# Seasonal variation in the diversity of soil microfungi of some grazinglands of Doon Valley of Uttarakhand Himalaya

#### \*\*SAURABH GULERI, \*SEEMA SAXENA AND BHUPENDRA SINGH BHANDARI

Department of Botany and Microbiology, HNB Garhwal University, Srinagar Garhwal, Uttarakhand, India \*Department of Botany, S.G.R.R. (P.G.) College, Dehradun, Uttarakhand 248001, India

Received : 17.12.2012	Accepted : 11.01.2013	Published : 28.10.2013

Soil is a dynamic living in which the biological activity is mostly regulated by microorganisms. Besides others, soil fungi play an important role as major decomposers in the soil ecosystem. Many soil fungi are biological control agents for plant pathogens and insect pests. In Uttarakhand Himalaya there has been a very little research regarding microfungi in general and soil microfungi in particular. Therefore, the present communication attempts at providing information on the composition, dominance and diversity of soil microfungi in some grazinglands of Doon Valley of Uttarakhand Himalaya. Four grazinglands viz., Chakrata (CHK), Lakhamandal (LKH), Sahiya (SAH) and Bhadraj (BHD) were selected to isolate the soil fungal diversity. Each grazingland was repeatedly surveyed seasonwise to collect soil samples to observe the impact of seasons" on richness and diversity of soil microfungi. In addition, soil from different depths viz., 0 cm (surface soil), 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, and 20-25 cm were also analysed to explore the distribution of these fungi at different depths. A total of 34 species belonging to 16 genera were isolated from grazingland soils. During the investigation, Deuteromycetes dominated the soil mycoflora with 9 genera and 24 species. Ascomycetes and Zygomycetes both were represented by 3 genera and 5 species. Oomycetes was monotypic with one genus. Highest number of species were recorded during winter season followed by rainy season. Surface soils were generally rich in mycoflora as compared to deeper profiles. Some species were recorded throughout the year, some appeared only after distinct seasonal interruption while others were season specific, Alternaria alternata and Mucor sp. encountered most commonly in all seasons and soils indicating the wider ecological amplitude of these species. Shannon-Wiever index of species diversity ranged from 1.96 to 2.54 for CHK, 1.97 to 2.76 for LKH, 2.12 to 2.74 for BHD and 1.94 to 2.69 for SAH grazinglands. Simpson's index of diversity for soil mycoflora also showed the identical trend i.e. BHD?LKH>SAH>CHK. Highest similarity (98.4%) was observed between CHK and LKH and lowest (93.7%) between CHK and BHD.

Key words: Doon Valley, grazingland, mycoflora, season, soil profile

#### INTRODUCTION

Soil is a complex and dynamic environment in which the biological activity is mostly governed by microorganisms. The beneficial effects of soil mi-

\*\*gulerisaurabh26@gmail.com

croorganisms are manifold and range from nitrogen-fixation and organic matter decomposition to breakdown of metabolic by-products and agrochemical, enhancing the bioavailability of nitrates, sulphate, phosphates and essential metals (Bridge and Spooner, 2001).

The fungi (pathogenic and non pathogenic) that

are associated in the soil are known as soilborne fungi. 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 and Bisby, 1995). The distribution of these organisms is influenced by the abundance and nature of the organic content of the soil as well as by other soil and climatic conditions, surface vegetation and soil texture (Marschner *et al.*, 2003).

Soil fungi play an important role as major decomposers in the soil ecosystem. Besides, they also provide mankind with very useful pharmaceutical products, such as antibiotics and other valuable substances, including organic acids, enzymes, pigments and secondary metabolites used in the food industry and fermentation. In addition, many soil fungi are biological control agents for plant pathogens and insect pests. On the other hand, some of them are very harmful causing food spoilage and diseases to plants, animals and humans with significant economic losses and produce mycotoxins in certain products (Pongsatorn *et. al.*, 2010).

Waksman (1916) has been the first to demonstrate that the soil is truly a dynamic ecological habitat with an active complex of fungal populations. Since then numerous workers all over the world have investigated the fungal flora of various types of soils. Among the recent contributions include the work of Marschner (2003), Dong *et al.* (2004), Gleason *et al.* (2004), Song *et al.* (2004), Yu *et al.* (2007), etc. In India appreciable work has been done by various workers from different places (Saksena and Sarbhoy, 1963; Dwivedi, 1966; Rama Rao, 1970; Manoharachary, 1977; Bilgrami 1979, 1981; Behera and Mukerji, 1985; Giri *et. al.*, 2005; Rane and Gandhe, 2006; Saravanakumar and Kaviyarasan, 2010).

With reference to Uttarakhand Himalaya there has been a very little research regarding microfungi in general and soil microfungi in particular (Guleri *etal.* 2010; 2011; Bhandari *et al.* 2012). Therefore, considering the importance of microfungi in dynamic, living and complex soil systems coupled with paucity of information, the present investigation has been undertaken to study the seasonal behaviour of soil microfungi in different grazinglands of Doon Valley of Uttarakhand Himalaya.

#### MATERIALS AND METHODS

#### Study site

The study was carried out in the Doon Valley of Uttarakhand Himalaya (77°45? to 78°15?E longitude and 30°00? to 30°35?N latitude) (Fig 1). The valley lies between the West Himalayan mountain in the North and the Shiwalik range running parallel to it in the South at a mean altitude of 485m. Four grazinglands namely Chakrata (CHK), Lakhamandal (LKH), Sahiya (SAH) and Bhadraj (BHD) were selected to explore the soil microfungal diversity. The climate is characterized by a delightful cold weather, hot summers followed by rainy season. The grazinglands were repeatedly surveyed seasonwise *viz.*, winter, summer and rainy to analyse the impact of different seasons on the dominance and diversity of soil microfungi.

#### Collection of soil samples

Soil samples were collected from different grazinglands during different seasons of the study period (2008-2009). For the collection of soil samples a soil profile was randomly selected and the surface of the profile was cleaned (Brown, 1958) in order to avoid extra contamination. Soils of different depths viz., 0 cm (Surface soil), 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm and 20-25 cm were taken aseptically with the help of sterilized steel tube. Five to six such cores of soil samples were collected randomly. The soil of each different depth was mixed with the soil of same depth of different cores of a spot to make composite samples. In the field sampling for the isolation of soil microfungi, six composite samples during summer, six during rainy and six during winter were collected from each of the grazingland ecosystem. As such, a total of 72 soil samples were collected from the four grazinglands during the investigation. Samples were kept in the sterile bottles and brought to laboratory for the isolation and study of soil microfungf alongwith the analysis of physico-chemical properties of soil.

#### Soil analysis

The soil pH was determined by using digital pH meter (model 153 P, Toshon, India). Moisture content of soil samples was calculated by oven drying

Saurabh Guleri and Others



तबहरू जुंगी फ्रिस्टन

Fig. 1 : Map of the study area and location of sampling sites

the soil and determining the weight loss (Garrett, 1963). Organic carbon was determined using standard method (Hooda and Kaur, 1999), and the texture was determined by sieving and weighing method (Piper, 1944).

#### Isolation of soil mycoflora

The soil microfungi were isolated by Soil Dilution (Waksman and Fred, 1992) and Soil Plate Method (Warcup, 1950) using different media viz., Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA) and Malt Extract Agar (MEA). Pure cultures of the fungi were obtained by single spore culture and sometimes by hyphal isolation methods (Warcup, 1955a). Identifications were confirmed by microscopic analysis using taxonomic guides, standard procedures and other relevant literature (Raper and Fennell, 1965; Ellis, 1971; Moubasher, 1993; Barnett and Hunter, 1998; Gilman, 2001). Rare genera like Aspergillus awamori (NFCCI 2216) and Mucor racemosus (NFCCI 2217) were deposited in National Fungal Culture Collection Centre of India- Agharkar Research Institute, Pune for obtaining Accession Number.

The term periodicity of occurrence was used in presenting the data. The periodicity of occurrence denotes the number of samplings 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 Kaviyarasan (2010):Common—recorded in 10-15 samplings; Frequent—recorded in 7-9 samplings; Moderate—recorded in 4-6 samplings; Rare—recorded in 1-3 samplings

### Species richness, similarity and dissimilarity index

Species diversity is an 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):

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

where, A= Number of species in community A, B= Number of species in community B; C= Number of species common to both of the communities Dissimilarity Index (D) was calculated as: D=1-S

#### Diversity and other parameters

Shannon-Wiever Index, Simpson Index, Total dominance and Evenness were analyzed using a computer software program (Bio-tool kit 320). Analysis of variance was calculated to compare the sites with respect to different soil properties like pH, moisture and organic content.

#### RESULTS

#### Analysis of soil parameters

Table 1 indicates that organic content of all soil samples taken from grazinglands varied greatly from 0.18 to 0.81% between different sites. The pH varied from  $6.04\pm0.84$  to  $8.35\pm1.98$  in summers,  $5.64\pm1.83$  to  $7.14\pm1.44$  in winters and  $5.46\pm0.77$  to  $8.57\pm2.16$  in rainy season. The moisture content of the soil samples ranged from  $5.64\pm1.41\%$  to  $7.92\pm0.85\%$  in summer,  $7.0\pm0.54\%$  to  $9.36\pm0.50\%$  in winter and  $8.61\pm0.57\%$  to  $11.35\pm2.42\%$  in rainy season. Soil texture was loam to sandy-loam except in Bhadraj (BHD) where it was clayey-loam.

#### Soil fungal diversity

Table 2 shows the fungal classes, seasonal variations in species richness and number of colonies with percentage contribution of individual species and the periodicity of occurrence of soil fungi at different grazingland sites. It is evident that a total of 1,178 colonies of soil fungi belonging to 16 genera and 34 species were isolated during different seasons. Deuteromycetes was the dominant class with 9 genera and 24 species. Zygomycetes and Ascomycetes both were represented by 3 genera and 5 species. Oomycetes was monotypic with single genus i.e. *Pythium* sp.

During the study period on all the grazinglands, maximum number of species were isolated during winter season followed by rainy season, whereas, minimum number of species were recorded during summer season (Table 2).

*Ulocladium* sp. was the most dominant during summer season with maximum contribution (16.94%)

udy sites.
s
the
ð
parameters
Soil

Table .

9.36±0.5 7.04±1.2 6.19 8.98 9.60 9.62 9.24 7.24 9.70 9.06 7.70 7.54 7.60 6.0 ≥ 0 11.35±2.4 7.3±1.53 SAH 10.94 10.25 38.50 30.00 31.00 Loam 13,29 11.6 12,2 9.86 6.76 6.28 8.45 7.29 6.98 0.81 8.04 œ 5.64±1.4 8,35±1.9 5.98 7.96 6.20 8.70 8,66 4.24 9.81 5.20 6.0 8.0 7.0 6.24 ഗ 7.14±1.4 8 81±0.7 6.98 6.26 B.98 7.92 7.98 8.04 7.60 9.04 8.92 9.01 6.0 9.0 ≥ 8.57±2.16 9.77±0.86 Clayey. GНВ 28.00 26.00 10.19 10.02 10.02 45.32 Loam 10.25 9.96 8.98 6.96 9.18 9.02 9.42 9.06 7.02 0.31 œ 6.68±1.3  $7.92\pm0.8$ 6.19 7.92 6.24 5.64 8.19 8.20 7.60 7.20 8.05 7.24 7.0 8.21 l o 5.64±1.8 7.0±0.54 7.20 5.92 6.98 7.02 6.60 6.08 6.24 6.52 7 29 7.0 5.0 4.0 ≥ ო Sandy-Loam 10.82±0.92 Grazingland/Season\* 5.46±0.77 LKH 27.00 13.50 11.12 11.07 10.84 70.00 11.17 9.86 5.02 10.9 6.12 5.46 5,12 5.26 0.27 5.81œ 6.71±0.7 6.04±0.8 6.19 7.03 6.92 7.03 6,38 5.18 6.30 6.24 6.26 6.0 7.04 6.01 S 5.98±2.9 8.14±0.2 4.16 8.40 7.98 8 01 8.24 8.24 7.29 8.19 6.24 4,0 8.0 6.0 ≥ 8 61±0 5 6.96±1.1 CHK 32.00 25.50 42.50 Loam 6.19 0.18 8.92 8,92 8,37 7.85 7.24 7.29 6.98 6.24 8.29 9.01 8.2 œ 7.18±0.9 6.38±1.4 \*S- Summer, R- Rainy, W- Winter 5.20 5.64 6.06 7.55 7,48 6.24 7.24 7.20 6.98 7.60 7.01 7.21 ശ S Depth 10-15 20-25 0 (SS) 10-15 15-20 20-25 0 (SS) 15-20 (cm) 5-10 5-10 0-5 0-2 Moisture Content % Sand Texture content Organic % Clay % Silt Class Mean Mean (%) Hd (%) 002862

: 51(2) October, 2013]

Saurabh Guleri and Others

217

Color No.Color No.Accomposition5cc30c3cc<	Fungal species	(cm)		Sur	mer				ĕ	ainy					Winter			
CHV         BHD         SAH         Percent         CHV         BHD         SAH         Centroling         CHV         BHD         SAH         CHV         BHD         SAH         Centroling         CHV         SAH         CHV         SAH         CHV				Colc	N Vuc	ó		8	Colc	NV VO				0	olonv A	0	Par cent	Periodicity
Acomycetes     Acomycetes     Acomycetes     To     Acomycetes     S			Ę	E E	ы т	-ID SAH	Percent Contribution (%)	CHK	Ŧ	GНB	SAH	Percent Contribution (%)	Ъ.	Ĕ	HO HO	D SAH	Contribution (%)	Occurrence
Automotoureso:     10     30     -	Ascomycetes																	
Transcriental ruguicues <ol> <li>i = 1</li> <lii 1<="" =="" li=""></lii></ol>	Arachniotus sp.	10	30	;	С	ę	15.25	£	Ð	1	ġ	ţ.	5	8	ł.	¢	ţ)	L
E. sp.         6         · <td>Emericella rugulosa</td> <td>ى ئ</td> <td>ł</td> <td>ł</td> <td>1</td> <td>ł</td> <td>4</td> <td>ा</td> <td>3</td> <td>ं</td> <td>1</td> <td>ł</td> <td>C)</td> <td>ຍ ດ</td> <td>20</td> <td>4</td> <td>8.10</td> <td>ш</td>	Emericella rugulosa	ى ئ	ł	ł	1	ł	4	ा	3	ं	1	ł	C)	ຍ ດ	20	4	8.10	ш
Coordinationals         12                101         101           austratiansis         8               1         20         743           austratiansis         8              1         20         7         20         743           Cuberculaus         8               1         20         6         20         743           Zuberculaus         8                 1         1         20         743           Zuberculaus         5         8                1         1         135           Mucorspus         55         8         5         10         1186           1         1         135           Mucorspus         55         1         1         1         1         1	E. sp.	G	ł	ł	£	ł	i.	9	12	4	N	6.81	1	8	N.	:	Î.	L
austrationation         B	Cochliobolus	12	1	;	a,	ŗ	ł	ः	3	a	1	1	υ Ω	30	9	20	10.81	L
Circinetias         S         -         -         -         -         -         -         -         -         -         -         -         -         -         -         3.37           Zjonnyceles         S         -         -         -         -         -         -         -         1         1.35         3.37           Circinella spinosa         S3         -         -         -         -         -         1         6         -         1         1.35           Mucorracemosus         S3         2         5         10         11.86         10         9         8         9         10.22         4         8         14         1.35           Mucorsp.         S3         2         5         3         6         6.77         -         -         -         1         1.35         -	australiensis	œ	ł	-	ŧ	ı	- 6	£:	40	t	i	1	-	Х	33	20	7.43	Ľ
Zygomycetes         B           -         -         -         -         -         -         -         3.37           Cirinella spinosa         SS         -         -         -         -         -         -         -         -         1         1         3.37           Cirinella spinosa         SS         -         -         -         -         -         -         1         1         1         1         1         1           Mucorracemosus         S         5         0         11.86         10         9         9         10.22         4         8         14         6         5         10           Mucorsp.         SS         2         5         10         11.86         10         9         10.22         4         8         14         6         5         10           Mucorsp.         SS         2         3         6         6.77         1         1         10.16         9         10.22         4         8         14         4         7.35           Mucorsp.         SS         2         1         1         10.16         9         9         10	C. tuberculatus																	
Chronella spinosa         SS         -         -         -         -         -         -         -         1         1.35         -           Mucorrazemosus         5         8         5         10         11.86         10         9         8         9         10.22         4         8         14         6         5.40           Mucorrazemosus         5         8         5         3         6         6.77         9         9         10.22         4         8         14         6         5.40           Mucorsp.         SS         2         5         3         6         6.77         9         9         10.22         4         8         14         6         5.40           Mucorsp.         SS         2         5         3         6         6.77         9         9         10.22         4         8         4         4         7           Hitzopus arriyzus         18         7         14         10.16         9         9         1         6         4         4         4         7           Printursp.         1         10.16         9         9         9         1         2<	Zygomycetes	σ	4	ŧ	а	ł	,	4	4	1	1	<u>9</u> 1		ω Ω	80	4	3.37	œ
Mucorracemosus         5         8         5         10         11.85         10         11.85         10         11.86         10         5         4         8         14         6         5.40           Mucorracemosus         SS         2         5         3         6         6.77         1         1         1         6         5.40           Mucorspus         SS         2         5         3         6         6.77         1         1         1         6         1         4         5         540           Mucorspus         SS         2         5         3         6         6.77         1         1         1         4         7         5         1         4         7         5         1         4         5         5         1         5         5         1         4         5         5         1         5         5         1         1         1         1         5         1         1         5         1         1         5         1         1         1         1         1         1         1         1         1         1         1         1         1         1	Circinella spinosa	SS	E)	£	Ē	ŧ	ĸ	Ð	Ð	f	ŝ	ı	ŕ	Q	1	-	1.35	а: •
Mucorsp.         SS         2         3         6         6.77         1         1         1         1         1         4         1 <i>Hizopus arthyzus</i> 8         -         -         -         -         -         -         -         -         4         472 <i>Hizopus arthyzus</i> 8         -         -         -         -         -         -         4         4         4 <i>Hizopus arthyzus</i> 12         7         14         2         1         4         1         4	Mucor racemosus	ß	œ	S	Ω	10	11.86	10	б -	8	6	10.22	v	æ	14	Q	5.40	O
Hizopus arrhyzus       8       -       -       -       -       -       -       -       4.72         Hizopus sp.       .       1       1       10.16       -       -       -       12       8       4       4       4.72         Omvoetes       12       7       14       2       1       10.16       -       -       6       4       2       4       2.70         Pythium sp.       .       .       .       10.16       .       .       .       6       4       2       4       2.70         Pythium sp.       .       .       .       .       .       .       .       .       .       .       2.70         Deuteromycetes       5       20       2       1       1       10.16       .	Mucor sp.	SS	N	S	ო	9	6.77	£5	ŧ	£	ŧ	Ĕ	5	5	Ę	Ĕ		Σ
<i>Hizopus.sp.</i> 12       7       14       2       1       10.16       -       6       4       2       4       2.70 <i>Pythiumsp.</i> 5       20       2       1       1       10.16       20       8       -       7       6       4       2       7       6.08 <i>Pythiumsp.</i> 5       20       2       1       10.16       20       8       -       7.95       7       6       4       19       6.08 <i>Deuteromycetes</i> 5       20       2       1       10.16       20       8       -       7.95       7       6       4       19       6.08 <i>Atternaria atternata</i> 8       3       5       3.38       -       1       19       6.08	Rhizopus arrhyzus	ω	đ	ł	1	ł	( <b>k</b>	3	з	a	1	ł	f	B N	4	4	4.72	U.
Omycetes         12         7         14         2         1         10.16         1         10.16         1         2         4         2         4         2.70           Pythiumsp.                   2.70           Pythiumsp.                   2.70           Deuteromycetes <td< td=""><td>Rhizopus. sp.</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>	Rhizopus. sp.																	
Pythiumsp.         Deuteromycetes       5       20       2       1       10.16       20       8       -       7       6       4       19       6.08         Meteromycetes       5       20       2       1       10.16       20       8       -       7       6       4       19       6.08         Atternaria afternata       8       3       -       5       3.38       -       -       4       14       8       2       4.72	Oomycetes	12	7	14	N	-	10.16	ं	а	4	1	ï	Ĵ	4	~≀	4	2.70	O
Deuteromycetes       5       20       2       1       10.16       20       8       -       7.95       7       6       4       19       6.08         Alternaria alternata       8       3       -       5       -       3.38       -       -       4       14       8       2       4.72	Pythium sp.																	
Afternaria afternata 8 3 5 5 3.38 1 1 1 4 14 8 2 4.72	Deuteromycetes	Q	20	N	-	-	10.16	22	80	æ	ţ.	7.95	17	9	4	19	6.08	O
	Alternaria alternata	80	ი	à	2	9	3.38	24	19	8		(j	V	1	8	0	4.72	O
		1,00																

218

On seasonal variation of soil microfungi

[J. Mycopathol. Res. :

P.T.O

(contd. table 2...)

ŝ

4.72 C	2.70 M	E.	W	3.37 C	Σ	6.75 C	4.72 M	u t	4.72 F		4.05 M	W	а.	ш. (112)	4.72 M	LL A	LL St	2.70 R	3.37 M	ш Э	W	8.10 C	
2	ę	;	ł	10	ı	0	8	ł	5		8	ł	1	1	7	١	ł	7	9	ł	ł	14	
8	9	ł	I	2	$\left\{ i \right\}$	12	თ	t	-		4	ł	ł	I	12	ł	1	S	8	)	Ű.	10	
14	ഹ	ŧ	3	Ð	9	ø	10	ł	ŝ		თ	:	I	;	9	;	ł	ი	4	ŧ	1	4	
4	2	ť,	0	ю	्व	18	-	÷	20		ო	ł	ł	;	ო	I	ł	-	N	3	<u>R</u>	20	
Ē	ł	t	7.95	10.22	1	13.63	J.	5.68	1		ŝ	7.95	10.22	Ę.	i)	4,54	60.6	4	9	I	5.68	ł	
ţ.	1	1	10	4	1	18	зţ;	N	4		1	8	σ	1	ſ	ß	4	I	:	I	10	1	
ŧ;	1	t	4	ഗ	3	9	E	¢	аr.		43	10	N	ł	:	4	9	1	ł	)i	Ω.	ł	
Ð	3	ъ	ო	φ	38	o	£5	9	12		£	9	თ	ł	1	S	2	1	1	I	2	I	
ŧ	94	30	÷	19	8 <b>1</b> 0	20	ß	4	ts		t	4	16	È	I.	2	20	ŧ	4	1	e	I	
3.38	1	3.38	9	i	6 77	ï	Ŭ)	ji	ŝ		£	ţ	(I)	8.47	1	I	I	I	1	6.77	1	16.94	
I	:	1	;	3	4	ł	1	ł	:		r	1	E	10	ŧ	x	0	3	3	4	đ	2	
£	1	I	ł	ł	4	t	È	ŧ	I.		ţ,	ŧ	- Ú	4	Î.	:	:	3	1	1	ł	4	
F.	54	4	4	æ	8	÷	C.	з	ю		t	а	10	4	30	:	а	1	31	4	1	18	
ო	1	4	1	$\tilde{E}$		Ŧ	ŧ	3	Ę		ł	3	ţ,	0	I	ł	ł	ł	l	Ø	đ	16	
ø	8	9	15	10	15	ŝ	Ŋ	വ	8		9	ъ	10	ß	ω	5	ß	9	SS	15	8	10	
A .tenuissima	A .humicola	Aspergillus awamori	A.chevalieri	A. fumigatus	A. nidulans	A. niger	A. repens	A.ustus	Cladosponium	herbarum	C.sp.	Curvularia interseminata	C.tuberculata	Csp.	Fusarium dimerum	F.moniliforme	F.oxysporum	Fsp.	Helminthosporium nodulosum	Penicilliumsp.	Rhizoctoniasp.	Ulocladiumsp.	

followed by Arachniotus sp. (15.25%) and Mucor sp. (11.86%). Alternaria alternata and Pythium sp. showed their presence by 10.16%. Alternaria tenuissima and Aspergillus awamori (3.38%) were the least important species followed by Aspergillus nidulans and Penicillium sp. (6.77%) (Table 2).

During rainy season, Aspergillus niger was the most dominant species with maximum contribution (13.63%) followed by Aspergillus fumigatus, Curvularia tuberculata and Mucor sp. (10.22%). Though maximum number of species were isolated during winter season, however, there was no sharp variation in percent contribution of individual species. Five species viz., Rhizopus sp., Aspergillus repens, Alternaria tenuissima, Cladosporium herbarum and Fusarium dimerum contributed equally by 4.72% (Table 2).

Season to season variation in species richness and diversity was observed during the study period. Some fungal species like Alternaria alternata and Mucor sp. were recorded throughout the year irrespective of seasons. While, Alternaria tenuissima, Aspergillus fumigatus, Aspergillus niger, Pythium sp. and Ulocladium appeared only after distinct seasonal interruption. Many species like Aspergillus awamori, Aspergillus ustus, Circinella spinosa, Arachniotus sp., Alternaria humicola, Cladosporium herbarum, Fusarium dimerum, Fusarium Fusarium oxysporum and moniliforme, Helminthosporium nodulosum, etc., were season specific. In general, higher number of fungal species were found at the superficial layers (0-10cm) than deeper soil profile (Table 2).

#### Periodicity of occurrence

Of the total 34 species recorded, seven species viz., Alternaria alternata, Alternaria tenuissima, Aspergillus fumigatus, Aspergillus niger, Mucor sp., Pythium sp., and Ulocladium sp. were of common occurrence. Thirteen species viz., Arachniotus sp., Emericella rugulosa, Emericella sp., Cochliobolus australiensis, Cochliobolus tuberculatus, Rhizopus sp., Aspergillus ustus, Cladosporium herbarum, Curvularia tuberculata, Curvularia sp., Fusarium moniliforme, Fusarium oxysporum and Penicillium sp., were of frequent occurrence while, ten species viz., Rhizopus arryhzus, Alternaria humicola, Aspergillus chevalieri, Aspergillus nidulans, As-

pergillus repens, Cladosporium sp., Curvularia interseminata, Fusarium dimerum, Helminthosporium nodulosum and Rhizoctonia sp. were of moderate occurrence. Remaining four species namely Circinella spinosa, Mucor racemosus, Aspergillus awamori and Fusarium sp. were of rare occurrence.

## Species richness, similarity and dissimilarity index

Remarkable difference in species richness was not observed during the study period on different grazinglands but, nevertheless, composition varied greatly from site to site in different seasons. The highest fungal richness was recorded for CHK (33) followed by LKH (32), SAH (32) and BHD (31) (Table 3). Highest similarity (98.4%) was observed between CHK and LKH and lowest (93.7%) between CHK and BHD (Table 3).

#### Diversity, dominance and other parameters

The Shannon Wiever Diversity index was highest (2.765) in LKH during winter followed by CHK (2.268) during rainy and BHD (2.12) during summer as the total richness and abundance was highest in LKH during winter season followed by CHK during rainy season. The species evenness was highest during rainy season in BHD (Table 4). The Simpson Index estimates are close to 1, meaning that probability is very low. The analysis of variance for the different values of soil physico-chemical properties of grazingland sites show that the mean values of soil physical properties (% sand and % silt) were insignificant (P>0.05). The mean value of a soil physical property (% clay) and different soil chemical properties (pH, moisture content and % organic content) were highly significant (P<0.01).

#### DISCUSSION

Morpho-functional diversity of the fungal assemblage increases with fungal biodiversity which suggests that morpho-functional diversity determines the capacity for soil C storage (a low soil C turnover rate in grazinglands reflects high fungal biodiversity). This is in keeping with the observations of changes in the composition and activity of saprotrophic fungi in grazinglands (Bardgett *et al.*, Table 3 : Similarity and dissimilarity index of soil fungi between the grazinglands

	СНК	LKH		BHD		SAH		
		IS	DS	IS	DS	1S	DS	
СНК		0.984	0.016	0,937	0.063	0.953	0.047	
LKH				0.952	0.048	0.984	0.016	
BHD						0.952	0.048	
SAH								

IS- Index of similarity, DS- Dissimilarity index

Table 4 :Diversity indices during different seasons

		Sumr	ner			Win	ler			Rai	ny	
Diversity	СНК	LKH	BHD	SAH	снк	LKH	BHD	SAH	снк	LKH	BHD	SAH
Shannon-Wiever Index	1.96	1.97	2.12	1.94	2.54	2.76	2.74	2.69	2.26	2.36	2.32	2.21
Species richness	10.0	9.0	9.0	9.0	20.0	20.0	19.0	19.0	12.0	12.0	11.0	11.0
Total Abundance	100	64	31	41	140	164	138	150	135	79	62	81
Simpson diversity Index (1-D)	0.823	0.832	0.874	0.831	0.899	0.919	0.925	0.917	0.883	0.899	0.895	0.875
Evenness	0.849	0.899	0.965	0.884	0.848	0.923	0.931	0.912	0.912	0.953	0.968	0.925

1999a,b; Brodie *et al.*, 2003). The conditions associated with higher depth and increased soil moisture in each site determine reduced plant diversity and increased fungal biodiversity (Persiani *et al.*, 2008).

It has been reported by many researchers that the soil moisture, soil pH (Rama Rao, 1970), and organic matter content (Behera and Mukerji, 1985) influence the activity of soil microorganisms. Fungi generally grow well in acidic conditions (Dix and Webster, 1995), but Jensen (1931) and Yamanaka (2003) have proved that fungi are also found abundantly in alkaline soils and play a dominant role in the microbiological activity of such soils (Waksman, 1927). From the present studies it is clear that pH of soil samples generally ranged between five and eight, being neither very acidic nor very alkaline. 34 different species of microfungi were isolated alongwith universal occurrence of bacteria as they are less tolerant to the acid condition (Waksman, 1932). The maximum numbers of fungi were isolated from acidic soil due to the occurrence of lesser number of bacterial colonies. There was no marked variation in soil texture of the sites, the soil texture of the research sites were loam to sandy loam with a few exceptions. The values of simpson index in the present investigation are close to 1 meaning that the probability of occurrence of different species is low. Further, low index of species diversity is due to the fact that in the present study only cultivable soil fungi have been isolated in the laboratory and these do not include several other fungi directly associated with trees and other plants as has been reported in other studies (Nilima satish *et. al.*, 2007). The species evenness was highest during rainy season due to adequate moisture and rotting dead materials on the ground. No significant effect of soil texture was observed on fungal populations as observed by Luitel and Koirala (2009).

The contribution of Zygomycetes was larger in superficial horizon, while that of Ascomycetes was larger in deeper horizons showing depth specific distribution. This specific distribution is ruled by the availability of organic matter and oxygen to CO, ratio in the soil atmosphere at various depths (Giri et al., 2005). It is well established fact that fungi exhibit a selective preference for various soil depths. The significant decrease in the number of species with increasing depth can be attributed to a lower content of organic matter, CO, and oxygen in deeper profile. These results are in agreement with the previous findings that biochemical activities tend to be greatly increased in the surface soil ... layer (0-8cm) alongwith nutrient concentration (Aon and Colaneri, 2001). Species common at lower depths are rarely found on the surface. The distribution and dominance of Deuteromycetes fungi suggested that the fungi belonging to this class are strong colonizers of the decaying substrate with better and wider adaptability coupled with high competitive ability, whereas, those of Zygomycetes

and Ascomycetes were weak colonizers (Rai *et al.,* 2001; Vibha and Sinha, 2007; Kumar *et al.*, 2011) with narrow and/or poor adaptability.

The changes in the soil moisture, organic content, temperature and pH are the important factors determining the fungal populations (Dwivedi, 1966; Dkhar and Mishra, 1987). Results of the present investigation are in accordance with these authors that some of the species appeared only sporadically after distinct seasonal interruption while other species were predominant in all seasons. These species were constant in their occurrence throughout the year and repeatedly isolated in all seasons indicating their wider ecological amplitude. Some species were restricted in specific season because of selective environmental preference and tolerance.

It is concluded from the present investigation that microfungal populations differ drastically season to season and these microorganisms are never uniformly distributed throughout a single horizon. Most of the microfungi are confined to a particular soil profile showing adaptability to a particular depth. The various factors like soil texture and depth, pH, moisture, organic and inorganic nutrients play an important role in determining the richness, diversity and overall growth of microfungi. Higher numbers of species are generally present at surface soils as compared to deeper profiles. It is due to the fact that surface soils of grazinglands are comparatively rich in decaying organic matter with majority of annual herbal constituents which support a variety of microbes including microfungi.

#### ACKNOWLEDGEMENTS

We thank Dr. S.K. Singh, Senior Scientist, Mycology and Plant Pathology Group, National Fungal Culture Collection of India, Agharkar Research Institute, Pune and T. Prameela Devi, Senior Scientist, ITCC New Delhi for proper identification and accession number. Thanks are also due to Dr. A.K. Saksena and Dr. A.P. Singh for help rendered in soil and statistical analysis.

#### REFERENCES

Ainsworth, G.C. and Bisby, G.R. 1995. Dictionary of the Fungi, 8<sup>th</sup> edition. Commonwealth Mycological Institute Kew, Surrey, p.445.

- Aon, M.A. and Colaneri, A.C. 2001. II. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. Applied Soil *Ecology*, **18** : 255-270.
- Bardgett, R.D., Lovell, R.D., Hobbs, P.J. and Jarvis, S.C. 1999a. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biology Biochemistry. 31: 1021-1030.
- Bardgett, R.D., Mawdsley, J.L., Edwards, S., Hobbs, P.J., Rodwell, J.S. and Davis, W.J.1999b. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Functional Ecology.* **13:** 650-660.
- Barnett, H.L. and Hunter, B.B. 1998. Illustrated Genera of Imperfect Fungi. IV ed. APS Press St. Paul, Minnesota, p.218.
- Behera, N., Mukerji, K.G. 1985. Seasonal variation and distribution of microfungi in forest soils of Delhi, India. *Folia Geobot Phytotaxon.* 20: 291-311.
- Bhandari, B.S., Saxena, S. and Guleri, S. 2012. Studies on the lungal flora in rhizosphere of pea plant in subtropical region. In Sati, S.C. and Belwal, M. (eds). *Microbes: Diversity and Biotechnology*. Daya Publishing House, New Delhi. Pp. 269-276.
- Bilgrami, K.S., Jamaluddin and Rizwi, M.A. 1979. Fungi of India. Pt. I. List & Ref., Today and Tomorrow Publ., New Delhi, pp.407.
- Bilgrami, K.S., Jamaluddin and Rizwi, M.A. 1981. Fungi of India. Pt. II. Host Index and Addenda. Today and Tomorrow Publ., New Delhi, pp.268.
- Bridge, P. and Spooner, B. 2001. Soil fungi: diversity and detection. *Plant and soil*, **232**: 147-154.
- Brodie, E., Edwards, S. and Clipson, N. 2003. Soil fungal community structure in a temperate upland grassland soil. *FEMS Mi*crobial Ecology. 45: 105-114.
- Brown, J.C. 1958. Soil fungi of some British sand dunes in relation to soil type and succession. *Ecology*. **46**: 641-664.
- Dix, N.J. and Webster, J. 1995. Fungal Ecology. Chapman and Hall, London, p.560.
- Dkhar, M.S. and Mishra, R.R. 1987, Microbial population, Fungal biomass and CO<sub>2</sub> evolution in maize (*Zea mays*) field soils. *Plant and Soil.* 99: 277-283.
- Dong, A.R., Lv GZ, Wu QY, Song, R.Q., Song, F.Q. 2004. Diversity of soil fungi in Liangshui natural reserve, Xiaoxinganling forest region. *Journal Northeast Forestry University.* 32: 8-10.
- Dwivedi, R.S. 1966. Ecology of soil fungi of some grasslands of Varanasi-II. Distribution of soil mycoflora. Bulletin of International Society of Tropical Ecology. 7: 84-99.
- Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Kew Surrey UK: p.608.
- Garrett, S.D. 1963. Soil Fungi and Soil Fertility. Pergamon Press, Oxford, p.105.
- Gilman, J.C. 2001. A Manual of Soil Fungi. 2<sup>nd</sup> Indian ed., Biotech Books, Delhi, p.392.
- Giri, B., Giang, P.H., Kumari, R., Prasad, R. and Varma, A. 2005. Microbial diversity in soils. In: *Microorganisms in soils: roles in genesis and functions* (eds.Buscot, F.and Varma, A.). Springer, Berlin, Heidelberg, New York: pp.19-55.
- Gleason, F.H., Letcher, P.M., McGee, P.A. 2004. Some Chytridiomycota in soil recovered from drying and high temperatures. *Mycological. Research.* **108**: 583-589.
- Guleri, S., Bhandari, B. S. and Saxena, S. 2010. Ecology of Rhizosphere soil mycoflora of Forest soils of Dehradun District Uttarakhand, *International Transaction in Applied Sciences*, 2: 69-77.
- Guleri, S., Saxena, S. and Bhandari, B. S. and 2011. Soil mycoflora of some selected agroforestry areas of Doon valley (Uttarakhand). *Annals of Forestry* (In Press).
- Hooda, S and Kaur, S. 1999. Laboratory manual for environmental chemistry. S Chand & Co,Ltd, New Delhi, pp 71-91.
- Jensen, H.L. 1931. The fungus flora of the soil. Soil Science.,31: 123-158.
- Kumar, R., Sinha, A., Srivastava, S. and Srivastava, M. 2011. Variation in Soil Mycobiota Associated with Decomposition of

Sesbania aculeate L.. Asian Journal of Plant Pathology 5: 37-45.

- Luitel, K.P. and Kolrala, U. 2009. Soil-borne Fungi of Cultivated Lands of Biratnagar, Morang District, Nepal. Our Nature, 7: 232-235.
- Manoharachary, C. 1977. Microbial ecology of scrub jungle and dry wasteland soils from Hyderabad district, Andhra Pradesh (India), Proceedings of Indian National Science Academy, 43: 6-18.
- Marschner, P., Kandelov, E. and Marschner, B. 2003. Structure and function of the soil microbial community in a long term fertilizer experiment, *Soil Biology Biochemistry*, **35**: 453-461.
- Moubasher, A.H. 1993. Soil Fungi in Qatar and Other Arab Countries, Doha University of Qatar, Center for Scientific and Applied Research, p.566.
- Nilima satish, Sadika Sultana and Vidyanand Nanjundiah (2007). Diversity of soil fungi in a tropical deciduous forest in Mudumalai, Southern India. *Current Science.*, **93:** 669-677.
- Oudernans, C.A.J.A. and Koning, C.J. 1902. Prodrome d'une flore mycologique obtenu parla culture sur gelatin prepare de laterre hummense du spanderswoud pres Bussum, Arch. Neerland, Sci. Exact. Et Nat. 7: 266-298.
- Persiani, A.M., Maggi, O., Montalvo, J., Casado, M.A. and Pineda, F.D. 2008. Mediterranean grassland soil fungi: Patterns of biodiversity, functional redundancy and soil carbon storage. *Plant Biosystems.* 142: 111-119.
- Piper, C.S. 1944. Soil and Plant Analysis. Interscience Pub., New York.
- Pongsatorn P., Uthaiwan S. and Dokrak M. 2010. Diversity of Soil Fungi in Different Land Use Types inTha Kum-Huai Raeng Forest Reserve, Trat Province. Kasetsart J. (Nat. Sci.) 44: 1162 – 1175.
- Rai, J.P., Sinha, A., and Govil, S.R. 2001. Litter decomposition mycoflora of rice straw. *Crop Research.*, **21:** 335-340.
- Rane, G. and Gandhe, R.V. 2006. Seasonal distribution of soil fungi from forest soils of Jalgaon district, Maharashtra. *Zoos Print Journal*, 21: 2407-2409.
- Rama Rao, P. 1970. Studies on soil fungi III. Seasonal variation and distribution of microfungi in some soils of Andhra Pradesh

(India), Mycopathol et Mycol. Appl. 40: 277-298.

- Raper, K.B. and Fennell, D.L. 1965. *The Genus Aspergillus*. Baltimore: Williams and Wilkins Company. P.685.
- Saksena, R.K. and Sarbhoy, A.K. 1963. Ecology of the soil fungi of Uttar Pradesh. I. fungi in different soils at Allahabad. Proceedings National Institute Science India. 29 B: 207-224.
- Saravanakumar, K. and Kaviyarasan, V. 2010. Seasonal distribution of soil fungi and chemical properties of montane wet temperate forest types of Tamil Nadu. African Journal of Plant Sciences. 4: 190-196.
- Song, F.Q., Tian, X.J., Li, Z.Q., Yang, C.L., Chen, B., Hao, J.J., Zhu, J. 2004. Diversity of filamentous fungi in organic layers of two forests in Zijn Mountain. *Journal of Forestry Research*, 15: 273-279.
- Sorenson, T. 1948. A method of establishing groups of equal amplitude in plant society based on similarity of species content. *K. Danske. Viendensk. Selsk.* 5: 1-34.
- Vibha and Sinha, A. 2007. Variation of soil mycoflora of rice stubble from rice wheat cropping system. *Mycobiology*, 35: 191-195.
- Waksman, S.A. 1916. Soil fungi and their activities. Soil Science. 2: 103-155.
- Waksman, S.A. 1927. *Principles of Soil Microbiology*. Bailliere Tindall & Co., London.
- Waksman, S.A. 1932. *Principles of Soil Microbiology*, 2<sup>rd</sup> Edition William and Wilkins Co., Baltimore, M.d., U.S.A.
- Waksman, S.A. and Fred, E.B. 1922. A tentative outline of plate's method for determining the number of microorganisms in soil. *Soil Science.* **14:** 27-28.
- Warcup, J.H. 1950. The soil plate method for isolation of fungi from soil. Nature, 166:117-118.
- Warcup, J.H. 1955a. Isolation of fungi from hyphae present in soil. *Nature*, Lond. **175**: 953.
- Yamanaka, T. 2003. The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi in vitro. Mycologia, 95: 584-589.
- Yu, C., Lv, D.G., Qin, S.J., Du, G.D., Liu, G.C. 2007. Microbial flora in *Cerasus sachalinensis* rhizosphere. *Chinese Journal of Applied Ecology.*, **18**: 2277-2281.