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Seasonal Variation in Mycoflora Population in Subtropical Forest Soil of Uttarakhand, India

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K e y w o r d s

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A B S T R A C T

Seasonal variation of soil mycoflora isolated from two forest ranges viz. Motichur and Chilla of Rajaji national park, Uttarakhand were studied for a period of twenty four months by using serial dilution agar plate technique. Results revealed that the higher CFU's (colony forming units) of mycoflora were recorded from Motichur forest range during rainy season followed by summer and winter season. Total 20 fungal species were reported and about 19 different species belongs to three groups *viz*. Ascomycotina, Zygomycotina and Deuteromycotina were identified with the help of relevant literatures and one was unidentified**.** The fungi isolated include one species of Acomycetes, one species of Zygomycetes and remaining species were Deuteromycetes. A marked seasonal variation in mycoflora has been found. Fungal flora was also correlated with varying ecological factors *viz.* temperature, moisture content, pH, organic carbon and nitrogen.

Introduction

Forest soil is a rich habitat for the growth of microorganisms than other microbial habitats. Among these microorganisms, fungi are one of the dominant groups present in soil. Soil fungi are microscopic plant-like cells that grow in long threadlike structures or hyphae that make a mass called mycelium. The mycelium absorbs nutrients from the roots it has colonised, surface organic matter or the soil. It produces special hyphae that create the reproductive spores.

Fungi live, multiply and die or disintegrate in the soil and thus they provide rich organic matter, which could be recycled as plant nutrition. This developed humus complex is a natural fertilizer mixed with soil and plays a very important role in the composition of soil (Rane and Gandhe, 2006).

Majority of the fungi are free living, saprophytic or parasitic widely distributed in soil, which may vary with the soil type .It has been estimated that 1.5 million fungal

species are present in natural ecosystems, but only 5 – 10% have been described formally (Hawksworth, 2001). Schmit and Mueller (2007) estimated that there is a minimum of 7, 12,000 fungal species worldwide. The actual number of fungi is still unknown; however, only 5-13 % of the total estimated global fungal species have been described (Wang *et al*., 2008).

Soil fungi are a major group of organisms responsible for controlling the amount of nutrient cycling and for controlling the amount of nutrient available to plants (Hernot & Robertson 1994; Singh & Rai 2004; Jain *et al*., 2005). They can decompose the plant and animal residues entering the soil and convert them into soil organic matter, which influences on soil physical, chemical and biological properties and on creating a complimentary medium for biological reactions and life support in the soil environment (Olson *et al*., 2000).

Soil fertility status is dependent upon soil microbial component and their mediated processes (Lynch, 1984). Therefore, it was considered imperative to investigate the seasonal variation in mycofloral population of forest soil and their relation with some important physico-chemical parameters of soil from Motichur and Chilla forest ranges of Rajaji national park, Uttarakhand.

Materials and Methods

Description of the Study Area

The Chilla and Motichur forest ranges together constitutes the study area. This is located in Rajaji National Park (29º15′ to 30º31′ north latitude, 77º52′ to 78º22′ east longitude) spread over an area of 820.42 km^2 in and around the shivalik foothills, which lies between the lesser Himalayas and the upper Gangetic plains . The shivalik hills offer the most prominent geomorphic features of this tract. The river Ganges has cut across these hills at Haridwar.

The Chilla forest range of Rajaji national park lies to the east of river Ganges and is attatched to the Garhwal forest division while the Motichur area of Rajaji lies to the west of the river. The study site falls in the subtropical moist deciduous forest type. The soil from both the forest ranges selected for this study. The climate of the study area is like the climatic conditions of plain areas of Uttarakhand. Because of its vicinity to outer Himalayan hills climate conditions become moderate. It varies from subtropical in the plains to temperate in higher hills. Important associated tree species, shrub and herbs found in the study area were: *Shorea robusta, Tectona grandis, Acacia catechu, Dalbergia sisso and Bombax ceiba, Zizyphus jujuba, Melotus* sp. and *Adathora vesica.* The climate of the area is quite distinct in a year and represents three different seasons viz. Winter**:** extends about November to February, Summer**:** starts from March and extends upto June and Monsoon**:** usually breaks during the middle June and continues till October.

Collection of Soil Samples

Soil sampling was performed over a period of 24 months from July 2011 to June 2013 at both the sites. Soil was sampled from 5 dug pits (25x25x40 cms) along a random transect on a regular monthly basis upto 30cm. The soil samples collected were air dried and sieved through a 2 mm sieve and stored for subsequent analysis (Jackson, 1958; Anderson and Ingram, 1993).

Analysis of Soil Physico-chemical Parameters

Standard procedures were followed in analyzing the soil samples. The average soil temperature was measured using soil

thermometer and moisture content of fresh soil samples was determined after oven drying them at 105°C and expressed as a percentage of weight of the soil samples. Soil pH was measured using digital pH meter. Organic carbon was determined following the wet digestion (Walkely and Black, 1934) and Total nitrogen was determined by Kjeldahl method (Trivedy and Goel, 1986).

Isolation and Identification of Soil Mycoflora

For isolation and estimation of fungal CFU's, serial dilution agar plating technique was adopted (Aneja, 1993) followed by Martin's agar and 10^5 dilution .Dilutions are usually made in multiples of ten.

A single dilution was calculated as follows:
Dilutions = Volume of the sample/ Total number of the sample and the dilutents

The culture plates were prepared using horizontal laminar air flow system for mycological analysis. The plates were incubated at 25±2°C for 4-7 days in incubator. Three replicates were maintained in each case.

Pure colonies were then transferred to PDA slants, overlaid with mineral oil and stored at 4°C in refrigerator for further identification. After incubation the colonies on plates were counted with naked eye or with the help of electronic colony counter.

Colony forming units and Relative occurrence were calculated as follows:

Colony forming units (CFU's)

$$\text{CFU's } (g^{-1} \text{ dry soil}) = \frac{\text{Average number of colonies}}{\text{Dry weight of soil}} \times \text{Dilution factor}$$

Relative occurrence

$$\text{Relative Occurrence (\%)} = \frac{\text{Average number of colonies of a species}}{\text{Average no of colonies of all the fungal species}} \times 100$$

The different fungal isolates were identified by the colour, shape, size, of their conidia, mycelium and other description given by Watanabe (2002); Barnett and Hunter (1998). Fungi that did not produce spores and did not show distinct morphological characters for identification were considered as 'unidentified sp'.

Statistical Analysis

The statistical analysis was carried out with the help of Microsoft Office Excel 2007. Correlation coefficients (r) between fungal population and various physico-chemical characteristics were analysed by using Pearson's correlation coefficient. *P* values <0.01 and <0.05 were considered as significant. The objective of statistical analysis was to determine any significant differences among the parameters analyzed during the experimental process.

Results and Discussion

Physico-Chemical Analysis

Soil physico-chemical properties have long been considered to exert great influence on the distribution, growth and development of trees. Tree cover in turn, influences the improvement of physico-chemical properties of soil. Important physico-chemical properties of forest soils include temperature, moisture content, pH, organic matter and nitrogen, etc. The physical properties of forest soils affect every aspect of soil fertility and productivity (Sheikh and Kumar, 2010). Results of the physico-chemical parameters are presented in Table 1.

Temperature

Soil temperature is an important physical property that regulates most of the physical, chemical, and biological processes of the soil, and the physiological processes of soil organisms and forest plants. In the present study, the mean values of temperature were recorded higher in summers season and lower in winter season in both the forest ranges .In the month of June, the temperature increased due to an increasing intensity of the sunshine .The reason for the decrease afterwards is that from the beginning of the second week of July, raining started due to that the temperature as well as the temperature of soil decreased.

Soil Moisture Content

Moisture content is known to be the primary contributing factor for the growth of fungi. Principle source of soil water is rainfall. Some of waterfalls during rain lost as runoff, the remaining goes on or percolates into the ground. Seasonally, in the present study the mean values of soil humidity and moisture content were reported higher in rainy season followed by winter and summer season in both the forest ranges.

This is due to the significant amount of rainfall. The rainfall affects the air temperature, the temperature of the soil surface and other parameters such as moisture contents of the soil and relative humidity. The values of moisture content (9.92% and 8.02%) recorded by Nazir and Netajini (2014) under *Shorea robusta* and *Tectona grandis* plantation in Dehradun region of Uttarakhand falls under the range recorded in the present study in both the forest ranges.

pH

It has been reported that forest soils should be slightly acidic for nutrient supply to be balanced (Leskiw, 1998). Soil pH influences nutrient uptake and tree growth. The availability of many plant nutrients in the soil changes as a result of reactions in the soil, which are largely controlled by soil pH. Trees may or may not be able to use nutrients because of these reactions. Soils with a pH of 6.0-7.0 typically have high concentrations of available nutrients (Williston and LaFayette, 1978).

In the present study, the pH was ranged from 6.20 ±0.249 to 6.29±0.251; 6.15±0.248 to 6.35±0.252 in Motichur and Chilla respectively. Value of pH (6.40±0.01) recorded by Chaudhary and Joshi (2013) in Motichur and Chilla forest range were similar to the results of the present study.

Organic Carbon

Soil organic carbon represents a major pool of carbon within the biosphere. According to Velayeutham *et al.*, (1998) to sustain the quality and productivity of soils, the knowledge of organic carbon in terms of its amount and quality in soils is essential. Soil organic carbon also serves as an important source of energy for soil mycoflora. The mean values of organic carbon were ranged from: 1.68±0.129% to 2.03±0.143% and from 1.35± 0.127% to 1.73±0.131% in Motichur and Chilla forest range respectively. Higher values of organic carbon were recorded in rainy season as the decomposition of leaf litter takes place at faster rates in rainy season and nutrients released into the soil.

Nitrogen

Nitrogen is an essential element for the development of cells in fungi. Soil N is supposed to be the most limiting nutrient in a majority of ecosystems (Fenn *et al.*, 1998). The values of total N varied significantly in

different forest types. Although N is mostly present in the form of nitrates in the soil, which is very mobile and get moved freely with moisture (Gupta and Sharma, 2008).

The values of total nitrogen in the study area were ranged from 0.21±0.045% to 0.24±0.049% and 0.25±0.050% to 0.28±0.053% in Motichur and Chilla forest range respectively. The availability of N depends to a large extent on the amount and properties of organic matter (de Hann, 1977). As C and N are intimately linked and primary source of C and N is found in the soil as an organic matter, in the form of plants and animal's debris (Aber and Melillo, 1991).

Table.1 Physico-chemical Characterization of Soil in Motichur and Chilla Forest Range (All Values are Mean± S.E. of 10 Observations each)

Sites	Motichur Forest Range			Chilla Forest Range		
Seasons / Parameters	Rainy	Winter	Summer	Rainy	Winter	Summer
Temperature (ºC)	21.87±0.467	17.88±0.422	22.37±0.472	21.77±0.466	17.97±0.423	23.85±0.487
Moisture Content (%)	12.27±0.350	9.15±0.313	9.62±0.310	10.15±0.321	8.65±0.293	9.25±0.303
pH	6.23±0.249	6.20±0.249	6.29±0.251	6.16±0.248	6.15±0.248	6.35±0.252
Organic Carbon (%)	2.03±0.143	1.68±0.129	1.78±0.133	1.73±0.131	1.55±0.124	6.35±0.127
Total Nitrogen (%)	0.24±0.049	0.21±0.045	0.22±0.047	0.28±0.053	0.25±0.050	0.27±0.052

S.E. = Standard Error

Table.2 Composition of Soil Mycoflora in Different Forest Ranges

Name of fungal species	Motichur Forest Range	Chilla Forest Range
Ascomycotina		
Botrytis cinerea	✓	-
Deuteromycotina		
Alternaria alternata	✓	✓
Aspergillus clavity	✓	✓
Aspergillus flavus	✓	✓
Aspergillus fumigatus	✓	✓
Aspergillus niger	✓	✓
Aspergillus parasiticus	✓	✓
Chrysosporium sp.	✓	-
Cladosporium sp.	✓	-
Curvularia affinis	✓	✓
Curvularia brachyspora	✓	✓
Fusarium trichothecioides	✓	-
Gliocladium roseum	✓	-
Gliocladium viride	✓	-
Helminthosporium sp.	✓	✓
Penicillium ochraceum	✓	-
Rhizoctonia sp.	-	✓
Trichoderma sp.	✓	✓
Zygomycotina	✓	-
Mucor jansseni		
***Unidentified* sp.**	✓	-
Total Present	**19**	**11**

Table.3 Relative Occurrence of the Fungal Species of Soil in Motichur and Chilla Forest Range (All values are Mean± S.E. of 24 Observations each)

Sites	Motichur Forest Range			Chilla Forest Range		
Seasons / Genera/Species	Rainy	Winter	Summer	Rainy	Winter	Summer
Alternaria alternata	13.12±0.166	7.40±0.113	12.50±0.147	2.86±0.070	-	-
Aspergillus clavity	7.89±0.117	7.40±0.113	16.75±0.171	5.71±0.100	3.22±0.075	-
Aspergillus flavus	7.89±0.117	7.40±0.113	10.50±0.135	8.57±0.122	9.67±0.130	3.03±0.073
Aspergillus fumigatus	10.52±0.135	3.70±0.080	9.37±0.128	22.85±0.199	16.12±0.167	15.15±0.162
Aspergillus niger	7.89±0.117	18.51±0.179	6.25±0.104	14.28±0.173	12.90±0.150	12.12±0.145
Aspergillus parasiticus	9.20±0.121	7.40±0.113	6.25±0.104	11.42±0.141	16.12±0.167	12.12±0.145
Botrytis cinerea	-	7.40±0.113	-	-	-	-
***Chrysosporium* sp.**	-	7.40±0.113	-	-	-	-
***Cladosporium* sp.**	-	3.70±0.080	-	-	-	-
Curvularia affinis	7.89±0.117	-	12.50±0.147	2.85±0.070	12.90±0.150	12.12±0.145
Curvularia brachyspora	7.89±0.117	3.70±0.080	12.50±0.147	8.57±0.122	12.90±0.150	12.12±0.145
Fusarium trichothecioides	-	3.70±0.080	-	-	-	-
Gliocladium roseum	3.95±0.085	-	-	-	-	-
Gliocladium viride	2.66±0.072	-	-	-	-	-
***Helminthosporium* sp.**	10.52±0.135	-	12.50±0.147	17.14±0.173	3.22±0.075	9.09±0.126
Mucor jansseni	-	7.40±0.113	-	-	-	-
Penicillium ochraceum	-	7.40±0.113	-	-	-	-
***Rhizoctonia* sp.**				-	9.67±0.130	15.15±0.162
***Trichoderma* sp.**	8.68±0.135	7.40±0.113	3.12±0.074	5.71±0.100	3.22±0.075	9.09±0.126

S.E. = Standard Error

Table.4 Correlation Analysis between Fungal Species and Some Physico-Chemical Parameters of Soil in Motichur Forest Range (n=3)

Parameters / Genera/Species	Temp	MC	pH	OC	N
Botrytis cinerea	-0.995**	-0.533	-0.756	-0.703	-0.756
Alternaria alternata	0.877	0.163	0.442	0.924	0.951*
Aspergillus clavity	0.622	1**	0.959*	-0.219	-0.143
Aspergillus flavus	0.689	0.994**	0.983*	-0.119	-0.043
Aspergillus fumigatus	0.966*	0.393	0.643	0.807	0.850
Aspergillus niger	-1**	-0.633	-0.831	-0.611	-0.670
Aspergillus parasiticus	0.263	-0.598	-0.339	0.916	0.882
***Chrysosporium* sp.**	-0.995**	-0.533	-0.756	-0.703	-0.756
***Cladosporium* sp.**	-0.995**	-0.533	-0.756	-0.703	-0.756
Curvularia affinis	0.963*	0.805	0.943	0.396	0.465
Curvularia brachyspora	0.901	0.897	0.987*	0.227	0.301
Fusarium trichothecioides	-0.995**	-0.533	-0.756	-0.703	-0.756
Gliocladium roseum	0.998**	0.762	0.427	0.217	0.029
Gliocladium viride	0.163	-0.467	-0.790	1**	0.982*
***Helminthosporium* sp.**	0.999**	0.652	0.844	0.591	0.651
Penicillium ochraceum	-0.995**	-0.533	-0.756	-0.703	-0.756
***Trichoderma* sp.**	-0.191	-0.891	-0.720	0.644	0.584
Mucor jansseni	-0.995**	-0.533	-0.756	-0.703	0.756

Temp= Temperature; MC= Moisture content; OC= Organic carbon; N= Nitrogen

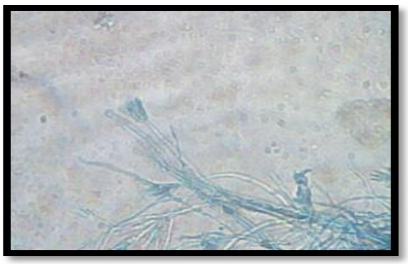
*Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level. Without asterisk statistically insignificant at these levels.

Table.5 Correlation Analysis Between Fungal Species and Some Physico-Chemical Parameters of Soil in Chilla Forest Range (n=3)

Parameters / Genera/Species	Temp	MC	pH	OC	N
Alternaria alternata	0.979*	0.877	-0.261	0.857	0.877
Aspergillus clavity	0.771	0.574	-0.900	0.492	0.257
Aspergillus flavus	0.271	0.006	-0.988*	-0.091	0.338
Aspergillus fumigatus	0.978*	0.888	-0.596	0.839	0.675
Aspergillus niger	0.972*	0.874	-0.619	0.823	0.653
Aspergillus parasiticus	-0.686	-0.854	-0.376	-0.901	-0.981*
Curvularia affinis	-1**	-0.957*	0.438	-0.925	-0.800
Curvularia brachyspora	-0.997**	-0.981*	-0.346	-0.958*	-0.856
***Helminthosporium* sp.**	0.943	0.997**	-0.090	1**	0.961*
***Rhizoctonia* sp.**	-0.897	-0.747	0.776	-0.679	-0.472
***Trichoderma* sp.**	0.006	0.272	0.907	0.364	0.586

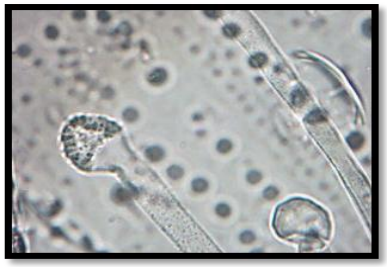
Temp= Temperature; MC= Moisture content; OC= Organic carbon; N= Nitrogen
*Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level. Without asterisk statistically insignificant at these levels.

Figure.1-20 Representatives of different genera/species of soil mycoflora



1. ***Botrytis cinerea***

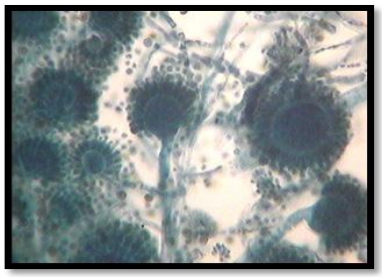
2. ***Alternaria alternata***



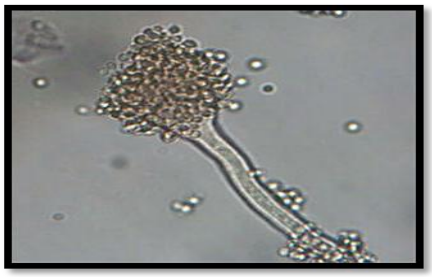
3. ***Aspergillus clavity***

4. ***Aspergillus flavus***

5. ***Aspergillus fumigatus***

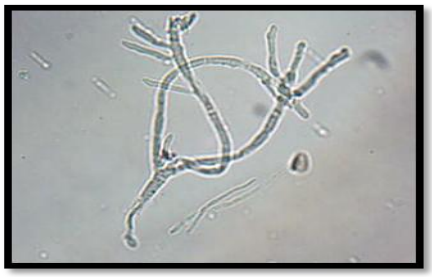


6. ***Aspergillus niger***

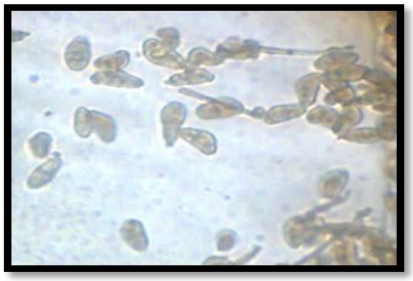


7. ***Aspergillus parasiticus***

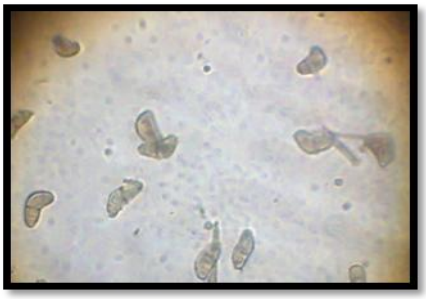
8. *Chrysosporium* sp.



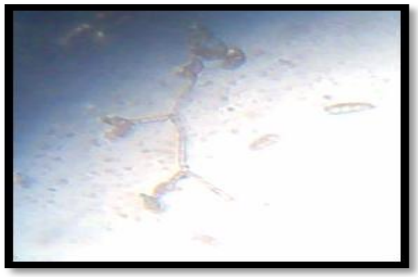
9. *Cladosporium* sp.



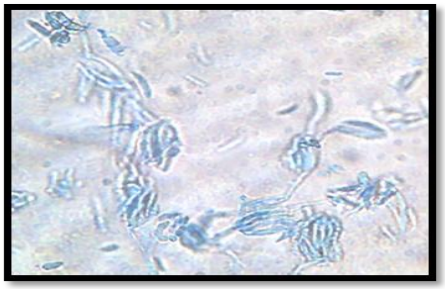
10. ***Curvularia affinis***



11. *Curvularia brachyspora*

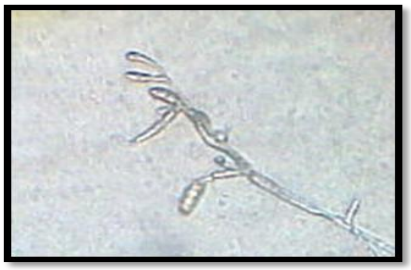


12. *Fusarium trichotheciodes*



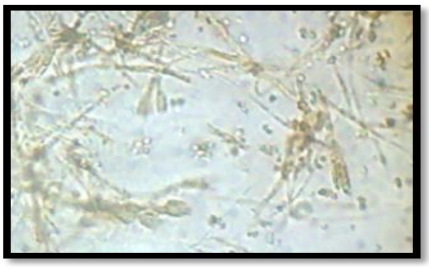
13. ***Gloicladium roseum***

14. *Gliocladium viride*



15. ***Helminthosporium* sp.**

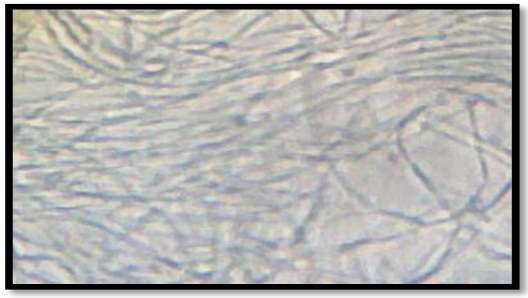
16. ***Mucor jansseni***



17. *Penicillium ochraceum*

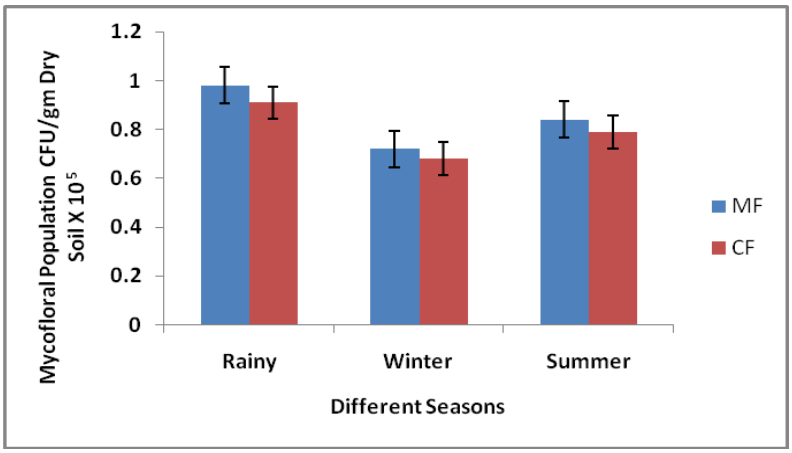
***18. Rhizoctonia* sp.**

19. ***Trichoderma* sp.**



20. *Unidentified* sp.

Figure.21 CFU's of Soil Fungi (10^5 Dilution) in Motichur (MF) and Chilla (CF) Forest Range



Species Composition of Soil Mycoflora

Total 20 fungal species were reported and about 19 different species belongs to three groups *viz*. Ascomycotina, Zygomycotina and Deuteromycotina were identified with the help of relevant literatures and one was unidentified (Figure 1-20). The fungi isolated include one species of Acomycetes, one species of Zygomycetes and remaining species were Deuteromycetes (Table 2). Out of twenty, nineteen species were isolated from Motichur forest range and eleven species were recorded from Chilla forest range.

In the present study, highest number of fungal species was recorded in soil of Motichur forest range as compared to Chilla forest range. This is due to the presence of favourable conditions required for the growth of fungi in soil of Motichur forest range. Saravanakumar and Kaviyarasan (2010) reported sixty seven species of soil fungi belongs to 23 genera in montane wet temperate forest types of Tamil Nadu. Among them six species of Zygomycotina, two of Ascomycotina, four species of Coelomycetes and remaining five were Deuteromycetes.

Colony Forming Units (CFU's)

Seasonally, the mean values CFU's of fungi in the study area were recorded higher in rainy season followed by summer and winter

season in both the forest ranges (Figure 21). Soil moisture content was related to the fungal CFU's and was responsible for the higher CFU's in the rainy season. Lower fungal CFU's in the dry winter season is due to the low soil moisture content. Mishra (1965) also observed that in summer months the fungal populations in soil decreases drastically, but it suddenly increases just after the onset of rain in July while April, May and June were unsuitable for the survival of fungi in soil. So, the population density of soil fungi is positively related to the moisture content of soil.

The population of fungi in the soil is quite sensitive to the amount of decomposable organic matter present. The fungi are prevalent in soils rich in plant residues where competition for food and energy is not too keen but decline rapidly as the readily decomposable material disappears (Troeh, 1993). Chaudhary and Sachar (1934), Miller *et al.,* (1957) and Saksena and Sarbhoy (1964) reported seasonal variation in soil mycoflora and fungal population which drastically differ from season to season in a particular soil.

Relative Occurrence of Soil Mycoflora

It was observed that the some species showed highest percentage of occurrence in rainy season, some in winter season and some in summers as presented in Table 3. This pattern of relative occurrence of mycoflora was similar in both the forest ranges. This is due to the different nutritional requirements of different fungal species for their growth in different seasons.

In all the three seasons, *Aspergillus* sp. was common to all sites. Dominance of the genus *Aspergillus* sp. in the present study sites may be due to their greater sporulation capacity. This fact is also supported by Schimel, 1995.

Saravanakumar and Kaviyarasan (2010) reported that *Penicillium* sp. was the predominant genera followed by *Aspergillus* with twelve species. Similarly, Asan (1997) studied the flora of *Penicillium* and *Aspergillus* in different habitat soils in Edrine and reported twenty three species and two varieties belonging to *Aspergillus* and sixteen species belonging to *Penicillium.* Earlier reports also indicate that *Aspergillus* was dominant in forest soils (Galloway, 1936) and Moubasher and El-Dohlob (1970).

Correlation Analysis

Correlation analysis was performed between physico-chemical parameters and fungal species isolated in10^5 dilution, as the pure cultures were obtained in this dilution. From the results given in Table 4 & 5, it was observed that most of the species were significantly and negatively correlated with temperature which suggested that as the temperature increases the occurrence of fungal species decreased in the study area.

Some species showed a significant positive correlation with temperature which indicates that the fungal species are highly influenced by soil temperature. Castro *et al*., (2010) also found that fungal abundance was affected by temperature.

The same relationship of fungal species was observed with other parameters also *viz*. moisture content, pH, organic carbon and nitrogen in both the forest ranges. As some species were positively correlated with these parameters and some were negatively correlated. Joshi *et al.,* (2013) suggested that the physico-chemical characteristics of soil serve as an important regulating factor for the growth of fungi.

The soil pH, organic content and water are the main factors affecting the fungal

population and diversity (Yu *et al.*,2007). Organic carbon largely controls microbial growth in the natural soil (Saravanakumar and Kaviyarasan, 2010).

This study clearly indicates a marked seasonal variation in mycofloral population. Higher fungal population during rainy season which perhaps is due to prevailing favourable moisture and temperature setting during the period and other plant residues decomposed faster during rainy season and sufficient soil organic matter and humus accumulates that may have enhanced the growth of soil mycoflora in subsequent period.

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