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Rhizospheric Soil Microfungal Diversity under *Hippophae salicifolia* D.Don ecosystems of Garhwal Himalayas

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Abstract

The present communication attempts to provide information on the distribution and diversity of rhizospheric soil mycoflora under *Hippophae salicifolia* D.Don of Garhwal Himalayas. To achieve the target, soil samples were collected from two different localities, *viz.*, Mana of Chamoli and Yamnotri of Uttarkashi districts of Garhwal Himalaya. Samples were also analysed for physicochemical properties, including texture, soil reaction (pH), and organic carbon. The serial dilution method was used to isolate the soil mycoflora. During the investigation, pH ranged from 5.95±1 to 6.67±1, and the moisture content was between 3.86±1 % and 1.63±1 %. Thirty-four species of microfungi belonging to 21 genera were isolated from all the sampling sites. *Cladosporium* was the most dominant genus, followed by *Aspergillus* and *Fusarium*. *Cladosporium cladosporiodes* was the most common fungi isolated from *Hippophae* growing sites with maximum contribution (7.43%) whereas, *Nigrospora sphaerica* was the least important species with minimum contribution (0.80%). The highest number of species (23) was isolated from the Mana site of Chamoli District of Garhwal Himalaya. Of the 34 species recorded, six were common, ten frequent, 12 moderate, and six rare occurrences. Similarity ranged from 16.21% to 96.26%. The Shannon-Wiener's diversity index was highest in Mana 2 (2.972), followed by Yamnotri 1 (2.217). Total richness and abundance were the highest in Mana 2 whereas lowest in Yamnotri 1. Evenness was highest in Mana 1 (0.966), followed by Mana 3 (0.954), while lowest observed in Yamnotri 3 (0.905). It was noteworthy in the present contribution that all the soil microfungi reported under *H. salicifolia* associated rhizospheric soils are new reports to such ecosystems of Garhwal Himalaya. Thus, it is hoped that further investigation of soils under different vegetation regimes of these altitudinal zones mainly dominated by *Cedrus deodara, Pinus wallichiana, Picea smithiana, Betula utilis, Alnus nepalensis, Abies pindrow* will definitely add to the knowledge of microfungal flora adapted to different ecosystems.

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Introduction

Soil is a complex heterogeneous habitat for a wide variety of organisms, including bacteria, fungi, protozoans, nematodes, and earthworms in which organisms interact with each other and with their physical environment contributing to plant nutrition, soil structure, soil fertility, decomposition of organic matter, cycling of nutrients, suppression of soil-borne pathogens and removal of toxins (Prescott et al. 2005). These are highly complex systems, with many components playing diverse functions mainly due to the activity of soil organisms (Chiang & Soudi 1994). Soil microflora plays a pivotal role in the evaluation of soil conditions and in stimulating plant growth.

Microorganisms are beneficial in increasing soil fertility and plant growth since they are involved in several soil biochemical transformations and mineralization activities. This involvement of soil microorganisms in a wide variety of metabolic and physiological activities influences the microhabitat. The type of cultivation and crop management practices were found to have a greater influence on the activity of soil microflora. Continuous use of chemical fertilizers over a long period may cause an imbalance in soil microflora and thereby indirectly affect the biological properties of soil, leading to soil degradation (Manickam et al. 1972).

Natural forests are sites of high biodiversity, where complex relationships among fauna, flora, and microflora are maintained due to the structural richness of the habitats (Tsai et al. 2007). In this environment, soil microorganisms are essential components of the biotic community, where they are responsible for the breakdown of organic materials, mobilization of nutrients, and maintenance of soil-plant quality and ecosystem biogeochemistry (Hackel et al. 2004). Soil microorganisms are also known to influence the physical, chemical, and biological properties of the soil (Tangjang & Arunachalam 2009).

Fungi are fundamental for soil ecosystem functioning (Warcup 1951). In forest and agricultural soils particularly, they play a key role in many essential processes such as organic matter decomposition and elemental release by mineralization (Christensen 1989). Fungi are an important component of soil microbiota, contributing more to soil biomass than bacteria (Ainsworth & Bisby 1995). Fungi are dominant in acid soil because the acidic environment is not conducive for the existence of either bacteria or actinomycetes, resulting in the monopoly of fungi for utilization of native substrates in soil. Isolation of fungi from different horizons of soil profile shows that these organisms exhibit selective preferences for various soil depths (Guleri et al. 2012). The plant species growing on the soil also exert influences on the population and species composition of the soil fungi (Mishra & Sharma 1977). The diversity of soil fungi is a reflection of multiple factors and is reduced by disturbances and manipulation activities (Christensen et al. 1981). Natural or anthropogenic disturbances can alter the species composition or may have a negative effect on the species diversity of the decomposer fungi (Lodge 1997).

The plant's root with a zone of intense microbial metabolic activity with a high concentration of carbon is called the rhizosphere. The rhizosphere is categorized into three portions: endorhizosphere (interior of the root), the rhizoplane (root surface), and the soil directly adjacent and adhering to the root surface (Barea et al. 2005). The rhizosphere soil has a lower pH, water potential, oxygen pressure, and high carbon dioxide levels than the bulk soil (Suresh & Bagyaraj 2002). A range of interactions occurs in the rhizosphere, from beneficial symbiotic relationships to detrimental pathogenic (Sylvia et al. 2005).

The rhizosphere represents one of the most diverse habitats on the planet and is essential in ecosystem functioning (Hinsinger et al. 2009). The maximum microbial population occurs in this microenvironment because of the secretion of several organic substrates (Garbaye 1991). The ecology and dynamics of rhizosphere soil microorganisms vary temporally and spatially due to its effect (Aguilera et al. 1999). In this area, microorganisms essentially protect plants against unfavorable soil and environmental conditions (Hrynkiewicz & Baum 2012).

Herbal plants are of immense medicinal value for the drugs and pharmaceutical industries. The growth of the herbal plants also depends on the population of soil microorganisms present in

their rhizosphere, and the rhizoplane area constitutes one of the important biotic and ecological factors responsible for plant growth. Seabuckthorn (*Hippophae* spp.), a member of the family Elaeagnaceae is a multipurpose, spinescent, non-leguminous nitrogen-fixing shrub. It is widely distributed in higher latitude and altitudes of the temperate zone of Asia and Europe. In India, its distribution occurs in Leh and Kargil districts of Jammu and Kashmir, dry temperate regions of Lahul and Spiti valley of Himachal Pradesh, and Garhwal and Kumoan divisions of Uttarakhand. In Uttarakhand, out of two endemic species of seabuckthorn, *H. salici- folia* D. Don is a widely distributed species. *H. salicifolia* is the most common and widely distributed species reported to exist in abundance in three districts of Uttarakhand *viz.,* Uttarkashi, Chamoli, and Pithoragarh (Yadav et al. 2009). Due to its wider adaptability, drought resistance, strong root system, and capacity to fix atmospheric nitrogen through *Frankia* present in its root nodules, considered a wonder or magical plant for the Indian Himalayan regions. The leaves, berries, and seeds of seabuckthorn have high nutritional and medicinal value and are an excellent source of vitamins, sugars, and organic acids. It has a great role in ecological management, restoration and biodiversity conservation. Seabuckthorn, being a multipurpose species, is widely used in fencing, fuel, fodder, and medicinal properties.

Gadgil (1965) observed the colonization of hyphomycetes fungi in the root region. An exhaustive work was done on the taxonomy of forest soil fungi in India. Manoharachary (1977), Reddy et al. (1987) and Mohanty & Panda (1994a) have studied the soil fungi of different forests. Sule & Oyeyiola (2012) work highlighted the different fungal isolates in the rhizosphere and rhizoplane of cassava cultivar. Similarly, fungal diversity in rhizosphere soil of several crops has been well documented by Gopal & Kurein (2013). Parkinson & Waid (1960) have discussed qualitatively as well as quantitative distribution of fungi in the rhizosphere and non-rhizosphere soil in detail, Burges & Raw (1967). In Uttarakhand, most of the studies are either concentrated on aquatic fungi and/or to some extent, on ecto or endomycorrhiza or macroscopic fungi (Sati & Pant 2000, Rawat et al. 2003, Belwal & Sati 2007, Vishwakarma 2010).

In view of the great economic and ecological potential of *Hippophae*, several studies were conducted in different regions to assess the morphological and biochemical variations in natural growing populations of seabuckthorn in Himachal Pradesh and Ladakh region of Jammu and Kashmir (Singh & Singh 2004, Sankhyan et al. 2004). However, no such studies are available from Garhwal Himalayas except biochemical and value-added work done by Gupta & Mukerji (2001), Maheshwari (2005) on plants like *Terminalia arjuna,* Thapar et al. (1992) on *Emblica officinalis,* and Suryanarayanan & Rajagopal (2000) on *Dalbergia ratifolia*.

Research on medicinal properties and other aspects of Seabuckthorn has received much attention in the recent past (Eccleston et al. 2002, Singh 2006), but there is little information available on the rhizosphere, vesicular-arbuscular mycorrhiza (VAM), and endophytic fungi of this plant (Kumar & Sagar 2007). An intensive survey of literature revealed that there is not much information on the soil mycoflora of Uttarakhand and particularly in the selected two districts *viz.* Uttarkashi and Chamoli. So far, a microbiological study of Seabuckthorn stand soil has not yet been carried out (Sharma et al. 2009). Recently work on microfungi of phyllosphere region of *H. salicifolia* by Saxena et al. (2015), Malik & Bhandari (2018a, b) with some interesting and new records from India. In the perusal of literature available, there is no record of the diversity of soil fungi in the study area. Therefore, the present investigation is an attempt to provide information on the documentation of rhizosphere soil mycoflora prevailing under *H. salicifolia* ecosystems of Garhwal Himalaya located in the two distant habitats.

Materials & Methods

Study area and study site

The study was carried out in the Mana and Yamnotri areas of Chamoli and Uttarakashi district of Garhwal Himalayas (Fig. 1).

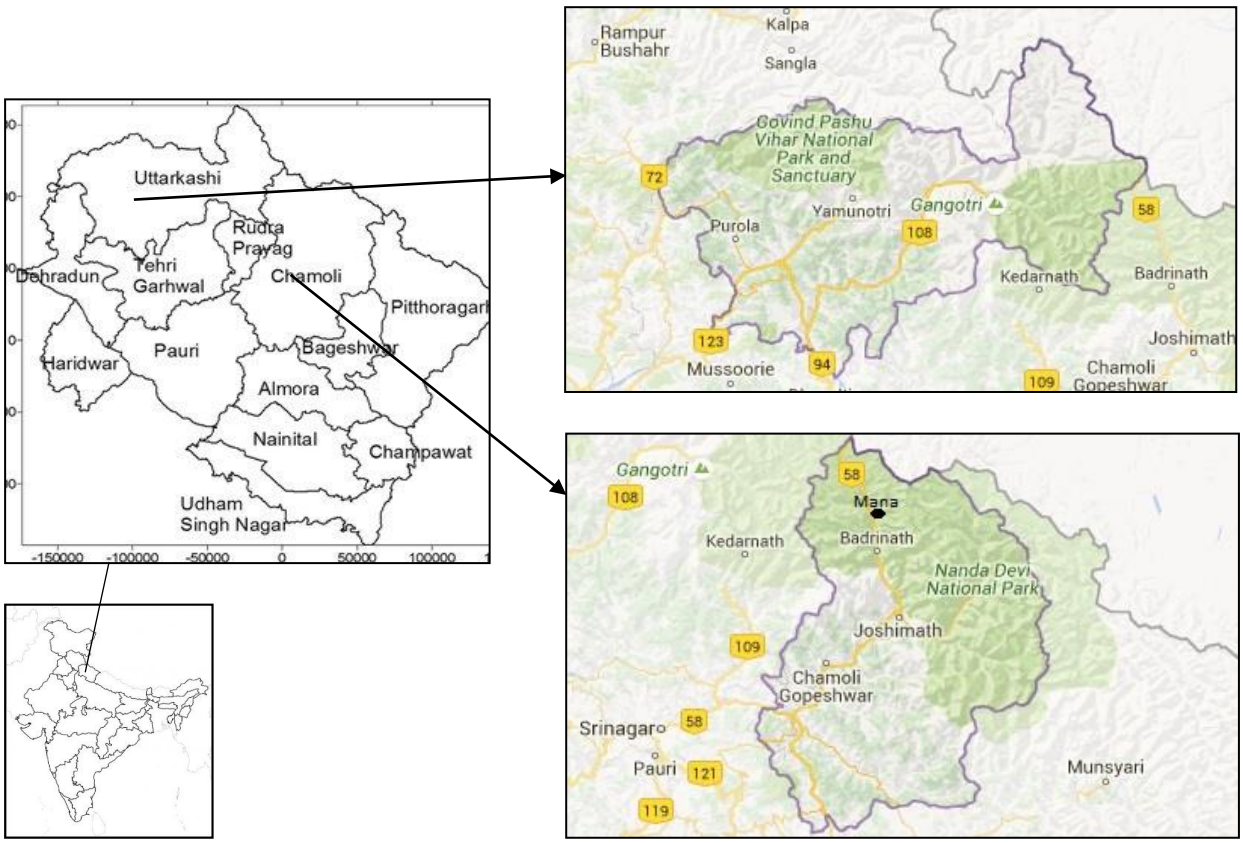


Fig. 1 – Location map of the study area (source: http//:www.nic.in)

Sampling sites

Bulk soil samples from the rhizosphere of *H. salicifolia* were collected from different altitudinal sites in different localities of the Garhwal Himalayas.

Collection of Soil Samples

For the collection of soil samples, a soil profile was randomly selected, and the surface of the soil profile was cleaned (Brown 1958) to avoid extra contamination. Soils of different sites of the selected area were taken aseptically with the help of a sterilized steel tube. Five to six such cores of soil samples were collected randomly. The soil of each different site was mixed with the soil of the same site of different cores of a spot to make composite samples. As such, 20 soil samples were collected from the two forestlands during the investigation. Samples were then brought to the laboratory for the isolation of soil microfungi along with the analysis of physicochemical properties of soil.

Physicochemical Properties of Soil

The moisture content of soil samples was calculated by oven drying the soil and determining the weight loss (Garrett 1963) and the percentage of the soil particles determined by Robinson's pipette method (Piper 1964). Soil reaction (pH) was determined by using a pH meter with a glass electrode in a mixed soil-water ratio of 1:2 and by electrometric method (Brady 1990) and the quantity of organic carbon of the soil estimated by the method of Walkley & Black (1934) as described by Jackson (1967).

Isolation of Soil Mycoflora

The soil samples were processed for isolation of rhizospheric mycoflora by using the soil dilution method (Waksman & Fred 1922) using different media *viz.,* CDA (Czapek's Dox Agar)

and MEA (Malt Extract Agar). Pure cultures of the fungi were obtained by single spore culture and by hyphal isolation methods (Warcup 1955).

Microscopic Observation and Identification

The microscopic observations, carried out at 400X and 1000X magnification using a compound light microscope. The colonies developed on the Petri plates were counted carefully, and individual colonies were identified and transferred to a separate agar plate.

Identification was done by following Smith (1971). For this purpose, pure colonies of isolates were obtained in CDA and MEA, followed by regular examining of the developed colonies, macroscopic and microscopic specifications studied, and identifications made. Macroscopically and microscopic specifications were studied, and identifications were made.

Further, identification of isolates was also confirmed by consulting various monographs, books, and papers available on fungal systematics (Raper & Fennell 1965, Barnett & Hunter 1998, Domsch et al. 1980, Moubasher 1993, Gilman 2001). The microphotography of different genera was identified during investigation using Magnus MIPS-USB (Olympus), DSCW320 (SONY). Further, the genera and species identified by consulting the reference cultures of **ITCC** (Indian Type Culture Collection) *viz., Aspergillus aculeatus* (ITCC2589), *Eurotium chevallieri* (ITCC2435), *C. oxysporum* (ITCC2797), *Fusarium dimerum* (ITCC3864), *F. oxysporum* (ITCC1635), *Penicillium janthinellum* (ITCC3014) and *P. oxalicum* (ITCC4933); **NFCCI** (National Fungal Culture Collection of India) *viz., A. ustus* (NFCCI5040); **MTCC** (Microbial Type Culture Collection) *C. cladosporoides* (MTCC2136); **QUCC** (Qatar University Culture Collection) and providing accession numbers *viz., Alternaria chlamydospora* (QUCC1120), *A. carbonarius* (QUCC128), *A. japonicas* (QUCC174), *Aspergillus tamari* (QUCC280), *C. sphaerospermum* (QUCC1950), *C. tenuissimum* (QUCC1960), *Cochliobolus hawaiiensis* (QUCC2530), *F. moniliforme* (QUCC2654), *Giberella intricans* (QUCC2620), *Mucor circinelloides* (QUCC4805), *N. sphaerica* (QUCC8010), *Talaromyces stipitatus* (QUCC8060), *Trichothecium roseum* (QUCC7410) and *Ulocladium chartarum* (QUCC7130).

Periodicity of Occurrence

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

$$S = \frac{2C}{spA + spB}$$

Where spA= Number of species in community A
spB= 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-Wiener Index, Simpson Index, Total dominance, and Evenness were analyzed using a computer software program (Bio-tool kit 320).

The Shannon-Wiener's diversity index was used instead of the direct number of soil fungal species to indicate the fungal diversity of each land use, and the index value was compared with other land use diversity indices due to the fact that it is logarithmic, thus preventing an overestimation of heavily sporulating species and taking into account the number of species and the number of isolates (colonies) of all species (Grishkan et al. 2005).

Results

Physical and Chemical Analysis of Soil Samples

The moisture content of soil samples collected from the rhizosphere of *H. salicifolia* varied greatly between different sites. The average soil moisture at both sites was recorded between 3.86±1 and 1.63±1, (Table 1). The pH (Hydrogen ion concentration) measures the hydrogen ion concentration, i.e., acidity or alkalinity of the soil. It can affect the availability of nutrients and the activity of many essential microorganisms. The pH of soil may influence plants grown in the forestland and the types of soil microbiota. The pH of all soil samples found in the range between 6.67±1 to 5.95±1 indicated the slight acidity of soils (Table 1). The observation revealed that in the forestland of the selected two sampling sites of Uttarakhand state, the texture of the soil samples of different sites varied from sandy to loam. The value of organic carbon was between 2.01 and 1.49 mg/kg.

Table 1. Moisture content, pH, Organic Carbon content, Colour, and Texture of two different sites and subsites**.**

Parameters	Sampling sites					
	Mana (M)			Yamnotri (Yam)		
	(1)	(2)	(3)	(1)	(2)	(3)
M.C.	3.36	3.82	4.41	1.67	1.96	1.28
Mean		3.86±1			1.63±1	
Ph	6.68	6.60	6.75	5.98	5.46	6.42
Mean		6.67±			5.95±1	
$C_{org.}$	1.76	2.31	1.97	1.07	1.87	1.54
Mean		2.01±1			1.49	
Colour		Greyed brown			Blackish brown	
Texture		Sandy			Loam	

*****M.C.= Moisture content; $C_{org.}$= Organic Carbon content.

Fungal Diversity from Rhizosphere Soil of *Hippophae salicifolia*

During the present investigation, 34 species of microfungi belonging to 21 genera were isolated from the *Hippophae* growing forestlands of Chamoli and Uttarkashi districts of Uttarakhand. *Cladosporium* was the most dominant genus, followed by *Aspergillus* and *Fusarium*. *C. cladosporiodes* was the most common fungi isolated from *Hippophae* growing sites with maximum contribution (7.43%), whereas *N. sphaerica* was the least important species with low contribution (0.80%). The highest number of species (23) was isolated from the Mana site of Chamoli (Table 2).

Periodicity of Occurrence

During the present investigation, six species *viz., C. cladosporiodes, C. oxysposum, C. sphaerospermum, Geotrichium sp., G. intricans,* and *U. chartarum* showed common occurrence. Ten species *viz., A. chlamydospora, A. humicola, A. aculeatus, A. carbonarius, A. japonicas, A. tamarii, C. hawaiiensis, F. dimerium, P. oxalicum, Verticillium sp.* showed frequent occurrence

while twelve species *viz., Absidia sp., Arachnoitus sp., A. ustus, Cl. teniussium, E. chevalieri, F. moniliforme, M. circinelloids, M. moelleri, Nectria sp., P. janthinellcum, Trichoderma sp.,* and *Zygorhynchus sp.* showed moderate occurrence. The rest of the six species *Acrophialophora, F. oxysporum, Fusarium* sp. *N. sphaerica Taloromyces stipitatus T. roseum* showed rare occurrences in all selected forest sites (Table 2, Fig. 2a, b, c, d, e, f).

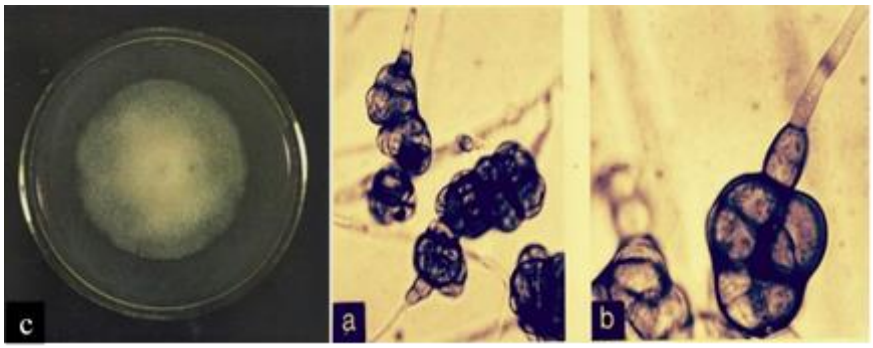
Comparative analysis of fungal diversity

Six hundred nineteen colonies of soil fungi belonging to 21 genera and 34 species were isolated from the selected rhizospheric soil of *H. salicifolia* growing sites. A total of 15 genera and 23 species were isolated from Mana and 12 genera and 14 species from Yamnotri (Fig. 3). Anamorphic fungi (Deuteromycota) were found to be dominant with 13 genera and 25 species, followed by Ascomycota with five genera and five species, and Zygomycota with three genera and four species. *Cladosporium* sp. was the most dominant genus with maximum contribution (7.43%), followed by *Geotrichium* sp. (6.00%).

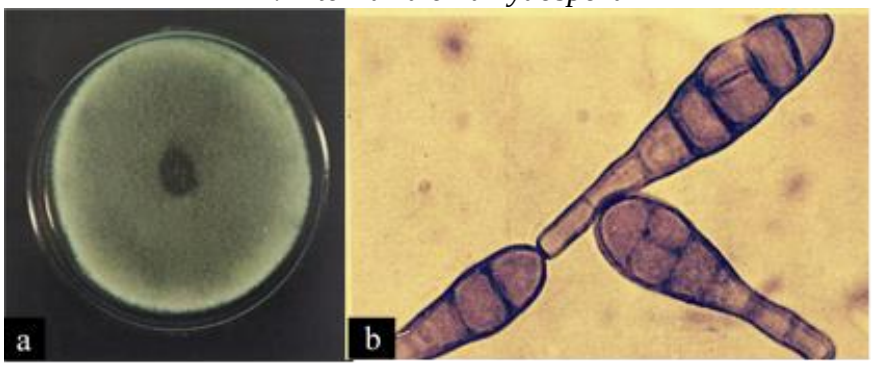
Table 2 Mycoflora of *Hippophae salicifolia* growing areas of Chamoli and Uttarakashi district of Uttarakhand.

S.N	Name of fungus	Mana (1)	Mana (2)	Mana (3)	Yam (1)	Yam (2)	Yam (3)	Percent contribution %	Periodicity of Occurrence
1	*Absidia* sp.	6	4	1	-	-	-	1.77	M
2	*Acrophialophora*	2	8	-	-	-	-	1.61	R
3	*Alternaria chlamydospora*	8	2	5	-	-	-	2.42	F
4	*Alternaria humicola*	-	-	-	3	5	7	2.42	F
5	*Arachniotus* sp.	-	-	-	-	4	6	1.61	M
6	*Aspergillus aculeatus*	7	9	2	-	-	-	2.90	F
7	*Aspergillus carbonarius*	6	15	2	-	-	-	3.71	F
8	*Aspergillus japonicas*	7	10	6	-	-	-	3.71	F
9	*Aspergillus tamari*	-	13	6	-	-	-	3.06	F
10	*Aspergillus ustus*	-	-	-	-	4	8	1.93	M
11	*Cladosporium cladosporiodes*	4	10	5	8	2	17	7.43	C
12	*Cladosporium oxysporum*	15	6	4	-	-	-	4.03	C
13	*Cladosporium sphaerospermum*	12	20	3	-	-	-	5.65	C
14	*Cladosporium tenuissimum*	-	8	8	-	-	-	2.58	M
15	*Cochliobolus hawaiiensis*	-	-	-	8	7	2	2.74	F
16	*Eurotium chevalieri*	-	-	-	1	12	2	2.42	M
17	*Fusarium dimerum*	5	17	2	-	-	-	3.87	F
18	*Fusarium moniliforme*	-	-	-	3	9	1	2.10	M
19	*Fusarium oxysporum*	-	-	-	-	1	5	0.96	R
20	*Fusarium* sp.	-	-	-	1	-	7	1.29	R
21	*Geotrichium* sp.	3	10	5	8	7	9	6.78	C
22	*Giberella intricans*	10	4	3	7	5	11	6.46	C
23	*Mucor circinelloides*	5	6	2	-	-	-	2.10	M
24	*Mucor moelleri*	9	4	2	-	-	-	2.42	M
25	*Nectria* sp.	-	-	-	9	5	2	2.58	M
26	*Nigrospora sphaerica*	-	5	-	-	-	-	0.80	R
27	*Penicillium janthinellum*	4	7	5	-	-	-	2.58	M
28	*Penicillium oxalicum*	8	10	5	-	-	-	3.71	F
29	*Taloromyces stipitatus*	6	3	-	-	-	-	1.45	R
30	*Trichoderma* sp.	7	1	4	-	-	-	1.93	M
31	*Trichothecium roseum*	-	6	2	-	-	-	1.29	R
32	*Ulocladium chartarum*	-	-	-	3	5	16	3.87	C
33	*Verticillium* sp.	-	-	-	8	11	3	3.55	F
34	*Zygorhynchus* sp.	-	11	2	-	-	-	2.10	M

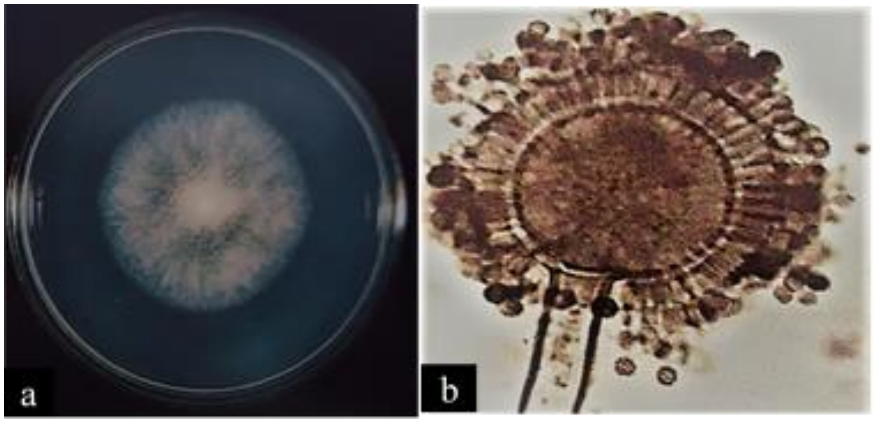
sp.- species; YAM-Yamnotri



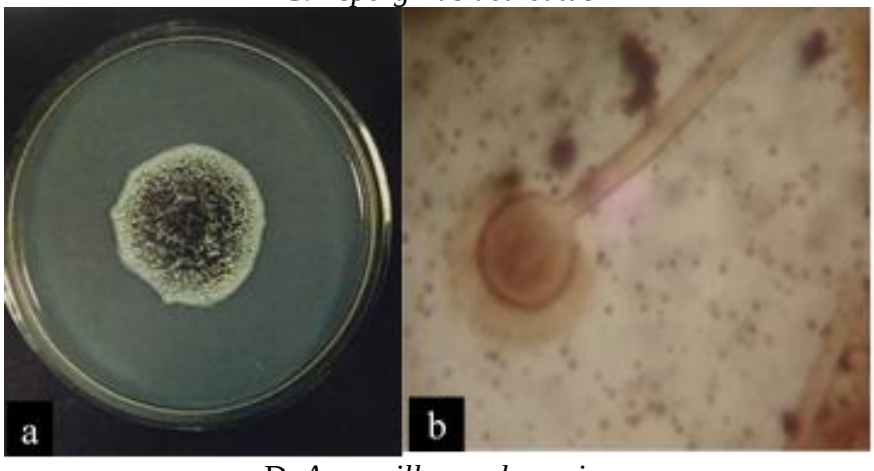
A. *Alternaria chlamydospora*



B. *Alternaria humicola*

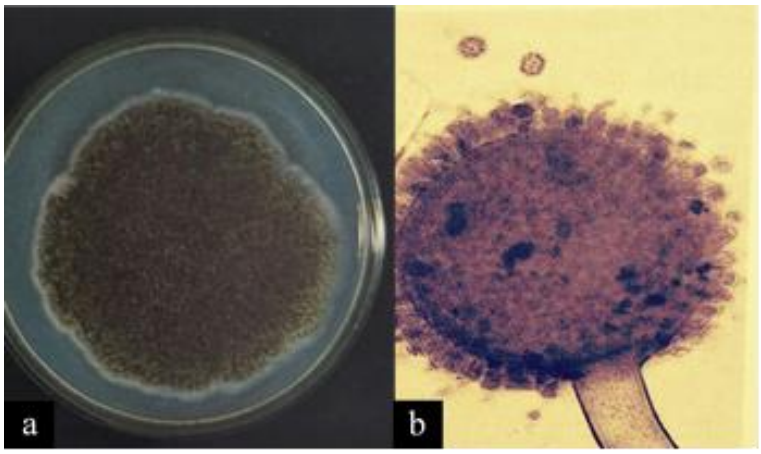


C. *Aspergillus aculeatus*

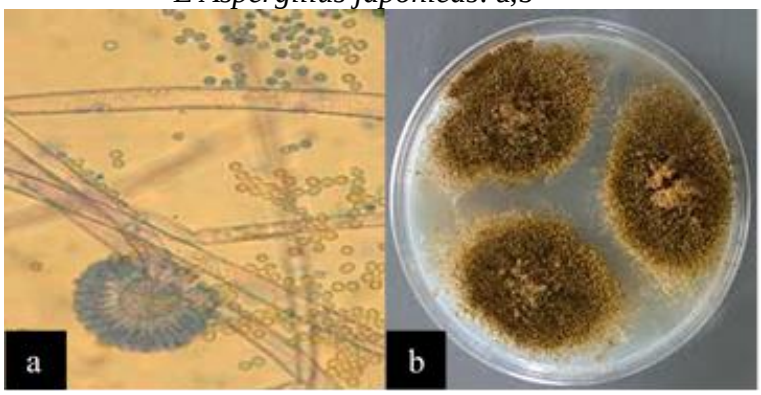


D. *Aspergillus carbonarius*

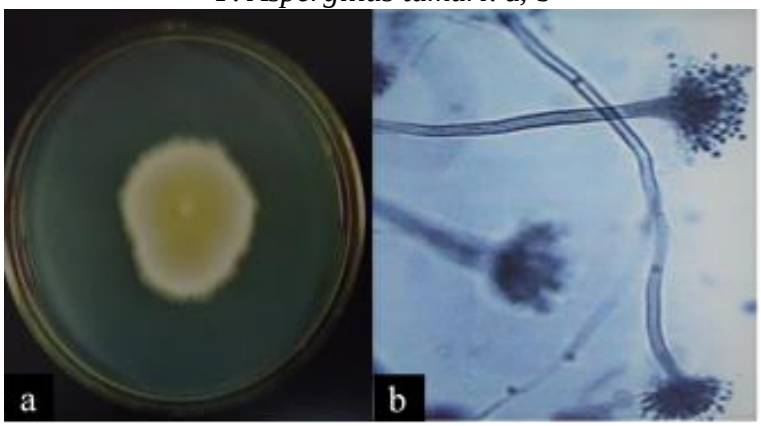
Fig. 2a – A *A. chlamydopora*: a, b-conidiophore and conidia (x1000), c: 7-day old colony on CDA. B *A. humicola*: a- 7-day old colony on CDA, b- conidia (x400). C *A. aculeatus*, b: showing conidial head and conidiophores (x400), a: 7-day old colony on MEA. D *A. carbonarius*, a: conidiophores and conidial heads (x400), b: 7-day old colony on MEA



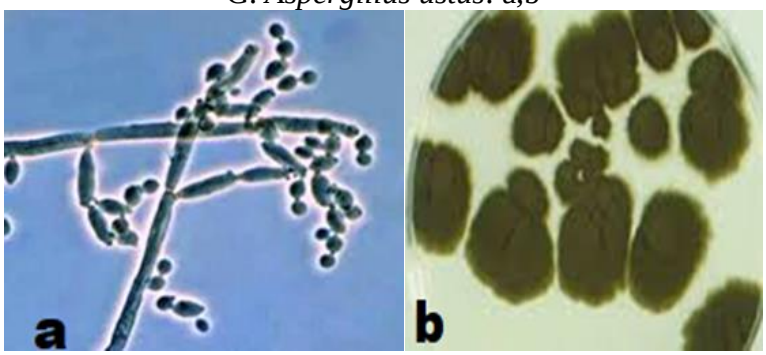
E *Aspergillus japonicas*: a,b



F. *Aspergillus tamari*: a, b

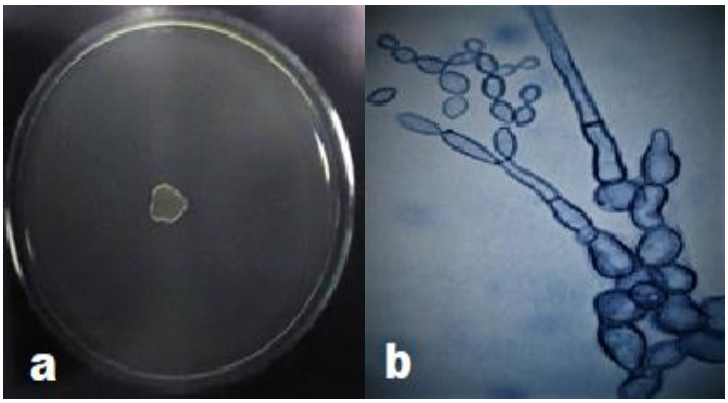


G. *Aspergillus ustus*: a,b

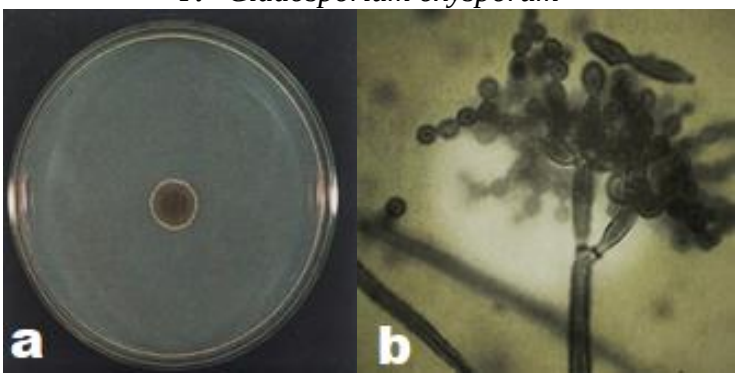


H. *Cladosporium cladosporoides*: a,b

Fig. 2b – E *Aspergillus japonicas*: a-7 day old colony on CDA, b-conidia head, and conidia (x400). F *Aspergillus tamari*:a- conidial heads (x400), b-7 day old colony on CDA. G *Aspergillus ustus*: a-7 day old on MEA, b-conidiophore, conidial head and conidia (x400). H *Cladosporium cladosporoids*: a-conidia and conidiophores [x400], b-7 day old colony on MEA.

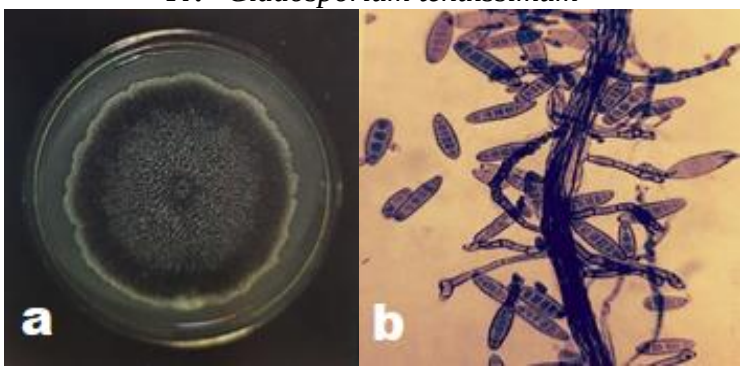


I. *Cladosporium oxysporum*



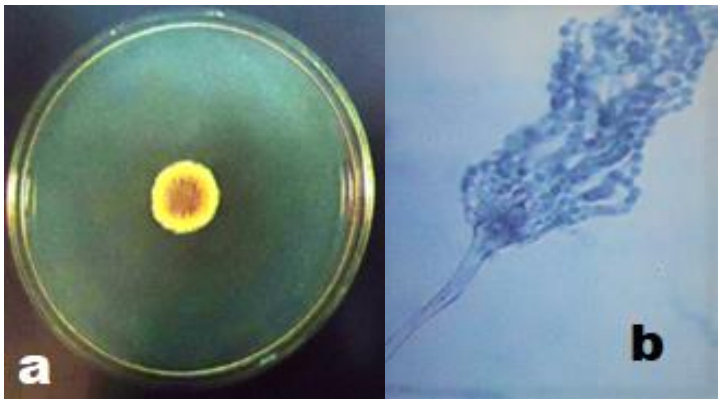
J. *Cladosporium sphaerospermum*

K. *Cladosporium tenuissimum*

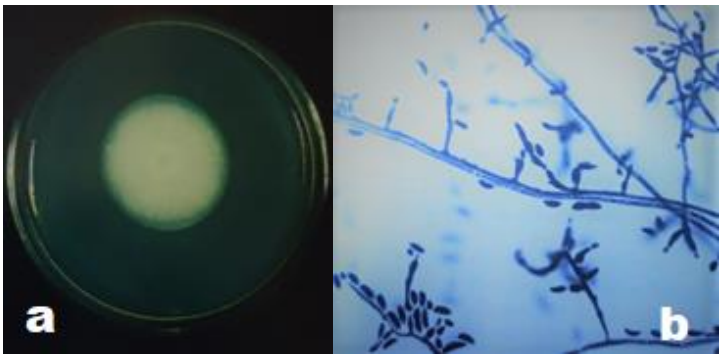


L. *Cochliobolus hawaiiensis*

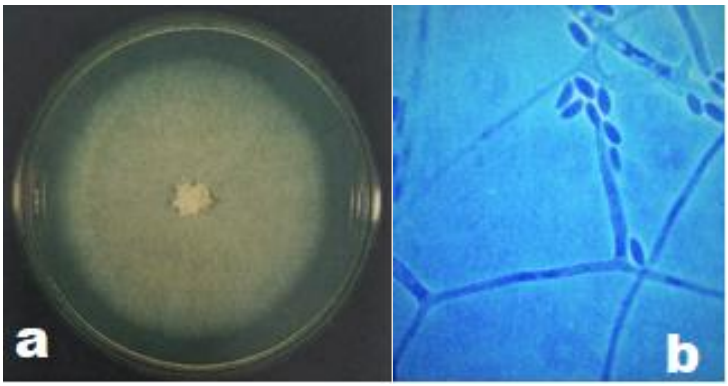
Fig. 2c – I *Cladosporium cladosporoides*: a-7 day old colony on MEA, b- conidiophore and conidia (x1000). J *Cladosporium sphaerospermum*: a-7 day old colony on MEA, b- conidiophores and condia (x1000). K *Cladosporium tenuissimum*: a-7 day old colony on MEA, b- conidiophore and conidia in chains (x1000). L *Cochliobolus hawaiiensis*: a- 7 day old colony on CDA, b- conidiophores and conidia (x400).



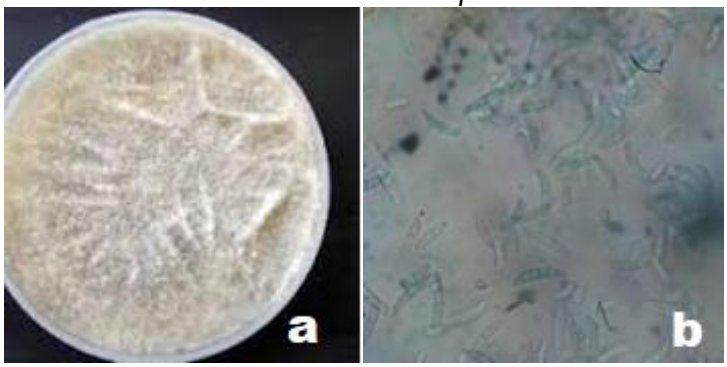
M. *Eurotium chevalieri*



N. *Fuasrium dimerum*

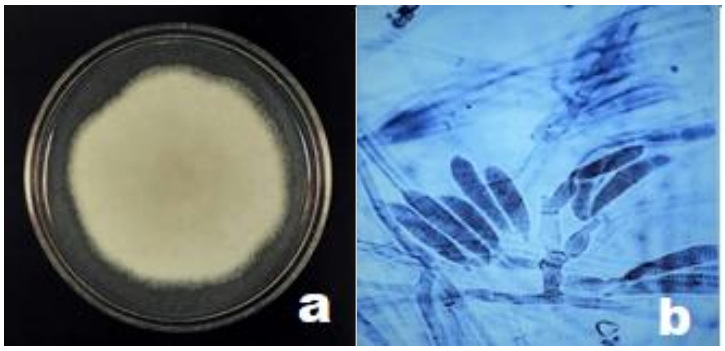


O. *Fusarium moniliforme*

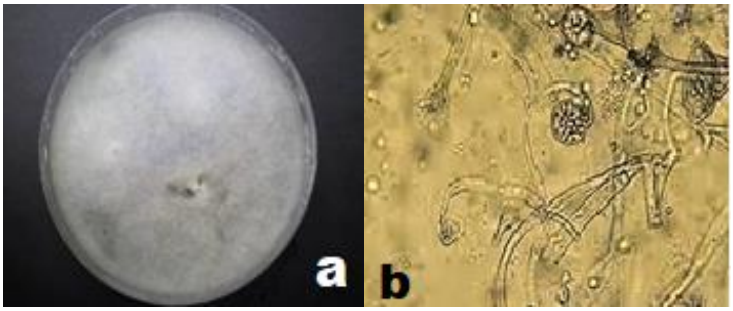


P. *Fusarium oxysporum*

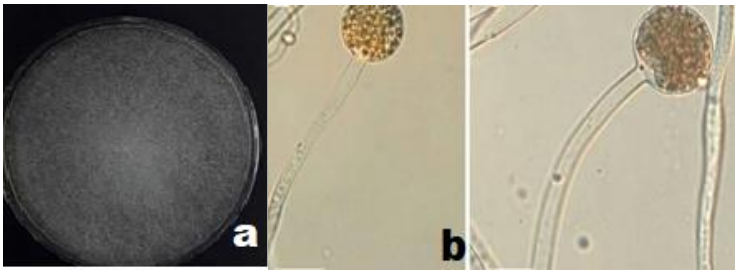
Fig. 2d – M *E. chevalieri*: a-7-day old colony on CDA, b- conidial heads (x400). N *F. dimerum*: a-7 day old colony on CDA, b- phialides and conidia (x400). O *F. moniliforme*: a- 7 day old colony on CDA, b- phialides and conidia (x400). P *F. oxysporum*: a-7 day old colony on CDA, b- conidia (x400).



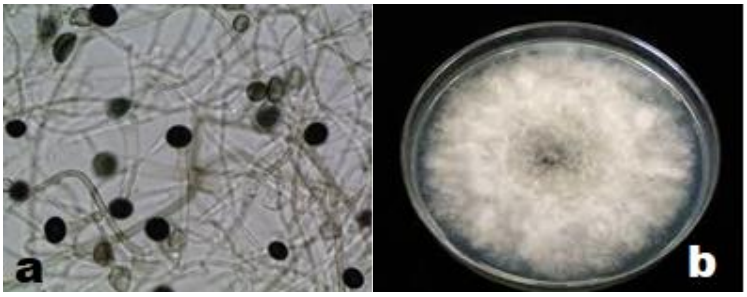
Q. *Giberella intricans*



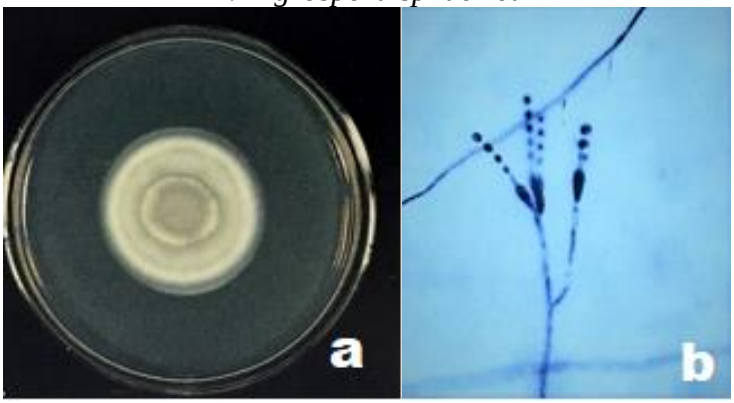
R. *Mucor circinelloides*



S. *Mucor molleri*



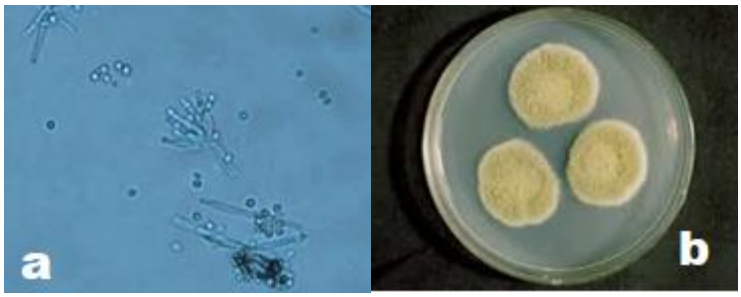
T. *Nigrospora sphaerica*



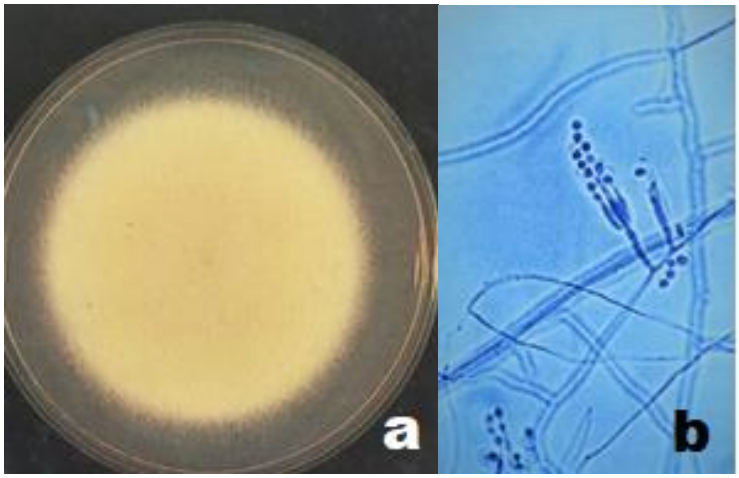
U. *Penicillium janthinellum*

Fig. 2e – Q *Giberella intricans*: a- 7 day old colony on MEA, b- phialides and conidia (x1000). R *Mucor circinelloides:* a-7 day old colony on CDA: b- sporangiophore, sporangia and sporangiospore (x100). S *Mucor molleri*: a- 7 day old colony on CDA, b- sporangiophore,

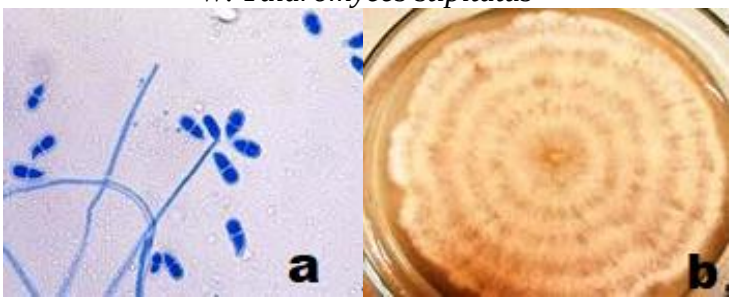
columella and sporangia (x400). T *Nigrospora sphaerica*: a-7 day old colony on MEA, b- conidia (x400). U *Penicillium janthinellum*: a-7 day old colony on MEA, b- penicili (x1000).



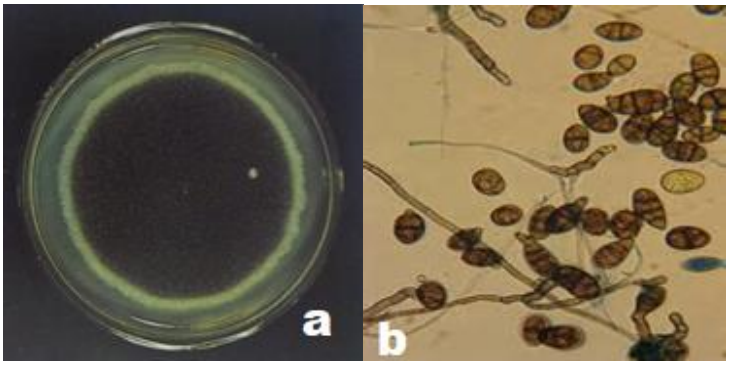
V. Penicillium *oxalicum*



W. Talaromyces stipitatus



X. Trichothecium roseum



Y. Ulocladium chartarum

Fig. 2f – V *Penicillium oxalicum*: a- phialides and conidia (x400), b- 7 day old colony on MEA. W *Talaromyces stipitatus*: a-7 day old colony on MEA, b- penicili (x400). X *Trichothecium roseum*: a- conidia (x400), b- 7 day old colony on MEA. Y *Ulocladium chartarum:* a- 7 day old colony on CDA, b- conidiophores and conidia (x400).

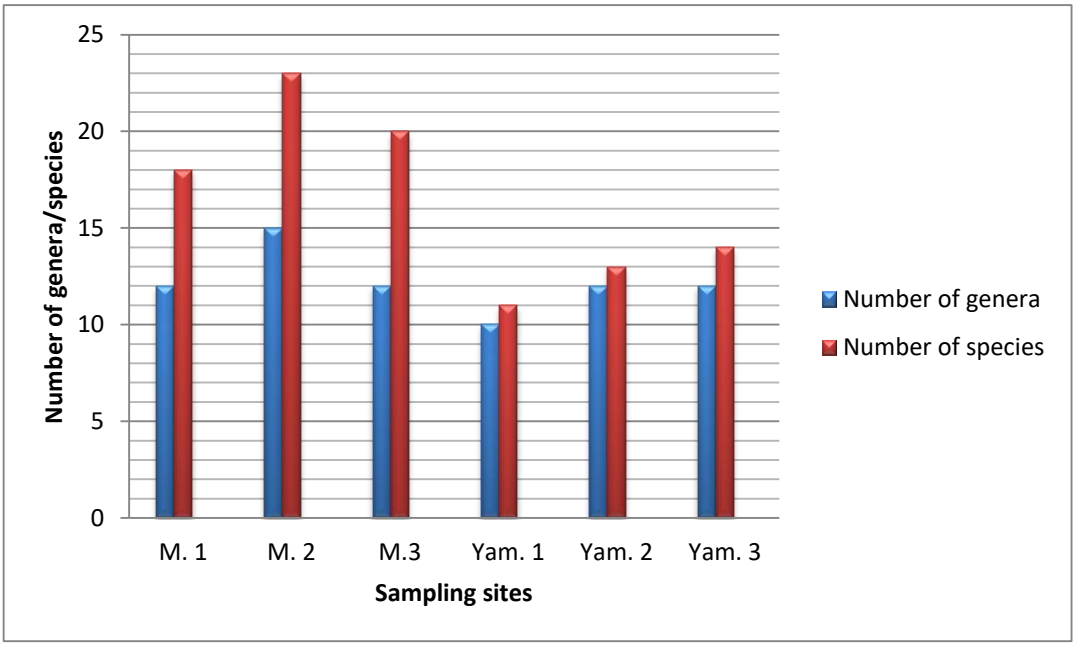


Fig. 3 – Total number of genera and species isolated from different *Hippophae salicifolia* growing sites

Species richness, similarity, and dissimilarity index

Mana site was richer with 23 species than Yamnotri with only 14 species. The highest similarity index (96.29%) was observed between Yam (2) and Yam (3), while the lowest similarity index (16.21%) was between Mana (2) and Yam (3) (Table 3).

Diversity index, Dominance, and Other Parameters

The Shannon-Wiener's diversity index was highest in Mana 2 (2.972), followed by Yamnotri 1 (2.217). Total richness and abundance were highest in Mana 2 and lowest in Yamnotri 1. Evenness was highest in Mana 1 (0.966), followed by Mana 3 (0.954), while lowest observed in Yamnotri 3 (0.905) (Table 4).

Table 3 Similarity and Dissimilarity Index of Soil Fungi.

		MANA				**YAMNOTRI**					
MANA	M1	M 2		M 3		YAM 1		YAM 2		YAM 3	
		IS	DS	IS	DS	IS	DS	IS	DS	IS	DS
1		0.878	0.122	0.842	0.158	0.206	0.794	0.193	0.807	0.187	0.813
2				0.930	0.07	0.176	0.824	0.166	0.834	0.162	0.838
3						0.193	0.807	0.181	0.819	0.176	0.824
YAM 1								0.833	0.167	0.88	0.12
2										0.962	0.038
3											

*M = MANA; YAM = YAMNOTRI; IS = INDEX SIMILARITY; DS = DISSIMILARITY INDEX

Table 4 Diversity Indices and Other Parameters.

PARAMETERS	**MANA**			**YAMNOTRI**		
	(1)	**(2)**	**(3)**	**(1)**	**(2)**	**(3)**
Shannon-Wiener Index	2.792	2.972	2.858	2.217	2.423	2.389
Species richness (S)	18.0	23.0	20.0	11.0	13.0	14.0
Total Abundance	124	189	77	56	77	96
Simpson diversity Index (D)	0.066	0.057	0.063	0.119	0.097	0.107

Table 4 Continued.

PARAMETERS	MANA			YAMNOTRI		
	(1)	(2)	(3)	(1)	(2)	(3)
(1-D)	0.933	0.942	0.936	0.880	0.902	0.892
(1/D)	14.95	17.36	15.64	8.34	10.20	9.29
Evenness	0.966	0.948	0.954	0.924	0.944	0.905

Discussion

Soil is the outer region of the earth's crust consisting of loose materials formed by the gradual weathering of rocks and provides both mechanical and nutritional support to plants. Thus, the soil is a complex product of parent material, topography, climate, and biological activity (Griffin 1972). The major external influences exposed to the microbial community include moisture content, pH, temperature, organic and inorganic fertilizers, soil profile position, and vegetation composition (Dubey & Dwivedi 1988)

The occurrence of the fungal population is also correlated with the availability of mineral nutrition and other factors, including temperature and moisture (Vanvuurde & Schippers 1980). The saprobic fungi represent the largest proportion of fungal species in soil. They perform a crucial role in the decomposition of plant structural polymers, such as cellulose, hemicelluloses, and lignin, thus contributing to the maintenance of the global carbon cycle. The distribution of these organisms is influenced by the abundance and nature of the organic content and soil texture (Marschner et al. 2003). The fungal identification is complicated by the fact that their life cycle in the soil and in the laboratory can be quite different. Fungi are nutritionally so diverse that there is no perfect medium, which can yield all the fungi. The technique used in the present study is a standard one (Warcup 1955a). *Aspergillus* and *Penicillium* usually appeared abundantly in collections because of their prolific sporulating capacity.

The co-relationship between soil pH, organic carbon, moisture content, total N, and available K with fungal population numbers reflects the major role played by these physicochemical factors in changing the population number and relative abundance of fungal species. Soil moisture enhances the microfungal activity by making organic carbon (C_{org}) available (Schreven 1967). The quantity and quality of organic carbon present in soil could govern the microbial population numbers by affecting the microbial activity (Tiwary et al. 1982, Jiménez-Morillo et al. 2016).

In the present investigation, the moisture content of all soil samples was very low. The soil moisture was found to correlate with spore density. The increase in spore population may be due to an increase in soil moisture. Mohankumar & Mahadevan (1999) reported similar reports. Khan (1974) reported low spore density May. It indicates that spore density correlated with moisture content. Besides, it is a fact that acid soils conditions favor the development of fungi. Fungi are much more resistant to the acidic environment than any other group of soil microorganisms (Johnson 1923). Therefore, on acidic media or habitats, luxuriant growth of soil fungi is generally recorded because of low competition for food.

Various studies have reported similar fungal genera from the rhizosphere of different plants (Sagar et al. 2006, Sagar & Chauhan 2009, Sagar & Kumari 2009). Visser & Parkinson (1975) stated that only a few fungal species were dominant for a given community, which might strongly affect the environmental conditions for other species. Maximum isolated genera belong to subdivision Deuteromycotina because these fungi imperfecti can tolerate wider environmental conditions compared to the other fungal populations (Behera & Mukherji 1984). Manoharachary (1977) reported a direct correlation of moisture and fungal members of various soils.

The serial dilution method is a stepwise method, and due to its simpler, cheaper, and helpful method, the results obtained are more manageable (Aneja 2001). The rhizospheric soil of wild plants supports an abundance of diverse saprophytic microorganisms. This could be due to the high input of organic carbon compounds into the soil through the process of rhizodeposition (Merckx et al. 1987). The present experimental result showed infection of many species of genus *Aspergillus, Penicillium,* and *Fusarium*. Our data suggested that methods used in detecting rhizosphere soil

mycoflora were effective in a large number of fungi isolation, and these play vital roles in biomass turnover and form an important part of the ecosystem (Jones & Hyde, 1988). However, Sharmir & Steinberger (2007) stated that it might be a higher amount of organic matter, which in the presence of adequate moisture supply acted upon by the microorganisms present in topsoil, resulting in higher fungal colony counts. In the present study, the species of *Aspergillus* were common and integral associates to the soils under study. Rama Rao (1970) and Domsch & 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 (Guleri et al. 2016).

The presence of one or a few dominant fungal species in the present study agreed with that of Jha et al. (1992), who pointed out that for a given community, only a few species are numerically predominant and may strongly affect a given community the environmental conditions for the others. The dominance of *Cladosporium, Aspergillus*, and *Fusarium* in the present study might be due to their greater rate of spore production and dispersal as well as their resistance to existing environmental conditions. Schimel (1995) made a similar observation. According to Entry & Emmingham (1996), the significant changes in soil microfungal population are attributed to the type of vegetation growing in a particular area. In addition, topography might influence the quantity and diversity of fungal population numbers in soil (Tsai et al. 2007). Also, the fungal population was observed more in rhizosphere regions of Seabuckthorn plant than in the soil away from the host plant. The root exudates contain sugars and amino acids, which are used by microbes as food or energy sources. That is the possible reason for a higher fungal population in the rhizospheric region of Seabuckthorn plant.

A distinct pattern of fungal community structure was observed in all the samples during the study period. The percentage contribution and abundances of different fungal species fluctuated. *Cladosporium* was the most dominant, followed by *Aspergillus, Fusarium, Penicillium,* and *Mucor*. Earlier reports have indicated that these genera appeared abundantly in soils (Mohanty et al. 1994b). This may be due to the faster growth rate of these fungi and their better intrinsic prolific sporulating capacity to utilize the substrate. Considering the dominant species, it is clear that fungal succession in the plantation site greatly differed. The species composition in soil showed marked differences with a change in habitat and surface vegetation. Their occurrence might be due to the ability of these groups of fungi to survive in adverse conditions. They have frequently been isolated in various soils in India (Behera et al. 1985, Manoharachary et al. 2005).

The purpose of categorizing soil fungi is based on their appearance in all or some sites and their abundance 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 *Hippophae* growing sites, while those that distribution was categorized as moderate or rare will disappear easily from the sites. In this study, Deuteromycotina was the dominant group. These observations have also been observed by several workers in their different studies from many parts of the world (Manoharachary et al. 2005, Rane & Gandhe 2006, Panda et al. 2009, Saravanakumar Kaviyarasan 2010).

Regarding rhizosphere fungi of Seabuckthorn, it is the first report of its kind. The present study is a preliminary attempt to explore the occurrence of soil mycoflora from the rhizosphere of the host plant of some selected sites of Garhwal Himalayas. The results show consistency with earlier findings of other workers (Odunfa & Oso 1979). The values of similarity and dissimilarity index also show the effect of the rhizosphere upon the quality and quantity of the fungal species present in the soil.

To conclude, the study is an attempt to explore the soil microfungal diversity of selected sites from two regions (Uttarkashi and Chamoli) of Garhwal Himalaya under the rhizospheric region of *H. salicifolia* growing areas. The investigation revealed the isolation and identification of 34 species belonging to 23 genera. It is believed that further investigation of these areas soils under different associated plant species of host plant like *Cedrus deodara, Pinus wallichiana, Picea smithiana, Betula utlis, Alnus nepalensis, Abies pindrow, Juniperus macropoda, Prinsepia utilis,*

Rosa webbiana, and Cotoneaster sp. will definitely add to the knowledge of microfungal flora adapted to different ecosystems of the temperate zones of Garhwal Himalayas.

Acknowledgements

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