**Distribution of endophytic Fungi in Tectona grandis Linn.F**

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**Abstract**

Isolation and enumeration of endophytic fungi from leaf samples of Tectona grandis L.f was carried out in Thanjavur District of Tamil Nadu India. During the study period, a total of 140 fungi were isolated from PDA medium and Different fungal species namely Alternaria sp., Colletotrichum sp., Phomopsis sp., Penicillium sp., Schizophyllum sp., Xylariaceae sp., Schizophyllum sp., Nigrospora sp., Fusarium sp.,

The lower portion of leaf sample was most frequently colonized Penicillium sp, Schizophyllum Sp., Fusarium sp., and Xylariaceae sp., (15%), Followed by Alternaria sp, Colletotrichum sp., and Phomopsis sp.(13.5%), whereas Nigrospora sp., was found only in young leaves. However, from the upper region was most frequently colonized by Alternaria sp (10%) followed by Xylariaceae sp., Colletotrichum sp, Phomopsis sp., (8.5%).% and Fusarium sp., (5.0%) and sterile mycelium(4.5%).

There was the significant differences in the type of colonization of fungus with reference to reason and location. However, from the upper region was most frequently colonized by Alternaria sp (10%) followed by Xylariaceae sp., Colletotrichum sp, and Phomopsis sp., (8.5%). Whereas at upper portion of leaf segment was mostly similar colonized by Alternaria sp(15%), Colletotrichum sp, and Phomopsis sp, Fusarium sp., (13.5%). It was noticed in general, that the colonization frequency of some of those fungi, Alternaria sp, Schizophyllum sp, Xylariaceae sp, and sterile mycelium was little higher percentage in lower portion of the leaf.

The findings in the present study is also agreement with finding of Bills (1996) who reported large number of fungal species isolated as entophytes from the leaves of this plant sp. The study of entophytic fungus in Ticino grandis suggests that there is a need of larger survey in relation to various seasons to study the diversity existing in this species and their seasonal variability on endophytes colonization. They showed colonization frequency was greater during rainy season than summer and winter.

**Key words:** Endophytic fungi, Medicinal plant, Tectona grandis
Introduction

Tectona grandis (teak) is a tall tree from Southeast Asia and is widely cultivated for its durable wood. It is a member of the mint family (Lamiaceae), which is perhaps better known for its aromatic members including culinary herbs such as basil, oregano and rosemary. Teak is widely cultivated in the tropics; the main producers are Burma, India, Thailand and Indonesia. Teak (Tectona grandis L.) is one of the most valuable timber resources in the tropics; it is also one of the most widely studied tropical plants in terms of its ecology and silviculture. This species has been planted in various areas outside of its natural distribution area (Tanaka et al. 1998). The period from November to February in Thanjavur is pleasant, with a climate full of warm days and cool nights. The onset of summer is from March, with the mercury reaching its peak by the end of May and June. The average temperatures range from 81 °F (27 °C) in January to 97 °F (36 °C) in May and June. Summer rains are sparse and the first monsoon, the South-West monsoon, sets in June and continues till September. North-East monsoon sets in October and continues till January. The rainfall during South-West monsoon period is much lower than that of North-East monsoon. North-East monsoon is beneficial to the district at large because of the heavy rainfall and the Western ghats feeding the river Cauvery. The average rainfall is 37 inches (940 mm), most of which is contributed

Like all forests, plantations of teak can act as carbon stores; plants and fungi are essential for human wellbeing; they provide us with food, medicine, fibres, fuel, building materials and many other products. In addition, plants hold great cultural meaning for people all over the world. Teak is a tree up to 40 m high. The bark is scaly and the leaves are opposite one another. The leaves are 6 – 75 cm long, 8 - 45 cm wide, and hairless on the upper surface when mature, with many star-shaped hairs below. The calyxes (sepals) form a balloon-like shape enveloping the fruit. The corolla (petals) is regular, and white to cream-coloured. The fruit is pale yellow, of 1.2 to 2 cm diameter, and covered with star-shaped hairs. Wood has been used as a building material since the Neolithic period (between 4,000 to 11,000 years ago) when humans first used trees to build shelters. The endophytic assemblages of rain tree leaves were composed of a Colletotrichum spp., N. sphaerica, a Penicillium sp., Phomopsis spp. and mycelia sterilia. Species of Phomopsis were the most frequency isolated endophytes. A number of these genera have been reported previously as endophytes in needles or evergreen leaves (Bussaban et al. 2001; Kumaresan & Suryanarayanan 2001; Hata et al. 2002; Suryanarayanan et al. 2002). A number of these genera have been reported previously as endophytes in needles or evergreen leaves (Bussaban et al. 2001; Kumaresan & Suryanarayanan 2001; Hata et al. 2002; Suryanarayanan et al. 2002). Teak (Tectona grandis L.f.) is considered to be an extraordinarily durable building timber with a worldwide reputation. It is indigenous to India, As teak is a species with a wide geographic distribution in South India, Rawat et al (1998) Identification of provenances based on leaf morphology in Tectona grandis. The natural populations develop heritable Its widespread use has entailed the overexploitation of natural forests and a large reduction in natural diversity. Isolation and analysis of endophytic fungal communities from woody plants have been studied
Bills (1996), Fisher et al (1994), observed the Fungal endophytes from the leaves and twigs of Quercus ilex L. Strobel G.A. (2002) studied Rainforest endophytes and bioactive products in teak plant. Mathew J, Vasudeva R (2003) Clonal variation for seed germination in teak. Priya PB and Bhat KM (1998) False ring formation in teak (Tectona grandis L.f.) and the influence of environmental factors. Analysis showed that in the teak natural area there were four main centers of genetic variability. Two clusters were in India and could be considered as main centers of genetic diversity in teak. Teak (Tectona grandis L.f.) is one of the most valuable timber trees in the world. The teak, a member of the Verbenaceae family, is a diploid species 2n = 36, Tectona grandis (teak) is one of the most important economic timbers worldwide. Limited studies exist on the potential entophytic fungus in this plant. Rao PS (2003) have been studied the Status of teak in Andhra Pradesh India and Proceedings of the international conference on quality timber products of teak from sustainable forest management. Consequently, the area of natural teak forests has drastically diminished over the last 50 years and the remaining forests are still under threat from illegal logging and other forms of forest destruction. Due to population pressure and unfavorable biotic factors, teak resources have considerably decreased both in extent as well as in density, quality and quantity over the natural range. To combat loss of biodiversity, programs for evolutionary in situ and ex situ gene resource conservation have been formulated in India, Laos, Myanmar and Thailand by Katwal (2003); Rao et al. (1991) The Present study was to important investigation the geographical diversity and population dynamics for use in sustainable management of teak in Thanjavur District of Tamilnadu india.

Materials and Methods

Sample Collection

Collection of plant samples The leaves of T.grandis were collected from (10° 47´ 00.99“ N latitude and 79°13´ 78.27”E longitude; Elivation 88 m (289 ft)), Thanjavur District of Tamilnadu, India. Trees free from insect and disease infestation were selected and marked. Healthy leaves from these healthy trees were collected and processed separately within 48 h of collection.

Description of the Plant

Class: Equisetopsida
Subclass: Magnoliidae
Superorder: Asteranae
Order: Lamiales
Family: Lamiaceae
Genus:Tectona

Surface sterilization of leaves

Isolation and identification of endophytic fungus The leaves were washed thoroughly in running water and segments of 1 cm2 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 1 min

**Culture of leaf samples on PDA plates**

Segment of leaf sample was placed in Petri dishes containing potato dextrose agar (with chloramphenicol 150 mg l–1). Two leaf segments were plated in each Petri dish, the dishes were sealed with parafilm and incubated in an incubator at 26 ± 1°C for 21 days. 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.

**Isolation of fungi and Identification**

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. The isolated fungus was numbered and sent to Dr. S. John Britto, St. Josephs College in Botany, Under Bharathidasan University, Trichy (India) for confirmation. Simultaneously, the isolates were stored in 15% (v/v) glycerol at −80°C in deep freeze, as spores and mycelium for further study.

**Result and discussion:**

Studies conducted on the Teak (Tectona grandis L.f.) in Thanjavur District of Tamilnadu, India. It healthy leaves were collected at Three-monthly intervals during January to December 2015. The number of genera and species, with their colonization frequency (CF%) in mature teak and rain tree leaves were greater than those in the young leaves. The low colonization during the summer months may attribute to reduce fungal activity. As tropical and subtropical climate harbor most of the worlds plants diversity so endophytic diversity in this subtropical zone is also more as to possess endophytic microorganisms in almost all vascular plant species examined to date. Cannon and Simmons, (2002) and Varghes et al (2000) confirmed that majority of endophytic flora are from leaves. It is also evident that in traditional practice. The present study, a total of 140 colonies were isolates belonging to class ascomycetes(33.3%) Coelomycetous (13.3%)Basidomycetes and Hyphomycetes (33.3%) and sterile mycelia (40.%). Lower portion of leaf sample was most frequently colonized by Alterneria sp., colletotrichum sp., Schizophyllum commune (15%) followed by Phomopsis sp. And Xylariaceae sp (13.5%). upper portion of leaf sample showed similar pattern with Colletotrichum sp., Phomopsis sp. And Xylariaceae sp (10%) being the highest, followed by Alterneria sp., penicillium sp., (8.5%) and Fusarium sp.,( 5.0%) and sterile mycelium(4.5%).There was the significant Differences in the type of colonization of fungus with reference to reason and location. However,from the upper region was most frequently colonized by Alternaria sp (10%) followed by Xylariaceae sp., , Colletotrichum sp,and Phomopsis sp., (8.5%),whereas at upper portion of leaf segment was mostly similar colonized by Alternaria sp(15%), Colletotrichum sp,and Phomopsis sp.,Fusarium sp., (13.5%) sterile mycelium was little higher percentage in lower portion of the leaf there was significant differences in the type of colonization of the fungus with reference to location. The
finding in the present study is also agreement with finding Bills (1996) who reported large number of fungal species isolated as endophytes from the leaves of single plant sp. There was a difference in occurrence of these fungi on the sites studied although few reports that site specific factors may cause the reason for the occurrence of these fungi at higher level in terms of colonization. The study of endophytic fungus in Tectona grandis suggests that there is a need of larger survey in relation to various seasons to study the diversity existing in this species and their seasonal variability on endophyte colonization and its showed colonization frequency was greater during rainy season than summer and winter.

Reference


**Table-I**

**Distribution of Endophytic Fungi Isolated from The Leaf of Tectino Grandis.L**

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>NAME OF THE FUNGUS</th>
<th>Frequency of colonization (% CF)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
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<tr>
<td>Ascomycetes</td>
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<tr>
<td>1.</td>
<td>Alternaria sp.,</td>
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</tr>
<tr>
<td>2.</td>
<td>Colletotrichum sp.,</td>
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</tr>
<tr>
<td>3.</td>
<td>Xylariceae sp.,</td>
<td>2.0</td>
</tr>
<tr>
<td>4.</td>
<td>Fusarium sp.,</td>
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<td></td>
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<tr>
<td>5.</td>
<td>Penicilium sp.,</td>
<td>1.5</td>
</tr>
<tr>
<td>6.</td>
<td>Phomopsis sp.,</td>
<td>5.5</td>
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</tbody>
</table>

**Hypomycetes and Basidomycetes**

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<tbody>
<tr>
<td>7.</td>
<td>Schizophyllaceae</td>
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</table>

**Sterile Mycelia Group**

<p>| | | |</p>
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<tr>
<td>8.</td>
<td>Sterile mycelium 1</td>
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<td>9.</td>
<td>Sterile mycelium 2</td>
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<td>10.</td>
<td>Sterile mycelium 3</td>
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<td>11.</td>
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<td>12.</td>
<td>Sterile mycelium 5</td>
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</tr>
<tr>
<td>13.</td>
<td>Sterile mycelium 6</td>
<td>1.5</td>
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