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DIVERSITY AND ABUNDANCE OF MICROFUNGI ON DECAYING LEAF- LITTER OF *FICUS BENGALENSIS* Linn.

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Abstract : Fallen and decaying leaves of *Ficus bengalensis* Linn., gathered at monthly intervals during a 3-month post-monsoon period, were subjected moist-chamber incubation and particle-plating techniques to isolate and analyse the diversity and abundance of microungi inhabiting the litter. Simultaneously, the fungus flora of soil and air in the proximity and fresh leaves of the tree were analyzed in the same site to understand the possible sources of fungi to ficus-litter. A total of 106 species belonging to 88 genera were recorded. Of these 54 were encountered on litter. The results indicate that the endophytic fungi found on fresh leaves often reach the litter pool; the ubiquitous fungi encountered in the isolation plates could be attributed to air-spora; majority of the recovered soil fungi overlapped qualitatively with the ficus-litter fungi. The fungi recovered exclusively from the decomposing litter with no other probable source or origin may be considered as 'true ficus-litter fungi'.

Key words : Microfungal diversity; *Ficus bengalensis;* moist-chamber incubation and particle- plating techniques; tropics; India.

INTRODUCTION :

Ficus bengalensis Linn. (Moraccae), the banyan tree, associated with many aspects of the Indian sociocultural milieu, is a multifariously useful and widely distributed plant species in the country. The tree is evergreen, spreads its branches extensively and attains a height of nearly 30 m. It is considered to be an ecosystem by itself as it harbours and plays host to a number of climbers, epiphytes, insects, reptiles, birds, mammals and microbes. The leaf fall is continuous and the rate of litter decomposition is slow. The plant parts exude a milky latex from the cut ends and it is said to be antibiotic in its properties (Fahn,1990). So far, about 30 species of fungi have been recorded from decaying leaves and twigs of *F. bengalensis* (Mukherji 'i & Juneja, 1974; Sarbhoy *et al.*, 1996).

Monthly analysis of the fungus-litter association was made for a 3-month duration on samples gathered from a *Ficus* tree found growing in the campus of Goa University. Besides moist-chamber incubation, particle-plating technique employed by Bills & Polishook (1994) was used for the recovery and observation of microfungi.

In order to understand the possible sources of fungi to the litter pool, simultaneous study of endophytic fungi associated with living leaves (Petrini et al, 1993) and saprophytic moulds present in the ambience of the tree and those associated with the root-traversed surface soil were done. The diversity and abundance of microfungi associated with *F. bengalensis* litter were analyzed.

MATERIALS AND METHODS :

The tree with its dense canopy and entanglement by a variety of perennial stranglers resulted with a conspicuous shade at its base. In all, the following four types of samples were made at monthly intervals between September to November 1997. (1) Fungal propagues from the air were trapped by exposing MEA petri dishes (malt extract 5 g, agar 20 g, 1 L distilled water) beneath the tree canopy approximately 1 m above ground level. (ii) Five fresh and disease-free leaves were randomly collected

at a height of 3.5 m above the ground level to source the endophytic fungi. (iii) Ten partially decomposed and dead leaves were picked from the ground surface to recover the litter fungi. (iv) Three g of surface soil from beneath the tree was gathered to isolate soil fungi. The samples were processed immediately or stored in fresh polythene bags in a refrigerator at 4°C until processed.

A mixture of antibiotics, viz. bacitracin 0.02 g, neomycin 0.02 g, penicillin G 0.02 g, polymixin 0.02 g, steptomycin 0.02 g and terramycin 0.04 g dissolved in (a) 10 ml of distilled water and (b) cyclosporin A 0. 0.001 g dissolved in 1 ml of methanol were filter-sterilized and added to 1 litre of Water agar medium. The test-tubes consisted of 2% without the antibiotics.

The leaf-litter was subjected to two methods of observation. For the moist- chamber incubation, five partially decomposed leaves were thoroughly washed in tap water and incubated in moist filter paper-lined sterile Petri dishes at 22°C. The fungi appeared on the substrate were regularly examined.

In the particle-plating method, 5 partially decayed leaves were thoroughly washed in tap water, cut into pieces and wet-homogenized in an electric blender for 4 min. The pulverized sample was filtered through two superimposed and alcohol disinfected sieves (250 μm and 100 μm # size). The particles trapped in the lower filter, of size between 100-250 μm, were thoroughly washed in sterile distilled water. About 1g of wet particles were resuspended in 5 ml sterile water and 0.05ml of the dilution was plated out into 5 petri dishes, each containing MEA with antibiotics. The plates were incubated at 22°C.

The soil sample was subjected to particle-plating. For recovery of endophytes, five fresh leaves were washed in deionised water and surface sterilized first, 1 min. in 96% ethanol, followed by 3 min. in 4% sodium hypochlorite and 30 sec. in 96% ethanol. Each surface-sterilized leaf was thoroughly rinsed in sterile distilled water and cut into 1 mm^2 bits with an alcohol disinfected razor blade. Seven pieces of each were placed in MEA petri plates with antibiotics. The plates were incubated at 22°C. Fungal colonies appearing in the plates with leaf-litter, soil and air samples were counted as total colony forming units (CFU) on the 2nd, 8th and 15th day. Of these, five colonies each were randomly transferred into MEA slants on the 2nd, 10 colonies on the 8th and 5 on the 15th day. Isolation of endophytes from fresh leaves were made on the 8th and 15th day.

The isolates were grouped into sporulating and non-sporulating forms, besides recognising various cultural characters. The sporulating isolates were identified using relavent taxonomic keys. The non-sporulating forms were regarded as 'morphotypes'.

RESULTS AND DISCUSSION :

A total of 240 isolates were recovered during the 3-month analysis, ranging 107, 74 and 59 during September, October and November respectively. The number of species per substrate was 20 in air, 11 on fresh leaves, 41 from soil and 54 on litter. The use of two techniques resulted in the isolation of 106 species belonging to 88 genera. (Table 1). The fungi isolated included diverse conidial and several pyc-

nidial fungi besides a number of sterile strains. Of these, 93 were isolated by particle plating technique and 20 were recovered from moist chamber incubation. The highest number of forms, 55, were obtained in September, followed by 36 in October and 30 in November.

Fungi belonging to various genera were isolated from the litter. These include *Acremonium*(3), *Beltrania* (1), *Botryodiploidea* (1), Beltraniella(1), Circinotrichum(1), Cylindrocarpon (1), *Cylindrotrichum* (1), *Cylindrocladium* (1), *Dictyosporium* (1), *Drechslera* (1), *Epicoccum* (1), *Fusarium* (1), *Helicomyces* (2), *Helminthosporium* (1), *Idriella* (2), *Pseudobotrytis* (1), *Phoma* (1), *Phaeoisaria* (1), *Stachybotrys* (1), *Scolecobasidium* (2), *Trichothecium* (1), *Weisneriomyces* (1) *Zygosporium* (1), undetermined pycnidial form(3), undetermined sporodochial form(1), undetermined ascomycete(1), undetermined hyphomycete(3), sterile strains(5).

Guignardia (1), *Nigrospora* (1), undetermined pycnidial form(S) and sterile strains(3), were isolated as endophytes. An unidentified sporodochial fungus predominant as an endophyte on *Ficus* during September was isolated on the fallen litter in October and November. The sterile strain-1 was isolated from the plates exposed to air in September, from soil in October and as an endophyte in November. The species abundance distribution (Fig. 1) shows that there were few abundant species and a high proportion of rare species. The most abundant species did not account for more than 7%, 8% and 12% of the total isolates for litter, soil and air respectively.

Forty-two percent of the isolates recovered during the study period were endophytes. For endophytes, as 7 leaf-bits were plated on each plate, the probability of isolating the same species was higher than that of for litter and soil where particle-plating technique was used in the recovery of fungi. Thus particle plating method was also found to be an efficient technique for the recovery of maximum species of fungi.

The high diversity of fungi observed in Ficus-litter and its ambience may be attributed to the high humidity and temperature of the months of September to November where the rate of litter decomposition in the tropical forests is also said to be high (Dickinson & Pugh, 1974).

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Table 1. Abundance of fungi isolated from *Ficus bengalensis* L. during a 3-month analysis (Sept- Nov.'97) from Taleigao Plateau, Goa

List of Fungi	Sept.	Oct.	Nov.	P.P.	M.C.
Aspergillus spl	A_1		S_1	+	
Aspergillus sp2		S_1		+	
Aspergillus sp3	S_1			+	
Aspergillus niger			A_1	+	
Acremonium spl			S_1	+	+
Acremonium sp2	L_1S_1				+
Acremonium sp3	L_1				+
Acremonium sp4	L_1				+
Beltrania rhombica	L_1			+	
Botryodiploidia theobromae		L_1	L_2	+	+
Beltraniella sp.	L_1				
Circinotrichum sp	L_1				
Chaetomium sp	S_1			+	
Cylindrocarpon sp	L_1			+	
Cylindrotrichum sp	L_1	L_1		+	
Cladosporium sp		S_1		+	+
Curvularia spl sp2		A_1, L_1		+	
Curvularia sp3			A_2	+	
Cylindrocladium sp			L_2	+	+
Dictyosporium sp	L_1				
Drechslera spl		L_1			
Drechslera sp2			A_1	+	
Epicoccum-like	L_2			+	
Fusarium semitectum	A_1			+	
Fusarium spl	A_2,S_2	S_2		+	
Fusarium decemcellulare	L_1			+	
Fusarium sp2		A_1, L_1		+	
Fusarium sp3			S_1	+	
Fusarium sp4			S_1	+	
Fusarium sp5	S_2			+	
Gonytrichum sp	A_1		A_1	+	
Gilmanielia sp	S_1			+	
Guignardia sp			E_{12}	+	
Helicomyces sp1		L_1			+
Helicomyces sp2		L_1			+
Heminthosporium sp		L_1			+
Idriella spl		L_1			+
Idriella sp2			L_1	+	
Memnoniella echinata		S_1		+	
Mucor sp		A_1		+	
Nigrospora sphaeilca			E_4	+	
Pseudobotrytis.gp	L_1			+	
Penicillium spl	L_1			+	
Penicillium sp2			A_1	+	
Pithomyces chartarum		A_1		+	+
Phoma sp		L_{18}		+	

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Pestalotiopsis sp			L_2S_1	+	+
Phaeoisaria phallinosa		L_1			+
Stachybotrys nephrospora		L_1			
Scolecobasidium		L_1		+	+
Scolecobasidium sp2		L_1	L_1	+	
Trichoderma sp	S_1			+	+
Trichothecium roseum		L_1			+
Weisneriomyces javanicus	L_1				+
Zygosporium masonii	L_1			+	
Undetermined pycnidial spl	L_1, S_1			+	
Undetermined pycnidial sp2	E_8			+	
Undetermined pycnidial sp3	E_1			+	
Undetermined pycnidial sp4	L_1			+	
Undetermined pycnidial sp5	L_1, S_1			+	
Undetermined pycnidial sp6	A_1, S_1			+	
Undetermined pycnidial sp7		E_1		+	
Undetermined pycnidial sp8		L_1		+	
Undetermined pycnidial sp9			L_1	+	
Undetermined pycnidial sp10			E_1	+	
Undetermined sporodochial sp	E_{23}	L18	L_3	+	
Undetermined ascomycete sp			L_1	+	
Sterile strain spl	A_1	S_1	E_1	+	
Sterile strain sp2	E_2			+	
Sterile strain sp3	E_1			+	
Sterile strain sp4	L_1		S_1	+	
Sterile strain sp5	L_1, S_1			+	
Sterile strain sp6	L_2, S_1			+	
Sterile strain sp7	L_1, S_1			+	
Sterile strain sp8	L_1, S_1			+	
Sterile strain sp9	S_3	L_1, S_1		+	
Sterile strain spl0	A_3	A_1	S_1	+	
Sterile strain spl1	S_1			+	
Sterile strain spl2	S_1			+	
Sterile strain spl 3	L_1, S_1	A_2		+	
Sterile strain spl4	L_2, S_1			+	
Sterile strain spl5		S_1	L_7		
Sterile strain spl6		L_1		+	
Sterile strain spl7		L_1		+	
Sterile strain spl 8		L_1		+	
Sterile strain spl 9		L_1		+	
Sterile strain sp20			A_1	+	
Sterile strain sp21			E_1	+	
Sterile strain sp22			S_1	+	
Undetermined hypho. spl	L_1			+	
Undetermined hypho sp2	S_1			+	
Undetermined hypho sp3	S_2			+	
Undetermined hypho sp4	S_1			+	
Undetermined hypho sp5	S_1			+	
Undetermined hypho sp6	S_1			+	
Undetermined hypho sp7	S_1			+	
Undetermined hypho sp8	S_1			+	

Undetermined hypho sp9	S_1			+
Undetermined hypho spl0	S_1			+
Undetermined hypho spl1	S_1			+
Undetermined hypho spl2		L_1		+
Undetermined hypho spl 3		L_1		+
Undetermined hypho sp14			A_2	+
Undetermined hypho sp 15			A_1	+
Undetermined hypho sp 16			A_1	+
TOTAL			93	20

P.P. = No. of fungi isolated using particle plating technique
M.C. = No. of fungi isolated using moist chamber incubation
A = No. of isolates of a single species from plates exposed to air
E = Total no. of a single endophytic species
L = No. of isolates of individual species isolated from leaf-litter
S = No. of isolates of individual species isolated from soil.

Fig. 1 Abundance of microfungi of four substrates from *Ficus bengalensis* L. Species in each sample in the order of most abundant at left and least abundant at right.