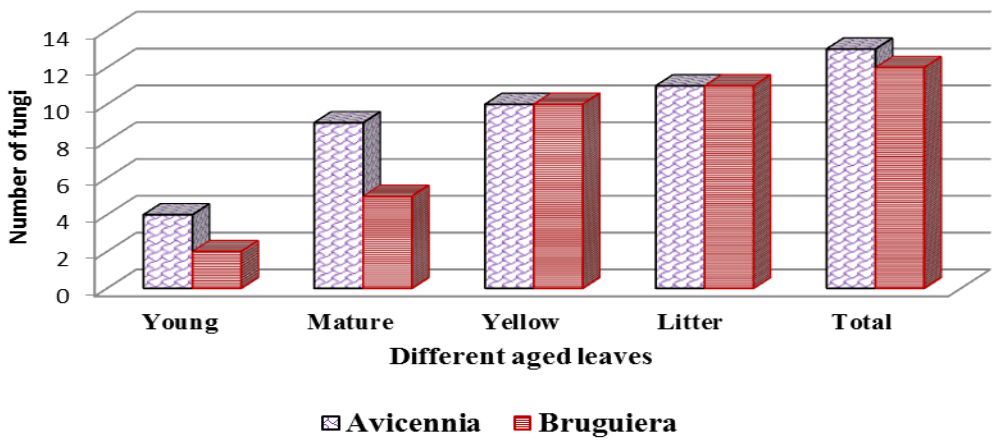
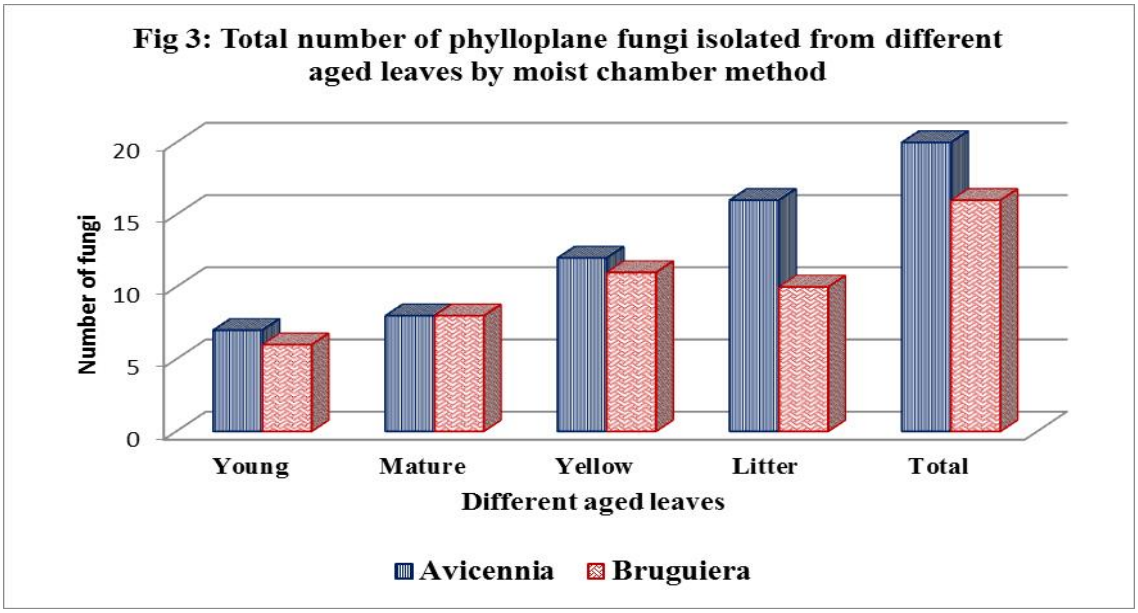


Fig 2: Total number of phylloplane fungi isolated from different aged leaves by agar plate method





The Present study revealed that despite the variation in physical, chemical and phenological properties in the leaf types of two mangrove plants, the fungal species isolated were more or less, similar and common [14]. Further investigations on the endophytic and epiphytic fungal species compositions associated with the same host leaves at other sites or during different seasons and increased sampling efforts could yield more fungal taxa and could further clarify the effect of host leaf on the fungal populations. The number of fungal taxa found without the leaf washing method was similar to some previous studies on *Acalypha indica* [15]. These studies might, however, include some endophytes, as they also recorded the fungi found on the washed leaves. Fungal taxa found in this study were also different from the study of *Solanum nigrum, Pongamia pinnata* and *Andrographis paniculata* in India [16, 17, 18], perhaps due to the variation in technique as a leaf impression method was used in the Indian study. Both of these previous studies are likely to only reveal the casuals, but not the true phylloplane fungi from the mangroves. We found that direct observation using the light microscope is the most suitable method for studying the abundance and species richness of true phylloplane fungi, while the leaf washing method is suitable for qualitative studies only.

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