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Isolation and identification of phylloplane and endophytic fungi from one ornamental plant, *Mangifera indica*

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Abstract : Biodiversity of phylloplane and endophytic fungi have been considered as one of the rich source of novel natural compounds of biological activities and a high level of structure diversity on the leaf surfaces. Bioactive components produced by phylloplane and endophytes have shown promising potentiality towards human health. In this juncture, it is necessary to understand and manipulate this important microbial resource and make it more beneficial for the mankind. In the present study isolation and enumeration of phylloplane and endophytic fungal diversity was carried out from one ornamental plant, *Mangifera indica* with the host relationship based on two methodologies, agar plate and moist chamber. Altogether, 17 fungal species were isolated from the mango plant. In agar plate method, *Cladosporium herbarum*, *Penicillium digitatum*, *Moniliella* sp., *Candida* sp., *Trichoderma* sp., *Aspergillus niger* were identified as phylloplane and *Aspergillus niger*, *Aspergillus flavus*, *Penicillium digitatum*, *Trichoderma* sp. were identified as endophytes. In moist chamber method, *Colletotrichum* sp., *Curvularia* sp., Aspergillus *niger*, *Stachybotrys* sp., *Trichoderma* sp., were identified as phylloplane fungi, *Aspergillus niger*, *Botrytis* sp., *Cladosporium resinae*, *Monascus*, Grey sterile mycelia, *Trichoderma* sp., *Sordaria fumicola* species were identified as endophytes. 6 species belonged to 4 genera of phylloplane and endophytic fungi were isolated and identified in agar plate, but by moist chamber method, 10 species belonging to 7 genera of phylloplane and endophytic fungi were isolated and identified. White sterile mycelia form was isolated from both methods only as phylloplane fungus and it was not isolated as endophytes. The evidence for host relative preference and tissue specification was found between the phylloplane and endophytes based on fungal community distribution and composition and thus the fungi isolated are dependent on the methodologies.
Key words: Phylloplane, Endophytic fungi, Biodiversity, Ornamental plant, *Mangifera indica*

Introduction

Vascular plants in general, harbor phylloplane and endophytic organisms. 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]. Endophytic fungi that are present in living tissue of varied plant parts viz., root, fruit, stem, seed, leaf etc. establishing a mutual relationship without making apparently any dysfunction or diseases in the host. Endophytic fungi are capable of living in host plant without causing any types of symptoms [2,3]. Endophytic fungi have been categorized into four classes based on host range type of tissue. 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[4]. 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]. Resistance to antimicrobial drugs used in the treatment of many infectious diseases not only of bacterial, but also of fungal origin is increasing which complicates and renders treatment of critical infectious illnesses more difficult. During the present study, isolation and identification of phylloplane and endophytic fungi were carried out from one ornamental plant, *Mangifera indica* collected from KMCPGS (Autonomous) campus, Lawspet, Pondicherry-605008, India.

Materials And Methods

Sample collection

Leaves of *Mangifera indica* were collected in fresh condition from our K. M. Centre for P. G. Studies (Autonomous) campus, Lawspet, Puducherry- 605008. Healthy and mature leaf samples 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 : *Mangifera indica*
Family : Anacardiaceae
Common name : Mango
Vernacular name : Mango

The **mango** is a juicy stone fruit belonging to the genus *Mangifera*, consisting of numerous tropical fruiting trees, cultivated mostly for edible fruit. The majority of these species are found in nature as wild mangoes. They all belong to the flowering plant family Anacardiaceae. The mango is native to South and Southeast Asia, from where it has been distributed worldwide to become one of the most cultivated fruits in the tropics. The center of biodiversity of the *Mangifera* genus is in India. It is the national fruit of India, Pakistan, and the Philippines, and the national tree of Bangladesh. In several cultures, its fruit and leaves are ritually used as floral decorations at weddings, public celebrations, and religious ceremonies. The mango trees in erect, 30 to 100 ft (roughly 10-30m) high, with a broad, rounded canopy which may, with age, attain 100 to 125 ft (30-38m) in width, or a more upright, oval, relatively slender crown.

Food value of mango

Mangoes are widely used in cuisine. Sour, unripe mangoes are used in chutneys, *athanu*, pickles, side dishes, or may be eaten raw with salt, chili, or soy sauce. A summer drink called *aam panna* comes from mangoes. Mango pulp made into jelly or cooked with red gram *dhal* and green chillies may be served with cooked rice. Mango lassi is popular throughout South Asia, prepared by mixing ripe mangoes or mango pulp with buttermilk and sugar. Ripe mangoes are also used to make curries. *Aamras* is a popular thick juice made of mangoes with sugar or milk, and is consumed with *chapatis* or *poors*. The pulp from ripe mangoes is also used to make jam called *mangada*. *Andhra aavakaaya* is a pickle made from raw, unripe, pulpy, and sour mango, mixed with chili powder, fenugreek seeds, mustard powder, salt, and groundnut oil. Mango is also used in Andhra to make *dahl* preparations. Gujaratis use mango to make *chunda*.

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 $1cm^2$) 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

Five (5) leaf segments of a centimeter square, both sterile an 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

Same like agar plates, five (5) leaf 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.

Isolation of fungi

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 Amoxillin as well as in 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. All the plates were incubated at 25±3ºC temperature in the incubation chamber. Incubation time was maintained differently since, 7-8 days is meantt 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 [7,8,9,10]. 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

During the present study, altogether 17 fungal species under 14 genera were isolated and identified from the healthy leaf of the mango plant, *Mangifera indica* by employing both agar plate and moist chamber methods. This plant were screened for the presence of phylloplane and endophytic fungi, of which 6 species belonged to 4 genera of phylloplane and endophytic fungi were isolated and identified in agar plate method but by moist chamber method, 10 species belonging to 7 genera of phylloplane and endophytic fungi were isolated and identified (Fig 1). Endophytic and Ectophytic/phylloplane fungi isolated from *Mangifera indica* is given in fig 1. In agar plate method, *Cladosporium herbarum*, *Penicillium digitatum*, *Moniliella* sp., *Candida* sp., *Trichoderma* sp., *Aspergillus niger* were identified as phylloplane and *Aspergillus niger*, *Aspergillus flavus*, *Penicillium digitatum*, *Trichoderma* sp. were identified as endophytes. in moist chamber method *Colletotrichum* sp., *Curvularia* sp., *Aspergillus niger* , *Stachybotrys* sp., *Trichoderma* sp., were identified as phylloplane fungi, *Aspergillus niger*, *Botrytis* sp., *Cladosporium resinae*, *Monascus*, Grey and white sterile mycelia, *Trichoderma* sp., *Sordaria fumicola* species were identified as endophytes. Total number of isolated phylloplane and endophytic fungi recorded from the mature leaf of *Mangifera indica* by two different methods are given in Table1. 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.

Table 1: Comparison of isolated fungi by agar plate and moist chamber methods from ornamental/medicinal plant, *Mangifera indica*.

Sl. No.	Name of the fungi	Agar plate method		Moist chamber method	
		Endophytic fungi	Ectophytic fungi	Endophytic fungi	Ectophytic fungi
1	*Aspergillus niger*	+	+	+	+
2	*Aspergillus flavus*	+	_	_	_
3	*Botrytis* sp.	_	_	+	+
4	*Candida* sp.	_	+	_	_
5	*Cladosporium herbarum*	_	+	_	+
6	*Cladosporium resinae*	_	_	+	+
7	*Colletotrichum* sp.	_	_	_	+
8	*Curvularia* sp.	_	_	_	+
9	Grey sterile mycelia	_	_	+	_
10	*Monascus* sp.	_	_	+	_
11	*Moniliella* sp.	_	+	_	_
12	*Penicillium citrirum*	_	+	_	+
13	*Penicillium digitatum*	+	_	_	_
14	*Sordaria fumicola*	_	_	+	_
15	*Stachybotrys atra*	_	_	_	+
16	*Trichoderma* sp.	-	+	-	+
17	White sterile mycelia	-	+	-	+

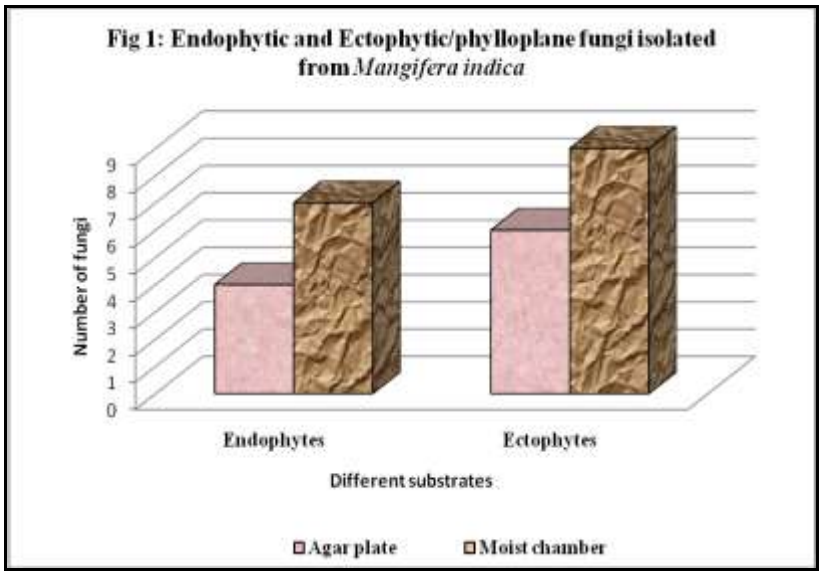

Fig 1: Endophytic and Ectophytic/phylloplane fungi isolated from *Mangifera indica*

Discussion

Endophytic and ectophytic organisms have received considerable attention as they are found to protect their host against pest pathogens and even domestic herbivorous. Most of the isolated fungi belonged to anamorphic fungi in particular to Deuteromycetes and Ascomycetes [11]. The isolated fungi of the endophytic and phylloplane origin may lead to the production of special compound within the host plant [6]. 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 occure only in the plant [12]. *Mangifera indica* 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 17 taxa of phylloplane and endophytic fungi showed that the medicinal property of the plant has some role to play in the colonization of fungi[12]. This low rate of colonization may be attributed to secretion of the phyto-chemicals since they contain antifungal and anti-bacterial compound[13]. In the present study selection of the

isolates, was based on the maximum number of fungi have been widely known as a source of bioactive components from the mature leaf samples of *Mangifera indica*. It was inferred that 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[11,12]. Moreover it was seen that the growth of endophytic and phylloplane fungi was very slow in the moist chamber than the agar plate method [14,15]. 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 presence of moisture than the agar plates where no humidity is prevailed[12].

Conclusion

Fungi have long been used as medicinal agents with natural products once serving as the basic source of most of the diseases. *Mangifera indica* is a ornamental plant with having broad spectrum medicinal properties. In the present study totally 15 fungal species were recorded in moist chamber and agar plate. The isolated fungi belonged to the class deuteromycetes and hyphomycetes. Among the phylloplane and endophytic fungal population, *Aspergillus niger, Trichoderma* sp. were the most dominant fungal species in both agar plate and moist chamber method. The data suggested that smaller and the more scattered plant fragment samples, the higher the probability of approaching real diversity values of phylloplane and endophytic fungal communities. *Aspergillus niger, Trichoderma* sp. isolated and reported frequently from the leaves of *Mangifera indica*. It was confirmed from the present study that, moist chamber method was superior than the agar plate method in order to isolate phylloplane and endophytic fungi from plant materials. The future work pertaining to isolation endophytic fungi from plant parts may kindly be carried out by moist chamber methods than agar plate method.

References

1. Leben C. Epiphytic microorganisms in relation to plant diseases. Annu. Rev. Phytopathol, 2 (1965) 209-230.
2. Norse D. Fungal populations of tobacco leaves and their effect on the growth of *Alternaria longipes*. Trans Br Mycol Soc. 59 (1972) 261-271.
3. Nakkeeran S., Krishnamoorthy A.S., Ramamoorthy V. and Renukadevi. Microbial inoculants in plant disease control. J. Eco. Boil. 14 (2002) 83-94.
4. Strobel G.A. Endophytes as source of bioactive product. Micro infects 5 (2003) 535-544.
5. Azevedo J. L., Ereira J. O. P. and Araiyo W. L. Endophytic microorganism: A review on insect control and recent advances on tropical plants Electronic journal of Biotechnology, 3(1) (2002) 40-65.
6. Carroll G. C. and Caroll F. E. Studies on the incidence of coniferous needle endophytes in the Pacific North-West. Can. J. Bot., 56 (1978) 3032-3043.
7. Ellis M B, J P Ellis. Microfungi on land plants, Biddles Ltd., Guildford and King'slynn, Great Britain. 1985.
8. Onion A H S, D Allsopp, HOW Eggins, Smith's introduction to industrial Mycology, London, Edward Arnold. 1986.
9. Ellis M B. Dematiaceous Hyphomycetes, Commonwealth Mycological Institute, Kew, 1971.
10. Ellis M B. More Dematiaceous Hyphomycetes, Commonwealth Mycological Institute, Kew, 1976.
11. Nayak B K. Studies on endophytic fungal diversity from different leaf samples of *Pongamia pinnata*, Int Journal of MediPharm Res, 1 (2015) 134-138.
12. Nayak B K. Endophytic fungal enumeration from various leaf samples of a medicinal plant: *Ziziphus mauritiana*, Int. Journal of PharmTech Res. 7(2) (2014) 344-348
13. Strobel G and Daisy B. Bioprospecting for microbial endophytes and their natural productes. Microbial Molecule biolo Rev. 67 (2003) 491-502.
14. Strobel G., Daisy B., Castillo U. and Harper J. Natural products from endophytic microorganisms. Journal of Natural products, 67 (2004) 257-268.
15. Strobel G.A. Endophytes as source of bioactive product, Micro infects, 5 (2003) 535-544.

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