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Diversity and abundance of endophytic fungi in four plant species of Western Ghat forests in Goa, Southern India

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ABSTRACT

Endophytic fungi associated with leaves of four plant species, viz. *Calamus thwaitesii, Careya arborea, Dendrocalamus strictus* and *Saraca asoca* of Western Ghats forests in Goa State, Southern India, were investigated during the pre-monsoon, monsoon and post-monsoon periods of 1999-2001. Features such as colony morphology and shape, size and colour of sporulating structures were used for species identification and recognition of morphotypes. From a total of 1200 leaf segments; 865 isolates belonging to 44 taxa [Ascomycetes (3), Coelomycetes (7), Hyphomycetes (10) and Sterile mycelia (24)] were recognized. Higher number of endophytes was isolated during the post-monsoon period; sterile forms were found to be prevalent in all seasons. Statistical analysis showed that the expected number of species of endophytes in all plants is maximum in post-monsoon except in *Saraca asoca* in which it was during the monsoon season. Jaccard Similarity Coefficient revealed that each plant species hosted a distinct endophytic assemblage.

INTRODUCTION

Fungal endophytes are characterized by their ability to produce apparantely harmless infections in living plant tissues (Carroll, 1988; Bills, 1995). Endophytic fungi have been isolated from a diverse range of plants and plant parts and studies indicate that they are ubiquitous (Fisher and Petrini, 1990; Petrini and Fisher, 1987, 1988; Siber, 1989; Azevedo *et al*, 2000). While some are widespread (Petrini, 1986), many of them have a restricted host range (Carroll and Carroll, 1978; Sherwoodpike *et al.*, 1986).

Although endophytic fungi are recognized as dormant saprobes (Chapela and Boddy, 1988) or latent pathogens (Carroll, 1986). properties such as pest deterrence, herbivory (Lactch *et al.*, 1985; Bacon *et al.*, 1986), release of growth promoting stimuli and increased competitive ability in the host (Clay, 1986) have been attributed to them. They are now known to produce metabolites useful in biocontrol of plant pests and pharmaceutical (Bills, 1995 Wagner and Lewis 2000; Benhamou *et al.* 2002; Stiele *et al.*, 1993; Lee *et al.*, 1995).

While the role played by individual fungus is said to be important, the significance of endophytic communities in plant ecology is yet to be assessed. Endophytes associated with temperate tree species have been studied since the middle of 1970's but not much is known on diversity, ecology and biology of those from the tropics (Bills, 1995). Rodrigues (1994) and Rodrigues and Samuels (1990) studied the foliar fungal endophytes of two tropical palm species, viz. *Euterpe oleraceae* Mart., in Brazil and *Licuala ramsayi* (Muell.) Domin., in Australia. The endophyte assemblage of *Stylosanthes guianensis* Swart, a Brazilian pasture legume (Periera *et al.*, 1993), *Gynoxis oleifolia* Muchler, a tree species of Ecuador (Fisher *et al.*, 1995) and *Parthenium hysterophorus* (Asteraceae), a tropical herb (Romero *et al.*, 2001) and several tropical plant species from India

(Suryanaryanan *et al.*, 1998; Suryanarayanan and Kumaresan, 2000; Suryanarayanan and Vijaykrishna, 2001; Suryanarayanan and Thennarasan, 2004), have been studied.

The Western Ghat forests in Southern India, one of the major biodiversity hotspots in the world, harbour a wide variety of tropical plant species. The present work was undertaken to recover a maximum number of endophytic fungi from four of the plant species and to estimate their diversity in this region.

MATERIALS AND METHODS

Study season, site and plants sampled

The study was carried out during the pre-monsoon (Feb-May), monsoon (June-Sept.) and post-monsoon (Oct-Jan.) seasons of 1999-2001. These seasons are unique in this part of the tropical world. The pre-monsoon is warm and humid (temp. 28 – 37°C; humidity (70 - 90%). The monsoon or rainy season has precipitation ranging between 200-350 cm, humidity always remaining 100% and temperatures seldom going above 23°C. The post-monsoon season has temperature range from 17 – 28°C and humidity between 40-70%.

Four widely distributed native plant species, namely *Calamus thwaitesii* Becc. & Hook. (Arecaceae), *Dendrocalamus strictus* (Roxb.) Nees. (Poaceae), *Saraca asoca* (Roxb.) de Wilde (Casesalpinaceae) and *Careya arborea* Roxb. (Lecythidaceae) were examined. Of these, *Saraca asoca* and *Calamus thwaitesii* were from Bondla Wildlife Sanctuary and *Careya arborea* and *Dendrocalamus strictus* from Mollem Wildlife Sanctuary, two sampling sites 30 km apart north-south and located along the Western Ghats in Goa State.

Sampling and Isolation

From each plant, a set of five fresh and disease-free young (from leaf bud), middle-aged (mature) and old (nearing senescence) leaves was considered as a sample. The samples were transported to the laboratory in collection bags and processed within 24 h of collection.

The 3-step surface sterilization process described by Fisher and Petrini (1987) was followed. The leaves were washed thoroughly under running tap water to remove any adhered debris from the surface, surface-sterilized by sequential immersion in 70% ethanol (1 min), 4% sodium hypochlorite (3 min) and 70% ethanol (0.5 min), repeatedly rinsed in sterile distilled water and cut in to segments of approximately 0.2-0.5 cm with a sterile blade. Three hundred segments of each plant species were plated on 2% Malt Extract Agar (MEA) plates incorporated with antibiotics (0.1 g Penicillin G, 0.1 g Streptomycin, 0.1 g Neomycin, 0.1 g Bacitracin and 0.1 g Polymyxin in 10 ml sterile distilled water and added to 500 ml MEA). The plates were incubated at 22°C for 7-28 days. Fungal hyphae emerging from within the leaf segments were transferred to fresh 2% MEA plates. Dissimilar colonies were considered as possible taxonomic entities and transferred to slants. Sporulating fungi were identified using standard taxonomic keys. Non-sporulating isolates were characterized in to different morphotypes based to cultural characters such as growth rate, surface texture, margin shape and hyphal pigmentation.

Statistical Analysis

The frequency of colonization (%) of each endophyte species was calculated by the method of Fisher and Petrini (1987) as, the number of colonized segments divided by the total number of segments plated × 100. The proportion of species belonging

to either the Ascomycetes, Coelomycetes, Hyphomycetes or the Mycelia sterilia in a single host plant species was calculated by the formula $(N_z/N_t \times 100)$, where N_z is the number of fungal species belonging to a group and N_t is the total number of species from a single host species. The Jaccard Similarity Coefficient was calculated for all possible pairs of hosts to compare the endophyte assemblages, according to the following formula:

Similarity Coefficient $= C/(A + B + C)$, where A and B are the total number of fungal species isolated from any two hosts and C the number of fungal species found in common (Sneath and Sokal, 1973). The results were expressed as percentages.

To estimate the expected number of species, $[E(S_n)]$ from the isolates obtained in the pre-monsoon, monsoon and post-monsoon season for each plant species, rare faction index was performed following the method of Ludwig & Reynolds (1988) as follows.

$$E(S_n) = \sum_{i=1}^{S} \left\{ 1 - \left[\left(\frac{N - n_i}{n} \right) / \left(\frac{N}{n} \right) \right] \right\}$$

where,

N = Total number of individuals of a community.

n = number of individuals drawn from N.

ni = number of individuals of the i^{th} species.

S = Total number of species of a population.

$E(S_n)$ = Expected number of species

RESULTS AND DISCUSSION

All the four plant species studied, viz. *Calamus thwaitesii*, *Careya arborea*, *Dendrocalamus strictus* and *Saraca asoca*, harboured fungal endophytes. From a large number of leaf segments screened 865 isolates belonging to 44 fungi (including sterile forms) were isolated. Of these, 9 were recovered from *Calamus thwaitesii*, 11 from *Saraca asoca*, 16 from *Careya arborea* and 8 from *Dendrocalamus strictus* (Table 1). However, only 30 were isolated more than once from the four plant species.

Similar studies conducted earlier indicated that the abundance of endophytic fungi can be affected by season (Petrini, 1991; Rodrigues 1994). In the present work, species such as *Acremoniella sarcinellae*, *Cochliobolus lunatus*, *Ramichloridium fasciculatum*, *Volutella roseola* and several nonsporulating forms were isolated only in one season and it is possible that these are of 'one-season participation'. Further, *Ramichloridium faciculatum*, *Volutella roseola* and nonsporulating forms 3, 10, 14, 19 and 20 appeared only once in the entire study period and this 'one-time appearance' by these fungi probably is very unique.

Maximum number of endophytes were isolated from *Careya arborea* (145) during the post monsoon season (Table 2). The least number of isolates were encountered in *Dendrocalamus strictus* (16) in the pre-monsoon season. In the post-monsoon season, the recovery of endophytes was highest where as it was 96 in *Saraca asoca* and 92 in *Dendrocalamus strictus*.

As indicated in the rarefaction curves (Fig. 1-4), with exception of *Saraca asoca* where it was highest during the monsoon season, most endophyte species were recovered during the post-monsoon season. The $E(s)$ was highest in *Careya arborea* in post-monsoon season as compared to other plant species (Fig. 3). The expected number of species $[E(s)]$ is in the gradation of post-monsoon > monsoon > pre-monsoon.

As in Table I, *Kumbhamaya indica* (46%) in *Dendrocalamus strictus* and *Vermiculariopsiella* and *Aspergillus*

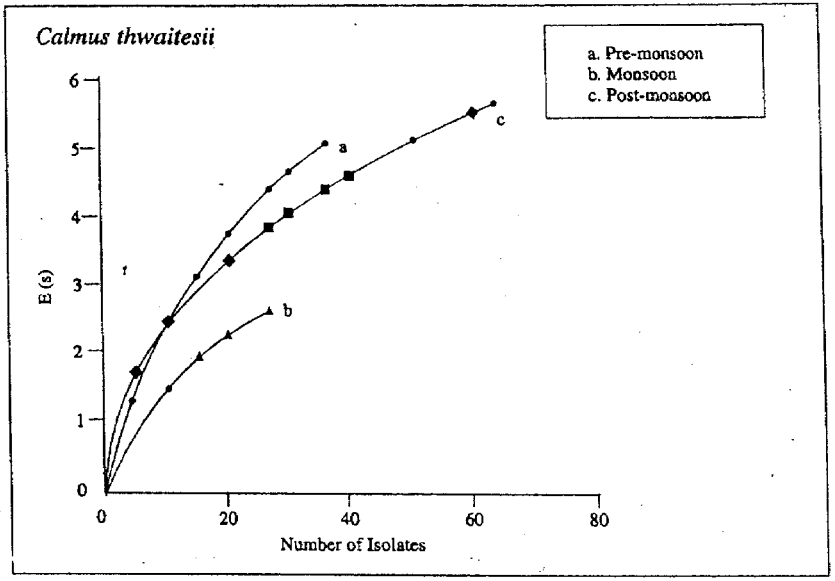

Fig 1: Rarefaction curve for *Calamus thwaitesii*

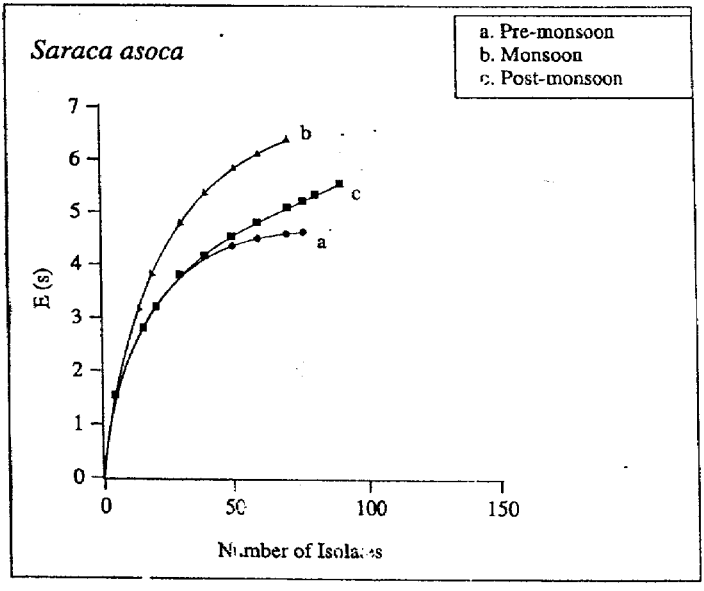

Fig 2: Rarefaction curve for *Saraca asoca*

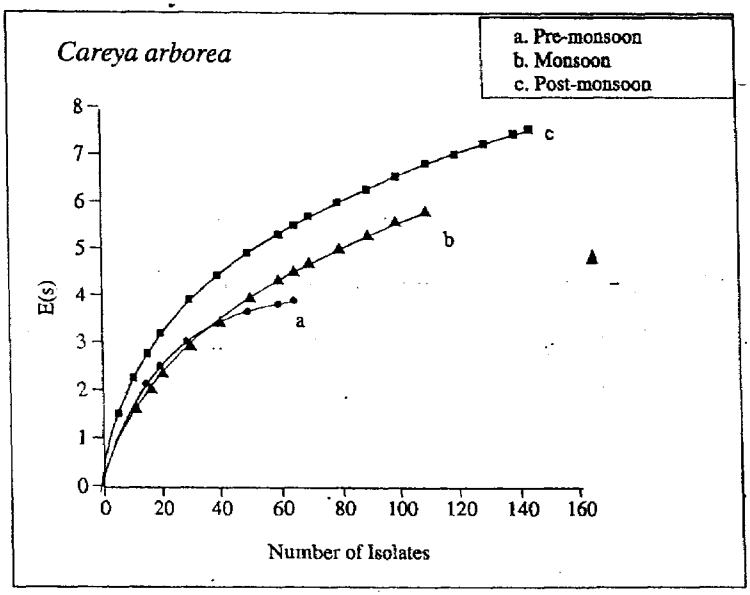

Fig 3: Rarefaction curve for *Careya aborea*

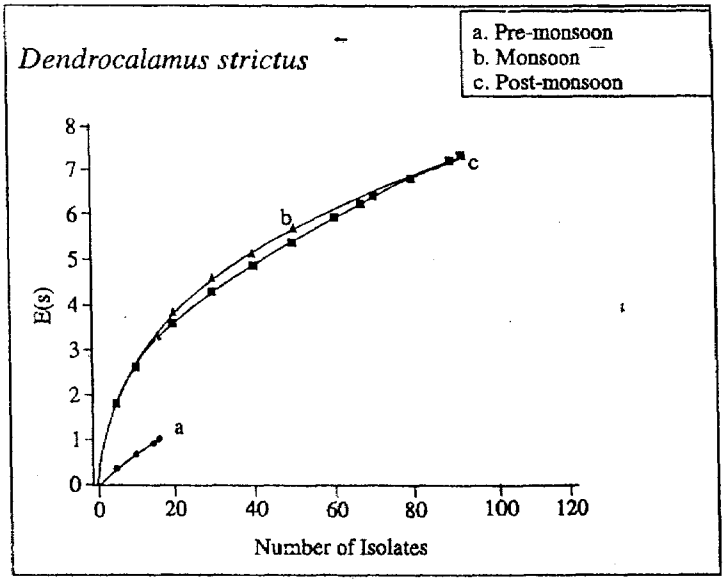

Fig 4: Rarefaction curve for *Dendrocalamus strictus*

Table 1: Frequency of occurrence of endophytic fungi in four plant species during different seasons

Fungi / Plant species	*Calamus thwaitesii*			*Saraca asoca*			*Careya arborea*			*Dendrocalamus strictus*		
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post
Hyphomycetes												
Acremoniella sarcinella	4	–	–	–	–	–	–	–	–	–	–	
Acremoniella sp.	–	–	–	–	–	–	–	–	–	–	1	1
Aspergillus restrictus	–	–	38	–	–	–	–	–	–	–	–	–
Cladosporium herbarum	–	–	–	–	–	–	–	–	2	1	–	–
Corynospora smithii	12	–	–	–	–	–	–	–	–	–	–	–
Corynospora cassicola	6	17	–	–	–	–	–	–	–	–	–	–
Kumbhamaya indica	–	–	–	–	–	–	–	–	–	13	25	46
Ramichloridum fasciculatum	–	–	–	–	–	–	–	–	–	–	–	1
Bharatheeya goanensis	–	–	–	16	4	–	–	–	–	–	–	–
Volutella roseola	–	–	–	–	–	–	–	–	–	–	1	–
Coelomycetes												
Pestalotiopsis versicolor	–	–	–	–	–	1	4	–	–	–	–	–
Macrophoma sp.	–	–	–	17	19	–	–	–	–	–	–	–
Vermiculariopsiella elegans	–	–	1	3	12	2	–	2	–	–	21	31
Undetermined sporodochial from 1	–	5	2	–	–	–	–	–	–	–	–	–
Undetermined sporodochial from 2	–	–	–	–	–	–	–	1	–	–	1	1
Undetermined pycnidial form	1	–	–	–	–	1	–	–	–	–	–	–
Undetermined acervular form	7	1	–	–	–	–	–	–	–	–	–	–
Ascomycetes												
Cochliobolus lunatus	–	–	–	9	4	19	–	86	–	–	–	1
Guignardia sp. 1	–	–	1	–	15	–	20	5	22	1	9	–
Xylaria sp. 1	3	3	1	–	19	2	–	–	–	–	–	2
Non-Sporulating												
NSM 1	3	1	4	–	–	–	–	–	–	–	–	–
NSM 2	–	–	15	–	–	–	–	–	1	–	–	–
NSM 3	–	–	1	–	–	–	–	–	10	–	–	3
NSM 4	–	–	–	31	–	–	–	–	–	1	–	–
NSM 5	–	–	–	–	8	–	–	–	–	–	–	–

Table 1 contd.,

Fungi / Plant species	*Calamus thwaitesii*			*Saraca asoca*			*Careya arborea*			*Dendrocalamus strictus*		
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post
NSM 6	–	–	–	–	–	2	–	–	–	–	–	–
NSM 7	–	–	–	–	–	–	23	–	–	–	–	–
NSM 8	–	–	–	–	–	–	16	–	–	–	–	–
NSM 9	–	–	–	–	–	–	2	–	–	–	–	–
NSM 10	–	–	–	–	–	–	–	1	–	–	–	–
NSM 11	–	–	–	–	–	–	–	9	–	–	–	–
NSM 12	–	–	–	–	–	–	–	4	–	–	–	–
NSM 13	–	–	–	–	–	–	–	2	–	–	–	–
NSM 14	–	–	–	–	–	–	–	1	–	–	–	–
NSM 15	–	–	–	–	–	–	–	–	96	–	–	–
NSM 16	–	–	–	–	–	–	–	–	7	–	–	–
NSM 17	–	–	–	–	–	–	–	–	3	–	–	–
NSM 18	–	–	–	–	–	–	–	–	2	–	–	–
NSM 19	–	–	–	–	–	–	–	–	1	–	–	–
NSM 20	–	–	–	–	–	–	–	–	1	–	–	–
NSM 21	–	–	–	–	–	–	–	–	–	–	2	–
NSM 22	–	–	–	–	–	–	–	–	–	–	5	–
NSM 23	–	–	–	–	–	–	–	–	–	–	2	–
NSM 24	–	–	–	–	–	15	–	–	–	–	–	–
Total no. of individuals	36	27	63	76	71	96	65	111	145	16	67	92
Total no of species	7	5	8	5	7	8	5	9	10	4	9	10

Note: Pre: Pre–Monsson [February–May]; Mon: Monsoon (June–September); Post: Post–Monsoon [October–January]

Table 2: Total number of isolates and species of endophytic fungi recorded from four plant species during different seasons

Plant species	Season	Total Isolates	Total Species	Asco-mycetes	Coelo-mycetes	Hypho-mycetes	Myce-liasterilia
Calamus thwaitesii	Pre	36	7	1	2	3	1
	Mon	27	5	1	2	1	1
	Post	63	8	2	2	1	3
Saraca asoca	Pre	76	5	1	2	1	1
	Mon	71	7	3	2	1	1
	Post	96	8	2	3	1	2
Careya arborea	Pre	65	5	1	1	0	3
	Mon	111	9	2	2	0	5
	Post	145	10	1	0	1	8
Dendrocalamus strictus	Pre	16	4	1	0	2	1
	Mon	67	9	1	2	3	3
	Post	92	10	2	3	3	3

restrictus (38% in *Calamus thwaitesti* and 54% in *Saraca asoca*) showed the highest frequency of occurrence in the post-monsoon season. It may be said that in the tropics, the post-monsoon is the best season for maximum recovery of endophytic fungi colonizing the plant species in their annual occurrence. Maximum colonization of endophytic fungi during the monsoon and post monsoon in the tropics may be due to high moisture content (humidity).

Carroll and Carroll (1978) and Sherwood-Pike *et al.* (1986) observed that the plant species were specific with regard to the number and type of endophytes harboured. This was found true in the present study. *Kumbhamaya indica*, originally reported as an endophyte from young leaves of *Carissa conjesta* in India (Jacob and Bhat, 200), was consistenly isolated from *Dendrocalamus strictus* during the period of investigation irrespective of the seasons. *Vermiculariopsiella elegans* and an undetermined ascomycete form were found common to all plant species. *Macrophoma* sp. and *Vermiculariopsiella elegans* were widespread in all plant species studied. This was in accordance with Petrini (1986) who showed that some fungi are widespread in their occurrence.

The Jaccard Similarity Coefficient showed that the composition of endophytes recovered did not overlap by more than 18% in *Calamus thwaitessii* and *Saraca asoca* and by 21% in *Careya arborea* and *Dendrocalamus strictus* though these four plant species were practically exposed to the similar environmental conditions and fungal inoculum (Table 3). This observation indicated that each plant species exhibited certain degree of distinctness in its endophyte composition.

Of the different groups of endophytic fungi colonizing the plant species, in *Careya arborea* the highest percentage of fungi were the nonsporulating forms (72.72%) and the lowest were the coelomycetes In contrast, in *Saraca asoca*, the proportion of hyphomycetes, coelomycetes and ascomycetes were uniform (Table 4). The fungal endophytes colonize the plant parts by adapting to its internal environment. Studies carried out

Table 3: Jaccard Similarity Coefficient for the endophytes isolated from four plant species (expressed as %)

Plant species	*Calamus thwaitesii*	*Saraca asoca*	*Careya arborea*	*Dendrocalamus strictus*
Calamus thwaitesii	100	18.18	12.9	15.38
Saraca asoca		100	12.9	10.71
Careya arborea			100	21.21
Dendrocalamus strictus				100

Table 4: Percent occurrence (%) of different groups of fungi as endophytes in four plant species

Host Plant	Hyphomycetes	Coelomycetes	Ascomycetes	Mycelia Sterilia
Calamus thwaitesii	46	15.5	15.5	23
Saraca asoca	23	23	23	31
Careya arborea	14	4	9	72.72
Dendrocalamus strictus	46.66	0	20	33.33

simultaneously on fungal colonization on leaf litter of these plant species (unpublished) in our laboratory, have shown that fungi such as *Pestalotiopsis versicolcr, Bharatheeya goanensis* and *Vermiculariopsiella elegans,* observed as endophytes were often recovered as litter fungi. As observed by Chapela (1989), these fungi might be the ones that act as dormant saprobes in the endophytic stage.

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