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Diversity of foliar endophytic fungi isolated from medicinal plants of Indian dry deciduous forest, Bhadrachalam, Telangana

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Abstract

Twenty Four foliar endophytic fungal species were isolated from the living leaves of 37 medicinal plants from Bhadrachalam dry deciduous forest localised along the river Godavari of Khammam district, Telangana state, India. Morphotypes of mycelia sterilia are predominant occurring among fungal endophytes encountered. Endophytes associated with *Andrographis paniculata* are more in number while *Holorrhina antidysentrica* supported minimum endophytic fungi. Species of *Acremonium, Cladosporium, Curvularia, Alternaria* and *Colletotrichum* were frequently isolated. *Arthrinium phaeospermum* is a rare and infrequent endophyte mostly confined to *Andrographis paniculata. Badarisima sojae, Emericella nidulans, Sordaria fimicola, Stachybotrys chartarum, Tretopileus sphaerophorus* are the new endophytes reported for the first time. The results indicate significant information on endophytic fungal diversity associated wide natural dry deciduous forest flora which is under the threat of inundation by the ongoing Polavaram irrigation project.

Key words :

INTRODUCTION

Endophytes commonly refer to a group of fungi that reside asymptomatically inside the living plant tissues [1, 2]. Earlier surveys of various hosts have demonstrated that fungal endophytes are ubiquitous [3, 4]. Endophytic fungi represent an important and quantifiable component of fungal diversity, and are known to affect plant community diversity and structure [5, 6]. Plants in general and particularly medicinal plants in partial provide a unique environment for endophytes as they have a repository of endophytes secreting novel metabolites of pharmaceutical importance [7, 8, 9]. Differences in fungal community compositions and frequencies of endophytes are reported in various host plants. The endophytic fungi may increase the growth response in inoculated host plants through nutrient cycling, by secreting plant growth promoting substances which can offer resistance to the host plants under unfavourable environmental conditions [10]. Investigations of endophytic fungi hold promise for conservation as well as utilizing fungi for novel drugs and other biotechnologically important industrial products. It is therefore a necessity to determine endophytic biodiversity to survive in adverse environmental conditions.

Natural habitats are fast degrading and disappearing due to anthropogenic intervention in the name of development. One such habit in southern India, a deciduous forest of Bhadrachalam is at the verge of disappearance due to the ongoing Polavaram irrigation project that was given national status in recent times. Bhadrachalam forest spread along the river Godavari in the state of Telangana, India, is a dry deciduous forest encompassing local pockets of moist deciduous forest elements which provides a special environment for diversify of endophytic fungi. The present study was taken up to investigate the endophytic diversity of certain medicinal plant species occurring in Bhadrachalam forests using standard techniques.

METHODOLOGY

Sources of Endophytes

Endophytic fungi were isolated from healthy medicinal plants of Bhadrachalam forest located along the river Godavari, Khammam dist., Telangana, India.

Isolation and culture of endophytic fungi

The leaf samples were collected using the perforated bags to avoid the desiccation of plant material. The samples were rinsed gently in running water to remove dust and debris. After proper washing, leaves were cut into 3-4 mm × 0.5-1 cm pieces under aseptic conditions. Plant material was treated with 75% ethanol for 1 min followed by immersion in sodium hypochlorite and again in 75% ethanol for 30 seconds [11]. They were finally rinsed with de-ionized sterile distilled water and blot dried on sterile tissue paper, sterilized leaves were cultured in Petri dishes containing potato dextrose agar medium (PDA) supplemented with 100 µg/ml of streptomycin. The Petri dishes were sealed with parafilm and incubated at 27±2°C for 15 days.

Fungi growing out of the plant explants were sub cultured on separate PDA slants and stored at 4°C for further identification. The fungi were identified based on the cultural characteristics and direct microscopic observations of the fruiting bodies and spores of fungi using standard manuals [12]. Non sporulating strains were induced for sporulation by culturing them on malt extract agar (MEA) and water agar (WA). Those cultures which failed to sporulate were grouped under mycelia sterilia.

Calculation of colonization frequency and Relative frequency

Colonization frequency (%) [13] of an endophyte species was equal to the number of segments colonized by a single endophyte divided by the total number of segments observed ×100.

Relative frequency (RF) of isolation, used to represent fungal density, was calculated as the number of isolates of a species

divided by the total number of isolates, and expressed also as a percentage [14,3].

RESULTS

A total of 335 endophytic fungal strains were isolated from 37 medicinal plants from a dry deciduous forest of Bhadrachalam, Khammam district of Telangana, India, were identified into 24 taxa based on morphological characters. The endophytes which failed to sporulate were also isolated more frequently from majority of plants and named as morphotypes under mycelia sterilia. These morphotypes are predominant among frequently isolated fungal endophytes with colonization frequency of 15% and relative frequency of 19 % (Fig 1, Fig 2) (Table 1). *Andrographis paniculata* has the highest number of isolates while *Holorrhina antidysentrica* recorded minimum number of endophytes with only two isolates. Species of *Acremonium*, *Cladosporium*, *Curvularia*, *Alternaria* and *Colletotrichum* were the frequently occuring endophytes along with many known but less frequently isolated fungi in this study. These results are in conformity with the earlier findings from tropical endophytic fungi [15]. *Arthrinium phaeospermum* is a rare and infrequent endophyte that was isolated very frequently from *Andrographis*. Five fungi, *viz*., *Badarisima sojae*, *Emericella nidulans*, *Sordaria fimicola*, *Stachybotrys chartarum* and *Tretopileus sphaerophorus* are reported as new endophytes for the first time. Each medicinal plant out of 37 medicinal plants harboured at least one, often many endophytic fungi with high endophytic diversity being in *Andrographis paniculata, Terminalia bellerica* and *Wrightia tinctoria* (Fig 3).

Mitosporic fungi were the most common endophytes isolated in the present study with a large number of non sporulating group of fungi categorized as mycelia sterilia. There is a considerable species diversity among the endophytic fungi from 37 different medicinal plants. Recent studies of endophytic fungi from tropical and temperate forests support the high density of species diversity [16, 17, 18]. The endophytes which failed to sporulate were commonly isolated from majority of plants and named as morphotypes under mycelia sterilia. Mycelia sterilia have shown wide diversity with 21 morphotypes distinguished based on colony and morphological growth parameters. Mycelia sterilia is a large group of fungi that fail to produce true spores and consists of fungi of various morphological types [19]. These mycelia sterilia are considerably prevalent among endophytic mycoflora [20]. These morphotypes are predominant among frequent fungal endophytes with colonization frequency of 15% and relative frequency of 19 % and this percentage agree with many earlier endophytic studies [21, 3]. Fungal taxa represented of hyphomycetes (46%), sterile fungi (20%), coelomycetes (18%), ascomycetes (9%), imperfect fungi (4%) and plectomycetes (3%) (Fig. 4), (Fig.5).

DISCUSSION

Acremonium species are frequently identified as endophytes [14]. These results are in conformity with the earlier findings reported from tropical endophytic fungi [5, 3, 20]. The endophytic fungi residing in 29 traditional Chinese medicinal plants and reported high biodiversity, host- recurrence, tissue specificity and spatial heterogeneity. The dominant fungal endophytes of their

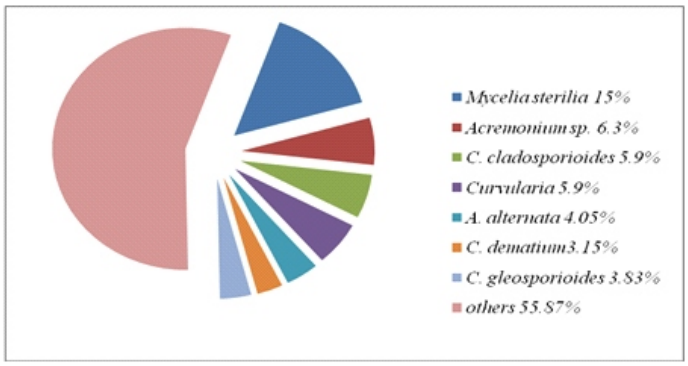

Fig. 1: Colonization frequencies of different endophytic taxa isolated from 37 medicinal plants of Bhadrachalam forest. "Others" include rare and infrequent endophytic fungal species

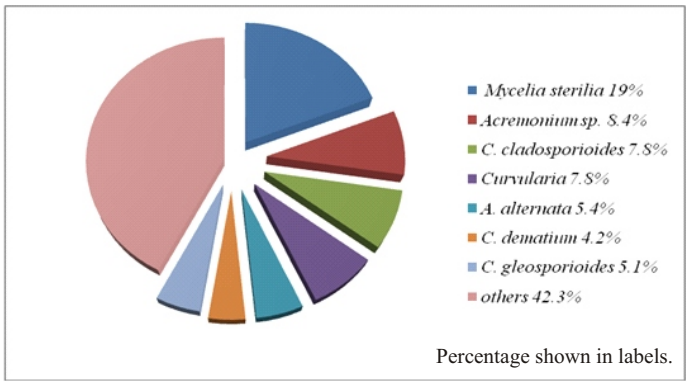

Fig. 2: Relative frequencies of different endophytic taxa isolated from 37 medicinal plants of Bhadrachalam forest. "Others" include rare and infrequent endophytic fungal species.

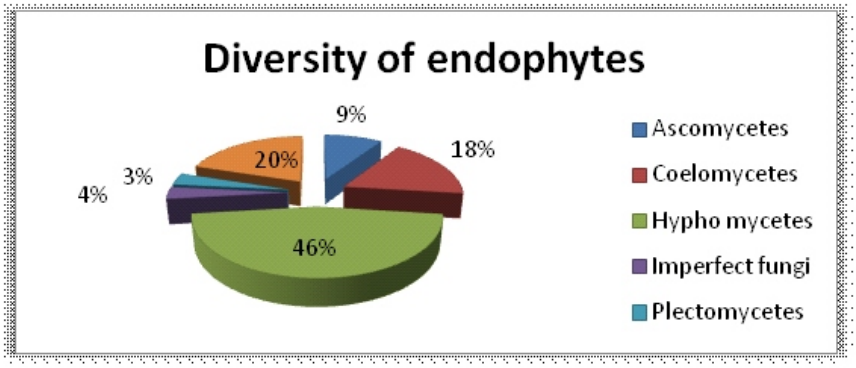

Fig. 3: Diversity of endophytic fungi isolated from medicinal plants of Bhadrachalam forest.

Table 1: Number of endophytic colonies isolated from 37 medicinal plants of Bhadrachalam forest

S.no	Host/Endophytic fungi	*Acremonium sp.*	*Alternaria alternata*	*Arthrinium pheospermum*	*Aspergillus sp.*	*Aspergillus ochraceus*	*Badarisoma soja*	*Chaetomium sp.*	*Cladosporium ladosporioides*	*Colletotrichum capsici*	*Colletotrichum dematium*	*Colletotrichum gloeosporioides*	*Curvularia sp.*	*Drechslera sp.*	*Emericella nidulans*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium sp.*	*Glomerella cingulata*	*Khuskia oryzae*	*Leptosphrulinae hartarum*	*Nigrospora sphaerica*	*penicillium frequentans*	*P. microspora*	*P. mangiferae*	*P. glandicola*	*Phomopsis sp.*	*Rhizoctonia sp.,*	*Sordaria fimicola*	*Stachybotrys chartarm*	*T. sphaerophorus*	*mycelia sterilia*	*Total*
1	*Acacia sundra*	2							3										6												3		14
2	*Aegle marmoelos*																															5	5
3	*Andrographis paniculata*		4	5									4											3	3	2						2	23
4	*Aristolochia indica*								2																							6	8
5	*Atylosia* sp.																															4	4
6	*Blumea* sp.										5									4						6							15
7	*Buchanania lazan*								3													5											8
8	*Casia absia*		5		3				2																			4				2	16
9	*Chlorophyton*		2																													3	5
10	*Chloroxylon swietenia*														4												2						6
11	*Clitoria ternatea*								3				5					2														2	12
12	*Coleus* sp.	3																														3	6
13	*Cryptostegia* sp.													2		3	3								2	3							13
14	*Dalbergia latifolia*			4		3			2																			2					11
15	*Diospyros* sp.										4								2						3								9
16	*Emblica* sp.	5				2	2											4									1						14
17	*Eupatorium* sp.						1				3												2										6
18	*Gardenia gummifera*				4				3																								7

19	*Gloriosa superba*												2	4															2					8
20	*Gymnosporia montana*																																	4	4
21	*Holarrhena antidysenterica*														2																				2
22	*Indigofera* sp.					2			2																									4	8
23	*Legustomia purviflora*	2	3																															3	8
24	*Lepidogathis cristata*																																	5	5
25	*Manikara* sp.				2							4							2																8
26	*Mitragyna parvifolia*																																	5	5
27	*Morinda* sp.																																	2	2
28	*Paciflora*												6	2																					8
29	*Pupalia lappacea*							3		2	2			5							2														14
30	*Pergularia daemia*																																	4	4
31	*Phaseolus trilobus*	2				1		2																										2	7
32	*Polygala arvensis*											3						2	1												3				9
33	*Sapindus* sp.								2				4																					4	10
34	*Strychnos* sp.																																	2	2
35	*Terminalia ballarica*	6	4																2											4					16
36	*Secruniga leucopyrosa*	8							4			2						4																3	21
37	*Wrightia tinctoria*											8	5																						13
	Total	28	18	9	9	8	2	5	26	2	14	17	26	13	6	3	3	12	13	4	2	5	2	3	8	11	3	8	4	3	3	65	335
	Colonization frequency %	6.3	4.0	2.0	2.0	1.8	0.4	1.1	5.8	0.4	3.1	3.8	5.8	2.9	1.3	0.6	0.6	2.7	2.9	1	0.4	1.1	0.4	0.7	1.8	2.5	0.7	1.8	1	0.7	0.7	15	75.5
	Relative frequency %	8.4	5.4	2.7	2.7	2.4	0.6	1.5	7.8	0.6	4.2	5.1	7.8	3.9	1.8	0.9	0.9	3.6	3.9	1.2	0.6	1.5	0.6	0.9	2.4	3.3	0.9	2.4	1.2	0.9	0.9	19	

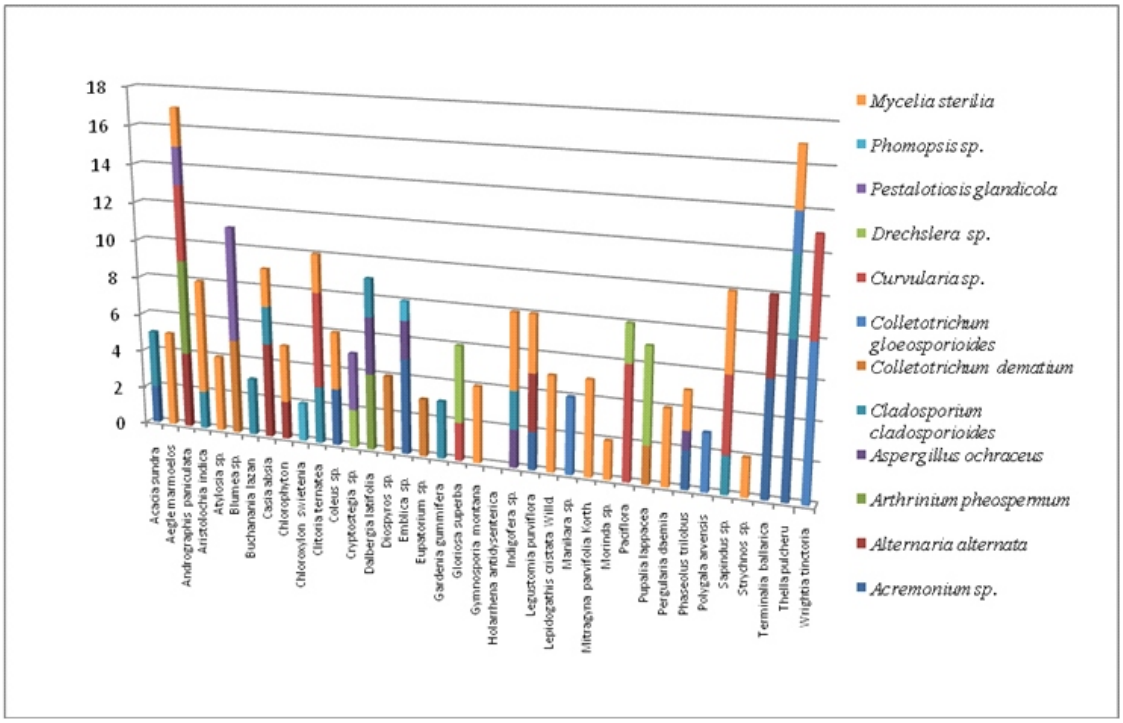

Fig. 4: Endophytic fungal diversity of medicinal plants from Bhadrachalam forest, Andhra Pradesh, India.

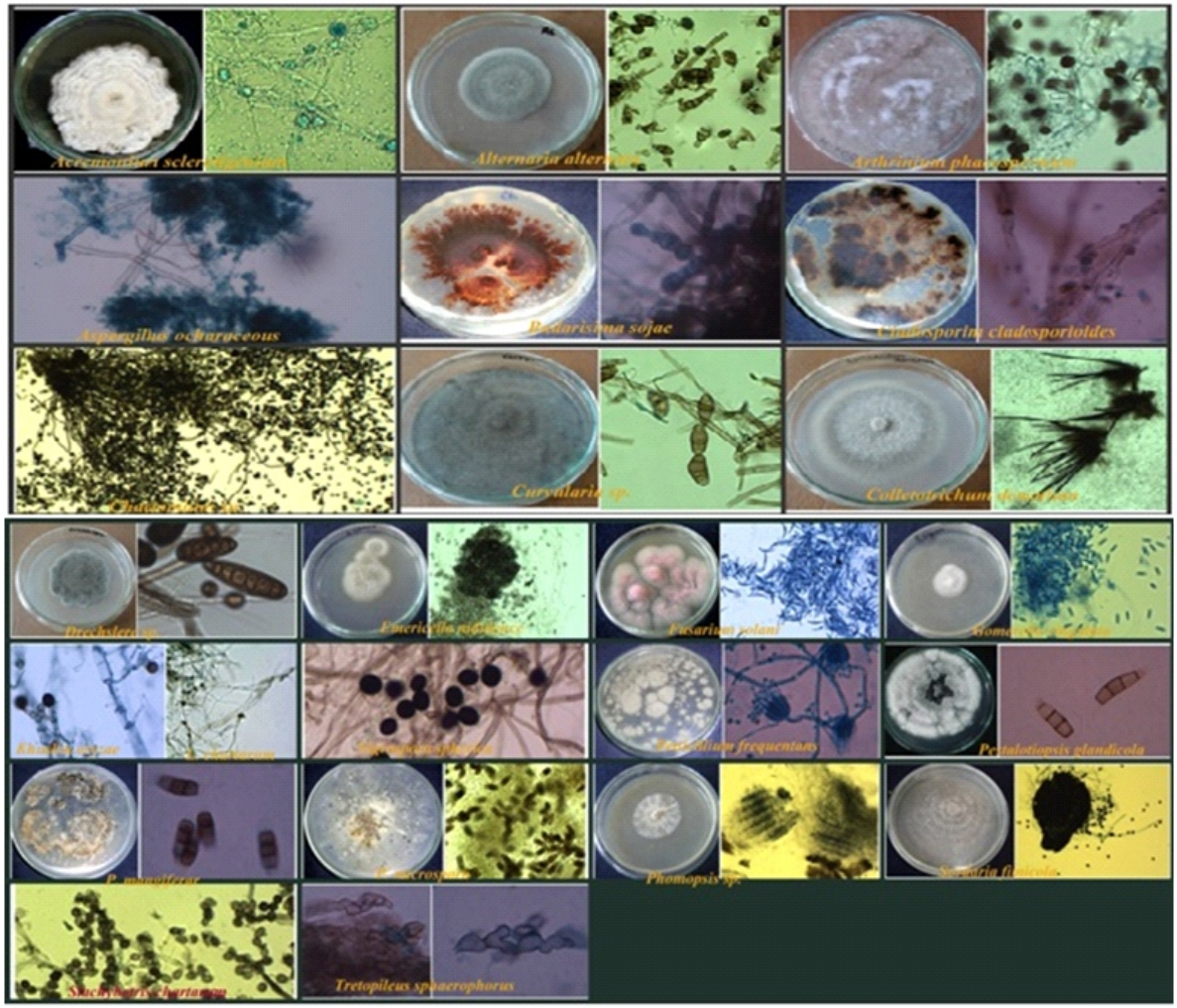

Fig. 5: Isolated endophytic fungi

study include taxa of *Alternaria*, *Colletotrichum*, *Phoma*, *Phomopsis*, Xylariales and Mycelia sterilia [3]. The frequent endophytes isolated from 37 medicinal plants in the present study are mycelia sterilia, *Cladosporium*, *Curvularia*, *Alternaria* and *Colletotrichum.*

The dynamics of endophyte-plant symbioses and their interaction is influenced by the ecological factors. The relationships of endophytes with single or multiple hosts have been described in terms of hostspecificity, hostrecurrence, host selectivity or host preference [22, 23]. Host-specificity is the relationship in which a fungus is restricted to a single host or a group of related species, but does not occur in other unrelated plants in the same habitat [24]. The frequent or predominant occurrence of an endophyte on a specific host or a range of plant hosts is considered as host- specificity in relation to endophytic fungi was not evident. Endophytic mycelia sterilia dominated both in colonization frequencies and relative frequencies covering almost 50% of the medicinal plants studied. On the other hand, relatively rare endophyte, *A. phaeospermum* was isolated only from two hosts, *D. latifolia* and *A. paniculata*. The results indicated no clear cut host preference by the endophytes either to a specific host or to a range of related hosts. For the first time in this study, *B. sojae*, *E. nidulans*, *Sordaria fimicola*, *Stachybotrys chartarum* and *Tretopileus sphaerophorus* are infrequently isolated from same habitat. The differences in endophyte assemblages of different hosts have been attributed to the host chemical make-up and the host specificity at the plant species level as endophyte influenced by environmental conditions [23]. Huang *et al.* (2008) have shown the coexistence of some phenolic compounds with certain endophytic fungi of Chinese traditional medicinal plants. Fungal endophytes of trees and woody plants have been reported to belong to ascomycetes and their anamorphic states [25, 26].

CONCLUSION

The present results on abundance and frequency of foliar endophytic fungi from medicinal plants of Bhadrachalam forests have to be supplemented by studying the nature of the chemicals produced by these plants to establish any relationship between host compounds vis-a-vis endophyte-plant symbiosis. The endophytes are mostly the anamorphic forms of ascomycetous fungi belonging to hyphomycetes and coelomycetes.

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