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Isolation and identification of soil fungi in Mattavara forest, Chikamagalur, Karnataka

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Abstract
Soil fungi are the important part of the terrestrial ecosystem and it plays a major role in nutrient cycle as decomposer. The diversity of soil fungi indicates the good or defective condition of the soil health. In the present work we aimed to study the isolation of fungi in unexplored forest soils in terms of percent occurrence and frequency and its relationship with physicochemical properties in scrub jungle with patches of moist deciduous forest of Mattavara forest, Chikkamagalur district, Karnataka which is situated eight km away from Chikkamagaluru. The study was conducted from Jan 2013 - Dec 2013. For the present study soil samples were collected from three different blocks of Mattavara forest once in a month. Total thirty six samples were collected randomly from 0-15 cm depth after removing the surface soil, from different sites of the forest for the isolation of fungi. Serial dilution methodology and PDA media was used for isolation. About eighty two sps belongs to thirty two genera were identified by using relevant literature. Out of these 22 genera and 58sps belongs to Deuteromycotina, 4 genera and nine sps belongs to Zygomycotina, 3genera and five sps of Oomycotina, three genera and nine sps of Ascomycotina and NSF. Among them *Penicillium* and *Aspergillu*are the dominant sps followed by *Chaetomium, Trichoderma*, *Fusarium, Absidia, Cladosporium, Phoma, Acremonium*, *Achyla*, *Alternaria*. The highest percent occurrence showed by *Phomafemeti* (83.33%), and lowest percent occurrence showed by *Myrothecium sps* (1.92%). The maximum fungal frequency of *Fusarium oxysporum* found to be 33.83%, and other sps showed minimum fungal frequency of 2.7 %. Physicochemical analysis reveals that soil is rich in mycoflora due to acidic pH of 4.63–5.05, rich organic matter and optimum moisture content. In conclusion the true purpose of presence of fungi in the nature is in recycling of dead organic matter, thus making them available for the next generation and maintained the ecological balance in the environment. The present finding is the first report on the diversity of soil fungi in Mattavara forest of Chikkamagaluru, Karnataka one of the biodiversity hot spot of Western Ghats.

Keywords: mattavara forest, soil fungi, diversity, physicochemical, unexplored

Introduction
Undisturbed forest soil harbors a great variety of microorganisms like Bacteria, Actinomycetes, Algae, Fungi and protozoa. Among these fungi is the dominant group present in the soil next to bacteria. Fungi determines the good or defective condition of the soil health as it acts as primary decomposer and some twenty functions of soil fungi were discovered by Christensen (1989). These microorganisms are essential for the purpose of recycling of dead organic matter and thus making them available for the next generation to maintain the ecological balance in the environment. There are over 1.5 million fungal sps distributed widely throughout the globe (Hawks worth.2004). Recent evidence suggests that out of 1.5 million fungi, about 1/3exist in India. Out of this only 50% are identified until now. Only fraction of hidden wealth has been subjected to scientific survey and mycologists have unraveled the hidden wealth of un- explored soil (Manoharachary- 2005). Inorder to obtain a fact idea of an endemic micro flora soil should be examined from the locality which is touched and untouched by man (Ling Youg 2007). It is disappointing that despite a long period of research many fungi are yet to be discovered. The patent literature on fungal diversity concluded that different habitat exhibited variation in different plant systems and also environment and edaphic factors greatly influence the growth and development of microorganisms (Gentry. 1998, Bohera. *et al*.1991, Nilima. *et al*. 2007). Nowadays more number of research papers was published regarding agriculture soil fungal diversity than the natural soil. So in the view of above there is a need to study the diversity of soil fungi in unexplored area. The present finding is the first report on the diversity of soil fungi in Mattavara forest of Chikkamagalur, Karnataka one of the biodiversity hot spot of Western Ghats.

Materials and Methods

Study area

Mattavara forest is located eight km away from Chikkmagaluru and is referred as "Boon of biodiversity" this has varieties of flora and fauna. Mattavara forest of Chikkmagaluru is situated between 12^0 54' 42" and 13 53'53" north latitude and between 75 04'46" and 76 21'50" east latitude. It has greatest length from east to west is about 138.4 km and from north to south 88.5 km. total area is about 7.201 sq km. The terrain is flat with scrub jungle and patches of moist deciduous forest. Soil is undisturbed for more than twenty five years. Mattavara village geographic area is about 718.17 hectare. The forest cover is about 224.23 hectare & is divided into 3 regions.

- Mattavara Forest-Block I- includes 55.00 hectare which is situated 1km north from Mattavara information Centre.
- Mattavara Forest – Block II – includes 68.79 ha situated half km North West from Mattavara information centre.
- Mattavara Forest - Block III – includes 100.44 ha situated both side of Mudigere Chikkmagaluru road near Mattavara information centre.

Regeneration of the plot includes, *Steriospermum personatum, Flacourtia indica, Asperagus gono, Pittospoerum decicolon, Carissa carandas, Ichnocarpus frutescence, Buchanania langan, Sapindus trifoliate, Allophylus cobbe, Olea dioica, Zanthoxylum ovalifolia, Carissa candra, Gymnema sylvestre, Hemidesmus indicus, Bridelia retusa, Mallotus philippinensis, Emblica officinalis, Breynia rhamnoides, Accacia pennata, Pterocarpus marsupiana, Vitex altissima, Clerodendron serratum, Cyclea peltata, Clematis gowriana, Ardisia, Todalia asiatica, Elephanto passcaber, Canthium dicocum, Rubia cordifolia, Carya arborea, Madhuca indica, Butea monosperma, Dalbergia sissoo, Crotalaria pallida, Pterocarpus marsupium, Albizia odoratissima, Disperis zylanica, Bryophytum sensitivum, Peristylus aristatus, Liparis viridiflora, Nervalia sps, Dioscoria bulbifera, Ventiligoma chapatana, Ziziphu sxylocarpa, Diospyrus montane, Santalum album,Solanum torvum, Solanum xanthocarpum, Solanum seaforthianum, Terminalia tomentosa, Terminalia bellerica, Terminalia paniculata, Bambusa arundinacea, Solanum torvum, Grewia tiliaefolia, Lagerstroemiami crocarpa, Lantana camara.*.

Climate and rain fall

During summer the temperature remains maximum 31 to 34^0 c and reaches minimum of 18 to 21^0 c. During winter the temperature reaches a maximum of 21 to 23^0 c and minimum of 14 to 17^0 c. During rainy season the temperature reaches a maximum of 25 to 29^0 c and minimum of 18 to 20^0 c. Average rain fall is 600 - 750 mm (mini) and maximum of 1650mm. Wind velocity is 2- 4km/hour, atmosphere pressure is 1489.8 - 1516 milli bars and RH is minimum of 60 -70% and maximum of 90 – 100%.

Collection of samples and isolation of fungi

The 224 ha block of forest is divided into 50ha sized units as study area. Simple random sampling (SRS) of 1×1m area is selected for the soil sampling to study the diversity of fungi. The study was conducted in different sampling sites. Sampling was made arbitrarily to collect bulk sample and from the bulk sample working sampling were derived. The soil samples were collected once in every month from each block from Jan 2013- Dec 2013, Soil sample collected out at the depth of 0 – 15 cm using steel borers after removing the surface organic matter, collected in sterilized bottles and then brought to the laboratory for the isolation of fungi. The soil fungi were enumerated by using serial dilution method [Waksman, 1994] on PDA media within 24 hours. The remaining soil samples were sieved through 2 mm mesh size to remove course material and used for physicochemical analysis. The Petri dishes are incubated in an inverted position for 3-7 days at room temperature 25 ± 2^0 c. There replicate plates are prepared for each sample. Slides of isolated fungi were prepared in lacto phenol cotton blue method and were examined under stereo –binocular microscope and compound microscope. Isolates were identified on the basis of colony morphology, cultural, colony character and spore structure by using relevant literature (Barnet., 1972; Gilman., 1957, Nagamani *et al*, 2006) [3, 11, 22].

Presentation of Data

The obtained data was presented in terms of CFU/gm. of soil ($10^{-2}/10^{-3}$), Percent contribution, and percentage of frequency by using following formula (Subha. *et al*. 2013)

- The number of colonies appearing in dilution plates are counted and multiplied by the dilution factor to determine the number of colony forming unit per gram of soil.

$$\text{CFU/gm. of soil} = \frac{\text{Number of colonies}}{\text{Dry weight of the soil}} \text{ X Dilution factor}$$

- The percent contribution of each isolate calculated by using the following formula.

$$\text{Percent contribution} = \frac{\text{No of colonies of an individual sps in a sample}}{\text{Total number of all colonies of all sps in a sample}} \text{ X } 100$$

- The frequency denotes the number of samplings in which a fungus is present as against the total number of samplings.

$$\text{Percentage of frequency} = \frac{\text{No of samples in which a particular fungi recorded}}{\text{Total number of samples recorded.}} \text{ X } 100$$

Result and Discussion

Thirty six soil samples were collected from three different blocks of Mattavara forest, i.e., 12 samples from each block] Chikkmagaluru, Karnataka. Soil fungi were isolated from Jan 2013 to Dec 2013. About eighty two sps belongs to thirty two genera were identified by using relevant literature. Out of these 22 genera and 58sps belongs to Deuteromycotina, 4 genera and nine sps belongs to Zygomycotina, 3genera and five sps of Oomycotina, three genera and nine sps of Ascomycotina and NSF. Among Eighty two sps 12 sps of *Penicillium*, 09 sps of *Aspergillus*, 7 sps of *Chaetomium*, 6 sps of *Trichoderma*, 4 sps of *Fusarium*, 4 sps of *Absidia*,4 sps 0f *Cladosporium*, and 3 sps of *Phoma* sps were recorded. The other genera represented by 2 sps are *Acremonium, Achyla, Alternaria* and other genera represented by one sps and NSFare listed in the table 1. During the course of study Deuteromycotina reported as dominant group. This observations have also been observed by several workers in their different study area from many parts of the world i.e. Ramarao, 1966; Saxsena, 1966; Bagga,1999; Manoharachary *et al.*, 2005; Rane and Gandhe, 2006; Panda *et al*., 2009; Saravankumar and kaviyarasan, 2010; Sharma, 2010) During our investigation period we have recorded more number of *Penicillium* sps and *Aspergillus* sps followed by *chaetomium*,

Trichoderma, Fusarium, Absidia and *Cladosporium.* Earlier reports also indicate that *Aspergillus* and *Penicillium* were dominant in forest soils (Galloway,1936; and Monbasher and El-Dohlob 1970) and same was reported by Saravanakumar *et al* 2012 in wet ever green forest soils of Tamilnadu. Asan (1997) recorded twenty three sps of *Aspergillus* and sixteen sps of *Penicillium* while studying the flora of *Penicillium and Aspergillus* in different habitat soils in Edrin and Hasenkeoglu (1993) studied the flora of *penicillium* and *Aspergillus* in North east Anatolia and recorded 20 sps of *Aspergillus* and 22 sps of *Penicillium*. Hasenkeoglu (1985) reported that the genus *Penicillium* was most common in terms of sps and intensity in his quantitative analysis of the micro fungi flora of forest, grass and field soils in vicinity of Sarikamis. The propagules of *Aspergillus niger* was most abundant, followed by *Trichoderma aureoviride, Aspergillus japonicus* and *A.flavus* (Saravanakumar *et al*. 2012.) [30]. The scrub jungle soil which has large amounts of organic matter added to the soil in the form of litter harbored a good qualitative and quantitative mycoflora of *Aspergillus, Penicillium, Fusarium, Curvularia, Phoma, Cladosporium, Alternaria and Pythium. Penicillium* is predominant in the winter, while *Aspergillus* occurred more frequently in the summer (Manoharachary 1976) [17]. *Aspergillus* was the dominant genus and repeatedly isolated from soils in all seasons and it occurs on all sorts of organic debris (Gauri Rane and R.V Gandhe, 2006). In the present study the sps of *Aspergillus* are not only dominant but also common in all soil samples. These observations in the present study are similar to Gangawane and Deshpande (1972) [9], Reddy *et al*. (1987) [27], Monaharachary. *et al.*, (1990) who have reported that *Aspergillus* occurs more frequently than *Penicillium* in warmer climate.

Colony forming units is a good estimate of fungal population in different ecosystem such as the soil and is one of the most common methods employed by researchers. Total number of colonies, CFU/gm of soil, Percent contribution and Percentage of frequency of fungi were showed in the Table 1. The colony forming units of fungi varied from minimum of 1.56 to maximum of 18.75x $10^{-2/-3}$CFU/gm. The percent contribution of the individual sps to the total fungal population showed variation. The maximum percent contribution showed by *Phoma femiti* with 83.3%, followed by *Chaetomium reflexum* with 81.8% *Penicillium restrictum* 61.5%, *Zygo rhynchus* 51.8%, *Aspergillus unguis* 51.6%, *Penicillium viridicarum* 47.91%, *Pythium sps* about 47%, *Cladosporium herbarum* 44.82% in order of dominance. Percentage of frequency showed that *Fusarium oxysporum* ranks first (33.82), then comes *Cladosporium cladosporioides* with 30.36%, *Penicillium chrysogenum* 16.67%, *Aspergillus niger* 13.8%, *Trichoderma aureovridae* 13.89%, *Penicillium aurentigratum* 11.1% and so on. Physico chemical parameters of forest soil such as P^H, electrical conductivity, moisture content, macro nutrients (nitrogen, organic carbon, phosphorous, potassium, sulphur) and micro nutrients (Ca, Mg) were carried out in detail which are represented in Table-2. Physicochemical analysis showed that pH of the soil is acidic and is rich with both macro and micro nutrients which is favorable for the growth of fungi. Fungal diversity of any soil depends on a large number of factors of the soil such as P^H, organic content and moisture (Rangaswami *et al*., 1998). Analysis of forest soils revealed very optimum moisture and organic contents. In all the soils investigated, the P^H, moisture content and electrical conductivity were almost same in block 1 and block 2, but in block 3 showed slight variation with pH of 4.63 and high moisture content. Total number of colonies obtained in B1 is 316, B2 310 with slight variation because of almost similar P^H 5.05 and 5.43 and moisture content about 13.05 and 12.30 % and but in B3 410 colonies were obtained because of high moisture content of 14.8 % and low P^H 0f 4.63. This shows that low P^H and optimum moisture content favors the growth of fungi. The organic carbon, nitrogen, phosphorous, sulphur and potassium were important for fungi. In the absence of any one of these, the growth and sporulation of moulds as well as the other microorganisms were hampered a lot. Calcium and Magnesium though needed but in very small quantities and are also essential (Saksena, 1955) [29]. The soil pH, organic content and water are the main factors affecting the fungal population and diversity and Organic carbon largely controls microbial growth in the natural soil. It is a key factor governing Nitrogen, Phosphorous and Sulphur cycles (Yu *et al*., 2007; Dong *et al*., 2004; Song *et al*., 2004; Zhang *et al*., 2001) [41, 7, 42]. Due to the accumulation of more litter in scrub and deciduous forest more percentage of fungi are present in the soil for the purpose of recycling of dead organic matter. It is known that the bacteria thrive well in neutral and alkaline soils, whereas fungi show the best activity under acidic conditions. (Ramann. *et al*. 1899) [24], Both acidic and alkaline soil condition support large number of fungi. A significant correlation was observed between the fungal numbers and P^H in scrub jungle and soils which is rich in organic matter showed the presence of a great variety of fungal sps. (Manoharachary 1976) [17]. Fungal flora may be vary depends on its native soils (Shi. *et al* 2002) [33]. Distribution of soil fungi depending upon the nature of the organic content, climatic condition, surface vegetation and soil texture (Waksmann. 1994, Marchner *et al*, 2003.). Direct relationship is observed between the soil Texture and Moisture content. Silt and Clay soil holds the highest moisture content that's why there is increased population of fungi is observed.

In conclusion Eighty two sps of fungi were isolated from undisturbed soils of Mattavara forest, in which *Cladosporium, Penicillium* and *Aspergillus, Fusarium, Trichoderma* sps are the most commonly occurring fungi due to heavy sporulation and production of secondary metabolites, which prevent the growth of other fungal sps. The diversity of fungi varies greatly in different areas of study area. Result reveals that acidic P^H and optimum moisture content and rich mineral content is most favorable condition for the growth of fungi. It is concluded from our findings that the soil which has large amounts of organic matter due to the accumulation of more litter in scrub jungle and moist deciduous forest, acidic soil pH and silt and clay soil texture holds good amount of moisture content harbored a good qualitative and quantitative mycoflora in the soil for the purpose of recycling of dead organic matter thus making them available for the next generation and maintained the ecological balance in the environment with dominant and sporulating genera.

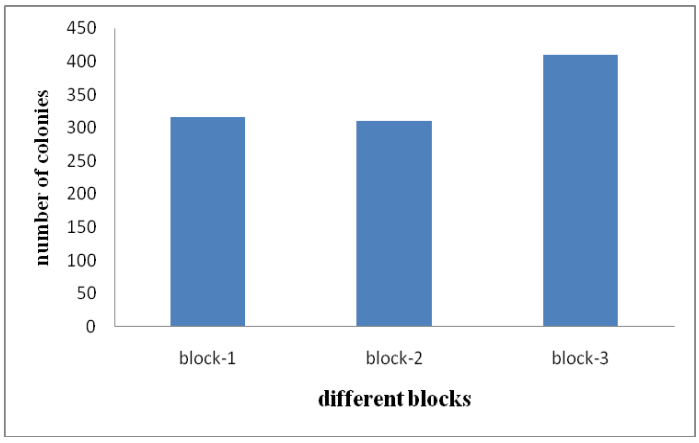
Table 1: List of fungi with total number of colonies, CFU/gm of soil (10^{-2} and 10^{-3}), Percent contribution, and percentage of Frequency recorded in the scrub jungle and moist deciduous forest of Mattavara forest. Chikkamagalur, Karanataka

S. No	*Name of the fungus*	TNC	CFU	% Contribution	% of Frequency
	Oomycotina				
1	*Achyla recurva*	05	6.25	13.1	2.78
2	*Achyla debaryana*	10	3.75	10.7	5.56
3	*Phytophthora sps*	02	2.5	10	2.78
4	*Pythium sps*	08	6.25	47.05	2.78
5	*Pythium spinosum*	01	1.56	1.53	2.78
	Zygomycotina				
6	*Absidia corymbifera*	03	3.75	8.5	2.7
7	*Absidia fusca*	29	6.25	13.8	11.1
8	*Absidia gluca*	12	10	41.7	2.7
9	*Absidia spinosa*	07	3.75	17.9	2.7
10	*Mucor meguroense*	01	1.56	3.1	2.78
11	*Mucor varians*	01	1.56	7.14	2.78
12	*Mucor hiemelina*	01	1.56	4.54	2.78
13	*Rhizopus stolonifer*	05	5	9.25	5.56
14	*Zygorhynchus moelleri*	29	15	51.78	2.78
	Ascomycotina				
15	*Amorphotheca resinae*	01	1.56	2.6	2.7
16	*Chaetomium solani*	07	2.5	7.8	5.5
17	*Chaetomium amberpetule*	01	1.56	4.16	2.7
18	*Chaetomium globosum*	01	1.56	2.6	2.7
19	*Chaetomium reflexum*	18	6.25	81.8	2.7
20	*Chaetomium homopilatum*	02	1.56	5.7	2.7
21	*Chaetomium sps*	03	1.56	15	2.7
22	*Chaetomium osmamae*	02	2.5	3.07	2.7
23	*Gymnascella sps*	01	1.56	4.16	2.78
	Deuteromycotina				
24	*Aspergillus kanagawaensis*	09	7.5	11.1	8.3
25	*Aspergillus flavus*	21	7.5	13.5	8.3
26	*Aspergillus niger*	58	12.5	23.7	13.8
27	*Aspergillus nidulans*	32	12.5	57.1	5.5
28	*Aspergillus versicolor*	10	12,5	28.5	2.7
29	*Aspergillus ochraceous*	03	2.5	14.2	5.5
30	*Aspergillus awamori*	02	2.5	08	2.7
31	*Aspergillus terreus*	02	2.5	11.6	2.7
32	*Aspergillus unguis*	02	2.5	51.6	2.7
33	*Acremonium strictum*	06	2.5	27.2	2.7
34	*Acremonium implicatum*	02	2.5	8.3	2.7
35	*Alternaria alternate*	01	2,5	4.16	2.7
36	*Alternaria brassicicola*	03	3.75	13.6	2.7
37	*Botrytrichum pilulifera*	06	3.75	24	2.7
38	*Chlamydomyces palmarum*	41	2.5	19.1	11.1
39	*Cladosporium cladosporioides*	83	3.75	20.4	30.56
40	*Cladosporium herbarum*	13	2.5	44.82	5.56
41	*Cladosporium sphaerospermum*	02	1.56	6.6	2.7
42	*Cladosporium variable*	02	1.56	5.26	2.7
43	*Cylindrocladium parvum*	07	2.5	13.2	5.56
44	*Dichobotrys abundance*	02	2.5	8.3	2.7
45	*Fusarium oxysporum*	23	3.75	5.56	33.82
46	*Fusarium incarnatum*	08	3.75	22.85	2.78
47	*Fusarium javanicum*	09	10	23.68	2.78
48	*Fusarium poae*	01	1.56	4.54	2.78
49	*Geocladium roseum*	19	3.75	17.27	8.34
50	*Geotrichum candidum*	05	1.56	7.04	8.34
51	*Geosmithia lavendula*	23	10	13.29	5.56
52	*Macrophomium phaseolina*	05	3.75	29.48	2.78
53	*Monodyctyus fluctuate*	23	3.75	25,84	5.56
54	*Myrothecium sps*	02	1.56	1.92	2.78
55	*Paceliomyces sps*	07	2.5	7.95	5.56
56	*Paceliomyces varioti*	15	12.5	23.02	2.78
57	*Penicillium chrysogenum*	39	5	13.68	16.67
58	*Penicillium digitatum*	09	2.5	10.58	5.56
59	*Penicillium sps*	72	5	38.91	11.1
60	*Penicillium islandicum*	06	3.75	17.94	2.78
61	*Penicillium citrinum*	08	2.5	5.56	2.78
62	*Penicillium admetzi*	53	25	44.91	8.34

63	*Penicillium aurantiogresium*	42	12.5	24.41	11.1
64	*Penicillium viridicarum*	10	10	47.61	2.78
65	*Penicillium combratum*	03	3.75	14.28	2.78
66	*Penicillium restrictum*	40	50	61.53	2.78
67	*Penicillium decumbence*	01	1.56	2.77	2.78
68	*Penicillium carylophylum*	02	3.75	5.12	2.78
69	*Phoma herbarum*	04	1.56	7.14	5.56
70	*Phoma sps*	02	2,5	6.25	2.78
71	*Phoma femeti*	15	13.75	83.33	2.78
72	*Pyrenochaetae sps*	08	8.75	57.14	2.78
73	*Trichoderma viridae*	20	18.75	6.89	2.78
74	*Trichoderma aureoviride*	45	7.5	15.15	13.89
75	*Trichodermalongibranchiatum*	02	2.5	8.69	2.78
76	*Trichoderma pseudokoningii*	08	3.75	7.54	8.34
77	*Trichoderma atroviride*	02	1.56	2.29	2.78
78	*Trichoderma harzianum*	08	1.56	20	5.56
79	*Tritrirachium sps*	02	2.5	6.89	2.78
80	*Tritrirachium dependence*	03	2.5	3.44	5.56
81	*Verticillium terrestre*	10	10	20.83	2.78
82	*NSF*	44	3.75	7.02	33.3

Table 2. Physicochemical analysis of forest soils in different blocks

P^H, EC, Macro and micro nutrients	B1	B2	B3
Soil P^H	5.05	5.43	4.63
Soil moisture	13.05	12.30	14.08
EC	0.021	0.023	0.018
Organic C	1.84	2.01	1.14
N ppm	1058	1182	670
P ppm	17.6	10.0	29.8
K ppm	110	145	130
CaC.mol/kg	6.0	6.5	3.5
Mg C.mol/kg	2.0	4.0	3.0
S ppm	220	15	Traces
Sand %	61.9	55.8	49.0
Silt %	16.1	16.0	19.0
Clay %	22.0	28.2	32.0



Graph 1: Total number of colonies obtained in different blocks Mattavara forest

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