Diversity and structure of fungal endophytes in some climbers and grass species of Malnad region, Western Ghats, Southern India

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A total of 3,732 fungal isolates were recovered from 6000 leaf segments incubated from ten medicinal climbers and grass species during the monsoon, winter and summer seasons. The fungi comprised Ascomycota (22.8%), Coelomycetes (17.7%), Hyphomycetes (44.7%), Zygomycetes (0.3%) and mycelia sterilia (14.5%). *Alternaria alternata*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *C. hebarum*, *Curvularia lunata*, *Gliocladium roseum*, *Nigrospora shaerica*, *and Phyllosticta* spp. were frequently isolated. Colonization frequency (%) differed significantly between the seasons (F=5.35). The colonization rate was higher during the winter than in the monsoon and summer seasons.

Key words – Tropics – symbiotic fungi – variation – species richness

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Introduction

Endophytic fungi are symbionts that live inside the above ground tissues of plants. They may enhance host resistance to herbivores through the production of various alkaloids (Clay & Shardl 2002), nutrient uptake (Malinowski et al. 2000) and play a key role in affecting host tolerance to stressful conditions such as heat (Redman et al. 2002) and salinity (Rodriguez et al. 2004), and affect evolution (Brundrett 2006) and plant biodiversity (Clay and Holah 1999). However, the diversity, geographic distribution and host specificity of endophytes remain largely unknown (Arnold & Lutzoni 2007) and most of the endophytic fungi are yet to be discovered (Hyde et al. 2007). Several authors have suggested that the majority of undiscovered endophyte diversity occurs in leaves of tropical trees (Promputtha et al. 2005). Endophytes are the potential producers of novel biologically active compounds of immense value in agriculture, medicine and industry (Tan et al. 2000, Tan & Zou 2001, Aly et al. 2010, Shankar & Krishnamurthy 2010) and some endophytic fungi have the potential to produce bioactive compounds similar to those of host plants that may have therapeutic purposes (Strobel 2002, Huang et al. 2008). Screening this diverse group of fungi is a promising approach for obtaining medicinal plant products on a commercial scale (Strobel & Daisy 2003, Kumar & Hyde 2004).The present work aimed to identify the composition of fungal endophytes in some medicinal climbers of Malnad region, Western Ghats and to determine their incidence in different seasons.

Materials and Methods

Sample collection and isolation of endophytes

The leaf samples of *Clematis gouriana* (Ranunculaceae), *Clitoria ternatea* (Fabaceae), *Cyclea peltata* (Menispermiaceae), *Cymbopogon citratus* (Poaceae), *Dioscorea pentaphylla* (Dioscoriaceae), *Naravelia zeylanica* (Ranunculaceae), *Oryza sativa* (Poaceae), *Piper nigrum* (Oxalidaceae), *Tinospora cordifolia* Menispermiaceae), *Zymnema sylvestris* (Asclepidiaceae) (Table 1) were collected in winter (December, 2007 – January, 2008), summer (April – May, 2008) and monsoon (Aug – September, 2008) seasons from Malnad region of Shimoga District, Karnataka state, Southren India, which is a part of Western Ghat region, a hot spot of global biodiversity. The samples were taken in sterile polythene bags to the laboratory and processed within 24 h of collection. For each host, 200 segments were randomly cut from the leaves of two individual plants; the two plants werelocated within a kilometre apart. Samples were washed under running tap water, cuttin to ≤ 1 cm^2 segments, and surface sterilised by stepwise washing with 70% ethanol for 2 min, sodium hypochlorite solution for 5 min and 70% ethanol for 30 s. The leaf segments were then allowed to surface dry under sterile conditions. This method of surface sterilization has been shown to effectively eliminate contaminants (Arnold et al. 2000). Leaf segments were placed on 9 cm Petri plates containing potato dextrose agar (PDA, Hi Media Laboratories, Mumbai, India) amended with streptomycin 250 (mg/L^{-1}) to suppress bacterial growth. The efficacy of surface sterilization was confirmed by pressing the sterilized leaf segments on to the surface of PDA medium. The absence of growth of any fungi on the medium confirmed that the surface sterilization procedure was effective (Schulz et al. 1993). The plates were incubated at 28 ± 1 ºC with a 12 h photoperiod. Fungi growing out from the leaf segments were subsequently transferred onto fresh PDA plates and sporulation was induced by incubation in a light chamber under near UV light for 1 to 12 d. Pure cultures were maintained on PDA slants. Endophytic fungal species were identified on the basis of cultural characteristics and morphology of fruiting bodies and spores by using standard texts and keys (Subramanian 1971, Sutton 1980, Barnett & Hunter 1998). Cultures that failed to sporulate were recorded as mycelia sterilia. All isolates were numbered and are maintained in Culture Collection Centre of Department of Applied Botany, Kuvempu University, Shankaraghatta, India.

Data analysis

The colonization rate of endophytic fungi was determined as the total number of segments yielding ≥ 1 isolate in a host sample divided by total number of segments incubated in that sample x 100. Frequency of colonization by individual taxa was calculated similarly. Significance of differences in the frequency of colonization among the host plants was determined by Kruskal Wallis method (Gibbons 1976). Differences between winter, monsoon and summer seasons were tested by ANOVA. Shannon diversity index (H'), Shannon evenness index (J') and Simpson diversity index (1/D) were used for evaluation of fungal species richness. (Zar 2004). Similarity of endophytic mycobiota was assessed among the host plants.

Results

A total of 3,732 fungal isolates were recovered from the total of 6000 leaf segments incubated from 10 medicinal plants, and comprised ascomycetes (14.1%), coelomycetes (10.9%), hypomycetes (27.6%), zygomycetes (0.2%) and sterile forms (9.0%), *Chaetomium globosum* (10.1%), *Colletotrichum gleosporioides* (7.7%), *Nigrospora sphaerica* (5.0%), *Phyllosticta* spp. (3.5%), *Alternaria alternata* (2.3%), *Cladosporium herbarum* (2.2%) were frequently isolated fungal species. The total colonization rate was higher in winter than in monsoon and summer seasons (Fig. 1). The colonization frequency of endophytic fungi varied and differed significantly between monsoon, winter and summer seasons (F=5.35).

The total colonization frequency ranged from a low of 52.2% in *Clitorea ternatea* to 68.1% in *Piper nigrum*. The frequency of colonization was highest in *Naravelia zeylanica* (86.5%) and lowest in *Oryza sativa* (43.5%) during monsoon, and it was high in *Tinospora cordifolia* (85.5%) and low in *Clitorea ternatea*

Table 1 Plants studied for the isolation of endophytic fungi from Malnad region of Karnataka and their medicinal uses.

Host Plant	Family	Collection site	Medicinal uses
Clematis gouriana	Ranunculaceae	Shankaraghatta	Antiseptic, cold
Clitoria ternatea	Fabaceae	Kumsi	Antibacterial
Cyclea peltata	Menispermiaceae	Thirthahalli	Bronchitis, heart ailments
Cymbopogon citratus	Poaceae	Lakkavalli	Gastrointestinal disorders
Dioscorea pentaphylla	Dioscoriaceae	Koppa	Rheumatism
Naravelia zeylanica	Rananculaceae	Shankaraghatta	Headache and toothache
Oryza sativa	Poaceae	Lakkavalli	Gastrointestinal disorders
Piper nigrum	Oxalidaceae	Koppa	Dyspepsia, piles, anemia, timpanist
Tinospora cordifolia	Menispermaceae	Shankaraghatta	Rheumatism, expectorant, diuretic
Zymnema sylvestris	Asclepidiaceae	Lakkavalli	Diabetics

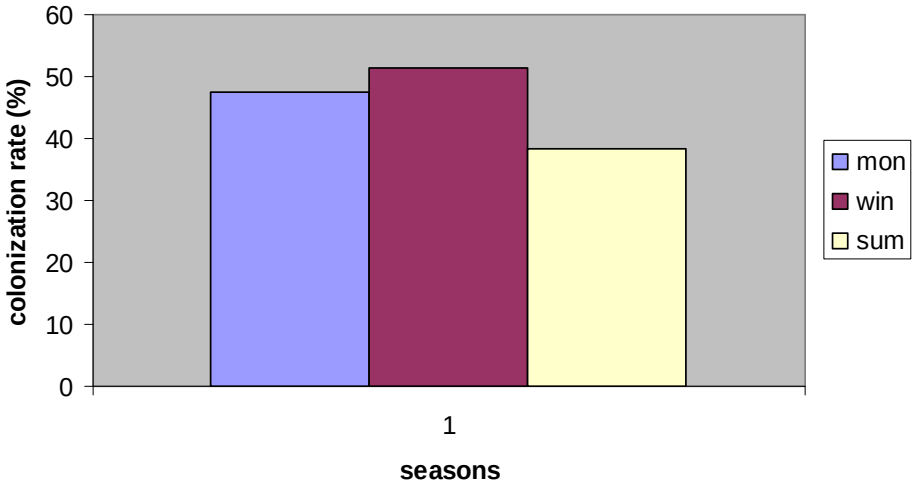

Fig. 1 – Colonization rate % of endophytic fungi in different medicinal plants collected from Malnad region of Western Ghats, Karnataka (*mon=monsoon, win=winter, sum=summer)

(64.0%) during the winter. The colonization was high in *P. nigrum* (55.0%) and low in *N. zeylanica* (27.5%) during the summer season (Table 2). Colonization frequency (%) did not differed significantly among medicinal plants studied. Maximum endophytic diversity was observed in *Clitoria ternatea* (H' = 1.04) and least diversity was found in *Clematis gouriana* (H' = 0.62). Evenness was maximum in *Clitoria ternatea* (J' = 0.91) and minimum in *Clematis gouriana* (J' = 0.59). According to Simpson's diversity index higher species abundance was recorded in *Clitoria ternatea* (1/D = 10.0) and lower in *Clematis gouriana* (J' = 3.12) (Table 3, 4). *Colletotrichum gloeosporioides*, *Chaetomium globosum*, *Phyllosticta* spp., *Xylaria* spp., *Penicillium* spp., and *Gliocladium roseum* were isolated more during winter and monsoon than summer season. *Nigrospora sphaerica*, *Aspergillus* spp., *Aureobasidium pullulans*, *Penicillium* spp., and *Cladosporium cladosporioides*, *C. herbarum* were isolated in higher numbers during the monsoon season. The incidence of *Septocylindrium septatum*, *C. cladosporioides*, *Penicillium* spp., and *Aspergillus* spp. was highest during the summer season. Similarity of endophytic assemblage shows the highest similarity between *Tinospora cordifolia* and *Dioscorea pentaphylla*, and *Cyclea peltata* and *Zymnema sylvestris* (Fig. 2).

Discussion

The colonization frequency of fungal endophytes was within the range of many host plants studied in the tropics (Fröhlich et al. 2000). The frequently isolated fungi such as *Chaetomium globosum Colletotrichum gloeosporioides*, *Phyllosticta* spp., *Cladosporium* spp. are generalist species which grow rapidly on culture medium (Fröhlich & Hyde 1999, Cannon & Simmons 2002, Suryanarayanan et al. 2003, Krishnamurthy et al. 2008). *Aspergillus*, *Penicillium* and *Cladosporium* species isolated in this study are common soil or air

Table 2 Percent colonization frequency, dominant genus and total number of species encountered in medicinal climbers and grasses.

Host Plants	Dominant Genus	Colonization frequency (%)				Number of endophytic species
		Monsoon	Winter	Summer	Total	
Clematis gouriana	*Xylaria*	61.0	80.0	53.0	64.6	11
Clitoria ternatea	*Curvularia*	47.5	64.0	46.0	52.5	14
Cyclea peltata	*Chaetomium*	59.0	77.0	45.5	60.5	11
Cymbopogon citratus	*Talaromyces*	67.5	83.0	43.5	64.6	14
Dioscorea pentaphylla	*Colletotrichum*	57.0	79.5	41.5	59.3	11
Naravelia zeylanica	Sterile form	86.5	78.0	27.5	64.0	10
Oryza sativa	*Chaetomium*	43.5	84.5	52.5	60.1	15
Piper nigrum	Sterile form	71.0	78.0	55.0	68.0	10
Tinospora cordifolia	*Colletotrichum*	68.0	85.5	37.0	63.5	11
Zymnema sylvestris	*Nigrospora*	74.0	84.5	43.0	67.1	14

Table 3 Diversity indices of endophytic fungi in some medicinal climbers and grass species of Malnad region, Karnataka.

Host Plant	Shannon Diversity Index	Shannon evenness (J)	Simpson Diversity Index (1/D)
Clematis gouriana	0.62	0.59	3.12
Clitoria ternatea	1.04	0.91	10.00
Cyclea peltata	0.90	0.86	6.25
Cymbopogon citratus	0.98	0.85	7.69
Dioscorea pentaphylla	0.90	0.86	6.25
Naravelia zeylanica	0.81	0.81	5.00
Oryza sativa	1.01	0.86	7.69
Piper nigrum	0.83	0.83	5.26
Tinospora cordifolia	0.92	0.88	7.14
Zymnema sylvestris	1.02	0.89	9.09

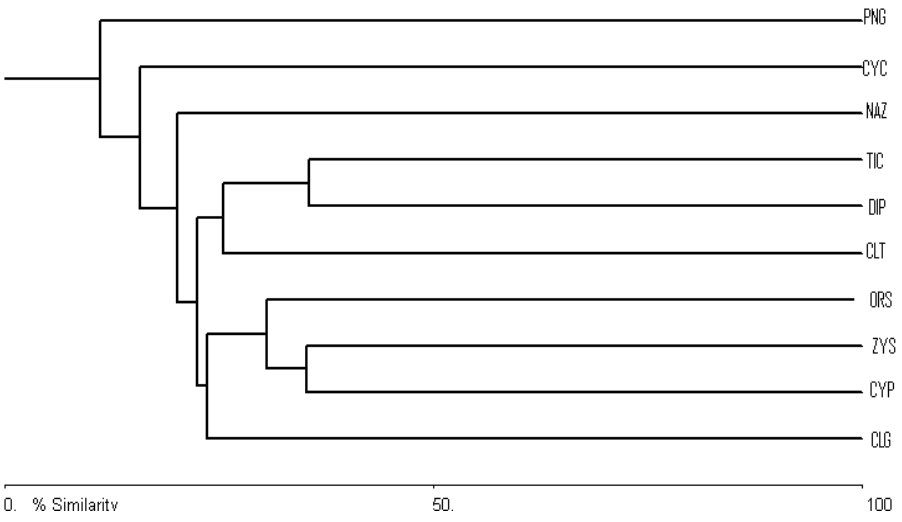

Fig. 2 – Dendrogram showing the similarity of endophytic fungi among different host plants (PNG=*Piper nigrum,* CYC=*Cymbopogon citratus,* NAZ=*Naravelia* zeylanica, TIC=*Tinospora cordifolia,* DIP=*Dioscoria pentaphylla,* CLT=*Clitorea ternatea,* ORS=*Oryza sativa*, ZYS=*Zymnema sylvestris,* CYP=*Cyclea peltata,* CLG=*Clematis gouriana*)

Table 4 Colonization frequency of endophytic fungi in some medicinal climbers and grasses.

Sl. No.	Endophytic fungi	*Clematis gouriana*			*Clitoria ternatia*			*Cyclea peltata*			*Cymbopogan citratus*			*Dioscorea pentaphylla*		
		Mon	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**
1	*Alternaria alternata*	-	-	-	-	-	-	-	5.5	4.5	-	-	-	-	9.5	-
2	*Arthobotrym nilgirense*	-	-	-	-	-	-	5.0	2.5	-	-	-	-	-	-	-
3	*Aspergillus niger*	3.5	4.5	-	3.0	2.0	-	-	-	-	3.0	2.0	-	3.5	13.5	-
4	*A. chraceous*	-	1.0	1.5	-	-	-	-	-	-	9.5	6.5	6.5	-	-	-
5	*Botryodiplodia theobromae*	-	-	-	-	5.5	-	-	-	-	-	-	-	-	-	-
6	*Chaetomium globosum*	9.5	16.0	-	-	-	-	14.5	36.0	11.5	-	7.0	-	-	-	-
7	*Cladosporium cladosporioides*	2.5	-	7.0	-	-	-	-	-	-	-	-	-	-	-	-
8	*C. herbarum*	-	-	-	-	-	-	-	-	-	-	15.5	6.5	-	6.5	12.5
9	*Colletotrichum gleosporioides*	6.0	6.5	-	4.5	9.5	-	-	-	-	-	-	-	17.5	31.0	13.0
10	*Curvularia lunata*	-	-	-	-	21.0	10.0	-	-	-	-	11.5	-	-	-	-
11	*Fusarium lateritrum*	-	-	-	-	-	-	-	-	-	-	5.5	5.5	-	-	-
12	*F. oxysporum*	-	-	-	7.0	-	-	-	-	-	-	-	14.5	-	-	-
13	*F. semitectum*	-	-	-	-	-	-	-	-	-	-	-	-	7.0	5.5	1.0
14	*Fusarium sporotrichioides*	-	-	6.0	-	-	-	-	-	-	-	-	-	-	-	-
15	*Gliocladium penicillioides*	-	-	-	-	-	-	-	-	-	1.5	8.5	-	-	-	-
16	*G. roseum*	-	-	-	9.0	6.5	1.0	-	-	-	-	-	-	-	-	-
17	*Humicola fusco atra*	-	-	3.5	-	-	5.5	-	-	-	-	-	-	-	-	-
18	*Lacellina graminicola*	-	-	-	-	-	-	3.5	16.0	-	-	-	-	-	-	-
19	*Mucor racemosus*	-	-	-	-	-	-	-	-	-	1.5	-	-	-	-	-
20	*Myrothecium rorrhidum*	6.0	4.5	-	-	-	-	-	-	-	-	-	-	-	-	-
21	*Nigrospora sphaerica*	-	-	-	-	3.5	3.5	-	9.5	14.5	-	-	-	-	-	-
22	*Penicillium chrysogenum*	-	-	-	-	-	-	-	-	-	5.5	-	-	-	-	-
23	*P. decumbens*	-	-	-	16.0	-	9.5	-	-	-	-	-	-	-	-	-
24	*P. digitatum*	-	-	-	-	1.0	7.0	-	-	-	-	-	-	-	-	-
25	*P. frequentans*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.5
26	*Periconia byssoides*	-	-	-	2.0	-	-	-	-	-	1.0	-	-	-	-	-
27	*Phoma glomerata*	-	-	-	-	-	-	8.5	-	5.5	-	-	-	-	-	-
28	*Phoma herbarum*	-	-	-	-	-	-	-	-	-	-	-	-	5.5	2.0	-
29	*Phyllosticta* spp.	-	5.5	-	6.0	9.5	-	-	-	-	-	-	-	-	-	-
30	*Rhizopus stolonifer*	-	-	-	-	-	-	-	1.0	3.5	-	-	-	-	-	-
31	*Sporormiella minima*	-	-	-	-	-	-	-	-	-	-	-	-	8.0	-	3.5
32	*Talaromyces*	-	-	-	-	-	-	-	-	-	30.0	16.0	10.5	-	-	-
33	*Trichoderma viride*	-	1.0	4.5	-	-	-	-	-	-	-	-	-	-	-	-
34	*Verticillium glaucum*	-	-	-	-	-	-	-	-	-	-	-	-	-	4.5	-
35	*Xylaria* spp.	33.5	41.0	30.5	-	-	-	-	-	-	-	-	-	-	-	-

Table 4 (Continued) Colonization frequency of endophytic fungi in some medicinal climbers and grasses.

Sl. No.	Endophytic fungi	*Clematis gouriana*			*Clitoria ternatia*			*Cyclea peltata*			*Cymbopogan citratus*			*Dioscorea pentaphylla*		
		Mon	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**
36	Sterile form- pink	-	-	-	-	-	-	7.0	6.5	1.5	6.5	10.5	-	-	-	-
37	Sterile form- white	-	-	-	-	-	9.5	6.0	-	4.5	9.0	-	-	15.5	-	6.0
38	Unidentified coelomycete	-	-	-	-	5.5	-	8.0	3.5	-	-	-	-	-	7.0	-

Sl. No.	Endophytic fungi	*Naravelia zeylanica*			*Oryza sativa*			*Pipernigrum*			*Tinospora cordifolia*			*Zymnema sylvestris*		
		Mon	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**
1	*Alternaria alternata*	1.0	6.0	-	-	-	-	-	-	-	6.0	10.0	1.5	-	-	-
2	*Amerosporium polynematoides*	-	-	-	-	-	-	-	-	-	-	-	-	-	2.0	-
3	*Aspergillus flavus*	-	-	-	2.5	-	8.5	-	-	-	-	-	-	-	-	7.0
4	*A. ochraceous*	-	-	-	-	-	10.0	-	-	-	-	-	-	-	-	-
5	*A. versicolor*	-	-	-	-	-	-	-	-	-	-	-	-	5.5	-	4.5
6	*Aureobasidium pullulans*	-	-	-	-	-	-	11.0	5.0	8.5	-	-	-	-	-	-
7	*Botryosphaeria subglobosa*	-	-	-	-	-	-	-	-	-	-	-	-	-	6.5	-
8	*Cyclothyrium juglandis*	6.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	*Chaetomium globosum*	-	-	-	13.5	28.0	11.0	-	-	-	-	-	-	15.5	20.5	6.0
10	*Chlamydomyces palmarum*	-	-	-	1.0	6.0	-	-	-	-	-	-	-	-	-	-
11	*Cladosporium cladosporioides*	-	-	-	4.5	-	16.0	-	-	-	-	-	-	-	-	-
12	*Coleophoma cylindrium*	2.0	3.0	-	-	-	-	-	-	-	-	-	-	-	3.5	-
13	*Colletotrichum gleosporioides*	-	-	-	-	-	-	-	-	-	19.5	20.5	16.0	-	-	-
14	*Coniella diploidiella*	-	-	-	-	-	-	-	-	-	-	-	-	9.5	-	7.0
15	*Coniothyrium fuckeli*	-	-	-	1.0	2.5	0.5	-	-	-	-	-	-	-	-	-
16	*Fusarium dimerum*	-	-	-	-	-	-	-	-	-	-	-	-	6.0	8.0	2.0
17	*F. oxysporum*	-	-	-	4.5	5.5	1.5	-	-	-	-	6.5	2.0	-	-	-
18	*F. semitectum*	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	-
19	*Fusarium sporotrichioides*	-	-	-	-	-	-	-	-	10.5	-	-	-	-		-
20	*Gliocladium roseum*	-	-	-	-	-	-	4.5	8.5	-	-	-	-	-	-	-
21	*Hyalostachybotrys bisbyi*	2.0	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-
22	*Humicola fuscoatra*	-	-	-	-	3.5	-	-	-	-	-	-	-	-	-	-
23	*Lacellina aginta*	-	-	-	-	-	-	-	-	-	6.0	-	-	-	-	-
24	*Nigrospora oryzae*	-	-	-	-	9.5	-	-	-	-	-	-	-	-	-	-
25	*N. sphaerica*	-	-	-	-	-	-	-	-	-	-	14.5	10.0	10	14.5	5.5
26	*Penicillium chrysogenum*	-	-	-	6.5	14.5	-	13.5	-	10.5	-	-	-	-	-	9.5
27	*P. citrinum*	-	-	-	-	-	-	10.0	18.0	9.5	-	-	-	-	-	-
28	*P. decumbens*	-	-	-	-	2.0	-	-	-	-	-	-	-	-	-	-
29	*P. frequentans*	-	-	-	-	-	-	3.5	-	-	-	-	-	-	-	-

Table 4 (Continued) Colonization frequency of endophytic fungi in some medicinal climbers and grasses.

Sl. No.	Endophytic fungi	*Naravelia zeylanica*			*Oryza sativa*			*Pipernigrum*			*Tinospora cordifolia*			*Zymnema sylvestris*		
		Mon	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**	**Mon**	**Win**	**Sum**
30	*Pestalotiopsis* spp.	9.0	9.5	5.0	-	-	-	-	-	-	4.0	6.5	-	-	-	-
31	*Phialophora verrucosa*	-	-	-	-	-	-	3.0	1.5	-	-	-	-	-	-	-
32	*Phyllosticta* spp.	-	-	-	-	-	-	-	-	-	7.5	19.5	-	3.0	16.0	-
33	*Rhizoctonia solani*	-	-	-	5.5	-	4.5	-	-	-	-	-	-	-	-	-
34	*Stachylidium bicolor*	-	-	-	-	-	-	-	6.0	-	-	-	-	-	-	-
35	*Septocylindrium septatum*	7.0	-	5.5	-	-	-	-	-	-	-	-	-	-	-	-
36	*Stemphylium botryosum*	-	-	-	-	3.5	-	-	-	-	-	-	-	-	-	-
37	*Tetracladium furcatum*	-	-	-	-	-	-	-	-	-	3.0	-	-	-	-	-
38	*Trichoderma viride*	-	-	-	1.5	3.0	-	4.5	5.5	-	-	-	-	-	-	-
39	*Verticillium chlamydosporium*	-	-	-	-	-	-	-	-	-	-	4.0	-	-	-	-
40	*Xylaria* spp.	30.5	8.0	-	-	-	-	-	-	-	-	-	-	-	-	-
41	Sterile form- grey	-	6.0	-	-	-	-	-	-	-	-	-	-	-	-	-
42	Sterile form- pink	19.5	34.5	17.0	-	-	-	-	-	-	-	-	-	-	-	-
43	Sterile form- white	9.0	8.5	-	3.0	6.5	0.5	21.5	33.5	16.0	9.5	-	5.5	-	-	-
44	Unidentified ascomycete	-	-	-	-	-	-	-	-	-	11.0	-	2.0	15.5	8.5	-

Data based on 200 leaf segments/plant in each season Mon- Monsoon; Win- Winter; Sum- Summer

borne fungi but they also have the potential to live endophytically, especially in leaves (Petrini 1984). Precipitation is a major factor influencing endophyte assemblage and changes of colonization frequency in different seasons. However, other factors that may contribute to changes in the endophytic community include leaf age, weathering of leaf cuticle, the presence of wounds, increased exposure to propagules with time and changes in leaf physiology, leaf chemistry and also soil characteristics (Lumyong et al. 2009, Marquez et al. 2010). These endophytic fungi can also switch their endophytic life style to saprobic with the progression of leaf decomposition (Hyde & Soytong 2008, Promputtha et al. 2010).

Mycelia sterilia have been frequently isolated as endophyes from a wide range of tropical and temperate host plants. Since the many sterile fungi do not sporulate in culture and due to the existence of non-culturable endophytes, the real number of endophytic species can be underestimated (Hyde & Soytong 2007). Recent studies have successfully used molecular techniques such as DNA cloning (Seena et al. 2008), DGGE (Duong et al. 2006), T-RLFP (Nikolcheva & Barlocher 2005) to give taxonomic placements for mycelia sterilia. In spite of these techniques the evaluation of fungal diversity is a major challenge to mycologists due to the scarcity of fungal and related eukaryotic sequences in databases (Hyde & Soytong 2008).

The screening of endophytic fungi in some medicinal plants helps provide knowledge on the fungal symbionts with these host plants. It has been hypothesized that microbial symbionts could affect plant nutrition, defensive chemistry and biodiversity (Rudgers et al. 2007). In antagonistic studies on rice endophytes, strains of *Chaetomium*, *Penicillium*, and *Streptomyces* exhibited antifungal activity against various phytopathogens *in vitro* (Shankar et al. 2007). Further investigation should include studies aimed at detecting ecological significance, production of antibiotics and secondary metabolites and potential use of these endophytes in biological control.

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