

Full Length Research Paper

## Prevalence and seasonal periodicity of endophytic coelomycetous fungi in Tamil Nadu, India

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The present study reports 2,644 endophytic isolates; only 74 different morphospecies of coelomycetes have been isolated and identified from 14,400 segments of healthy tissues such as leaves (4,800), stem (4,800) and bark (4,800). Each plant tissue with 300 segments was studied in a Petri dish containing 10 segments; totally, 30 plates were studied with four seasons. Totally, 74 coelomycetes were isolated and identified to belong to 16 genera by classical taxonomy. Interestingly, the *Pestalotiopsis* spp. was found to be dominant as compared to other genera in all the collection spots in the four different seasons.

**Key words:** Coelomycetes, endophytes, parasites, morphospecies, colony frequency, *pestalotiopsis*, relative percentage occurrence.

### INTRODUCTION

About 100,000 species of fungi have been formally described by taxonomists (Kirk et al., 2008), but the global biodiversity of the fungal kingdom is not fully understood (Mueller and Schmit, 2006). 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 and Zou, 2001; Aly et al., 2010; Shankar and Krishnamurthy, 2010). Coelomycetes are anamorphic fungi producing asexual spore (conidia) in a pycnidium, acervulus or stroma (Sutton, 1980; Nag Raj, 1993). Currently, 1000 genera comprising 7000 species have been described (Kirk et al., 2001). The previous reports strongly indicated that endophytic coelomycetous fungi were studied for over three decades (Webber and Gibbs, 1984; Hudson, 1986; Johnson and Whitney, 1989a, b; Stone et al., 1996; Suriyanarayanan and Vijayakrishna, 2001). The diversity of endophytic coelomycetes from the tree species was

subjected to biotechnological approach because of the colony frequencies accumulated over the time; the age of leaves and positively correlated needles with the fungal colonization (Bernstein and Carroll, 1977b; Petrini and Carroll, 1981; Stone, 1987; Helander et al., 1993; 1994; Carroll, 1995; Magan et al., 1996; Gaylord et al., 1996; Faeth and Hammon, 1997; Hata et al., 1998; Poteri et al., 2001; Arnold and Herre, 2003). Many reports confirmed that the endophytic fungi such as *Pestalotiopsis* spp., *Pestalotia* spp., *Phomopsis* spp., *Phyllosticta* spp., *Melanconium* spp., *Colletotrichum* spp., *Phoma* spp., *Chaetomium* spp., *Botryodiplodia* spp., *Bartalinia* spp. and *Monochaetia* spp. can produce medicinally important bioactive secondary metabolites (Firakova et al., 2007). In which, interestingly, more endophytic coelomycetous fungi have been reported (Petrini, 1986; Wang et al., 2000; Zhou et al., 2001; Yuan et al., 2006; Muthumary and Sashirekha, 2007).

The delicate balance between host and endophytic organism seems to be controlled in part by chemical

factors, for example, herbicidal natural products produced by the fungus versus antifungal metabolites biosynthesized by the host plant (Tadych and White, 2009). The coelomycetous fungi are considered to have evolved from saprophytes (Zavos et al., 1988), and in turn, parasites are assumed to have given rise to endophytes (Clay, 1988). In the present study, first endophytic diversity of coelomycetes was studied in *Mangifera indica* Linn. (Anacardiaceae), *Rhododendron arboreum* Sm. (Ericaceae), *Taxodium mucronatum* Tenore. (Cupressaceae) and *Taxodium distichum* Rich. (Cupressaceae) in India.

## MATERIALS AND METHODS

### Study area

*R. arboretum* Sm., *T. distichum* Rich. and *T. mucronatum* Ten. were collected from the forests at Tamil Nadu Botanical Garden (2623 m above sea level) in Tamil Nadu National Park, Ooty, Nilgiri District of Tamil Nadu, South India (Lat. 10° 38' 11.49''; Long. 76° 0' 77.15'') which is an evergreen hill forest with much canopy, and *M. indica* Linn. was collected from Botany Field Laboratory, University of Madras, Maduravoyal, Chennai, Tamil Nadu.

### Isolation of endophytic fungi

The collected samples were washed thoroughly by running tap water. From 300 segments (approximately 0.5 cm<sup>2</sup>) of each 50 leaves, stem and bark tissues from each plant were surface sterilized and placed on PDA medium poured Petri plates for the study of fungal endophytes (Fisher et al., 1993; Dobranic et al., 1995; Schulz et al., 1998). The Petri plates were incubated in a light chamber for a period of 3 to 4 weeks (Bills and Polishook, 1992) at 23 ± 2°C. The light regime was 12 h light followed by 12 h darkness (24 h/day). Throughout the experiment, two Philips "Cool white" (6500 K) florescent lamps (Philips, India) providing energy of 50 µE/m<sup>2</sup>/S was maintained. A Philips 100 W lamp served as the source of incandescent light. The Petri dishes were observed at regular intervals starting from the second day onwards for the fungal growth. The emerging fungal propagules were isolated, purified and maintained by subsequent subcultures (Bills 1996).

### Preparation of semipermanent slides

After the sporulation of each fungus, the conidiomata of the coelomycetes were mounted, cleared and examined; the preparation was tapped vigorously to break the conidiomata and then reheated. In this way, the conidia would be released and if one is diligent, some conidiogenous cells will be evident with the developing conidia attached. The vertical sections of conidiomata were taken to confirm the nature of the fructification. Specimens with fruit bodies were sectioned with the help of sterile scalpel blade. The selected sections (10 to 15 µm) were observed under light microscope by using lactophenol mount. Lactophenol and lactophenol with cotton blue stain were used for the preparation of semipermanent slides. The slides were sealed with DPX mountant (BDH Chemical Division, Glaxo Laboratories Ltd. Mumbai, India).

### Colonization frequency (CF%)

The colonization frequency for endophytic coelomycetes species in the leaf, stem and bark tissue was calculated by using the following formula (Hata and Futai 1995):

$$CF (\%) = \frac{\text{The number of segments colonized by an endophytic species}}{\text{Total number of segments}} \times 100$$

### Relative percentage occurrence (RPO%) of each group of fungi

Relative percentage occurrence of different genera of species was calculated using the following formula:

$$RPO (\%) = \frac{\text{Density of colonization of one group}}{\text{Total density of colonization of all groups}} \times 100$$

## RESULTS

### Isolation of endophytic mycobiota

The results indicated the colony frequencies of endophytic coelomycetous species from 4 plants belonging to three families. The number of endophytic coelomycetes in barks of *T. mucronatum* and *T. distichum* were significantly higher than stem and leaves. Moreover, the highest colonization frequency was found in leaves of *M. indica* and *R. arboreum* as compared to stem and bark.

This study was undertaken for four different seasonal variations, that is, South West Monsoon (SWM), North East Monsoon (NEM), Winter Season (WS) and Summer Season (SS). The colonization frequency of leaves, stem and bark significantly increased during monsoon and decreased during summer and winter seasons (Figure 1).

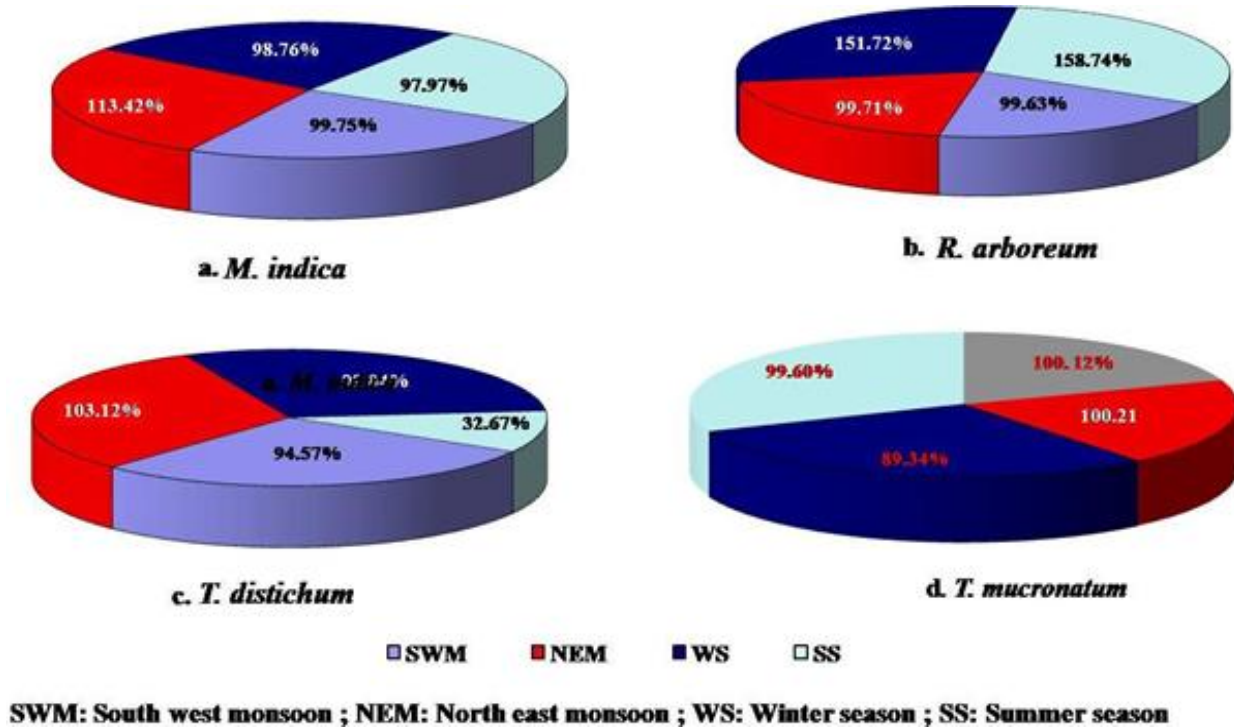
### Identification

The characteristics of the fungi were observed by making microslide preparations of the fruit bodies and conidia. They were identified on the basis of the structure of fruit body (conidiomata), conidiogenous cells and conidia (Sutton, 1980). The presence of appendage on the conidia which is now being recognized as an important taxonomic criterion was studied critically (Guba, 1961; Ellis, 1971; Sutton, 1973a, b, 1980; Onions et al., 1981; Nag, Raj 1993) and measurement of the conidia was done. Identification of coelomycetes up to different morpho-species levels were carried out using standard manuals (Guba, 1961; Sutton, 1980; Nag Raj, 1993).

### Isolation of endophytic coelomycetous mycobiota at four different seasons

#### South west monsoon

A total of 189 isolates, 15 different morphospecies belonging to 7 genera were isolated and identified from *M. indica*. The most frequently isolated genera were *Colletotrichum*, *Pestalotiopsis*, *Phomopsis* and *Phyllosticta*. Single species of the genera *Botryodiplodia*, *Pestalotia* and *Phoma* were also recorded (Table 1a). The



**Figure 1.** Total relative percentage occurrence of endophytic coelomycetous fungi on different seasons

The maximum RPO was found in *Pestalotiopsis* spp. (48.61%) followed by *Phomopsis* spp. (14.2%).

In the case of *R. arboreum*, 129 isolates existed at 16 different morphospecies belonging to 8 genera which were isolated and identified. Among them, *Colletotrichum*, *Pestalotiopsis*, *Pestalotia* and *Phomopsis* occurred predominantly. Single species of the genera *Bartalinia*, *Botryodiplodia*, *Camarosporium* and *Fusamen* were also observed (Table 1b). In this study, the maximum RPO was found in *Pestalotiopsis* spp. (47.11%) followed by *Colletotrichum* spp. (13.5%) and *Phomopsis* spp. (9.57%).

Totally, 215 isolates were recorded from *T. distichum*. Of these, 22 different morphospecies under 11 genera were isolated and identified. Among them, *Botryodiplodia*, *Colletotrichum*, *Fusicoccum*, *Monochaetia*, *Pestalotiopsis*, *Pestalotia*, *Pestalozziella*, *Phomopsis*, *Phyllosticta*, *Seimatosporium* and *Septoria* occurred predominantly. Single species of the genera *Bartalinia*, *Botryodiplodia*, *Camarosporium* and *Fusamen* spp. were also recorded (Table 1c). In this study, the maximum RPO was found in *Pestalotiopsis* spp. (37.6%) followed by *Pestalotia* spp. (9.72%) and *Seimatosporium* sp. (8.83%).

Similarly, a total of 209 isolates were recorded. Of these, 20 different morphospecies that existed in the leaves, stem and bark belonging to 10 genera were isolated and identified from *T. mucronatum*. *Colletotrichum*, *Pestalotiopsis*, *Phomopsis*, *Phyllosticta* and *Septoria* were isolated. Single species of the genera *Botryodiplodia*, *Fusamen*, *Monochaetia*, *Truncatella* and *Pestalotia* were

also observed (Table 1d). The maximum RPO was found in *Pestalotiopsis* spp. (43.40%) followed by *Phyllosticta* spp. (10.62%) and *Colletotrichum* spp. (10.11%).

#### **North east monsoon**

From *M. indica*, a total of 195 isolates were obtained. Among these, 15 different morphospecies under 7 genera such as *Colletotrichum*, *Pestalotiopsis*, *Phomopsis* and *Phyllosticta* occurred predominantly. Single species of the genera *Botryodiplodia*, *Pestalotia* and *Phoma* were also recorded. In this study, the maximum RPO was found in *Pestalotiopsis* spp. (46.6%) followed by *Colletotrichum* spp. (17.89%).

A total of 171 isolates were recorded from *R. arboreum*. Of these, 16 different morphospecies under 8 genera were isolated and identified (Table 1b). Among them, *Colletotrichum*, *Pestalotiopsis*, *Pestalotia* and *Phomopsis* occurred predominantly. Single species of the genera *Bartalinia*, *Botryodiplodia*, *Camarosporium* and *Fusamen* were also observed. In this study, the maximum RPO was found in *Pestalotiopsis* spp. (57.47%) followed by *Colletotrichum* spp. (15.75%).

A total of 173 isolates were recorded from *T. distichum*. Of these, 22 different morphospecies that existed in the leaves, stem and bark belonging to 11 genera were isolated and identified. *Botryodiplodia*, *Colletotrichum*, *Pestalotiopsis*, *Pestalotia*, *Phomopsis* and *Phyllosticta* occurred predominantly. Single species of the genera *Seimatosporium*, *Septoria*, *Fusicoccum*, *Monochaetia*

**Table 1a.** CF and RPO of endophytic coelomycetous fungi isolated from different samples of *M. indica* during various seasons between 2006 and 2007

Endophyte	Number of segments colonized															
	South west monsoon				North east monsoon				Winter season				Summer season			
	(June - Sep)				(Oct - Nov)				(Dec - Feb)				(Mar - May)			
	L	S	B	RPO (%)	L	S	B	RPO (%)	L	S	B	RPO (%)	L	S	B	RPO (%)
<i>Botryodiplodia</i> sp. MUBL1086	0.33	0.66	0.66	2.61	0.66	0.66	0.66	3.04	0.66	0.33	0.66	2.82	0.33	0.33	0.33	2.47
<i>Colletotrichum</i> sp. MUBL1042	1.33	1.0	0.66		2.0	0.66	1.33		2.0	0.66	2.66		0.66	0.66	2.0	
<i>Colletotrichum</i> sp. MUBL1043	2.0	2.66	3.0	20.04	2.33	1.33	0.66	17.89	2.33	1.0	0.66	18.22	0.66	0.33	0.33	12.4
<i>Colletotrichum</i> sp. MUBL1044	-	0.33	0.66		2.0	0.33	0.66		0.33	0.33	0.33		0.33	-	-	
<i>Colletotrichum</i> sp. MUBL1045	0.33	0.33	0.33		0.33	-	-		-	-	0.33		-	-	-	
<i>Pestalotiopsis</i> sp. MUBL1000	2.0	1.0	1.66		1.33	2.0	3.0		3.33	1.33	2.0		1.0	2.0	2.66	
<i>Pestalotiopsis</i> sp. MUBL1001	3.0	3.33	2.0		2.33	1.33	2.66		1.0	2.0	1.66		0.33	0.66	2.0	
<i>Pestalotiopsis</i> sp. MUBL1002	3.0	3.66	2.66	48.61	2.0	3.0	3.66	46.6	2.33	2.66	1.33	44.5	2.0	1.33	3.0	51.5
<i>Pestalotiopsis</i> sp. MUBL1003	2.0	0.66	1.33		1.0	2.33	2.0		1.66	1.33	1.0		1.33	0.66	0.33	
<i>Pestalotiopsis</i> sp. MUBL1004	1.33	2.0	1.0		1.33	1.66	0.66		1.0	2.33	1.0		2.0	0.66	0.66	
<i>Pestalotia</i> sp. MUBL1062	0.33	-	-	0.52	-	-	0.33	0.50	-	-	0.33	0.56	-	-	-	-
<i>Phomopsis</i> sp. MUBL1070	1.0	2.0	0.66	14.25	2.33	2.0	0.66		3.0	1.33	2.0	20.5	2.33	2.0	2.0	28.3
<i>Phomopsis</i> sp. MUBL1071	1.33	1.66	2.33		2.33	1.0	2.0	15.87	2.66	1.0	2.0		2.0	1.0	2.0	
<i>Phoma</i> sp. MUBL1106	0.33	0.33	0.33	1.57	0.33	0.33	0.33	15.2	0.33	-	-	0.56	-	-	-	-
<i>Phyllosticta</i> sp. MUBL1078	2.0	0.66	1.0	12.15	2.66	0.66	2.0	14.32	1.33	1.66	2.0	11.9	0.33	-	0.33	3.3
<i>Phyllosticta</i> sp. MUBL1079	1.0	2.0	1.0		1.0	1.33	1.66		1.0	0.66	0.33		0.33	0.66	0.33	
Number of coelomycetes species recorded	15	15	15	-	15	14	15	-	14	14	15	-	13	11	12	-
Total Number of coelomycetes isolates observed	64	67	58	-	72	56	67	-	70	50	55	-	41	31	48	-

L: Leaves; S: stem; B: bark; RPO: Relative percentage occurrence; CF: colony frequency.

and *Pestalozziella* were also observed. The maximum RPO was found in *Pestalotiopsis* spp. (41.5%) followed by *Colletotrichum* spp. (13.2%) and *Phomopsis* spp. (11.55%) (Table 1c).

From *T. mucronatum*, a total of 205 isolates were recorded. Of these, 20 different morphospecies belonging to 10 genera were isolated and identified. *Colletotrichum*, *Pestalotiopsis*, *Phomopsis*, *Phyllosticta*, *Septoria* and *Truncatella* were recorded predominantly. Single species of the genera *Botryodiplodia*, *Fusamen*, *Monochaetia* and *Pestalotia* were also observed (Table 1d). The maximum RPO was

found in *Pestalotiopsis* spp. (37.04%) followed by *Phyllosticta* spp. (10.62%) and *Colletotrichum* spp. (10.11%).

#### Winter season

Totally, 175 isolates were obtained from *M. indica*. Among them, 15 different morphospecies belonging to 7 genera were isolated and identified. Of these, *Colletotrichum*, *Pestalotiopsis*, *Phomopsis* and *Phyllosticta* were routinely served.

Single species of the genera *Botryodiplodia*, *Pestalotia* and *Phoma* were also recorded (Table

1a). In this study, the maximum RPO was found in *Pestalotiopsis* spp. (44.5%) followed by *Colletotrichum* spp. (20.5%). Among the 185 isolates from *R. arboretum*, 15 different morphospecies under 7 genera were isolated and identified. In which, *Pestalotiopsis*, *Pestalotia* and *Phomopsis* species occurred predominantly. Single species of the genera *Bartalinia*, *Botryodiplodia*, *Camarosporium* and *Colletotrichum* were also recorded (Table 1b). In this study, the maximum RPO was found in *Pestalotiopsis* spp. (60.49%) followed by *Colletotrichum* spp. (18.35%).

From *T. distichum*, 164 isolates were recorded

**Table 1b.** CF and RPO of endophytic coelomycetous fungi isolated from different samples of *R. arboreum* during various seasons between 2006 and 2007.

Endophyte	Number of segments colonized															
	South west monsoon				North east monsoon				Winter season				Summer season			
	(June – Sep)				(Oct – Nov)				(Dec – Feb)				(Mar – May)			
	L	S	B	RPO (%)	L	S	B	RPO (%)	L	S	B	RPO (%)	L	S	B	RPO (%)
<i>Bartalinia</i> sp. MUBL1096	0.33	0.33	0.33	2.37	-	1.0	-	1.75	-	0.33	0.33	1.07	-	-	-	-
<i>Botryodiplodia</i> sp. MUBL1087	0.66	0.66	-	3.16	0.66	1.33	1.66	5.24	0.33	1.0	1.33	3.77	0.66	0.33	1.0	3.77
<i>Colletotrichum</i> sp. MUBL1046	0.33	1.0	0.66		0.33	3.0	1.33		-	-	-		1.33	2.0	0.33	
<i>Colletotrichum</i> sp. MUBL1047	0.33	0.66	2.0	13.5	2.0	-	1.0	15.75	2.66	2.0	1.0	18.35	2.66	1.0	0.66	18.93
<i>Colletotrichum</i> sp. MUBL1048	0.33	0.33	-		0.66	-	0.66		2.0	1.33	2.33		1.0	0.33	0.66	
<i>Camarosporium</i> sp. MUBL1102	-	1.33	2.0	7.99	-	0.33	2.0	4.0	0.33	-	-	0.53	0.33	-	-	0.62
<i>Fusamen</i> sp. MUBL1098	-	0.33	2.66	7.17	-	1.0	1.33	3.3	-	0.33	0.33	1.07	2.66	2.0	3.33	15.17
<i>Pestalotiopsis</i> sp. MUBL1005	0.66	1.0	3.00		1.33	2.66	2.66		2.0	4.0	3.0		2.33	1.0	0.66	
<i>Pestalotiopsis</i> sp. MUBL1006	-	0.33	2.33		0.66	2.0	2.33		1.0	1.33	2.0		3.33	3.0	1.33	
<i>Pestalotiopsis</i> sp. MUBL1007	-	0.66	2.0	47.11	2.0	2.33	1.0	57.47	3.33	2.66	2.0	60.49	2.0	0.66	1.30	53.66
<i>Pestalotiopsis</i> sp. MUBL1008	0.66	0.33	3.00		0.33	3.0	3.33		3.66	4.0	2.66		1.33	3.0	2.66	
<i>Pestalotiopsis</i> sp. MUBL1009	-	2.0	3.66		1.00	3.66	3.33		2.66	2.0	1.0		2.0	0.66	3.0	
<i>Pestalotia</i> sp. MUBL1063	-	0.33	1.33	8.76	-	0.66	1.0	6.40	1.0	0.66	0.66	5.90	1.33	0.16	0.33	4.59
<i>Pestalotia</i> sp. MUBL1064	0.33	1.0	0.66		0.33	0.33	1.33		0.66	0.33	0.33		-	0.33	0.33	
<i>Phomopsis</i> sp. MUBL1072	0.33	1.0	1.0	9.57	-	-	0.33	5.80	0.33	0.66	1.0	8.07	0.33	-	-	0.62
<i>Phomopsis</i> sp. MUBL1073	-	1.33	0.33		0.66	1.66	0.66		1.33	1.0	0.66		-	-	-	
Number of coelomycetes species recorded	09	16	14	-	11	13	15	-	16	14	14	-	12	12	12	-
Total number of coelomycetes isolates observed	12	38	75	-	30	69	72	-	64	65	56	-	64	47	47	-

L: Leaves; S: stem; B: bark; RPO: relative percentage occurrence; CF: colony frequency.

in this season. Of these, 22 different morpho-species belonging to 11 genera were isolated and identified such as *Colletotrichum*, *Fusicoccum*, *Monochaetia*, *Pestalotiopsis*, *Pestalotia*, *Phomopsis* and *Phyllosticta*. Single species of the genera *Pestalotziella*, *Seimatosporium*, *Septoria* and *Botryodiplodia* were also observed (Table 1c). The maximum RPO was found in *Pestalotiopsis* spp. (45.0%) followed by *Pestalotia* spp. (10.95%).

A total of 173 isolates were recorded. Of these, 19 different morphospecies that existed in the leaves, stem and bark belonging to 10 genera such as *Colletotrichum*, *Pestalotiopsis*,

*Phomopsis*, *Phyllosticta*, *Septoria* and *Truncatella* were isolated from *T. mucronatum*. The single species of the genera *Botryodiplodia*, *Fusamen*, *Monochaetia* and *Pestalotia* species were also observed (Table 1d). The maximum RPO was found in *Pestalotiopsis* spp. (35.20%) followed by *Colletotrichum* spp. (12.10%) and *Phomopsis* spp. (10.96%).

#### Summer season

A total of 120 isolates were obtained. Of these, 13 different morphospecies that existed in the bark,

leaves and stem belonging to 5 genera were isolated and identified from *M. indica*. Among them, *Colletotrichum*, *Pestalotiopsis*, *Phomopsis* and *Phyllosticta* occurred predominantly. Single species of the genus *Botryodiplodia* was also recorded. In this study, the maximum RPO was found in *Pestalotiopsis* spp. (51.5%) followed by *Colletotrichum* spp. (28.3%).

A total of 158 isolates were recorded. Of these, 12 different morphospecies that existed in the leaves, stem and bark belonging to 7 genera were isolated and identified in *R. arboretum*. Among them, *Colletotrichum*, *Pestalotiopsis*, *Pestalotia*

**Table 1c.** CF and RPO of endophytic coelomycetous fungi isolated from different samples of *T. distichum* during various seasons between 2006 and 2007.

Endophyte	Number of segments colonized															
	South west monsoon				North east monsoon				Winter season				Summer season			
	(June - Sep)				(Oct - Nov)				(Dec - Feb)				(Mar - May)			
	L	S	B	RPO (%)	L	S	B	RPO (%)	L	S	B	RPO (%)	L	S	B	RPO (%)
<i>Botryodiplodia</i> sp. MUBL1089	0.66	0.33	1.66	3.69	0.33	0.66	2.0	5.18	-	2.33	2.0	7.92	-	1.33	0.66	2.28
<i>Colletotrichum</i> sp. MUBL1052	0.33	1.0	0.66		0.33	1.33	0.66		-	1.66	0.66		-	0.66	0.33	
<i>Colletotrichum</i> sp. MUBL1053	-	0.66	0.33	9.26	-	2.66	1.0		-	2.0	1.0		0.66	0.33	1.0	
<i>Colletotrichum</i> sp. MUBL1054	-	2.33	1.33		-	1.0	0.66	13.2	-	1.0	0.66	12.76	0.66	0.33	1.0	5.71
<i>Fusicoccum</i> sp. MUBL1104	0.33	0.66	0.33	1.84	-	0.33	0.33	1.14	0.33	0.33	0.33	1.20	-	-	-	-
<i>Monochaetia</i> sp. MUBL1101	-	0.33	0.33	0.92	-	0.66	0.66	2.28	-	0.33	0.66	1.81	-	-	-	-
<i>Pestalotiopsis</i> sp. MUBL1016	-	2.66	3.0		-	1.0	2.0		-	2.33	2.0		-	2.0	2.33	
<i>Pestalotiopsis</i> sp. MUBL1017	-	0.66	1.33		-	1.33	2.0		-	3.0	2.0		-	1.33	1.66	
<i>Pestalotiopsis</i> sp. MUBL1018	-	2.33	1.33		-	0.66	2.0		-	1.0	2.0		-	1.0	0.66	
<i>Pestalotiopsis</i> sp. MUBL1019	-	3.0	3.33	37.6	-	1.0	2.0	41.5	-	1.0	2.0	45.0	0.33	0.66	0.33	17.5
<i>Pestalotiopsis</i> sp. MUBL1020	-	2.66	2.33		-	3.33	1.33		-	2.0	1.33		-	0.66	1.33	
<i>Pestalotiopsis</i> sp. MUBL1021	-	3.66	3.33		-	0.66	3.66		-	1.0	3.66		-	1.33	0.66	
<i>Pestalotiopsis</i> sp. MUBL1022	-	0.66	0.33		0.33	2.0	1.0		-	0.33	1.0		0.33	0.33	0.33	
<i>Pestalotia</i> sp. MUBL1066	0.33	0.66	2.66	9.72	-	1.66	2.33		-	0.66	2.33		-	-	-	
<i>Pestalotia</i> sp. MUBL1067	-	0.66	2.66		0.33	0.66	1.0	9.79	-	2.0	1.0	10.95	-	0.66	0.33	1.13
<i>Pestalozziella</i> sp. MUBL1105	-	3.0	0.66	5.10	-	1.0	2.33	5.77	-	0.66	0.66	2.41	-	0.33	0.33	0.75
<i>Phomopsis</i> sp. MUBL1076	-	1.0	1.66		-	3.0	1.0		-	-	0.33		-	-	-	
<i>Phomopsis</i> sp. MUBL1077	-	1.0	2.33	8.35	0.33	2.0	0.66	11.55	-	0.66	2.0	5.47	-	0.66	0.33	1.13
<i>Phyllosticta</i> sp. MUBL1082	-	1.33	3.0	7.88	-	0.66	0.33		-	1.0	2.33		-	-	0.33	
<i>Phyllosticta</i> sp. MUBL1083	-	0.66	0.66		0.33	0.66	2.0	6.33	-	0.33	0.33	7.29	-	1.33	0.33	1.90
<i>Seimatosporium</i> sp. MUBL1107	-	3.33	3.0	8.83	-	0.33	2.33	4.61	-	0.66	1.0	3.03	-	-	1.33	1.52
<i>Septoria</i> sp. MUBL1093	-	0.66	0.33	1.38	-	0.33	0.33	1.14	-	0.33	0.33	1.20	-	0.33	0.33	0.75
Number of coelomycetes species recorded	4	22	22	-	6	22	22	-	1	21	22	-	4	16	18	-
Total number of coelomycetes isolates observed	5	100	110	-	6	81	86	-	1	74	89	-	6	40	41	-

L: Leaves; S: stem; B: bark; RPO: relative percentage occurrence; CF: colony frequency.

and *Phomopsis* occurred predominantly. Single species of the genera *Botryodiplodia*, *Camarosporium* and *Fusamen* were also recorded (Table 1b). In this study, the maximum RPO was found in *Pestalotiopsis* spp. (53.66%) followed by *Colletotrichum* spp. (18.93%). A total of 87 isolates were recorded. Of these, 20 different morpho-

species that existed in the leaves, stem and bark belonging to 9 genera were isolated and identified in *T. distichum*. *Colletotrichum*, *Pestalotiopsis* and *Phyllosticta* were obtained. Single species of the genera *Pestalozziella*, *Phomopsis*, *Pestalotia*, *Seimatosporium*, *Septoria* and *Botryodiplodia* were also observed (Table 1c). The maximum RPO

was found in *Pestalotiopsis* spp. (17.5%) followed by *Colletotrichum* spp. (5.71%).

A total of 100 isolates were recorded in *T. mucronatum*. Of these, 20 different morpho-species that existed in the leaves, stem and bark belonging to 10 genera such as *Colletotrichum*, *Pestalotiopsis*, *Phomopsis*, *Phyllosticta*, *Septoria*

**Table 1d.** CF and RPO of endophytic coelomycetous fungi isolated from different samples of *T. mucronatum* during various seasons between 2006 and 2007.

Endophyte	Number of segments colonized															
	South west monsoon				North east monsoon				Winter season				Summer season			
	(June-Sep)				(Oct-Nov)				(Dec-Feb)				(Mar-May)			
	L	S	B	RPO (%)	L	S	B	RPO (%)	L	S	B	RPO (%)	L	S	B	RPO (%)
<i>Botryodiplodia</i> sp. MUBL1088	-	0.66	0.33	1.43	-	1.33	1.0	3.41	-	1.0	0.66	2.87	-	0.33	0.33	1.98%
<i>Colletotrichum</i> sp. MUBL1049	0.33	1.0	0.66		-	2.66	2.33		0.33	1.0	1.66		-	1.0	0.66	
<i>Colletotrichum</i> sp. MUBL1050	-	2.33	1.0		-	1.33	2.0		-	1.66	2.33		-	1.66	1.33	
<i>Colletotrichum</i> sp. MUBL1051	-	0.66	1.0	10.11	0.33	0.33	0.33	13.63	-	-	-	12.10	-	0.66	0.33	16.92%
<i>Fusamen</i> sp. MUBL1099	-	1.33	3.0	5.67	-	1.66	1.33	4.37	-	0.66	0.33	1.71	-	-	0.33	0.99%
<i>Monochaetia</i> sp. MUBL1100	-	2.0	3.33	7.72	-	2.66	2.33	7.30	-	1.0	2.33	5.77	-	1.0	0.66	4.98%
<i>Pestalotiopsis</i> sp. MUBL1010	0.33	2.66	3.0		-	4.0	2.33		-	2.0	1.66		0.33	2.0	1.0	
<i>Pestalotiopsis</i> sp. MUBL1011	-	3.66	2.0		-	2.33	1.33		-	1.0	0.66		-	1.66	0.66	
<i>Pestalotiopsis</i> sp. MUBL1012	-	2.66	1.66	43.40	-	1.33	2.0	37.04	-	3.33	3.0	35.20	-	2.0	1.33	49.89%
<i>Pestalotiopsis</i> sp. MUBL1013	-	1.0	2.33		-	2.66	1.0		-	2.33	2.0		-	1.0	1.33	
<i>Pestalotiopsis</i> sp. MUBL1014	-	3.66	2.33		-	1.33	2.0		-	1.66	2.66		-	1.0	1.33	
<i>Pestalotiopsis</i> sp. MUBL1015	-	2.66	2.0		-	2.66	2.33		0.33	1.33	1.0		-	1.66	1.33	
<i>Pestalotia</i> sp. MUBL1065	0.33	2.0	1.66	5.78	-	2.0	3.66	8.28	-	2.0	3.0	8.67	-	1.0	0.66	4.98%
<i>Phomopsis</i> sp. MUBL1074	-	1.0	1.66		-	3.0	1.0		-	1.0	2.3		-	0.33	0.33	
<i>Phomopsis</i> sp. MUBL1075	-	1.33	0.66	6.73	-	2.66	0.33	9.25	-	2.66	3.66	10.96	-	1.0	0.33	5.97%
<i>Phyllosticta</i> sp. MUBL1080	-	1.0	1.33		-	2.0	1.33		-	1.33	0.33		-	0.33	0.33	
<i>Phyllosticta</i> sp. MUBL1081	-	3.0	2.0	10.62	0.33	1.33	0.33	7.78	-	0.66	1.33	33	-	0.33	0.33	3.96%
<i>Septoria</i> sp. MUBL1091	-	1.0	2.0		-	1.66	1.0		-	1.0	0.33		-	1.0	0.66	
<i>Septoria</i> sp. MUBL1092	-	0.66	1.33	7.23	-	1.0	1.66	7.78	0.33	0.33	0.66	4.59	-	0.33	0.33	6.96%
<i>Truncatella</i> sp. MUBL1108	-	0.33	0.66	1.43	-	0.33	0.66	1.44	-	0.33	0.33	1.14	-	0.66	0.33	2.97%
Number of coelomycetes species recorded	3	20	20	-	2	20	20	-	3	19	19	-	1	19	20	-
Total number of coelomycetes isolates observed	3	104	102	-	2	112	91	-	3	79	91	-	1	57	42	-

L: Leaves; S: stem; B: bark; RPO: relative percentage occurrence; CF: colony frequency.

and *Truncatella* were isolated. The single species of the genera *Botryodiplodia*, *Fusamen*, *Monochaetia* and *Pestalotia* species were also observed (Table 1d). The maximum RPO was found in *Pestalotiopsis* spp. (49.89%) followed by *Colletotrichum* spp. (16.92%) and *Septoria* spp. (6.96%).

In the results, 74 endophytic coelomycetous fungi were isolated and successfully cultured in PDA medium. The identified coelomycetes were

deposited at Madras University Botany Laboratory (MUBL), University of Madras, Chennai 600 025, Tamil Nadu, India. They were also maintained on PDA slants under refrigeration.

## DISCUSSION

The fungal endophytes were a diverse group of organisms forming associations almost ubiqui-

tously throughout the plant kingdom (Vega et al., 2008).

The range of associated plants and fungi may be altered as climate changes, and not necessarily in a synchronous fashion. Plants may lose or gain endophytes, with as of yet unknown impacts on defense and fitness, although generalist species may provide indirect defense in new habitats more often than not (Van and Putten, 2003).

Changes in distribution may bring plants into competition with previously established local species, making the fungal community and particularly the pathogenic role of fungus important in determining outcomes of competition with non-native invasive species (Dighton, 2003; Molinari and Knight, 2010).

The colonization frequency was used to compare diversity (Orachaiapunlap, 2009). In this present study, the colonization frequency in four different seasonal variations of endophytes was recorded. 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).

In this study, the maximum isolates were observed with highest total CF% in SWM, NEM and WS, whereas, the meager amount of total CF% was observed in SS. Similarly, the higher colonization frequency and greater diversity of endophytes were observed during the monsoon and winter seasons than in summer by Thalavaipandian et al. (2011). The greater number of fungal isolates suggested that colonization by endophytes is correlated with climatic factors, such as rainfall and atmospheric humidity, which might influence the occurrence of some endophytic species (Wilson and Carroll, 1994).

In the present study, since the leaf lamina of *M. indica* and *R. arboreum* was broader as compared to the needles of gymnosperms, it has higher moisture content; hence, it anchors large number of endophytic colonization (Bernstein and Carroll, 1977b). An evaluation of the endophytic coelomycetes in *T. distichum* and *T. mucronatum* showed that since leaves are needle in shape, the lesser number of coelomycetes were found. Moreover, the diversity and ecological role of endophytes in tropical angiosperms are almost entirely unexplored (Hawksworth, 1993; Rodrigues and Petrini, 1997). The colonization frequency of endophytes in this study was within the range of many host plants studied in the tropics (Fröhlich et al., 2000; Photita et al., 2001; Suryanarayanan et al., 2003).

The frequently isolated fungi such as *Pestalotiopsis* spp., among the 74 different morphospecies belonging to 16 genera where observed in all the seasons in the four different plants. One of the most commonly found endophyte of Yew tree was *Pestalotiopsis* spp. Generally, this genus was among the most commonly isolated endophytic fungi of tropical plants (Strobel and Daisy, 2003; Cannon and Simmons, 2002). Moreover, Mahesh et al. (2005) isolated and identified 18.2% of coelomycete endophytes which includes *Pestalotiopsis* spp. and *Phoma eupyrena* from the living inner bark tissues of Neem tree. Interestingly, the present studies on the endophytic coelomycetes diversity in bark, leaf and stem of *M. indica*, revealed that *Pestalotiopsis* spp. were most dominant. Similarly, these results also supported that *Pestalotiopsis* spp. occurred most dominantly in tropical

plants collected from various parts of South India and followed by the *Colletotrichum* spp. (Cannon and Simmons, 2002; Shankar and Shashikala, 2011).

Goveas et al. (2011) observed the percentage frequency of colonization of the endophytic fungi in leaves as compared to stem. Similarly, in this study, the most colony frequency was observed in leaves as compare to stem and bark in *M. indica* and *R. arboreum* than the other two plants at different climatic conditions.

Therefore, the present investigation was carried in the coelomycetes diversity for discovering and identifying novel strains. In the future, investigation should continue with these isolates obtained to recover the potential novel compound for human beings.

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