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Biodiversity of Soil Fungi of Achanakmar Forest of Bilaspur

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***Abstract* -** The fertility status of soil depends not only on its chemical and physical composition but also depends of the occurrence of micro-organisms. During the course of study an extensive survey of area of forest of Achanakmar Bilaspur District (Chhattisgarh) was made to investigate the total microbial flora in relation to the seasonal trends in the forest appearance.

Forty one fungal species were recorded from Achankmar forest of Bilaspur District of Chhattisgarh state. The fungi from these soil samples were isolated in season I (June – July) and in season II (November – December). Both seasons different species belongs to various groups viz; Ascomycotina, Zygomycotina and Deuteromycotina were identified with the help of relevant literatures. Dominance of *Penicillium* and *Aspergillus* were also recorded from both seasons. The diversity indices of forest soil fungi over the two seasons were 3.061, 2.861 (Shannon- Weinner), 0.9011, 0.9032 (Simpson index) and 16.72, 14.92 (Fishers's alpha), respectively. The soil nutrients were also analyzed . The macro nutrients such as N, P, K content were rich in after the raining season and organic content of natural soil was also increased.

***Keywords*:** Soil Fungi, Diversity, Forest Soil, Seasonal Distribution, Soil Nutrients.

I. INTRODUCTION

Soil surface of any forest is a good platform for microorganisms. Forest surface provide detritus based micro habitats for soil dwelling microorganisms in the above ground parts of the soil. (Pandey *et al.,* 2011).
The relationship between biodiversity of soil fungi and ecosystem function is an issue of paramount importance, particularly in the chemical properties and humus alteration of ecosystem process. Fungi are an important component of the soil micro biota constituting more of the soil micro biota depending on soil physico chemical properties and soil depth (Ainsworth and Bisby, 1995) the distribution of soil microorganism influenced by many factors such as abundance of the soil as well as by other edaphic and climatic conditions surface vegetation and soil texture (Manchner *et al.,*2008). While the general nature of the soil fungal flora has become recognized details.

Fig. 1: Achankmar deciduous forest

From the numerous studies on soil fungal flora Shi *et al.,*2002; Gleason *et al.,*2004; Pandey 2011,2014; Tilak, 2000, Yadav, 2011, Dwivedi *et al.,*2009, Rane and Gandhi, 2003. Therefore the present study was undertaken to find the relationship between soil micro flora and their climate.

II. MATERIAL AND METHODS

Present study were carried out in natural mixed deciduous forests of Achankamar, Bilaspur District it lies between 22.5^0N latitude and 82.12^0 E at 262 meters above mean level. The average annual rainfall was recorded 1369.71mm the average relative humidity was 62% and 41.2% in the morning and evening respectively. The population status of micro flora recorded to 2013-14. Soil samples were collected from 0-10 cm depth after scraping the surface soil layer by means of sterilized agaur of mixed composite soil for the conventional dilution plate method (Diwedi, 1965, Mishra, 1964) was used for fungal isolation and soil plate method (Warcup, 1950) were also used.

The different media such as Potato Dextrose Agar, Czpek's Dox and Rose Bengal Agar media at 6.5 pH were used. All the petri plates were incubated at room temperature. Temperature 24 ±3 ^{0}C for a period of 4-7 days and then examined.

The Achankmar deciduous forest exhibit high biodiversity and the major plant species i.e. *Shorea robusta***,** (family-

Depterocarpaceae), *Butea monosperma* (family- Fabaceae), *Dulbergia sissoo* (family – Fabaceae), *Accacia nilotica* (family- Fabaceae) and *Bombusa vulgaris* (family- Poaceae) are endemic to this region.

Determination of physicochemical properties of soil samples

The pH values, electrical conductivity, soil moisture, organic carbon, nitrogen, phosphorous, potassium, iron, manganese, copper and zinc were analyzed (Table 1). The macro nutrients such as Nitrogen (Alkali permanganate method), phosphorous (Olsen method), potassium (neutral normal ammonium acetate method), organic carbon (Walkley and Block method) and micro nutrients such as copper, manganese and zinc were analyzed by DTPA extract method using atomic absorption spectrophotometer.

Isolation of soil mycoflora

The soil micro fungi were enumerated by two methods, namely, Soil dilution, (Waksman, 1927), and Soil plate method (Warcup, 1950) on different media such as Potato Dextrose Agar, Czapek's Dox and Rose Bengal Agar at pH 6.5. All the Petri dishes were incubated at room temperature 24 ± 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 grown excessively to interfere with observations of other species. Second observation was made when these had come to an advanced stage to enable identification. The number of colonies per plate in 1 g of soil was calculated.

Identification

Identification of the organisms was made by microscopic analysis using taxonomic guides, standard procedures and relevant literature . While presenting the data two terms, viz; periodicity of occurrence and 'percent contribution and statistical analysis were used.

The following indices were analyzed;

Shannon–Wiener index: 2

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

Where S is the number of OTUs (Operational Taxonomic Units) 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)

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

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, that is 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')

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

Where S is the number of OTUs in the sample, N is the number of individuals in the sample and $\propto$ 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, $\propto$ 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

III. RESULT AND DISCUSSIONS

The soil moisture, soil pH, organic content and water are the main factors for the growth and development of fungal population and diversity (Yu *et al.,* 2007; Dong *et al.,* 2004; Zhang., 2001). Nutrient analysis of forest soils revealed very high moisture and organic contents. In all the soil samples the pH and electrical conductivity were almost same in both seasons, The Nitrogen, Phosphorous and Potassium contents were very rich in the season two viz; 189.6, 13.13 and 49.4%, respectively. Organic Carbon was also slightly increased than the season one. Organic carbon controls the microbial growth in the deciduous forest. It is a key factor governed nitrogen, phosphorous, potassium cycle. micro nutrients such as Fe, Mn, Cu and Zn required in small quantity were recorded 1 – 25 ppm concentration is higher in season two. Similar findings was also observed by Saravan Kumar and Kaviyarasan (2010) in temparate forests of Tamil Nadu.

Mean value frequency is more in season two then season one.The diversity indices of forest soil fungi over the two seasons were 3.061, 2.861 (Shannon- Weinner), 0.9011, 0.9032 (Simpson index) and 16.72, 14.92 (Fishers's alpha), respectively. Vraious diversity indices indicates that biodiversity of fungal flora was higher as compared to season I. The soil nutrients were also analyzed . The macro nutrients such as N, P, K content were rich in after the raining season and organic content of natural soil was also increased.

Among all isolated fungal species, the *Deuteromycetes Penicillium* spp., *Aspergillus* sp. were dominent in both seasons. *Curvularia* sp., *Trichoderma* sp., *Fusarium* sp., *Cladosporium* sp. were constant sp. found in season I and season II. *Penicillium* spp., *Aspergillus* sp. were dominent in forest soil.

Aspergillus niger , *A. terreus* occured in highest contribution in season II. *Curvularia* sp. *F. oxysporum, A. flavus, Penicillium* spp. and *Nigrospora sphaerica* were recorded as second dominant group of species in the above forest.

Table 1: Physico chemical properties of mixed deciduous forest of Achanakmar, Bilaspur

S.N.	Parameter	Season I	Season II
1	Soil moisture	21.56	23.28
2	Soil pH	6.5	7.00
3	Electrical conductivity (Dsm-1)	0.066	0.65
4	Nitrogen Kg/ac	179.7	180.76
5	Organic carbon %	4.00	4.22
6	Phosphorus Kg/ac	08.89	08.99
7	Potessium Kg/ac	36.22	36.22
8	Zinc (ppm conc)	0.99	0.89
9	Copper (ppm conc)	0.65	0.67
10	Manganese (ppm conc)	5.5	5.6
11	Soil Temperature ^{0}C	26.6^{0}C	24.2^{0}C

Table 2: Diversity indices of two seasons of mixed deciduous forest of Achanakmar, Bilaspur

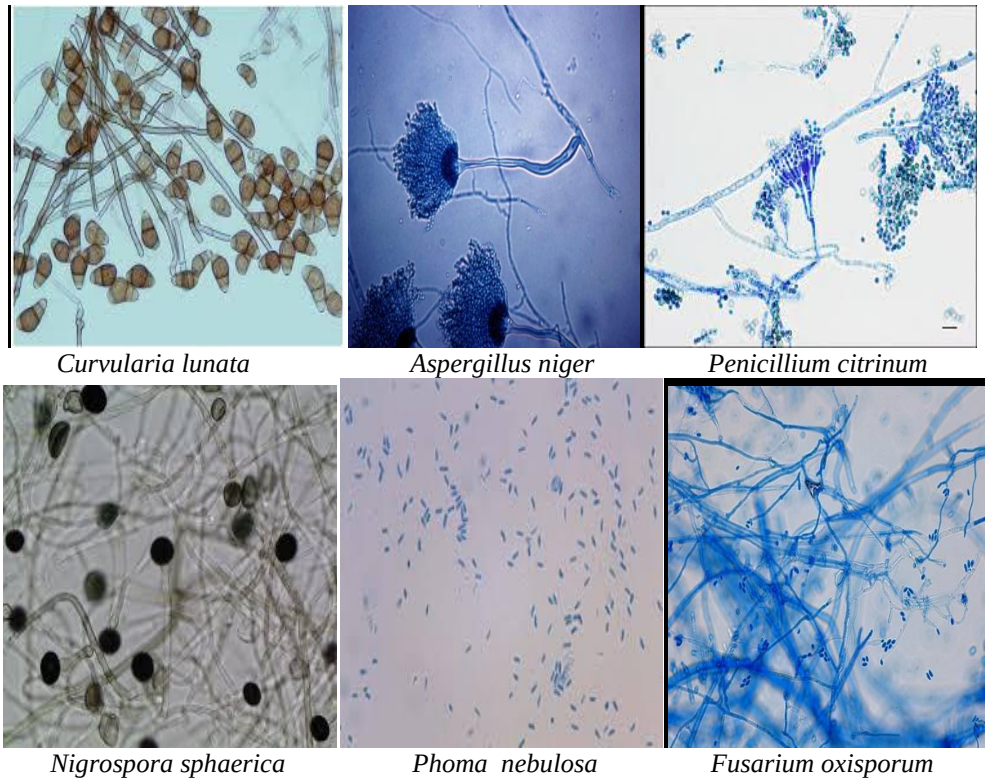
S.N.	Diversity indices	Season I	Season II
1	Mean value of frequency %	55.97	59.12
2	Shannon_H	3.061	2.861
3	Simpson_1-D	0.9011	0.9032
4	Evenness_H/S	0.1156	0.2214
5	Fisher_alpha diversity	16.72	14.92

Table 3: Diversity of fungal isolates in mixed deciduous forest of Achanakmar, Bilaspur

S.N.	Fungal isolated	Season I	Season II
1.	*Absidia cylindrospora*	+	+
2.	*Aspergillus niger* Van Tighem	+	+
3.	*A. candida* Fink	+	+
4.	*A. flavus*	-	+
5.	*A. flaviceps* Toom and church.	-	+
6.	*A. nireus* Blocharitz	-	+
7.	*A. terreus* Thom	-	+
8.	*Chaetomium globosum*	+	+
9.	*Cladosporium* sp.	-	+
10.	*Curvularia lunata* Boedijn.	+++	++++
11.	*Fusarium oxysporum* Schl.ex fries.	++	+++
12.	*Humicola grisea* Traean	++	+++
13.	*Monolia stiphila* (Mont.) Succ.	+	+++
14.	*Mucor racemosus* Fresinius.	++	+++
15.	*Mucor circinelloides*	+	+++
16.	*Phoma nebulosa* Berk.	+++	+++
17.	*Rhizopus stolonifer* Kuhn.	+++	+++
18.	*Penicillium citrinum* Thom.	+++	+++
19.	*Trichoderma viridae Pers & Fries.*	+++	+++
20.	*Torula herbarum* Scc.	+++	+++
21.	*Alternaria alternata*	+++	+++
22.	*Aspergillus glucus*	+++	+++
23.	*Prechstera australienstis*	++	++
24.	*Hypomyces sp.*	+	+
25.	*Helminthosporium* sp.	++	++
26.	*Nigrospora sphaerica*	++	++

27.	*Gliotricham candiderm*	+	+
28.	*Fusarium solani*	+	++
29.	*Glomus viscosum*	-	+
30.	*Aspergillus oryzae*	+	+
31.	*Aspergillus fumigatus* Fresinus	+	+
32.	*Sterile mycelium white*	+	+
33.	*Rhizoctonia solani* Kuchh.	+	+
34.	*Curvularia clavata*	++	++
35.	*Curvularia covoidea*	++	++
36.	*Aspergillus nidulans*	+++	+++
37.	*A. japonicus*	+++	+++
38.	*Cunninghamella echinulata*	++	++
39.	*Aspergillus oryzae*	++	++
40.	*Curvularia tuberculata*	++	++
41.	*Curvularia ceragrostidis*	++	++

Table 2 showing various parameters of Achankmar forest recorded higher moisture percentage in both seasons, soil temperature range between 28- 29^{0}C shows better symbiotic association in both the seasons. Organic carbon , Nitrogen and phosphorus shows positive and significant influence on the distribution pattern of fungal colonies. Micronutrients such as Copper, Manganese, Zinc ad Potassium recorded quite similar in both the seasons.



Curvularia lunata *Aspergillus niger* *Penicillium citrinum*

Nigrospora sphaerica *Phoma nebulosa* *Fusarium oxisporum*

Fig. 2-7: Some isolated fungal species

Among all 41 isolated species, the *Duteromycetes* were dominant in both seasons. *Aspergillus, Fusarium, Curvularia, Alternaria* was found dominantly in both seasons. Similar findings obtained by Rane and Gandhe, 2016; Reddy *et al.*, 1987; Manoharachary *et al.*, 1990 reported that *Aspergillus* occur more frequently than *Penicilium* in soil of warmer climate.

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