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BIODIVERSITY OF ENDOPHYTIC FUNGUS FROM *TECTONA GRANDIS* - LITTER LEAVES AND SOIL IN COMPARISON

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ABSTRACT

Study revels to characterize the endophytic fungus isolated from litter leaves and soil from Perungalathur hill for their diversity and ecosystem function. Totally 45 species were isolated for two years. They comprised of Zygomycotina (6), Ascomycota (4), Hypomycetes (28) and Coelomycetes (7).The litter samples was divided into 2 active decompositions (sample 1) Leaves and (sample 2) Soil. Litter leaves are advanced stage of decomposition, usually fragmentary, very thin and tightly compressed, were the funguses are abundant during the South West Monsoon (Jan to Aug). The fungal community is a heterogeneous assembly of species derived a homogenous habitat with a long normal pattern of distribution formed due to the interplay of many independent factors governing the relative abundance of the species. The greater variation in the species composition was due to the North West Monsoon. Taking the average percentage occurrence as colonization efficiency of a taxon it was found that Deuteromycetes were active in that order.

Key words: Biodiversity, Endophytes, Litter soil, Litter leaf, Serial dilution, pour plate.

INTRODUCTION

The biodiversity of fungus associated with leaf litter of *Tectona grandis* and soil from Perungalathur hill, Chennai was studied 4 seasons. Leaf litter decomposition is one of the important processes of terrestrial ecosystem. Even though the last decade has seen the biodiversity and ecosystem function studies which emerge the major topics in ecology, fewis known about the effect of biodiversity on decomposition. Fungi play fundamental roles in leaf litter decomposition processes within forest ecosystem [1, 2]. The decomposition of leaves is not entirely confined to the litter layer on the forest floor [3, 4]. In fact, decay processes are initiated as soon as the leaf is formed, and the large surface of this plant organ is exposed to microbial and faunal attack during its entire life, senescence and death. Relatively few studies have been designed to follow the colonization of leaves from their initial expansion to their incorporation into humus [5, 6, and 7]. The focus of this study is to enumerate the diversity of fungus inhabiting the different layers of the litter in natural ecosystem and also to record the seasonal changes in the litter soil and in the leaf litter of *Tectona grandis.*

MATERIALS AND METHOD

Sample Characterization: Litter leaves and litter soil of *Tectona grandis* (fig.1) were collected from Perungalathur Hill for two years (January 2012 to May 2013) to check the Seasonal variation and to record Biodiversity. The temperature is 28.4°C (24.0–32.8°C) and annual rainfall of 685 mm. The monthly mean relative humidity is 78.6%. The first showers of the South West monsoon occur during the middle of January- August. Showery weather continues through September and ceases in the middle of October, when the North East Monsoon occurs. Litter samples were collected at random from the study site and taken to the laboratory in sterile polythene bags.


Figure 1. Collected samples for inoculation -Teak (*Tectona grandis*) litter leaf and Soil

Experimental Methods: Sample 1 and Sample 2 were inoculated in different methods. The Sample 1 in platting technique and Sample 2 in Pour Plate method and in Serial Dilution method.

Platting technique (Sample 1): Leaves of *Tectona grandis* were collected. Leaves from the litter layer were divided into five stages of decomposition [8] according to their morphological criteria such as colour, thickness and hardness from the leaf; 1 cm 2 pieces were cut with a pair of sterile scissors. The sample was washed in 100 mL of sterile water and was placed in PDA medium.

Pour Plate Method (Sample 2): 1 gram of soil is weighed and it is directly spread onto the PDA medium for the fungal growth.

Serial Dilution Method (Sample 2): 1gm of soil sample was diluted in 9 mL of sterile distilled water from 10^{-1} to 10^{-7} (fig.2) from this initial suspension, serial dilutions were prepared. One mL of the required dilution (1/1000) from 10^{-3} to 10^{-7} was pipetted into each of five replicate plates. Potato dextrose agar (PDA) media supplemented with chloramphenicol (50µg/ml). The plates were incubated at 28ºc for 7-14 days [9]. Fungi growing on agar plates were sub cultured in fresh PDA medium until pure colonies were observed. The endophytic fungi grown on plates were transferred to sterile PDA slants to maintain culture purity. Fungal colonies were identified by their morphological characteristics [10, 11, 12, 13, and 14]. For the purpose of identification, the isolated fungal species were grown and made to sporulate on PDA. All fungal growth were recorded, isolated and identified. The periodicity of occurrence of each species of fungus was calculated based on the number of samples in which they occurred against the total number of samples collected into the four groups: most common (76-100%), common (51-75%), occasional (26-50%) and rare (1-25%).

Figure 2. Serial dilution method

RESULT

Fungi are known to colonize and survive in diversified habitats, viz. water, soil, air, litter, dung, foam etc. Total of 50 species were isolated from the litter samples. These were made up of 6 species of Zygomycota, 4 species of Ascomycotina, 28 species of Hypomycetes and 7 species of Coelomycetes. The number of taxa recorded per sampling was higher in litter leaf (11-38). Although many of the species were recored in sample 2 soils (Table 1). At litter stage high colonization rates for soil were registered for the genera *Trichoderma* sp, *Penicillium* sp and *Asperillus* sp. The few fungal species such as *Aspergillus niger*, *Rhizopus* sp, *Mucor* sp, *Pathogenic* sp and *Aspergillus japonicas* are more common. These fungi have been detected by all methods and are grouped into common primary saprophytes [15]. Isolation methods showed that these fungi were both superficial growers and internal colonies of leaves. However, the present investigation and others [15, 16, 17, 6] have shown that these fungi are active prior to leaf senescence. Among the fungi reported as endophytes by several authors, a number of taxa known to be frequent epiphytes are able to live endophytically within plant tissue [18]. *Alternaria alternate* is a phyllosphere colonizer able to penetrate into leaving leaf tissue at the onset if the senescence process.

Table 1 Frequencies of fungus from two litter samples.

S.No	Species	Litter Leaves	Soil serial dilution	Soil pour plate method
	ZYCOMYCOTINA			
1	*Absidia corymbifera*	Occasional	-	-
2	*Cunninghamella echinulata*	Occasonal	-	-
3	*C. elegans*	Occasional	Rare	-
4	*Mucor racemosus*	Most common	Occasional	-
5	*Rhizopus stolnifer*	Commmon	Common	Rare
6	*Syncephalastrum racemosum*	Occasional	Rare	-
	ASCOMYCOTINA			
7	*Anthestoma simplex*	Rare	Rare	-
8	*Ascotricha chartarum*	Rare	-	-
9	*Chaetorium longirostre*	Rare	Occasional	-
10	*C.globosum*	Rare	-	-
	DEUTEROMYCOTINA			
11	*Acremonium strctum*	Occasional	Rare	-
12	*Alternaria alternate*	Common	Common	-
13	*Longipes*	Rare	-	-
14	*Aspergillus candida*	Rare	-	-
15	*Flavus*	Most Common	Most Commin	Common
16	*Fumigates*	Common	Occasional	-
17	*Japonius*	Most Common	MC	-
18	*Nidulans*	Occasional	Rare	-
19	*A. niger*	Most Common	Most common	-
20	*Ochraceus*	Most Common	-	-
21	*Sydouiri*	Rare	-	-
22	*Tamari*	-	-	-
23	*Terreus*	Most Common	Common	-
24	*Versicolar*	Occasional	-	-
25	*Aureobasidium pullane*	Rare	-	-
26	*Beltrania manifera*	Rare	-	-
27	*Cladasporium oxysporum*	Rare	Rare	-
28	*Curvalaria lunata*	Common	Occasional	-
29	*Emericella nidulans*	Occasional	Occasional	-
30	*Fusarium oxysporum*	Common	Occasional	-
31	*Fusarium solani*	Rare	-	-
32	*Pathogenic* sp	Most Common	Most Common	-
33	*Penicillum oxalicum*	Common	-	-

34	*Penicillium citrinum*	Most Common	Common	Rare
35	*Trichoderma viride*	Occasional	Occasional	Rare
36	*Trichoderma basicola*	Rare	-	-
37	*Trichoderma koningii*	Rare	Rare	Occasional
38	*Torula herbarum*	Occasional	-	-
	COELOMYCETES			
39	*Botriyodiplodia*	Most Common	Common	-
40	*Colletotrichum dematium*	Rare	-	-
41	*Phyllostica*	Occasional	Rare	-
42	*Pestalotiopsis mangiferae*	Common	Rare	-
43	*Phoma betae*	Rare	-	-
44	*Phomompsis eugenese*	Rare	-	-
45	*Gleospora*	Most common	-	-

The genus *Trichoderma* sp. are known to be secondary colonizer of wide variety of forest litters and have been mostly recorded in relatively well decomposed materials [19] but species differed in their affinity for temperature and moisture [20], which may explain *Trichoderma* sp were found. *Trichoderma* sp are also known for their ability to produce cellulose [21]. In addition to the seasonal variation in colonization, there are changes in the diversity of fungus, with higher diversity at early and late time points within a crop rotation. The data presented in this work demonstrates the impact modern agriculture practice can have on a key functional group in the soil. Therefore hypomycetes are more common in both the litter samples.

SEASONAL CHANGES:

Number of species of fungi inhibiting the litter sample was grouped into 4 which were represented in 4 seasons (fig.3). In this study litter leaves have the rich species followed by the soil. The trend indicates that the habitat was highest in fungal species during South West Monsoon (JAN-AUG).

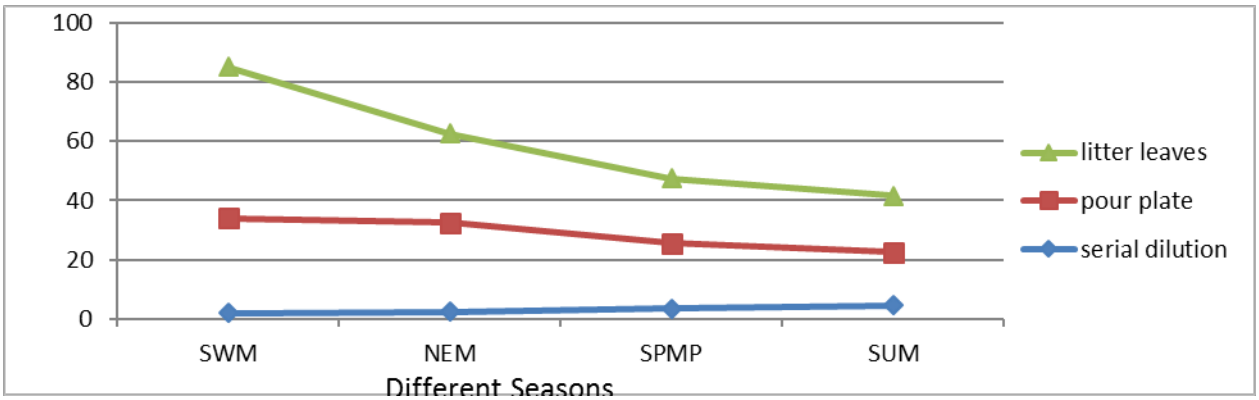

Figure 3. Shows the highest peak value during South West Monsoon (jan-aug) in x-axis and the y-axis it shows the percentage of culture grown.

DISCUSSION

All the species were identified from Perungalathur hill and these species were capable of growing in alkaline pH. Indicating the specific functional role they play in the process of litter degradation and also play role in community and signify the species niche requirements of species. Niches of species are dependent on a multitude of different factors and that the amount of resource used by each species is normally distributed. During decomposition the initial colonizers gradually disappears and were

replaced by new colonizers. This could be due to the availability of different kinds of nutrients from the substrate at various stages of fungal species in the litter. This supports the hypothesis of that fungi capable of infecting living leaf could be the initiations of litter degradation.

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