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Research Article

SEASONAL DISTRIBUTION AND ABUNDANCE OF SOIL FUNGI FROM FOREST SOILS OF WET EVERGREEN FOREST OF TAMIL NADU

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Abstract

Twenty four soil samples were collected from wet evergreen forest of Tamil Nadu, Southern India; the fungi from these soil samples were isolated in both seasons. About – different species belongs to various groups viz. Ascomycotina and Deuteromycotina were identified with the help of relevant literatures. Out of these five species belongs to Zygomycetes, one species of Ascomycetes, one species of Coelomycetes remaining were Hyphomycetes. Twenty one species of *Penicillium* and 10 of *Aspergillus* were also recorded from both collections. None of the basidiomycetes could be isolated from these soils in spite of our best efforts. The diversity indices of forest soil fungi over the two seasons were 2.57, 2.49 (Shannon-Weinner), 0.87, 0.84 (Simpson index) and 10.51, 11.1 (Fishers's alpha) respectively. The results are discussed in detail.

Keywords: Soil fungi, forest soil, seasonal distribution, diversity

INTRODUCTION

The beginning of the study of soil fungi was made as early as 1886 when Adametz in Germany isolated several species of fungi in the course of his biochemical studies on soils. This work was, however, not followed up till 1902 when Oudemans and Koning isolated and described 45 species of soil fungi from Holland, the majority of which were new to science. After this came a large number of contributions from different parts of the world-Hagem (1907), Lendner (1908), Jensen(1912), Gray and McMaster (1933),Gray and Taylor (1935), Campbell (1938), Garret (1938), Warcup (1955b), Treser et al.(1954) and Durrel and Shields (1960). Amongst the Indian workers the names of Shaw (1915), Thakur and Naurris (1928), Ravi Prakash and Saksena (1952), Saksena and Mehrotra (1952), Saksena and Murthy (1953), Shetye (1954), Saksena (1955), Raizada (1957), Dwivedhi (1958), Rai and Tewari (1960), Ghosh and Dutta (1960), Mehrotra and Agnihotri (1961), Mehrothra and Kumar (1961) and Rai and Mukerji (1961) may be mentioned.

Soils are a rich habitat for the growth of microorganisms and among these fungi are abundant in soil next to bacteria. Soil fungi provide an excellent indicator group for determining changes in ecosystem function arising from natural succession events, climate change and poll Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth & Bisby, 1995). Many important plant pathogens (e.g. smuts and rusts) and plant growth promoting microorganisms (e.g., ecto- and endo-mycorrhizae) are fungi. The saprobic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in the decomposition of plant structural polymers, such as, cellulose, hemicellulose, and lignin, thus contributing to the maintenance of the global carbon cycle.

Tamil Nadu has a vast area of forests which are rich in bio-diversity. Forests are classified in to different ways depending on climate, soil type, topography, and elevation. Studies of soil fungi have

been engaging the attention of a large number of workers, the ecological factors, which govern their distribution, have not been seriously studied still recently, particularly in India. The state of Tamil Nadu is still unexplored. In view of this fact the present work was undertaken by the authors. This paper deals mainly with abundance and distribution of fungi from both seasons and their possible inter-relations.

MATERIALS AND METHODS

Study site and location

Tropical Wet evergreen forest occurred at an elevation of 2000 to 2,980 meters above MSL, during summer the temperature remains maximum of 11°C to 15°C and reaches a minimum of 2°C to 5°C. During winter the temperature reaches a maximum of -2°C to 2°C and a minimum of -12°C. Canopy is extremely dense without any stratification, generally climber are not conspicuous, epiphytes are numerous and ground vegetation almost absent.

Methods for the collection of soil samples

The soil samples were collected before and after summer rains during 2007- 2008. The flora of all the three forest areas remain covered for a long time by the plant litter. The method used for taking soil samples was a slight modification as that used by Goddard (1913). It was slightly modified by Saksena and Mehrotra (1952) for their studies. In the present case the pit was 12 inches (30 cm) deep instead of 9 inches from surface layer and rich samples collected from 10 different sites were kept in sterile polythene bags and brought to the laboratory. All random soil samples of each site were put together to make a single sample for each forest.

Isolation of soil mycoflora

The soil micro fungi were enumerated by two methods, namely, Soil dilution method (Waksman 1927), and Soil plate method (Warcup 1950) on two different media such as Czapek's Dox and Rose Bengal agar at pH 6.5. All the Petri dish were incubated at room temperature 27± 3°C for a period of 4–7 days and then examined.

The first set of observations were made at the end of two days to make sure that the fast growing flocculent types such as *Rhizopus, Mucor* and *Trichoderma,* etc., has not grown excessively to interfere with observations of other species. Second observation was made when these had come to an advanced stage to enable identification. Finally, the slow growing organisms has to be subculture in different media for the purpose of further growth to save them from being overrun by the more aggressive types. The number of colonies per plate in 1 gram of soil was counted.

Identification of the fungi

Identification of the organisms were made by microscopic analysis by using taxonomic guides, standard procedures and relevant literature (Raper and Fennel 1965; 1968), Domsch *et al.,* (1980) and Ellis (1971).

The percent contribution of each isolate was calculated by using following formula:

$$\frac{\text{Total no. of CFU of an individual species}}{\text{Total no. of CFU of all species}} \times 100$$

Shannon–Wiener index: 2

$$\mathbf{H}' = -\sum_{i=1}^{S} p_i \log_2 p_i$$

where S is the number of OTUs and p_i is the proportion of total samples belonging to the i^{th} OTU. H' varies between 0 and log2 S is the information content of the relevant sample (units, bits per OTU). H' close to 0 indicates low diversity; whereas a value closes to $\log_2 S$ indicates high diversity.

Simpson's index (modified by Pielou):

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

where 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 minimum when only one OTU exists, i.e., if $ni = N$ for some i and $ni = 0$ otherwise, $1 - D = 0$. It is a maximum when all species are represented equally (each $ni = N/S$). Then $1 - D = (1 - 1/S)$ approximately for large values of N.

Fisher's index ('alpha diversity'):

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

where S is the number of OTUs in the sample, N is 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. If so, α is a measure of the rate of increase of the number of OTUs with respect to increasing (logarithmic) population size when the size is large.

Evenness index (1)

$E = H'/\ln(S)$,

Where H' is the Shannon–Wiener index of diversity and S is the number of OTU.

RESULT AND DISCUSSION

The number of species found after combining data from both the collection was 56. Forty four and forty six species of fungi were recorded from first and second season of Wet evergreen forest, Mariyan Shola of Nilgiris district (12 samples) and Anadikundi Shola of (12 samples) Pollachi near Coimbatore. Among five species which belonged to *Zygomycotina*, one to *Coelomycetes* and one to Ascomycetes, all others were members of *Deuteromycotina.* The genus *Penicillium* was represented by more number of species were (21) followed by *Aspergillus* (10). The other genera which were represented by more than one species viz. *Trichoderma* (4), *Curvularia* (3), *Fusarium* (2),and *Paecilomyces* (2) represents to the total biodiversity (H = 2.571). The fungi are listed in Table 1.

Our results in the present study were significantly higher than that reports previously. The propagules of *Aspergillus niger*, (22.98 %) was most abundant, this was closely followed by *Trichoderma aureoviride* (17.88 %) *Aspergillus japonicus* and *Aspergillus flavus* were ranked third and fourth in the order of dominance. Most of the genera were occurred insignificant in numbers during first season. Earlier reports also indicate that *Aspergillus* and *Penicillium* were dominant in forest soils (Galloway, 1936; and Moubasher and El-Dohlob 1970). In our case the pattern of distribution of common forms, though not of the rare forms, repeat itself over the two seasons.

Table 1: Total CFU and percentage of contribution of Wet evergreen forest of Tamil Nadu

S. No	Name of Fungi	Wet Evergreen Forest			
		Season I (North East monsoon)		Season II (South west monsoon)	
		Total CFU	% contribution	Total CFU	% contribution
	Ascomycotina				
1	*Chaetomium globosum*	--	--	8	2.46
	Zygomycotina				
2	*Absidia cylindrospora*	1	0.16	2	0.29
3	*Cunninghamella elegans*	1	0.16	4	0.58
4	*Mucor racemosus*	1	0.16	5	0.73
5	*Rhizopus stolonifer*	3	0.43	2	0.29
6	*Syncephalastrum racemosum*	3	0.43	4	0.58
	Deuteromycotina				
7	*Alternaria alternata*	10	1.48	2	0.29
8	*Aspergillus flavus*	85	12.44	19	2.79
9	*A. fumigatus*	8	1.17	2	0.29
10	*A. japonicus*	100	14.64	88	12.94
11	*A. chevalieri*	--	--	1	0.14
12	*A. nidulans*	1	0.16	1	0.14
13	*A. niger*	157	22.98	217	31.91
14	*A. ochraceous*	3	0.43	4	0.58
15	*A. tamarii*	1	0.16	3	0.44
16	*A. terreus*	25	3.66	4	0.58
17	*A. versicolor*	6	0.87	6	0.88

18	*Cladosporium cladosporioides*	1	0.16	4	0.58
19	*C. oxysporum*	2	0.29	--	--
20	*Curvularia lunata*	14	2.04	1	0.14
21	*C. ovoidea*	2	0.29	1	0.14
22	*Fusarium laterium*	--	--	64	9.41
23	*F. oxysporum*	--	--	12	1.76
24	*F. redolens*	1	0.16	1	0.14
25	*F. solani*	2	0.29	--	--
26	*Myrothecium sp*	5	0.73	1	0.14
27	*Paecilomyces variotii*	4	0.58	2	0.29
28	*Penicillium brevicompactum*	7	1.04	--	--
29	*P. citrinum*	--	--	1	0.14
30	*P. charlessi*	8	1.17	--	--
31	*P. cyaneum*	--	--	5	0.73
32	*P. duclauxi*	--	--	21	3.08
33	*P. expansum*	1	0.16	--	--
34	*P. fellutanum*	3	0.43	1	0.14
35	*P. frequentans*	--	--	1	0.14
36	*P. fumorosens*	2	0.29	--	--
37	*P. funiculosum*	7	1.04	--	--
38	*P. islanticum*	3	0.43	2	0.29
39	*P. janthinellum*	5	0.73	--	--
40	*P.jensenii*	2	0.29	4	0.58
41	*P. oxalicum*	3	0.45	--	--
42	*P. piceum*	6	0.87	3	0.44
43	*P. purpurogenum*	2	0.29	3	0.44
44	*P. variabile*	13	1.90	--	--
45	*P. waksmanii*	--	--	3	0.44
46	*Penicillium sp 6*	21	3.07	23	3.38
47	*Penicillium sp. 11*	2	0.29	2	0.29
48	*Penicillium sp. (white)*	--	--	8	1.17
49	*Trichoderma areoviride*	122	17.88	101	14.85
50	*T. harzianum*	3	0.43	1	0.14
51	*T. koningii*	1	0.16	--	--
52	*T. viride*	--	--	24	3.52
53	*Trichoderma sp. 5*	4	0.58	1	0.14
54	*Trichrus sp*	2	0.29	1	0.14
55	*Unidentified sp1*	--	--	2	0.29
56	*Pestalotiopsis sp*	--	--	1	0.14
57	*Nonsporulating*	29	4.24	22	3.23

Asan (1997) studied the flora of *Penicillium* and *Aspergillus* in different habitat soils in Edrine. He found 23 species and 2 varieties belonging to *Aspergillus* and 16 species belonging to *Penicillium*. Sulun and Hasenekoglu (1993) researched the flora of *Penicillium* and *Aspergillus* in North-east Anatolia. In their research they found 20 species of *Aspergillus* and 22 species of *Penicillium.* Hasenekoglu (1985) performed quantitative analysis of the micro fungi flora of forest, grass and field soils in vicinity of Sarikamis. He reported that the genus *Penicillium* was most common in terms of species and intensity in his research.

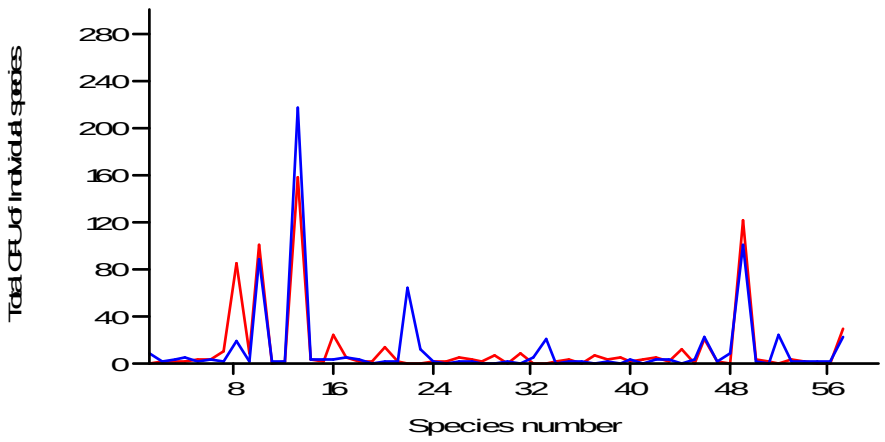
In the second season forty five fungi were recorded. Of the total biodiversity (H= 2.497), *Aspergillus niger* (31.91%) dominates others and followed by *Trichoderma aureoviride* (14.85%). *A. japonicus* (12.94%), *Fusarium lateritium* (9.41%) which rank third and fourth in the order of dominance respectively. *Syncephalastrum racemosum* (0.58%), *Cunninghamella elegans* (0.58%) *Rhizopus stolonifer* (0.29%) *and Absidia cylindrospora* (0.29%) were exhibited less representation (Figure 1).

Most of the *Zygomycotina* members were recorded from both season of Wet evergreen forest. In the case of *Ascomycetes* and *Coelomycetes* were represented only from the second season.

Schoenlein *et al.* (2006) reported 76 taxa in the aquatic and terrestrial environments of specifically in

the island "Ilha dos Eucaliptos. Among them seven species of *Mucorales* and four *Ascomycetes* and remaining 65 belonged *Hyphomycetes*. Warcup (1955) reported the common species of fungi isolated from grassland soil, *Penicillium, Absidia, Mucor* and *Mortierella*, followed by the members of, *Thilaria, Trichoderma*, *Cephalosporium*, *Fusarium*, *Gliomastix*, and *Zygorrhynchus* were the members of genera.

The highest total number of taxa_S was recorded in the second season of Wet evergreen forest when compared to first season. The Fisher alpha test was more or less same in the both seasons (Table 2).



Blue line- Total CFU of second season (South west monsoon)

Red line-Total CFU of First season (North East monsoon)

Figure 1: Distribution of Fungal flora of Wet evergreen forest of Tamil Nadu

Table 2: Various measures of diversity indices over the two seasons

S.No	Diversity indices	Wet Ever Green Forest	
		Season - I	Season -II
1	Taxa_S	44	46
3	Dominance_D	0.1282	0.1522
4	Shannon_H	2.571	2.497
5	Simpson_1-D	0.8718	0.8478
6	Evenness_e^H/S	0.297	0.264
7	Fisher_alpha	10.51	11.1

All our estimates of Simpson index are close to 1, meaning that the probability is very low. Further, a number of indices of fungal diversity – number of genera, Fisher's alpha, Shannon – Weiner index and Simpson index were almost similar to both seasons. This may be a reflection of the fact that we have monitored only culturable living soil fungi and not from fungi associated with plants and trees (Nilima satish *et al*., 2007). The species Evenness_e^H/S was least in second season of wet evergreen forest, but in the case of total taxa _ S was higher. The total number of fungal genera increases with the area monitored. Thomas and Shattock (1986) also found that the logarithmic and log normal distributions were best studied for a description of the abundance of 33 genera of filamentous fungi (Krebs, 1989).

Reported values of soil fungal diversity and population density are often a reflection of the methods used to recover the fungi, with optimal sampling methods differing from organism to organism Brock (1987) and Schlegal and Jannasch (1972). Identification is complicated by the fact that fungal life cycles in the soil and in the laboratory can be quite different. Therefore, instead of attempting species identification, many researchers classify individuals at the generic level (Donnel *et al*., 1994). Fungi are so nutritionally diverse that there is no single medium that can be used to isolate all of them. The technique of direct isolation from particles of soil would have yielded more counts, but the fast growing fungi would still be favored (Galloway, 1936).

In conclusions fifty six species of fungi were isolated from wet evergreen forest of Tamil Nadu, *Penicillium* and *Aspergillus* spp were dominated in both seasons, these fungi produce various types of aflotoxin and some kind of antibiotics it may prevent the growth of other fungal species especially heavy sporulating capacity. Here there was no significant difference in abundance and fungal distributions of both seasons, but in the case of pathogenic species, *Fusarium laterium* was highest percent contribution and followed by *F. oxysporum* in the second season. While the first season, *Curvularia lunata* and *Alternaria alternata* were contributed as high.

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