

## DIVERSITY OF ENDOPHYTIC FUNGI ASSOCIATED WITH BARK TISSUES IN IMPORTANCE MEDICINAL PLANTS FROM DELTA REGION OF TAMIL NADU

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### ABSTRACT

**Background and Objectives:** Endophytes are microorganisms that generally reside within plant tissues. These microorganisms interact with the host plants and that part of life cycle that could disclose protective functions towards the host. The present study involves a diversity of the endophytic fungal community from five medicinal tree species growing in the territorial area of Tamil Nadu was examined to both dry and wet seasons for the presence of fungi species. **Materials and Methods:** These medicinal plants belong to four families, such as Fabaceae, Moraceae, Sapotaceae and Combretaceae. The fungal diversity was found in different plant tissue specificities of diverse. The bark fungi were more varied, we assessed the completeness of the sampling of this group of fungi by using the Colonization Frequency, Relative Percentage of Occurrence of fungal groups and Principal Component Analysis performed for the endophytes fungi. **Results:** These trees are recorded with 2420 phellyphytes (bark fungi) isolates from 3000 bark segments during dry and wet seasons. These isolates varied seasonally, with 1024 fungal species during the dry season, and 924 during the wet season. Some ascomycetes, coelomycetes, hyphomycetes, and zygomycetes of the fungal species were routinely isolated, the coelomycetes species were recovered to the high diversity, and these dominated. **Conclusion:** The fungal assemblages were varied for the tissues' specificity. Even though, the fungal diversity was increased during the wet and dry seasons. These results indicated that a diverse endophytic fungal population has presences in medicinal plants' bark.

**KEYWORDS:** Plant-fungi interaction, bark tissues, fungal diversity, medicinal plants.

### INTRODUCTION

Endophytes are microorganisms that live within plant tissues without causing symptoms of the disease. Also, the fungi that live inside the bark tissue are called phellyphytes. Plant-associated fungi are usually divided into different ecological niches groups based on mycorrhizal, pathogenic, epiphytic, endophytic, and saprotrophic fungi. Most studies focus on only one of these groups, and very few consider interactions among them, or between fungi and bacteria.<sup>[1]</sup> The tissues of higher plants resource complicated fungal species-rich diverse assemblages of microorganisms. Filamentous fungi colonizing leaf and twig surfaces (epiphytes), internal leaf (leaf endophytes), young and old bark (bark fungi or phellyphyte), and fungi on wood (epixylic and wood decomposers) are recognized as dominant components of these assemblages.<sup>[2]</sup> Filamentous and non-pathogenic colonization of healthy plant tissues by microfungi is a widespread and potency documentation event, and increasing interest in the ecological functions of these fungi has energizing research in present years.

Fungi causing such infections in certain grasses, sedges and rushes hosts are both often referred to as endophytes or endophytic fungi, although the two groups have little in common.<sup>[3]</sup> It considered of the early use of the terms "endophyte" and "endophytic fungi" about fungal pathogens and plant disease.<sup>[4]</sup>

Rhytidome or out layered bark is the dead part of the bark consisting of the periderm and tissues external to it.<sup>[5]</sup> The living part of the bark inside is frequently mentioned as the inner bark. Usually, the bark is a presence to prominent structure in the stems and roots of mature woody plants in dicotyledons and gymnosperms. Monocotyledonous stem and root generally lack bark. It is developed during the secondary growth of the plant and by the activities of the cork cambium, which are phellogen. Its regions are living filamentous fungi known phellyphytes. The fungal colonists of inner bark were isolated an ascomycete taxa such as *Trybliodopsis pinastri* and *Phialocephala scopiformis* are common on twigs and branches of *Picea* spp. are termed

“phellophytes”.<sup>[6]</sup> A few fungi species are associated with the healthy outer bark of trees.<sup>[7,8]</sup>

Fungi, as associates of higher plants in the form of mycorrhizas, endophytes and pathogens, and as decomposers of plant litter, play a crucial role in structuring and maintaining plant communities.<sup>[9,10]</sup> However, only a few have been explored for their fungal endophytic diversity including crop plants,<sup>[11,12]</sup> tropical forest plants,<sup>[13,14]</sup> forest trees in bark fungi,<sup>[15,16]</sup> structure and diversity of endophytic fungi were found to be different in each plant due to canopy cover, leaf age, and location of plant growth,<sup>[18]</sup> plant health<sup>[19]</sup> and climate.<sup>[20]</sup> Recently, greater progress has been made in fungal endophytic research. Fungal endophytes have been found to colonize land plants worldwide on earth. Those fungal species have been isolated from boreal forests, arctic environments, tropical and temperate climates, hydric and xeric environments, mosses, ferns, gymnosperms, and angiosperms.<sup>[21,22]</sup> Fungal diversity to date, completely described fungi have considered for only 7% of the 1.5 million species hypotheses, which is a relatively conserved estimate.<sup>[24]</sup> Average numbers of species are newly described every year and estimated fungal taxonomists within a decade to a great range.<sup>[25,26]</sup> According to this, the average numbers of new species increased to calculate each year from 2008 to 2012.<sup>[27]</sup> However, it was undertaken that the number of fungal species varies between 3.5 and 5.1 million based on the generation deed.<sup>[28,29]</sup> The present work aimed to survey of diversity and seasonal variations in fungal endophytes assemblage of five medicinal trees' inner bark tissues.

Fungal species were identified to the morphological character.

## MATERIALS AND METHODS

### Study Area

Five medicinal plant species of the tropical trees, in this, studied of bark fungi, this tree plant's bark collected from Mannargudi, Tamil Nadu and India. This city is 6 m above sea level and it's located at 10.67° N 79.43° E. As the temperature distinctive vary from 21°C to 38°C. Hence, the season varying from heavy rain and air humidity effect year by year from October to December. The Northeast monsoon which starts in October to December contributes about 60 - 80% of the total annual rainfall. The Southwest monsoon rains from June to September and summer rains from March to June contribute about 20 to 40% of rainfall. Bark in plants was collected from individuals of each host species and their phellogen fungi were isolated during both seasons such as a summer (April to June 2019) and winter (October to December 2019) seasons were studied.

For these studies, five medicinally important plant species in *Erythrina variegata*, *Ficus racemosa*, *Madhuca longifolia*, *Pterocarpus santalinus* and *Terminalia arjuna* are four different members of the families i.e. Fabaceae, Moraceae, Sapotaceae, and Combretaceae (Table 1). Each bark samples were brought to the laboratory in sterile polythene bags and processed within 24 hours of collection.

**Table 1: Details of host trees studied for the presence of endophytes/phellophytes.**

Sl. No.	Hosts	Family	Host Code	
			Dry season	Wet season
1.	<i>Erythrina variegata</i> Linn.	Fabaceae	EVD	EVW
2.	<i>Ficus racemosa</i> Linn.	Moraceae	FRD	FRW
3.	<i>Madhuca longifolia</i> (L.) J. F. Macbr.	Sapotaceae	MLD	MLW
4.	<i>Pterocarpus santalinus</i> Linn.	Fabaceae	PSD	PSW
5.	<i>Terminalia arjuna</i> (Roxb.)	Combretaceae	TAD	TAW

**Note:** EVD- *Erythrina Variegata* Dry season; EVW- *Erythrina Variegata* Wet season; FRD- *Ficus Racemosa* Dry season; FRW- *Ficus Racemosa* Wet season; MLD- *Madhuca Longifolia* Dry season; MLW- *Madhuca Longifolia* Wet season; PSD- *Pterocarpus Santalinus* Dry season; PSW- *Pterocarpus Santalinus* Wet season; TAD- *Terminalia Arjuna* Dry season; TAW- *Terminalia Arjuna* Wet season.

### Surface Sterilization and isolation of endophytes/phellophytes

The bark samples surface sterilization was using ethanol and or sodium hypochlorite. The bark tissues of selected plants sample were cut into 1cm<sup>2</sup> segments, and the segments were surface-sterilized. Surfaces sterilized tissues were gently pressed onto antibiotic-amended agar medium and incubated. The bark segments were dipped

in 75% ethanol for 60 secs immersed in 4% sodium hypochlorite for 180 secs and dipped in 75% ethanol for 30 secs and inoculated.<sup>[30]</sup> Three hundred tissue segments (bark) from each host species were distributed in Petri dishes containing PDA medium (with chloramphenicol 150mg<sup>-1</sup>). Ten segments were placed on each Petri dish and incubated in a light chamber at 26±1°C for 21 days.

### Statistical and other analysis

The Colonization Frequency (CF %) of an endophyte/phellophyte species was calculated by the method.<sup>[31]</sup> CF% = N colonies/ N total x100. Where N colonies and N total are the number of segments colonized by each phellophyte and the total number of segments observed respectively. The Relative Percentage of Occurrence (RPO) of each group (viz. Ascomycetes, Coelomycetes, Hyphomycetes, Zycomycetes and Sterile

forms) of fungal species in each plant species was calculated as follows.  $RPO = \frac{\text{Total Colonization frequency of one group}}{\text{Total colonization frequency for all the groups of fungi}} \times 100$ . The Principal Component Analysis (PCA) was performed for the phellophyte fungi. For this analysis, each host and both seasons of phellophyte fungi with higher colonization frequencies were considered. This analysis was performed as described.<sup>[32]</sup> The diversity index was calculated using the method.<sup>[33]</sup>

## RESULTS AND DISCUSSION

In the present study, we screened five tree species for the presence of phellophytes during dry and wet seasons. For the five tree species, we recorded 2420 phellophytes fungi (bark fungi) isolates from 3000 bark segments during dry and wet seasons, such as 1429 phellophyte fungi isolates during the dry season from 1500 bark segments and 991 fungi isolates from bark during the wet season from 1500 bark tissue segments (Table 2). In most of the cases, each tissue segment was infected by more than one fungal species (multiple infections) substantiating the view that tropical trees have high rates of endophyte colonization.<sup>[34]</sup> In the present investigation also, more phellophyte (more than 100%) isolates were recorded from two (EVD and TAD) of the five trees during the dry season. The number of phellophyte isolates increased by 95% in the dry season compared to the wet season, 66% of isolates for the wet season. A total of 178 fungal species was recorded during both seasons. Such as 74 fungal species from the dry season, 104 fungal species from the wet season. The fungal species recovered higher in the wet season than in the dry season.

These fungi taxonomically were found out 64 species belonging to 30 genera and sterile (2), yeast forms were isolated to the five host and both seasons. These fungi are related to Ascomycetes, Coelomycetes,

Hyphomycetes, sterile forms and yeast forms, respectively (Table 2). Besides, in all the five hosts and both season, irrespective of the season, hyphomycetes dominated the phellophyte assemblages in all the hosts. (Table 2 and Figure 3). We recorded are individually analyzed for these hosts, a total of 15 fungal species during dry, 24 fungal species during the wet season belonging to 14 genera from the dry season, 17 genera from the wet season were isolated from the (Phellogen) bark of *Erythrina variegata*. Thirteen and 15 species during dry, wet seasons beheld to 12 genera from during both seasons were recorded from the bark of *Ficus racemosa*. Fourteen and 22 species during dry, wet seasons look to 11 and 16 genera from during dry and wet seasons were recorded from the bark of *Madhuca longifolia*. Nine species during dry, 22 species during the wet season belonging to nine genera from the dry season, 14 genera from the wet season were isolated from the bark of *Pterocarpus santalinus*. 24 and 21 species during dry, wet seasons belonging to 11 and 16 genera from during dry and wet seasons were recorded from the bark of *Terminalia arjuna*. The colonization frequency of phellophytes has shown results in five trees during wet and dry seasons (Table 2).

Though, the show the result, sterile and yeast colonizes is no record during the dry and wet seasons. Here, inconsiderate the dominant fungus. On the contrary, occurs in more than 10% of the fungal species were examined in various groups; they frequently have isolated from ascomycetes species are *Sporormiella minima*, *Talaromyces* sp. Xylariaceous forms, Coelomycetes group of fungal species in *Lasiodiplodia theobromae*, *Pestalotiopsis* sp. *Phoma* spp, *Phomopsis* spp, *Hyphomycetes* group of fungi in *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium* spp, *Nigrospora oryzae*, *Paecilomyces* spp, *Penicillium* spp, *Torulomyces* sp. *Trichoderma* sp. and sterile form was isolated during the dry and wet season.

**Table 2: Number of isolates, species and diversity index for endophytes/phellophytes isolated from five tree species during dry and wet seasons.**

Sl. No	Seasons Plant Name Code	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
		EVD	EVW	FRD	FRW	MLD	MLW	PSD	PSW	TAD	TAW
1.	Total no. of segments	300	300	300	300	300	300	300	300	300	300
2.	Total no. of colonizes	340	271	236	145	275	263	186	174	392	138
3.	Total Colonization Frequency (CF %)	113.5	90.5	82.0	48.2	91.6	87.7	62.0	57.8	130.6	45.9
4.	Total no. of species (64 species)	15	24	13	15	14	22	9	22	24	21
5.	Relative Percentage of Occurrence (RPO) of each group of fungal species										
	Ascomycetes (13 species / 4 genera)	33.3	12.5	23.0	13.3	14.3	18.1	22.2	22.7	12.5	24.0
	Coelomycetes (9 species / 5 genera)	13.3	12.5	30.8	13.3	21.4	13.6	33.3	13.6	29.1	14.3
	Hyphomycetes (38 species / 21 genera)	40.0	75.0	38.5	73.3	50.0	64.0	33.3	54.5	54.1	57.1
	Sterile forms (2 form)	6.6	0.0	7.7	0.0	7.1	4.5	11.1	9.0	4.1	4.7
	Yeast forms (2 form)	6.6	0.0	0.0	0.0	7.1	0.0	0.0	0.0	0.0	0.0
6.	Fisher's Alpha	4.63	10.67	4.35	7.47	4.61	9.44	2.89	12.96	8.63	14.97

A few tropical plant communities have been investigated for their phellophyte association,<sup>[15,16]</sup> single host studied. Kowalski and Kehrwas proposed the term phellophyte to

circumscribe fungi that colonize the dead tissues of bark from those that colonize the living tissues, several conifers have been screened for the presence of fungi in

their bark.<sup>[35]</sup> Each plant harbored at least one or more fungal endophytes.<sup>[36]</sup> Some of them have a wide range of hosts and the rest have specific hosts, thus fungal endophytes have high biodiversity. A species accumulation curve of the phellophytes fungi obtained

for the five medicinal trees and dry and wet seasons showed that the number of endophytes fungal species increased up to a certain extent but reached a plateau rather early unlike the progressive curve obtained (Figures 1, 2).

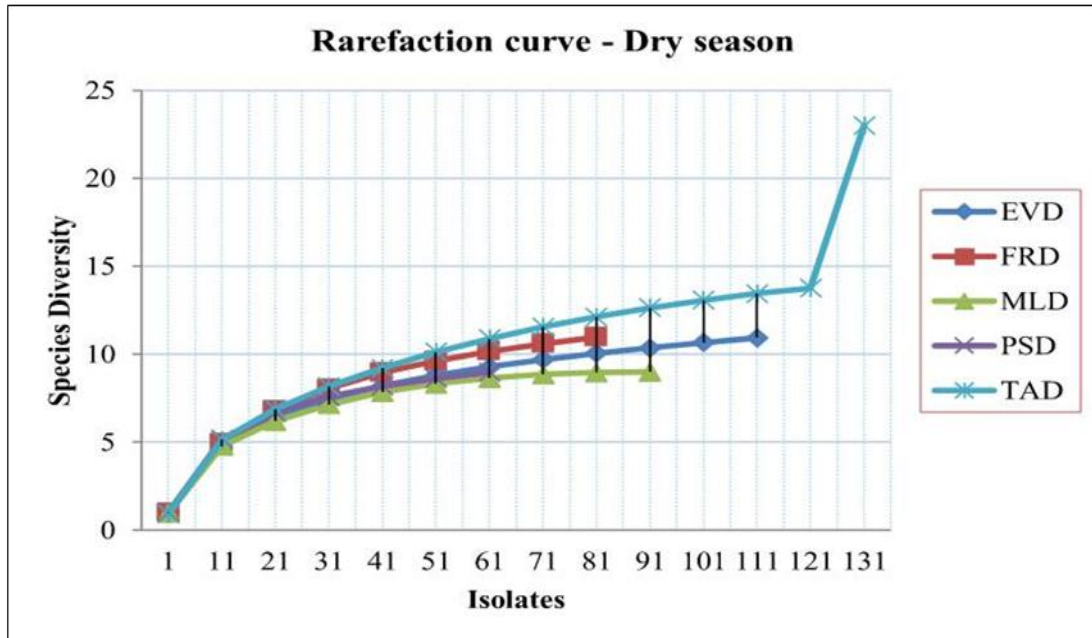


Figure 1: Isolates and species accumulation curve for the five trees from the dry season.

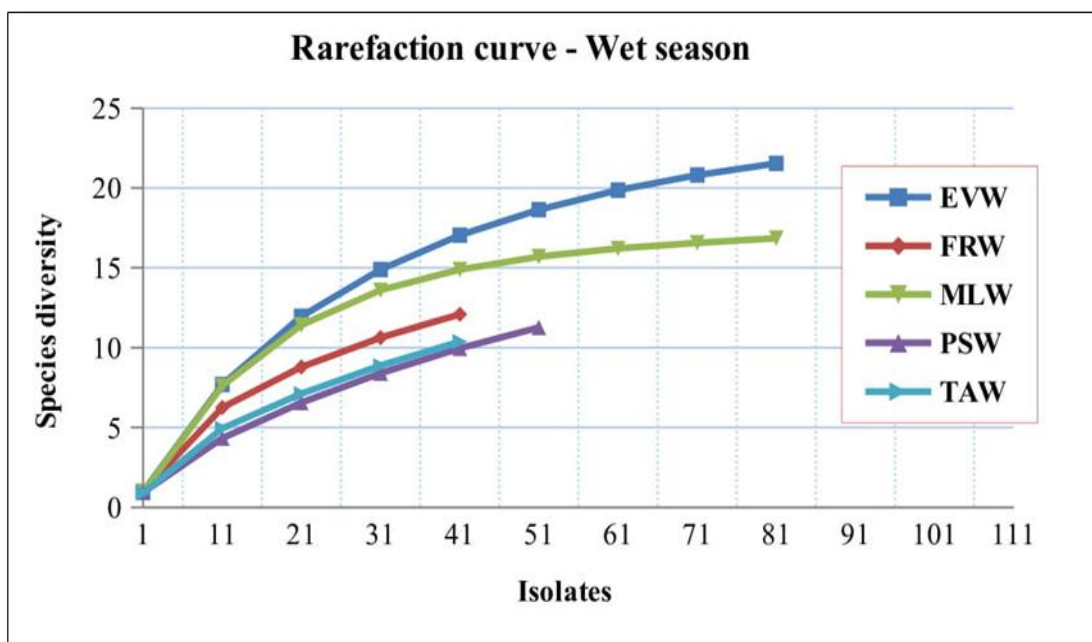
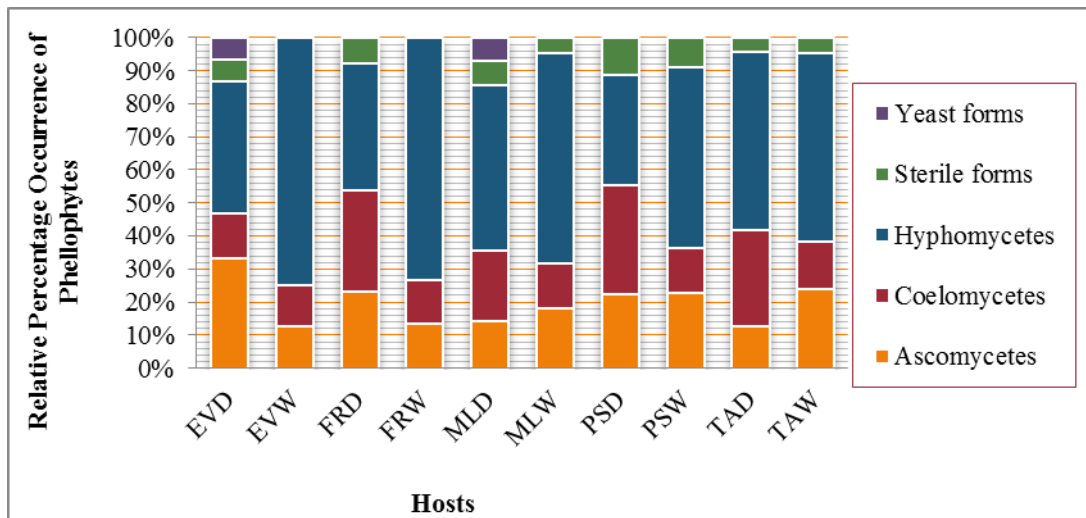


Figure 2: Isolates and species accumulation curve for the five trees from the wet season.

Earlier studies reveal that hyphomycetes, coelomycetes, ascomycetes and sterile forms invariably constitute the endophyte assemblages of trees; basidiomycetes and oomycetes are rarely encountered.<sup>[37]</sup> This trend was noticed in the present study also, although the number of sterile forms was relatively less. Interestingly, hyphomycete members contributed more to the

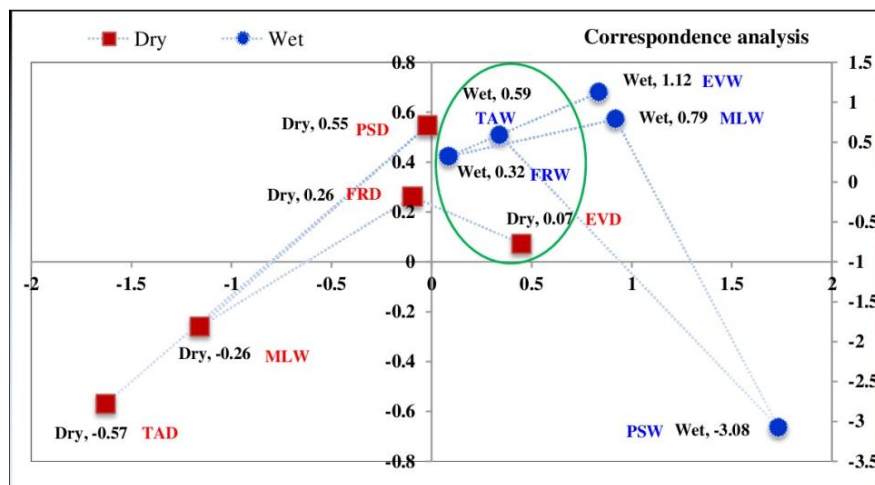
assemblage of all the hosts irrespective of the seasons and also coelomycetes were dominant endophytes in all the trees and seasons followed than ascomycetes. All the hosts were isolated belonging to ascomycetes, coelomycetes, hyphomycetes, yeast forms and sterile forms during wet and dry seasons respectively (Figure 3).



**Figure 3: Relative Percentage Occurrence of endophytes/phellophytes belonging to different groups of fungi during dry and wet seasons.**

In the present study also, tissue preference among endophytes was evident when bark endophytes were compared between different plant species. Ordination of the samples by PCA revealed strong differences between endophytes (phellophytes) of plant species. Generally, the diversity of fungal species found inner bark is less than that species found in outer bark. Fungal colonizes of outer bark contain many species with common host distributions against the species that colonize inner bark. Here, the endophytes of *Erythrina variegata*, *Ficus racemosa* and *Terminalia arjuna* had high rich diversity while these types of trees have shown greater host tissues

specificity. These plants are well covered in live bark (Phellogen) tissues (Figure 4). A few fungi that failed to sporulation were designated as “mycelia sterile”, can be become identified later with different incubation such as sporulation in UV, so for colony characteristics, the mycelia were transferred into PDA agar media. The bark’s lignicolous fungi are fibrous structures and cause decay in the end-stage. So the endophytes diversity is low presenting in some species. While the coelomycetes fungi were dominated by the endophytic assemblages.



**Figure 4: Principal Component Analysis (PCA) for tissues specificity in the endophytes/phellophytes isolated from five trees during dry and wet seasons.**

**CONCLUSION**

Most of the medicinal value often produced or induced either the plant bioactive compounds or antimicrobial. Therefore, we selected these five medicinal trees for the present study and isolated a great number of fungi which were distributed in the right vogue of this medicinal plant during the dry and wet season. The colonization

frequency of endophytes was a higher frequency during the dry season and also, the number of species increased. The endophytic fungi were done with the distinct few host tissue specificities. The results of our investigation suggest that the prediction of the global fungal diversity could be increasing based on the studies of a few hosts or a particular region to the representative.

**Significance statement**

We take this study in consideration from medicinal bark, particularly, these endophytes fungi are not more studied in the delta region of Tamil Nadu for about the fungal diversity estimate. I have believed to this study is not recorded to yet for this region. Endophytic fungi are rich sources of natural products that can be used to gratify of Pharmaceutical, medicinal, agriculture and industries.

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