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Research Article

DIVERSITY OF SOIL FUNGI IN KUMARAKOM BIRD SANCTUARY, KERALA

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ABSTRACT

In the present investigation, a total of 28 species belonging to 13 genera of fungi were isolated from Kumarakom Bird Sanctuary, Kerala during February 2016. The whole area was divided into 3 plots (I, II and III) and the mycoflora were isolated by using soil dilution plate method on potato dextrose agar medium supplemented with antibiotic ambistryn. The maximum number of fungal isolates belonging to the class Ascomycetes (8 genera and 23 species), followed by Zygomycetes (3 genera and 3 species) and Oomycetes (2 genus and 2 species) were recorded. Maximum species diversity was shown by the genus *Penicillium* (12 species) followed by *Aspergillus* (3 species). The fungal density and relative frequency was highest for *Aspergillus* and least for *Isoachlya*. The fungal abundance was maximum for *Cunninghamella* and minimum for *Scopulariopsis*. Maximum similarity of species was shown between plots I and II & II and III and least between I and III. Maximum species diversity was shown by plot 3 followed by plot 2 and then by plot 1.

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INTRODUCTION

India ranks under world's twelve mega biodiversity zones. The diverse physical features and climatic situations have formed ecological habitats like forests, grasslands, wetlands, coastal and marine ecosystems and desert ecosystems, which harbor and sustain immense biodiversity. The biodiversity in our country is unique in nature and its *in-situ* and *ex-situ* conservation is very well needed.

In-situ conservation is a set of conservation techniques involving the designation, management and monitoring of biodiversity in the same area where it is encountered. This requires conservation of the components of the natural system (populations, species, communities and biophysical systems) as well as the ecological and evolutionary processes occurring within that system.

The strategies for conservation and sustainable utilization of biodiversity have comprised providing special status and protection to biodiversity - rich areas by declaring them as national parks, wildlife sanctuaries, biosphere reserves, ecologically fragile and sensitive areas. India now has 14 biosphere reserves, 90, 448 wildlife sanctuaries.

A sanctuary is a protected area which is reserved for the conservation of only animals and human activities like harvesting of timber, collecting minor forest products and private ownership rights are allowed as long as they do not interfere with the well-being of animals.

Kumarakom Bird Sanctuary (KBS) (9 37'46.97''N & 76 25'25.56''E/ 44 ft alt) a green patch of land with mangrove forests criss-crossed with channels connected to the nearby backwater which is famous for its wetland vegetation and birdlife. This area that encompasses the Kerala Tourism Development Corporation (KTDC) Complex is 90.199 acres (36.4869 hectares) in extent and forms a part of the Baker Estate. The Kumarakom Bird sanctuary is situated at Kavanattinkara in Kottayam District of Kerala on the western side of the Kumarakom - Vechoor road on the southern banks of the river Kavanar, a branch of the Meenachil river system. This area is known for its avian fauna which includes a variety of local resident birds and a number of migratory birds. Many of them use this place as the breeding ground (Jobi *et al*., 2014).

There is a vast microbial flora inheriting the earth and they are found in all types of soils. Microbial communities are controlled primarily by plant species composition, soil type, seasonal variability, temperature and availability of organic substances. These microbes may interact with the plants resulting sometimes in useful effect and other times in harmful consequences. Fungi are an important component of the soil microbiota and are present as mycelia bits, rhizomorphs or as spores. The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem. They are geographically widely distributed and have been observed in a broad range of habitats principally in soils and decaying vegetation. Fungi are saprophytic i.e., they live on dead and decaying organic matter, thus breaking it down and converting

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it to forms that are available to higher plants, as they excrete a wide range of degradative enzymes that attack virtually any organic material. Such degradative activities make fungi essential participants in recycling natural waste in our environment.

Around 1.5 million fungal species are estimated to occur in the world. However, till now mycologists have got limited success and reported the presence of 93843 fungal species from various parts of the world (Kirk *et al*., 2008). Furthermore, a total of 29000 fungal species are reported from India (Manoharachary *et al*., 2005). Fungi plays an important role in the agriculture, industry, medicine, waste recycling, environment management, food industry, biofertilizers, biopesticides, paper industry, pharmaceuticals and others such activities of human welfare. Still, there is a hidden wealth of fungi which needs to be explored from India (Manoharachary *et al*., 2009). The habitats/substrates that occur around some sanctuaries and water bodies include litter with high moisture content, humid soils and marginal soils around water bodies are the important site which needs still a lot of attention. An extensive survey of literature indicates that habitats around water-bodies and sanctuaries have not been explored for fungi.

MATERIALS AND METHODS

Study area

The whole area of Kumarakom Bird Sanctuary was divided in to three plots as shown in the plot map (Fig. 1). Soil samples were collected from these three sampling plots – plot 1, plot 2 and plot 3.

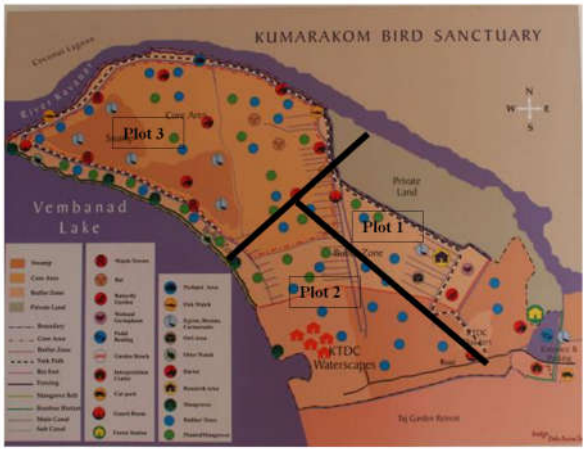


Fig. 1 Plot map of Kumarakom Bird Sanctuary

Collection of Soil Samples

Soil samples were collected during the month of February 2016. During each sampling, four soil samples were collected at random from a depth of 0-10 cm and mixed together to get one composite soil sample. Three such composite samples were collected from the area. The samples were stored at 4 C till they were processed for isolation of fungi (Sankaran *et al*., 2000).

Isolation of fungi from the soil samples

The soil dilution method on Potato Dextrose Agar was used as isolation technique (Waksman, 1922).

Identification of the Soil Fungi

Identification of the fungal species was based on morphological characteristics of the colony (developing degree of cultures, color of colonies and changes in color, reverse color of the plate and changes in its color, texture of colony surface) and microscopic (hyphae, conidia, conidiophores, arrangement of spores and resting bodies) examinations (Aneja, 2001). The fungi were identified with the help of authentic manuals of fungi (Gillman, 1998).

Quantitative analysis

Percentage frequency

% frequency = Number of plots in which a particular fungus occurred / Total number of plots examined X 100

Based on the frequency occurrences the fungi were grouped as rare (0–25% frequency), Occasional (26–50% frequency), Frequent (51–75% frequency) and Common (76-100% frequency) species.

Percent contribution

The percent contribution of each isolate was calculated.
% contribution = Total No. of CFU of an individual species / Total No. of CFU of all species X 100

Density, Abundance and Relative Frequency

The density, abundance, and relative frequency was calculated
Density = Total number of individuals of the species/Total number of plots studied

Abundance = Total number of individuals of the species/ number of plots in which the species occurred

Relative Frequency (RF) = (N col /N total) x 100

Similarity and Dissimilarity Index

Similarity index was used to compare the 3 plots. In the present approach, the index was calculated (Sorenson, 1948).

$S=2C/ A+B$

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
When the sites are similar in species composition, the index of similarity approaches 1.

Species diversity

The diversity of fungi in these 3 plots were assessed on the basis of Brillouin diversity index (Stiling, 2012).

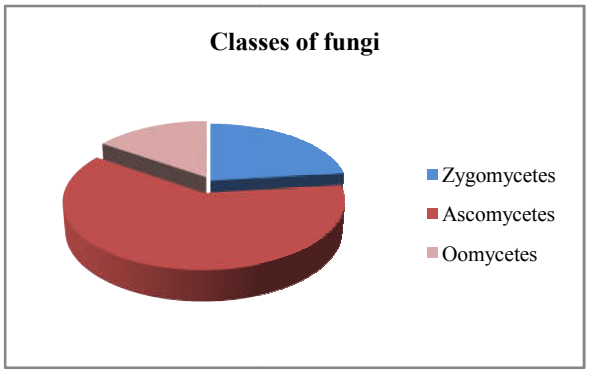
$HB = \ln N! - \Sigma \ln n! / N$

Where,
N = Total no. of individuals of all species in the community
n = No. of individuals in each species

RESULTS AND DISCUSSION

In the present investigation a total of 137 colonies of soil fungi were obtained from 3 different plots of Kumarakom Bird Sanctuary during February 2016 through soil dilution plate method (Fig. 2). They were identified to 13 genera and 28 species, which belonged to the Class Zygomycetes (3 genera

and 3 species), Class Ascomycetes (8 genera and 23 species) and Class Oomycetes (2 genera and 2 species) (Table 1). The maximal fungal species belong to Ascomycetes followed by Zygomycetes and Oomycetes (Graph-1) The abundance of this group of fungi on marine and mangrove substrates has been reported by Hyde and Jones (1988) and this might be due to their spores show adaptation to the marine ecosystem by way of production of appendages, which provide buoyancy in water, entrapment and adherence to the substrates, as reported in mangrove wood driftwood and animal substrates. Among the longest mangrove belt (ten species of mangroves) of Kerala, eight species were recorded from the Kumarakom Tourist Complex namely *Avicennia officinalis*, *Bruguiera gymnorrhiza*, *Rhizophor aapiculata*, *Rhizophora mucronata*, *Sonneratia caseolaris* and *Kandelia candel*. Of these, three are found only at Kumarakom (Jobi *et al*., 2014).



Graph.1 Diversity of Fungal classes at kumarakom bird sanctuary

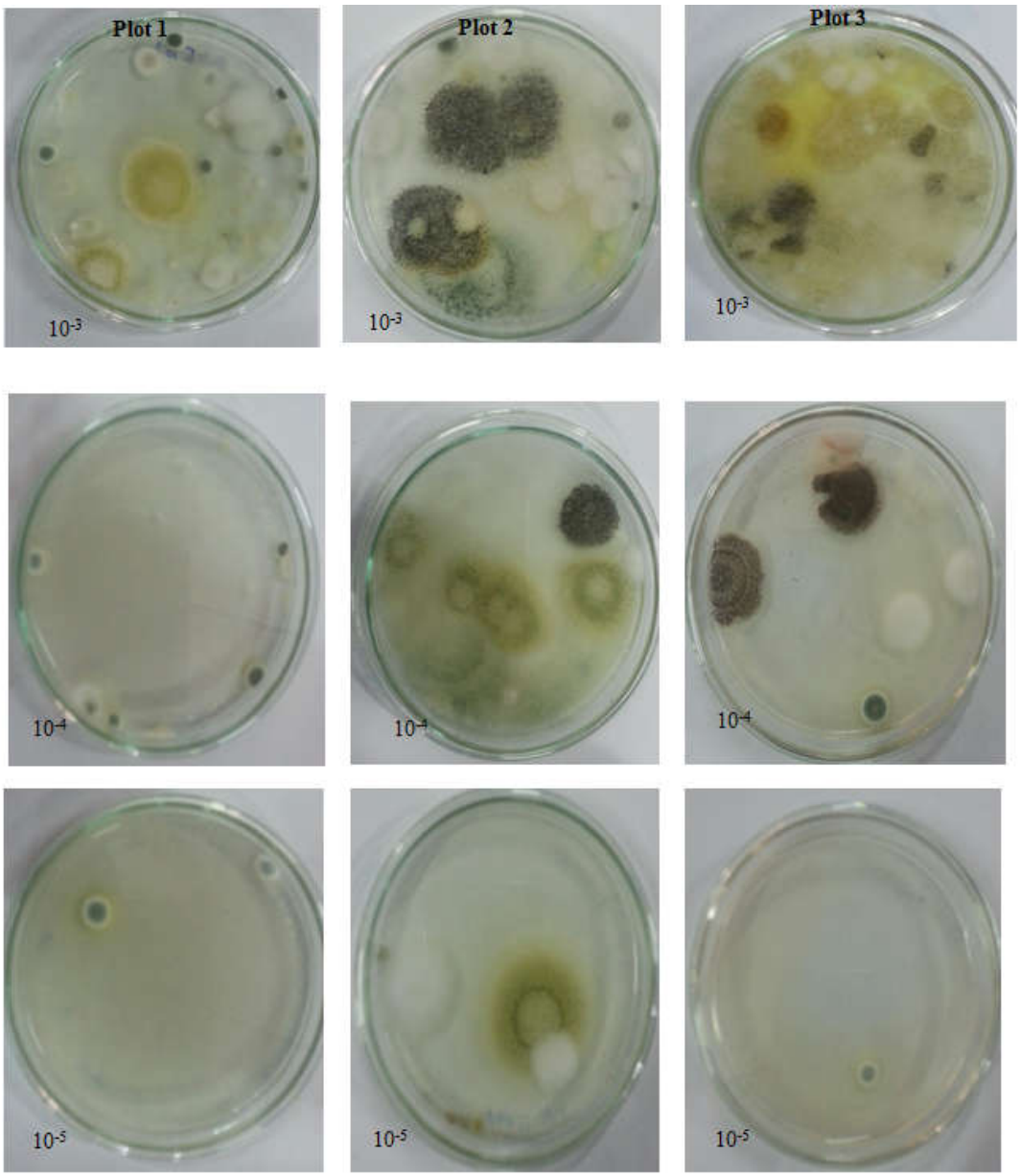


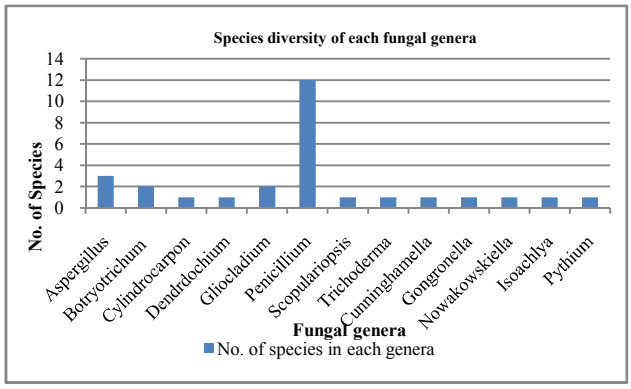
Fig. 2 Colonies isolated by serial dilution plate method

The identified fungi were as follows:

Table 1 List of mycoflora isolated

Class Ascomycetes	Class Zygomycetes	Class Oomycetes
1. *Aspergillus flavus*	*Cunninghamella* sp.	*Isoachlya*
2. *Aspergillus niger*	*Gongronella butleri*	*subterranean*
3. *Aspergillus parasiticus*	*Nowakowskiella* sp.	*Pythium* sp.
4. *Botryotrichum piluliferum*		
5. *Botryotrichum* sp.		
6. *Cylindrocarpon* sp.		
7. *Dendrodochium* sp.		
8. *Gliocladium catenulatum*		
9. *Gliocladium fimbriatum*		
10. *Penicillium chrysogenum*		
11. *Penicillium citrinum*		
12. *Penicillium diversum*		
13. *Penicillium expansum*		
14. *Penicillium glabrum*		
15. *Penicillium minioluteum*		
16. *Penicillium pallidum*		
17. *Penicillium rubrum*		
18. *Penicillium terrestre*		
19. *Penicillium waksmani*		
20. *Penicillium* sp.		
21. *Penicillium* sp.		
22. *Scopulariopsis* sp.		
23. *Trichoderma* sp.		

Among the fungus isolated in the present study, the maximum number of species belonged to the genus *Penicillium* followed by *Aspergillus*, *Botryotrichum*, and *Gliocladium*(Graph-2). Most of the *Penicillium* sp.are saprophytic with few nutritional requirements. In addition, the genus *Penicillium* is extremely important in nature because some of its 300 species are highly active in the recycling of organic matter (Kirk *et al*., 2008).



Graph 2 Species diversity of each fungal genera

The numbers of fungal genera identified from the soils in plots I, II, and III were 7, 8 and 7 respectively and their percentage frequencies were also calculated (Table 2)**.** The occurrence of abundance of species in genus *Aspergillus* and *Penicillium* were probably due to their capability of producing a diverse range of antibiotics and mycotoxins which protect them from other soil organisms and may also hinder the growth of other fungal species (Thavaselvi *et al*., 2015).

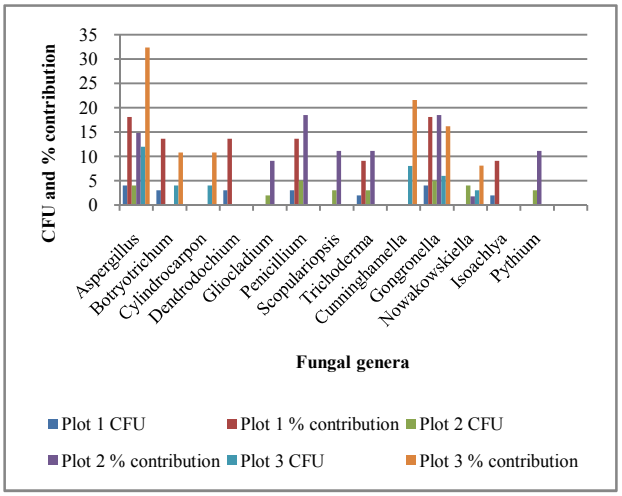
The colonies formed by each fungal genera in 10^{-3}, 10^{-4} and 10^{-5} dilutions were recorded (Table 3). The probable reason for the higher fungal population in plot 3 may be because, plot 3 is the perching area of birds in Kumarakom bird Sanctuary. Behera *et al.* (2012) reported that among the two study sites of mangrove area in the Mahanadi delta, Site 1 (Mangrove soil) harbors comparatively a large number of fungal population than Site 2 (River flat soil).

Table 2 Distribution of fungi in different plots of Kumarakom bird sanctuary

Sl. No	Fungal Genera	Plots 1	2	3	% frequency	Frequency class
	Class Ascomycetes					
1	*Aspergillus*	+	+	+	100%	C
2	*Botryotrichum*	+	-	+	66%	F
3	*Cylindrocarpon*	-	-	+	33%	O
4	*Dendrodochium*	+	-	-	33%	O
5	*Gliocladium*	-	+	+	66%	F
6	*Penicillium*	+	+	-	66%	F
7	*Scopulariosis*	-	+	-	33%	O
8	*Trichoderma*	+	+	-	66%	F
	Class Zygomycetes					
9	*Cunninghamella*	-	-	+	33%	O
10	*Gongronella*	+	+	+	100%	C
11	*Nowakowskiella*	-	+	+	66%	F
	Class Oomycetes					
12	*Isoachlya*	+	-	-	33%	O
13	*Pythium*	-	+	-	33%	O
	Total	**7**	**8**	**7**		

C = Common; F= frequent; O = Occasional; R= Rare

Colonies formed in 10^{-3} dilution was taken as Colony forming Unit (CFU) and the percent contribution of the isolated fungal genera in the 3 plots were calculated (Graph-3). The dominant fungi of Kumarakom Bird Sanctuary was *Aspergillus* followed by *Gongronella*. Kalaiselvi and Panneerselvam (2011) reported that *A. niger* was dominant fungal species isolated from agricultural field soil of Thanjavur Dt. and Ramgarh, India respectively. The ability of *A. niger* to dominate other fungal species could be linked to its high sporulating capacity.

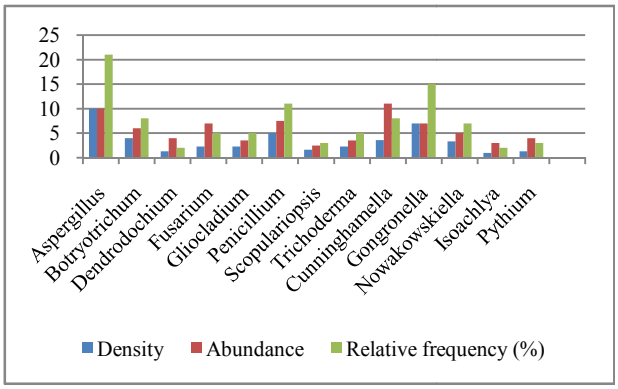


Graph 3 Comparative analysis of CFU and percentage contribution of different fungal genera

In this study, the fungal density, abundance and relative frequency were calculated. Fungal density and relative frequency were highest for *Aspergillus* and least for *Isoachlya*. The fungal abundance was highest for *Cunninghamella* and least for *Scopulariopsis* (Graph-4). Sivakumar (2007) reported that the fungal densities in Muthupet mangroves, Tamilnadu was highest for *Aspergillus niger*(32.2) followed by *A. terreus*(29.8), *A. ochraceus*(29.4), *A. erythrocephalus*(27.2),*A. fumigatus* (21.8). *A. flavus*(26.8), *A. oryzae*(20.6), *A. funiculosus*(17.4),*A. clavatus*(19.2), *A. sulphureus*(19.2), *A. nidulans*(17.0).He also reported that the fungal abundance was maximum for *A. niger*(32.2) followed by 29.8 for *A. terreus*, 29.4 for *A. ochraceus*, 27.2 for *A. erythrocephalus,* 26.8 for *A. flavus,* 20.6 for *A. oryzae*, 19.2 for *A. clavatus*.

Table 3 Number of colonies of fungal genera

Sl. No	Fungal genera	Plot I			Plot II			Plot III		
		No. of Colonies			No. of Colonies			No. of Colonies		
		10^{-3}	10^{-4}	10^{-5}	10^{-3}	10^{-4}	10^{-5}	10^{-3}	10^{-4}	10^{-5}
1	Aspergillus	4	2	1	4	2	0	12	4	1
2	Botryotrichum	3	2	0	-	-	-	4	2	1
3	Cylindrocarpon	-	-	-	-	-	-	4	3	1
4	Dendrodochium	3	1	0	-	-	-	-	-	-
5	Gliocladium	-	-	-	2	1	0	3	1	0
6	Penicillium	3	2	1	5	3	1	-	-	-
7	Scopulariopsis	-	-	-	3	2	0	-	-	-
8	Trichoderma	2	1	0	3	1	0	-	-	-
9	Cunninghamella	-	-	-	-	-	-	8	3	0
10	Gongronella	4	1	0	5	1	0	6	3	1
11	Nowakowskiella	-	-	-	4	1	0	3	2	0
12	Isoachlya	2	1	0	-	-	-	-	-	-
13	Pythium	-	-	-	3	1	0	-	-	-
	Total no. of colonies	21	10	2	29	12	1	40	18	4



Graph 4 Density, abundance and Relative frequency of fungal genera

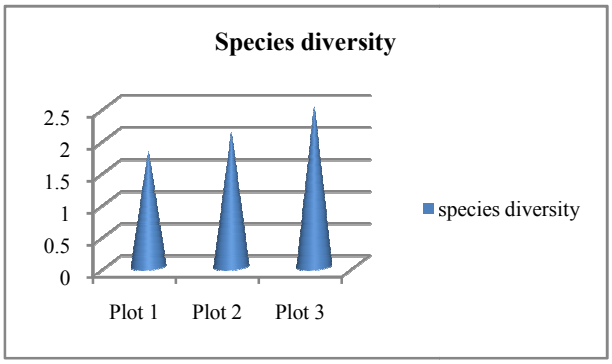
Maximum similarity of species was shown between plots I and II & II and III and least between I and III (Table: 4).

Table 4 Similarity and dissimilarity index of soil fungi.

PLOTS	I		II		III	
	IS	DS	IS	DS	IS	DS
I			0.53	0.43	0.28	0.72
II					0.53	0.43
III						

*IS = Similarity Index
*DS = Dissimilarity Index

Maximum species diversity was shown by plot 3 followed by plot 2 and then by plot 1(Graph-5).



Graph 5 Species Diversity of 3 plots

This result indicates that plot 3 is different from the other plots in number of colonies produced and thereby the species diversity.

This may be because of high moisture content and presence of bird droppings. Guleri *et al.* (2016) reported that highest similarity of soil mycoflora (84.2%) was observed between Rajawala and Bahadarpur and lowest (55.6%) between Rajawala and Telpura, agricultural Fields of Dehradun District of Uttarakhand Himalaya.

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