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Soil mycofloral diversity under wheat cultivation in Doon Valley, Uttarkhand

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The present communication attempts to provide information on the distribution and diversity of soil mycoflora under wheat (*Triticum aestivum*) cultivation in Doon Valley of Uttarakhand Himalaya. To achieve the target, soil samples were collected from five agricultural fields *viz.,* Doiwala (DOI), Harawala (HAR), Tunwala (TUN), Mohakampur (MOH) and Subhashnagar (SUBH). Samples were also analysed for physico-chemical properties including texture, soil reaction (pH) and moisture content. Serial Dilution and Direct Plate Methods were used to isolate the soil mycoflora. During investigation, pH ranged from 7.10 to 7.80 and the moisture content between 7.23 and 10.01 (%). Texture varied from clay to loam. A total of 23 species of microfungi belonging to 11 genera were isolated from all the sampling sites. Surface soils were richer in microfungal diversity as compared to deeper profile. *Aspergillus* was the most dominant genus followed by *Alternaria, Cochliobolus* and *Rhizopus*. Of the total 23 species, 6 each were of common and frequent, 5 species moderate and rest of the species showed rare occurrence. *Aspergillus* encountered in all the wheat croplands indicating uniform ecological amplitude. Highest similarily (84.6%) was observed between HAR and TUN while lowest (53.8%) between TUN and MOH.

Key words: Soil mycoflora, microfungi, diversity, Doon Valley

INTRODUCTION

Soil is a dynamic and species-rich habitat containing all major groups of microorganisms (Hagvar, 1998). The soil micro-community plays a vital role as the global element cycles and thus for life on earth because 60-90% of the whole terrestrial primary production is decomposed in the soil and furthermore, many waste products of human society are detoxified there (Giller, 1996). Microorganisms in the soil form a part of the biomass and contribute to reserve the soil nutrients and are generally referred to as the microbial biomass. Microbial biomass regulates the transformation and storage of nutrients; these processes affect many nutrient cycling functions including soil fertility and soil organic matter turnover (Horwath and Paul, 1994). There is a relationship between microbial diversity and soil functionality, by considering that 80-90% of the processes in soil are reactions mediated by microbes (Coleman and Crossley, 1996; Nannipeiri and Badalucco, 2003).

The fungi (pathogenic and non pathogenic) associated with the soil are known as soil borne fungi and play a fundamental role for the functioning of the soil ecosystem (Doron and Parkin, 1994, 1996; Hawksworth *et al.*, 1996). Due to their ability to

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decompose complex macro molecules like lignin or chitin they are essential for making the locked-up nutrients like C, N, P, S easily available. Moreover, the fungal mycelia play an important role for the stabilization of the soil because it binds soil aggregates and thus reduces erosion and helps to increase the water holding capacity (Kennedy and Gewin, 1997). Fungi are dominant in acid soils because an acidic environment is not suitable for the existence of either bacteria or actinomycetes, resulting in the monopoly of fungi for the utilization of organic substrates (Bolton *et al.*, 1993). In the frame of agriculture, the micro flora is of great significance because it has both beneficial and detrimental role in overall productions (Whitelaw, 2000). Farm practices including crop rotations and fertilizer or pesticide applications influence the nature and dominance of fungal species (Pelczar and Reid, 1972). Similar to Plant Growth Promoting Rhizobacteria (PGPR), some rhizosphere fungi able to promote plant growth upon root colonization are functionally designated as Plant-Growth-Promoting-Fungi (PGPF) (Hyakumachi, 1994). Macroscopic edible and poisonous forest fungi have been reported and studied in detail from this Himalayan region time to time by different workers (Moncalvo *et al.*, 2004; Semwal *et al.*, 2007; Vishwakarma, 2010; Joshi, 2010; Vishwakarma and Bhatt, 2013), but references pertaining to soil microfungi are scanty. An intensive survey of literature revealed that there are only selective references available pertaining to soil mycoflora of Garhwal Himalaya in general and Doon Valley in particular (Guleri *et al.*, 2010, 2011, 2012, 2013). Therefore, it is an attempt to explore the soil mycoflora in some selected wheat growing agricultural lands of Doon Valley of Garhwal Himalayas.

MATERIALS AND METHODS

Sampling sites

The study was conducted in the Doon Valley during 2012-2013. The area lies between 30°00'-30°35' N latitude and 77°45'-78°15'E longitude. Soils from five wheat growing agroecosystems *viz.*, Doiwala (DOI), Harrawala (HAR), Mohkampur (MOH), Subhashnagar (SUBH), Tunwala (TUN) were analyzed to explore the mycoflora (Fig 1).

Collection of soil samples

Soil sample was collected from different agricultural areas in three replicates. In the collection of soil samples, first a soil profile was extracted and the surface of the profile was cleaned (Brown, 1958). Vertical samples were taken from 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm depths with a disinfected spatula. The spatula was applied perpendicular to the vertical surface of the profile. Samples were kept in sterile polythene in refrigerator.

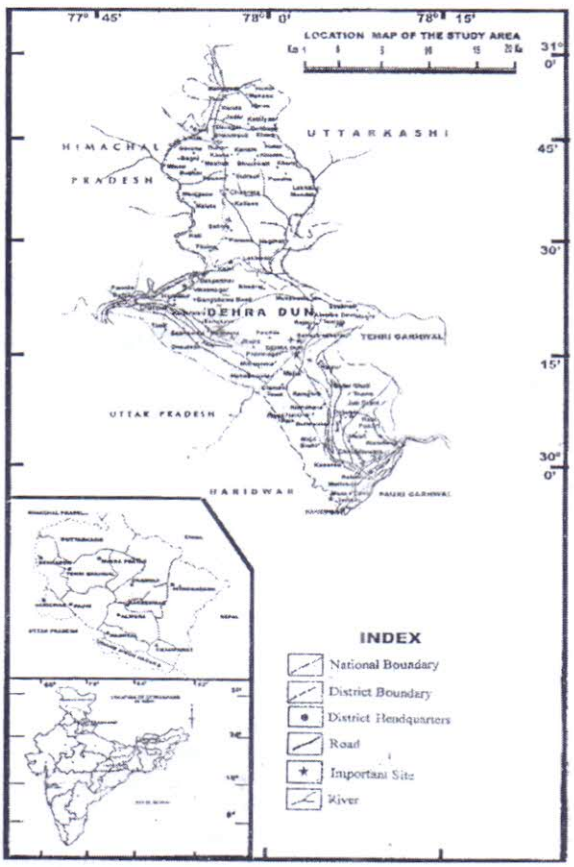

Fig. 1 : Location Map of Doon Valley

Soil analysis

The soil texture was determined by wet sieving technique (Barbour *et al,* 1980). Soil reaction (pH) was determined by using digital pH meter (Model 153P, Toshon, India). Moisture content of soil samples was calculated by oven drying the soil and determining the weight loss (Garrett, 1963).

Isolation of soil mycoflora

The soil microfungi were isolated by Soil Dilution (Waksman, 1927) and Soil Plate Methods (Warcup, 1950) using Czapek's Dox Agar and Malt Extract Agar media.

With the Soil Plate Method, soil samples (0.01 g) were dispersed in 1ml of sterile distilled water in a sterilized Petri dish and then approximately 10 ml of molten, cooled sterile agar was added and mixed. The soil particles were distributed throughout the medium by rotating the Petri dish. The Petridishes were incubated for 5-7 days at 25± 2°C.

For Serial Dilution, soil samples (2.0 g) were suspended in 18 ml of sterilized water, giving a dilution of 1:10. Serial dilutions of 1:100, 1:1000, and 1:10,000 were prepared, and then a 1 ml aliquot from the 1:1000 dilution was added to a Petridish containing penicillin (20,000 units/l) and streptomycin (200 µg/l). Thereafter, approximately 10 ml of molten, cooled sterile agar medium was added to each dish. Each dilution was replicated three times and the dishes were incubated for 5-7 days at 25± 2°C.

Identification was done by microscopic analysis using taxonomic guides, standard procedures and other relevant literature available on fungal systematic (Raper and Fennell, 1965; Ellis, 1971; Moubasher, 1993; Barnett, 1967; Gilman, 2001). Further, the identification of isolates was also confirmed by Reference Cultures of ITCC (*Alternaria alternata*; 1774, *Alternaria tenuissima*; 1744, *Aspergillus flavus*; 1419, *Cladosporium herbarum*; 3137, *Fusarium oxysporum*; 1635, *Rhizopus arrhizus*; 3069), MTCC (*Aspergillus fumigatus*; 3070, *Aspergillus niger*; 2196, *Rhizopus oryzae*; 2233), QUCC (*Rhizopus stolonifer*; 5710) and NFCCI (*Mucor racemosus*; 2217).

Data analysis

Periodicity of occurrence, species richness, similarity and dissimilarity index

The term periodicity of occurrence was used in presenting the data. The periodicity of occurrence denotes the number of samples in which a fungus was present against the total number of samplings. The periodicity of occurrence of fungi was arbitrarily classified as per Saravanakumar and Kaviyarasaran (2010): Common- recorded in 5-7; Frequent in 4-5; Moderate in 2-3 samplings and Rare in 1-2 samplings

Species diversity is a statistical abstraction with two components *viz.*, species richness and evenness. Total number of species on sites/locations was considered as species richness. Similarity index of populations/communities was used to compare the sites. In order to determine this parameter, any quantitative character is taken into consideration. In the present approach, the index(s) was calculated using species richness following Sorenson (1948) as:

$$S = \frac{2C}{A+B}$$

where A- Number of species in community A, B- Number of species in community B and C- Number of species common to both of the communities

Dissimilarity Index (D) was calculated as: D- 1-S

Table 1 : Soil pH, texture and moisture content of different sampling sites

Sampling sites	Parameters		
	pH	MC	Texture
Doiwala	7.42	7.23	clay
Harawala	7.10	9.01	clay
Tunwala	7.80	9.84	loam
Mohkampur	7.59	9.89	loam
Subhashnagar	7.26	10.01	loam

RESULTS AND DISCUSSION

Physico-chemical properties of soil

Physically the textures of soil samples were loam to clay dominating. Moisture content varied from 7.23 to 10.01. The pH values ranged from 7.10 to 7.80 and differed significant statistically (P> 0.05) (Table 1).

Isolation of soil mycoflora

A total of 23 species of microfungi belonging to 11 genera were isolated from all the wheat growing agricultural lands of Doon Valley of Garhwal Himalaya. Highest number of species were isolated from upper soil (0-10 cm depth) while the lowest number of species were isolated from deeper profile (10-20 cm). *Aspergillus* was the most dominant genus followed by *Alternaria, Cochliobolus* and *Rhizopus*. *Aspergillus niger* was the most common fungus isolated from wheat fields with maximum contribution (91.30%), whereas, *Cochliobolus*

Table 2 : Mycoflora of different wheat (*Triticum aestivum*) croplands of Doon Valley

Name of fungus	Depth (cm)	DOI(1)	HAR(2)	TUN (3)	MOH (4)	SUBH (5)	Periodicity of Occ.	Percent contribution
Alternaria alternata	5	1	3	4	6	2	C	69.56
Alternaria chlamydospora	5	2	0	0	8	0	M	43.47
Alternaria tenuissima	10	0	4	0	3	0	M	30.43
Aspergillus flavus	5	0	2	3	0	5	F	43.47
Aspergillus fumigatus	10	3	8	1	3	5	C	86.95
Aspergillus niger	5	7	4	2	4	4	C	91.30
Cladosporium herbarum	15	0	0	3	0	0	R	13.04
Cladosporium sphaerospermum	20	0	5	8	0	0	M	56.52
Cochliobolus australiansis	15	1	0	0	0	0	R	4.34
Cochliobolus sativus	5	5	7	3	0	0	F	65.21
Cochliobolus spicifer	10	0	0	0	0	6	R	26.08
Eurotium repens	5	3	0	0	0	3	M	26.08
Fusarium graminearum	10	0	0	0	2	0	R	8.69
Fusarium oxysporum	10	6	4	3	1	2	C	69.56
Helminthosporum rostrata	5	1	0	0	0	5	M	26.08
Heliminthosporium tetramera	5	1	2	1	0	7	F	47.82
Humicola sp	15	0	0	0	6	0	R	26.08
Mucor circinelloides	10	0	3	0	5	1	F	39.13
Mucor racemosus	SS	5	4	4	1	2	C	69.56
Penicillium sp	5	0	0	0	7	0	R	30.43
Rhizopus arrhyzus	10	7	1	4	1	1	C	60.86
Rhizopus oryzae	15	1	0	2	1	0	F	17.39
Rhizopus stolonifer	5	12	5	2	0	0	F	82.60

SS- Surface soil; 0- not present C-Common, M- Moderae, F-Frequent, R-Rare

austarliensis (4.34%) was the least important species (Table 2).

Periodicity of Occurrence

Of the total 23 species recorded, six species *viz., Alternaria alternata* (69.56%), *Aspergillus fumigatus* (86.95%), *Aspergillus niger* (91.30%), *Fusarium oxysporum* (69.56%), *Mucor racemosus* (69.56%) and *Rhizopus arrhyzus* (60. 86%) were of common occurrence, whereas, six species namely *Aspergillus flavus, Cochliobolus sativus, Helminthosporium tetramera, Rhizopus stolonifer, Mucor circinelloides* and *Rhizopus oryzae* showed frequent occurrence. *Alternaria chlamydosporum, Alternaria tenuissima, Cladosporium sphaerospermum, Eurotium repens* and *Helminthosporium rostrata* were of moderate occurrence. Rest of the genera showed rare occurrence in all the agricultural sites (Table 2, Fig. 2 & 3).

Comparative analysis of fungal diversity

A total of 23 species belonging to 11 genera were

Table3 : Similarity and dissimilarity Index

Sites	DOI		HAR		TUN		MOH		SUBH	
	IS	DS	IS	DS	IS	DS	IS	DS	IS	DS
DOI	0.667	0.330	0.704	0.296	0.592	0.408	0.692	0.308		
HAR			0.846	0.154	0.615	0.385	0.720	0.280		
TUN					0.538	0.462	0.640	0.360		
MOH							0.560	0.440		
SUBH										

IS-Index of similarity, DS- Dissimilarity index

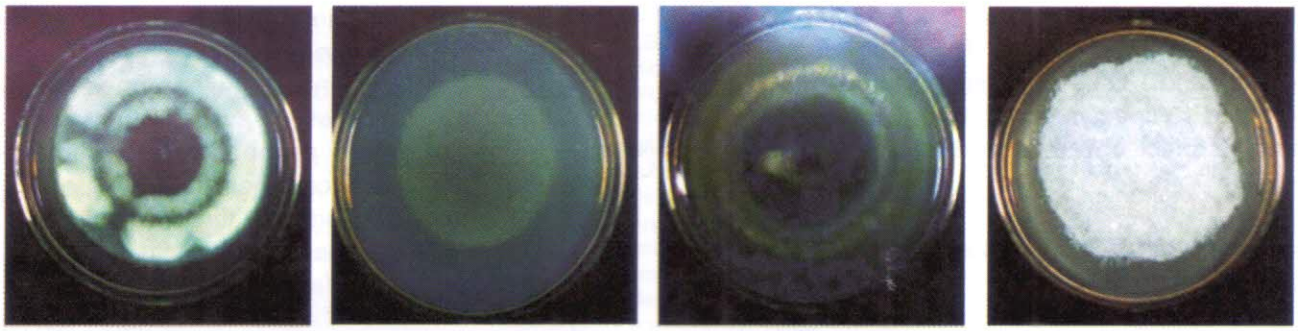

Fig. 2 : a- *Alternaria alternata*; 7-day-old colony on MEA, b- *Aspergillus fumigatus*; 7-day-old colony on CDA, c- *Cochliobolus australiensis*; 5-day-old colony on MEA, d- *Mucor racemosus*; 5-day-old colony on MEA.

Fig. 3 : a- *Alternaria alternata*; 7-day-old colony on MEA, b- *Aspergillus fumigatus*; 7-day-old colony on CDA, c- *Cochliobolus australiensis*; 5-day-old colony on MEA, d- *Mucor racemosus*; 5-day-old colony on MEA.

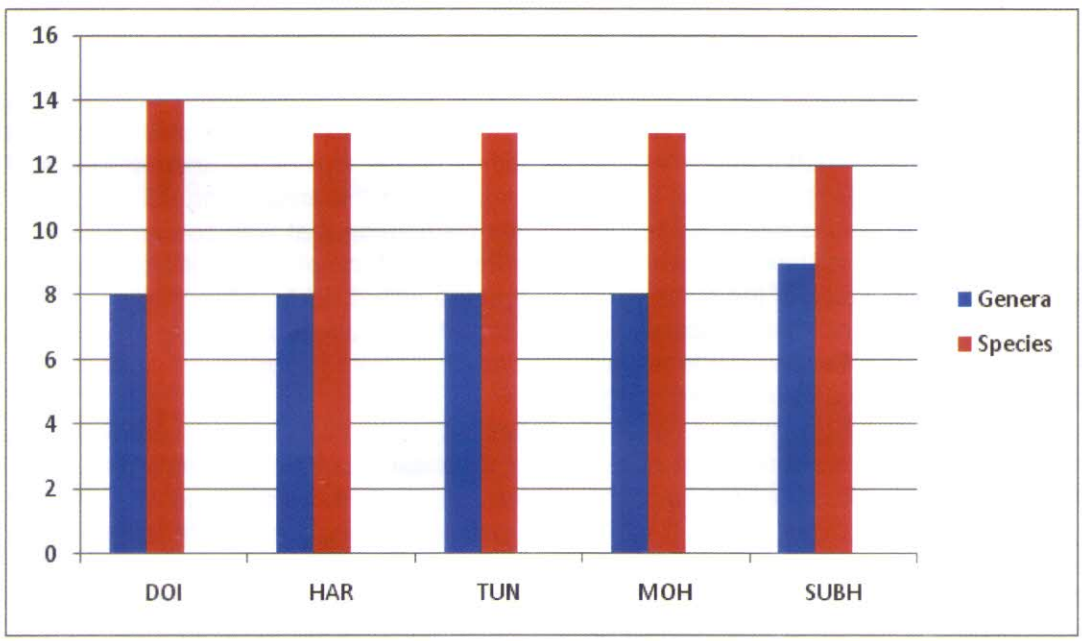

Fig. 4 : Total number of genera and species isolated from different sites.

isolated from all agricultural sites during the study period. Eight genera and 14 species from DOI, 8 genera and 13 species from TUN and HAR, 7 genera and 13 species from MOH, whereas, 8 genera and 12 species were isolated from SUBH soil (Fig 4)

Species richness, similarity and dissimilarity index

Highest species richness was observed in DOI (14) while the lowest was found in SUBH (12). Highest similarity index (84.6%) was found between HAR and TUN while lowest similarity index (53.8%) was found between TUN and MOH (Table 3).

It has been reported by many researchers that the soil moisture, pH and organic matter content influence the activity of soil microorganisms (Rama Rao, 1970; Behera and Mukerji, 1985). Fungi generally grow well in acidic conditions (Dix and Webster, 1995), but Jensen (1931) and Yamanaka (2003) have proved that fungi are abundantly found in alkaline soils and play a dominant role in the microbiological activity of such soils (Waksman, 1927).

In the present study soil pH was near neutral to alkaline which favours microfungal growth. There was no marked variation in soil texture of the different sites and there is no significant effect of texture on fungal populations (Luitel and Koirala, 2009).

Species confined to upper layers were rarely found in deeper soils. This specific distribution is ruled by the availability of organic matter and oxygen to CO_2 ratio in the soil atmosphere of various depths (Giri *et al.*, 2005). These results are in agreement with the previous findings that biochemical activities tend to be greatly increased in the surface soil layer (0-10 cm) along with nutrient concentration (Aon and Colaneri, 2001). Further, surface soils of grazing lands and croplands are comparatively rich in decaying organic matter with majority of annual herbal constituents which support a variety of microbes including microfungi (Guleri *et al.*, 2013).

The majority of the taxa showed qualitative variation with increasing soil depth. In agriculture systems, wide changes in community structure take place at the surface layer due to activity of herbaceous undergrowth. At greater depth, differences of distribution level or patterns are usually reversed according to the prevailing conditions (moisture, potential oxygen and substrate availability) at various intervals after the respective operations (Domsch, 1986). The occurrence of fungal populations are also correlated with the availability of mineral nutrition and other factors including temperature, moisture, etc. (Vanvuurde and Schippers, 1980).

In the present study also the species of *Aspergillus* are not only dominant but are common and integral associates to the soils under study. Rama Rao (1970) and Domsch and Gams (1972) suggested that species of *Aspergillus* are more common in tropical soils. *Penicillium* predominated in the winter, while *Aspergillus* occurred more frequently in summer showing season specific adaptability.

For a given community, it is generally observed that one or a few species are numerically predominant and may strongly affect environmental conditions for other species (Durall and Parkinson, 1991). In the present study, few species were regularly isolated at relatively high frequencies. These species also had the most widespread and least aggregated distribution. The low levels of aggregation observed for these species may reflect a relatively broad or diverse niche space that may be the result of successful adaptation to many dimensions in the system.

The purpose in categorizing soil fungi based on their appearance in all or some sites and on their abundance is to indicate their chances of disappearing from the areas. The soil fungi with a species distribution that was categorized as common or frequent, but not rare, will not disappear easily from the wheat fields, while those that distribution was categorized as moderate or rare will disappear easily from the sites.

Similarity is considered as an index of homogeneity of habitat (Clarke and Christensen, 1981). However, same does not hold true when we look at the population of fungi for a particular sampling depth. It appears that during digging or ploughing the soil is mixed, thus the fungal species are distributed almost uniformly up to depth taken into consideration. To conclude, the study is an attempt to explore the soil microfungal diversity of Doon Valley

under wheat cultivation. The investigation revealed the isolation and identification of 23 species belonging to 11 genera. It is hoped that further investigation of Doon Valley soils under different cultivation practices like paddy (*Oryza sativa*), sugarcane (*Saccharum officinarum*), pulses and other horticultural crops will definitely add to the knowledge of microfungal flora adapted to different agro-ecosystems.

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