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Studies on endophytic fungi of commercially important tropical tree species in India

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ABSTRACT

India targets to increase the forest cover to 33% by 2020 through programmes like Tree outside Forests (TOF) and Tree Cultivation in Private lands (TCPL). Cultivation of fast growing tree species for fuel and fodder in cultivable wastelands and uncultivable barren land has been encouraged. While planting efforts are on full swing to increase green cover, however, it is reported that about 1 million ha of forest area is being destroyed by insect pests annually, hence management of insect pests in domesticated forestry is much needed. Synthetic organic insecticides have emerged as major tools in pest management. About 3% of the total pesticides used in the world are utilized in India. It has been reported that about 2.5 million tons of chemical pesticides are used on crops each year. The toxicity of these chemicals results in residues in soil, water resources and crops that affect public health, hence there is a need to develop ecologically sound, environmentally safe and economically viable insect pest management strategies. Biological control has become an important tool for Integrated Pest Management (IPM). Use of microorganisms for the management of insect pests and diseases is risk free. Endophytic fungi are microorganisms that offer great-untapped effective solution for insect pest management. Several endophytic microbes are known to have anti-insect properties. The fungal endophytes protect their hosts from infectious agents and adverse conditions through secretion of bioactive secondary metabolites. In the present study, an attempt was made to isolate and identify the diversity status of endophytic fungi from commercially important tropical tree species viz., *Tectona grandis* (Teak), *Ailanthus excelsa* (Ailanthus) and *Gmelina arborea* (Gmelina). A total of 18 species of endophytic fungi belonging to 13 genera and 58 strains were isolated from young and mature leaves of Teak, *Ailanthus* and *Gmelina*. Diversity and richness of endophytic fungi was found high in Teak followed by *Ailanthus* and *Gmelina.* Population of endophytic fungal species isolated from Teak, *Ailanthus* and *Gmelina* was found to be low and has not attained the expected population. It is also expressed in the rarefaction curve that it didn't reach asymptote curve, hence chances of encountering more number of species will be possible if the number of sampling unit increases. Of the 18 species, five species with one strain each were found to possess entomopathogenic significance based on preliminary bioassay studies. Future study will focus on biopesticidal properties of these fungi to develop novel biopesticide.

Keywords: Endophytic fungi, rhizosphere, phyllosphere, tropical tree species.

INTRODUCTION

The losses of crops and yield caused by insects are quite high due to which synthetic organic insecticides have emerged as chief major tools of insect pest management and about 3 per cent of the total pesticides used in the world are utilized in India and reported that about 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches at high cost annually due to the high toxicity to insect pests (www.indiastat.com). This leads to residual infiltration in soil, water resources and crops that affect public health. But still the pesticide consumption in India increased to manifold, accounting for 61 per cent of total consumption. However, a sharp reduction during 2011-2012 has been witnessed due to realization of the fact that the indiscriminate use of pesticides has created numerous problems, even causes more deaths than infectious diseases (Eddleston *et al*., 2002). A study of pesticide poisoning in South India demonstrated that two compounds, monocrotophos and endosulfan, accounted for majority of deaths (Srinivasa Rao *et al*., 2005). Therefore, there is a need to develop ecologically sound, environmentally safe and economically viable insect pest management strategies. New and useful compounds to provide protection to crop plants from insect pests and diseases are very much essential. Natural and biological control of insect pests and diseases affecting cultivated plants has gained considerable attention since it helps to reduce the use of chemical pesticides (Srinivasa Rao *et al*., 2005). Use of microorganisms that antagonize plant pathogens and insects as biological control agent results in enhancement of resident antagonist and is risk free. The microorganisms most frequently are from the rhizosphere or the phyllosphere, while few are also endophytes. Endophytic fungi offer great-untapped potential, which can be utilized for insect pest management (Schneider *et al*., 2008).

Many synthetic agricultural agents have been targeted for removal from the market, because of profound harmful effects on human health and environment. Thus, India needs newer group of biopesticides which are secondary metabolites that include thousands of alkaloids, terpenoids, phenolics and minor secondary chemicals, derived from plants, microbes, animals, and certain minerals. Perhaps endophytic fungi could serve as reservoir of untapped biological compounds that may present alternative ways to control farm insect pests and pathogens (Demain, 2000; Strobel, 2002a; b). Several endophytic microbes are known to have anti-insect properties which colonize living, internal tissues of plants without causing any harm to their host, protect the hosts from infectious agents and adverse conditions by eliciting bioactive secondary metabolites. For example, the secondary metabolites like peramine and nodulosporic acid isolated from the cultures of *Neotyphodium coenophialum*, *N. lolli*, *Epichloë festucae* and *E. typhina* associated with tall fescue were found to exhibit potent insecticidal properties against the larvae of the blowfly without any harmful impact on mammals (Demain, 2000). Endophytic fungus, *Muscodor vitigenus* isolated from *Paullina paullinioides*, was found to yield naphthalene as its major product, heptelidic acid and hydroheptelidic acid from *Phyllosticta* sp. have been shown to be toxic to spruce bud worm (*Choristoneura fumiferana*) larvae (Calhoun *et al.,* 1992). Endophytes are obviously a rich and reliable source of bioactive and chemically novel compounds with huge medicinal and agricultural potential. The quantity and diversity of microbial endophytes depends heavily on the plant species, the environment, and many other biotic and abiotic factors. Approximately 3,00,000 plant species that exist in unexplored area on the earth are host to one or more endophytes (Strobel and Daisy, 2003), however only handful of them are described (Guo *et al*., 2008).

Endophytic fungi offer great potential in plant protection, imparting tolerance against several biotic and abiotic stress factors. Endophytes of entomopathogenic significance may be exploited for crop protection from insect pests (Ren *et al*., 2008). Moreover, the metabolites responsible for the beneficial effect can be isolated and exploited. Structural elucidation of secondary metabolites will help in defining modes of action as well as in preparation of right formulations for field application. Standard protocols for isolation of bioactive molecules will be of great importance for production on large scale by fermentation technology. This will reduce the extra expenditure incurred in synthesis of chemical compounds. More plant species need to be explored for their endophytic fungi and their corresponding secondary metabolites. Endophytes that have not been investigated for their natural products so far should be studied for their bioactive metabolites in order to tap the rich biodiversity of endophytes. Hence, the present study aims at exploration, isolation, identification and diversity of endophytic fungi of *Tectona grandis, Ailanthus excelsa* and *Gmelina arborea* for entomopathogenic significance and use as novel biological control mechanism against the insect pests of forestry importance.

MATERIALS AND METHODS

Collection of plant samples

The leaves of *Tectona grandis, Ailanthus excelsa* and *Gmelina arborea* were collected from different parts of Tamilnadu (8.04° N to 21.50° N latitude and 76.40° E to 80.30° E Longitude). Each location and the geo-coordinates are given in the **Table 1**. At each location, trees free from insect pests and disease infestation were selected and marked. Healthy leaves from these healthy trees were collected and processed separately within 48 h of collection.

Rationale for tree selection

It is important to understand the methods and rationale used to provide the best opportunities to isolate novel endophytic microorganisms as well as ones making novel bioactive products. Plant selection strategies are as follows. (i) Plants from unique environmental settings,. (ii) Plants that have an ethnobotanical history, (iii) Plants that are endemic, (iv) Plants growing in areas of great biodiversity and (v) Plants that are of economic value.

Isolation and identification of endophytic fungi

The leaves were washed thoroughly in running water and segments of 1 cm^2 were cut from the midrib portion of each leaf and surface sterilized by immersing in 70% ethanol for 1 min, followed by 4% sodium hypochlorite (v/v) for 2 min, and finally washed in sterile water for 1min (Suryanarayanan *et al*., 1998). Each segment from each individual was placed in Petri dishes containing potato

Table 1. Samples collected from different locations in Tamilnadu.

Teak (*Tectona grandis*)	**GPS co-ordinates**	**Ailanthus (*Ailanthus excelsa*)**	**GPS co-ordinates**	**Gmelina (*Gmelina arborea*)**	**GPS co-ordinates**
Coimbatore- Boluvampatti	10º59'38.56" N 76º42'39.32" E	Coimbatore- Boluvampatti	10°58'13.58"N 76°43'32.60"E	Kothamangalam -Pudukottai	10 º18'56.3"N 79 º02'28.6"E
Jayankondam- Periyavalayam	11º11'43.09" N 79º24'27.52"E	Pudukottai-Varappur	10°28'19.6"N 79°00'49.3"E	Trichy	10º46'07.4"N 78º 8'54.6"E
Cuddalore – Kattumannarkoil	11º16' 32.81" N 79º 33' 08.67" E	Jeyankondam- Vettiyaarvettu	11°15'15.28"N 79°24'30.74"E	Sathyamangalam	77º6'50.8"E 11º36'37.4"N
Tanjore- Cholapuram	11º 03' 02.55"N 79º 24' 55.97" E	Karaikudi- Singampunari	10°11'06.33"N 78°25'34.43"E	Siruvani	76º42'05.4"E 10º57'03.8"N
Karaikudi- Thiripathur	10º 06' 32.41"N 78º 35' 47.57"E	Dindigul- Natham	10°15'59.47"N 78°05'30.55"E	Poondi hills	76º42'05.5"E 10º57'48.8"N
Dindigul-Natham	10º 15' 42.36"N 78º 06' 37. 46"E	Dharmapuri- Pennagaram	12°05'7.29"N 77°55'32.23"E	Keelapalayam- Ariyalur	79º25'19.2"E 11º28'03.0"N
Dharmapuri- Pennagaram	12º07' 40. 79"N 77º 53' 52. 22"E	Salem-Kurumpapatti	11°45'08.98"N 78°09'54.39"E	Kourtalam	77º13'02.6"E 08º56'35.4"N
Salem- Kurumpapatti	11º 45'11.11" N 78 º09' 51.47"E	Salem- Ayothiyapattanam	11°40'16.90"N 78°14'28.72"E	Kourtalam	77º12'36.5"E 08º56'37.7"N
Mudumalai- Theppakadu	11º 34' 32.38"N 76 º 34' 03.37"E	Thiruppur- Udumalaipettai	10°34'14.96"N 77°14'15.36"E	Kumuli	77º60'9.36"E 9º21'54.72"N
Mayiladudurai	11º 06'01.25"N 79 º39' 09.79"E	Viruthunagar- Kallikudi	9º40'45.50"N 77°57'59.29"E	Kodaikanal	77º31'42.2"E 10º15'18.7"N
Walajabad	12º 47' 43.7"N 79 º 48'24.2"E	Ramapuram – Melmaruvathur	12º 27' 58.0"N 79º 45' 38.5"E	Yercaud	11º46'45.66"N 78º11'50.90"E
Kanchipuram- Mettukadu	12 º 54' 56.2"N 79 º 43' 38.1"E	Pidagam	11°42'48.81"N 79°12'48.03"E	Ulunthurpettai	11º41'05.21"N 79º18'28. 42"E

dextrose agar (with chloramphenicol 150 mg l1). Two leaf segments were plated in each Petri dish and the dishes were sealed with parafilm and incubated in an incubator at 26 ± 1°C for 21 days. The efficacy of the sterilization procedure was ascertained with the method of Schulz *et al*. (2002). In addition, 10 ml of the last rinsing water were centrifuged for 10 min at 5000 rpm. The supernatant was removed and added 500 µl sterilized water in the centrifugal tube; 100 µl of this volume were then plated onto PDAS. The surface sterilization was validated because no mycelial growth occurred. The fungi that grew out from the segments were isolated and identified. Pure fungal cultures of the endophytic isolates were obtained by the hyphal tip method in test tube slants. Simultaneously, the isolates were stored in 15% (v/v) glycerol at - 80 °C in deep freezer (VWR Scientific) as spores and mycelium for further study.

RESULTS

Plants are associated with different kinds of microorganisms in different parts. The endophytic fungi are such microorganism, which live inter and intra-cellularly in plants without inducing pathogenic symptoms, while interacting with the host biochemically and genetically. These endophytic microorganisms may function as plant growth and defense promoters by synthesising phytohormones, producing biosurfactants, enzymes or precursors for secondary plant metabolites, fixing atmospheric nitrogen and CO_2, or controlling plant diseases, as well as providing a source for new bioactive natural products with utility in pharmaceutical, agrochemical and other Life Science applications. The use of endophytic microorganisms to control insect pests and pathogens is receiving increased attention as a sustainable alternative to synthetic pesticides and antibiotics. Furthermore, endophytes may be adapted to the presence and metabolism of complex organic molecules and therefore can show useful biodegradation properties. In order to reduce inputs of pesticides and fertilizers and add value to eco-friendly agriculture, it will be important to

Table 2. Endophytic fungi isolated from Teak, *Ailanthus* and *Gmelina* leaves collected at different locations in Tamilnadu.

Teak (Locations)	Endophytic fungal species	*Ailanthus* (Locations)	Endophytic fungal species	Gmelina (Locations)	Endophytic fungal species
Coimbatore-Boluvampatti	*Colletotrichum gleosporidies* 1 *Phoma eupyrena* *Phoma* sp. 1 *Alternaria* sp.1	Coimbatore-Boluvampatti	*Botryodiplodia theobromae* 3 *Phoma* sp.3 *Alternaria* sp.2	Kothamangalam -Pudukottai	*Alternaria* sp.4
Jayankondam-Periyavalayam	*Fusarium* sp.1	Pudukottai-Varappur	*Fusarium* sp.5 *Alternaria* sp.3	Tiruchi	*Phomopsis* sp.6
Cuddalore - Kattumannarkoil	*Pestalotiopsis* sp. 1	Jeyankondam-Vettiyaarvettu	*Pestalotiopsis* sp.2	Sathyamangalam	*Clasporium cladosporioids* 2
Tanjore-Cholapuram	*Fusarium* sp.2	Karaikudi-Singampunari	*Aspergillus niger* 4	Siruvani	*Phomopsis* sp.4
Karaikudi-Thiripathur	*Fusarium* sp.3	Dindigul- Natham	*Fusarium clamydosporium* 2	Poondi hills	*Phomopsis* sp. 5
Dindigul-Natham	*Fusarium clamydosporium* 1	Dharmapuri-Pennagaram	*Aspergillus fumigatus* 2	Keelapalayam-Ariyalur	*Aspergillus niger* 3
Dharmapuri-Pennagaram	*Aspergillus fumigatus* 1	Salem-Kurumpapatti	*Phomopsis* sp.2 *Botryodiplodia theobromae* 2 *Phoma* sp.4	Kourtalam	*Fusarium* sp.7
Salem-Kurumpapatti	*Botryodiplodia theobromae* 1 *Phomopsis* sp.1 *Phoma* sp. 2	Salem-Ayothiyapattanam	*Phomopsis* sp.3 *Aspergillus flavus* 6	Kourtalam	*Fusarium* sp.8
Mudumalai-Theppakadu	*Botryodiplodia theobromae* 2 *Colletotrichum gleosporidies*2 *Phyllostica* sp. *Clasporium cladosporioids* 1	Thiruppur-Udumalaipettai	*Aspergillus flavus* 5	Kumuli	*Aspergillus flavus* 7
Mayiladudurai	*Fusarium* sp.4	Viruthunagar-Kallikudi	*Fusarium* sp.6	Kodaikanal	*Phoma* sp. 5
Walajabad	*Nigrospora sphaerica* 2 *Aspergillus flavus* 3			Yercaud	*Aspergillus niger* 2
Kanchipuram-Mettukadu	*Nigrospora sphaerica* 1 *Phomopsis longicola* *Aspergillus flavus* 4				
Ramapuram – Melmaruvathur	*Aspergillus flavus* 1				
Pidagam	*Aspergillus flavus* 2 *Chloridium* sp. *Aspergillus niger* 5				
Ulunthurpettai	*Xylaria* sp. *Aspergillus niger*1				

develop inocula of biofertilizers, stress protection and biocontrol agents. Synthetic organic insecticides have emerged as chief major tools of pest management. However, due to indiscriminate use of synthetic chemicals, insect pests have developed resistance to insecticides, resurgence of secondary pests, reduction in the population of natural enemies, and harmful residues in food, feed and fodder. These concerns have led to the surge of alternative pest control technologies. The pesticide formulations based on chemicals from living organisms have attracted particular attention because of their specificity to insect pests, their biodegradable nature and a potential for commercialization. Hence, attempt has been made to isolate and identify endophytic fungi of entomopathogenic significance from commercially important native tree species viz., *Tectona grandis, Ailanthus excelsa* and *Gmelina arborea* to develop novel insecticides.

Diversity of endophytic fungi

A total of 18 species of endophytic fungi belonging to 13 genera and 58 strains were isolated from young and mature leaves of Teak, *Ailanthus* and *Gmelina*. Teak leaves harboured 18 species of endophytic fungi belonging to 13 genera and 29 strains (**Table 2**) and *Ailanthus* leaves harboured 10 species with 7 genera and 17 strains. However, 7 species with 5 genera and 12 strains were isolated from *Gmelina* leaves. It is also reflected in diversity indices *viz.,* Shannon's index, Simpson's diversity index, Richness and equitability index (**Table 3**). Diversity indices gave more values to Teak with 3.96 followed by *Ailanthus* with 3.90 and *Gmelina* with 2.85. Richness index was also high in Teak with 5.05 followed by *Ailanthus* with 3.25 and *Gmelina* with 2.82. Equitability index (Evenness index) was also found to be high in the entire environment 0.9. It clearly reflected that the species are evenly distributed with high diversity. Interestingly, mature leaves of Teak, *Ailanthus* and *Gmelina* at different locations harboured maximum number of endophytic fungi than the young leaves.

The dominant fungi are *Fusarium* sp. with an index of 8.4 followed by *Aspergillus flavus* with an index of 6.2 and *Aspergillus niger* and *Phomopsis* sp. with an index of 4.0 each (**Table 3**). Of the 18 species, five species with one strain each were found to possess entomopathogenic significance based on preliminary bioassay studies (**Table 4**).

Population of endophytic fungal species isolated from Teak, *Ailanthus* and *Gmelina* was found to be low. It is really regarded that Teak has more diversity of endophytic fungal species than *Ailanthus* and *Gmelina* since R´enyi diversities are higher in Teak than that of *Ailanthus* and *Gmelina* (**Figs.1a-c**). Population trend of the species encountered during the survey has not attained the expected population as depicted in Lorenz graph (**Figs. 2a-c**). There was a slight decrease in population trend in all the

Table 3. Colonization frequency and dominant endophytic fungi of Teak, *Ailanthus* and *Gmelina*.

Endophytic fungi species	*Tectona grandis*	*Ailanthus excels*	*Gmelina arborea*	Colonization frequency (200 segments)	Dominant fungi
Botryodiplodia theobromae	2	2	-	2.0	2.4
Phoma eupyrena	1	-	-	0.5	0.8
Colletotrichum gleosporidies.	2	-	1	1.5	2.4
Phomopsis sp.	1	2	3	3.0	4.0
Fusarium sp.	4	2	2	4.0	8.4
Phoma sp.	2	2	1	2.5	4.0
Phomopsis longicola	1	-	-	0.5	0.8
Nigrospora sphaerica	2	1	-	1.5	3.2
Aspergillus flavus	4	2	1	3.5	6.2
Aspergillus fumigates	1	1	-	1.0	1.6
Phyllostica sp.	1	-	-	0.5	0.8
Alternaria sp.	1	2	1	1.5	2.4
Xylaria sp.	1	-	-	0.5	0.3
Aspergillus niger	2	1	2	2.5	4.0
Chloridium sp.	1	-	-	0.5	0.8
Clasporium cladosporioids	1	-	1	1.0	1.6
Fusarium clamydosporium	1	1	-	1.0	1.6
Pestalotiopsis sp.	1	1	-	1.0	1.6
Total	29	17	12		
Diversity indices					
Shannon index	3.96	3.25	2.86		
Simpson index	0.08	0.1	0.15		
Dominance index	0.92	0.90	0.85		
Menhinick index	3.34	2.5	2.30		
Buzas and Gibson's index	0.87	0.95	0.90		
Equitability index	0.95	0.97	0.95		
Malgalef Richness index	5.05	3.25	2.81		

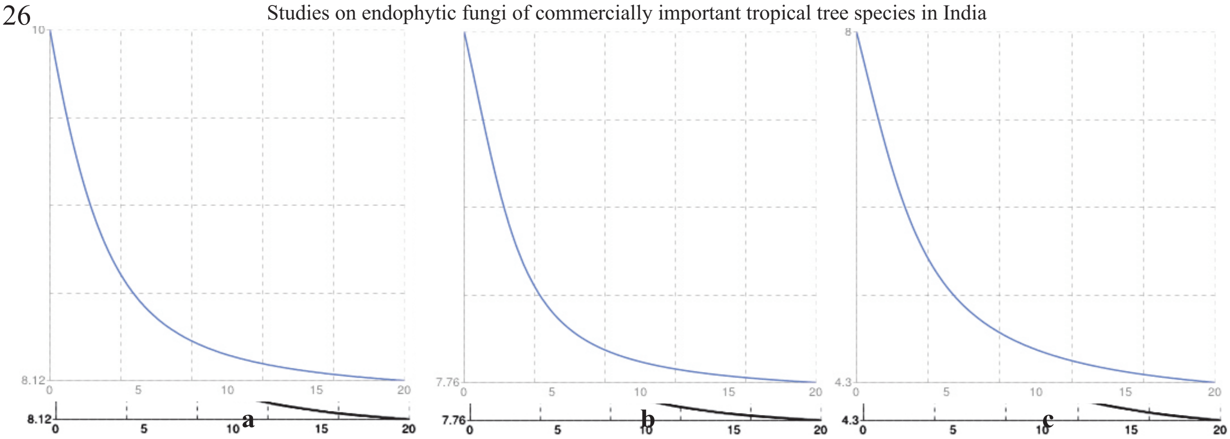


Fig. 1. Renyi/Hill graph a) Teak b) *Ailanthus* c) *Gmelina*

Fig. 1. Renyi/Hill graph a) Teak b) *Ailanthus* c) *Gmelina*

species studied. Number of species encountered were also found to be less and it is expressed in the rarefaction curve that it didn't reach asymptote curve and the same trend was observed in *Ailanthus* and *Gmelina* also (**Fig. 3**), hence chances of encountering more number of species will be possible if the number of sampling unit increases. Of the 18 species of endophytes, eight species were found to have very low population and eight species are moderate in population size and two species have higher population size (**Fig. 4a.**). The same trend was also observed in *Ailanthus* and *Gmelina* (**Figs. 4b-c**).

DISCUSSION

All microorganisms which inhabit the interior of a plant for at least one period of their life cycle are considered as endophytes (Arnold and Herre, 2003). Endophytic fungi are widespread in all phyla of the kingdom Fungi. Webber (1981) was probably the first researcher to report an example of plant protection giving endophytic fungus, in which the endophyte *Phomopsis oblonga* protected Elm trees against the beetle *Physocnemum brevilineum*. He also reported that the repellent effect observed towards the insect to toxic compounds produced by the fungi. This was also confirmed by Claydon *et al*. (1985), who showed that endophytic fungi belonging to the *Xylariaceae* family synthesize secondary metabolites in hosts of the genus *Fagus* and that these substances affect the beetle larvae. These examples show the importance of microorganisms in controlling insects-pests in agriculture. Kanda *et al*. (1994) reported that endophyte-free plants were severely attacked by insects, whereas those harboured endophytes almost free of insect larvae. Hence the present study gives emphasis on endophytes of entomopthogenic significance in commercially important tropical tree species *viz., Tectona grandis*, *Ailanthus excelsa* and *Gmelina arborea* for insect pest management of forestry importance by developing novel insecticides.

Endophytic fungal diversity

Microbial endophytes form integral part of microbial community that is commonly associated with plants. They have many ecological roles that include mutualism, commensalism and parasitism (Sieber, 2007). They occur on a variety of hosts that include trees, shrubs, herbs, grasses, lichens, mosses and ferns (Zhang *et al*., 2006). Although there is abundant evidence for the positive effects that endophytes confer on grasses, there is little information on endophytes of trees. The host genotype plays an important role in the host-endophyte association. An endophyte thus acts as mutualist, anatagonist or commensalist based on the host genotype. For example, Redman *et al*. (2001) demonstrated that a well known

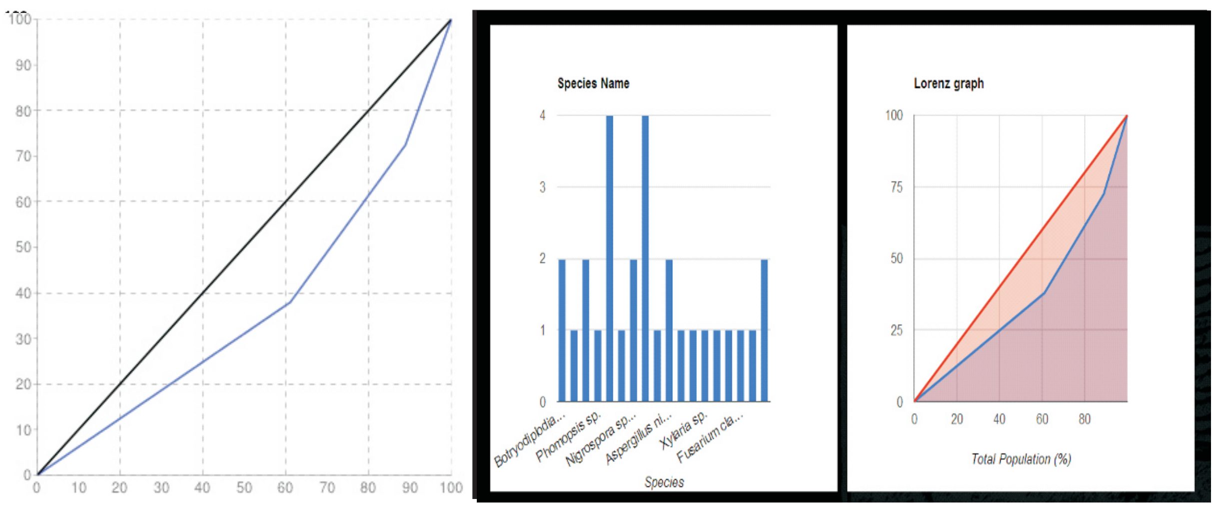


Fig. 2a. Lorenz graph indicating the population trend of endophytes in Teak.

Table 4. Preliminary evaluation of endomopathogenic endophytic fungi against Ailanthus and Teak defoliators.

S. No.	Endophytic fungal species	Targeted insect pests		
		H. purea	***E. narcissus***	***A. fabricella***
1	*Botryodiplodia theobromae* (Teak)	+	+	+
2	*Phoma eupyrena*	-	-	-
3	*Colletotrichum gleosporidies.*	-	-	-
4	*Phomopsis* sp. (Ailanthus)	+	+	+
5	*Fusarium* sp.	-	-	-
6	*Phoma* sp. (Teak)	+	+	+
7	*Phomopsis longicola*	-	-	-
8	*Nigrospora sphaerica* (Teak)	+	+	+
9	*Aspergillus flavus* (Teak)	+	+	+
10	*Aspergillus fumigates*	-	-	-
11	*Phyllostica* sp.	-	-	-
12	*Alternaria* sp.	-	-	-
13	*Xylaria* sp.	-	-	-
14	*Aspergillus niger*	-	-	-
15	*Chloridium* sp.	-	-	-
16	*Clasporium cladosporioids*	-	-	-
17	*Fusarium clamydosporium*	-	-	-
18	*Pestalotiopsis* sp.	-	-	-

+: Effective against tested insect pests
-: Non effectiveness against tested insect pests
(Within paranthesis given host from which the endophytic fungi isolated)

pathogenic genus, *Colletotrichum* is not pathogenic to pepper and tomato cultivars. Hence it is essential to understand the role and spread of endophytes in trees. In the present study, a sum of 18 species of endophytic fungi belonging to 13 genera and 58 strains from young and mature leaves of *Tectona grandis*, *Ailanthus excelsa* and *Gmelina arborea* were isolated and identified. The present work analyzed fungal endophytes found in leaves of different tree species. Most studies have focused on endophytes that colonize leaves of different plants. According to Arnold *et al.* (2000), the endophytes are especially rich and abundant in leaves. In this study, Teak leaves harboured 18 species of endophytic fungi belonging to 13 genera and 29 strains. The fungal genera found in Teak leaves were *Alternaria, Colletotrichum, Nigrospora, Aspergillus* and *Phomopsis*. *Aspergillus, Nigrospora* and *Phomopsis* were the dominant genera found in both young and mature leaves. *Fusarium* sp. and *Aspergillus* spp. were found only in mature leaves. Chareprasert *et al.* (2006) isolated *Alternaria, Colletotrichum, Nigrospora* and *Phomopsis* species from Teak in Thailand. Similar to the findings reported previously in *Calotropis procera* and *Withania somnifera* by Khan *et al.* (2007). Shekhawat *et al.* (2010) isolated *Aspergillus* spp. and *Nigrsopora* sp. from *Melia azedarach*. Krittapong *et al.* (2009) reported that endophytic genera such as *Phomopsis*, *Nigrospora*, *Fusarium*, *Pestalothiopsis* and *Xylaria* were commonly

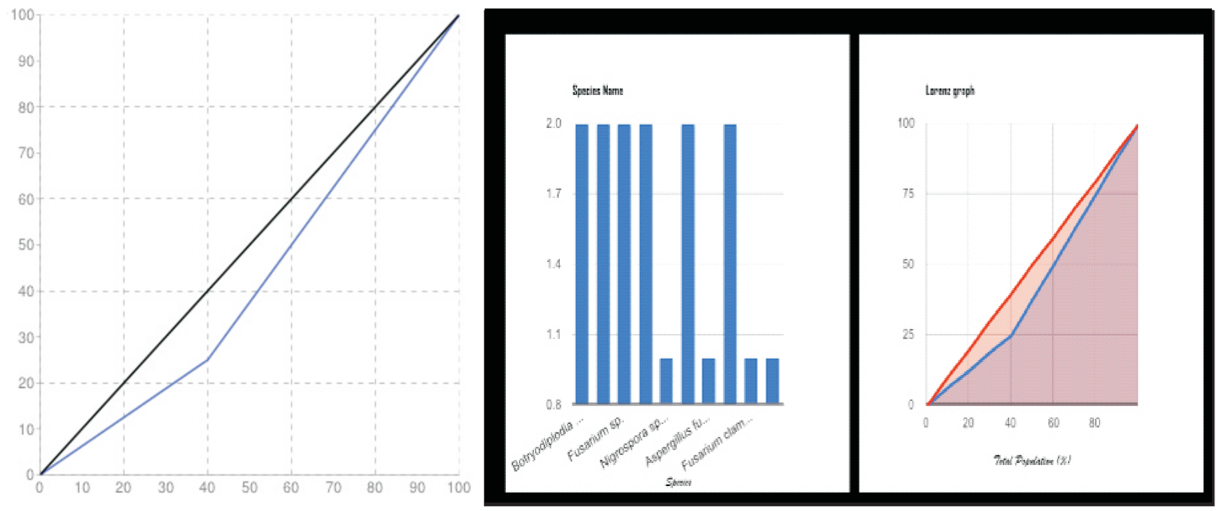


Fig. 2b . Lorenz graph indicating the population trend of endophytes in *Ailanthus*.

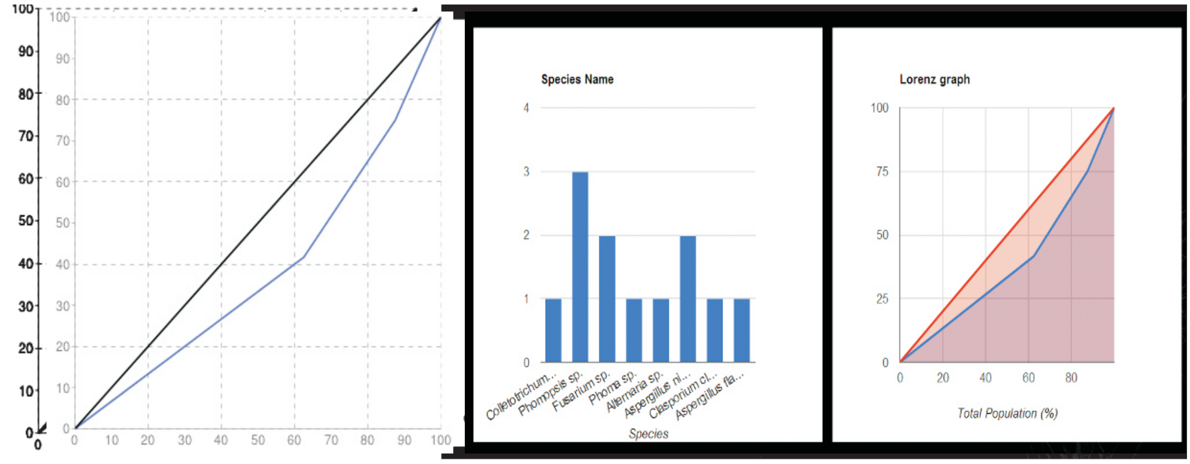
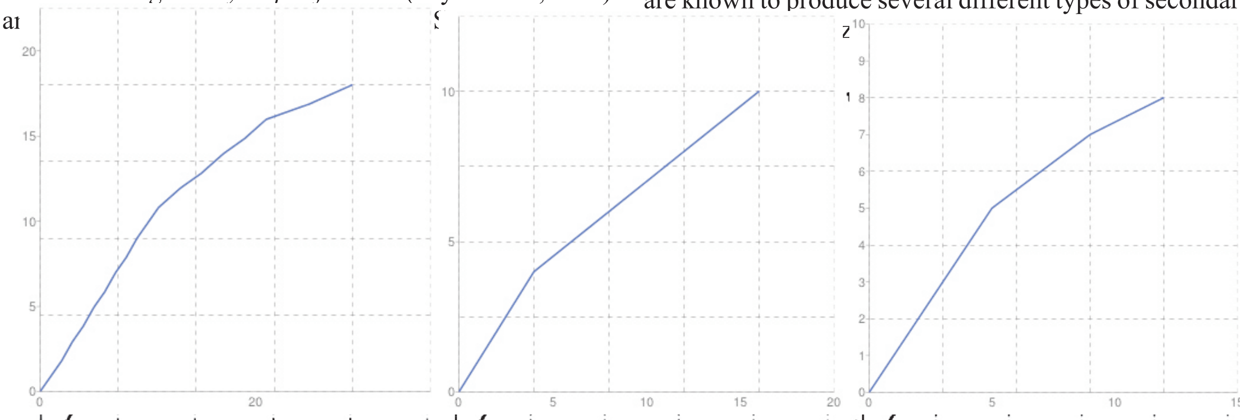


Fig. 2c . Lorenz graph indicating the population trend of endophytes in *Gmelina*.

found in the deciduous forest. In this study, total of 10 species with 7 genera and 17 strains of endophytic fungi were isolated from *Ailanthus* leaves and the dominant species were *Aspergillus flavus*, *Fusarium* sp. and *Nigrsopora sphareica*. However, total of 7 species with 5 genera and 12 strains were isolated from *Gmelina* leaves. In this study, mature leaves of Teak, *Ailanthus* and *Gmelina* at different locations harboured maximum number of endophytic fungi than the young leaves. As pointed out by Mekkamol (1998), the mature Teak leaves attain a greater size, growing to approximately 40 cm in length and 30 cm at their widest point. The young leaves were around one third the size of the mature leaves. Therefore in Teak the mature leaves offer a much greater surface area for inoculum capture. In addition, older leaves would have been exposed longer than younger leaves and therefore received higher amounts of inoculum (Wilson and Carroll, 1994). The occurrence and distribution of endophytic fungi in the leaves of tropical plants have been relatively well-studied by many earlier researchers (Suryanarayanan *et al*., 2002; Kumaresan and Suryanarayanan, 2002). The colonization frequency of the endophytes increased with the age of the leaf and reached a maximum when the leaves attained senescence. The number of endophytes that can be recovered from the leaf tissue increases with the age of the leaf in several plant hosts including *Trachycarpus fortunei* (Taylor *et al*., 1999)

2000). This increased colonization of old leaves is due to superinfection of the leaves over time by air-borne inoculum (Suryanarayanan and Vijaykrishna, 2001). Kumaresan and Suryanarayanan (2002) showed that the endophyte assemblage of senescent leaves of *Rhizophora apiculata* is more diverse than that of the young leaves indicating that the susceptibility to endophytes and saprobic fungi increases with leaf age. This indicates that although the susceptibility of the leaves to endophytes increases with age, the leaves continue to recruit only some species of fungi as endophytes.

The endophytic assemblages of the *Tectona grandis* trees were composed of a number of cosmopolitan species such as *Alternaria* sp. *Fusarium* sp. and *Aspergillus* spp. which have been recorded as endophytes in both temperate and tropical areas (Kumaresan and Suryanarayanan, 2001). There were also a large number of coelomycetous taxa such as *Phomopsis* spp. and a *Colletotrichum* sp. Species of *Phomopsis* were the most frequently isolated endophytes. A number of species of this genus have been reported previously as endophytes in needles or evergreen leaves by Kumaresan and Suryanarayanan (2001) and Suryanarayanan *et al*. (2002). This indicates that endophytic fungi may preferentially colonize plant hosts. Leaves of tropical plants are densely colonized by endophytes (Suryanarayanan *et al*., 2002) and endophytes are known to produce several different types of secondary



a) Teak b) *Ailanthus* c) *Gmelina*

Fig. 3. Rarefaction curve for species richness extrapolation

a) Teak b) *Ailanthus* c) *Gmelina*

Fig. 3. Rarefaction curve for species richness extrapolation

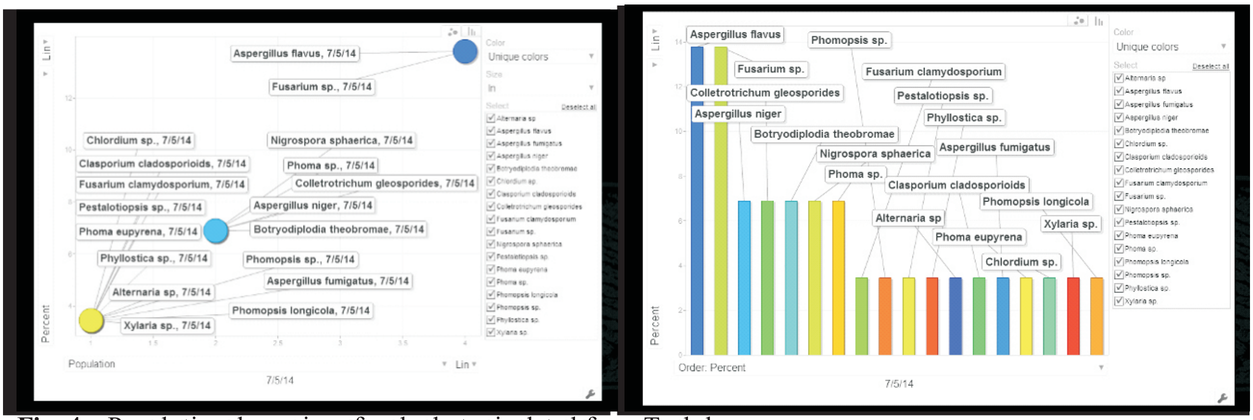


Fig. 4a. Population dynamics of endophytes isolated from Teak leaves

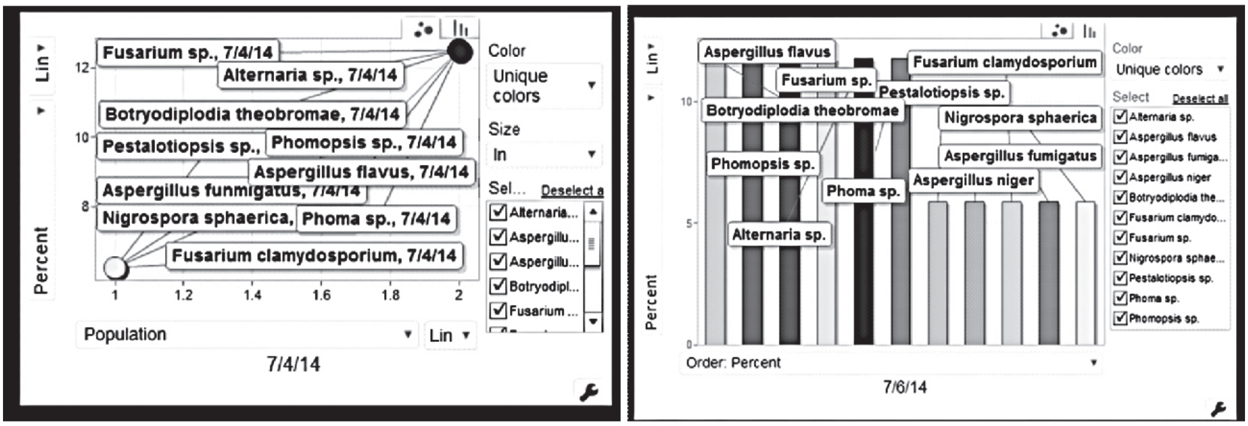


Fig. 4b. Population dynamics of endophytes isolated from *Ailanthus* leaves

Fig. 4c. Population dynamics of endophytes isolated from *Gmelina* leaves

In the present study, species of *Colletotrichum* and *Phyllosticta* were the dominant endophytes recorded in Teak and *Ailanthus* leaves. These two genera are ubiquitous endophytes and have been reported from several plant hosts (Suryanarayanan *et al.*, 2002). Magalhães *et al.* (2008), studied the diversity of endophytic fungi isolated from *Eremanthus erythropappus* and obtained variation in colonization rates from different tissues (seeds, leaves and stem fragments). Using macro and microscopic identification, genera *Xylaria* and *Phomopsis* were documented in all the sampled tissues. In *Eremanthus erythropappus*, the genera with the highest specificity, *Alternaria* and *Fusarium* were found in seeds, *Nigrospora* and *Aspergillus* in leaves and *Dothiorella* in stems. Gómez-Vidal *et al.* (2006) studied endophytic colonization of palm leaves (*Phoenix dactylifera*) by entomopathogenic fungi and observed the colonizing of *Beauveria bassiana* in the parenchyma, especially in intracellular spaces. It is also reflected in diversity indices *viz.,* Shannon's index, Simpson's diversity index, Richness and equitability index. Diversity indices gave more values to Teak with 3.96 followed by *Ailanthus* with 3.90 and *Gmelina* with 2.85. Richness index was also high in Teak with 5.05, followed by *Ailanthus* with 3.25 and *Gmelina* with 2.82. Equitability index (Evenness index) was also found to be high in the entire environment (0.9). It clearly reflected that the species are evenly distributed with high diversity. Suryanarayanan *et al.* (2002) showed that the endophyte diversity in a tropical forest in southern India was positively correlated with host abundance. In this study, endophytes such as *Alternaria* sp., *Fusarium* sp. *Nigrospora* sp. *Aspergillus* spp. and *Phoma* spp. were isolated from Teak, *Ailanthus* and *Gmelina* and this indicates that the endophyte assemblages of the majority of tropical trees are dominated by a single species. The results of the present study, coupled with those of Fisher and Pertini (1992), strengthens the view that endophyte colonization depends more on the availability of inoculum and host plants than on the geographical location of the host plants (Suryanarayanan *et al*., 2002).

A species accumulation curve was plotted using the cumulative numbers of recorded endophyte species against individuals of host trees. The software estimate was used to randomize the data 100 times and to obtain a mean species accumulation curve. The number of species of endophytes increased with the number of isolates initially, but the slope flattened with further addition of isolates. In addition, when unique taxa were plotted against the number of samples, the unique species increased with increase in samples. This shows that as the sample size increased some of the new additions may be observed. These results suggested that some more species might be encountered if we do more samples with reference to endophytes. Evenness of the endophyte assemblage was more towards 1 (normal range 0-1), this indicated the species are evenly distributed. This index decreased when any assemblage is dominated by a single genus. Petrini (1984) was of the opinion that host specificity among endophytes is expressed at the family level. However, some endophytic genera such as *Phyllosticta*, *Phomopsis* and *Colletotrichum* occur in a wide variety of distantly related host plants. We suggest that host abundance should be considered while studying a tropical plant community for endophytes. Moreover, while attempting to study tropical forests for their endophyte assemblages, it is suggested to identify the generalists first in order to rapidly calculate the diversity. Host specificity is critical for extrapolation on global biodiversity of fungi since this estimate heavily depends on fungus: plant ratios. *Colletotrichum* species has worldwide occurrence as a plant pathogen, causing diseases of several plants including soybean, *Pisum sativum*. However, it has been reported as an endophyte from *Jatropha curcas* (Kumar and Kaushik, 2013). Despite the pathogenic nature of the fungus, it has been utilized for management of several weeds, including *Sesbania exaltata* a noxious weed of soybean. In this study, we found this fungus as predominant endophyte of Teak and *Ailanthus* trees. During the present investigation, it was found that the colonies represented a very wide spectrum of colour and the endophytes *viz., Alternaria, Aspergillus, Nigrospora, Bortryo* and *Pestalotiopsis* are characterized by colored colonies and were presented in abundance than the white color colonies of *Fusarium* species. The findings are in accordance with the observations made by Bohra (1990).

CONCLUSION

The study indicates that several of these plants support wide spectrum of endophytes with significant bioactive potential. Thus, concerted efforts should be carried out for bioprospection in the Western Ghats to tap and conserve the microbial resources of this important biodiversity hotspot and utilize their potential for human welfare. In addition, the endophytic populations of these tree species may be studied in detail with an ecological perspective which may help to understand community structure of their endophytes and warrant isolation of diverse endophytic fungi with useful bioactivities. Investigations on the interactions of *Tectona grandis, Ailanthus excelsa* and *Gmelina arborea* and their endophytes would be the next direction for future research.

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