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Soil nutrients dynamics and status of Mycoflora Population in a tropical Forest Soil of Uttarakhand Himalaya

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Abstract

Tropical forests have a great diversity of microorganisms. Fungi are the most important group of microbes. They play an essential role in tropical forest ecosystem as saprotrophs, mutualists and pathogens. In the present study, we attempted to study the soil nutrient dynamics and status of the mycoflora population, along with the effect of soil nutrient dynamics on the fungal population. Fourteen species of fungi within five genera belonging to three families were isolated and identified from the six forest sites in the Chilla forest division under Rajaji tiger reserve. They belong to the class *Zygomycetes* and form class *Deuteromycetes*. The soil of the study area was acidic without much variation among all the six study sites. Higher nutrient concentrations were recorded in the top layer of the soil, which supports the maximum number of fungi population in the study area. Further, fungal flora was also correlated with varying ecological factors, *viz.* pH, organic carbon and nitrogen and phosphorus.

Keywords – Chilla – Fungi – Microorganism – Rajaji tiger reserve – Soil fungi

Introduction

Soil harbor huge diversity of fungi with various ecological functions (Bridge & Spooner, 2001). It is the dynamic environment in which various biological activities are accelerated or controlled by microorganisms. These microorganisms play significant role for decomposition of organic matter and nitrogen fixation, which further enhance the bioavailability of various metals ions, sulphates, as well as nitrates (Bridge & Spooner 2001). Microorganisms are the important part of all ecosystems. The ubiquity of microbes is just because of their population size rather than any inherent property (Fenchel & Finlay 2004). As the environmental requirement of microbes are met, they can be found everywhere (Brock 1961).

Tropical forest soil provides rich habitat for the growth of microbes. Fungi are an important group that could survive at low level of pH (Mohammadi & Balasjin 2014). Soil fungi are microscopic, thread-like structures of hyphae that constitute the mycelium, which helps to absorb nutrients from the colonized roots and surface organic matter. Soil fungi are responsible for controlling nutrient cycling and avails nutrients in soil through degradation of organic matter (Singh & Rai 2004). Further, they developed humus in the soil and play essential role in soil composition (Rane & Gandhe 2006). They changed the biological properties of soil, creating a medium for biological reactions and different life-supporting processes in soil (Olson et al. 2000).

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The Chilla forest range of Rajaji Tiger reserve lies to the east of the river Ganges and is attached to the Garhwal forest division, while the Motichur range of Rajaji lies to the west of the river. The study area comes under subtropical moist deciduous forest in Shivalik Himalaya (Akash et al. 2020a). Due to wide variation in the topography and climatic conditions, soil of the Tiger reserve show variation in the texture, colour, drainage, organic matter content and cation exchange capacity (Akash et al. 2018). The dense vegetation is contiguous with the Chilla forest range, making it a unique repository of biodiversity in Northern India (Akash et al. 2018). Climatic conditions are generally hot with high humid conditions. The temperature rises to 40–45^{0}C in summer and drops to 20–25^{0}C in the winter. The annual rainfall ranges from 1200–1500 mm in the study area. The highest rainfall occurs from May to July during the Southeast monsoon and October to November during the Northeast monsoon (Akash et al. 2018, 2019). There is limited data on herbal remedies used by local inhabitants of Rajaji. The Gujjars, a forest-dwelling tribe, live in and around the forest of Rajaji Tiger reserve. Gujjars live together as a small community in Chilla and Motichur forest ranges in the reserve. In addition, some of the Gujjars are now rehabilitated (Akash et al. 2018, 2019). The traditional occupation of Gujjars includes rearing cattle for milk and meat and supplying it to the market areas (Akash et al. 2020b).They also prepare medicines at a small level and sell them into the market. Further, they also used to collect various non-timber forest products, such as bee wax, honey, ethnomedicinal plants, raisin and gum (Akash et al. 2020a, b, c, d, Akash et al. 2021).

In Himalayan forests, due to anthropogenic activities like deforestation, lopping, scraping and trampling well-furnished ecosystems have converted into degraded lands, leading to the continuous loss of soil (Akash & Navneet 2020). This condition arises during the last few years due to higher demands, in which a huge area of the forest cover has been converted into a plane land, resulting in the reduction of organic carbon in soil (Lal 2002, Steenwerth et al. 2005), as well as changes in microbial flora (Ding et al. 2013). Himalayan soil has various nutrients due to degrading activities of the litter, so it is best suited for the high production rate and sustainability (Akash et al. 2020b). Due to the degradation of litter, the soil of this region is rich in nutrients, which is helpful for plants growing around the Himalayan region (Akash et al. 2020). As per the study, enzymes play important role in the degradation of organic matter released by microbial biomass in the soil (Ajwa et al. 1999, Klose & Tabatabai 2000).

Material & Methods

For microbial analysis, composite soil samples from the depth of 0-10 were collected from the six forest sites, namely Lalsroth (LS, 378.6 m, Latitude: 30° 00' 35.9'' N, Longitude: 78° 16' 21.8 E), Kodiya ridge (KR, 350 m, Latitude: 30° 0' 35.8'' N, Longitude: 78° 15' 45.7 E), Kodiyabelt 6-8 (KB 6-8, 458.1 m, Latitude: 29°59' 41.0'' N, Longitude: 78° 17' 41.1 E), Sofuti SF, (482.7 m, Latitude: 29°59'28.8'' N, Longitude: 78° 17' 44.2 E), Ghasiram sroth (GS, 304 m, Latitude: 29°57' 44.92''N, Longitude: 78° 11' 33.81 E), Kharasroth (KS, 318 m, Latitude: 29°56' 51.9'' N, Longitude: 78° 10' 46.17 E) of the Chilla range of Rajaji Tiger reserve in polybags. (Fig. 1). The surface litter were removed from the soil samples. On the other hand, for soil analysis, the composite soil samples were collected at 0–10 cm (upper layer), 15–25 cm (middle layer) and 30–40 cm (lower layer) depths and packed into poly bags. The pH of soil samples was determined by pH meter, and the total organic carbon was determined using Walkley and Black method (Maiti 2003). The total nitrogen was calculated using the Kjeldahl method (Maiti 2003, Bahera 2014) whereas the total phosphorus content was analyzed using the Molybdenum-blue method (Singh & Chauhan 2014, Maiti 2003). The total potassium was determined by the method described in Maiti (2003) and Bahera (2014).

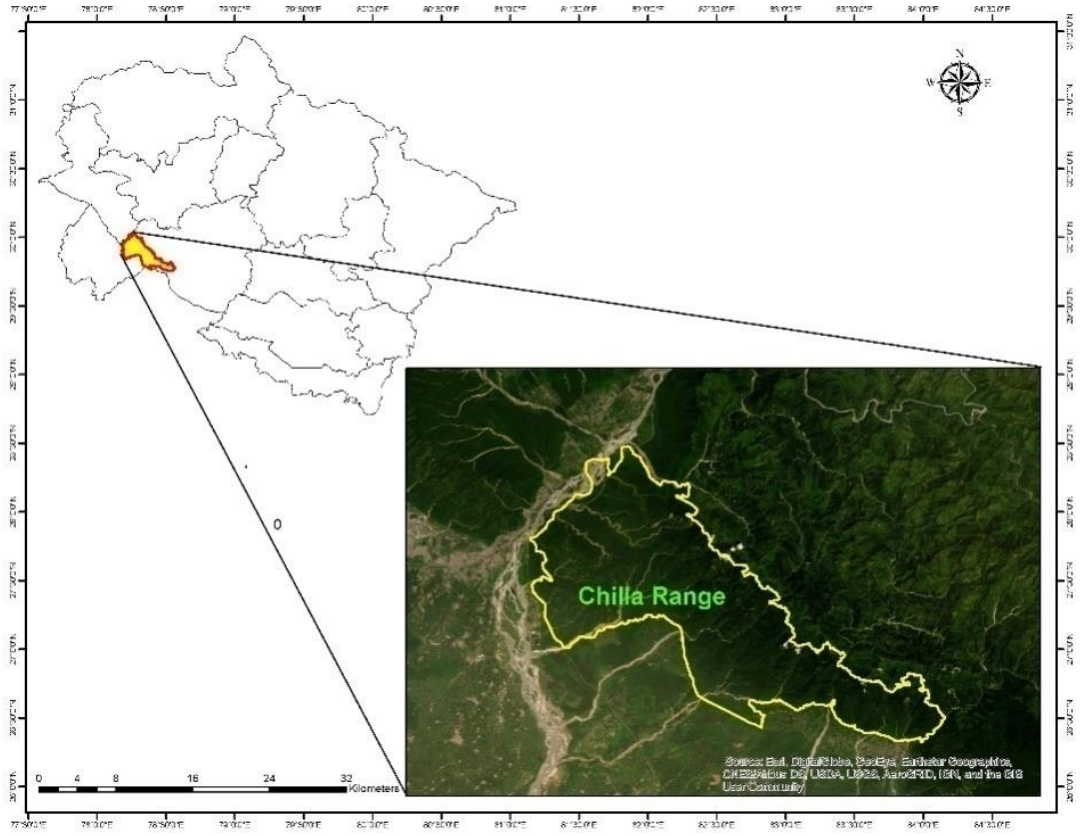

Fig. 1 – Map of the study area

Isolation of Fungi and Bacteria from soil

Martin's Rose Bengal Agar medium was used for qualitative and quantitative estimation of fungi and Nutrient agar medium was used for quantitative estimation of bacteria. The enumeration of fungi and bacteria was done by the serial dilution plate method. The quantitative estimation was done by following formulas:

$$\text{CFUs } g^{-1} \text{ dry soil} = \frac{\text{Average number of colonies}}{\text{Dry weight of soil}} \text{ X Dilution factor}$$

Identification of fungi

All fungal species were identified on the basis of their morphological characteristics with the help of Thom & Raper (1945), Raper & Thom (1949) and Gilman (1957). Finally, relative occurrence was calculated by using the following formula:

$$\text{Relative occurrence (\%)} = \frac{\text{Average number of colonies of a species X 100}}{\text{Average no of colonies of all the fungal species}}$$

Diversity indices

The Shannon-Wiener's diversity index (H') was used to determine the fungal species diversity among all the six sites of the Chilla forest division in Rajaji Tiger reserve (Magurran 1988).

$$H' = - \Sigma \, pi \ln p_i$$

Where, pi = ni/N, is the proportion of the number of colonies of individual species to the total number of colonies

ni = number of colony for individuals species "i", N = total number of colonies

The concentration of dominance or Simpson diversity index was calculated using the following formula:

$$Cd = \Sigma p_i^2 \quad \text{(Simpson 1949).}$$

Where Cd = Concentration of dominance

The species richness of fungi in each of the six sites of the Chilla forest range was calculated from the method of Margalef (1968)

$$R = S\text{-}1/\ln (N)$$

Where S = Number of the fungal species,

N = Total number of Individuals

The fungal diversity profile and Individual rarefaction curve of species were constructed in Paleontological Statistics (PAST) Software Program Version 3.14 based on the presence and absence of fungal species and the number of individual of fungal species in all the six sites, while ANOVA for soil parameter was in SPSS software version 20.

Results

Fourteen species of fungi within five genera under three families were isolated and identified from the Chilla range of Rajaji Tiger reserve. They belongs to the class *Zygomycetes* and form class *Deuteromycetes*. The fungal species were *Aspergillus niger, A. flavus, A. oryzae, A. terreus, A. luchuensis, Penicillium notatum, P. citrinum, P. oxallicum, Penicillium* spp., *Cladosporium cladosporides, Fusarium* spp., *Rhizopus stolonifer,* white sterile form and black sterile (Fig. 5a-d,6a-b,9a-d,10a-d). There were variations in fungal species among all the soil samples from six forest sites. The genera *Aspergillus* has been recorded with the highest number of species in all the sites (Table 1,2). In LS, the highest value of relative occurrence was recorded for *A. niger* and *A. terreus* (12.16), followed by *P. citrinum* (9.77), *C. cladosporioides* (9.77). However, in sterile fungi, the highest value of relative occurrence was recorded for white sterile fungi (46.38) and lower relative occurrence for black sterile fungi (9.77). In KR, the highest relative occurrence was observed for *P. citrinum* (19.1), followed by *A. niger* (12.77), *A. luchuensis* (12.77), *Penicillium* spp. (10.06) and *A. terreus* (8.49). In the case of sterile fungi, only white sterile fungi (36.17) were observed in KR. In KB 6-8, *A. niger* (12.65) has been recorded with the highest relative occurrence, followed by *P. notatum* (11.39), *A. flavus* (6.32), *A. terreus* (5.06), *P. citrinum* (5.06), *Fusarium* spp. (5.06), *C. cladosporides* (3.79), whereas only white sterile fungi (50.63) were recorded from KB 6-8. In SF, *A. niger* (21.73) has been recorded with the highest relative occurrence, followed by *C. cladosporioides* (18.83), *A. flavus* (13.04), *A. terreus* (7.24), *A. oryzae* (5.79), *P. citrium* (5.79), *R. stolonifer* (5.79), whereas the highest relative occurrence was observed for white sterile fungi (15.93) and lowest for Black sterile form (5.79). In GS, *A. terreus* (9.72) has been observed with the highest relative occurrence, followed by *Penicillium citrinum* (6.94), *A. niger* (6.94), *A. flavus* (5.56), *P. notatum* (5.55), *C. cladosporides* (5.55), whereas in sterile fungi highest relative occurrence was observed for white sterile form (54.17) and lowest for black sterile form (5.55). In KS, the highest relative occurrence was observed for *Aspergillus niger* (32.08), followed by *C. cladosporioides* (26.1), *P.notatum* (8.95), *A. flavus* (8.20), *A. terreus* (4.48), *A. oryzae* (3.73) and *P. oxallicum* (2.98), whereas the highest relative value was observed for black sterile form (10.44) and the lowest for white sterile form (2.98). The CFUs of bacteria did not vary significantly among all sites. The maximum bacterial count was recorded among the six study sites, viz. Kr and KS (160). Few colonies were observed in KB 6-8 and SF (42) while others have shown a moderate number of bacterial colonies (Fig. 7a, b, Fig. 8a-d).

Fungal species diversity and richness

The highest value of the Shannon-Wiener index in an area represents the higher diversity of fungal species in that area. The value of the Shannon-Wiener index in six sites of Chilla range of Rajaji tiger reserve varied from 1.39 (GS) – 1.98 (SF), representing GS, the most diverse site for fungal species. The Simpson index varied from 0.59 (GS) – 0.84 (SF) among the sites, whereas the value of the Evenness index varied from 0.50 (GS) – 0.85 (KR). On the other hand, the Richness index varied from 1.89 (KR) – 2.67 (SF) among all the sites.

The longest individual rarefaction curve of an area represents the maximum number of individuals present in that area. The longest individual rarefaction of tree species in the study area revealed that the highest number of individuals is in KS, followed by KB, 6-8, GS, KR. At the same time, LS and SF have an equal number of fungal species (Fig 3). In addition, the diversity profile of fungal species on the basis of the number of species revealed that KS has the highest number of species, followed by SF, GS, KB 6-8, KR and LS in the study area (Fig 4).

Table 1 Relative occurrence of the soil fungi in all the six forest sites of Chilla forest division in Rajaji tiger reserve.

Genera/Species	**LS**	**KR**	**KB 6-8**	**SF**	**GS**	**KS**
A. niger	12.16	12.77	12.65	21.73	6.94	33.08
A. oryzae	-	-	-	5.79	-	3.73
A. luchuensis	-	12.77		-	-	-
A. flavus	-	-	6.32	13.04	5.56	8.20
A. terreus	12.16	8.49	5.06	7.24	9.72	4.48
P. notatum	-	-	11.39	-	5.55	8.95
P.citrinum	9.77	19.1	5.06	5.79	6.94	-
Penicillium spp.	-	10.6	-	-	-	-
P.oxallicum	-	-	-	-	-	2.98
C. cladosporioides	9.77	-	3.79	18.83	5.55	26.1
White sterile form	46.38	36.17	50.63	15.93	54.17	2.98
Black sterile form	9.77	-	-	5.79	5.55	10.44
Fusarium spp.	-	-	5.06	-	-	-
R. stolonifer	-	-		5.79	-	-

Table 2 Abundance of soil fungi in all the six sites of Chilla forest division of Rajaji tiger reserve.

Genera/Species	**LS**	**KR**	**KB6-8**	**SF**	**GS**	**KS**
A. niger	$1.66\pm0.88\times10^3$	$2.00\pm0.57\times10^3$	$3.33\pm0.33\times10^3$	$5.00\pm0.58\times10^3$	$1.66\pm0.66\times10^3$	$14.33\pm0.88\times10^3$
A. oryzae	-	-	-	$1.33\pm0.33\times10^3$	-	$1.67\pm0.33\times10^3$
A. luchuensis	-	$2.00\pm0.58\times10^3$	-	-	-	-
A. flavus	-	-	$1.66\pm0.67\times10^3$	$3.00\pm0.57\times10^3$	$1.33\pm0.33\times10^3$	$3.67\pm0.88\times10^3$
A. terreus	$1.66\pm0.34\times10^3$	$1.33\pm0.34\times10^3$	$1.33\pm0.34\times10^3$	$1.66\pm0.34\times10^3$	$2.33\pm0.88\times10^3$	$2.00\pm0.57\times10^3$
P. notatum	-	-	$3.00\pm0.58\times10^3$	-	$1.33\pm0.33\times10^3$	$4.00\pm0.58\times10^3$
P. citrinum	$1.33\pm0.33\times10^3$	$3.00\pm0.57\times10^3$	$1.33\pm0.34\times10^3$	$1.33\pm0.34\times10^3$	$1.66\pm0.66\times10^3$	-
Penicillium spp.	-	$1.66\pm0.68\times10^3$	-	-	-	-
P.oxallicum	-	-	-	-	-	$1.34\pm0.33\times10^3$
C.cladosporioides	$1.33\pm0.34\times10^3$	-	$1.00\pm0.58\times10^3$	$4.33\pm0.58\times10^3$	$1.33\pm0.34\times10^3$	$11.67\pm0.88\times10^3$
White sterile form	$6.33\pm0.33\times10^3$	$5.66\pm0.89\times10^3$	$13.33\pm0.89\times0^3$	$3.67\pm0.33\times10^3$	$13.00\pm0.57\pm\times10^3$	$1.34\pm0.34\times10^3$
Black sterile form	$1.33\pm0.34\times10^3$	-	-	$1.33\pm0.33\times10^3$	$1.33\pm0.34\times10^3$	$4.66\pm0.67\times10^3$
Fusarium spp.	-	-	$1.33\pm0.03\times10^3$	-	-	-
R. stolonifer	-	-	-	$1.33\pm0.34\times10^3$	-	-
Total	13.64×10^3	15.65×10^3	26.31×10^3	23.00×10^3	24.00×10^3	44.67×10^3
R. stolonifer	-	-	-	+	-	-

Table: 3. Soil parameter in the six forest sites of Rajaji tiger reserve (Mean ± SE)

Parameters	Depth (Cm)	LS	KR	KB 6-8	SF	GS	KS
Organic carbon (%)	0-10	1.36 ± 0.01	1.39 ±0.005	1.33 ±0.005	1.35 ±0.008	1.36 ±0.01	1.37 ±0.01
	15-25	1.24 ± 0.01	1.36 ± 0.02	1.32 ±0.01	1.30 ±0.008	1.24 ±0.02	1.33 ±0.01
	30-40	1.24 ± 0.008	1.23 ± 0.01	1.27 ±0.01	1.24 ±0.01	1.23 ±0.005	1.25 ±0.02
Nitrogen (%)	0-10	0.25 ± 0.01	0.29 ±0.001	0.27 ±0.005	0.24 ±0.01	0.22 ±0.008	0.22 ±0.01
	15-25	0.26 ± 0.003	0.26 ±0.001	0.24 ±0.005	0.24 ±0.01	0.21 ±0.003	0.22 ±0.01
	30-40	0.26 ± 0.008	0.26 ±0.001	0.23 ±0.008	0.25 ±0.005	0.19 ±0.005	0.21 ±0.01
Phosphorus (kg/ha)	0-10	22.83 ± 0.33	15.41±0.96	10.33 ±0.88	14.5 ±0.57	21.5 ±0.57	15 ±0.57
	15-25	18.33 ± 0.33	19.33 ±0.88	14.41 ±0.50	24.1 ±0.88	16 ±0.57	13 ±0.28
	30-40	14.16 ± 0.33	24.1 ±0.88	19.33 ±0.88	15.1 ±0.88	16.1 ±0.88	11.1 ±0.66
Potassium (kg/ha)	0-10	219.71 ± 0.51	159.4 ±0.52	245.63 ±0.86	208.7 ±0.87	220.96 ±0.62	241.46 ±0.57
	15-25	189.73 ± 0.56	285.4 ±0.58	165.55 ±0.70	155.3 ±0.66	180.06 ±0.56	160.83 ±0.33
	30-40	159.1 ±s0.6	181.3 ±0.58	195.35 ±0.65	145.35 ±0.56	150.9 ±0.7	169.05 ±0.55
pH	0-10	6.28 ± 0.07	6.28 ±0.33	5.36 ±0.01	6.53 ±0.04	6.28 ±0.11	5.45 ±0.05
	15-25	5.43 ± 0.02	6.16 ±0.02	5.35 ±0.02	6.42 ±0.014	5.35 ±0.07	5.21 ±0.04
	30-40	6.16 ± 0.33	6.19 ±0.029	5.53 ±0.04	6.25 ±0.02	6.45 ±0.02	5.33 ±0.01

LS=Lalsroth, KR=Kodiya ridge,KB= Kodiyabelt 6-8, SF= Sofuti, GS=Ghasiramsroath, KS=Kharasroth

Soil nutrients dynamics

The pH value of soil showed an acidic nature at different sites and ranged from 5.36 ± 0.01(KB 6-8) - 6.53± 0.04 (SF) in top layer (0-10cm) of soil, 5.21 ±0.04 (KS) - 6.42 ±0.014 (SF) in middle layer, and (30-40),5.33 ± 0.01 (KS) - 6.45 ± 0.02 (GS) in lower depth. The pH value was also acidic and did not vary much from the top most (0-10 cm) layer. ANOVA showed that soil pH varied significantly ($P<0.05$) among all sites. The value of organic carbon was ranged from 1.33 ± 0.005 % (KB 6-8) - 1.39 ± 0.005 (KR) in top layer (0-10 cm), 1.24 ± 0.01(LS), 1.24±0.02 (KS) - 1.36 ± 0.02 (KR) in middle layer (15-25 cm) and 1.23 ±0.01 (KR), 1.23 ± 0.005 (GS) - 1.27 ± 0.01 (KB 6-8) in lower layer (30-40 cm). In all the study sites, the value of organic carbon was highest in the top layer. Further, KR has more carbon while the least carbon was recorded in the lower depth of KR (1.23±0.01) and GS (1.23±0.005). ANOVA showed that soil organic carbon varies significantly ($P<0.05$) among all the study sites. Although total nitrogen did not exhibit higher levels in either the top and sub-layers among all the sites whereas the decreasing level of nitrogen was observed in all the lower depths of the study sites. The highest level of nitrogen was recorded in KR (0.29 ± 0.001 %) at the top layer whereas lowest in GS (0.19 ± 0.005) at the lower layer (30-40 cm). In top layer, nitrogen varied from 0.22 ± 0.008 (GS), 0.22 ± 0.01 (KS) - 0.29 ± 0.001 (KR), 0.21 ± 0.003 (GS) – 0.26 ± 0.003 (LS), 0.26 ±0.001 (KR) in middle layer and 0.19 ± 0.005 (GS) - 0.26 ±0.008 (LS), 0.26 ±0.001 (KR), Table (5.3). ANOVA showed that nitrogen of soil varies significantly ($P<0.05$) in KB 6-8 and GS, but the variation was non-significant ($P>0.05$) in LS, KR, SF and KS. Phosphorus content ranged from 10.33 ± 0.88 kg/ha (KB 6-8) - 24.1 ± 0.88. kg/ha (KR and SF). In upper layer, phosphorus ranged from 10.33 ± 0.88 kg/ha (KB 6-8) -22.83 ± 0.33 kg/ha (LS), 13. 0 ± 0.28 kg/ha – 24.1 ± 0.88 kg/ha (SF) in middle layer and 11.1 ± 0.66 kg/ha (KS) - 24.1 ±0.88 kg/ha (KR). These values also increase as the depth increases in all study sites of the Rajaji tiger reserve. Higher content of phosphorus viz. 24.1 ± 0.88 kg/ha was recorded in the lower depth

of KR (30-40cm) and middle depth of SF (15-25 cm). ANOVA showed that phosphorus in the soil varies significantly (P<0.05) among all sites. The potassium content ranged from 145.35 ± 0.56 kg/ha (SF 6-8) - 285.4 ± 0.58 kg/ha (KR). The value of potassium varies significantly among all the sites at each depth in the study area. The value of potassium varied from 159.4 ± 0.52 kg/ha (KR) – 245.63 ±0.86 kg/ha (KB) in top layer, 155.3 ± 0.66 kg/ha (SF) - 285.4 ±0.58 kg/ha (KR) in middle layer and 145.35 ±0.56 kg/ha (SF) - 195.35 ± kg/ha (KB) in lower layer. In all three except KR, KB 6-8, GS, potassium content decreases as the depth increases. ANOVA showed that the potassium of soil varies significantly (P<0.05) among the sites (Table. 3).

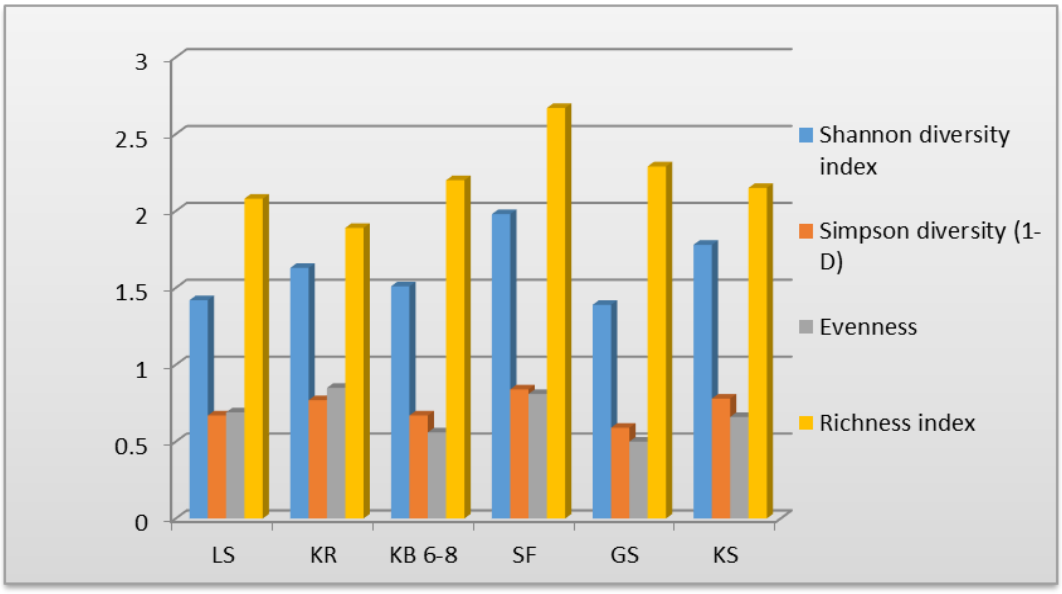

Fig. 2 – Diversity indices among all study sites in Chilla forest division. LS = Lalsroth, KR= Kodiya ridge, KB= Kodiyabelt 6-8, SF= Sofuti, GS= Ghasiramsroath, KS=Kharasroth

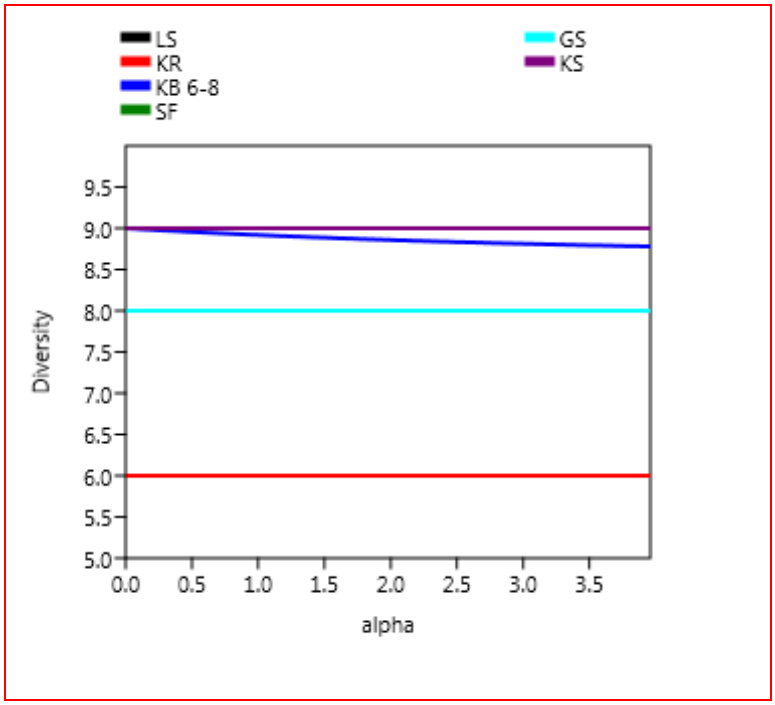

Fig. 3 – Diversity profile in the six forest sites of the tiger reserve LS = Lalsroth, KR= Kodiya ridge, KB= Kodiyabelt 6-8, SF= Sofuti, GS= Ghasiramsroath, KS=Kharasroth

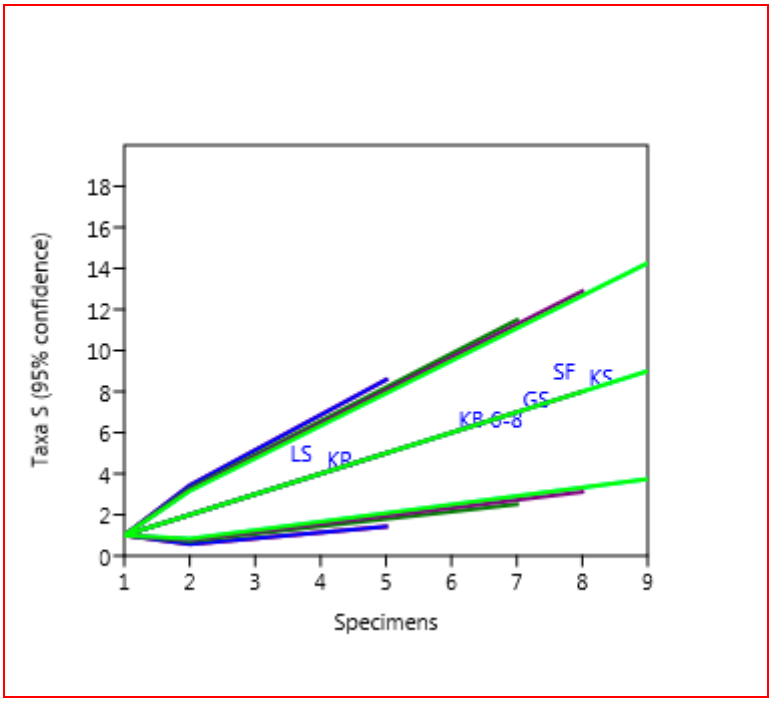

Fig. 4 – Individual rarefaction curve in the six forest sites of the tiger reserve. LS = Lalsroth, KR= Kodiya ridge, KB= Kodiyabelt 6-8, SF= Sofuti, GS= Ghasiramsroath, KS=Kharasroth

Discussion

Rajaji tiger reserve occupied with great floristic diversity of *Dalberigia sissoo, Holoptelea integrifolia, Shorea robusta, Mallotus philippensis, Cassia fistula, Trewia nudiflora*, whose greater canopy and litter from the decomposed leaves provide ample nutrients to soil microflora, flavouring their growth. In present study, most of the fungal species belonging to the class *Deuteromycetes* and class *Zygomycetes* are ubiquitous and very common in forests and agricultural lands (Manoch 1998, Ramanwong et al. 2000). The serial dilution plate method used in the present study is one of the best methods for isolating fungi between these two classes because the dilution plate method supports the mixing and releasing of various spores from these fungi in a diluted suspension of soil **(**Kosol 1999, Manoch et al. 1998). On the other hand, this method is also suitable for the isolation of bacteria from soil. The list of fungi includes *A. niger, A. flavus, A. terreus, A. oryzae, Penicillium notatum, P. oxallicum, P. citrinum, Penicillium* spp., *Fusarium* spp., *C. cladosporioides, R. stolonifer,* white sterile form and black sterile form. These results might be an underestimation of the fungal species in the six study sites because we have applied only one isolation method. However, the dilution method remains the most, judicious, trustable and simplest isolation method among all the other methods through which fungi assemblage might be screened (Keller & Bidochka 1998).

There are about 75,000 species of soil fungi in the world (Finlay 2007). The contribution of various classes like *Ascomycetes* and *Zygomycetes* shows the depth-specific distribution. The number of fungi is generally found higher in the upper depth of soil. In the present study, we have isolated fungi only from the upper layer (0–10 cm depth) of soil. The specific occurrence of fungi is governed by organic matter availability and the ratio of carbon to oxygen at different depths (Giri et al. 2005). A lower amount of nitrogen, organic matter and phosphorus in deeper soil layers decrease fungal species (Giri et al. 2005, Guleri et al. 2013). On the other hand, fungi belonging to the class *Deuteromycetes* strongly colonizes the decomposable substrates with greater adaptability but those of *Zygomycetes* and *Ascomycetes* colonizes very weak to the degraded substrates (Kumar et al. 2011, Rai et al. 2001, Vibha & Sinha, 2007). In the present study, the former class *Deuteromycetes* was the most dominant in all the sites. The genera *Aspergillus* and *Penicillium* were recorded in all the six sites of the Chilla forest division. This could be due to the sporulation

capacity of these two genera among all the sites. The Sporulation capacity of fungal species affects their occurrence from habitat to habitat (Schimel 1995). The earlier study by Chaudhary et al. (2016) from the Chilla and Motichur range of Rajaji Tiger reserve also supports our results. They have recorded 20 species of fungi in which *Aspergillus* was one of the most dominant genera in both Chilla and Motichur range of Rajaji tiger reserve. The other studies from Galloway (1936), Moubasher & El-Dohlob (1970), Saravanakumar & Kaviyarasan (2010) also have recorded *Penicillium* and *Aspergillus* as the most dominant genera in different forests of India. The results from our study did not vary significantly from the study of Saravanakumar & Kaviyarasan (2010) and Galloway (1936).

Joshi et al. (2015) suggested that the physico-chemical characteristics of soil, like pH and moisture content, serve essential roles for the growth of fungi and bacteria. High moisture content supports the availability of organic carbon in the soil, which is an essential source of nutrient for bacteria, fungi and other microbes. The highest abundance of fungi and the bacteria was observed at KS (44.67) and KB 6-8 (26.31), which may be due to better moisture absorbance in these two sites. Secondly, the overall soil pH was acidic in all study sites of Chilla forest division, which varied from 5.36 ± 0.01 (KB 6-8) - 6.53 ± 0.04 (SF) in the top layer (0-10cm), and also favors the overall growth of fungi, bacteria and their spores in the soil.

The present study is only confined to the upper layer (0-10 cm) of the soil as most of the fungi and bacteria are present in the upper surface of the soil. Carbon in soil represents an essential pool atmosphere and knowledge of soil organic carbon is an important factor in determining the nutrient cycling and quality of soil (Velayeutham et al. 2019). Secondly, soil organic carbon also serves as an important source for microbes, which is further used by plants. The value of soil organic carbon varied from 1.33 ± 005 (KB 6-8) - 1.39±0.005 (KR). Further, it seemed that organic carbon content was greater in the top layer (0–10 cm) in comparison to the middle (15–25 cm) and deeper depths (30–40 cm). In other words, organic carbon decreases significantly as the depth increases. Moderate carbon content in KB 6-8 and KS also favors the growth of the highest number of fungi in the top layer (0–10 cm), where a higher level of organic carbon was observed.

The diversity indices were used to determine the overall diversity status of soil fungi among the six different sites of the forest division. The Shannon-Weiner diversity index from our study varied from 1.39 (GS) – 1.98 (SF), representing SF as the most diverse site for fungal species. The Simpson index varied from 0.59 (GS) – 0.84 (SF) among the sites, whereas the value of the Evenness index varied from 0.50 (GS) – 0.85 (KR) among all the sites in the study area. Earlier studies on soil mycoflora support the value of the Shannon-Weiner diversity index from our results. A study from Guleri et al. (2013) at Uttarakhand Himalaya (Dehradun region) found Shannon diversity of 1.92 –2.76, Simpson index of 0.82 – 0.92, and Evenness value varied from 0.84 – 0.96 among different forest sites. In another study carried out by Guleri et al. (2010), the value of the Simpson index ranged from 0.18 – 0.39 in different seasons. Our results are comparable with Guleri et al. (2016), where Shannon diversity index varied from 2.54–2.73, Simpson index 0.90–0.92, and Evenness index 0.91–0.92 in the forests of Uttarakhand Himalaya.

The Chilla range of Rajaji Tiger reserve lies to the east of the river Ganges. This forest division is attached to the forest of Garhwal, which has a subtropical moist deciduous forest type. These forest types support huge wealth of plant species, whose huge canopy and litter support the growth of microorganisms in the study area. These microbes play an important role in ecosystem health by degrading the organic matter that releases nutrients into the soil. The released nutrients in the soil are utilized by the plants, which improves the diversity and regeneration potential of plant species.

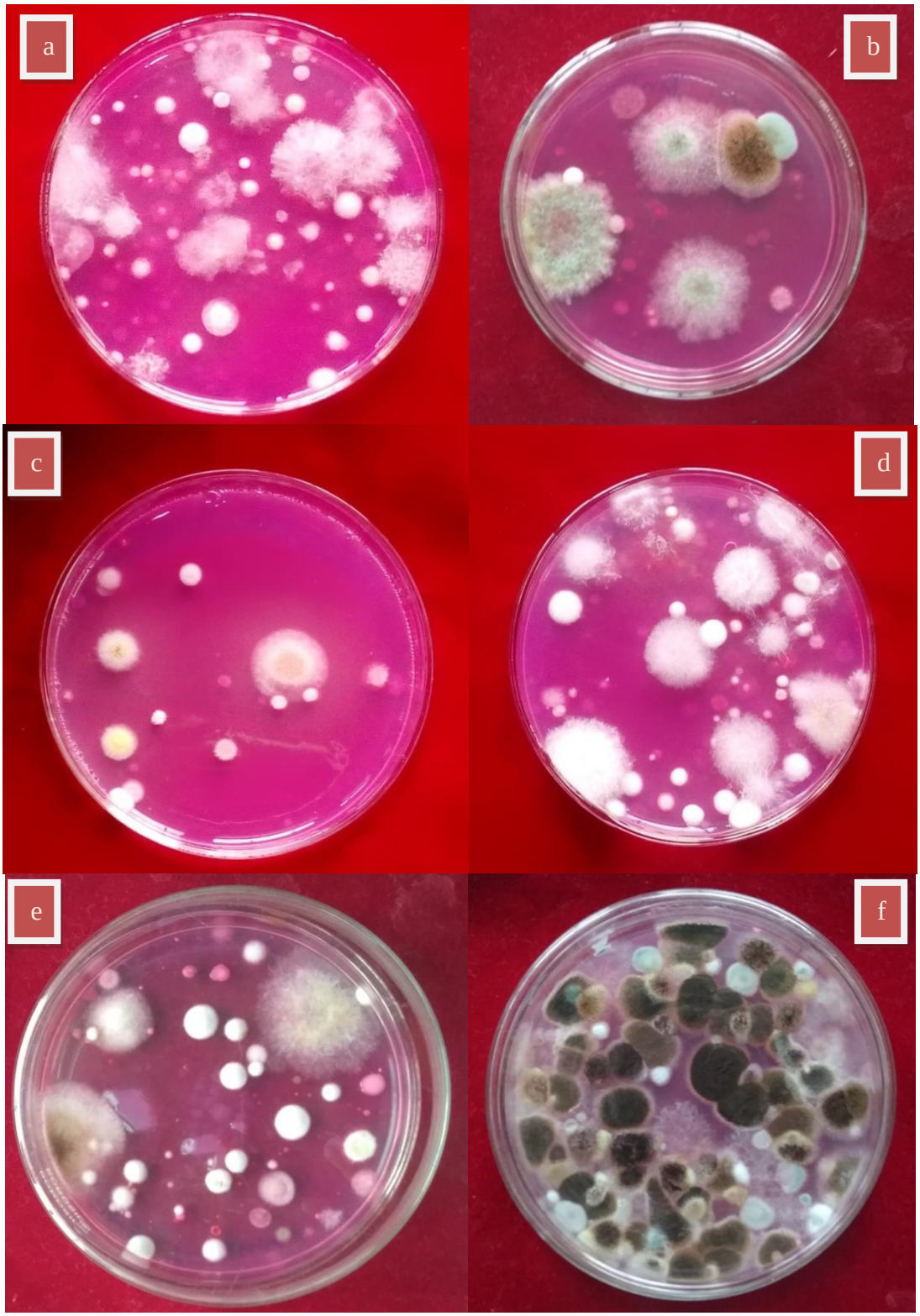

Fig. 5 – Isolation of soil mycoflora from the study sites. a Lalsroath, b Kodiya ridge, c Kodiyabelt 6-8, d Sofuti, e Ghasiramsroath, f Kharasroath.

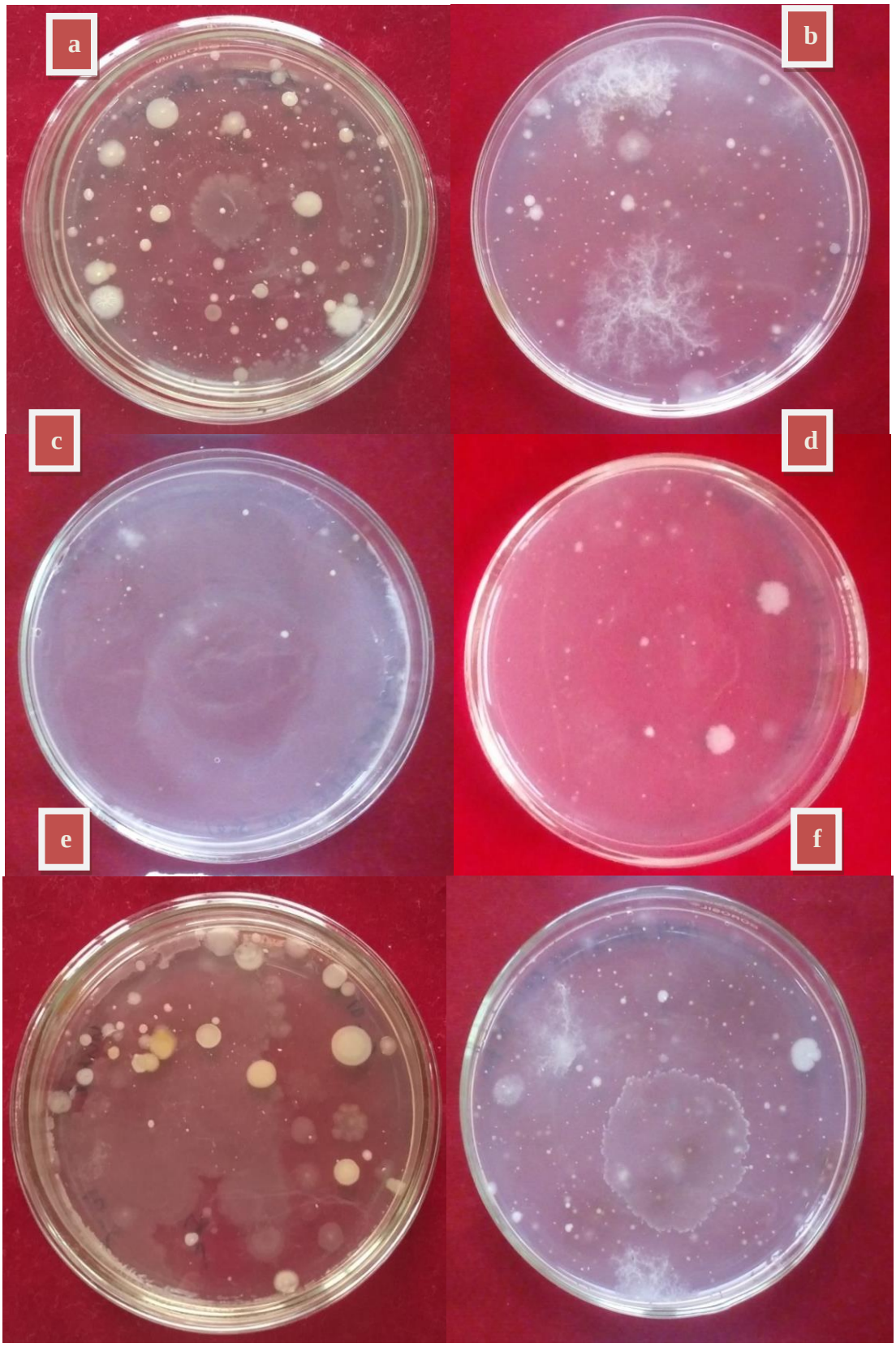

Fig. 6 – Isolation of bacteria from the soil in study sites. a Lalsroath, b Kodiya ridge, c Kodiyabelt 6-8, d Sofuti, e Ghasiramsroath, f Kharasroath.

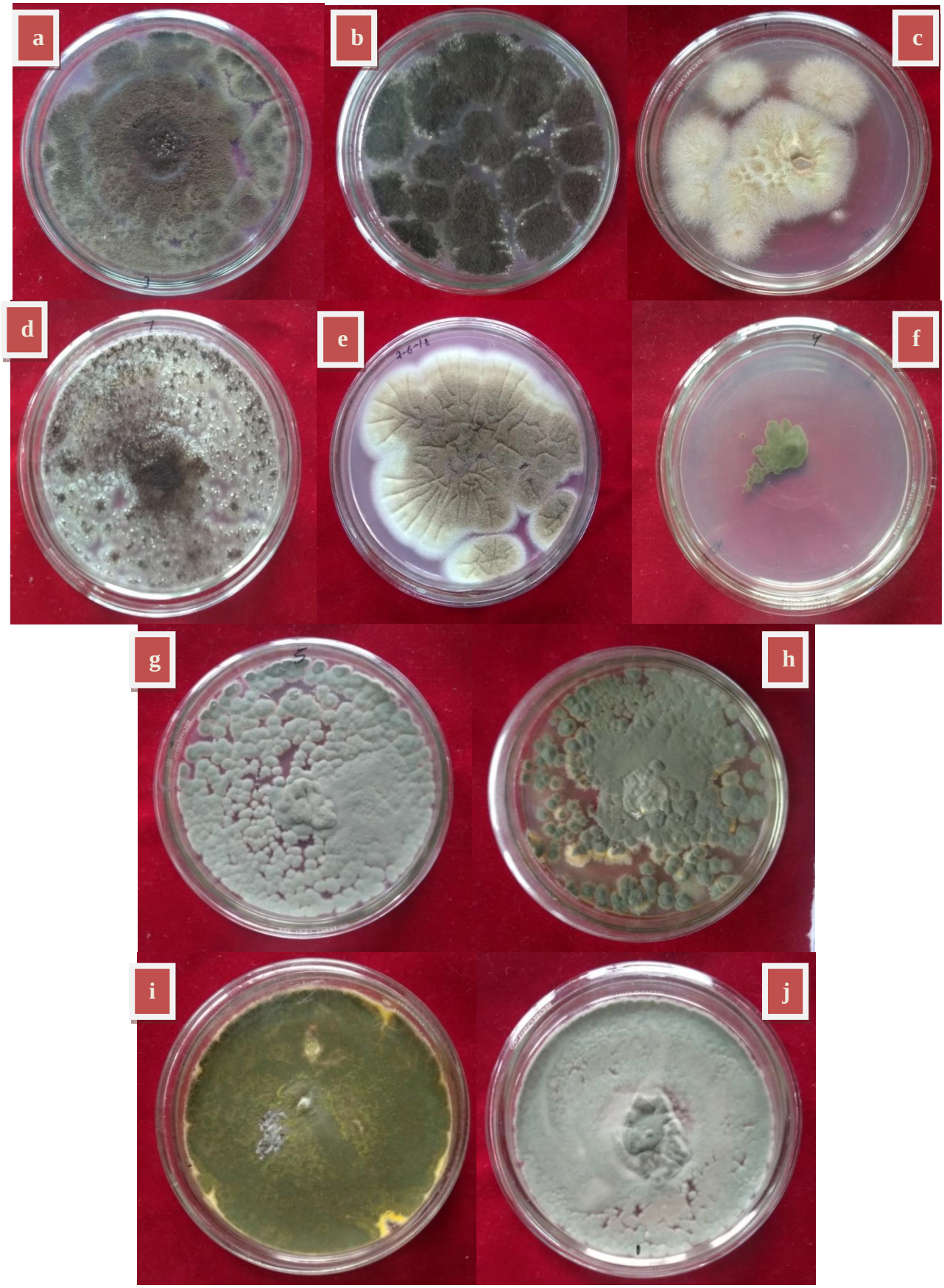

Fig. 7 – Cultural characteristics of fungi. a *Aspergillus flavus*, b *Aspergillus luchuensis*, c *Aspergillus terreus*, d *Aspergillus niger*, e *Aspergillus oryzae*, f *Cladosporium cladosporioides*, g *Penicillium notatum*, h *Penicillium oxallicum*, i *Penicillium citrinum*, j *Penicillium* sp.

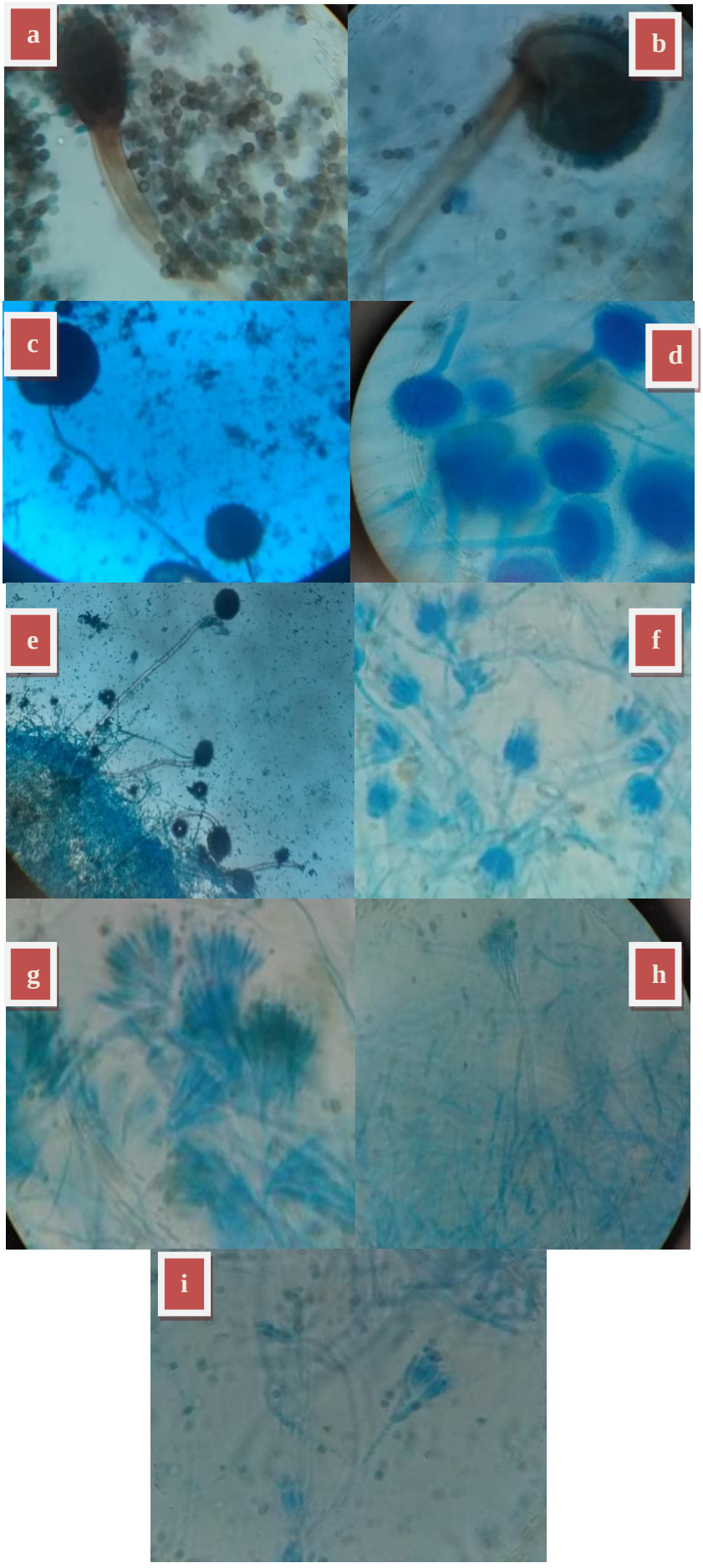

Fig. 8 – Morphological characteristics of fungi. a *Aspergillus flavus*, b *Aspergillus luchuensis*,

c *Aspergillus niger*, d *Aspergillus terreus*, e *Aspergillus oryzae*, f *Penicillium oxallicum*, g *Penicillium citrinum*, h *Penicillium* sp., i *Penicillium notatum.*

Conclusion

The Chilla range of Rajaji Tiger supports different forest associations, such as Sal, Shisham, Teak, etc, whose litter and huge canopy release different organic matter to the soil, which supports fungal species, like *A. niger*, *A. flavus*, *A. oryzae*, *P. notatum*, *P. oxallicum*, *Fusarium* spp., *Cladosporium* spp, etc. The highest number of fungal species was recorded in the soil from the GS site due to the thriving status of organic carbon, phosphorus, nitrogen, and higher moisture absorbance ability of soil. Further, the acidic pH in the soil in all the study sites of the Chilla range also favours the overall growth of fungi, bacteria and their spores in the soil. The fungal species are helping the forest ecosystem health by degrading organic matter and releasing various nutrients into the soil, which are utilized by the plants and improve their diversity and overall forest structure. Therefore, the greater abundance of the microbial population would lead to good soil fertility resulting in many floral species.

Conflict of Interest

All the authors declare that they have no conflict of interest.

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References

Ajwa HA, Dell CJ, Rice CW. 1999 – Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. Soil Biology and Biochemistry 1 31, 769–77.

Akash, Navneet, Bhandari BS. 2018 – Tree diversity, stand structure and community composition in tropical forest of Rajaji tiger reserve, Northern India. Journal of applied science and Natural Sciences 10, 945–953.

Akash, Navneet, Bhandari BS. 2019 – A Community Analysis of Woody Species in a Tropical Forest of Rajaji Tiger Reserve. Environment and Ecology 37, 48–55.

Akash, Navneet, Bhandari BS. 2020a – Natural regeneration dynamics of tree species along the altitudinal gradient in a Sub tropical moist deciduous forest of Northern India. Current Science 119, 2019–2023.

Akash, Zakir M, Navneet, Bhandari BS, Mansotra DK. 2020b – Uses and Knowledge of Wild and Cultivated Edible Medicinal Plants by Indigenous Groups of Rajaji Tiger Reserve, Uttarakhand, India. Plant Archives 20, 5393–5402.

Akash, Navneet, Bhandari BS, Bisht SS, Mansotra DK. 2020c – Ethnobotany: The traditional medical science for alleviating human ailments and suffering. In: Ethnomedicinal Plant Use and Practice in Traditional Medicine, Akash, Navneet and B.S. Bhandari (eds). IGI Global USA, 38–57.

Akash, Navneet, Bhandari BS. 2020d – Ethnomedicinal plant use and practice in traditional medicine, Akash, Navneet and B.S. Bhandari (eds). IGI Global USA, 1–300.

Akash, Zakir M, Navneet. 2021 – Quantitative ethnomedicinal study of plants used to cure different ailments in Rajaji tiger reserve, Uttarakhand, India. Herba Polonica 66, 1–5.

Bahera RK. 2014 – Soil and solid waste analysis. Dominant publishers and distributors pvt ltd, New Delhi, India, 1–124.

Brock TD. 1961 – Milestones in Microbiology. Englewood Cliffs (NJ): Prentice-Hall.

Bridge P, Spooner B. 2001 – Soil fungi: diversity and detection. Plant and Soil 232, 147–154.

Chaudhary P, Joshi N, Bora M. 2016 – Seasonal Variation in mycoflora population in subtropical forest soil of Uttarakhand, India. International Journal of Current Microbiology and Applied Science 5, 654–666.

Ding C, Shen Q, Zhang R, Chen W. 2013 – Evaluation of rhizosphere bacteria and derived bio-organic fertilizers as potential biocontrol agents against bacterial wilt (*Ralstonia solanacearum*) of potato. Plant and soil 366, 453–466.

Fenchel T, Finlay BJ. 2004 – The ubiquity of small species: patterns of local and global diversity. Bioscience 54, 777–784.

Finlay RD. 2007 – The fungi in soil. In: Modern Soil Microbiology (2nd edition). Van JD, Elsas JK. Jansson JT.(eds.). CRC Press, New York, 107–146.

Galloway LD. 1936 – Indian soil fungi. Indian Journal of Agricultural Science 6, 578–585.

Gilman JC. 1957 – A manual of soil fungi. Iowa State College Press, Iowa, U.S.A., 1–325.

Giri B, Giang PH, Kumari R, Prasad R, Varma A. 2005 – Microbial diversity in soils. In Microorganisms in soils: roles in genesis and functions. Pp. 19–55. Springer, Berlin, Heidelberg.

Guleri S, Bhandari BS, Saxena S. 2010 – Ecology of rhizosphere and non-rhizosphere soil mycoflora of forest soils of Dehradun district, Uttarakhand. International Transactions in Applied Sciences 2, 69–77.

Guleri S, Saxena S, Bhandari BS. 2013 – Seasonal variation in the diversity of soil microfungi of some grazinglands of Doon Valley of Uttarakhand Himalaya. Journal of Mycopathological Research 51, 213–223.

Guleri S, Saxena S, Sharma P, Malik N, Thapliyal M. 2016 – Occurrence and diversity of soil mycoflora in some selected *Brassica* growing agricultural fields of Dehradun District of Uttarakhand Himalaya. International Journal of Pure Applied Bioscience 4, 253–264.

Joshi G, Negi GCS. 2015 – Physico-chemical properties along soil profiles of two dominant forest types in Western Himalaya. Current Science, 798–803.

Keller L, Bidochka MJ. 1998 – Habitat and temporal differences among soil micro fungi among soil microfungi assemblages in Ontario. Canadian Journal of Botany 76, 1798–1805.

Klose S, Tabatabai MA. 2000 – Urease activity of microbial biomass in soils as affected by cropping systems. Biology and fertility of soils 31, 191–199.

Kosol S. 1999 – Biodiversity of micro-fungi in soil, water and plant under teak plantation of linthin watershed, Kanchanaburi province. M.Sc. Thesis, Kasetsart University. Bangkok, 1-208.

Kumar S. 2011 – Biotechnological approach for bioremediation of pesticide contaminated soil (Doctoral dissertation, Saurashtra University), 1–222.

Lal R. 2002 – Soil carbon sequestration in China through agricultural intensification and restoration of degraded and desertified ecosystems. Land Degradation and Development 13, 469–478.

Maiti SK. 2003 – Hand book of methods in environmental studies: air, noise, soil and overburden analysis (2nd edition), ABD publishers, Jaipur, India, 1–240.

Magurran AE. 1988 – Ecological diversity and its measurement. Princeton university press; Nov 21.

Manoch L. 1998 – Biodiversity of soil fungi in Thailand. In Proceedings of the Asia-Pacific mycological conference on biodiversity and biotechnology, Hua Hin, 126–140.

Margalef R. 1968 – Perspectives in ecological theory, 1–444.

Mohammadi P, Maghboli-Balasjin N. 2014 – Isolation and molecular identification of deteriorating fungi from Cyrus the Great tomb stones. Iranian Journal of Microbiology 6, 361.

Moubasher AH, El-Dohlob SM 1970 – Seasonal fluctuation of Egyptian soil fungi. Transaction of British Mycological Society 54, 45–51.

Olson RK, Schoeneberger MM, Aschmann SG. 2000 – An ecological foundation for temperate agroforestry. In: North American Agroforestry, an integrated science and practice. Garrett HE. Rietveld W.J, Fisher RF. (eds.). Springer Netherland, 31–61.

Ramanwong KW, Himamarn J, Ayawong S, Maneerat. 2000 – Preliminary survey on soil fungi at eastern Thungyai Naresuan wildlife sanctuary. Journal of Thai Forestry Research 2, 117–124.

Rane G, Gandhe RV. 2006 – Seasonal distribution of soil fungi from forest soils of Jalgaon District, Maharashtra. Zoos Print Journal 21, 2407–2409.

Raper KB, Thom C. 1949 – A Manual of Penicilia. William and Wilkins Co., Baltimore, 1–878.

Rai MK. 2001 – Current advances in mycorrhization in microporopagation. In Vitro Cellular and Developmental Biology-Plant 37, 158–167.

Saravanakumar K, Kaviyarasan V. 2010 – Seasonal distribution of soil fungi and chemical properties of montane wet temperate forest types of Tamil Nadu. African Journal of Plant Science 4, 190–196.

Schimel J. 1995 – Ecosystem consequences of microbial diversity and community structure. In: Arctic and alpine biodiversity: patterns, causes and ecosystem consequences. Schimel, L. (ed). Springer, Berlin, Heidelberg, 239–254.

Simpson EH. 1949 – Measurement of diversity. Nature 163, 1–688.

Singh SK, Rai JP. 2004 – Soil microbial population and enzyme activity related to grazing pressure in alpine meadows of Nanda Devi Biosphere Reserve. Journal of Environmental Biology 25, 103–107.

Singh RBP, Chauhan P. 2014 – A handbook on soil quality testing manual and their interpretation, Technical institute of Mountain Ecology pvt ltd, 1–106.

Steenwerth KL, Jackson LE, Calderón FJ, Scow KM, Rolston DE. 2005 – Response of microbial community composition and activity in agricultural and grassland soils after a simulated rainfall. Soil Biology and Biochemistry 37, 2249–2262.

Thom C, Raper KB. 1945 – A Manual of the Aspergilli. LWW.

Velayutham M, Pal DK, Bhattacharyya T. 2019 – Organic carbon stock in soils of India. In: Global climate change and tropical ecosystems, CRC Press, 71–95.

Vibha, Sinha A. 2007 – Variation of soil mycoflora of rice stubble from rice wheat cropping system. Mycobiology 35, 191–195.