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ORIGINAL PAPER

Diversity of soil fungi in dry deciduous forest of Bhadra Wildlife Sanctuary, Western Ghats of southern India

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Abstract: We assessed soil fungal diversity in the dry deciduous forest of a Bhadra Wildlife Sanctuary of the Western Ghats (210.31 m a.s.l.; N 13°44' and E75°37'). Soil samples were collected by random mixed sampling during winter (November, 2008), summer (March, 2009) and monsoon (August, 2009) seasons, and physico-chemical parameters were recorded. During winter, summer, and monsoon seasons, 49, 45 and 49 of fungal species belongs to 20, 18 and 19 of genera were isolated, respectively. Isolated soil fungi were mainly of the Mitosporic fungi, followed by Zygomycotina, Ascomycotina, Oomycotina and Coelomycetes. Indices of diversity, dominance and fisher alpha during winter, summer and monsoon seasons were 3.756, 3.638 and 3.738 (H'), 0.9737, 0.9694 and 0.9726 ($1\text{-}D$) and 18.84, 29.83 and 19.46 (α), respectively. Spearman's (r) correlation coefficient of fungal population with physico–chemical parameters of soils showed significantly positive and negative correlations ($p<0.01$) during winter, summer and monsoon seasons. Physico-chemical soil parameters played an important role in the occurrence, diversity, distribution, and relative abundance of fungal species in the tropical dry deciduous forest soil.

Keywords: forest soil; physico-chemical parameters; seasonal; soil fungi; Western Ghats

Introduction

Soil is the natural medium for the growth of various types of saprophytic, parasitic, and antagonistic mycota. Forest soils are rich in nutrients and soil-associated fungi are involved in nutrient cycling. Saprophytic fungi have been adapted for decomposing woody debris and leaf litter in forest ecosystem as they can produce enzymes capable of breaking down recalcitrant substances (Hyde et al. 2001). Turnover of the plant detritus including plant structural polymers like cellulose, hemicelluloses and lignin by saprophytic fungi is one of the major ecological services in global carbon cycling. This helps to improve soil structure and fertility. Saprophytic basidiomycotina are dominant as they produce lignin modifying enzymes (Behera and Mukerji 1985; Rane and Gandhe 2006; Wahegaonkar et al. 2009). Distribution of fungal species across soil horizons may be due to the influence of vegetation, pedological factors and soil microorganisms (Vardavakis 1990). Saprobic microfungal assemblages in leaf litter are strongly influenced by host phylogeny and affected by seasonal and site factors, such as, the nutrient status of substrata, and climatic and microclimatic conditions (Fryar et al. 2005; Paulus et al. 2006). Identification of fungi is complicated as the fungal life cycle in natural habitats differs from that in the laboratory. Furthermore, fungi are so nutritionally diverse that there is no one medium that can isolate all of them (Domsch and Gams 1972; Panda et al. 2010). The classical methods of examining soil samples involve studying the species composition in quadrats and transects. Such approaches are not feasible for fungal inventory as it requires meticulous observation and repeated sampling (Cannon 1997). Among the estimated total of 1.5 million fungal species, only about 7% have been detected so far (Hawksworth 2001; Manoharachary et al. 2005). Different habitats exhibit variation in plant systems and both environment and edaphic factors greatly influence the growth and development of microbes (Gentry 1988; Behera et al. 1991; Satish et al. 2007). Organic carbon, nitrogen, phosphorous and potassium are important for fungi. In the absence of any of these, the growth and sporulation of moulds as well as other micro organisms are hampered (Saksena 1955; Saravanakumar and Kaviyarasan 2010).

Earlier studies reported that *Aspergillus* and *Penicillium* species are more dominant species in forest soils (Galloway 1936; Moubasher and El-Dohlob 1970; Saravanakumar and Kaviyarasan 2010). Soil fungal diversity and population density are often the reflection of the methods used to recover the fungi (Brock 1987). Instead of attempting species identification, many

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researchers classify individuals at the generic level (O'Donnel et al. 1994; Satish et al. 2007). Fungal population was always higher in surface soil, which might be due to high amounts of organic carbon, higher aeration and greater moisture, and similar observations were also reported by Yamamoto and Glenn (1985). Behera and Mukerji (1985) estimated the number of fungal propagules per gram of dry soil recorded in dilution plates for twenty four months in different layers of the soil profile. It was evident that number of fungi was higher at the surface layer at all sampling sites, and declined with soil depth (Vardavakis 1990; Bhattachryya and Jha 2011). Changes in soil microfungal populations might be attributed to the types of vegetation growing on a particular area (Entry and Emmingham 1996) and the complex vegetation provides various kinds of substrata, thereby allowing different fungal species to coexist (Christensen 1984, 1989; Ogawa et al. 1996). In addition, topography might influence the quantity, diversity and pattern of distribution of fungal populations in soil (Tsai et al. 2007; Tangjang et al. 2009; Bhattacharyya and Jha 2011). Human activities, such as habitat destruction, urban development, industrialization, agriculture, pollution, and the use of pesticides can potentially affect soil fungal diversity (Tsui et al. 1998).

Recent studies from tropical forests suggest that fungal diversity is greater in the tropics than that in temperate regions (Suryanarayanan et al. 2003). Fungal species are especially important components of biodiversity in tropical forests, where they are major contributors to the maintenance of the earth's ecosystem, biosphere and biogeochemical cycle (Satish et al. 2007; Panda et al. 2010). Fungi have beneficial roles in nutrient cycling, agriculture, biofertilizers, antibiotics, food and biotechnological industries (Hawksworth 1991; Hawksworth and Colwell 1992; Lodge 1997; Pointing and Hyde 2001; Manoharachary et al. 2005). The objective of this study was to evaluate the fungal resource and its pattern of distribution and diversity in soil samples of Bhadra Wildlife sanctuary of the Western Ghats of Southern India.

Materials and methods

Study area

Bhadra Wildlife Sanctuary is located in Karnataka State, India, between N 13°25' and 13°50' latitude to E 75°15' and 75°50' longitude and covers an area of 492.46 km^2. This is a hot spot of biological diversity in the Western Ghats. The sanctuary is surrounded by the Bababudan Hills, Bhadra reservoir and teak forest patches adjoining plantations opening a corridor into Kudremukh National Park. Bhadra Wildlife Sanctuary includes four forest types, dry and moist deciduous, semi-evergreen, and evergreen forests (Champion and Seth 1968). Temperatures in the sanctuary range between 9ºC (mean minimum temperature in December) and 36ºC (mean maximum temperature in March). Annual precipitation ranges from 2,000–2,500 mm most of which falls during the southwest monsoon (July to September).

The study site was located near Lakkavalli at N 13º44' latitude and E 75º37'30" longitude in the northern region of the sanctuary at 690 m above a.s.l. (Fig. 1). The site received mean annual precipitation of 1,018.7 mm and 1,222.2 mm during 2008 and 2009, respectively (data from Chikmagalur Meteorological Station, 2009). The temperature in the area varies little and has a monthly mean of 23.93 ºC. The most common and dominant tree species in the study area include *Butea monosperma* (Lam.) Taub., *Canthium parviflorum* Lam., *Dalbergia latifolia* Roxb., *Eucalyptus globules* Labill., *Gmelina arborea* Roxb., *Grewia tiliifolia* Vahl., *Lagerstroemia lanceolata* Wall., *Madhuca latifolia* Roxb., *Santalum album* L., *Schefflera venulosa* (Wight & Arn.) Harms., *Tamarindus indica* L., *Tectona grandis* Linn., *Terminalia bellirica* (Gaertner) Roxb., and *Terminalia paniculata* Roth. Common herbs, shrubs and climbers include: *Carissa carandas* Lour., *Chromolaena odorata* L., King & H.E. Robins., *Cryptolepis buchanani* Roem. & Schult., *Cynodon dactylon* Pers., *Gymnema sylvestre* R. Br., *Hemidesmus indicus* L., R. Br., *Lantana camara* L., *Parthenium hysterophorus* L., *Sida acuta* Burm. f., *Sida cordifolia* L., *Solanum* spp. L. and *Tinospora cordifolia* (Thunb.) Miers., (Table 1), (Prakasha 2007).

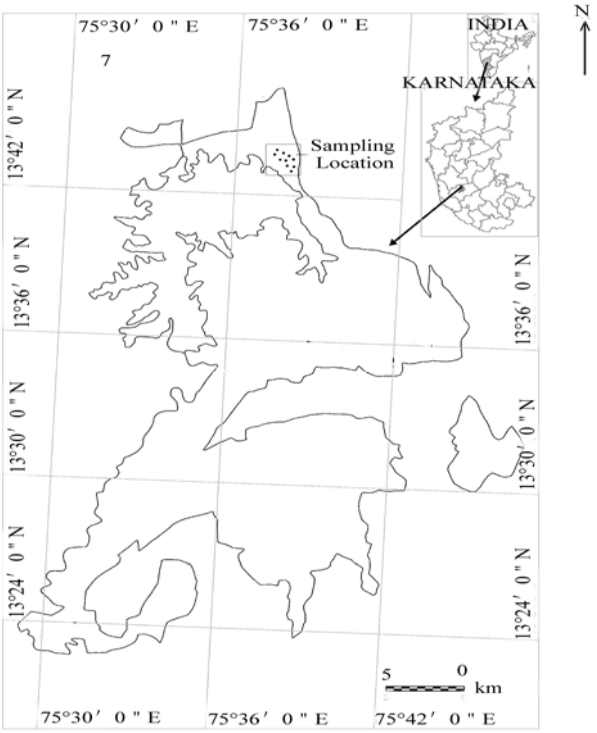

Fig. 1 Study location and map of Bhadra Wildlife Sanctuary, Western Ghats of Southern India

Sample collection

Soil samples were collected in winter (November, 2008), summer (March, 2009) and monsoon (August, 2009) seasons from the study area. Random sampling was done at a soil depth of 5-10 cm. Soil samples of 200 g were collected in sterile polythene bags after removing the upper litter layer and brought to the laboratory (10 subsamples). Samples were air dried to have random mixed single sample for microfungal isolation (Table 1), (Fig. 1).

Table 1. Soil sampling locations and major kinds of trees, herbs, shrubs and climbers in the dry deciduous forest of Bhadra Wildlife Sanctuary

SL. No.	Sampling sites	Locations		Trees	Herbs, Shrubs and Climbers
		Elevation (m.l.s.)	Altitude and Latitude		
1	Site-1	705.61	13º 44' 00.50" N 75º 37' 34.04" E	*Terminalia paniculata*, *Tectona grandis*, *Lagerstroemia lanceolata*, *Santalum album*	*Carissa carandas*, *Hemidesmus indicus*
2	Site-2	707.44	13º 44' 02.01" N 75º 37' 32.94" E	*Terminalia paniculata*, *Dalbergia latifolia*, *Grewia tiliifolia*	*Lantana camara*, *Hemidesmus indicus*, *Cryptolepis buchanani*
3	Site-3	710.18	13º 44' 04.02" N 75º 37' 32.25" E	*Terminalia paniculata*, *Terminalia bellirica*, *Canthium parviflorum*	*Lantana camara*, *Sida acuta*, *Parthenium hysterophorus*
4	Site-4	710.48	13º 44' 05.09" N 75º 37' 30.97" E	*Terminalia paniculata*, *Tectona grandis*, *Eucalyptus globules*, *Santalum album*	*Carissa carandas*, *Sida cordifolia*, *Tinospora cordifolia*
5	Site-5	708.96	13º 44' 06.41" N 75º 37' 30.27" E	*Terminalia paniculata*, *Lagerstroemia lanceolata*, *Madhuca latifolia*	*Lantana camara*, *Gymnema sylvestre*
6	Site-6	709.87	13º 44' 06.98" N 75º 37' 27.51" E	*Tectona grandis*, *Dalbergia latifolia*, *Schefflera venulosa*	*Lantana camara*, *Sida cordifolia*
7	Site-7	707.13	13º 44' 07.04" N 75º 37' 29.95" E	*Terminalia paniculata*, *Tamarindus indica*, *Butea monosperma*	*Cynodon dactylon*, *Hemidesmus indicus*
8	Site-8	703.17	13º 44' 08.39" N 75º 37' 29.93" E	*Terminalia paniculata*, *Terminalia bellirica*, *Gmelina arborea*	*Sida acuta*, *Chromolaena odorata*
9	Site-9	705.91	13º 44' 09.35" N 75º 37' 27.37" E	*Tectona grandis*, *Lagerstroemia lanceolata*, *Butea monosperma*	*Solanum* spp. *Chromolaena odorata*
10	Site-10	711.70	13º 44' 12.64" N 75º 37' 23.29" E	*Terminalia paniculata*, *Grewia tiliifolia*, *Canthium parviflorum*	*Cynodon dactylon*, *Chromolaena odorata*

Fungal isolation

Serial dilution was used to isolate fungi on Czapek Dox Agar (CDA), Rose Bengal Agar (RBA) and Potato Dextrose Agar (PDA) media supplemented with streptomycin sulfate. The spread plate method was done with 10^{-3} dilution after standardization of the dilution samples. The fungi samples were inoculated in replicates and incubated at (27±2) ºC for 3 to 5 days (Aneja 2004; Satish et al. 2007). The fungi grown on the plates were identified based on morphological properties (color, shape, surface, margin and pigmentation) by using standard manuals (Ellis 1971, 1976; Pitt 1979; Domsch et al. 1980; Ellis and Ellis 1997; Gilman 2001; Nagamani et al. 2006).

We identified fungi species by following two methods, based on their morphological and cultural characteristics (culture dependent). Fungi are highly diverse and most species remain uncultured in the media. Non-sporulating or mycelia sterilia fungi were isolated from the forest soils. The non-sporulating mycelia sterilia cannot be provided with taxonomic names without reproductive structures in conventional classification methods and they are now generally categorized as "morphotypes" based on similar cultural and morphological characteristics (Guo et al. 2003; Naik et al. 2008). A major contributing factor has been the tendency of mycologists to rely upon culture-based methods in ecological investigations of soil fungi.

Physico-chemical parameters of forest soil sample

The pH of the soil samples was measured with an electrical digital pH meter in 1:5 (w/v) soil-water suspensions. Soil temperature was recorded with a soil thermometer at the time of sampling. Soil moisture content was determined by drying 10 g of fresh soil overnight in a hot air oven at 105–110°C. For chemical analysis, samples were air dried, ground, and sieved through a 0.2 mm sieve. Electrical conductivity, the macro nutrients such as phosphorous (Olsen method), potassium (neutral normal ammonium acetate method), organic carbon (Walkley and Block method) and micro nutrients such as copper, iron, manganese and zinc were analyzed by DTPA extract method using atomic absorption spectrophotometer (Jackson 1973; DACMA 2011).

Statistical data analysis

Relative abundance (R, %) of fungi on forest soil sample was calculated as follows during winter, summer and monsoon seasons (Elmholt 1996; Mueller et al. 2004):

$$R(\%) = \frac{N}{T} \times 100 \quad (1)$$

where, N is the number of isolates for each species; T is the total number of isolates.

The following indices of diversity were computed with the species as the operational taxonomic unit (OTU). Ideally, the indices should be based on species level identification (Krebs 1989; Panda et al. 2010). This problem was addressed by estimating the number of species corresponding to each genus in the survey during winter, summer and monsoon seasons. A colony obtained on the primary growth plate was identified with a single spore in the soil sample.

$$H' = -\sum_{i=1}^{s} - p_i \log_2 p_i \quad (2)$$

where, H' is Shannon–Wiener index, S the number of OTU, and p_i is the proportion of total samples belonging to the i th OTU. H' varies between 0 and $\log_2 S$ is the information content of the relevant sample (units, bits per OTU). H' is closed to 0 and indicates low diversity; whereas a value closes to $\log_2 S$ indicates high diversity.

$$1 - D = 1 - \sum_{i=1}^{s} n_i (n_i - 1) / [N(N-1)] \quad (3)$$

where, (1-D) is Simpson's index, n_i is the number of individuals in the i th OTU, S is the total number of OTUs, and N is the total number of individuals. The diversity is the minimum when only one OTU exists. If n_i is N for some i and n_i is 0 otherwise, 1–D is 0. The index reaches its maximum when all species are represented equally (each $n_i = N/S$). Then 1–D = (1–1/S) is approximate for large values of N.

$$S = \alpha \ln(1 + N/\alpha) \quad (4)$$

where, (α) is Fisher's index, S the number of OTUs in the sample, N the number of individuals in the sample, and α is the Fisher's index of diversity. The assumption here is that the number of OTUs increases logarithmically with the number of individuals. So α shows an increase in the number of OTUs with respect to increasing (logarithmic) population size when the size is large.

$$S_{ab} = S_{AB} / (S_A + S_B - S_{AB}) \quad (5)$$

where, (S_{ab}) is Jaccard's index, S_{AB} the number of OTUs shared by two locations (A and B), S_A the number of OTUs in location A, and S_B is the number of OTUs in location B. S_{ab} is a measure of the extent of overlap between the OTUs in locations A and B. S_{ab} is 0, if S_{AB} is 0 (no overlap), and S_{ab} is 1 if the OTUs in A and B are identical ($S_A = S_B = S_{AB}$).

$$E = H' / \ln(S) \quad (6)$$

where, E is Evenness, H' is the Shannon–Wiener index of diversity and S is the number of OTUs.

We considered fungal diversity and soil physico-chemical parameters to differ significantly between winter, monsoon and summer seasons when ANOVA results showed $p<0.05$. Statistical analysis was performed by PAST, Version 2.01 (Hammer et al. 2001).

Physical and chemical parameters such as soil pH, soil moisture, soil temperature, atmospheric temperature, relative humidity, electric conductivity and macro nutrients (Organic Carbon, Phosphorous, Potassium) and micro nutrients (Fe, Mn, Cu, and Zn), were quantified. Correlation coefficients (r) between fungal population and various physico-chemical characteristics were analyzed by using Spearman's correlation coefficient (r) for randomly collected and mixed forest soil samples in three seasons (winter, summer and monsoon). The value of $p<0.01$ was considered as correlated significantly. Statistical analysis was performed by SPSS, Version 10.0 (Morgan et al. 2004).

Results

Analysis of fungal diversity

Species diversity varied seasonally. Forty-nine species of 20 genera were recorded in winter (Fig. 2). Among these were two species of Ascomycotina, seven species of Zygomycotina, one species of Oomycotina, thirty eight species of mitosporic fungi and one species of Coelomycetes. *Penicillium* sp. 1 (5.11%) was the dominant species and *Penicillium* sp. 5 (4.68%) ranked second. The dominant species of these fungi were *Aspergillus fumigatus* (4.26%), *Verticillium* sp. (3.83%), *Mucor hiemalis, Cladosporium herbarum, Penicillium herquei*, *Penicillium* sp. 3 and *Penicillium* sp. 6 contributed equally (3.40%) followed by the other species during winter (Appendix 1).

Forty-five species of 18 genera were recorded during summer (Fig. 2). Among these were two species of Ascomycotina, seven species of Zygomycotina, one species of Oomycotina, thirty four species of Mitosporic fungi and one species of Coelomycetes. *Penicillium herquei* (6.67%) was dominant, followed by *Aspergillus fumigatus* (5.71%) and *Verticillium* sp. (4.76%) during summer (Appendix 1).

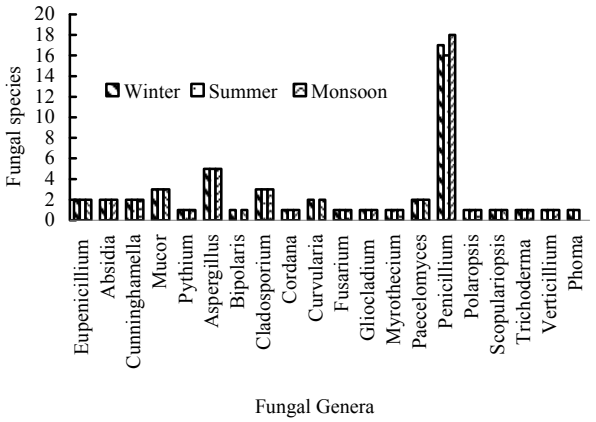

Fig. 2 Graphical representation shows the occurrence of fungal species over different seasons (winter, summer and monsoon) in a dry deciduous forest, Bhadra Wildlife Sanctuary

Forty-nine species of 19 genera were recorded during monsoon season (Fig. 2). Among these were two species of Ascomycotina, seven species of Zygomycotina, one species of Oomycotina and thirty nine species of mitosporic fungi. *Penicillium* sp. 3 (5.41%) was dominant, followed by *Verticillium* sp. (4.95%) during monsoon (Appendix 1).

Indices of diversity in winter, summer and monsoon seasons were 3.756, 3.638 and 3.738 (Shannon H'), 0.9737, 0.9694 and

0.9726 (Simpson 1/*D*) and 18.84, 29.83 and 19.46 (Fisher alpha), respectively (Table 2).

Table 2. Diversity indices over three seasons during 2008 –2009

Diversity indices	Dry deciduous forest		
	Winter	Summer	Monsoon
Taxa	49	45	49
Individuals	235	105	222
Dominance	0.026	0.031	0.027
Shannon	3.756	3.638	3.738
Simpson	0.974	0.969	0.973
Evenness	0.873	0.845	0.857
Fisher alpha	18.84	29.83	19.46
Equitability	0.965	0.956	0.960

Diversity and dominance of fungal species were significantly correlated with soil physicochemical parameters during winter, summer and monsoon seasons. Diversity was significantly and positively correlated with soil temperature, relative humidity, soil pH, potassium and iron during winter, with zinc and iron during summer and with soil temperature, potassium and Mn during monsoon season. Diversity was significantly and negatively correlated with atmospheric temperature, soil moisture, EC, OC, phosphorous, Zn, cu and Mn during winter, soil temperature, soil moisture, soil pH, EC, OC, P, K, Cu and Mn during summer and soil moisture, soil pH, EC, OC, P, Zn, Cu and Fe during monsoon season (Table 3). Dominance was significantly and positively correlated with atmospheric temperature, relative humidity, EC, Zn and Cu during winter, with soil moisture, EC and P and Mn during summer, and with soil temperature, soil pH, K, Cu and Iron during monsoon season. Dominance was significantly and negatively correlated with soil temperature, soil moisture, soil pH, OC, P, K and iron during winter, with atmospheric temperature, relative humidity, Zn and iron during summer, and with atmospheric temperature, relative humidity, soil moisture, EC, OC, P, Zn and Mn during monsoon season (Table 3).

Table 3. Spearman's (*r*) correlation coefficient between diversity (*H'*) and dominance (*D*) with soil physico-chemical parameters during winter, summer and monsoon seasons

Index	Seasons	AT	ST	RH	SM	SpH	EC	OC	P	K	Zn	Cu	Mn	Fe
Diversity (*H'*)	Winter	-1.000**	1.000**	0.500	-0.500	1.000**	-1.000**	-0.500	-0.500	1.000**	-1.000**	-1.000**	-0.866	1.000**
	Summer	0.000	-0.500	0.000	-0.500	-0.500	-1.000**	-0.500	-1.000**	0-.500	0.866	-0.500	-0.866	0.500
	Monsoon	0.000	1.000**	0.000	-1.000**	-0.500	-1.000**	-1.000**	-1.000**	0.500	-1.000**	-0.500	0.500	-0.500
Dominance (*D*)	Winter	0.500	-0.500	0.500	-0.500	-0.500	0.500	-0.500	-0.500	-0.500	0.500	0.500	0.000	-0.500
	Summer	-0.500	0.000	-0.500	0.866	0.000	0.866	0.000	0.866	0.000	-1.000**	0.000	1.000**	-0.866
	Monsoon	-0.866	0.500	-0.866	-0.500	0.500	-0.500	-0.500	-0.500	1.000**	-0.500	0.500	-0.500	0.500

Note: ** is Correlation is significant at the 0.01 level (2-tailed) (p<0.01). AT is Atmospheric Temperature (ºC), ST is Soil Temperature (ºC), RH is Relative Humidity (%), SM is Soil Moisture (%), pH is Soil pH, EC is Electric Conductivity (mS·cm^{-1}), OC is Organic Carbon (%), P is Phosphorous (kg·ha-1), K is Potassium (kg·ha^{-1}), Zn is Zinc (kg·ha^{-1}), Cu is Copper (kg·ha^{-1}), Mn is Manganese (kg·ha^{-1}) and Fe is Iron (kg·ha^{-1}).

Soil physico-chemical parameters

Soil parameters varied significantly by season (Table 4). In forest soils, atmospheric temperature was high (35.67ºC) in summer but, medium ((26–27.67) ºC) in monsoon and winter seasons. Relative humidity was high (80%) in monsoon season but medium (64.67%–68%) in summer and winter. Soil temperature was high (33.5ºC) in summer but medium ((23.5–24.67) ºC) in monsoon and winter seasons. Soil moisture showed a seasonal trend, which was maximum (29.67%) in monsoon season, followed by winter (28.33%) and summer (15.71%). Soil pH was highly acidic (5.5) in winter and acidic (6.13) in monsoon and in summer (6.83) seasons. Electrical conductivity of soil was lower than the critical value ((0–2) mS·cm^{-1}) in winter (0.42 mS·cm^{-1}), summer (0.24 mS·cm^{-1}) and monsoon (0.17 mS·cm^{-1}) seasons. Soil organic carbon was very high in summer (1.8%), followed by winter (1.62%) and monsoon (1.56%) seasons. The amount of organic carbon was higher than the critical values (ICAR 2009). Available phosphorous was much lower in winter (1.82 kg·ha^{-1}), summer (1.73 kg·ha^{-1}) and monsoon (1.78 kg·ha^{-1}) seasons, compared with the critical value ((10.11–24.32) kg·ha^{-1}). Available potassium was much lower in winter (22.86 kg·ha^{-1}), summer (33.72 kg·ha^{-1}) and monsoon (65.15 kg·ha^{-1}) seasons, compared with the critical value ((108–280) kg·ha^{-1}). Concentration of zinc was higher (5.8 kg·ha^{-1}) in the monsoon but much lower in winter (0.36 kg·ha^{-1}) and summer (0.74 kg·ha^{-1}), compared with the critical value ((0.89–2.7) kg·ha^{-1}). Concentration of copper was higher (3.11 kg·ha^{-1}) in winter and in summer (0.94 kg·ha^{-1}) and very low (0.18 kg·ha^{-1}) in monsoon season compared to the critical value (0.45 kg·ha^{-1}). Concentration of manganese was higher in the monsoon (98.09 kg·ha^{-1}), summer (11.73 kg·ha^{-1}) and winter (5.15 kg·ha^{-1}) compared to the critical value (4.5 kg·ha^{-1}). Concentration of iron was higher in monsoon (83.6 kg·ha^{-1}), winter (63.65 kg·ha^{-1}) and summer (22.01 kg·ha^{-1}) than the critical value ((5.6–10.1) kg·ha^{-1}), (Table 4).

Statistical data analysis

The total fungal population was significantly positively correlated with atmospheric temperature, relative humidity, electric conductivity, zinc and iron, and significantly negatively correlated with soil temperature, soil moisture, soil pH, organic carbon, phosphorous, potassium, copper and manganese (Table 5).

Table 4. Physico-chemical parameters* over three seasons during 2008 – 2009

Season	Atmospheric temperature (ºC)	Soil temperature (°C)	Relative humidity (RH %)	Soil moisture (%)	Soil pH	Conductivity ($mS.cm^{-1}$)	Organic carbon (%)	P_2O_5 ($kg.ha^{-1}$)	K_2O ($kg.ha^{-1}$)	Zinc ($kg.ha^{-1}$)	Copper ($kg.ha^{-1}$)	Manganese ($kg.ha^{-1}$)	Iron ($kg.ha^{-1}$)
Winter	27.67 ± 0.58	24.67 ± 0.58	68.00 ± 1.0	28.33 ± 0.58	5.50 ± 0.1	0.42 ± 0.02	1.62 ± 0.02	4.52 ± 0.02	56.50 ± 0.5	0.32 ± 0.01	2.78 ± 0.02	4.60 ± 0.02	56.84 ± 0.07
Summer	35.67 ± 0.58	33.50 ± 0.5	64.67 ± 0.58	15.71 ± 0.26	6.83 ± 0.06	0.24 ± 0.02	1.80 ± 0.1	4.28 ± 0.03	83.33 ± 1.53	0.66 ± 0.01	0.84 ± 0.02	10.48 ± 0.02	19.66 ± 0.06
Monsoon	26.00 ± 0.5	23.50 ± 0.5	80.00 ± 1.0	29.67 ± 0.29	6.13 ± 0.06	0.17 ± 0.01	1.56 ± 0.02	4.40 ± 0.05	161.00 ± 1.0	5.18 ± 0.01	0.16 ± 0.01	87.6 ± 0.1	74.66 ± 0.58

Note: Mean ± Standard Deviation (SD) (*p>0.5494)

Table 5. Spearman's (r) correlation coefficient between fungal population and physico-chemical parameters of forest soils by season

Seasons	AT	ST	RH	SM	SpH	EC	OC	P	K	Zn	Cu	Mn	Fe
Winter	0.500	-0.500	0.500	-0.500	-0.500	0.500	-0.500	-0.500	-1.000**	0.500	-0.500	-0.866	0.500
Summer	-0.866	-0.500	-0.866	1.000**	-0.500	0.500	-0.500	1.000**	0.500	0.500	1.000**	0.500	0.500
Monsoon	1.000**	0.000	1.000**	0.000	-0.866	0.000	0.000	0.000	0.000	0.000	-0.866	0.000	0.000

Notes: ** Correlation is significant at the 0.01 level (2-tailed) (p<0.01). AT is Atmospheric Temperature (ºC), ST is Soil Temperature (ºC), RH is Relative Humidity (%), SM is Soil Moisture (%), pH is Soil pH, EC is Electric Conductivity ($mS{\cdot}cm^{-1}$), OC is Organic Carbon (%), P is Phosphorous ($kg{\cdot}ha^{-1}$), K is Potassium ($kg{\cdot}ha^{-1}$), Zn is Zinc ($kg{\cdot}ha^{-1}$), Cu is Copper ($kg{\cdot}ha^{-1}$), Mn is Manganese ($kg{\cdot}ha^{-1}$) and Fe is Iron ($kg{\cdot}ha^{-1}$).

The fungal population was significantly positively correlated with soil moisture electric conductivity, phosphorous, potassium, zinc, copper, manganese and iron, significantly negatively correlated with atmospheric temperature, soil temperature, relative humidity, soil pH and organic carbon during summer (Table 5).

The fungal population was significantly positively correlated with atmospheric temperature and relative humidity, and significantly negatively correlated with soil pH and copper. But there is no correlation was observed with soil temperature, soil moisture, electric conductivity, organic carbon, phosphorous, potassium, zinc, manganese and iron during monsoon season (Table 5).

Discussion

Microfungal diversity in the tropical dry deciduous forest was dominated by mitosporic fungi of the Zygomycotina, Ascomycotina, Oomycotina and Coelomycetes during winter, monsoon and summer seasons. Similar observations were reported by Satish et al. (2007) and Saravanakumar and Kaviyarasan (2010). Significant variation in microbial quantity was recorded during different seasons of the year. We found diversity, pattern of distribution and abundance of fungal species to be greater in winter, followed by the monsoon and summer seasons. Similar results were reported in earlier studies (Saksena and Sarbhoy 1964; Behera and Mukerji 1985; Rane and Gandhe 2006). We found the diversity of fungal species in the dry deciduous forest during winter to be higher than that during the monsoon and summer. Dominance was greatest in summer, followed by winter and monsoon seasons. Thus, both common and rare fungi were more diverse in winter and monsoon seasons than in summer. Seasonal variation in diversity and abundance or frequency of fungal species in dry deciduous forest may be due to seasonal fluctuations in environmental factors and soil physico-chemical parameters. We observed greatest diversity of soil microfungi in optimum conditions for these parameters during different seasons. Schimel (1995) and Tangjang et al. (2009) reported that only a few fungal species were dominant in different seasons of the year across their sites. Species such as *Aspergillus*, *Botrytis, Geotrichum, Penicillium* and *Rhizopus* were common to all sites. Some fungal species were rare and restricted to particular sites. Dominance of the genus *Penicillium* and *Aspergillus* in their study sites may have been due to the greater rate of spore production and dispersal and partly due to their resistance over extreme environmental conditions. All evaluated soil physico-chemical parameters varied seasonally as did fungal populations. Fungal population parameters were significantly correlated with soil physico-chemical parameters. Seasonal variations in fungal populations were due to changes in micro and macro nutrients, water holding capacity, temperature and pH. Some of the species appeared only sporadically after distinct seasonal interruption while other species were predominant in all seasons.

Atmospheric temperature and fungal population

In the present study, the fungal population showed significantly positive correlation with atmospheric temperature during monsoon and winter seasons but significantly negative correlation during summer season due to the occurrence of high temperature and low atmospheric humidity. A marked difference in qualitative and quantitative data during summer months is referred to the high temperature and low atmospheric humidity together with the affected soil parameters like moisture content and other nutrients, which may restrict vegetative growth, formation of resting spores and inactivation of viable vegetative prolagules (Behera and Mukharji 1985).

Soil temperature and relative humidity with fungal population

We found significantly negative correlation between soil temperatures and fungal populations during winter and summer and insignificant correlation during monsoon season. There was significantly positive correlation between relative humidity and fungal population during winter and monsoon seasons but significantly negative correlation during summer. Atmospheric temperature plays an important role on the variation of soil temperature and relative humidity by season.

Soil moisture and fungal population

We found significant positive correlation between soil moisture and fungal populations during summer but significantly negative correlation during winter and insignificant correlation during monsoon season. Moisture, which is important for the growth of soil microfungi, has a pronounced effect on the distribution of soil fungi (Saksena 1955; Zoberi 1979; Joshi and Chauhan 1982; Behera and Mukharji 1985) though in some cases such a clear correlation was not observed (Ramakrishnan 1955 and Orpurt and Curtis 1957). Accumulation of organic matter on the soil surface and vegetation cover in the forest is highly effective in checking soil evaporation, which increases soil moisture (Swer et al. 2011). Moisture requirement, desiccation, or water loggings are important determinants of fungi abundance. There is a great reduction in active growth of fungi whenever the moisture content is sufficiently high to reduce aeration. Soil moisture showed a fixed seasonal trend, which was maximum in monsoon followed by winter and summer seasons, showing direct relationships with precipitation. Soil moisture favored the growth of soil microfungi and has a pronounced effect on the distribution and the rate of biodegradation (Saksena and Sarbhoy 1964).

Soil pH and fungal population

We found significant negative correlation between the fungal population and soil pH by season. Increased pH might be the reason for less abundant fungi in the soil (Behera and Mukerji 1985). Marginal variations in soil pH fail to influence fungal populations (Behera et al. 1991; Panda et al. 2010). The acidic nature of the soil is influenced by rainfall as heavy rainfall causes leaching of the basic forming cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) leaving mostly H^{+} and Al^{3+} cations, which are largely responsible for soil acidity (Swer et al. 2011). Acidic soil facilitates the rate of growth of soil fungi and biodegradation.

Electric conductivity and fungal population

We found significantly positive correlation between electrical conductivity and fungal populations during winter and summer, but insignificant correlation during monsoon season. Rainfall, soil moisture and salts played an important role in the variation of electrical conductivity. Electrical conductivity of soils was lower than the critical value, indicating that the soils were good for the growth of fungi, all types of plants, and seeds.

Organic carbon and fungal population

We found significant negative correlation between organic carbon content and fungal population during winter and summer and insignificant correlation during monsoon season. Saksena (1955) and Kanazawa (1979) confirmed that organic matter had a great influence on fungal abundance. Vandecavveye and Katznelson (1940) and Zoberi (1979), however, did not report any correlation between fungal population and carbon content of the soil. Fresh organic matter has a stimulatory effect on the first colonizing fungi (Huber and Watson 1970; Jackson 1958). Plant residues are returned to the soil and the death and decay of organisms provides the necessary organic carbon for fungal communities (Swer et al. 2011).

Available phosphorous and fungal population

We found significantly positive correlation between available phosphorous and fungal population during summer but significantly negative correlation during winter and insignificant correlation during the monsoon season. Others have reported positive correlation between fungal populations and the phosphorous content of soils (Ramakrishnan 1955; Rao 1970).

Potassium and fungal population

We found significantly negative correlation between potassium and fungal populations during winter and summer seasons, but insignificant correlation during monsoon season. The stimulatory effects of potassium on soil fungi have been confirmed by many mycologists (Saksena 1955; Rao 1970; Joshi and Chauhan 1982).

Micronutrients (Zn, Cu, Mn & Fe) and fungal population

We found significantly positive correlation between zinc and fungal populations during winter and summerseasons, but insignificant correlation during monsoon season. Correlation between copper and fungal populations was significantly positive during summer and significantly negative during winter and monsoon seasons. Correlation between manganese and fungal populations was significantly positive during summer and significantly negative during monsoon seasons, but insignificant during winter. Correlation between iron and fungal populations was significantly positive during winter and summer seasons, but insignificant during monsoon. Manganese, zinc, copper and iron though needed in very small quantities, are also essential (Saksena 1955; Rao 1970).

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Appendix 1. Abundance and relative abundance of mycoflora in different seasons

Species*	Winter		Summer		Monsoon	
	Abundance	Relative abundance (%)	Abundance	Relative abundance (%)	Abundance	Relative abundance (%)
Ascomycotina						
Eupenicillium meloforme	6	2.55	3	2.86	4	1.8
Eupenicillium sp.	7	2.98	4	3.81	5	2.25
Zygomycotina						
Absidia cylindrospora Hagem	3	1.28	1	0.95	2	0.9
Absidia fusca Linnem	4	1.7	2	1.9	3	1.35
Cunninghamella blakesleeana	4	1.7	2	1.9	2	0.9
Cunninghamella echinulata	2	0.85	1	0.95	4	1.8
Mucor hiemalis Wehmer	8	3.4	5	4.76	7	3.15
Mucor racemosus	6	2.55	4	3.81	4	1.8
Mucor sp.	6	2.55	2	1.9	5	2.25
Oomycotina						
Pythium intermedium	4	1.7	2	1.9	6	2.7
Mitosporic fungi						
Aspergillus candidus	2	0.85	1	0.95	2	0.9
Aspergillus fumigatus	10	4.26	6	5.71	8	3.6
Aspergillus niger Tiegh	4	1.7	2	1.9	3	1.35
Aspergillus parasiticus	2	0.85	1	0.95	4	1.8
Aspergillus terreus	4	1.7	1	0.95	2	0.9
Bipolaris sacchari	3	1.28	-	-	2	0.9
Cladosporium herbarum	8	3.4	3	2.86	10	4.5

Continue Appendix 1.

Species*	Winter		Summer		Monsoon	
	Abundance	Relative abundance (%)	Abundance	Relative abundance (%)	Abundance	Relative abundance (%)
Cladosporium sphaerospermum Penz	5	2.13	3	2.86	4	1.8
Cladosporium sp.	4	1.7	2	1.9	3	1.35
Cordana pauciseptata Preuss	3	1.28	1	0.95	1	0.45
Curvularia clavata	1	0.43	-	-	3	1.35
Curvularia lunata	3	1.28	-	-	2	0.9
Fusarium oxysporum	4	1.7	3	2.86	2	0.9
Gliocladium roseum Bainier	3	1.28	1	0.95	4	1.8
Myrothecium sp.	3	1.28	2	1.9	4	1.8
Paecelomyces sp. 1	6	2.55	3	2.86	4	1.8
Paecilomyces sp. 2	3	1.28	1	0.95	3	1.35
Penicillium canescens	2	0.85	1	0.95	4	1.8
Penicillium decumbens	7	2.98	2	1.9	5	2.25
Penicillium digitatum	3	1.28	2	1.9	5	2.25
Penicillium expansum Link (1809)	5	2.13	1	0.95	3	1.35
Penicillium griseofulvum Dierckx	5	2.13	3	2.86	4	1.8
Penicillium herquei	8	3.4	7	6.67	10	4.5
Penicillium javanicum	6	2.55	1	0.95	3	1.35
Penicillium lilacinum Thom	3	1.28	1	0.95	1	0.45
Penicillium pallidum	2	0.85	-	-	3	1.35
Penicillium restrictum	4	1.7	1	0.95	3	1.35
Penicillium westlingii	3	1.28	-	-	2	0.9
Penicillium sp. 1	12	5.11	4	3.81	8	3.6
Penicillium sp. 2	7	2.98	2	1.9	5	2.25
Penicillium sp. 3	8	3.4	3	2.86	12	5.41
Penicillium sp. 4	4	1.7	1	0.95	4	1.8
Penicillium sp. 5	11	4.68	3	2.86	10	4.5
Penicillium sp. 6	8	3.4	2	1.9	9	4.05
Penicillium sp. 7	-	-	2	1.9	4	1.8
Polaropsis sp.	2	0.85	1	0.95	3	1.35
Scopulariopsis acremonium Vuill.	2	0.85	3	2.86	5	2.25
Trichoderma viride	4	1.7	3	2.86	5	2.25
Verticillium sp.	9	3.83	5	4.76	11	4.95
Coelomycetes						
Phoma sp.	2	0.85	1	0.95	-	-
Total	235	-	105	-	222	-

Note: *Data shown are mean of triplicates (*$p \geq 0.05$).