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Studies on endophytic fungal diversity from different leaf samples of *Pongamia pinnata*

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Abstract: Biodiversity of endophytic fungi of different leaf samples viz., young, mature, dry, yellow and infected of *Pongamia pinnata* was carried out in the Microbiology laboratory, K. M. Centre for P. G. Studies (Autonomous), Pondicherry during 2011. Agar plate and moist chamber techniques were used to isolate the endophytic fungi. During the study period, a total of 15 fungi were isolated under 13 genera from both agar plate and moist chamber method of which, 13 species of 10 genera were recorded from agar plate and 15 species of 12 genera were recorded from moist chamber. Incidence of endophytic fungi isolated from *Pongamia pinnata* in agar plate was less than moist chamber. It was found that, white sterile mycelia was recorded in all the leaf samples starting from young to infected of the medicinal plant *Pongamia pinnata*. In agar plate technique, *Alternaria alternata, Cladosporium cladosporiodes, Penicillium funiculosum*, white sterile mycelia, *Curvularia lunata*, green sterile mycelia were recorded from all the leaf samples. In moist chamber, *Alternaria alternata, Curvularia lunata, Colletotrichum falcatum* sterile mycelia were recorded in all the leaf samples. It showed that infected and yellow leaves of the plant harboured maximum number of endophytic fungi followed by mature and young leaves. Moist chamber method was suitable to isolate and record the endophytic fungi correctly in comparison to agar plate method. White sterile mycelia and *Curvularia* were predominant in the agar plate method. But in moist chamber technique, *Colletotrichum* sp., *Curvularia*, *Penicillium citrinum* and white sterile mycelia were predominant.
Keywords: Endophytic fungi, Medicinal plant, *Pongamia pinnata*, Moist chamber.

Introduction

Fungi are ubiquitous and morphologically diverse in nature, they occur in wide spectacular array of shapes, sizes and colors. They have unique physiological and biochemical properties. Studies on endophytic fungi over the past twenty years indicate that they occupy a unique ecological niche and are thought to influence plant distribution, Ecology, Physiology and Biochemistry. There is a general call for new antibiotics, chemotherapeutic agents and agrochemicals that are highly effective possess low toxicity, and all have a minor environmental impact. Endophytes commonly refer to a group of fungi that reside asymptomatically inside the living Plant tissues. At least one million species of endophytic fungi which make as an important genetic resource for the biotechnological work globally [1,2]. Endophytes have been recognized as potential source of novel natural products for pharmaceutical, agricultural and industrial uses, especially those secondary metabolites produced by fungal endophytes colonizing medicinal plants. Among the phyllosphere and phylloplane fungi, a few works on ectophytic and endophytic fungi are known so far but the fungal endophytes, which are ubiquitous, diverse and ecologically specialized group of fungi that grow asymptotically within aerial plant tissue such as leaves and stems [2,3,4]. A number of works pertain to endophytic fungi of different medicinal plants in and around of India were carried out by various workers, but there is no work in the same field in

Puducherry State. The present work is an attempt to screen out and identify the endophytic fungi by employing two techniques from one medicinal plant, *Pongamia pinanta* collected from our P.G. Centre campus, Puducherry, India.

Materials and Methods

Collection of plant samples

The leaf samples viz., young, mature, infected, yellow and dry of the medicinal plant, *Pongamia pinanta* were carefully chosen, collected from the KMCPGS campus, Pondicherry and brought to the Microbiology Laboratory, Department of Botany in aseptic condition and kept in the refrigerator at 4-8^{o}C up to the completion of the experiment.

Isolation of endophytic fungi

The leaf samples were rinsed gently in running tap water to remove dusts and debris. The leaves were cut into segments (0.5 – 1cm). The samples were surface sterilized by modified method [5]. The samples were immersed in 70% ethanol for 5 seconds, followed by 4% sodium hypochlorite for 90 seconds and then rinsed in sterile distilled water for 10 seconds/ three times in a way. The excess moisture was blotted in a sterile filter paper. The surface sterilized segments were placed in Petridishes containing PDA medium as well as in moist chamber plates. The Petridishes were sealed using parafilm and incubated at 25 ± 3^{o}C at 12-h light/dark cycle. After incubation of three day, the Petridishes were monitored every day to check the growth of endophytic fungal colonies from the segments and were identified separately based on the availability of Laboratory manuals and references. The sterile endophytes i.e., the non-sporulating sterile forms that could not be assigned to any taxonomic group were given separate numbers and maintained in pure cultures. They were distinguished from each other by their cultural characteristics such as colony morphology, growth rates, hyphal mat characteristics and pigmentation of the fungal colony and medium. All the endophytic isolates were documented and maintained in PDA slants. Tables and figures were made based on the presence and absence of endophytic fungi on leaf samples.

Results and Discussion

Isolation, Culture, and Identification of different Endophytic fungi were made from one Medicinal plant viz. *Pongamia pinnata*. Altogether 15 Endophytic fungal species were isolated under 13 genera from both Agar plate and Moist chamber method of which, 13 species of 10 genera were recorded from Agar plate and 15 species of 12 genera were recorded from moist chamber. Incidence of Endophytic fungi isolated from Pongamia pinnata in Agar plate is given in Table 1. Table 2 showed the incidence of endophytic fungi from moist chamber. It was found that, white sterile mycelia was recorded in all the leaf samples starting from young to infected of the medicinal plant *Pongamia pinnata*. In Agar plate (Table1), *Alternaria alternata, Cladosporium cladosporiodes, Penicillium funiculosum*, White sterile mycelia, *Curvularia lunata*, Green sterile mycelia were recorded in all the leaf samples. Starting from young to infected of the Medicinal plant *Pongamia pinnata*. In moist chamber, *Alternaria alternata, Curvularia lunata, Colletotrichum falcatum*, White sterile mycelium were recorded in all the leaf samples. Starting from young to infected of the Medicinal plant *Pongamia pinnata*.

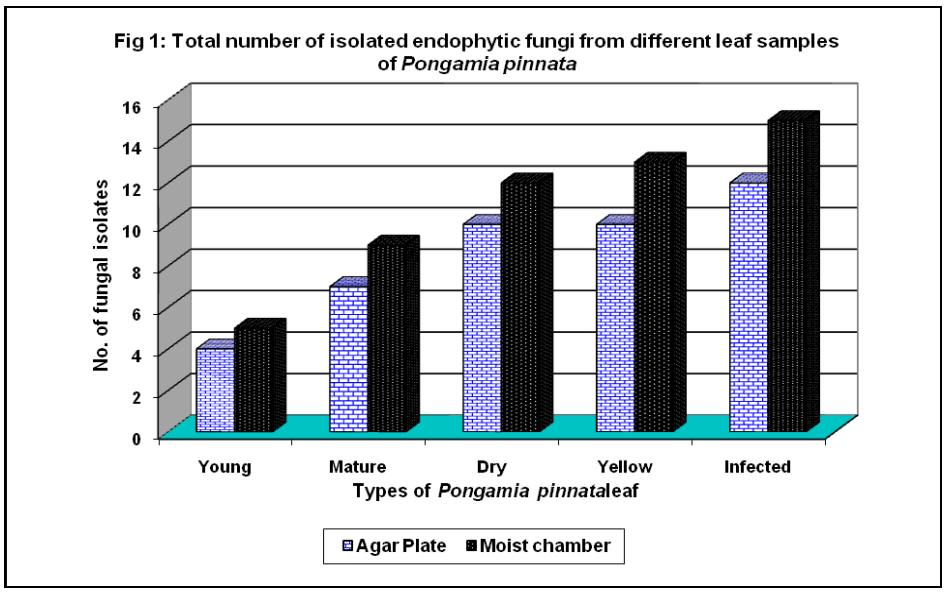
Total number of endophytic fungi isolated from different leaf samples of *Pongamia pinnata* by two methods is given in Fig 1. It showed that infected and yellow leaves of the plant harbored maximum number of endophytic fungi followed by mature and young leaves. Moist chamber method was suitable to isolate and record the endophytic fungi correctly in comparison to agar plate method. White sterile mycelia and *Curvularia* were predominant in the agar plate method. But in moist chamber technique, *Colletotrichum* sp., *Curvularia*, *Penicillium citrinum* and White sterile mycelia were predominant.

Table 1: Incidence of endophytic fungi isolated from different leaf samples of *Pongamia pinnata* by agar plate method.

Sl. No.	Endophytic fungi	Leaf samples				
		Young	Mature	Dry	Yellow	Infected
1.	Alternaria alternata	+	+	+	+	+
2.	Aspergillus fumigatus	_	_	+	+	+
3.	Aureobasidium sp.	_	_	+	_	+
4.	Cladosporioides sp.	_	+	+	_	+
5.	Curvularia lunata	+	+	+	+	+
6.	C. geniculata	_	_	+	-	+
7.	C. herbarum	_	+	+	+	+
8.	C. palescens	-	-	-	+	+
9.	Helminthosporium cyndontis	–	–	–	+	+
10.	Nigrospora sp.	_	+	+	+	+
11.	Pink sterile mycelium	_	_	_	+	_
12.	White sterile mycelium	+	+	+	+	+
13.	Green sterile mycelium	+	+	+	+	+

Table 2: Incidence of endophytic fungi isolated from different leaf samples of *Pongamia pinnata* by moist chamber method.

Sl. No.	Endophytic fungi	Leaf samples				
		Young	Mature	Dry	Yellow	Infected
1.	Alternaria alternata	+	+	+	+	+
2.	Aspergillus fumigatus	_	+	+	+	+
3.	Aureobasidium sp.	_	_	+	+	+
4.	Cladosporioides sp.	+	_	_	+	+
5.	Colletotrichum falcatum	+	+	+	+	+
6.	Curvularia lunata	+	+	+	+	+
7.	C. geniculata	_	_	+	_	+
8.	C. palescens	_	_	+	_	+
9.	C. catenulata	_	+	_	+	+
10.	Helminthosporium cyndontis	_	_	+	+	+
11.	Nigrospora sp.	_	+	+	+	+
12.	Trichoderma sp.	_	+	+	+	+
13.	Pink sterile mycelium	_	_	_	+	+
14.	White sterile mycelium	+	+	+	+	+
15.	Green sterile mycelium	_	+	+	+	+



The aim of this study was to establish any patterns in the distribution of endophytic fungal species from different leaf samples of *Pongamia pinnata as well as the succession of endophytic fungi* adhered to the leaves based on the ageing of the plant. The ultimate intent is to isolate widespread fungi that may be specific *Pongamia pinnata.* The approach to isolation may have influenced the results. Use of a severe surface sterilisation, while removing most epiphytes, may have also removed some vascular endophytes. In this experiment leaf segments were discarded as fungi were sub cultured from them. The purpose of this approach was to prevent fast growing fungi from overgrowing the plate and to prevent any other fungi present in the leaf segments contaminating the cultures. The approach selects for rapidly emerging and fast growing isolates and masks slow growing fungi. The approach also possibly reduces the potential for some fungi to form fruiting structures. Different fungi emerged from some leaf segments indicating that segments may be occupied by more than one fungus. Fungi that emerge late, and these were usually also slow growing, may have been discarded prior to detection. Endophytes are microorganisms, particularly fungi that live inside plant tissues without causing symptoms of disease[6]. They are a largely unexplored component of biodiversity, especially in the tropics. This study, in particular to the various leaves from the medicinal plant, *Pongamia pinnata* in the coastal region of Pondicherry region, was screened for diversity and composition of endophytic fungal communities[7]. Communities had a few abundant species and many species with few individuals. The fungal community from the *Guarea* population in a forest preserve was more diverse than that from a disturbed area. Fungal communities were stratified according to height within a tree, but no differences were found between blade, petiole, and rachis. The data suggest that the smaller and the more scattered the plant fragments sampled, the higher the probability of approaching real diversity values of endophytic fungal communities. *Alternaria alternata, Aspergillus, Cladosporidium* spp isolated from *Vinca rosea* is agreed with the previous workers who had also reported the same endophytic fungi in their study[6,7]. These common endophtes were isolated frequently from the leaves of medicinal plant. Petrini[8] reported that *Alternaria* spp, *Cladosporium* spp were not host specific fungi, but they used be recorded from tissues of different host plants. Certain endophytic fungi may be highly host specific while others are generally distributed[7]. Petrini and Carroll[9] contended that fungal endophytes exhibit some degrees of host specificity at least for families of host plant and that this specificity determines endophytic distribution more than the geographic location of the host plant. The occurrence of the endophytes is influenced by the age of leaf tissues[9]. Generally their colonization frequency and species richness increase with the age of leaf tissue[10], which was proved in our study since the endophytic flora generally increased according to the aging of the leaves. Studies on endophytic microbes over the past 25 years indicate that they occupy a unique ecological niche and are thought to influence plant distribution, ecology, physiology and biochemistry[10]. During the present study, Moist chamber method was found suitable to isolate and record the host specific as well as systemic endophytic fungi correctly in comparison to agar plate method.

Conclusion

Total number of endophytic fungi isolated from different leaf samples of medicinal plant, *Pongamia pinnata* plant by two methods showed that the infected and dry leaves of the plants harbored maximum number of endophytic fungi followed by yellow and mature. Moist chamber method was found suitable for isolation and enumeration of endophytic fungi correctly in comparison to agar plates. The highest value of similarity coefficient was found in moist chamber methods than the agar plate method.

References

1. Gangadevi V. and J. Muthumary. Preliminary studies on Cytotoxic effect on fungal taxol on cancer cell lines. African Journal of Biotechnology 6 (2007) 1382-1386.
2. Hawksworth DL. The magnitude of fungal diversity; the 1.5 million species estimate revisited. Mycological Research. 105 (2001) 1422-1432.
3. Vinton MA, ES. Kathol, KP Vogel and A.A Hopkins, 2001. Endophytic fungi in Canada wild rye in natural grasses lands.J.Range Manag. 54 (2001) 385-390.
4. Mehrotra, R. S. and K. R. Aneja. An Introduction to Mycology. New age international (P) limited publishers (1998) 613-637.
5. Schultz BS, U Wank, Draeger. and HJ Aust. Endophytes from herbaceous plants and shrubs: effecticveness of surface sterilization methods. Mycology Research 97 (1993) 1447-1450.
6. Lodge, D.J., P.J. Fisher and B.C. Button. Endophytic fungi of Manilkara bidentata leaves in Puerto Rico. Mycologia, 88 (1998) 733-738.
7. Suryanaryanan TS and V.Kumaresan. Endophytic fungi of some halophytes from an estuarine mangrove forest . Mycol. Res.104 (2000)1465 -1467.
8. Petrini O Fungal endophytes of tree leaves. In: Andrew JH Hirano SS (Eds.), Microbial ecology of leaves, Springer Verlag, New York. (1992) 179-197,
9. Petrini O and G. Carroll, 1981. Endophytic fungi in foliage of some Cuppressaceae in Oregon. Can. J. Bot. 59 (1981) 629-636.
10. Petrini O Ecological and physiological aspects of host-specificity in endophytic fungi. In. Redlin S.C., Carris L..M., eds. Endophytic Fungi in Grasses and Woody Plants, APS Press. St. Paul (USA). (1996) 87-100.

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