Biodiversity of microfungi associated with litter of *Pavetta indica*

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The biodiversity of fungi associated with leaf litter of *Pavetta indica* from jungle scrub at Tambaram, Chennai, India was studied from October 1999 to September 2000. The litter was divided into freshly fallen senescent leaves (grade 1) and leaves already undergoing active decomposition (grade 2). Moist chamber incubation of the litter revealed 54 fungal taxa belonging in 40 genera. More taxa were found on grade 1 than on grade 2 litter. *Alternaria alternata*, *Chaetomium seminudam*, *Circinotrichum fertile*, *Cladosporium oxysporum*, *Drechslera halodes*, *Fusarium lateritium*, *F. oxysporum*, *Torula herbarum*, *Tretopileus* sp., *Zygosporium echinosporum* and *Z. gibbum* were some species recorded exclusively on grade 1 litter. *Acremonium* sp., *Bartalinia robillardoides*, *Curvularia brachyspora*, *C. intermedia*, *Helicosporium vegetum* and *Harknessia* sp. were specific to grade 2 litter. Although 37 taxa were common to both grades, there were differences in percentage occurrence between the two grades of litter. Taking the average percentage occurrence as an index of colonizing efficiency of a taxon, it was found that *Euantennaria* sp., *Circinotrichum falcatisporum*, *Wiesneriomyces javanicus*, *Meliola* sp., *Zygosporium oscheoides*, *Beltraniella portoricensis*, *Selenosporella curvispora* and *Zygosporium masonii* were active in that order. In addition to the identification of fungi after moist incubation, a washing technique was also performed. Fresh leaves attached to the plant were also collected and studied for microfungi to understand if carryover of the species from the phylloplane to the litter occurred after the leaves senesced and fell. The phylloplane study revealed 29 species, among which 19 species were carried to the grade 1 litter and 12 species thereafter to the grade 2 litter.

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Introduction

Fungi play an indispensable role in decomposition of plant substrates in various ecosystems (Kshattriya et al. 1992, Bills & Polishook 1994, Aoki & Tokumasu 1995, Subramanian & Vittal 1980). The fungal diversity on decaying plant material has previously been studied in temperate regions (Hudson 1968, Dickinson & Pugh 1974, Frankland 1981, Swift 1982, Cooke & Rayner 1984) and tropical or sub-tropical regions (Aoki 1987, Promputtha et al. 2002, Osono et al. 2002, 2005, 2009, Tang et al. 2005, Pasqualetti et al. 2006, Paulus et al. 2006, Pinnoi et al. 2006, Duong et al. 2008, Kodsueb et al. 2008, Shirouzu 2009). Most of the studies have focused at the substratum level (e.g. petiole: Frankland 1969, 1976; terrestrial wood: Lange 1992; rust-infected plum leaves and wheat stems: McKenzie & Hudson 1976; dung: Nagy & Harrower 1979; pine cones: Kasai et al. 1995 and wool: Ghawana et al. 1997; leaf sheaths: Van Ryckegem & Verbeken 2005; fronds: Yanna & Hyde 2002; pods: Somrithipol

2002; decaying leaves: Bonet et al. 2004, Osono 2005, Twieg et al. 2007). There are also studies regarding the composition, succession and specific pattern of colonization of different fungal species on litter of a single plant species (Sherwood & Carroll 1974, Sieber-Canavesi & Sieber 1993, Pasqualetti et al. 1999, Duong et al. 2008) and of mixed leaf litter (Kshattriya et al. 1992*,* 1994, Gesser et al. 1993).

In India the vegetation is tropical evergreen forest, tropical deciduous forest, tropical thorn forest, scrub jungle, montane forest and mangroves. There have been a series of pioneering studies of saprobic fungal succession on the plants pertaining to each ecosystem, except for the montane and the scrub jungles; (Manoharachary et al. (1976) on *Cassia glauca*, Sudha (1978) on *Glycosmis* and *Ixora*, Mehrotra & Aneja (1979) on *Chenopodium album*, Subramanian & Vittal (1979, 1980) on *Atlantia monophylla* and *Gymnosporia emarginata*, Vittal & Sukumaran (1981) on *Cymbopogon flexuosus,* Sankaran (1993) on *Paraserianthes falcataria*, *Eucalyptus tereticornis* and *Tectona grandis,* Tiwari et al. (1994) on *Ananas comosus*, Sadhana-Srivastava et al. (1998) on *Saccharum officinarum*). For the present study, a scrub jungle ecosystem was chosen in South India. A plant from the family Rubiaceae, *Pavetta indica* was chosen to study the microfungal colonization associated with the decomposition stages of leaf litter.

Material and Methods

Characterization of the study site

A typical scrub jungle near Tambaram, a suburb 20 miles to the south of Chennai, India was selected. It lies in the Chengelput district of the state of Tamil Nadu and has a mean temperature of 28.4°C (24.0–32.8°C) and an annual rainfall of 685 mm. The monthly mean relative humidity is 78.6%. The first showers of the South West monsoon occur during the middle of June. Showery weather continues through August and September and ceases in the middle of October, when the North East monsoon sets in. The soil is a mixture of clay and loam with a proportion of sand.

Experimental material

The biodiversity and succession of the microfungal communities on leaf litter of *Pavetta indica* (Rubiaceae) was surveyed from October 1999 to September 2000 using a moist chamber incubation and leaf litter washing techniques. Twelve samplings were made during the period of study. Litter samples were collected at random from the study site and taken to the laboratory in sterile polythene bags. The litter was sorted into two grades representing the two stages of decomposition. These were 'grade 1' representing freshly fallen and senescent leaves and 'grade 2' representing leaves in an advanced stages of decomposition, usually thin, fragmentary and tightly compressed. Mould growth was conspicuous at this stage.

Moist chamber incubation technique

Twenty leaves of each grade of leaf litter were randomly selected and incubated in sterile moist chambers at 25±2°C. Petri plates (20 cm diam.) were sterilized and used as moist chambers with sterilized filter paper, which was periodically moistened with sterile distilled water. Precautions were taken not to over flood the chambers with water. Leaves were incubated for 48 hours and then examined under a binocular stereomicroscope for the fungi sporulating on them. All the fungi found sporulating were isolated, examined and identified to species level.

Isolation frequency and percentage occurrence were used to explain the colonization efficiency of the microfungi on the leaf litter. Isolation frequency denotes the number of samplings in which a particular fungus was recorded as against the total number of samplings (12). Based on this, the fungi were categorized into 5 groups; 81–100%—'most common'; 61–80%—'common'; 41–60%—'frequent'; 21–40%—'occasional'; 1–20%—'rare'. Percentage occurrence was used to denote the number of leaves on which a particular fungus was present as against the total number of leaves (20) examined per grade by moist chamber incubation.

Leaf litter washing technique

In addition to the moist chamber incubation, a second technique of washing fresh leaves removed from the plant and leaf litter was performed (Subramanian & Vittal

1979). Ten fresh leaves and ten litter leaves were randomly selected from each grade of litter and the washing technique was performed. From each leaf, five 1 cm^2 pieces were cut with a pair of sterile scissors. The samples were washed in 100 mL of sterile water in a 250 mL Erlenmeyer flask for 30 minutes on a shaker. From this initial suspension, serial dilutions were prepared. One mL of the required dilution (1/1000) was pipetted into each of six replicate plates. Potato dextrose agar (potato 200 g, dextrose 20 g, agar 20 g, distilled water 1 L) with streptomycin sulfate (300 µg/mL) was cooled to 45°C and poured into each Petri dish. The plates were incubated at room temperature in glass chambers under aseptic conditions for 4 days and then examined for fungal growth. All fungal colonies were recorded and the fungi were subcultured and identified. Frequency of occurrence and percentage contribution were calculated where frequency of occurrence refers to the number of samplings in which a fungus was recorded out of the total number of samplings made during the period of study. This was converted to a percentage and on this basis the fungi were classified as most common—81–100%; common—61–80%; frequent—41–60%; occasional—21–40%; rare—1–20%.

Results

Altogether 54 fungal species assignable to 40 genera were recorded and identified from dead and decaying litter of *Pavetta indica* (Table 1) following moist chamber incubation. Of these, 48 species belonging to 36 genera were from grade 1 litter and 43 species belonging to 33 genera were isolated from grade 2 litter.

Mycota from grade 1 litter

Only four species belonged to Ascomycotina and the remaining were anamorphic taxa. The number of species recorded per sampling varied from 9–21. The maximum number of species was recorded in the January 1999 sampling (Fig. 1). *Wiesneriomyces javanicus* (22.5%), *Zygosporium oscheoides* (19.6%) and *Circinotrichum falcatisporum* (23.3%) were 'most common' and *Meliola* sp., *Zygosporium masonii* were 'common'. Nine species, viz. *Beltraniella portoricensis*, *Botryodiplodia theobromae*, *Cladosporium cladosporioides*, *Cylindrocladium quinquiseptatum*, *Euantennaria* sp., *Selenosporella curvispora*, *Helicosporium helicosporum*, *Trichoderma viride* and *Verticillium* sp. were 'frequent'. Among the nine 'occasional' species, *Cylindrocladium parvum* and *Stachybotrys parvispora* showed relatively higher frequency and the remaining 26 species were 'rare'.

Taking the percentage occurrence as an index of the activity of the species recorded, *Wiesneriomyces javanicus* was highest in November 1999 and then gradually decreased (Fig. 2). A similar trend was recorded for *Zygosporium oscheoides* except the peak was recorded in December 1999. *Circinotrichum falcatisporum* appeared continuously except the last two samplings. *Meliola* sp. showed peak percentage occurrence during August 2000. *Beltraniella portoricensis* was recorded in only four of the twelve samplings and its percentage occurrence was relatively low. *Helicosporium helicosporum* reached a peak during November 1999 and thereafter declined and became absent until it reappeared during the final sampling (September 2000).

Mycota from grade 2 litter

Of the 43 species recorded, three belonged to Ascomycotina and the remaining were anamorphic taxa. The number of species in individual samplings varied from 7–19 with most species being recorded in January to March samplings, with maximum number recorded in January 2000 (Fig. 1). *Wiesneriomyces javanicus* (16.7%) was 'most common' while *Beltraniella portoricensis* (18.3%) and *Zygosporium oscheoides* (10.5%) were 'common'. Among the eleven species which were frequent, the isolation frequency of *Botryodiplodia theobromae* and *Circinotrichum falcatisporum* was relatively high. These were followed by *Circinotrichum maculiforme*, *Cylindrocladium quinquiseptatum*, *Euantennaria* sp., *Gyrothrix podosperma*, *Meliola* sp., *Selenosporella curvispora*, *Stachybotrys parvispora*, *Thysanophora asymmetrica* and *Zygosporium masonii*, in that order. Eight species were 'occasional', all with the same isolation frequency. The remaining 22 species were 'rare'. Of these, it was noteworthy that *Acremonium* sp., *Circinotrichum papakurae*,

Table 1. Average percentage occurrence and isolation frequency of species isolated from two grades of litter

Serial #	Species	Average % occurrence		Isolation frequency	
		Grade 1	Grade 2	Grade 1	Grade 2
	ASCOMYCOTINA				
1	*Chaetomium seminudam*	1.7	-	R	-
2	*C. spirale*	2	0.8	C	F
3	*Euantennaria* sp.	20.4	21.7	F	F
4	*Meliola* sp.	28.3	10.8	C	F
	MITOSPORIC FUNGI HYPHOMYCETES				
5	*Acremonium* sp.	-	1.3	-	R
6	*Alternaria alternata*	0.8	-	R	-
7	*Ardhachandra selenoides*	0.8	1.3	R	R
8	*Aspergillus flavus*	2.5	0.8	O	R
9	*A. japonicus*	1.3	1.7	R	O
10	*Beltrania rhombica*	2.9	2.1	R	R
11	*Beltraniella portoricensis*	7.9	18.3	F	C
12	*Beltraniella* sp.	1.3	0.4	R	R
13	*Circinotrichum falcatisporum*	23.3	18.3	MC	F
14	*C. fertile*	1.7	-	R	-
15	*C. maculiforme*	2.5	7.1	O	F
16	*C. papakurae*	1.7	0.8	R	R
17	*Cladosporium cladosporioides*	3.8	1.3	F	O
18	*C. oxysporum*	0.8	-	R	-
19	*Corynespora cassiicola*	1.3	0.4	R	R
20	*Curvularia brachyspora*	-	0.8	-	R
21	*C. intermedia*	-	0.8	-	R
22	*C. lunata*	5.4	0.8	O	R
23	*Cylindrocladium parvum*	3.3	0.8	O	R
24	*C. quinqueseptatum*	11.3	10	F	F
25	*Drechslera halodes*	0.4	-	R	-
26	*Fusarium lateritium*	0.4	-	R	-
27	*F. oxysporum*	0.4	-	R	-
28	*Gyrothrix circinata*	2.9	2.9	O	O
29	*G. podosperma*	2.5	7.5	O	F
30	*Helicosporium helicosporum*	10	2.5	F	O
31	*H. vegetum*	-	2.1	-	R
32	*Hermatomyces sphaericus*	1.3	1.7	R	R
33	*Idriella* sp.	3.3	0.8	R	R
34	*Leptoxyphium* sp.	0.8	0.4	R	R
35	*Penicillium* sp.	0.4	0.4	R	R
36	*Selenosporella curvispora*	12.9	11	F	F
37	*Sesquicillium setosum*	0.8	0.8	R	R
38	*Stachybotrys parvispora*	5	5.4	O	F
39	*Thysanophora assymetrica*	1.7	3.3	R	F
40	*Torula herbarum*	0.8	-	R	-

Table 1 (Continued). Average percentage occurrence and isolation frequency of species isolated from two grades of litter

Serial #	Species	Average % occurrence		Isolation frequency	
		Grade 1	Grade 2	Grade 1	Grade 2
41	*Tretopileus* sp.	0.4	-	R	-
42	*Trichoderma viride*	4.2	0.8	F	R
43	*Verticillium* sp.	5.8	2.1	F	O
44	*Volutella ciliata*	2.1	0.4	O	R
45	*Wiesneriomyces javanicus*	22.5	16.7	MC	MC
46	*Zygosporium echinosporum*	1.7	-	R	-
47	*Z. gibbum*	0.8	-	R	-
48	*Z. masonii*	11.7	9.6	C	F
49	*Z. oscheoides*	19.6	10.8	MC	C
	COELOMYCETES				
50	*Bartalinia robillardoides*	-	2.1	-	O
51	*Botryodiplodia theobromae*	7	5	F	F
52	*Colletotrichum falcatum*	1.7	4.2	R	R
53	*Harknessia* sp.	-	1.7	-	R
54	*Pestalotiopsis theae*	3	3	O	O

Note: Rare, O: Occasional, F: Frequent, C: Common, MC: Most Common

Corynespora cassiicola, *Curvularia brachyspora*, *C. intermedia*, *C. lunata*, *Cylindrocladium parvum*, *Idriella* sp., *Leptoxyphium* sp., *Penicillium* sp., *Sesquicillium setosum*, *Trichoderma* sp. and *Volutella ciliata* occurred in a single sampling. The percent occurrence of *Wiesneriomyces javanicus* was low until December 1999 and then increased reaching a peak in March 2000 followed by a sudden decrease (Fig. 3). Its occurrence was, however, relatively higher during June. *Beltraniella portoricensis* showed discontinuous occurrence. Its occurrence was relatively high in November 1999, April 2000 and July 2000 with a peak in July 2000. The occurrence of *Zygosporium oscheoides* was low in April 2000, increased in May and June 2000 and then decreased gradually. For *Circinotrichum falcatisporum* the peak was recorded in the November 1999 sampling. *Circinotrichum maculiforme* appeared only from January 2000. The peak activity of *Cylindrocladium quinqueseptatum* occurred during February 2000 but it was absent in the next five samplings and reappeared during August 2000. *Wiesneriomyces javanicus* occurred in all the 12 samplings from grade 1 litter and 11 samplings from grade 2 litter. A comparison of occurrence of some selected species on both grades of litter is shown in Fig. 4, where *Wiesneriomyces javanicus* shows higher percentage occurrence in both grades of litter when compared with the other taxa.

Mycoflora from the leaves and litter by washing technique

The fungi isolated from litter surfaces are listed along with their percent contribution and frequency occurrence in Table 2. A total of 38 species belonging to 22 genera were isolated out of which 25 species were from the phylloplane, 26 species from grade 1 and 21 species from grade 2 litter. *Aspergillus japonicus* was the 'most common' among phylloplane and litter fungi. *Aspergillus niger* was found as a 'common' species among the phylloplane fungi and as a 'most common' species on both the grades of litter.

Discussion

The litter samples were collected from two different layers of litter based on the stages of decomposition. The layer definitions are similar to those described for hardwood litter, where the litter layers were distinguished based on the decomposition (Watson et al. 1974). The

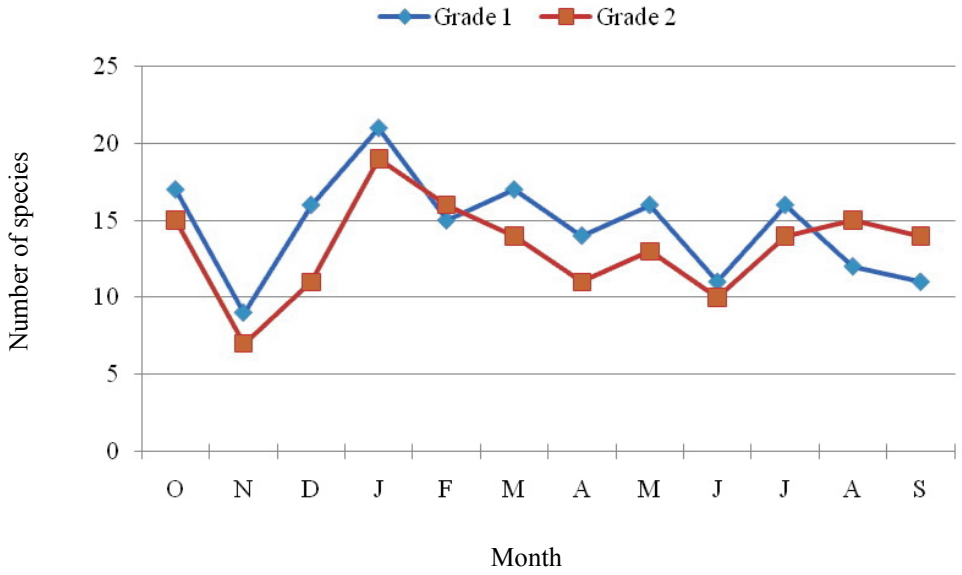

Fig. 1. Comparison of the number of species recorded in different samplings on two grades of litter

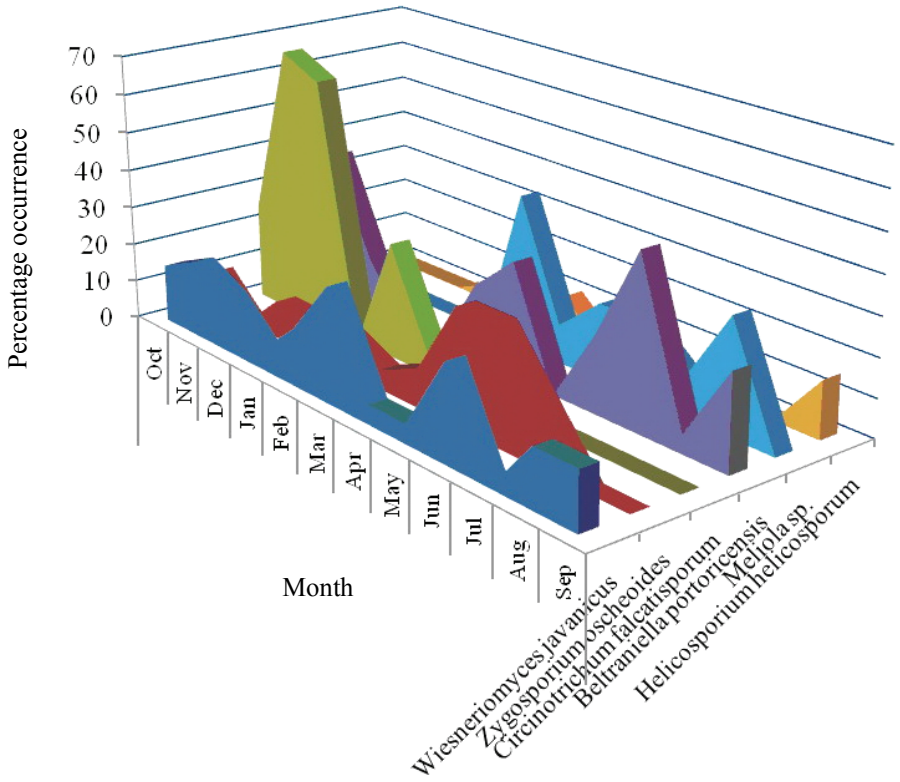

Fig. 2. Comparison of percentage occurrence of selected fungi on 'grade 1' litter layer over the sampling period

L layer consisted of recently fallen dry senescent leaves; F1 layer consisted of leaf litter under active decomposition; and in F2 layer, litter was in advanced stage of decomposition. These correspond to early, middle and advanced stages proposed by Heredia (1993). The grade 1 layer can be compared to that of the L layer and the grade 2 with that of the F1 layer. The objective was to know the nature of the mycota of the green foliage and in the two layers of litter in order to facilitate an understanding of the sequence of fungal colonization of the living leaves and dead leaves after they are shed. This approach is not unique to this study as in earlier studies litter was collected from different horizons

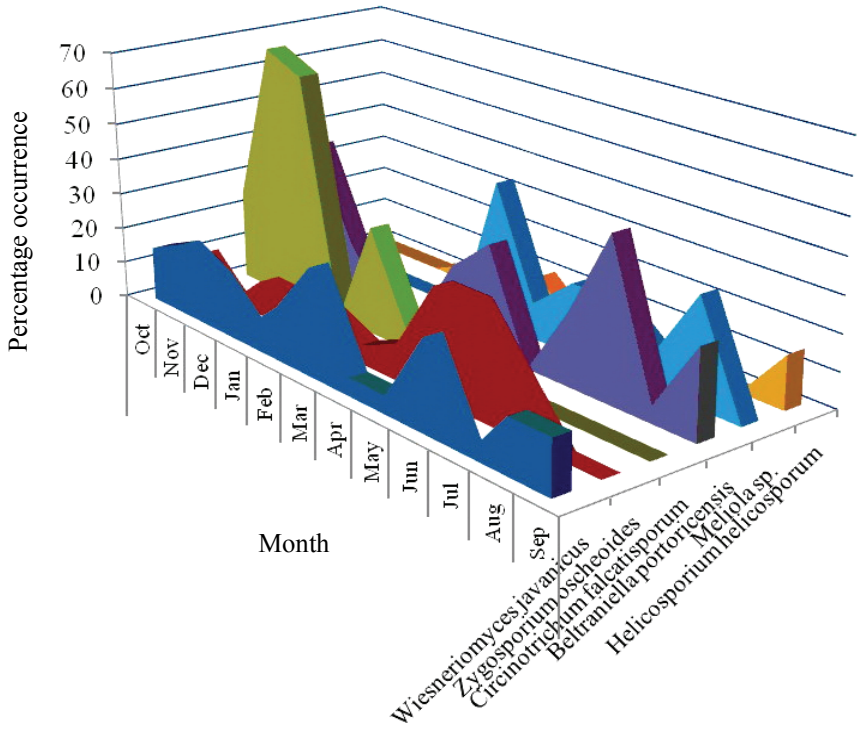

Fig. 3. Comparison of percentage occurrence of selected fungi on 'grade 2' litter layer over the sampling period.

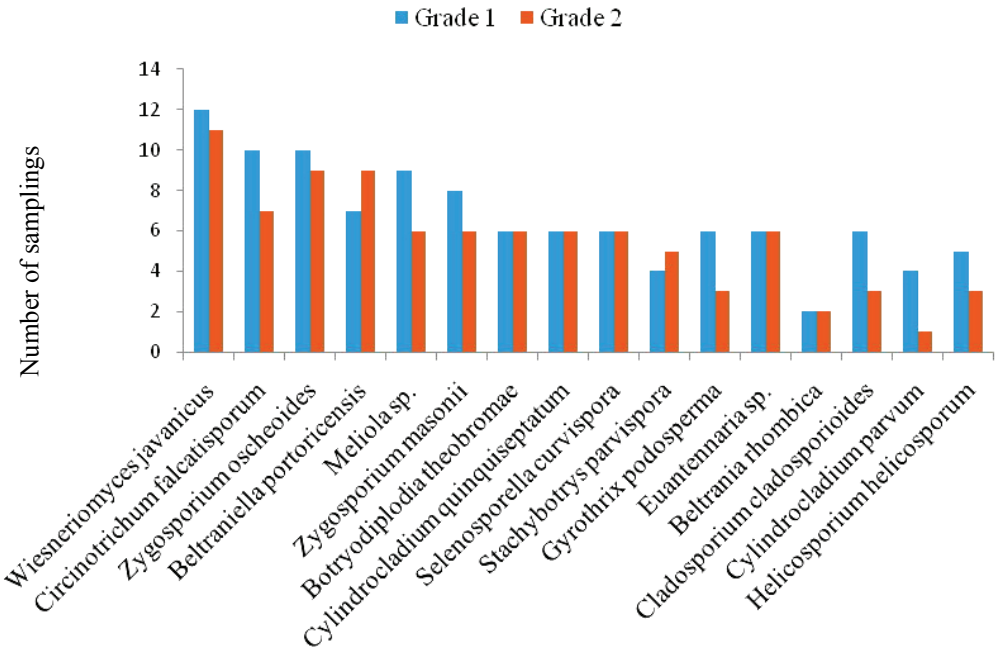

Fig. 4. Comparison of occurrence of selected species on 2 grades of litter.

several times in a single year (Watson et al. 1974, Visser & Parkinson 1975) and examined for fungal colonization. It was obvious that periodical samplings and analysis of the mycota are essential for obtaining a reliable picture of the spectrum of species colonizing the litter. In order to study the fungal colonization and succession, Shirouzu et al. (2009) collected decayed leaves of *Quercus myrsinaefolia*, a dominant evergreen oak in the northern broadleaf-evergreen forest in Japan at bi-monthly intervals. Kodseub et al. (2008) studied the fungal succession of the woody litter of *Magnolia liliifera* for 35 months at bi-monthly samplings. Promputtha et al. (2002) studied the fungal succession on senescent

Table 2. Average percentage contribution (average of 12 samplings) and frequency occurrence of species isolated by washing technique from phylloplane and two grades of litter.

Serial #	Species	Percent Contribution			Frequency occurrence		
		Phylloplane	Grade 1	Grade 2	Phylloplane	Grade 1	Grade 2
	ZYGOMYCOTINA						
1	*Absidia corymbifera*	1.4	-	2.46	O	-	O
2	*Mortierella ramanniana*	-	0.53	-	-	R	-
3	*Mucor racemosus*	0.6	0.25	0.38	O	R	R
4	*Rhizopus stolonifer*	1.9	0.3	1.19	O	R	R
5	*Syncephalastrum racemosum*	1.1	-	1.13	R	-	R
	MITOSPORIC FUNGI HYPHOMYCETES						
6	*Alternaria alternata*	0.9	-	-	O	-	-
7	*Aspergillus chevalieri*	-	-	0.15	-	-	R
8	*A. flavipes*	-	0.3	-	-	R	-
9	*A. flavus*	4.6	2.97	-	C	C	-
10	*A. fumigatus*	3.4	0.68	-	O	R	-
11	*A. japonicus*	17.8	19.45	20.62	MC	MC	MC
12	*A. nidulans*	0.1	0.39	0.24	R	R	R
13	*A. niger*	10.4	17.73	16.9	C	MC	MC
14	*A. ochraceus*	0.6	0.68	-	R	R	-
15	*A. tamarii*	-	-	0.75	-	-	R
16	*A. versicolor*	0.3	-	-	R	-	-
17	*Cladosporium cladosporioides*	0.9	-	-	R	-	-
18	*Curvularia brachyspora*	-	0.5	0.3	-	R	R
19	*C. lunata*	2.8	-	1.27	R	-	O
20	*C. ovoidea*	0.3	-	-	R	-	-
21	*C. pallescens*	0.2	1.2	1.9	R	O	O
22	*C. robusta*	0.6	-	-	R	-	-
23	*Drechslera australiensis*	-	0.14	0.23	-	R	R
24	*D. halodes*	1.2	1.28	1.1	O	R	R
25	*Fusarium oxysporum*	1.7	1.02	-	O	O	-

Table 2 (Continued). Average percentage contribution (average of 12 samplings) and frequency occurrence of species isolated by washing technique from phylloplane and two grades of litter.

Serial #	Species	Percent Contribution			Frequency occurrence		
		Phylloplane	Grade 1	Grade 2	Phylloplane	Grade 1	Grade 2
26	*Fusarium moniliforme*	-	-	0.3	-	-	R
27	*Humicola grisea*	-	0.3	-	-	R	-
28	*Monilia sitophila*	0.9	0.3	0.3	F	R	R
29	*Monodictys glauca*	-	0.1	0.3	-	R	R
30	*Phaeotrichoconis crotalariae*	0.6	-	-	R	-	-
31	*Penicillium citrinum*	10.2	8.9	7.6	C	C	C
32	*P. oxalicum*	2.8	1.2	-	R	F	-
33	*Scolecobasidium variabile*	0.2	3.02	-	R	O	-
34	*Trichoderma viride*	3.7	2.93	2.5	F	F	F
35	*Torula herbarum*	0.3	2.4	-	R	R	-
	COELOMYCETES						
36	*Bartalinia robillardoides*	1.7	2.45	3.47	R	R	R
37	*Pestalotiopsis theae*	6.4	-	1.36	F	-	O
38	*Robillardoides sessilis*	-	2.64	-	-	R	-
	Non-sporulating	22	1.26	-	-	-	-

R: Rare, O: Occasional, F: Frequent, C: Common, MC: Most Common
'-' indicates absence

leaves of *Mangletia garrettti* for 56 days. In the present study, monthly samplings were made for a year to analyze and study the fungal colonizers on the decomposing leaf litter of *Pavetta indica*.

Some interesting conclusions emerged from the results of this investigation. Altogether 54 species belonging to 40 genera were recorded. Of these, 48 species belonging to 36 genera were from grade 1 litter and 43 species belonging to 33 genera from grade 2 litter. The 54 species recorded included 4 ascomycetes, 5 coelomycetes and the rest were hyphomycetes. Among the 48 species appearing on grade 1 litter, 37 species continued to persist on grade 2 litter along with an additional 6 species (Table 3).

The fungal community composition was found to be distinct at each stage of succession by Promputtha et al. (2002). In the pioneer community stage, fungal communities were low in species number and in percentage occurrence. The dominant species at this stage was *Volutella* sp. The highest species diversity was present during the mature community stage. Certain species colonize only in a particular period of the year and if a sampling is not made at that time, there is a chance of missing these species altogether. For example, in the present study *Curvularia lunata* was recorded on grade 1 litter from October 1999 to December 1999. It is noteworthy that this species was recorded on grade 2 litter during May 2000. However, most of the important colonizers like *Wiesneriomyces javanicus*, *Circinotrichum falcatisporum* and *Zygosporium oscheoides* did not show such restricted seasonal occurrence.

In the present study a somewhat arbitrary classification of the various species of fungi had to be made on the basis of their isolation frequency. Yadav (1966) made a similar arbitrary classification, categorizing the fungi as 'rare', 'occasional', 'frequent', 'common' and 'most common'. Although several fungal species were common components of the mycota of both the litter grades, it was noticed that each grade had its own characteristic mycota with regard to percentage frequency. There were 37 species found to be present in both litter layers. The grade 1 and grade 2 litter had 11 and 6 species specific to them, respectively. Moreover, the isolation frequency of a particular taxon either remained the same or varied between the two grades of litter. For example, *Wiesneriomyces javanicus* was recorded as the 'most common' species in both grades of litter, whereas taxa like *Circinotrichum falcatisporum* and *Zygosporium oscheoides* were recorded as 'most common' species from grade 1 litter but their isolation frequency remained as 'common' and 'frequent' in grade 2 litter, respectively.

The data on the distribution of the mycoflora of *Pavetta indica* litter suggests that the first fungi to colonize the leaves soon after they fall are *Wiesneriomyces javanicus*, *Circinotrichum falcatisporum*, *Zygosporium oscheoides* (all considered as 'most common'), *Beltraniella portoricensis*, *Helicosporium helicosporum*, *Selenosporella curvispora*, *Cylindrocladium quinqueseptatum* (all 'frequent' species), *Gyrothrix podosperma*, *Volutella ciliata* (all 'occasional' species) and a few other 'rare' species. These may be considered the primary colonizers. In the course of further decomposition, some of the primary colonizers such as *Helicosporium helicosporum*, *Gyrothrix podosperma*, *Volutella ciliata* and *Cylindrocladium quinqueseptatum* disappeared, but the others continued to occur. At this stage they were joined by fungi such as *Meliola* sp. and *Euantennaria* sp. These may be considered as 'secondary' colonizers. Other species, which were recorded frequently, included *Cladosporium cladosporioides*, *Cylindrocladium parvum*, *Trichothecium roseum* and *Verticillium* sp. In the final stages of decomposition many of the primary and the secondary colonizers either disappeared or persisted, as evident from the mycota found on grade 2 litter. *Circinotrichum falcatisporum*, *Wiesneriomyces javanicus* and *Zygosporium masonii* persisted; *Acremonium* sp., *Bartalinia robillardoides*, *Curvularia brachyspora*, *C. intermedia* and *Helicosporium vegetum* appeared at this stage.

Colonization of phylloplane and different grades of litter

In addition to using the moist chamber incubation technique, leaf litter washing was also performed. The advantages in using more than one technique were highlighted by Lindsey & Pugh (1976) in their studies on

Table 3. Species appearing afresh in 'grade 1' layer, their persistence in 'grade 2' layer, newly appearing species in 'grade 2' layer

S.No.	Species appearing afresh in Grade 1 layer	Species in Grade 1 layer occurring in Grade 2 layer	Species of fungi appearing afresh in Grade 2 layer
1	*Chaetomium seminudam*	-	*Acremonium* sp.
2	*C. spirale*	+	*Curvularia brachyspora*
3	*Euantennaria* sp.	+	*C. intermedia*
4	*Meliola* sp.	+	*Helicosporium vegetum*
5	*Alternaria alternata*	-	*Bartalinia robillardoides*
6	*Ardhachandra selenoides*	+	*Harknessia* sp.
7	*Aspergillus flavus*	+	
8	*A. japonicus*	+	
9	*Beltrania rhombica*	+	
10	*Beltraniella portoricensis*	+	
11	*Beltraniella* sp.	+	
12	*Circinotrichum falcatisporum*	+	
13	*C. fertile*	-	
14	*C. maculiforme*	+	
15	*C. papakurae*	+	
16	*Cladosporium cladosporioides*	+	
17	*C. oxysporum*	-	
18	*Corynespora cassiicola*	+	
19	*C. lunata*	+	
20	*Cylindrocladium parvum*	+	
21	*C. quinqueseptatum*	+	
22	*Drechslera halodes*	-	
23	*Fusarium lateritium*	-	
24	*F. oxysporum*	-	
25	*Gyrothrix circinata*	+	
26	*G. podosperma*	+	
27	*Helicosporium helicosporum*	+	
28	*Hermatomyces sphaericus*	+	
29	*Idriella* sp.	+	
30	*Leptoxyphium* sp.	+	
31	*Penicillium* sp.	+	
32	*Selenosporella curvispora*	+	
33	*Sesquicillium setosum*	+	
34	*Stachybotrys parvispora*	+	
35	*Thysanophora assymetrica*	+	
36	*Torula herbarum*	-	
37	*Tretopileus* sp.	-	
38	*Trichoderma viride*	+	
39	*Verticillium* sp.	+	
40	*Volutella ciliata*	+	
41	*Wiesneriomyces javanicus*	+	
42	*Zygosporium echinosporum*	-	
43	*Z. gibbum*	-	
44	*Z. masonii*	+	
45	*Z. oscheoides*	+	

Table 3 (Continued). Species appearing afresh in 'grade 1' layer, their persistence in 'grade 2' layer, newly appearing species in 'grade 2' layer

S.No.	Species appearing afresh in Grade 1 layer	Species in Grade 1 layer occurring in Grade 2 layer	Species of fungi appearing afresh in Grade 2 layer
46	*Botryodiplodia theobromae*	+	
47	*Colletotrichum falcatum*	+	
48	*Pestalotiopsis theae*	+	

'+' indicates the presence of the particular taxon; '-' indicates the absence of the particular taxon

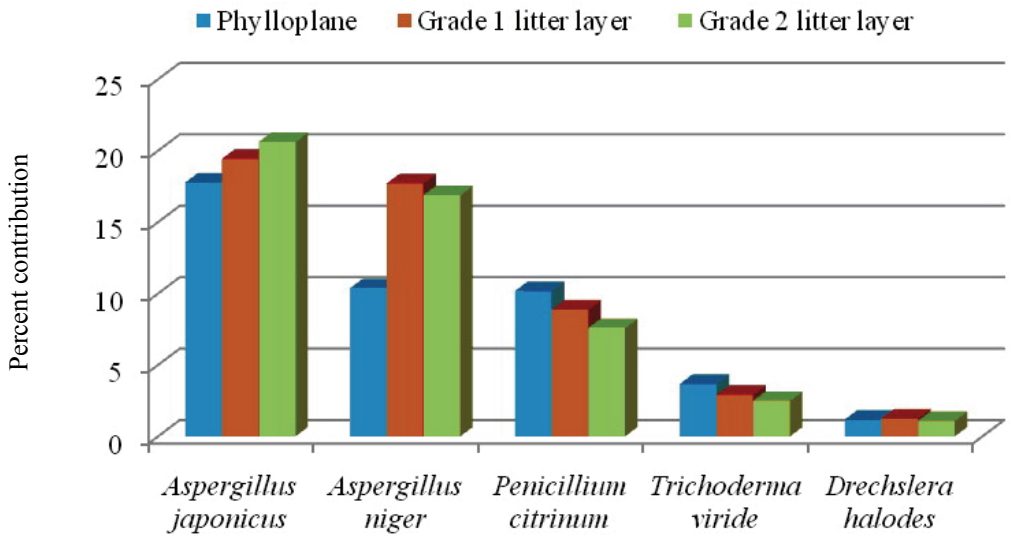

Fig. **5**. Comparison of the percent contribution of selected fungi isolated from green leaves and litter layers (grade 1 and 2)

microfungi on attached leaves of *Hippophae rhamnoides*. For instance, *Aureobasidium pullulans* was recorded mainly from washed leaf disks and only rarely from leaves in moist chambers. The presence of several other fungi was revealed by only a limited range of techniques, e.g. *Phoma* sp. was mainly found by spore fall, and *Penicillium* spp. mainly in damp chambers. Lindsey & Pugh (1976) emphasized that a failure to use these techniques would have resulted in the apparent absence of these fungi from the leaf surface. The method used for assessing the phylloplane mycota of green as well as litter leaves in the present study was also a washing technique, which has been used by several earlier workers (Dickinson 1965, 1967, Hering 1965, Hogg & Hudson 1966, Tokumasu 1980, Shirouzu et al. 2009). The reason for using these techniques was to establish if any fungi that were missed by the direct observation would be found.

Many species colonized the phylloplane of *P. indica* and several of these were also found on litter. About 62% of the phylloplane fungi were found on litter and therefore it appears that the phylloplane is an ideal substrate for colonization. The percent contribution of individual taxa differs among the phylloplane and the litter layers (Fig 5). The phylloplane mycota comprises certain casual and residential fungi that may be present as propagules incapable of further activity and certain other species which seem to have the potential for further development, growth and even sporulation, on or within the leaves, even after they are shed and in various stages of decomposition. By washing technique 29 species were recorded from the fresh leaves, of which 18 species were carried over to the grade 1 litter and 12 species thereafter to grade 2 litter. In the grade 1 litter, seven species newly appeared and two of these carried over to the

grade 2 litter. Three species appeared for the first time in the grade 2 litter.

Thus, it is clear that in different grades of litter shifts in activity of the various species of the mycota occurred indicating peaks and troughs of activity for some of the species. As assessment of such activity is based on percentage occurrence of these fungi in different grades of litter, computed on the basis of sporulating colonies on the litter, and not on dilution plate counts, the data so obtained may be considered sufficiently reliable. It is obvious that the fungi colonizing the phylloplane or litter must be already present in that area. The phylloplane serves as a settling area for propagules of numerous fungi, several of which are components of the air spora. The host leaf allows the development of only a few species and inhibits others. Those fungi which are able to establish on living leaves are foliicolous. These can, in turn, be classified in to: (i) those whose activity is confined to living leaves and (ii) those that continue to be active after colonizing a living leaf even after it is shed. The true litter fungi are perhaps those that colonize the leaves after they are shed and show activity for varying periods.

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