*Available online at* www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2222

**ISSN: 2320 – 7051**

*Int. J. Pure App. Biosci.* **4 (1):** 253-264 (2016)

International Journal of Pure & Applied Bioscience

*Research Article*

OPEN ACCESS

# Occurrence and Diversity of Soil Mycoflora in Some Selected *Brassica* Growing Agricultural Fields of Dehradun District of Uttarakhand Himalaya

**Saurabh Guleri*, Seema Saxena, Poonam Sharma, Nivedita Malik and Meena Thapliyal**

Shri Guru Ram Rai P.G. College, Dehradun, Uttarakhand-248001 (India)

*Corresponding Author E-mail: gulerisaurabh26@gmail.com

Received: 17.02.2016 | Revised: 22.02.2016 | Accepted: 26.02.2016

**ABSTRACT**

*Soil is an oligotrophic medium for fungal growth. Fungi are important component in soil microbiota. Depending on soil depth and nutrient conditions, the fungal biomass exceeds that of bacteria in almost every soil except rhizosphere soil which is dominated by bacteria. Filamentous fungi in soil degrade organic matter and help in soil aggregation. Some produce substances similar to humic substances and some are capable of forming ectotrophic associations on the root system of forest trees and help in mobilisation of soil phosphorus and nitrogen into plants. Many fungi are biological control agents for plant pathogens and insect pests. In Uttarakhand especially in Dehradun District very scanty information is available regarding soil inhabiting microfungi of Agricultural fields. Therefore some agricultural lands growing Brassica crop were selected to study the composition, dominance and diversity of microfungi and physico-chemical properties of the soil inhabited by these fungi.*

*Five agricultural lands viz. Rajawala (RAJ), Bahadarpur(BAH), Bhauwala (BHAU), Telpura (TLP) and Sudhowala (SUDH) were selected. Each land was surveyed repeatedly to collect soil samples. Soil samples were collected from different depths viz. 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm. and 20-25 cm and composite samples were made for each agricultural land.*

*For all soil samples examined, pH varied from 6.42±1.24 to 7.84±1.56; moisture content varied from8.42±1.68 to 10.84±2.16; organic contents varie d from 1.55 to 3.00 and texture varied from sandy to sandy loam. Micronutrients were very high in all the soil samples. A total of 383 colonies of microfungi belonging to 11 genera and 26 species were isolated. Anamorphic fungi (Deuteromycota) were dominant and represented by 9 genera and 22 species followed by Zygomycota represented by 2 genera and 4 species. Fungal populations were always highest in surface soil and decreased along with soil-depth. Maximum fungal species were isolated from BAH at the depth of 0-10 cm followed by RAZ, whereas the minimum fungal species were isolated from BHAU, TPR and SUDH at the depth of 15-20 cm. Of the total 26 species recorded, 8 species were of common occurrence, 9 species of frequent, 8 species of moderate and 1 species of rare occurrence respectively.*

*Shanon- Wiever index of species diversity was highest (2.734) in BAH followed by RAZ (2.686), TLP (2.583) and SUDH (2.553) while it was lowest for BHAU (2.540). Simpson index of diversity also showed identical trends. Highest similarity (84.2%) was observed between RAJ and BAH and lowest (55.6%) between RAJ and TLP.*

*The present investigation will be helpful in documentation and conservation as well as in controlling soil borne pathogens. Further, it certainly opens up a new horizon for the researchers in this interesting and challenging field of soil microfungal biodiversity.*

***Key words:*** *Diversity, Soil mycoflora, Brassica, Agriculture, Dehradun District*

**Copyright © February, 2016; IJPAB**

## INTRODUCTION

Soil not only provides a very conducive habitat for fungi but a major part of soil microbial biomass is comprised of fungi. Clark and Paul[7] reported about twice as much fungal biomass as bacterial biomass. Depending on soil depth and nutrient conditions, the fungal biomass exceeds that of bacteria in almost every soil except rhizosphere soil[21]. Fungi are known to colonize, multiply and survive in diverse ecological habitats, covering tropics to pole and mountain to deep oceans, but they grow best at temperature between 6˚C to 50˚C. Hundreds of different species of fungi inhabit the soil and exist in both the mycelia and spore stage. Many fungi commonly isolated from soil some under the class Deuteromycetes by virtue of the fact that they produce abundant asexual spores and lack sexual stages. The members of Basidiomycetes are difficult to isolate from soil on agar plates for the simple reason that the nutritional requirements of many of the Basidiomycetes are exact and conventional method of soil-dilution plating is inadequate to isolate them in pure culture[44].

The physical structure of soil is improved by the accumulation of mold mycelium within it[29]. The mycelium of the fungi penetrates through the soil forming a network which entangles the small soil particles[31]. Fungi play a crucial role role in the decomposition of plant structural polymers, such as cellulose, hemicelluloses and lignin thus contributing to the maintenance of the global carbon cycle. Since 95% of the plant tissue is composed of carbon, hydrogen, oxygen, nitrogen, phosphorous and sulphur; the decomposition activities of fungi are clearly important in relation to the redistribution of these elements between organisms and environmental compartments[12]. Many soil fungi are biological agents for plant pathogens and insect pests. On the other hand, some of them are very harmful causes diseases to plants, animals and humans with significant economic losses and produce mycotoxins in certain products[30]. Soil mycoflora thus exerts considerable influences on the soil fertility and plant growth. In agricultural soils, ploughing, tillage, applications of fertilizers and biocides and type of cultivation affect the mycoflora. The plant species growing in the soil also exerts very important influence on the population and species composition on the soil fungi[25].

In Uttarakhand, most of the studies are either concentrated on aquatic fungi and/or to some extent on ecto and endomycorrhiza and macroscopic fungi[14,5,20,35,40,47] but information regarding diversity of ruderals and stress tolerant fungi of *Brassica* field are scanty especially from Dehradun district. Recently work on agricultural, forest and grazingland of Doon Valley had been done by Guleri *et al.*[16-19]. Therefore the present study was undertaken: 1) To study the fungal species richness and diversity indices of the given area at various soil depths in *Brassica* growing agricultural field and 2) To study the effect of pH, temp and moisture content on soil mycoflora.

## MATERIALS AND METHODS

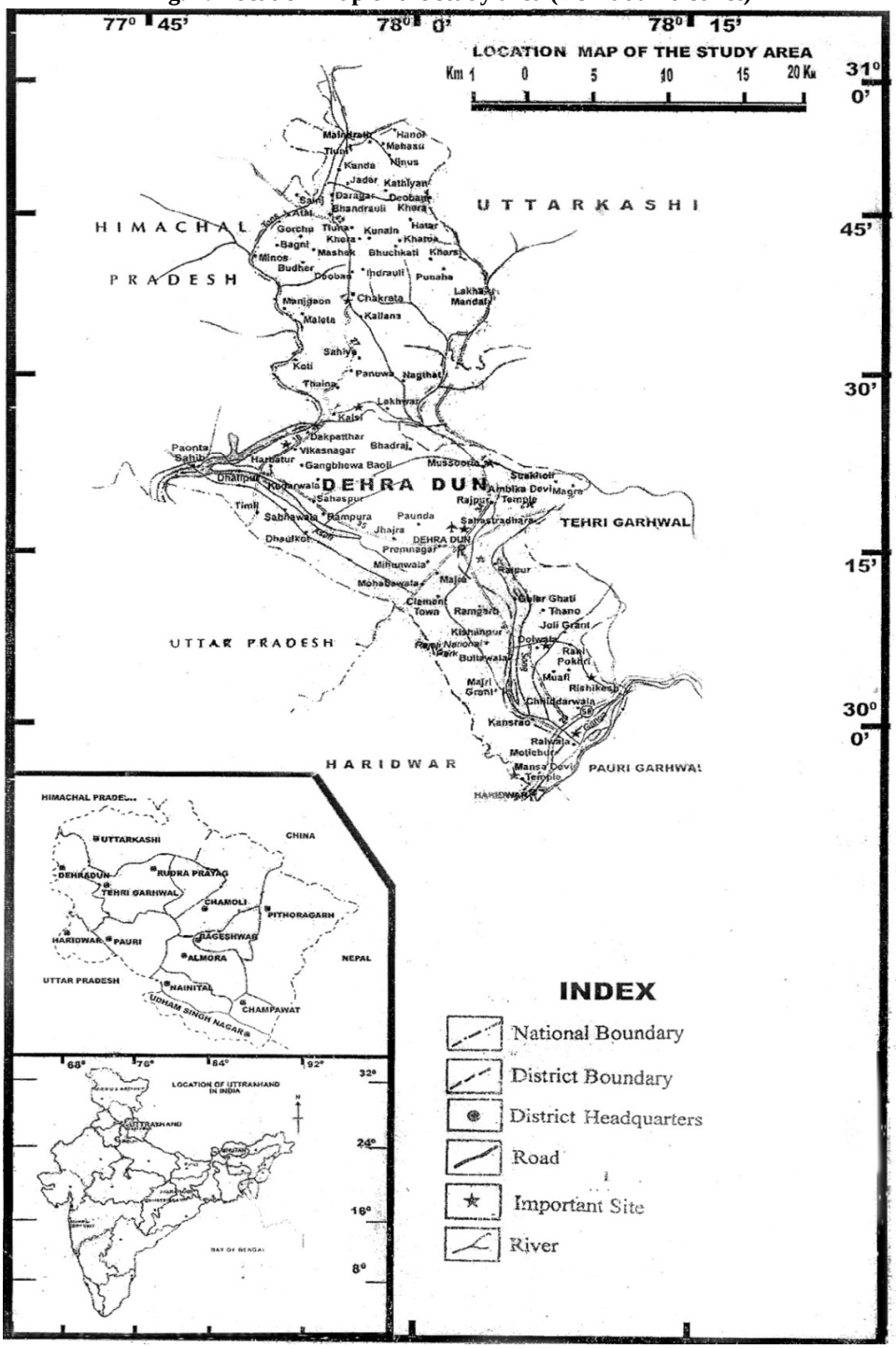
### Study Area

Located in the north of India in the Himalayan region, Uttarakhand state has a geographical area of 53, 483km$^2$ which lies between 77˚34′ to 81˚2′E longitude and 28˚53′N to 31˚27′N latitude. The state comprises high Himalayan region (3000 to 8000m asl), Sub Himalayan region (1000m to 3000m), Shiwalik range and Valley Region including Dehradun.

The study was conducted in Dehradun District (77˚45′ to 78˚15′E and 30˚00′N to 30˚35′N) of Uttarakhand which lies between the west Himalayan mountain ranges in North and the Shiwalik range running parallel to it in the south at a mean altitude of 485m (Fig 1).

**Cite this article:** Guleri, S., Saxena, S., Sharma, P., Malik, N. and Thapliyal, M., Occurrence and Diversity of Soil Mycoflora in Some Selected *Brassica* Growing Agricultural Fields of Dehradun District of Uttarakhand Himalaya, *Int. J. Pure App. Biosci.* **4(1):** 253-264 (2016). doi: http://dx.doi.org/10.18782/2320-7051.2222

**Fig. 1: Location Map of the study area (Dehradun district)**




**Collection of soil samples**

Sampling was initiated in the month of December 2012 and continued till May 2014. Soil samples were collected repeatedly during December 2012 and in Jan, March, April and May 2013. Samples were collected from some selected *Brassica* growing regions of Rajawala, Bahadarpur, Bhauwala, Telpura and Sudhowala. Soil samples were collected from Fifteen places at each locality and at various depths from each place. Six samples were collected from each place, from the surface of the soil and at various depths *viz.,* 0-5cm, 5-10cm, 10-15cm, 15-20cm and at 20-25cm. Samples of various depths were mixed to make it a composite sample which was further divided into two parts one for estimating physicochemical properties and the other for the isolation of fungi. Soil samples were collected by using the method of Brown[6].

**Soil Test and Soil Analysis**

Moisture content

It was calculated by oven drying the soil and determining the weight loss[13]. The percentage soil moisture was calculated by using the following formula:

$$Soil\ moisture\ (\%) = \frac{(FW - DW)}{DW} \times 100$$

FW= Fresh weight of soil sample

DW= Dry weight of soil sample

**Soil texture**

50gm of soil was sieved; organic matter (cementary agent) was removed by oxidizing the sample with hot $H_2O_2$. The volume of treated soil samples were made 1000ml. Percentage of clay in the sample was calculated by dividing the number of grams of clay by the total weight of the sample. Percentage of silt in the sample was calculated by substracting the sum of the percentage of sand and caly from 100. The class number or texture of the soil was determined by using the textural triangle.

**Detections of ions**

200gm soil sample was mixed with homogenized soil paste and kept for 24hrs. Soil extraction was done using blotting paper. The soil extract was further used for detection of Ca, Mg, Na and K.

Ca and Mg were detected by adding 1-3 drops of NaOH in the solution along with small amount of $NH_3$. Mixture was titrated against EDTA (ethylene-di-amine-tetra-acetate). Green, blackish, bluish coloration appeared as end point. To test for magnesium ion only, 3 drops of buffer solution with 3-4 drops of ereocrome black was added to 2ml soil extract, this gave a crimson color. Mixture was titrated against N/100 EDTA. Purple color appeared as end point. For Na concentration a series of sodium standard were run a calibration curve was drawn. Sodium ion in the soil extract was measured by taking the emission reading on the flame photometer[2] at 589 nm wavelength. $Na^+$ concentration was calculated by inferring to the calibration curve. For K analysis, 5g air dried soil was weighed into a 50ml centrifuge tube, ammonium acetate solution was added and shaked for 5 min on a shaker. Mixture was centrifuge until the supernatant liquid was clear. Extract was filtered and collected in a 100ml volumetric flask. Extract was diluted to 100ml with 1N ammonium acetate solution. A series of suitable potassium standards were run to draw a calibration curve. The concentration was measured by taking the emission readings on the flame photometer at 767nm wavelength[45].

Organic C was determined by using Walkley and Black's method and calculated by using the following formula

$$\%\ \text{organic matter} = 10[1(S \div B)] \times 0.67$$

S= Sample titration

B= Blank titration

Soil reaction (pH) was determined by using a pH meter with a glass electrode in mixed soil water ration of 1:2 by electrometric method following Brady[5].

**Isolation of fungi**

PDA (Potato Dextrose Agar), CDA (Czapek's Dox Agar) and MEA (Malt Extract Agar) were used for the isolation and maintenance of fungi. The soil samples were processed for isolation of *Brassica* associated soil mycoflora using Soil Dilution Plate Method[48] and Direct Plate Method[49]. Plates were incubated at 20 to 25˚C for 7-15 days. Fungal isolates were purified for identification. The microscopic observations were carried out at 400x and 1000x magnification using compound light microscope. The colonies developed on the plates were carefully counted and individual colonies were identified and transferred to a separate agar plate. Identification of the isolates was done by following Smith. Identification was also confirmed by consulting various monographs, books and papers available on fungal systematic: Raper and Thom[33], Raper and Fennell[34], Barnett and Hunter[3], Gilman[14], Ellis[10,11], Domsch *et al*.,[9], Moubasher[27]. The microphotography of different genera identified during investigation was carried out using Magnus MIPS-USB (Olympus), DSCW320 (Sony).

**Periodicity of Occurrence, Species richness, Similarity and Dissimilarity Index**

The periodicity of occurrence denotes the number of samplings in which a fungus is present against the total number of samplings. The periodicity of occurrence of fungi was arbitrarily classified as per Saravanakumar and Kaviyarasaran[39]:

Common—recorded in 5-7 samplings

Frequent—recorded in 4-5 samplings

Moderate—recorded in 2-3 samplings

Rare—recorded in 1-2 samplings

Species diversity is a statistical abastraction 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 campare the sites. In the present approach, the index (s) was calculated using species richness following Sorenson[43] as:

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

Where A= Number of species in Community A

B= Number of species in community B

C= Number of species common to both 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.

## RESULT AND DISCUSSION

Soil samples were analyzed for moisture content, pH, organic carbon, Ca+, Mg+, Na+, K+ and soil texture and colour. Organic content of all soil samples were greatly varied from 1.55 to 3.00. The pH varied from 6.42±1.24 to 7.84±1.56. The moisture content of all soil samples ranged from 8.42±1.68 to 10.84±2.16. Soil texture was sandy to sandy loam except in Bahadarpur, where it was sandy clay loam. The micronutrients are very high in all soil samples (Table 1).

**Table 1 Physico-chemical analysis of different agricultural sites**

| **Sampling sites** | **Parameters** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | MC | pH | C | Ca | Mg | Na | K | Color | Texture |
| **RAZ** | 8.42 | 6.42 | 1.55 | 14 | 18 | 24 | 0.4 | Greyed brown | Sandy |
| **BAH** | 9.01 | 6.60 | 2.90 | 13 | 14 | 21.7 | 0.6 | Light brown | Sandy clay loam |
| **BHAU** | 10.84 | 7.84 | 3.00 | 11 | 12 | 9.8 | 0.18 | Brown | Sandy loam |
| **TPR** | 9.89 | 7.59 | 1.90 | 10 | 13 | 22 | 0.9 | Brown | Sandy loam |
| **SUDH** | 10.78 | 5.43 | 2.62 | 9 | 11 | 8.2 | 2.1 | Black brown | Loam |

**Soil fungal Diversity**

A total of 383 colonies of soil fungi belonging to 11 genera and 26 species were isolated (Table 2). Anamorphic fungi (Deuteromycota) were dominant and represented by 9 genera and 22 species followed by Zygomycota represented by 2 genera and 4 species. Fungal population was always highest in surface soil and decreased along with soil depth. Maximum fungal species was harboured from BAH at the depth of 0-10cm followed by RAZ, whereas the minimum fungal species were isolated from BHAU, TPR, and SUDH at the depth of 15-20cm.

*Alternaria alternata, Acrophialophora, Aspergillus carbonarius, Aspergillus flavus, Aspergillus nidulans, Aspergillus oryzae, Emericella* sp., *Fusarium oxysporum, Fusarium moniliforme, Mucor racemosus, Rhizopus oryzae* and *Curvularia tetramera* were isolated only from 0-5cm depth and *Alternaria* sp., *Aspergillus flavus, Aspergillus niger, Aspergillus tamari, Aspergillus violaceus, Eurotium chevalieri,*

*Humicola insolens* were isolated only from the depth 5-10cm. Similarly, *Aspergillus fumigatus, Aspergillus humicola, Aspergillus japonicas, Eurotium amstelodomi, Mucor* sp., *Penicillium funiculosum* and *Rhizopus arrhyzus* were isolated only from 15-20cm depth (Plate 1; Plate 2).

*Aspergillus* was the most dominant genus with 11 species followed by *Alternaria, Eurotium, Fusarium, Mucor* and *Rhizopus* equally contributed 2 sp. *Alternaria alternata, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Fusarium moniliforme* and *Rhizopus arrhyzus* showed maximum contribution (19.23%) followed *Aspergillus nidulans, Penicillium funiculosum* and *Curvularia tetramera* showed their presence by 15.38%. *Aspergillus carbonarius, Alternaria* sp., *Aspergillus humicola, Aspergillus tamari, Aspergillus violaceus* and *Eurotium chevalieri* equally contributed (11.53%) followed by *Acrophialophora, Aspergillus japonicas, Aspergillus oryzae, Emericella* sp., *Eurotium amstelodomi, Humicola insolens, Mucor* sp., and *Rhizopus oryzae* (7.69%) *Aspergillus flaviceps* (3.84%) were the least important species (Table 2).

**Table 2 Mycoflora of different *Brassica* growing areas of dehradun district**

| S.No. | Name of fungus | Depth (cm) | RAZ (1) | BAH (2) | BHAU (3) | TLP (4) | SUDH (5) | Percent contribution % | Periodicity of Occurence |
|---|---|---|---|---|---|---|---|---|---|
| 3 | *Acrophialophora* | 5 | 2 | 0 | 0 | 0 | 4 | 7.69 | M |
| 1 | *Alternaria alternata* | 5 | 5 | 8 | 5 | 5 | 10 | 19.23 | C |
| 2 | *Alternaria sp.* | 10 | 0 | 2 | 3 | 1 | 0 | 11.53 | F |
| 4 | *Aspergillus carbonarius* | 5 | 4 | 6 | 0 | 3 | 0 | 11.53 | F |
| 5 | *Aspergillus flaviceps* | 10 | 0 | 0 | 8 | 0 | 0 | 3.84 | R |
| 6 | *Aspergillus flavus* | 5 | 5 | 2 | 7 | 3 | 6 | 19.23 | C |
| 7 | *Aspergillus fumigatus* | 15 | 12 | 7 | 14 | 2 | 1 | 19.23 | C |
| 8 | *Aspergillus Humicola* | 20 | 2 | 1 | 0 | 0 | 4 | 11.53 | F |
| 9 | *Aspergillus japonicus* | 15 | 0 | 0 | 0 | 2 | 1 | 7.69 | M |
| 10 | *Aspergillus nidulans* | 5 | 3 | 7 | 2 | 0 | 1 | 15.38 | F |
| 11 | *Aspergillus niger* | 10 | 2 | 6 | 8 | 10 | 12 | 19.23 | C |
| 12 | *Aspergillus oryzae* | 5 | 1 | 2 | 0 | 0 | 0 | 7.69 | M |
| 13 | *Aspergillus tamari* | 10 | 0 | 0 | 2 | 3 | 4 | 11.53 | F |
| 14 | *Aspergillus violaceus* | 10 | 2 | 8 | 0 | 6 | 0 | 11.53 | F |
| 15 | *Curvularia tetramera* | 5 | 2 | 6 | 4 | 0 | 4 | 15.38 | F |
| 16 | *Emericella sp.* | 5 | 0 | 0 | 1 | 2 | 0 | 7.69 | M |
| 17 | *Eurotium amstelodami* | 15 | 0 | 2 | 0 | 1 | 0 | 7.69 | M |
| 18 | *Eurotium chevallierii* | 10 | 4 | 6 | 0 | 0 | 4 | 11.53 | F |
| 19 | *Fusarium moniliforme* | 5 | 3 | 3 | 4 | 6 | 8 | 19.23 | C |
| 20 | *Fusarium oxysporum* | SS | 12 | 14 | 8 | 10 | 5 | 19.23 | C |
| 21 | *Humicola insolens* | 10 | 2 | 0 | 1 | 0 | 0 | 7.69 | M |
| 22 | *Mucor racemosus* | 5 | 5 | 8 | 5 | 3 | 4 | 19.23 | C |
| 23 | *Mucor sp.* | 15 | 1 | 0 | 0 | 2 | 0 | 7.69 | M |
| 24 | *Penicillium funiculosum* | 20 | 2 | 1 | 0 | 1 | 6 | 15.38 | F |
| 25 | *Rhizopus arrhyzus* | 20 | 3 | 4 | 6 | 2 | 1 | 19.23 | C |
| 26 | *Rhizopus oryzae* | 5 | 0 | 2 | 1 | 0 | 0 | 7.69 | M |

sp-species; SS- Surface soil; 0- not present; RAZ- Razawala; BAH-Bahadarpur; BHAU-Bhauwala; TLP- Telpura; SUDH-Sudhowala; F-Frequent;M-Moderate;C-Common;R-Rare

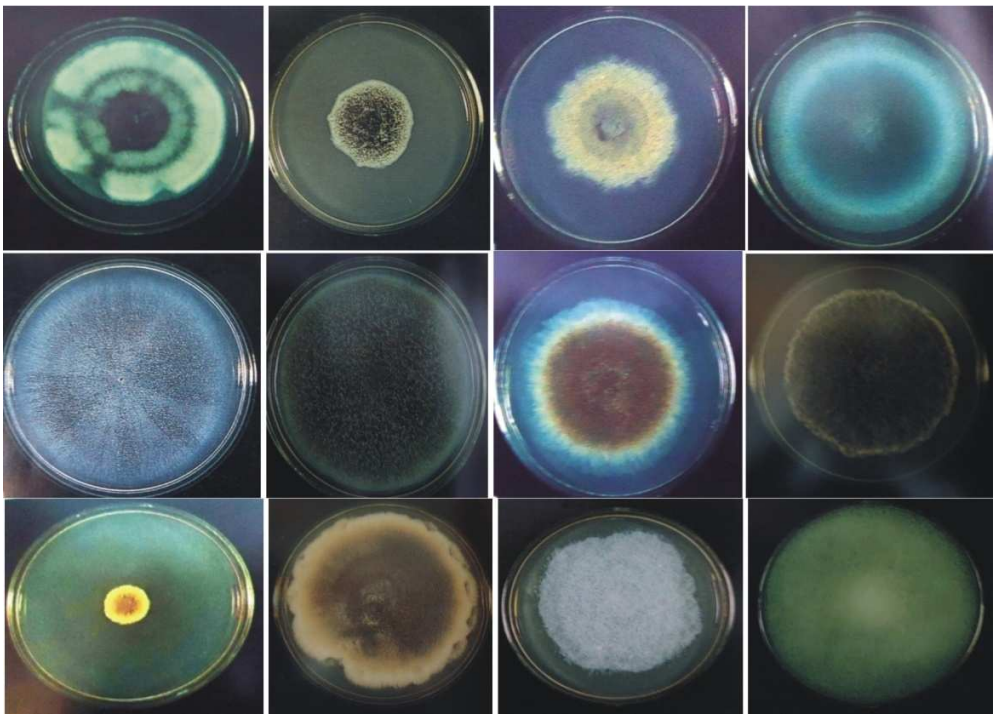


**Plate 1:** *Cultural morphology of different species: a- Alternaria alternata, 7-day-old colony on MEA; b- Aspergillus carbonarius, 7-day-old colony, on –MEA c- Aspergillus flavus, 7-day-old colony, -CDA d- Aspergillus fumigatus, 7-day-old colony on -MEA a hyaline isolate e- Aspergillus japonicas, 7-day-old colony on CDA f- Aspergillus niger, 7-day-old colony, on -MEA g- Aspergillus oryzae, 7-day-old colony, on –CDA h- Curvularia tetramera, 7-day-old colony on MEA i- Eurotium chevalieri, 7-day-old colony on CDA j- Humicola insolens, 7-day-old colony on MEA, k- Mucor racemosus, 5-day-old colony, on MEA; L- Rhizopus oryzae, 2-day-old colony on MEA*

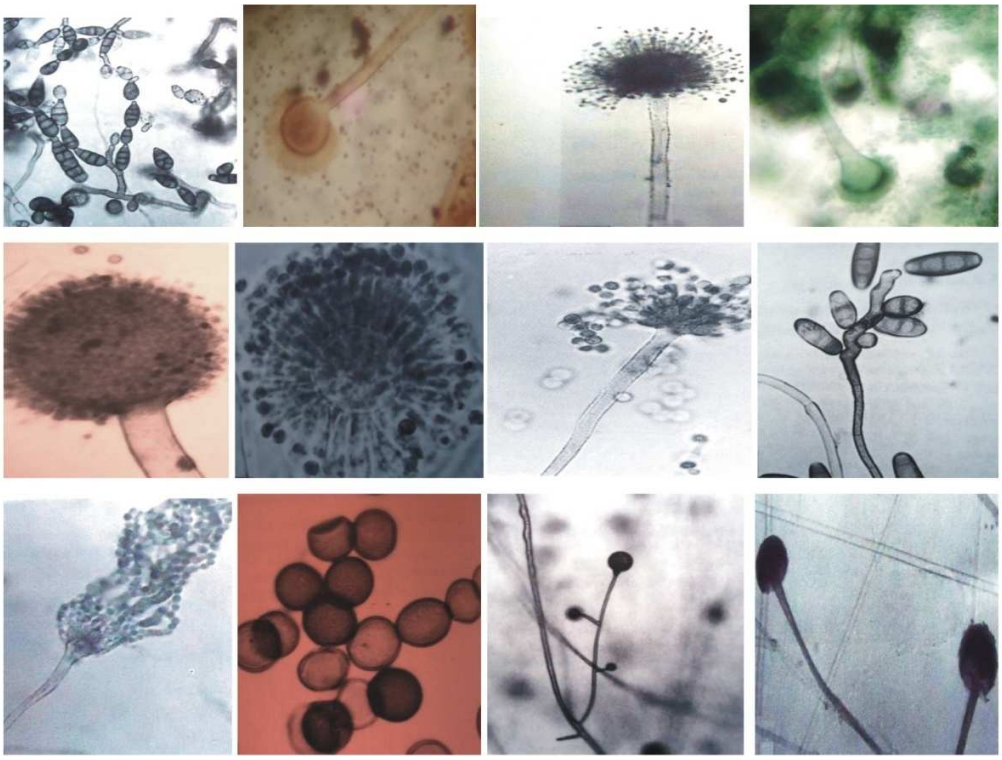


**Plate 2:** *a - Alternaria alternata, conidiophores [x400]; b- Aspergillus carbonarius, conidiophores and conidial heads [x400] c- Aspergillus flavus, conidial heads and conidia [x400] d- Aspergillus fumigatus, conidial heads [x400]; e- Aspergillus japonicus, conidiophore, vesicle, phialides and conidia [x400]; f- Aspergillus niger conidia, [x1000], g- Aspergillus oryzae, conidial heads [x400]; h-Curvularia tetrampra, conidiophores [x400]; i- Eurotium chevalieri, conidial heads [x400]; j- Humicola insolens, conidia[x1000]; k- Mucor racemosus, sporangiophores [x100]; l- Rhizopus oryzae sporangiophores [x100]*

**Periodicity of Occurrence**

Of the total 26 species recorded, eight species *viz., Alternaria alternata, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Fusarium moniliforme, Mucor racemosus* and *Rhizopus arrhyzus*, were of common occurrence. Nine species *viz., Alternaria* sp., *Aspergillus carbonarius, Aspergillus humicola, Aspergillus nidulans, Aspergillus tamari, Aspergillus violaceus, Curvularia tetramera, Eurotium chevallierii* and *Penicillium funiculosum* were of frequent occurrence while, eight species *viz., Acrophialophora, Aspergillus japonicus, Aspergillus oryzae, Emericella* sp., *Eurotium amsteladomi, Humicola insolens, Mucor* sp., and *Rhizopus oryzae* were of moderate occurrence. Remaining one species *viz., Aspergillus flaviceps* were of rare occurrence (Table 2).

**Similarity and dissimilarity index**

Highest similarity (84.2%) was observed between RAH AND BAH and lowest (55.6%) between RAZ and TLP (Table 3).

**Diversity, dominance and other parameters**

Remarkable difference in species richness was not observed during the study period on different *Brassica* growing agricultural land, but, nevertheless, composition varied greatly from site to site. Highest species richness was found in BAH and RAZ (19) followed TPR (17) and then BHAU and SUDH have the same composition (16). The Shannon-Wiever Diversity index was highest (2.734) in BAH followed by RAZ (2.686), TLP (2.583) and SUDH (2.553) while, the lowest diversity index was found in BAH (2.540) as the total abundance and richness was lowest in comparison to BAH. The species evenness was highest in BAH followed by SUDH. The value of Simpson index is close to 1 which indicates the probability of different species is very low (Table 4).

**Table 3 Similarity and dissimilarity index of soil fungi**

| **Sites** | **RAZ** | **BAH** | | **BHAU** | | **TLP** | | **SUDH** | |
|---|---|---|---|---|---|---|---|---|---|
| | | IS | DS | IS | DS | IS | DS | IS | DS |
| **RAZ** | | 0.842 | 0.158 | 0.571 | 0.429 | 0.556 | 0.444 | 0.80 | 0.20 |
| **BAH** | | | | 0.685 | 0.315 | 0.722 | 0.278 | 0.742 | 0.258 |
| **BHAU** | | | | | | 0.666 | 0.334 | 0.687 | 0.313 |
| **TLP** | | | | | | | | 0.666 | 0.334 |
| **SUDH** | | | | | | | | | |

IS-Index of similarity, DS- Dissimilarity index

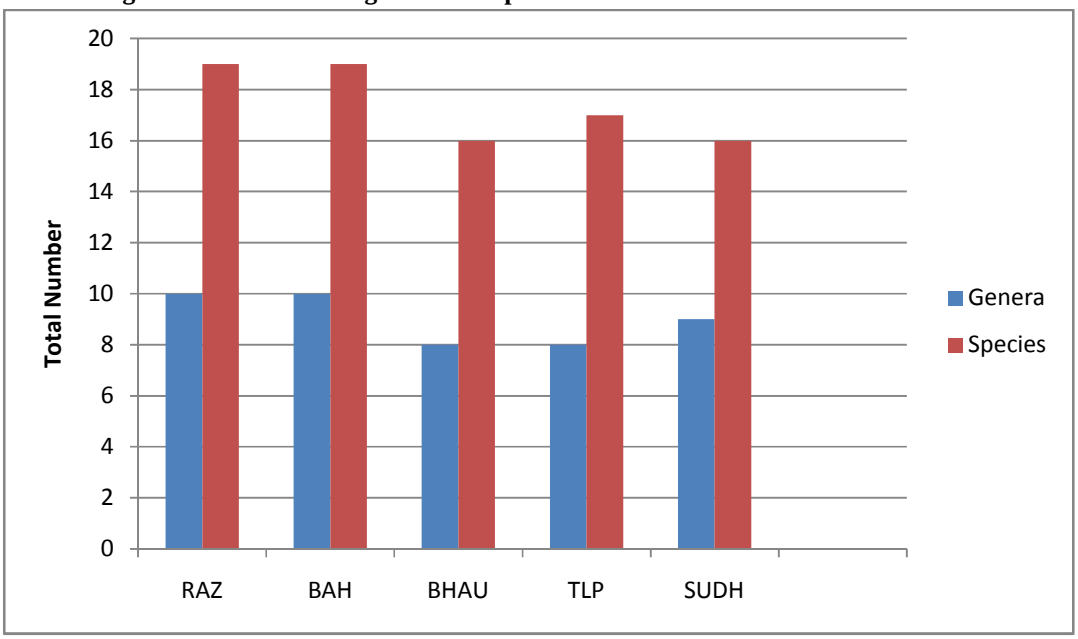
**Table 4 Diversity indices and other parameters**

| **Parameters** | **Sites** | | | | |
|---|---|---|---|---|---|
| | RAZ | BAH | BHAU | TLP | SUDH |
| **Shanon-Wiever Index** | 2.686 | 2.734 | 2.540 | 2.583 | 2.553 |
| **Species richness** | 19 | 19 | 16 | 17 | 16 |
| **Total Abundance** | 72 | 95 | 79 | 62 | 75 |
| **Simpson diversity Index (1-D)** | 0.913 | 0.925 | 0.908 | 0.907 | 0.910 |
| **Evenness** | 0.912 | 0.928 | 0.916 | 0.911 | 0.920 |

**Comparative analysis of fungal diversity**

A total of 26 species belonging to 11 genera were isolated from all agricultural sites during the whole study period. 10 genera and 19 species each from RAZ and BAH soil, 8 genera and 16 species from BHAU soil, 8 genera and 17 species from TLP soil, whereas, 9 genera and 16 species were isolated from SUDH soil (Fig 2).

**Fig. 2: Total number of genera and species isolated from different *Brassica* sites**




In the present study growth and species richness of soil mycoflora and the physic-chemical factors affecting the diversity of fungi are taken into consideration from the designated *Brassica* growing sites of Dehradun district.

The soil in the area varies in colour, texture, drainage, moisture content, pH, organic matter and micro or macro nutrients[17] thereby effecting the diversity of mycoflora. Nutrients in soil are strongly affected by soil pH due to reactions with soil particles and other nutrients[50] soils of different sites are neither too acidic, nor too alkaline because of more Mg and Ca ions available. Other factors affecting soil pH are fertilizers, organic matter, rain and soil microorganisms. There was no marked variation in soil texture of the sites, the soil texture of different sites were sandy to sandy loam with a few exceptions.

26 different species of microfungi were isolated from the *Brassica* cultivated soil. Out of 26 fungal species isolated, 8 species *viz.*, *Alternaria alternata*, *Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Mucor racemosus* and *Rhizopus arrhyzus* showed universal distribution occurring in all sites. The diverse distribution of these species is attributed to cumulative effect of different edaphic, climatic and other biotic factors including the wider adaptability of these species. Mishra and Kanaujia[24] isolated more diverse microflora from cultivated soil and they found that the *Aspergillus flavus* and *Aspergillus niger* were the most dominant fungi due to cold climate and acidic nature of soils. In the present study *Aspergillus* sp. predominantly found in relatively high temperatures, whereas species of *Penicillium* predominated at low temperatures. This is in accordance with the studies of Guleri *et al.*[17], Manoharachary *et al.*[22], Reddy *et al.*[36], Saksena *et al.*[38] and Saxena and Sarbhoy[37].

The number of fungi decreased with increase in depth. The drop in number of fungi in lower depth could be attributed to the high moisture content of the deeper soil resulting into reduced aeration of soil[42]. Surface soil usually provide high organic matter content which in presence of adequate moisture supply is acted upon the microfungi to decompose the complex organic residue into simple forms, hence the microfungi are higher in upper layer of soil[1].

The months with moderate temperature were regularly the richest (in counts and spectra) while the summer months were poorest. These observations were similar to Moubasher and El. Dohlob[26]. In summer, fungi are subjected to unfavourable condition, the soil dries up quickly and the temperature becomes so high as to affect severely the inhabitants. Taking similarity index of the fungal community is considered as an index of homogeneity of habitat[8]. Different vegetation supporting different communities of soil fungi have also been reported by other workers[28].

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 earlier reported in other studies[41]. The species evenness was highest in BAH due to adequate moisture and rotting dead materials on the ground. The distribution of dominance of anamorphic fungi suggested that the fungi belonging to Deuteromycota are strong colonizers of the decaying substrate with better and wider adaptability coupled with high competitive ability, whereas those of Zygomycota were weak colonizers[32,46] with narrow or poor adaptability.

## REFERENCES


1. Acea, M.J. and Carballos, T., First result of study of the microbial population of humid zone garmatic soils. *Edafol. Agrobiol*, **44:** 395-412 (1985).
2. Akhtar, N., Tufail, M. and Ashraf, M., Natural environmental radioactivity and estimation of radiation exposure from saline soils. *Inter. J. Environ. Sci. Technol*., **1(4):** 279-285 (2004).
3. Barnett, H.L. and Hunter, B.B. (1972) *Illustrated Genera of Imperfect Fungi*. Burges Publishing Company, Minnesota.
4. Belwal, M. and Sati, S.C. (2007) *Fungal flora of two altitudinally different streams of Kumaun Himalaya*, In: Recent Mycological Researches (eds) I.K. International Publ. House Pvt. Ltd., New Delhi, 3-21.
5. Brady, N.C. (1990) *The Nature and Properties of Soils*. 10th ed. Macmillan Pub. Co. New York, USA.
6. Brown, J.C. (1958) Soil fungi of some British sand dunes in relation to soil type and succession. *Ecology*. **46:** 641-664.
7. Clark, F.E. and Paul, E.A. (1970) The microflora of grasslands. In: N.C. Brady (eds) *Advances in Agronomy*, **22:** 375-435.
8. Clarke, D.C. and Christensen, M., The soil microfungal community of a South Dacota grassland. *Canad. J. Bot*., **59:** 1950-1960 (1981).
9. Domsch, K.W., Gams, W. and Anderson, T. (1980) *Compendium of soil fungi*. Academic Press, London.
10. Ellis, M.B. (1971) *Dematiaceous Hyphomycetes*. Kew Surrey UK: pp. 608
11. Ellis, M.B. (1976) *More Dematiaceous Hyphomycetes*. CMI, Kew, pp. 628.
12. Gadd, G.M. (2004) Myco-transformation of Organic and Inorganic substrates. *Mycologist*, **18:** 60-70.
13. Garrett, S.D. (1963) *Soil fungi and Soil fertility*. Pergamon Press, Oxford, pp.105.
14. Gilman, J.C. (2001) *A manual of soil fungi*. 2nd Indian ed., Biotech Books, Delhi, pp. 392.
15. Ginwal, H.S., Dubey, R.C. and Singh, R.P., Diversity of ectomycorrhizal fungi in different Himalayan Oak forests. *Annals Forestry*. **4(1)**: 65-69 (1996).
16. Guleri, S., Bhandari, B.S. and Saxena, S., Ecology of Rhizosphere and Non-Rhizosphere soil mycoflora of forest soils of Dehradun district, Uttarakhand. *Int. Trans. Appl. Sci.* **2(1)**: 69-77 (2010).
17. Guleri, S., Bhandari, B.S. and Saxena, S., Soil mycoflora of some selected agroforestry areas of Doon Valley, *Annals of Forestry*, **20:** 193-199 (2012).
18. Guleri, S., Saxena, S. and Bhandari, B.S., Seasonal variation in the diversity of soil microfungi of some grazinglands of Doon Valley of Uttarakhand Himalaya, *Journal of Mycopathological Research*, **51:** 213-223 (2013).
19. Guleri, S., Saxena, S.,Bhandari, B.S. and verma, N., Soil mycofloral diversity under wheat cultivation in Doon Valley, Uttarkhand *J. Mycopathol, Res*, **52(2) :** 245-252 (2014).
20. Joshi, Sweta. (2010) Systematics and Ecological Studies on the Genus *Lactarius* of Garhwal Himalaya. Unpublished. D.Phil. Thesis. HNB Garhwal University, Srinagar Garhwal.
21. Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A. (2001) *Dictionary of fungi*, 9th edition. CABI Publishing Surrey, UK.
22. Manoharachary, C., Reddy, V.R.T. and Prasad, U., Distribution and phenology of soil fungi in supporting forest vegetation. *Geophytology*, **20(2)**: 58-70 (1990).

23. Manoharachary, C., Sridhar, K., Singh, R., Adholeya, A., Suryanarayanan, T.S., Rawat, S. and Johri, B.N. 2005. Fungal biodiversity: Distribution, Conservation and prospecting of fungi from India. *Current Science*, **89(1):** 58-71 (1990).
24. Mishra, R.R. and Kanaujia, R.S., Studies on certain ecological aspects of soil fungi II. *Edafol. Agrobiol.*, **32:** 21-39 (1973).
25. Mishra, R.R. and Sharma, G.D., Ecology of soil fungi: population variation in relation to varying cover vegetation and soil factors. *Sydowia Ann. Mycol*., **30:** 134-140 (1977).
26. Moubasher, A.H. and El-Dohlob, S.M., Seasonal fluctuation of Egyptian soil fungi. *Trans. Brit. Mycol. Soc.* **54:** 45-51 (1970).
27. Moubasher, A.H. (1993) *Soil fungi in Qatar and Other Arab Countries*, Doha University of Qatar, Centre for Scientific and Applied Research. pp.566.
28. Orput, P.A., Curtis, J.T. Soil microfungi in relation to the Prairie continuum in Wisconsin. *Ecology*, **38:** 628-637 (1957).
29. Pelczar, M.J. Jr. and Reid, R.D. (1972) *Microbiology*. Mc Graw-Hill Book Company, New Delhi pp. 936.
30. Pongsatorn P., Uthaiwan, S. and Dokrak, M., Diversity of Soil fungi in Different Land Use Types in Tha Kum-Hui Raeng Forest Reserve, Trat Province. *Kaestsart J. (Nat. Sci.),* **44:** 1162-1175 (2010).
31. Powar, C.B. and Daginawal, H.F. (1986) *General Microbiology*. Vol II. 2nd Ed. Himalaya Publishing House, Bombay.
32. Rai, J.P., Sinha, A. and Govil, S.R., Litter decomposition mycoflora of rice straw. *Crop Res.*, **21:** 335-340 (2001).
33. Raper, K.B. and Thom, C. (1949) A manual of the *Penicillia*. Williams and Wilkins Co., Baltimore. pp. 875.
34. Raper, K.B. and Fennell, D.L. (1965) The Genus *Aspergillus*. Baltimore: Williams and Wilkins Company. pp. 685.
35. Rawat, P.S., Ginwal, H.S., Singh, R.P. and Dubey, R., Vertical distribution of ectomycorrhizae in deodar and chir pine forests in relation to their soil characteristics. *Indian Forester* **129:** 624-630 (2003).
36. Reddy, V.N., Prakash, P. and Manoharachary, C., Fungistasis in forest soil using the dominant soil fungi. *Biome* **2(1):** 126-128 (1987).
37. Saksena, R.K. and Sarbhoy, A.K., Ecology of the soil fungi of Uttar Pradesh. I. fungi in different soils at Allahabad. *Proc. Nat. Inst. Sci. India.* **29 B**: 207-224 (1963).
38. Saksena, R.K., Nanda, K. and Sarbhoy, A.K., Ecology of the soil fungi of Uttar Pradesh II. Soils of the Himalayan forests and their microfungi. *Proc. Nat. Inst. Sci. India* **33B (3-4)**: 144-153 (1967).
39. Saravanakumar, K. and Kaviyarasan, V., Seasonal distribution of soil fungi and chemical properties of montane wet temperate forest types of Tamil Nadu. *African Journal of Plant Sciences.* **4(6):** 190-196 (2010).
40. Sati, S.C. and Khulbe, R.D., Occurrence of aquatic fungi in soils of Nainital. *Geobios.* **7:** 42-43 (1980).
41. Satish, N., Sultanas, S., Nanjundiah, V., Diversity of soil fungi in a tropical deciduous forest in Madumalai, Southern India, *Curr. Sci.* **93:** 669-677 (2007).
42. Shukla, A.K. and Mishra, R.R., Influence of soil management systems on the microfungal communities of potato field. *Cryptog. Mycol.*, **13:** 135-144 (1992).
43. 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.
44. Subba Rao, N.S. (1999) *Soil Microbiology.* 4th Ed., of Soil microorganisms and Plant Growth, Oxford & IBH Publ., Co., Pvt. Ltd., New Delhi, pp. 407.
45. Tahira, J.J., Khana, S.N., Suliman, R. and Anwar, W., Evaluation of soil quality on the basis of chemical and microbial health for potential use in agriculture. *African J. Agri. Res.*, **6(16):** 3713-3717 (2011).

46. Vibha and Sinha, A., Variation of soil mycoflora of rice stubble from rice wheat cropping system. *Mycobiology*, **35:** 191-195 (2007).
47. Vishwakarma, M.P., (2010) Systematics and Ecological Studies on Agaricaceae and Coprinaceae of Garhwal Himalaya. *Unpublished D.Phil. Thesis.* HNB Garhwal University, Srinagar Garhwal.
48. Waksman, S.A. and Fred, E.B., A tentative outline of plate's method for determining the number of microorganisms in soil. *Soil Sci.* **14:** 27-28 (1922).
49. Warcup, J.H., The soil plate method for isolation of fungi from soil. *Nature*, **166:** 117-118 (1950).
50. Wright, A.L., Hanlon, E.A., Sui, D. and Rice, R., (2009) *Soil pH, Effects on Nutrient Availability in the Everglades Agricultural Area*. SL287, Soil and Water Science Department. Florida Cooperative Extension Service, Institute of Food and Agricultural Science, University of Florida, **287:** 1-5.