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Diversity of fungal endophytes in a few medicinal plants of fertile areas of Tamil Nadu

Hemalatha Kumar*, Venkatesan Govindan

PG and Research Department of Botany, Mannai Rajagopalaswamy Government Arts College, (Affiliated to Bharathidasan University, Tiruchirappalli), Mannargudi, Tamil Nadu, India

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Abstract

Endophytic fungi are important biotechnological tools for exploring the diversity of endophytic fungi and their species richness in different ecosystems. Endophytic fungi associated with three medicinal plant species in the region of Delta in Tamil Nadu that is, the *Erythrina variegata* (coral tree) from Fabaceae, *Vitex negundo* (Chinese chaste tree) from Lamiaceae, *Melia dubia* (Malabar neem) from Meliaceae were studied. These endophytic fungi are isolated in the delta region, and the fungal colonization is rich in tropical lands. We aimed to identify endophytic fungi using morphological taxonomy; to explore the richness estimated diversity, and colonization frequency in a few medicinal plants. Fungal endophytes were isolated using leaf surface sterilization standard methods. One hundred fifty plant leaf segments are used in endophytes isolated in each plant. A total of three plant samples were analyzed and 293 endophytes were isolated from 450 leaf segments. The 16 species and 13 genera were present in *Erythrina variegata* 15 species and 12 genera were present in *Vitex negundo* and 14 species and 14 genera were present in *Melia dubia*. We thus identified 48 endophytic fungi species that were isolated from these plant tissues. In total, 26 endophytic fungal species strains belonging to Ascomycetes (03), Coelomycetes (04), Hyphomycetes (17), sterile forms (02), taxa were obtained. There results of detection and identification of endophytic fungi, different endophyte diversity and colonization frequency of a few medicinal plants in the Delta region were recorded.

***Corresponding Author:** Hemalatha Kumar ✉ hemalathathilaga1998@gmail.com

Introduction

De Bary (1866) coined the term "endophyte" to describe any creature that lives inside plant tissues as opposed to epiphytes, which are found on the surfaces of plants. According to Petrini (1991), each organism that lives in plant organs has the ability to colonies internal plant tissues at some point in its life without appearing to harm the host. Endophytic fungi have been associated with plants for over 400 million years (Krings *et al.*, 2007), and have been widely studied in various geographical, climatic and Tropical regions. Plants generally have multiple endophytes, and those may develop multiple endophytes of the host. Endophytic fungi form a major role in plant physiology and absorb nutrients from the host but at the same time, plants gain by symbiotic relationship with endophytes (Clay and Schardi, 2002; Thrower and Lewis, 1973). The Endophytic fungi of medicinal plants are considered an attractive source of novel bioactive compounds. This ability is of great importance, it provides an alternative strategy for reducing the need to harvest growing and possibly rare plants and also helps to preserve the world's diminishing biodiversity. Endophytic fungi inhabit a unique biological niche because of their ability to symbiotically colonize plant tissues (Jia *et al.*, 2016). It is estimated that there are more than 420,000 plant species in nature; however, the endophytic community has only been catalogued in a few of these. Modern mycologists generally agree that endophytes are organisms that colonize internal plant tissues without causing apparent harm to their host. Different groups of organisms such as fungi, bacteria, actinomycetes and mycoplasma are reported as endophytes of plants (Arnold, 2007). Endophytic fungi live within healthy tissues of plants and can encourage host species to different environmental stresses. The endophytic fungi from the plants were an important source for the production of various secondary metabolites, and bioactive compounds which are useful for pharmaceutical industries (Stroble, 2002; Krishnamurthy *et al.*, 2008; Khan *et al.*, 2010).

The endophytic fungi production of unique bioactive metabolites such as alkaloids, benzoquinones, flavonoids, phenols, steroids, terpenoids, tetralones, xanthones etc has been identified from endophytic fungi. Endophytes produce secondary metabolites, act as biocontrol agents, anti-bacterial agents, anti-neoplastic agents and immunosuppressive agents, produce anti-viral compounds and endophytes produce natural anti-oxidants, anti-diabetics and antibiotics compounds (Gouda *et al.*, 2016). They are taxonomically and ecologically heterogeneous groups of organisms that belong mainly to types Ascomycetes, Coelomycetes, Hyphomycetes and Zygomycetes (Verma *et al.*, 2011).

In recent years, it has been widely recognized that endophytes have a significant impact on the quality and quantity of medicines through specific microbial-host interactions. It also suggests greater information on the relationships between endophytic fungi and medicinal plants (Faeth *et al.*, 2002). This study was conducted to investigate the endophytic fungi diversity and colonization frequency of these medicinal important plants. We isolated various endophytic fungi from the different medicinal plants comparable to *Erythrina variegate* from Fabaceae and there are Ethnomedicinal applications in Chinese and Indian medicine. It is used to treat joint pain and parasitic infections (Kumar *et al.*, 2010), *Vitex negundo* parts are used as medicine in the Indigenous system of medicine, and the leaves are most potent for the treatment of inflammation, eye disease. (Alam and Gomes, 2003; Dharmasiri, 2006), *Melia dubia* were growth regulating activity on insects as well as anti-microbial, anti-fungal, anti-malarial, anti-cancer, anti-viral and a number of their pharmacological properties (Koul *et al.*, 2004).

Endophytic microorganisms have been isolated from different parts of plants like leaves, roots, stems, barks, and petioles (Hata and Sone, 2001; Venkatesan, 2013). Some researchers reported that endophytic fungal colonization is higher in leaf

segments rather than stem segments of some tropical medicinal plants (Raviraja, 2005; Banerjee and Mahapatra, 2010). Environmental factors, such as rainfall and atmospheric humidity might influence the occurrence of some fungal endophyte species (Petrini, 1991; Selvanathan *et al*., 2001). This study aimed to investigate the diversity, distribution and colonization frequency of the endophytic fungi associated with a few medicinal plants in the Delta region of Tamil Nadu.

Materials and methods

Study area

From October 2024 to December 2024 (the wet season), these plants were investigated in Tamil Nadu's Delta region (Mannargudi in the city). Both the northeast and southwest monsoons provide rain to the district. In this region, the chilly season runs from December to February, whereas the hot season spans five months, from April to August.

Collection of plants

In the Delta region of Tamil Nadu, endophytic fungi linked to three medicinal plant species of *Erythrina variegate* from the Fabaceae, *Vitex negundo* from the Lamiaceae, and *Melia dubia* from the Meliaceae were investigated (Table 1, Figs 1-3). The fungal colonization richness in tropical regions and the delta region were the sites of these endophytic fungi's isolation in Mannargudi, Tiruvarur district. After being collected, each leaf sample was processed within 24 hours after being transported to the lab in sterile polythene bags. Samples of medicinal plants that have been obtained should be in good health and carefully cleansed under running water to get rid of any dirt or debris. The entire endophytic fungal process must be conducted in an aseptic environment.

Table 1. Details of host trees studied for the presence of endophytic fungi

Sl	Host name	Family	Host code
1	*Erythrina variegata* L.	Fabeaceae	EV
2	*Vitex negundo* Linn.	Lamiaceae	VN
3	*Melia dubia* Cav.	Meliaceae	MD

Fig. 1. *Erythrina variegata*

Fig. 2. *Vitex negundo*

Fig. 3. *Melia dubia*

Medicinal uses

Below is an outline of some of these plants' significant therapeutic uses. Pain, inflammation, and other ailments can be treated with *Erythrina variegata* tree in China, India, and Southeast Asia, it is utilized in traditional medicine. Traditional medicine uses the *Vitex negundo*, to cure several ailments, including pain, cancer, inflammation, diabetes, antimicrobials, and cardiovascular protection. *Melia dubia*, often known as Malabar neem, is used to cure several conditions, such as diabetes, cancer, infections, and congestion.

Sterilization and isolation of endophytes

The leaf samples were surface sterilized using sodium hypochlorite or ethanol. The tissues of the chosen plant samples leaves were divided into 1 cm2 pieces, which were then surface sterilized.

Sterilized tissues were carefully placed on agar medium that had been modified with antibiotics, and they were then incubated. After 60 seconds of immersion in 4% sodium hypochlorite, the leaf segments were dipped in 75% ethanol for 30 seconds and then infected. Each host species three hundred tissue segments were divided across Petri dishes with PDA medium and chloramphenicol added. Each Petri dish had ten segments, which were then nurtured for 21 days at 26+1°C in a light environment.

Statistical and other analysis

The colonization frequency

The colonization frequency (CF %) of an endophyte species was determined using the specified method.

Colonization frequency (CF %) = (Number of colonies/ Number of totals) ×100

The number of colonies and total segments observed were recorded for each endophyte, with n representing the number of segments colonized by each endophyte and n representing the total number of segments observed.

The relative percentage of occurrence (RPO)

The relative percentage of occurrence (RPO) for each group viz. The fungal species in each plant species were counted and categorized into four groups: Ascomycetes, coelomycetes, hyphomycetes, and sterile forms.

RPO = (Total colonization frequency of one group/ Total colonization frequency for all the groups of fungi)×100

Results and discussion

Endophytic fungi were isolated from leaves of *Erythrina variegata* (EV), *Viitex negundo* (VN), *Melia dubia* (MD) the Host code of the plants. In the investigation, we recorded 293 endophytes isolates during the wet season from 450 leaf bits. In most of the cases, each tissue segment was infected by than one fungal species (multiple infection) substantiating the view that tropical trees have high rates of endophytes colonization (Suryanarayanan *et al.*, 2003). A total of 25 species of fungi were isolated from three medicinal plants. In total 17 fungal species and 118 individuals were isolated from *Erythrina variegata*, 15 fungal species and 102 individuals were isolated from *Viitex negundo*and 14 fungal species and 73 individuals were isolated from *Melia dubia*.

The fungi were associated with Ascomytes, Coelomycetes, Hyphomycetes and sterile forms, respectively (Table 2). In addition, endophytic communities predominated in all hosts, independent of Coelomycetes, in the three hosts. However, Coelomycetes dominate the fungal species Colletotrichum gloeosporioides (VN, EV and MD) in plants. All of these differ to a lesser frequency Extent. These endophyte communities of Coelomycetes species were more isolated in all hosts and fever in Ascomycetes.

As only very few tropical communities have been investigated for their endophyte association. We concentrated on the endophyte states of a fertile area in Tamil Nadu in an effort to better understand the interactions between endophytes, hosts, and their environments in defining the distribution pattern of endophytes in tropical plant communities.

In the present study, after the initial morphological feature study of 26 fungal endophytes, we identified the fungal endophytes group from leaves of medicinal plants such as Ascomycetes, Coelomycetes, Hyphomycetes and Sterile form. Endophytic species diversity indices calculated with colonization frequency for all host plants. These endophytic fungi species were calculated for relative percentage of occurrence from three medicinal plants (Table 3). The morphological character has discovered in different colonization in fungal endophytes (Fig. 4).

Table 2. Number of isolates, species and diversity index for endophytes isolated from three plant species during wet season

Sl	Fungus	Host code					
		EV	CF (%)	VN	CF (%)	MD	CF (%)
1.	Ascomycetes						
2.	*Glomerella cingulata*	1	0.6	2	1.3	3	2
3.	*Sporormiella intermedia*	1	0.6			1	0.6
4.	*Xylariaceous* form 1			2	1.3	3	2
5.	Coelomycetes						
6.	*Colletotrichum gloeosporioides*	41	27.3	32	21.3	19	12.6
7.	*Colletotrichum carssipes*			8	5.3		
8.	*Phoma* sp. 1	2	1.3				
9.	*Phyllostica capitalensis*	3	2	1	0.6	1	0.6
10.	Hyphomycetes						
11.	*Alternaria alternata*	9	6	5	3.3	5	3.3
12.	*Aspergillus nidulance*					1	0.6
13.	*Aspergillus flavipes*	2	1.3				
14.	*Aspergillus flavus*			2	1.3		
15.	*Aspergillus niger*	4	2.6	5	3.3	3	2
16.	*Cladosporium cladosporioides*	1	0.6			1	0.6
17.	*Cladosporium candidum*			2	1.3		
18.	*Curvularia lunata*	26	17.3	21	14	17	11.3
19.	*Curvularia vermiformis*	2	1.3	1	0.6		
20.	*Cylindocarpon radicicola*	3	2	3	2	2	1.3
21.	*Drechslera maydis*	2	1.3			2	1.3
22.	*Nigrospora oryzae*			1	0.6		
23.	*Fusarium oxysporum*	16	10.6	13	8.6	09	06
24.	*Fusarium equiseti*	2	1.3				
25.	*Fusarium solani*	1	0.6				
26.	*Penicillium citrinum*			2	1.3	1	0.6
27.	*Verticillium nubilum*	2	1.3			1	0.6
28.	Sterile form						
29.	Black Sterile mycelium	3	2.3	2	1.3	3	2.3
30.	White sterile mycelium	2	1.3			1	0.6
	Total No. CF%		81.6%		67.4%		50.9%
	Total No. of species	17		15		14	
	Total No of Isolates	118		102		73	

Table 3. Number of isolates, species and diversity index for endophytes isolated from three plant species during wet season

SL	Information	EV	VN	MD
1	Species	17	15	14
2	Individuals	114	102	73
Relative percentage of occurrence (RPO) of each group of fungal species				
3	Ascomycetes	10.1	12.5	13.9
4	Coelomycetes	60.3	55.8	50.1
5	Hyphomycetes	25. 7	29.3	30.2
6	Sterile form	4.5	3.9	4.5

Although the numbers of endophytic species recovered in these hosts were not significantly different, the endophytic communities of the Coelomycetes fungal group were more diverse.

Endophyte isolates from *Erythrina variegata* (EV), *Vitex negundo* (VN), *Melia dubia* (MD) hosts were more species rich than isolates from other host plants. Comparatively to the other endophytes, *C. gloeosporioides*, *A. alternata*, *C. lunata*, *F. oxysporum* and *A. niger* had higher abundance rates and frequencies. While the Coelomycetes fungal group remaining isolated in all medicinal plants at the predominant frequency (Fig. 4). Endophytic fungal genera such as *Colletotrichum*, *Alternaria*, *Curvularia*, and *Fusarium* are host-related and colonize; taxonomically distinct plant hosts. Moreover, the morphological conditions used are not sufficient to distinguish species from complex genera such as *Colletotrichum* and *Curvularia*.

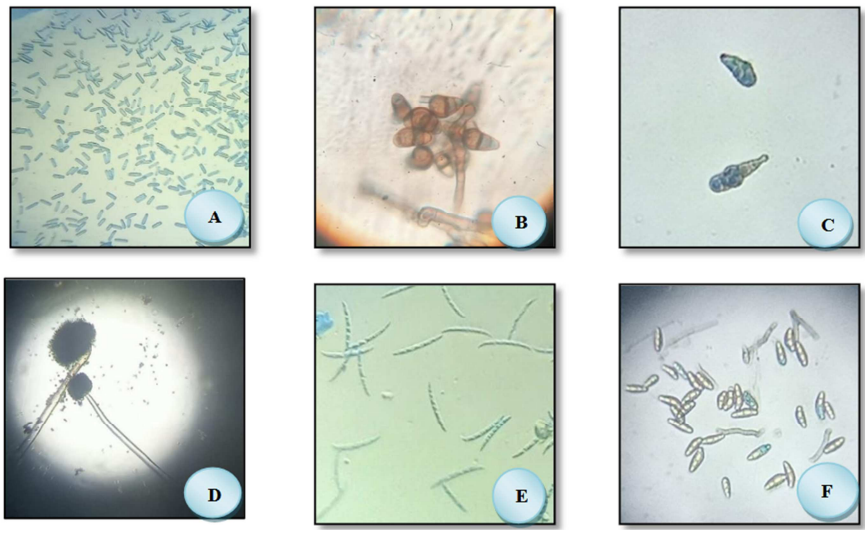

Fig. 4. A. *Colletotrichum gloeosporioides* B. *Curvularia lunata* C. *Alternaria alternata* D. *Aspergillus niger* E. *Fusarium solani* F. *Collectotrichum* sp

The number of isolated species described in each plant and compared to those recorded in the species richness. Here we point out that and *Colletotrichum*, *Alternaria*, *Curvularia*, and *Fusarium* were found in enormous numbers and that *A. nidulance* and *F. solani* were also in least numbers but gradually decreased.

In several host plant species in nature may be inhabited by a complex of pathogenic and endophytic fungi from genera such as *Colletotrichum* sp and *Curvularia* sp plant-pathogenic fungi are frequently isolated as endophytes from apparently healthy plant organs. Woody plants typically have a greater diversity of endophytes than grasses with their "mutualistic" associates.

Endophytes typically have a more limited distribution inside their host's tissue and may or may not produce secondary metabolites in plants. Fungi have a wide range of medical uses, either directly or indirectly, through the biochemicals they produce during their metabolism, which are identical to the medicinal uses of plants in human development. Several studies have shown that while endophytic fungus does not affect the plant, it may indirectly support plant tissues. In addition, many studies have shown that when fungi are isolated, a huge number of biochemicals are produced. They have applications in industry, agriculture, and medicine. On the basis of this, we investigated medicinal plants and discovered a diversity of fungi. According to our theories, these fungi may also produce biochemicals.

Conclusion

Plant endophytic fungi as an abundant microbes reserve from their host plants as well as some bioactive compounds, this report many investigators interesting in many basic research and applied fields. In the past few decades, many researchers mainly focused on the investigation of fungal endophytes for diversities and their relationships with their host plants. Recently interest has been produced in searching for medicinal plants and their bioactive compounds patented from the endophytic fungi. As a result, we selected a few important and traditional used plants from Delta region in Tamil Nadu, studied the plant tissues fungus in them, and discovered a wide diversity of fungal species. We discovered that only certain of these fungal species were more abundant than others and that some species were less common. The plant segments are multiple infection of fungal endophytes were studied in the research. Several of the species listed here are well-known for producing biochemical that is used in medicine, as far as we are concerned. In the future, we'll be consistent to conduct our own research.

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