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Enumeration and identification of endophytic fungi from various aged leaves of mangrove plant species, *Bruguiera cylindrica*

R. Anandhu[1], B K Nayak[1]* and Anima Nanda[2]

[1]Department of Botany, K. M. Centre for P.G. Studies (Autonomous), Lawspet, Pondicherry, India
[2]Department of Biomedical Engineering, Sathyabama University, Rajiv Gandhi Salai, Chennai - 600119, India

Abstract : Mangrove forests play an important role in tropical and subtropical coastal ecosystems. Endophytic fungi are widely distributed in various ecosystems and have great contribution to global biodiversity. In order to better understand the effects of mangrove species and tissue types on endophytic fungal community, we investigated cultivable endophyticand phylloplane fungi in different aged leaves of mangrove plant viz.,*Bruguieracyl indrica* by employing moist chamber and agar plate methods. The four types of aged leaves had similar overall colonization rates of endophytic and phylloplane fungi. The colonization rates of endophytic fungi were higher in litter leaves followed by yellow, mature and young ones. A total of 23 endophytic fungal taxa were identified under 16 genera based on morphological characteristics. Fungi belongs to Deuteromycetes were found higher in their occurrence in all the leaf samples followed by Zygomycetes and Ascomycetes. *Aspergillus* was dominated followed by *Phoma, Curvularia, Colletotrichum, Drechslera, Ulocladium, Helminthosporium* and white sterile mycelia. The diversity of phylloplane fungi were more in comparison to endophytic fungi in all the leaves of the plant species. Some endophytic fungi showed host and tissue preference. The endophytic fungal community composition was different among four leaves and between the moist chamber and agar plate methods.
Key words : Phylloplane, Endophytic fungi, Mangrove, Diversity, Aged leaves, *Bruguiera cylindrica.*

Introduction

Fungi are the cluster of individuals has a boundless biodiversity. They are the largest group of microbes after insects and the key component of tropical ecosystems throughout the world. They are prevailing in most of the plant parts, especially the leaves, where the tissue is apparently healthy. They may be endophytes, ectophytes or latent pathogens. Endophytes are microorganisms who are present in living tissue of various plants (root, fruit, stem, seed, leaf etc.) establishing mutual relationship without apparently any symptom of diseases [1, 2]. These endophytes guard their hosts from infectious causes and adverse conditions by secreting bioactive secondary metabolites [3, 4]. The endophytic fungi play important physiological and ecological roles in their host life. The recent investigations have been exaggerated by the potentialities of endophytic fungal strains in the production of bioactive metabolites like taxol, pestaloside, torreyanic acid and enzymes, i.e.,Xylanase**,** Isoflavonoids**,** sparaginase[5,6,7,8]. Natural products from entophytic microorganisms have been observed to inhabit or kill a wide variety of harmful disease causing agents but not limited to phytopathogens, as well as

bacteria, fungi, viruses and protozoan that affect humans and animals. Endophytic fungi are also capable to produce antimicrobial metabolites. The production of Hypericin, a naphthodianthrone derivative and Emodin believed to be the main precursor of hypericin, by the endophytic fungus isolated from an Indian medicinal plant, having an antimicrobial activity against several bacteria and fungi, including *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella enteric and Escherichia coli, and fungal organisms Aspergillus niger and Candida albicans*[9].Other compound polyketidecitrinin produced by endophyticfungus,*Penicillium janthinellum* from fruits of *Melia azedarach*, presented 100% antibacterial activity against *Leishmania*sp.[10, 11]. Medicinal plants are well known to harbor endophytic fungi that are believed to be associated with the production of pharmaceutical products[12,13,14, 15,16].Henceforth it is important to explore endophytic mycoflora of different plants in particular to mangrove, where more noble compounds would to come out. The present study was carried out to isolate and identify of endophytic and phylloplane fungi from *Bruguiera cylindrica* collected from Murugampakkam, Ariyankuppam and Nonankuppam, Pondicherry- 605008, India.

Materials and Methods

Collection of leaf sample

Different leaves of the mangrove plant, *Bruguiera cylindrica* were collected in fresh condition from Murugampakkam, Ariyankuppam and Nonankuppam, Puducherry- 605008. Young, Mature, Yellow and Litter leaves were brought to the Microbiology Laboratory, Department of Botany with utmost care, carefully segregated and kept in room temperature for further experiments.

Description of the plant

Binomial name**:***Bruguieracylindrica*
Family : Rhizophoraceae
Common name : Whiteburma mangrove
Vernacular name : Kaakkandal(Tamil name)

Bruguiera cylindrica

Bruguiera cylindrica is a small tree growing up to 20 meters tall but often grows as a bush. The bark is smooth and grey, with corky raised patches containing lenticels which are used in gas exchange and the trunk is buttressed by roots. The aerial roots or pneumatophores project from the soil in knee-shaped loops and have many lenticels which allow air into the interconnecting roots while excluding water. The roots spread out widely to provide stability in the waterlogged soil. The glossy green leaves are opposite, simple and elliptical with pointed ends. The flowers are in small bunches of 2-5 in the axils of the leaves. They have 8 long green sepals and 8 smaller, greenish-white petals with several little bristles on the tip. The flowers are pollinated by insects and release a cloud of pollen when probed at the base by the insect's mouthparts. The seed does not detach itself from the flower stalk but germinates where it is and is known as a propagule. It grows into a slightly curved cylinder up to 15 cm (6 in) long, with the upturned calyx still attached, and looks rather like a slender, dangling cucumber.https://en.wikipedia.org/wiki/Bruguiera_cylindrica - cite_note-Bakau-2 The propagules later drop off and float horizontally at first. The roots (lower part) absorb water and become heavier and after a few weeks the propagules float vertically and are ready to root into the substrate.*Bruguiera cylindrica* can be confused with *Bruguieragymnorhiza*, but that has larger, red flowers and red sepals which remain attached to the propagules, which is a straight cylinder in shape rather than being slightly curved.

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 segmentsof 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. 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 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.

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 [17,18]. 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 23endophytic fungal species under 16 genera were isolated and identified from the healthy leaf of the mangrove plant, *Bruguiera cylindrica* by employing both agar plate and moist chamber methods. 16 endophytic fungal species of 12 genera were isolated by agar plate method, whereas, by moist chamber method, 18 species of 13 genera were recorded. *Aspergillus awamori*, *Aspergillus niger*, *Aspergillus flavipes*, *Aspergillus flavus*, *Phoma* sp., *Cuvularia lunata*, *Drechslera*sp., *Fusarium oxysporum*, *Penicillium fellutanum*, *Ulocladium sp.*, *Helminthosporium sp.*, *Rhizopus stolonifer* , and three sterile forms viz., white sterile mycelia, pink sterile mycelia and grey sterile mycelia species were identified as endophytes. *Aspergillus awamori*, *A. fumigatus*, *Cladosporium* spp, *Gliocladium* sp.,*Helminthosporium* sp.and *Ulocladium* sp.were isolated by moist chamber method only. Likewise, Grey and pink sterile mycelia, *Aspergillus versicolar* and *Aspergillus tamari* were recorded exclusively by agar plate method. Incidence of endophytic fungi recorded from the different leaf samples of *Bruguiera cylindrica* by moist chamber method is given in Table1and by agar plate method in Table 2.Litter leaves harbored more number of endophytic fungi

than other leaves like yellow, mature, young due to the saprophytic nature of the fungi to get attraction towards decomposed leaf litter (Fig 1). Total number of endophytic fungi isolated from different aged leaves by moist chamber and agar plate methods in Fig 1.Moreover it is opined by previous authors that saprophytic fungi are more prominent and available in leaf litter than other leaves [13,14]. 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. Moreover it was seen that the growth of endophytic fungi was very slow in the moist chamber than the agar plate method. All obligate 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.

Table 1: Incidence of endophytic fungi isolated by moist chamber method from the mangrove plant, *Bruguiera cylindrica.*

Sl. No.	Fungal species	Different aged leaves			
		Young	Mature	Yellow	Litter
1	*Aspergillus awamori*	+	+	-	-
2	*Aspergillus flavipes*	-	+	-	-
3	*Aspergillus flavus*	-	+	-	+
4	*Aspergillus fumigatus*	-	+	-	-
5	*Aspergillus niger*	-	+	+	+
6	*Aureobasidium pullulans*	-	-	+	+
7	*Cladosporium herbarum*	-	+	+	+
8	*Cladosporium* sp.	+	-	-	-
9	*Curvularia lunata*	+	-	-	+
10	*Drechslera* sp.	+	-	+	+
11	*Fusarium oxysporum*	-	-	+	+
12	*Gliocladium* sp.	-	+	+	+
13	*Helminthosporium sp.*	-	-	+	+
14	*Penicillium fellutanum*	-	+	+	-
15	*Phoma* sp.	-	-	+	+
16	*Rhizopus stolonifer*	-	-	+	+
17	*Ulocladium* sp.	+	-	+	+
18	White sterile mycelia	-	-	+	+
Total		5	8	12	13

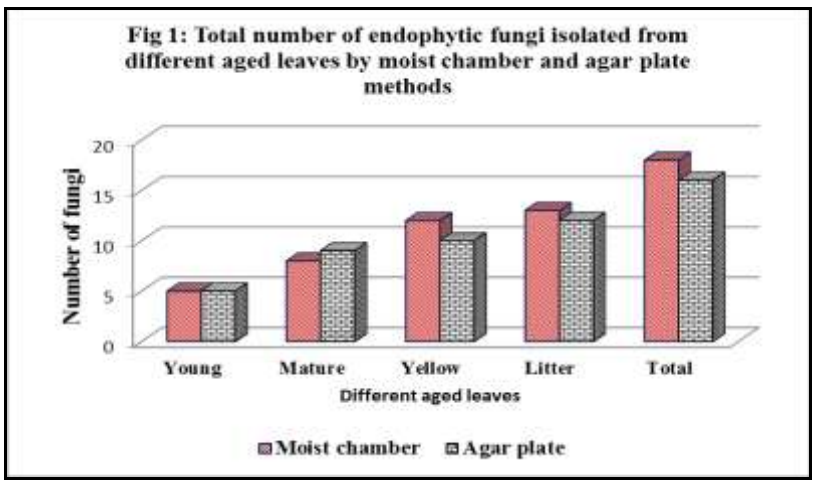

Table 2: Incidence of endophytic fungi isolated by agar plate method from the mangrove plant, *Bruguiera cylindrica.*

Sl. No.	Fungal species	Different aged leaves			
		Young	Mature	Yellow	Litter
1	*Aspergillus flavipes*	+	+	-	-
2	*Aspergillus flavus*	-	-	+	+
3	*Aspergillus niger*	-	+	+	-
4	*Aspergillus versicolar*	+	-	-	+
5	*Aspergillus tamari*	-	-	+	+
6	*Aureobasidium pullulans*	-	-	+	+
7	*Candida* sp.	-	+	-	+
8	*Curvularia lunata*	-	+	+	+
9	*Drechslera* sp.	-	-	+	+
10	*Fusarium oxysporum*	-	-	+	+
11	*Penicillium fellutanum*	+	+	-	-
12	*Phoma* sp.	-	+	+	+
13	*Rhizopus stolonifer*	-	-	+	+
14	Grey sterile mycelia	-	+	-	+
15	Pink sterile mycelia	+	+	-	-
16	White sterile mycelia	+	+	+	+
Total		5	9	10	12

Endophytic organisms have received considerable attention as they are found to protect their host against pest pathogens and even domestic herbivorous[15]. Most of the isolated fungi belonged to anamorphic fungi in particular to Deuteromycetes and Ascomycetes[16]. The recorded fungi of the endophytic origin may lead to the production of special compound within the host plant [13]. Fungi are 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 [15]. *Bruguiera cylindrica* is a plant having a broad spectrum of medicinal properties. Isolation of only23 taxa of endophytic fungi showed that the medicinal property of the plant has some role to play in the colonization of fungi[16]. Here we found a high rate of colonization may be attributed to secretion of the phyto-chemicals since they contain antifungal and anti-bacterial compound[19]. During the present study selection of theisolates, was based on the maximum number of fungi have been widely known as a source of bioactive components from the mature leaf samples of *Bruguiera cylindrica*. It was inferred that 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[2,13,14].

Conclusion

Fungi are used as medicinal agents with natural products once serving as the basic source of most of the diseases. *Bruguiera cylindrica* is a mangrove plant having broad spectrum medicinal properties used in this research for the isolation of endophytic fungi, and a total of23 fungal species of 16 genera were recorded in moist chamber and agar plate methods. The isolated fungi belonged to the class deuteromycetes, Zygomycetes and ascomycetes. Among the endophytic fungal population, *Aspergillus* was dominated followed by *Phoma, Curvularia, Colletotrichum, Drechslera, Ulocladium, Helminthosporium* and white sterile mycelia. Moist chamber method was superseded the agar plate method in order to isolate and identify endophytic fungi. The future work relating to segregation endophytic fungi from plant portions may kindly be approved out by moist chamber procedures than agar plate method.

References

1. NayakB. K., Endophytic fungal enumeration from various leaf samples of a medicinal plant: Ziziphusmauritiana, Int. Journal of PharmTech Res. 2014,7(2),344-348.
2. PetriniO., Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS, eds. *Microbial Ecology of Leaves*. New York: Spring Verlag, 1991, 179-197.
3. NayakB. K, and R. Anandhu, Biodiversity of Phylloplane and Endophytic Fungi from Different Aged Leaves of Medicinal Mangrove Plant Species, Avicennia marina, J. Pharm. Sci. & Res. 2017, 9(1), 6-9
4. Nayak B K, and Anandhu R. Comparative study of phylloplane fungi recorded from various aged leaves of two mangrove plant species collected from Puducherry coastal area.Res JPharm Bio ChemSc, 2017, 8(2), 543-548
5. StrobelG., Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms. *Journal of Natural Products*. 2004, 67, 257-268.
6. GunatilakaA A L, Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *Journal of Natural Products*. 2006, 69, 505-526.
7. VermaVC,Kharmar RN, Strobel GA. Chemical and functional diversity of natural products from plant associated endophytic fungi. *Natural Product Communications*. 2009, 4, 1511-1532.
8. StierleA.,Strobel G, Stierle D. Taxol and taxane production by *Taxomycesandreanae*, an endophytic fungus of Pacific yew.*Science*. 1993, 260, 214-216.
9. XuL., Zhou L, Zhao J, Jiang W. Recent studies on the antimicrobial compounds produced by plant endophytic fungi. *NaturalProduct Research and Development*. 2008, 20, 731-740.
10. SilviaF.,Sturdikova M, Muckova M. Bioactive secondary metabolites produced by microorganisms associated with plants.*Biologia*. 2007, 62, 251-257.
11. TanR X, Zhou WX. Endophytes: a rich source of functional metabolites. *Natural Product Reports*. 2001, 18, 448-459.
12. ZhangH. W., Song YC, Tan RX. Biology and chemistry of endophytes. *Natural Product Reports*. 2006, 23, 753-771.
13. NandaA, B. K. Nayak, Endophytic fungal community study of varied aging leaves of *Acalypha indica*.*Der Pharmacia Lettre*, 2015, 7 (5), 250-254
14. NayakB. K., Comparative assessment of two methods for isolation of endophytic fungi from varied leaves of *Andrographispaniculata, International Journal of ChemTech Research,* 2015, 7(4), 2085-2089
15. NayakB. K., Studies on endophytic fungal diversity from different leaf samples of Pongamiapinnata, Int Journal of MediPharm Res, 2015, 1,134-138.
16. NayakB. K., Enumeration of phylloplane and endophytic fungi from medicinal plant, *Solanumnigrum* by two different techniques.International Journal of Chemical Concepts. 2015, 1(3): 103-108.
17. EllisM B, J P Ellis. Microfungi on land plants, Biddles Ltd., Guildford and King'slynn, Great Britain. 1985.
18. OnionA H S, D Allsopp, HOW Eggins, Smith's introduction to industrial Mycology, London, Edward Arnold. 1986.
19. StrobelG., Daisy B., Castillo U. and Harper J. Natural products from endophytic microorganisms. Journal of Natural products, 2004, 67, 257-268.

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