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Rhizoplane mycoflora of some species of Myristicaceae of the Western Ghats, India

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Abstract: Rhizoplane mycoflora of *Gymnacranthera farquhariana, Knema attenuata, Myristica dactyloides, M. fatua* var. *magnifica* and *M. malabarica* was studied by root washing and root plating methods. The root samples along with adhering soil were collected from the rhizosphere regions of each tree species in three seasons – summer, monsoon and post-monsoon - over a period of two years from the forests of Gersoppa, Uttara Kannada district of Karnataka. Soil physico-chemical properties, including moisture, temperature, pH, electrical conductivity, and organic matter and total N, P, K, Ca, Cu, Fe, Mn, Mg, Na and Zn content, were also analyzed. A total of 37 species of fungi were isolated from the rhizoplane region of five tree species. Sørensen's index showed the similarity between the mycoflora of rhizoplane regions to range from 34.6 to 54.6 %. Among the five tree species, a maximum of 24 fungal species were isolated from the rhizoplane of *K. attenuata* and *M. fatua* var. *magnifica*, followed by 22 species in *M. dactyloides* and *M. malabarica*, and 20 species in *G. farquhariana.* Nine species were common to the rhizoplane of all the five tree species and nine species were observed in the rhizoplane of only one of the five tree species. The maximum number of fungal species was recorded during monsoon in all plant species and the minimum during post-monsoon and summer. The physico-chemical properties of the rhizosphere soil differed among plant species in different seasons. The dominant genera in terms of number of fungal species were *Penicillium* and *Aspergillus*. The rhizoplane fungal population for each plant species in different seasons was correlated with physico-chemical properties of respective seasons, and found that it was significant in some of the seasons or for some parameters only.

Resumen: Se estudió la micoflora de la rizosfera de *Gymnacranthera farquhariana, Knema attenuata, Myristica dactyloides, M. fatua var. magnifica* y *M. malabarica* por medio de los métodos de lavado y montado de raíces. Se recolectaron las muestras de raíces, junto con el suelo adherido a ellas, de la región de la rizosfera de cada especie arbórea en tres estaciones – verano, mozón y post-monzón – durante un periodo de dos años, de los bosques de Gersoppa, distrito Uttara Kannada, Karnataka. También fueron analizadas las propiedades fisico-químicas del suelo, incluyendo humedad, temperatura, pH, conductividad eléctrica, así como materia orgánica y contenidos totales de N, P, K, Ca, Cu, Fe, Mn, Mg, Na y Zn. En total fueron aisladas 37 especies de hongos de la región de la rizosfera de las cinco especies arbóreas. El índice de Sørensen mostró que la similitud entre la micoflora de las regiones rizosféricas varió de 34.6 de 54.6 %. Entre las cinco especies arbóreas el número máximo de especies de hongos asiladas fue de 24 en la rizosfera de *K. attenuata* y *M. fatua* var. *magnifica*, seguido por 22 especies en *M. dactyloides* y *M. malabarica*, y 20 especies en *G. farquhariana.* Nueve especies fueron comunes a la rizosfera de las cinco especies arbóreas, y nueve especies fueron observadas en la rizosfera de sólo una de ellas. En todas las especies de plantas se registraron más especies de especies de hongos durante el monzón, y menos durante el post-monzón y el verano. Las

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propiedades fisico-químicas del suelo de la rizosfera difirieron entre especies de plantas en las diferentes estaciones. Los géneros de hongos dominantes por número de especies fueron *Penicillium* y *Aspergillus*. La población fúngica de la rizosfera para cada especie de planta en diferentes estaciones estuvo correlacionada con las propiedades fisico-químicas de las estaciones respectivas, aunque ésta fue significativa sólo en algunas estaciones o para algunos parámetros.

Resumo: A micoflora radicular da *Gymnacranthera farquhariana, Knema attenuata, Myristica dactyloides, M. fatua var.* magnifica e *M. malabarica* foi estudada mediante lavagem das raízes e métodos de cobertura das raízes. As amostras de raízes, juntamente com o solo aderente, foram recolhidas das regiões da rizosfera de cada espécie arbórea em três estações – verão, monção e pós-monção – durante um período de dois anos nas florestas de Gersoppa e Uttara Kannada no distrito de Karnataka. As propriedades físico-químicas do solo, incluindo a humidade, temperatura, pH, condutividade eléctrica, matéria orgânica e os teores totais de N, P, K, Ca, Cu, Fe, Mn, Mg, Na e Zn foram também analisados. Da região da rizosfera das cinco espécies arbóreas foi isolado um total de 37 espécies de fungos. O índice de Sørensen mostrou que a semelhança da micoflora dos planos da rizosfera oscilava entre os 34,6 e os 54,6 %. Entre as cinco espécies arbóreas, isolou-se um máximo de 24 espécies de fungos do plano da rizosfera da *K. attenuata* e *M. fatua var. Magnific*, seguida por 22 espécies na *M. dactyloides* e *M. malabarica*, e de 20 espécies na *G. farquhariana*. Nove espécies eram comuns no plano da rizosfera de todas a cinco espécies arbóreas sendo nove espécies só observadas no plano da rizosfera de uma única espécie arbórea do conjunto das cinco estudadas. O número máximo de espécies fúngicas foi registado durante a monção em todas as espécies e o mínimo durante a pós-monção e verão. As propriedades físico-químicas do solo da rizosfera diferiam entre as espécies nas diferentes estações. O género dominante em termos do número de espécies fúngicas eram o *Penicillium* e o *Aspergillus*. A população fúngica da rizosfera para cada espécie estudada estava correlacionada com as propriedades físico-químicas das respectivas estações. Encontrou-se, também, que era significativa em algumas das estações ou só para alguns dos parâmetros.

Key words: Dominant fungi, rhizoplane mycoflora, Western Ghats.

Introduction

The Western Ghats (8° 20' - 20° 40' N and 73°-77° E) extend from Tapti in Gujarat to Kanniyakumari in Tamil Nadu, traverse through Maharashtra, Goa, Karnataka and Kerala along the west coast and form a practically unbroken relief for about 1600 km, with the exception of the Palghat Gap. They are a magnificent stretch of hill ranges presenting a rich and varied flora and fauna. Different types of vegetation occur here, including scrub jungle, grasslands at lower altitudes, moist and dry deciduous forests, tropical evergreen forests and montane grasslands and sholas. About 4500 species, of the estimated 17000 species of flowering plants found in India, are found in the Western Ghats (Nayar 1996). It is one of the 25 biodiversity 'Hotspots' identified in the world (Myeres *et al.* 2000). A large proportion of the plants found here - 54 genera, and 1720 species and 135 infraspecific taxa - are endemic. Nearly a third of the endemic plant taxa found here are rare or threatened, and several are believed to be extinct or at serious risk of becoming extinct (Shetty & Kaveriappa 1991). The region is also known for its fungal diversity. Although about 13000 species of fungi have been recorded from the Western Ghats (Bhat 1994).

Myristicaceae are one of the important families of flowering plants, with some species like *Myristica fragrans* Houtt. and *M. malabarica* Lam. having medicinal value (Hussain *et al.* 1992). The medicinal value of the other species is yet to be assessed. We studied five species of Myristicaceae belonging to three genera in the Western Ghats region of Karnataka: *Gymnacranthera farquhariana* (Hook. f. & Thomson) Warb., *Knema attenuata* (Wallich ex Hook. f. & Thomson) Warb.,

Myristica dactyloides Gaertner, *M. fatua* Houtt. var. *magnifica* (Beddome) Sinclair and *M. malabarica* Lam. All five tree species are found in Gersoppa, about 45 km from Honnavar (North West), Uttara Kannada district, Karnataka. One of the rarest forest types of the region, *Myristica* swamp, is found here. Most of the fresh water swamps in the Western Ghats region have been converted into paddy fields and arecanut gardens, and some taxa like *M. fatua* var. *magnifica* found only in these habitats have become rare and threatened (Rama Bhat & Kaveriappa 2009a & b). Of the five tree species chosen for the study, four species - *Gymnacranthera farquhariana, Knema attenuata*, *Myristica fatua* var. *magnifica* and *M. malabarica* - are endemic to the Western Ghats. Of these, the two taxa of *Myristica* are reported to be rare and threatened (Ahmedullah & Nayar 1986).

Microorganisms play a vital role in plant ecosystems. While some soil organisms are detrimental to the growth and development of plants, some others are beneficial. Fungi are prominent among such microorganisms. They exist in the rhizosphere region or in the rhizoplane, and others occur as endophytes. The term 'rhizosphere' refers to the narrow zone of soil which is subjected to the influence of the living root system. The actual surface of the plant root, together with any closely adhering particles of soil or debris, is distinguished as the rhizoplane region (Curl & Truelove 1986).

Root exudates can promote or inhibit microbial activity within the rhizoplane. The rhizoplane provides a highly favourable nutrient base for many species of actinomycetes, bacteria, and fungi. The growth and reproduction of root pathogens may be affected directly by root exudates of the host or indirectly by other microflora. Thus, the rhizosphere-rhizoplane zone is an environment created by the interactions between chemical substances released into the soil by living roots and microorganisms that can utilize these substances as nutrient sources or be inhibited by them (Curl 1982; Rengel & Marchner 2005).

The present investigation was undertaken to assess the fungal diversity of rhizoplane regions of five tree species, seasonal variations in the occurrence of rhizoplane mycoflora, and the physico-chemical properties of rhizosphere soil in relation to the fungal population.

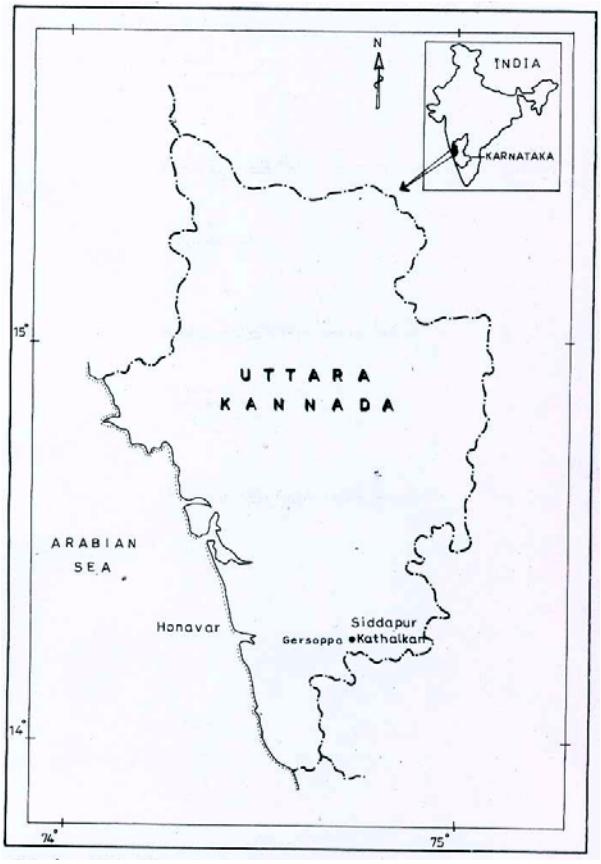

Fig. 1. Map showing the study location.

Materials and methods

Plants selected for the study

The following five tree species belonging to the family Myristicaceae were chosen for the study: *Gymnacranthera farquhariana* (Hook. f. & Thomson) Warb., *Knema attenuata* (Wallich ex Hook. f. & Thomson) Warb., *Myristica dactyloides* Gaertner, *Myristica fatua* Houtt. var. *magnifica* (Beddome) Sinclair and *Myristica malabarica* Lam.

Study area

The study was conducted in 1994 and 1995 in Kathalkan (14° 15' 40" to 14° 15' 50" N latitude and 74° 45' 25" to 74° 45' 35" E longitude) at an elevation of 500 - 530 m in Gersoppa, about 45 km from Honnavar, in Uttara Kannada district of Karnataka state (Fig. 1). There is an evergreen forest

of about 2 ha in this area, with a *Myristica* swamp of 0.5 ha (North East). Of the five species studied, two (*G. farquhariana* and *M. fatua* var. *magnifica*) are found in the swamp, with the remaining three species distributed in the rest of the forest.

The maximum rainfall occurred between the end of May and the end of August and the total rainfall during 1994 and 1995 were 3679 mm and 3731 mm respectively. The monthly maximum temperature varied from 28.2 to 33.5 °C and the monthly minimum from 20.4 to 26.2 °C. The temperature increased steadily by the end of February and reached the peak during April-May, and began to decline by the end of May due to pre-monsoon showers. The temperature again increased during August-September and reached its peak in December. The monthly maximum temperature of 33.5 °C, the hottest during the study period, was recorded in December, even though April/May was the hottest period observed in prior years (Karnataka Meteorological Survey, Bangalore). The monthly minimum of 20.4 °C was recorded in February, the lowest during the study period.

Collection of soil and root samples

Soil and root samples were collected from the study area in the months of March, July and November over a period of two years during 1994 - 1996 to represent the three seasons namely, summer (February - May), monsoon (June-September) and post-monsoon (October-January), respectively. During each trip rhizosphere soil, non-rhizosphere soil and feeder roots of the adult trees were collected. About 500 g of rhizosphere soil was collected in sterile polythene bags at a point of 1 - 2 m from the main stem of each tree at 10 - 15 cm depth. The samples collected from ten different plants of each species were mixed to form a composite sample of about 5 kg. Soil samples collected from ten open places 20 m away from the tree species in the study area, at a depth of 10 - 15 cm constituted the non-rhizosphere soil. They were also mixed to form a composite sample. The composite soil samples were air-dried in the laboratory and stored at 4 °C for further experiments. The feeder roots of each plant species were collected by retaining soil particles adhering to them and used for studying the rhizoplane mycoflora.

Soil analysis

The soil moisture content was determined gravimetrically by drying soil at 110 °C in an oven for 10 - 12 h.

Soil pH and electrical conductivity were determined using a soil suspension in water (1:10, w/v) kept overnight at room temperature to allow the solids to settle down. The pH and conductivity of the soil suspension were measured using a pH meter and conductivity meter (Systronics, Ahmedabad).

The soil temperature was measured at the spot using a digital soil thermometer.

To determine soil nitrogen content one gram of soil was mixed with 5 ml of conc. H_2SO_4, one gram of catalyst mixture (Se, $CuSO_4$ and K_2SO_4 in a 1:1:20 ratio) was added, and the mixture digested at 80 - 90 °C till the solution became colorless. The nitrogen content of the sample was determined using the Kjeldahl method (Sadasivam & Manickam 2008).

To determine the concentration of Na, K, P, Mg, Mn, Fe, Zn, Ca and Cu in the soil, one gram of soil was digested at 80 - 90 °C in 8 ml of a mixture of conc. HNO_3 acid and perchloric acid ($HClO_4$) (3:1, v/v) until the solution became colorless. The digested sample was diluted using double-distilled water before analysis. The concentration of Na and K was determined using a flame photometer (Elico, Hyderabad), while the concentration of P was estimated colorimetrically using a Shimadzu UV-160A Spectrophotometer. An atomic absorption spectrometer was used to determine the concentration of the remaining elements. The organic matter (OM) content of soil was estimated by the method of Walkley & Black (1934).

Isolation and enumeration of rhizoplane mycoflora

Two methods were used for isolation of rhizoplane mycoflora. For the root plating method (Harley & Waid 1955), the soil adhering to the root samples of each species was dislodged by shaking the roots and the roots were cut into 10 mm segments. One hundred segments were randomly picked, weighed and washed in sterile distilled water by changing the water 10 - 15 times, and the roots were plated on media containing potato dextrose agar (PDA) and Czapek-Dox agar (CDA). For the root washing method (Louw & Webley 1959), after removal of adhering soil, the roots were immersed in pre-weighed flasks of sterile water plus glass beads. The flasks were re-weighed, shaken for 10 minutes and the supernatant solution

Table 1. Analysis of rhizosphere soil (RS) of five tree species at Kathalkan in Gersoppa, Karnataka.

Plant species	Month	MC (%)	Temp (°C)	pH	EC (mmhos)	OM (%)	N(%)	P(%)	K(%)	Na(%)	Ca(%)	Cu(%)	Fe(%)	Mg(%)	Mn(%)	Zn(%)
	March	24.62	27.1	6.15	3	3.9	1.21	0.57	0.061	0.3	0.24	0.002	0.72	0.171	0.003	0.003
Gf	July	41.56	23.4	5.8	5	3.93	0.35	1.37	0.028	0.23	0.073	0.003	0.97	0.065	0.006	0.003
	November	35.89	23.1	5.86	2	4.1	0.63	0.56	0.054	0.3	0.082	0.003	1.44	0.035	0.035	0.004
	March	12.79	27.9	6.37	2	3.93	1.09	0.68	0.102	0.39	0.133	0.003	1.18	0.187	0.011	0.006
Ka	July	36.67	22.6	5.42	7	3.97	0.35	0.88	0.139	0.3	0.161	0.002	1.04	0.137	0.021	0.004
	November	24.42	22.4	5.56	5	4.28	0.58	0.56	0.123	0.3	0.013	0.001	3.63	0.195	0.029	0.005
	March	13.38	27.9	6.08	2	4	1.09	0.53	0.098	0.3	0.115	0.001	0.9	0.189	0.025	0.005
Md	July	36.19	23.6	5.33	5	4.1	0.42	0.66	0.069	0.21	0.06	0.001	0.94	0.126	0.027	0.004
	November	31.84	23.4	5.39	3	4.48	0.7	0.94	0.195	0.27	0.059	0.001	0.88	0.135	0.023	0.004
	March	24.01	25.4	6.01	4	3.79	0.72	0.52	0.065	0.43	0.062	0.003	1.4	0.198	0.021	0.006
Mf	July	42.27	22.2	5.73	6	4.07	0.42	1.87	0.054	0.37	0.054	0.003	1.63	0.128	0.011	0.005
	November	38.95	21.5	5.75	2	3.86	0.7	1.4	0.072	0.27	0.068	0.003	1.26	0.179	0.016	0.006
	March	14.38	26.9	6.18	3	4	0.75	0.54	0.095	0.36	0.201	0.004	1.06	0.071	0.018	0.004
Mm	July	33.66	23.9	5.67	4	4.07	0.63	0.83	0.142	0.47	0.106	0.007	3.83	0.131	0.029	0.007
	November	29.3	23.5	5.87	5	3.79	0.79	0.52	0.148	0.53	0.175	0.007	2.91	0.153	0.051	0.005
	March	12.07	28.9	5.84	4	1.47	0.49	0.34	0.156	0.3	0.081	0.005	0.68	0.128	0.037	0.004
NRS	July	23.9	25.6	5.62	5	1.72	0.46	0.43	0.089	0.4	0.054	0.002	1.25	0.058	0.013	0.003
	November	22.72	24.2	5.73	3	2.24	0.58	0.6	0.19	0.23	0.054	0.004	0.94	0.052	0.01	0.006

Gf- *Gymnacranthera farquhariana*; Ka - *Knema attenuata*; Md - *Myristica dactyloides*; Mf - *M. fatua* var. *magnifica*; Mm - *M. malabarica*; NRS- Non-rhizosphere soil 20 m away from rhizosphere region of plant species.

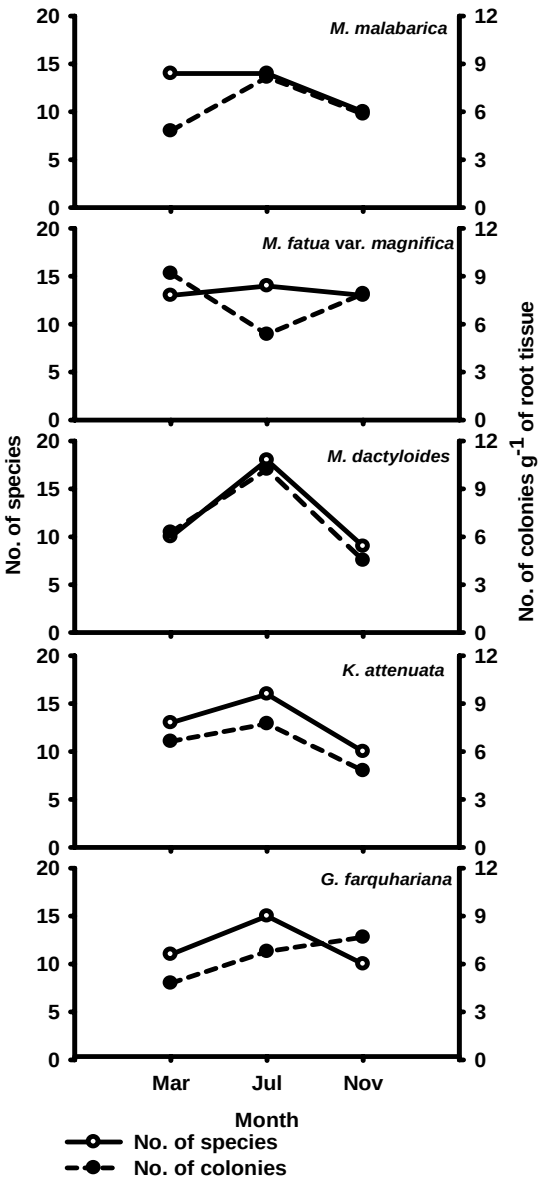

Fig. 2. Variation in rhizoplane fungal species and population in the three months of samplings representing summer, monsoon and post-monsoon seasons respectively in the five tree species (Data are average of two years).

serially diluted. Ten replicates of one ml of diluted suspension were spread over the PDA and CDA medium. The Petri dishes were incubated at 25±3 °C up to seven days. The colonies were identified using standard manuals, monographs and journals (Barnet, Ellis, C.V. Subramanian) and counted and the result was expressed as number of colonies per g of root tissue. During identification, each of the fungal isolates were subcultured and maintained in a slant and deposited in the Mangalore University fungal collections. For the authentic confirmation of some fungi, experts from Microbiology Dept. of CPCRI, Kasaragod; Dept. of Botany, Goa University and Microbiology Department of Mangalore University have helped.

The percent frequency (PF) and percent abundance (PA) of each species were calculated from the data obtained for rhizosphere and rhizoplane mycoflora as follows:

$$PF = \frac{\text{No. of replicates in which a fungal species was observed}}{\text{Total number of replicates}} \times 100$$

$$PA = \frac{\text{Total no. of colonies of each species in all replicates}}{\text{Total no. of colonies of all the species}} \times 100$$

The similarity between the rhizosphere mycoflora of each plant species was compared with other plant species taken up for study by calculating a similarity index (SI; Sørensen 1948) as follows:

$$SI = \frac{2C}{S_1 + S_2} \times 100$$

where,

C = Number of common fungal species in plant species 1 and 2

S_1, S_2 = Total number of fungal species in plant species 1 and 2

Results and discussion

The soil analysis showed that the physico-chemical properties of non-rhizosphere and rhizosphere soil were varied in different seasons in different plant species (Table 1). The soil moisture content, pH and electrical conductivity of both non-rhizosphere and rhizosphere soils were maximum during monsoon and minimum in summer seasons. The soil temperature was maximum during summer and minimum in post-monsoon season in both the soils. The organic matter content was maximum in post-monsoon, nitrogen content in summer, phosphorus in monsoon in both the rhizosphere and non-rhizopshere soils. The amount of other elements varied without specificity to the seasons.

The variation in fungal species and population in the rhizoplane of five tree species is given in

Table 2. The seasonal variation in the occurrence of rhizoplane mycoflora of five tree species.

Fungi	Gf			Ka			Md			Mf			Mm		
	Mar	July	Nov	Mar	July	Nov	Mar	July	Nov	Mar	July	Nov	Mar	July	Nov
Acremonium rutilum Gams.					+										
Alternaria alternata (Fr.) Keissler.		+			+	+		+	+		+				
Aspergillus flavus Link					+			+			+	+			
A. niger van Tieghem	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A. ochraceus Wilhelm						+									
A. sydowii (Bain. & Sart.) Thom & Church		+		+	+	+		+			+				
Cephalosporium acremonium Corda					+					+	+				
Chaetomium globosum Kunze											+				
Cladosporium oxysporum Berk. & Curt.				+				+	+			+			+
Cunnighamella echinulata Thaxt.	+	+		+	+	+	+	+	+	+	+	+	+	+	
Cylindrocarpon tenue Bugnicourt		+				+	+	+	+	+					
Emericella variecolor Berk. & Br.		+	+	+								+			
Epicoccum nigrum Link															+
Fusarium moniliforme Scheld.										+	+				
F. oxysporum Scheld.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F. roseum Link		+	+		+		+	+	+			+	+	+	+
F. solani (Mart.) Sacc.					+										
Gliocladium roseum (Link & Fr.) Bain.							+					+		+	
Gliomastix inflata Dickinson		+			+		+							+	
Mucor hiemalis Wehmer.	+			+				+		+	+		+		
Nectria rolfsii Berk. & Br.	+			+				+		+		+	+		+
Paecilomyces varioti Bain.					+			+			+		+	+	
Penicillium chrysogenum Thom	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. puberulum Bainer	+	+	+	+	+	+		+	+						+
P. restrictum Gilman & Abbott			+	+							+		+	+	
P. thomii Maire	+	+			+				+	+			+	+	
Phoma arcuata (Cke) Sacc.	+		+											+	
Phytophthora parasitica Buttler															+
Rhizopus nigricans Ehrenb.				+				+					+		
Sclerotium rolfsii Sacc.	+	+													
Stachybotrys parvispora Hughes			+										+		
Thielaviopsis state of *Ceratocystis paradoxa* (Dade) C. Maureau		+							+						
Trichoderma harzianum Rifai									+		+				
T. viride Pers. ex Fr.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ulocladium atrum Preuss							+			+					
Wiesneriomyces laurinus (Tassi) P.M. Kirk										+					
Zygosprium oscheoides Mont.														+	

Gf - *Gymnacranthera farquhariana;* Ka - *Knema attenuata;* Md - *Myristica dactyloides;* Mf - *M. fatua* var. *magnifica;* Mm - *M. malabarica.*

Table 2. The population of rhizoplane fungi was maximum in July in *Knema attenuata*, *Myristica dactyloides* and *M. malabarica*, whereas in the case of *M. fatua* var. *magnifica* it was maximum in March, and for *Gymnacranthera farquhariana* it was in November (Fig. 2). The minimum population of fungi was recorded in March in *G. faquhariana* and *M. malabarica*, in November in *K. attenuata* and *M. dactyloides*, whereas for *M. fatua* var. *magnifica* the minimum population was recorded in July.

Table 3. Pearson's correlation coefficient (r) between rhizoplane fungal population and soil properties of *Gymnacranthera farquhariana.*

Parameters	March	July	November
MC	-0.282	0.386	0.319
Temperature	0.087	-0.408	0.402
pH	0.188	0.799***	0.322
EC	0.236	-0.058	-0.413
OM	0.672**	0.286	-0.305
N	-0.892***	0.470	0.644**
P	0.747***	0.464	-0.363
K	0.236	-0.458	-0.094
Na	0.705**	0.765***	-0.888***
Ca	-0.404	0.489	0.170
Cu	0.698**	0.378	0.606**
Fe	-0.064	0.254	-0.686**
Mg	0.622**	0.073	0.494
Mn	0.499	-0.434	-0.876***
Zn	-0.724	-0.411	0.779***

*,**,***- Significant at $P < 0.05$, 0.01 and 0.001, respectively.

Gymnacranthera farquhariana: Of the 20 species of fungi isolated from the rhizoplane of *G. farquhariana*, six that were isolated by root washing method were not found using the root plating method. Likewise, four species isolated by root plating method were not found using the root washing method. The order of the more common species of fungi, based on their percent frequency and percent abundance, was as follows:

Percent frequency:

March - *T. viride* > *F. oxysporum* > *Sclerotium rolfsii*

July - *T. viride* > *Cunninghamella echinulata* and *P. chrysogenum*

November - *T. viride* > *P. chrysogenum* > *F. roseum*

Percent abundance:

March - *T. viride* > *F. oxysporum* > *C. echinulata*, *Nectria rolfsii, Phoma arcuta* and *S. rolfsii*

July - *T. viride* > *F. oxysporum* > *C. echinulata*

November - *T. viride* > *F. oxysporum* > *P. chrysogenum*

When the variations in the fungal population in different months were correlated with soil properties, it was observed that OM content, N, P, Na, Cu and Mg were significant at $P < 0.01$ and 0.001 in March; pH and Na content were significant $P <$ at 0.001 in the July month; N, Na, Cu, Fe, Mn and Zn contents were significant at $P <$ 0.01 and 0.001 in the month of November samplings (Table 3).

Knema attenuata: Of the 24 species of fungi isolated from the rhizoplane of *K. attenuata*, seven species isolated by root washing method were not observed using the root plating method. Likewise, five species isolated by root plating method were not found using the root washing method. The order of the more common species of fungi, based on their percent frequency and percent abundance, was as follows:

Percent frequency:

March - *T. viride* > *C. echinulata* > *Emericella variecolor* and *P. chrysogenum*

July - *T. viride* > *C. echinulata*, *F. roseum*, *P. chrysogenum* and *P. thomii*

November - *T. viride* > *F. oxysporum* > *A. niger*

Percent abundance:

March - *T. viride* > *C. echinulata* > *N. rolfsii*

July - *T. viride* > *P. chrysogenum* > *P. thomii*

November - *T. viride* > *F. oxysporum* > *P. chrysogenum*

When the variations in the fungal population in different months were correlated with soil properties, it was observed that the soil Cu, Mn and Zn content were significant at $P < 0.001$ and 0.05 in all the three sampling months, while the remaining soil properties were significant in the sampling of some months only (Table 4).

Myristica dactyloides: A total of 22 species of fungi were isolated from the rhizoplane of *M. dactyloides*, of which nine species isolated by root washing method were not observed using the root plating method. Likewise, one species isolated by root plating method was not found using the root washing method. The order of the more common

Table 4. Pearson's correlation coefficient (r) between rhizoplane fungal population and soil properties of *Knema attenuata.*

Parameters	March	July	November
MC	-0.094	0.179	0.127
Temperature	0.407	0.075	0.360
pH	0.294	0.717**	0.298
EC	0.143	0.375	-0.502*
OM	0.609**	-0.809**	0.380
N	-0.711**	0.661**	0.794***
P	0.601*	0.612**	0.830***
K	0.851***	0.380	0.452
Na	-0.265	0.404	0.817***
Ca	-0.403	0.726***	0.435
Cu	-0.541*	0.506*	-0.890***
Fe	-0.200	0.373	-0.126
Mg	0.294	0.251	-0.320
Mn	-0.737***	0.785***	0.752***
Zn	0.505*	0.836***	0.815***

*,**,***- Significant at $P < 0.05$, 0.01 and 0.001, respectively.

species of fungi, based on their percent frequency and percent abundance, was as follows:

Percent frequency:
March - *T. viride* > *C. echinulata*, *F. oxysporum* and *P. chrysogenum*
July - *T. viride* > *C. echinulata* > *F. oxysporum*
November - *T. viride* > *F. oxysporum* and *P. puberulum*
Percent abundance:
March - *T. viride* > *F. oxysporum* > *P. chrysogenum*
July - *T. viride* > *F. oxysporum* > *Nectria rolfsii*
November - *T. viride* > *F. oxysporum* > *P. puberulum*

When the variations in the fungal population in different months were correlated with soil properties, it was observed that soil temperature and Mg content were significant at $P < 0.05$ and 0.01 in March month; moisture content, EC, K, Ca, Fe, Mg and Zn contents were significant at 0.01 and 0.001 in July month; moisture content, P, K, Fe and Mn content were significant at $P < 0.05$, 0.01 and 0.001 in November month samplings (Table 5).

Myristica fatua var. *magnifica*: A total of 24 species of fungi were isolated from the rhizoplane of *M. fatua* var. *magnifica*, of which 10 species isolated by root washing method were not observed using the root plating method. Likewise, five

Table 5. Pearson's correlation coefficient (r) between rhizoplane fungal population and soil properties of *Myristica dactyloides.*

Parameters	March	July	November
MC	0.287	-0.789***	0.701**
Temperature	-0.531*	0.294	-0.330
pH	-0.266	0.256	-0.388
EC	0.327	0.699**	-0.292
OM	0.165	-0.245	-0.331
N	0.446	-0.409	-0.410
P	0.285	0.315	0.720**
K	-0.299	0.679**	0.698**
Na	0.134	0.422	0.236
Ca	-0.099	0.866***	-0.084
Cu	0.386	-0.389	-0.277
Fe	-0.459	-0.764***	0.488*
Mg	0.707**	-0.801***	-0.105
Mn	0.286	-0.489	-0.706**
Zn	0.124	0.796***	-0.080

*,**,***- Significant at $P < 0.05$, 0.01 and 0.001, respectively.

species isolated by the root plating method were not found using the root washing method. The order of the more common species of fungi, based on their percent frequency and percent abundance, was as follows:

Percent frequency:
March - *T. viride* > *Cephalosporium acremonium* and *F. moniliforme*
July - *T. viride* > *C. acremonium* and *P. chrysogenum* > *F. moniliforme*
November - *T. viride* > *F. oxysporum* and *A. niger*
Percent abundance:
March - *T. viride* > *Ulocladium atrum* > *Cunninghamella echinulata*
July - *T. viride* > *F. oxysporum* > *A. niger*
November - *T. viride* > *A. niger* > *F. oxysporum*

When the variations in the fungal population in different months were correlated with soil properties, it was observed that the moisture content was significant at $P < 0.001$, 0.01 and 0.05 in all the three sampling months, while the remaining factors were significant in the sampling of some months only (Table 6).

Myristica malabarica: A total of 22 species of fungi were isolated from the rhizoplane *M. mala-*

Table 6. Pearson's correlation coefficient (r) between rhizoplane fungal population and soil properties of *Myristica fatua* var. *magnifica.*

Parameter	March	July	November
MC	0.500*	-0.886***	-0.785***
Temperature	0.184	-0.514*	0.640**
pH	0.065	-0.394	-0.799***
EC	0.786***	0.734***	0.609**
OM	-0.325	0.888***	0.381
N	0.353	0.371	-0.777***
P	0.370	0.434	0.871***
K	0.331	0.188	0.381
Na	-0.235	-0.533*	0.285
Ca	0.180	-0.287	-0.780***
Cu	0.292	-0.792***	-0.285
Fe	0.706**	-0.533	-0.095
Mg	0.256	-0.287	-0.481
Mn	-0.740***	0.455	0.610**
Zn	-0.287	-0.061	-0.725***

*,**,***- Significant at $P < 0.05$, 0.01 and 0.001, respectively.

barica, of which nine species isolated by the root washing method were not observed using the root plating method. Likewise, two species isolated by the root plating method were not found using the root washing method. The order of the more common species of fungi, based on their percent frequency and percent abundance, was as follows:

Percent frequency:

March - *T. viride* > *P. chrysogenum* > *M. hiemalis*

July - *T. viride* > *P. chrysogenum* > *A. niger* and *P. restrictum*

November - *T. viride* > *F. oxysporum* and *A. niger*

Percent abundance:

March - *T. viride* > *P. chysogenum* > *P. puberulum*

July - *T. viride* > *P. chrysogenum* > *G. roseum*

November - *T. viride* > *F. oxysporum* > *A. niger*

When the variation in the fungal population in different months was correlated with soil properties, it was observed that EC, OM, P, Ca, Cu, Mg and Zn contents were significant at $P < 0.05$, 0.01 and 0.001 in the March month; soil temperature, EC, P, Na, Mn and Zn contents were significant at $P < 0.05$, 0.01 and 0.001 in the July month; moisture content, P, K, Na, Fe, Mg and Mn contents were significant at $P < 0.05$, 0.01 and 0.001 in the November month samplings (Table 7).

Table 7. Pearson's correlation coefficient (r) between rhizoplane fungal population and soil properties of *Myristica malabarica.*

Parameter	March	July	November
MC	-0.078	-0.317	0.866***
Temperature	0.153	0.699**	0.145
pH	0.231	0.107	0.371
EC	0.893***	0.859***	0.305
OM	0.619**	0.219	0.096
N	0.303	0.267	0.410
P	0.764***	0.667**	0.817***
K	0.181	0.418	-0.730***
Na	-0.285	0.643**	0.666**
Ca	-0.577*	0.069	-0.249
Cu	0.619**	0.178	-0.325
Fe	-0.277	0.249	-0.754***
Mg	0.731***	0.055	-0.637**
Mn	0.188	-0.569*	-0.484*
Zn	0.484*	0.485*	-0.265

*,**,***- Significant at $P < 0.05$, 0.01 and 0.001, respectively.

A total of 37 species of fungi were isolated from the rhizoplane of the five tree species. Of these, as many as nine species (24.3 %) were common to the rhizoplane of all the tree species. The variation in the occurrence of rhizoplane mycoflora of the five tree species is given in Table 2. A maximum of 24 fungal species each were isolated from the rhizoplane of *K. attenuata* and *M. fatua* var. *magnifica*, followed by 22 species each in *M. dactyloides* and *M. malabarica*, and 20 species in *G. farquhariana*. The mycoflora varied from plant species to plant species, and within the species also there was variation in the mycoflora between the seasons. The maximum number of fungal species was recorded in July in all the tree species except *M. malabarica*, where the maximum was in both March and July. The minimum number of species was recorded in November in *G. farquhariana*, *K. attenuata* and *M. malabarica*. In *M. dactyloides* and *M. fatua* var. *magnifica* the minimum number of fungal species were both in March and November. The fungal population was maximum in July in *K. attenuata*, *M. dactyloides* and *M. malabarica*, in March in *M. fatua* var. *magnifica* and in November in *G. farquhariana*. The minimum fungal population was in March in *G. farquhariana* and *M. malabarica*, in November in *K. attenuata* and *M. dactyloides* and in July in *M. fatua* var. *magnifica*. Dillon & Anderson (1994)

Table 8. Index of similarity between rhizoplane mycoflora of five tree species.

	Gf	Ka	Md	Mf	Mm
Gf	0	72.73	71.43	63.64	66.67
	Ka	0	78.26	75	69.57
		Md	0	73.91	63.64
			Mf	0	56.52
				Mm	0

observed higher population of fungi from June to August and minimum in the May (summer). As in the case of rhizosphere, the variations in the microbial population in the rhizoplane have often been attributed to the wide range of soluble metabolites exudated by the roots, age of the plant, temperature of the soil etc. (Dillon & Anderson 1994; Grayston *et al*. 1998; Ivarson *et al*. 1970; Khan & Prakash 1982; Nelson & Mele 2006; Parkinson 1967; Rovira 1969; Rengel & Marchner 2005; Singh & Khara 1991).

Generally, the number of species of fungi found in the rhizoplane is less than the number of species in the rhizosphere (Bali *et al*. 1987; Gangwane & Deshpande 1973; Khan & Prakash 1982; Mishra 1967; Rama Bhat & Kaveriappa 2009a & b; Singh & Khara 1991). This trend was also observed in the present study. Mishra (1967) attributes the higher count of fungi in the rhizosphere to the liberation of wide range of metabolites by the actively growing roots. On the other hand, the fungi associated with rhizoplane, according to Mishra (1967), have restriction of space on the surface of the roots and are affected by root specificity and condition of roots.

The fungal species common to the rhizoplane of all the tree species were *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Trichoderma viride*. In the present study, the most abundant fungal species in the rhizoplane region of all the five plant species in all the seasons was *Trichoderma viride*. Rovira (1965) and Parkinson (1967) observed greater frequency of species of *Rhizopus*, *Trichoderma*, *Aspergillus* and *Fusarium* in the rhizoplane region. Gangwane & Deshpande (1973) observed high abundance of *Rhizoctonia* and *Trichoderma* species in the rhizoplane of groundnut. Taylor & Parkinson (1964) observed that the frequency of *T. viride* on the root surface of dwarf bean was not affected by variation in temperature, whereas low or high soil moisture and increased pH (> 3.2) of the soil did. In the present study the moisture content of the rhizosphere soil varied between 12 and 41 %, temperature between 21 and 29 °C and pH between 5.3 - 6.3; nevertheless, the population of *T. viride*, appeared to be unaffected by these variations. Species of *Trichoderma* are well-known antagonists of root pathogens (Dennis & Webster 1971 a, 1971b), and the abundance of *Trichoderma* species in the rhizoplane helps to control the fungal root pathogens.

Table 9. Index of similarity between rhizosphere and rhizoplane mycoflora of five tree species.

	Gf RP	Ka RP	Md RP	Mf RP	Mm RP
Gf RS	38.1	50	46.51	47.73	39.53
Ka RS	37.21	48.89	40.9	46.67	38.64
Md RS	34.57	42.35	43.37	40	38.55
Mf RS	37.36	46.32	43.01	42.11	38.71
Mm RS	40.48	54.55	48.84	47.43	41.86

Index of similarity

The index of similarity between the rhizoplane mycoflora of the five tree species was > 50 % (Table 8). The highest similarity was between the rhizoplane mycoflora of *K. attenuata* and *M. dactyloides* (78.3 %) and lowest was between the rhizoplane mycoflora of *M. fatua* var. *magnifica* and *M. malabarica* (56.5 %). Out of the 37 species of fungi isolated from the rhizoplane of the five plants in the present study, as many as nine species (24.32 %) were exclusively found in the rhizoplane of one of the plant species only, mostly in July. Sorensen's similarity index revealed 21.74 to 43.28 % dissimilarity in the fungal flora between the rhizoplane of the five plant species. Such dissimilarity occurs due to the specific association between fungi and the plant species. The index of similarity between the rhizosphere and rhizoplane mycoflora of the five tree species ranged between 34.57 and 54.55 % (Table 9). Between the rhizosphere and rhizoplane mycoflora of the same tree species the similarity was in the range of 38 - 49 %. The similarity was maximum (54.55 %) between the rhizosphere mycoflora of *M. malabarica* and rhizoplane mycoflora of *K. attenuata* and minimum (34.57 %) between rhizosphere mycoflora of *M. dactyloides* and rhizoplane mycoflora of *G. farquhariana* (Rama Bhat & Kaveriappa 2009a & b). The above observation suggests that while the rhizoplane of each plant species supports the growth of some common mycoflora, there are some specific ones as well.

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