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Fungal Diversity of Ancient Caves Ecosystem in Doon Valley, Uttarakhand, Himalayas

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This correspondence aims to furnish details concerning the fungal diversity records of Pandav and Tiger Cave, located in the Chakrata forest sections of the Doon Valley in the Uttarakhand Himalayas. Samples from the entrance, dark areas, and dark sections of the Pandav and Tiger caves were taken to isolate soil fungi and examine their physicochemical characteristics.Malt Extract Agar and Czapek's Dox Agar were used for the maintenance and isolation of fungus. Soil dilution plate method was used to separate the samples of soil. The pH of the study varied from 6.60 to 7.61. There were variations in the moisture level between 6.23 and 11.23 percent, phosphorus between 10.42 and 192.64 kg/ha, sulfur between 11.02 and 35.2 ppm, zinc below 1.49 ppm, manganese between 0.36 and 5.46 ppm, copper between 0.36 and 5.46 ppm, and fossil carbon between 0.10 and 0.83%.Two hundred and nine colonies of soil fungi representing fifteen genera and twenty-four species were isolated. Of these, 12 genera and 21 species (EZ), 11 genera and 20 species (TW), 10 genera and 19 species (DZ), and 9 genera and 18 species (TW), were separated from Cave 1 and 11 genera and 20 species (DZ) from Cave 2.Four species of *Aspergillus*- the most common genera were identified from both the caves- *Aspergillus wentii, A. niger, A. flavus, and Aspergillus fumigatus. Fusarium* and *Penicillium,* with their species—*F.dimerum, Fusarium solani, f. sp., P. raistrickii, P. steckii, and P. maximae*—were next. Ten common species, ten moderate species, three frequent species, and one rare species are found in Pandav Cave. In contrast, 10 common species, 6 moderate species, 5 frequent species, and 3 uncommon species have been found in the tiger cave. The Pandav Cave's entrance zone and the Tiger Cave's Twilight zone showed the greatest resemblance, at 0.9047 (90.47%), while the Pandav Cave's Dark zone and the Tiger Cave's Dark zone showed the largest dissimilarity, at 0.3877 (38.77%). The EZ of Pandav Cave had the greatest Shannon-wiener Diversity Index (2.97), followed by the EZ of Tiger Cave (2.94). This study will provide important data regarding the distribution and diversity of cave ecology, which will also give researchers a platform to investigate the fundamental taxonomy of cave microfungi and the impact of fungal spores on the human population and vice versa.

Keywords : Cave ecology; diversity, Himalayas, microfungi, Uttarakhand

INTRODUCTION

Culver and Pipan (2009) define a cave as a big, easily accessible hole in the ground that is usually filled with dark. More than merely holes in the ground, caves have deeper meaning for people. For people of many cultures, caves are places of mystery, terror, and safety.

They have drawn a diverse range of visitors throughout history, from explorers hoping to reach the lowest point on Earth to those looking for a spiritual connection with God. Caves have also been repositories for some people. Revealing the beginnings of civilization and providing some of the earliest indications of contemporary human behavior (Tollefson, 2012). Generations of biologists have also been interested in distinctive settings, but not only because of their cultural and anthropological relevance.For reasons unrelated to the cultural and anthropological relevance of caves, these distinctive settings have also captured the interest of generations of biologists.

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In caves, microscopic fungi can be found on a variety of substrates, including soil, vermiculation, decomposing organic matter, and cave sediments. They constitute an essential component of the microflora within caves. Fungi are essential decomposers of ecosystems that are important for local and global biogeochemical transformation. Additionally, they have a significant effect on bioremediation, the bio weathering of rocks and minerals, and elemental cycling. Total darkness in cave areas interferes with circadian cycles, which affect many surface-level ecological processes (Langecker, 2000). Moreover, creatures inhabiting caves that lack any photosynthetic activity in their shadowy regions depend on outside carbon sources (Culver and Pipan, 2009).It is not possible for many fungi that are occasionally or frequently found in caves to develop there due to spores carried by water, air currents, or animals. It has been noted that conidia of *Aspergillus, Penicillium*, and *Mucor* species multiply swiftly in the organic debris present in caves. Fungi in caves are typically undernourished and dependent on organic matter inputs from the surrounding environment because organic matter is scarce in these environments(Jurado *et al*. 2000). Most likely, novel spore introductions and organic inputs (hair, dander, and lint) carried in by human visitors are the cause of changes in the abundance and variety of cave mycota (Cheliuse*t al*. 2009).

Not all caves contain the same assortment of mushrooms.The host material's susceptibility to colonization, the microenvironmental factors (water availability, temperature, pH, and nutrient sources), and the mineral composition, porosity, and rock permeability all affect the spread of cave microbiota colonies (Gorbushina, 2007).Fungi have been found in practically every type of environment, including caverns. The microorganisms in caves play several important roles. Benzene sulfonic acid and benzothiazole are two environmentally hazardous compounds, and the fungus plays a major role in their degradation. These pollutants can be eliminated by these microbes, preserving the natural order. A few specialized fungal species functions as the primary catalysts for decomposition, an essential process for both natural recycling and the continued existence of life on Earth. Fungi are essential to biodegradation because they can break down complex organic materials like lignin and cellulose. Bacteria can carry on the breakdown process after they have eaten the majority of the cellulose and lignin. The primary organisms that decompose and recycle nutrients from forest debris are fungi. In contrast to *Absidia, Rhizopus, Mucor, Chaetomium,* and*Sepedonium,* which were more frequent inside the cave. According to Fernandez Cortés *et al.* (2011), *Penicillium* has been demonstrated to display a distinct characteristic, and the air near the cave entrance is more densely populated with *Cladosporium* spores than it is within.There has been very little research on soil microfungiin particular with regards to the Uttarakhand Himalaya (Guleri*et al.* 2010, 2011, 2012, 2013, 2014, 2016). In recent years, Baskar *et al.*(2014) have attempted to study speleothems of Sahastradhara caves but no information on the diversity of soil microfungi has been provided in cave ecosystems. The existence of intriguing fungi in the Chakrata cave environment and the first-ever documented occurrence of *Clonostachys* in Indian cave ecosystems were the subjects of a recent study by Singh *et al*. (2022). The purpose of this investigation is therefore to investigate microfungi in cave environments.

MATERIALS AND METHODS

Study Area

At an elevation of 2118 meters, it is situated between the Tons and Yamuna rivers, 98 kilometers away from Dehradun, the state capital.In Uttarakhand's Dehradun district, Chakrata is a cantonment town as well as a subdistrict and tehsil. Originally stationed at Chakrata was the British Indian army. Westwards is Himachal Pradesh; eastwards are Tehri Garhwal and Mussoorie (73 km). As per the 2011 India census, Chakrata town is less than 15.70 square kilometers, with a latitude of 30º42'5.84" N and a longitude of 77º52'10.56" E, or 30.701621 and 77.8696, respectively (Fig.1).

Collection of soil sample

Sampling was started in November and December 2020. In the lakhamandal region, soil

samples were randomly collected from the Pandav and Tiger caves' entrance, twilight, and dark sections. To make a combined specimen, four samples from each zone were taken and sent to the lab. Samples were divided into two equal parts (one for physico chemical analysis for each zone and one half for the isolation and identification).

Soil Test and Soil Analysis
Moisture content

To compute it, the soil was dried in an oven and the weight loss was determined (Garrett 1963). The percentage of soil moisture has been determined using the following formula:

$$\text{Soil moisture percentage } (\%) = \frac{FW - DW}{DW} \text{ x } 100$$

FW = is the sample of fresh soil weight.
DW = Dry weight of sampled soil

Texture of the soil

After 50 g of soil were sieved, hot H_2O_2 was used to oxidize the sample.To remove organic components. from the soils undergoing treatment, a 1000 ml sample volume has been generated. The total weight of the specimen was divided by the grams of clay to determine the percentage of clay in the sample. Sand and clay proportions have been subtracted from 100 to determine the percentage of silt in the sample. Soil texture numbers have been calculated using a textural triangle.

About 200 g of soil was made into a paste with a homogenized soil paste was stored for 24 hrs. Using a blotting paper, the soil was removed. Ca, Mg, Na, and K were also found using the soil extraction process. By adding one to three drops of NaoH and a tiny quantity of NH_3 to the solution, Ca and Mg were found.EDTA (ethylene-diamine-tetra-acetate) was used to titrate the mixture. As the end point, colorations of green, black, and blue emerged. To test for magnesium ion solely, 2 ml of soil extract was mixed with three drops of buffer solution containing four ereocrome blacks. This produced a crimson color. The mixture's end point was reached when it was titrated against N/100EDTA and turned purple.A range of reference sodium values have been used to create a calibration curve for the NA concentration. Emission measurements from the flame photometer reported by Akhtar *et al.* (2004) at a wavelength of 589 nm have been used to analyze soil extracts containing sodium ions.

The calibration curve was considered while calculating the Na+ concentration. Five grams of air-dry soil were weighed for the K analysis, and then a 50 ml centrifuge tube was filled with an ammonium acetate solution that had been agitated for five minutes.The mixture was centrifuged until the liquid supernatant was removed. The extract was collected and filtered through a 100-milliliter solution of 1N ammonium acetate. A number of suitable potassium standards have been run in order to compute the calibration curve. Emission measurements from a flame photometer operating at 767 nm wavelength were used to calculate the concentration (Takahashi *et al*. 2011). The following formula was used to calculate organic C, which was determined using the method described by Walkley and Black (1934):

% organic matter =10[1(S÷B)] × 0.67
S=Sample titration
B=Blank titration

Using an electrometric approach and a pH meter with a glass electrode in a 1:2 mixed soil to water ratio, the soil response (pH) was measured (Brady, 1990).

Isolation of fungi

MEA (Malt Extract Agar) and CDA (Czapek's Dox Agar) were used for fungal isolation and maintenance. To isolate the soil samples, Waksman and Fred (1922) used soil dilution plates that were incubated for seven to fifteen days at 20 to 25!. Single spore culture and occasionally hyphal separation techniques were used to create pure cultures of the fungi (Barnet and Hunter, 1972). The 400x and 1000x magnification micrographs were captured with binocular microscopes. The plates' colonies have been detected and moved to different agar plates. The use of Mycokeys*viz.,*Moubasher (1993),

Gilman (2001),Watnabe(2002)were also used to identify them. Microphotography of the various genera identified in the course of the investigation has been carried out using the Magnus MIPSUSB), DS CW320 Sony. The use of reference culture samples from ITCC, MTCC and NFCCI has also confirmed the identification viz., *Aspergillus fumigatus* (MTCC3070), *Aspergillus niger* (MTCC2196), *Aspergillus wentii* (NFCCI3195), *Cladosporium* (MTCC2136), *Fusarium dimerum* (ITCC3864), *Aspergillus sp.* aff. *Aspergillus ustus*gr. (NFCCI5040), *Fusarium sp.* (NFCCI5041), *Aspergillus flavus* (NFCCI5042), *Fusarium sp.*aff.*Fusariumsolani* (NFCCI5043), *Gliocladium sp.* aff.*G. catennulatum*(NFCCI5044).

Periodicity of occurrence, species richness, similarity and dissimilarity index

The frequency of occurrence will indicate the proportion of samples containing the fungus relative to the total number of samples. According to Guleri*et al*. (2016), fungal periodicity was arbitrarily classified.

Common: found in five to seven samplings
Regular – noted in four to five samplings
Moderate; seen in two to three samplings
Seldom – seen in 1-2 samples

The statistical abstraction known as species diversity is composed of two elements: species richness and consistency. Species richness was defined as the total number of species absent from a place or location. The sites were compared using a population similarity measure of the Communities. The current approach employed species richness indices in accordance with Sorenson's (1948).

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

Where- A and B represent the number of species and species count, respectively, within the community and C= Total number of species that both groups share

The Dissimilarity Index (D) was computed as follows: D= 1-S.

Variability and addition elements

Bio Tool Kit 320 is a computer program that has been used to analyze the Shannon-Wiever Index, Simpson's index of dominance, and evenness.

RESULTS AND DISCUSSION

Physico chemical properties

Soil samples have been analyzed for moisture content, pH, organic carbon, Ca+, Mg+, Na+, and other soil micronutrients, as well as texture and color.The organic content of each soil sample ranged greatly from 0.10 to 221.7. The pH scale is 6.60–7.61. The moisture content of each soil sample ranged from 9.62 to 11.04. There were rocky, clayey, and sandy particles in the soil. Table1 displays the physicochemical parameters listed below.

Soil fungal Diversity

A total of 209 colonies comprising 24 species and 15 genera of soil fungi were isolated. 13 genera and 22 species (EZ), 9 genera and 18 species (TW), 11 genera and 20 species (DZ) were separated from Cave 1, whereas 12 genera and 21 species (EZ), 11 genera and 20 species (TW), and 10 genera and 19 species (DZ) were isolated from Cave 2. In both the caves 1 and 2 (Pandav cave and Tiger cave) *Aspergillus* was the most dominant genus having 5 species, viz. *Aspergillus flavus* (25% and 20.83%), *Aspergillus fumigatus* (16.66%), *Aspergillus wentii* (20.83% and 29.16%), *Aspergillus niger* (37.5% and 41.6%), *Aspergillus ustus* (37.5% and 20.83%); followed by *Fusarium* and *Penicillium* species viz. *Fusariumsp*. (41.6% and 20.83%), *Fusarium dimerum* (33.33% and 16.66%), *Fusarium solani* (25% and 29.16%), *Penicillium raistrickii* (12.5% and 20.83%), *Penicillium steckii* (8.33%), *Penicillium maximae* (25% and 29.16%), followed by *Humicola* having 2 species viz. *Humicola sp*. (8.33%), *Humicola tuberculata* (12.5% and 16.66%). *Acrophialophora fusifora* (16.66% and 8.33%); *Botryodiplodia theobromae* (12.5% and 8.33%) *Cladosporium sp.*(12.5% and 16.66%); *Chaetomium globossum* (20.83%), *Gliocladium sp.* (12.5% and 8.33%), *Geotrichum sp.* (12.5% and 16.66%), *Mucor hiemalis* (20.83% and

Table 1: Physico-chemical properties of Entrance (EZ), Twilight (TZ) and Dark zone (DZ) of Pandav and tiger cave

Location	Zone	pH	EC (ds m/m)	Fossil carbon in %	Phosphorous in kg/ha	Potash in kg/ha	Sulfur in ppm	Boron in ppm	Zinc in ppm	Iron in ppm	Mn in ppm	Copper in ppm	Moisture content	Soil Texture	**Soil color**
Tiger cave	EZ	6.60	0.19	0.76	76.16	189.82	11.05	0.62	Low	14.80	0.44	0.70	10.3	Sandy, clayey and rocky elements	Brown soil
	TZ	7.03	0.19	0.83	192.64	207.3	11.02	0.58	0.09	15.61	0.52	0.65	9.62		
	DZ	7.50	0.18	0.55	10.45	179.7	35.2	0.81	0.26	18.85	5.46	0.76	11.04		
Pandav cave	EZ	6.86	0.15	0.15	76.16	197.0	21.06	0.71	0.27	16.25	0.68	0.68	11.02	Sandy, clayey and rocky elements	Brown soil
	TZ	7.31	0.62	0.14	53.76	209.4	13.06	0.53	0.84	10.75	0.36	0.85	10.03		
	DZ	7.61	0.62	0.10	40.32	221.7	22.06	0.52	1.49	18.46	0.98	0.76	11.63		

Table 2: Fungal Diversity of Soil Fungi in Pandav and Tiger caves.

Name of Fungal sp.	Pandav cave				Periodicity of occurrence	Tiger cave				Periodicity of occurrence
	Entrance zone	Twilight zone	Dark zone	% contribution		Entrance zone	Twilight zone	Dark zone	% contribution	
Aspergillus flavus	1	3	2	25	C	2	2	1	20.83	C
Aspergillus fumigatus	1	2	1	16.66	F	3	1	0	16.66	F
Aspergillus wentii	1	1	2	20.83	C	4	2	1	29.16	C
Aspergillus niger	3	3	3	37.5	C	3	3	4	41.6	C
Aspergillus ustus	2	4	3	37.5	C	2	1	2	20.83	C
Acrophialophorafusifora	1	2	1	16.66	F	1	0	1	8.33	M
Botryodiplodia thiodermae	2	0	1	12.5	M	1	1	0	8.33	M
Cladosporium sp	1	2	0	12.5	M	2	1	1	16.66	F
Chaetomium globosum	2	2	1	20.83	C	2	2	1	20.83	C
Fusarium dimerum	3	3	2	33.33	C	1	1	2	16.66	F
Fusarium sp.	4	3	3	41.6	C	0	3	2	20.83	C
Fusarium solani	2	2	2	25	C	2	2	3	29.16	C
Gilocladium sp.	1	0	2	12.5	M	0	1	1	8.33	M
Geotrichum sp.	0	2	1	12.5	M	1	2	1	16.66	F
Humicola sp.	1	0	1	8.33	M	1	1	0	8.33	M
Humicola tuberculata	2	1	0	12.5	M	2	1	1	16.66	F
Mucor hiemalis	3	1	1	20.83	C	1	0	0	4.16	R
Penicillium raistriickii	1	0	2	12.5	M	2	2	1	20.83	C
Penicillium steckii	1	1	0	8.33	M	0	1	1	8.33	M
Penicillium maximae	3	2	1	25	C	3	2	2	29.16	C

Paecilomycessp	2	1	1	16.66	F	2	1	3	25	C
Trichoderma harzianum	1	0	1	8.33	M	1	0	1	8.33	R
Ulocladium sp.	1	0	0	4.16	R	1	1	1	12.5	M
Verticillium dahlieae	0	2	1	12.5	M	1	0	0	4.16	R

Table 3: Soil fungi Similarity and Dissimilarity Index

Pandav Cave			Pandav Cave				Tiger Cave					
	EZ		TZ		DZ		EZ		TZ		DZ	
	IS	DS	IS	DS	IS	DS	IS	DS	IS	DS	IS	DS
EZ			0.8	0.2	0.8571	0.1428	0.8837	0.1162	0.9047	0.0952	0.878	0.1219
TZ					0.7894	0.2105	0.8205	0.1794	0.7894	0.2105	0.8108	0.1891
DZ							0.7058	0.2941	0.64	0.36	0.6122	0.9877
Tiger Cave												
EZ									0.8292	0.1707	0.75	0.25
TZ											0.8717	0.1282
DZ												

Table 4: Parameters and diversity indices

Specifications	Pandav cave			Tiger cave		
	EZ	TZ	DZ	EZ	TZ	DZ
Shannon Wiener diversity Index	2.97	2.81	2.9	2.94	2.91	2.81
Species richness	22.0	18.0	20.0	24.0	21.0	20.0
Total abundance	39	37	32	38	31	30
Simpson's Index	0.0365	0.0370	0.0303	0.0345	0.0262	0.0344
Simpson's Diversity Index(I-D)	0.9635	0.9630	0.9697	0.9655	0.9738	0.9656
Simpson's reciprocal index (I/D)	27.39	27.02	33.00	28.98	38.16	29.06
Evenness	0.961	0.97	0.967	0.966	0.971	0.955

4.16%), *Paecilomyces sp.* (16.66% and 25%), *Trichoderma harzianum*(8.33%), *Ulocladium-microsporum* (4.16% and 12.5%) and *Verticillium dahlieae* (12.5% and 4.16%) represented by 1 species each respectively (Table 2). The maximum diversity in the entrance zone of Cave 1 was 22 species, with 20 occurring in dark and twilight zones respectively, as a result of various resources. In cave 2, 21 species from the entrance zone have been identified in this order of 20 between twilight zones and 19 among darkness zones while maximum diversity has been observed (Figs. 2 to 5).

Periodicity of occurrence

Out of the 24 species that have been identified, 10 were common in cave 1, including *Aspergillus flavus, Aspergillus niger, Aspergillus ustus, Chaetomium globossum, Fusarium dimerum, Fusarium sp., Fusarium solani, Mucor hiemalis, and Penicillium maximae;* 10 were common in cave 2, including *Botryodiplodia theobromae, Cladosporium sp., Gilocladium sp., Humicola tuberculata, Penicillium raistriickii, Penicillium steckii, Trichoderma harzianum,* and *Verticillium dahlieae;* 3 were frequent in nature, and the final

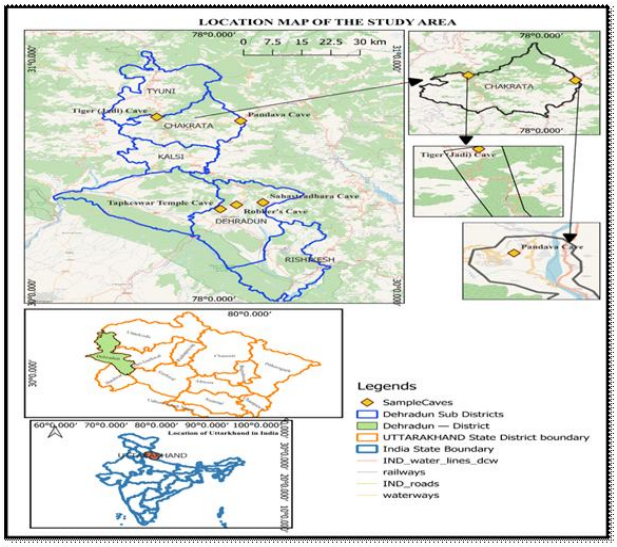

Fig.1. Location Map of the study area (Doon Valley)

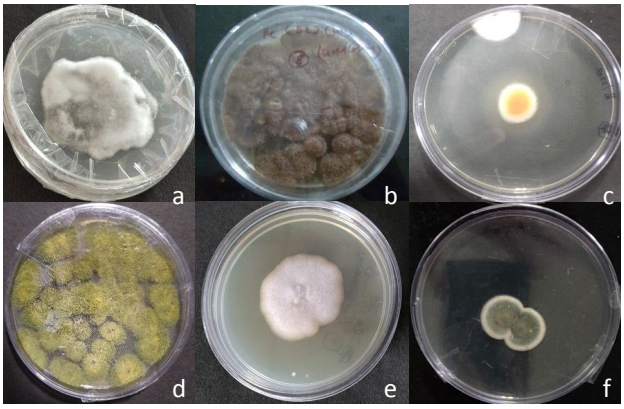

Fig. 2 : (a-f). Cultural morphology of various species: a. 7-day-old colony of *Acrophialophorafusifora* on CDA; b. 7-day-old colony of *Chaetomium globossum* on MEA; c. 5-day-old colony of *Aspergillus wentii* on MEA; d. 7-day-old colony of *Aspergillus flavus* on CDA; e. 5-day-old colony of *Fusarium solani* on MEA; f. 5-day-old colony of *Aspergillus ustus* on CDA

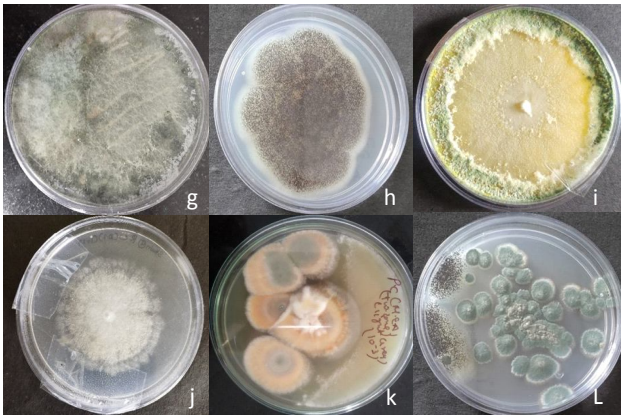

Fig. 3 : Cultural morphology of various species: g. *Verticillium dahlieae*, a 7-day-old colony on MEA; h. *Aspergillus niger*, a 5-day-old colony on CDA; i. *Trichoderma harzianum*, a 7-day-old colony on MEA; j. *Mucor hiemalis*, a 5-day-old colony on MEA; k. *Penicillium maximae*, a 10-day-old colony on MEA; l. *Penicillium steckii*, a 5-day-old colony on CDA.

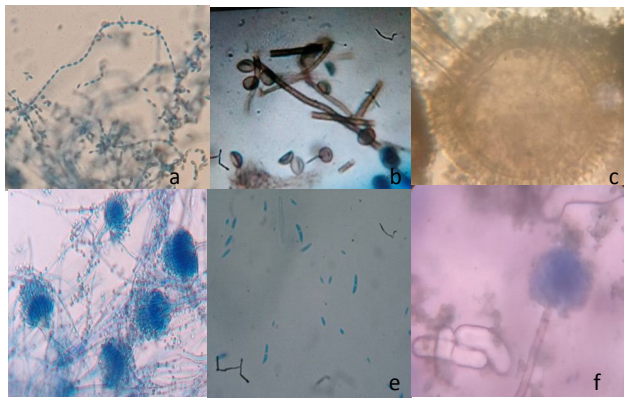

Fig. 4: a) Long chain of *Acrophialophora fusifora* conidia (x400); b) Ascospores of *Chaetomium globossum* (x1000); c) *Aspergillus wentii* conidiophores and conidial head (x400); d) *Aspergillus flavus* conidiophores and conidial heads with conidia (x400); e) Conidia of *Fusarium solani* (x400); f) *Aspergillus ustus* conidiophores with Hulle cells (x400).

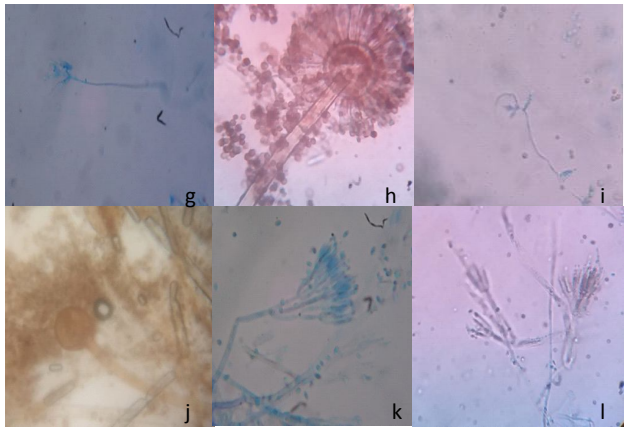

Fig. 5: g- Conidia and phialides of *Verticillium dahlieae* (x400); h -*Aspergillus niger* conidiophore, conidial head and conidia(x400); i- *Trichoderma harzianum* conidiophores phialides and conidia (x400); j- *Mucor hiemalis* and sporangiophores sporangiospores (x400); k- *Penicillium maximae* conidiophores and conidia (x1000); l- *Penicillium steckii* conidiophores and conidia (x1000).

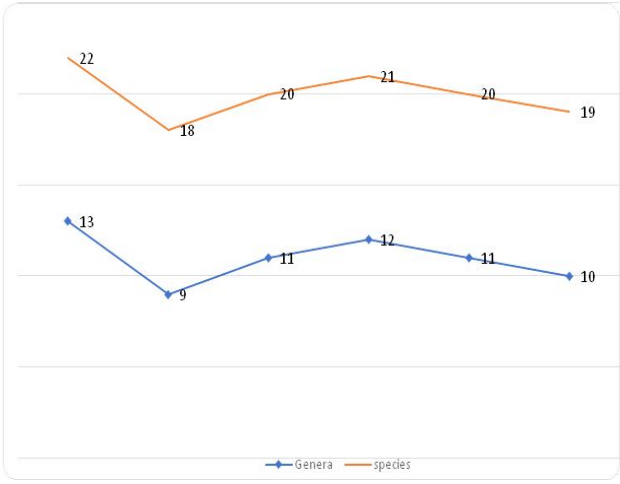

Fig 6: Number of genera and species isolated from all zones of Pandav and Tiger cave.

species,*Ulocladium microsporum,* was rare. *Aspergillus flavus, Aspergillus wentii, Aspergillus niger, Chaetomium globosum, Fusarium sp., Fusarium solani, Penicillium raistriickii, Penicillium maximae*, and *Paecilomyces sp.* were among the ten species that were frequently found present in Cave 2. Six species, namely *Gliocladium sp., Humicola sp., Penicillium steckii, Ulocladium microsporum, Acrophialophora fusifora*, and *Botryodiplodia theobromae,* were of moderate prevalence. *Aspergillus fumigatus, Fusarium dimerum, Cladosporium sp., Geotrichum sp., and Humicola tuberculata* were the five species that were frequently found; the remaining three species, *Mucor hiemalis, Trichoderma harzianum,* and *Verticillium dahlieae*, were rare.

Index of Dissimilarity and Similarity

Table3 shows that the highest similarity was observed between Entrance zone of Pandav cave and Twilight zone of Tiger cave, i.e. 0.9047 (90.47%) and highest dissimilarity was observed between Dark zone of Pandav cave and Dark zone of Tiger cave, i.e. 0.3877 (38.77%).

Diversity dominance and other parameters

Shannon Wiener's Diversity Index has been the highest in EZ of Pandav cave (2.97) followed by EZ of Tiger cave (2.94). The total richness was highest in Entrance zone of Pandav cave 22, followed by Entrance zone of Tiger cave 21. Whereas abundance was highest in Entrance zone of Pandav cave 39, followed by Entrance zone of Tiger cave. The species evenness was highest in Twilight zone of both Pandav and Tiger cave (Table 4)

Comparison of the diversity of fungi

Between Caves 1 and 2, a total of 24 species and 15 genera were isolated; in Cave 1, these included 13 genera and 22 species through the entrance zone, 9 genera and 18 species through the twilight zone, and 11 genera and 20 species from the dark zone. In Cave 2, on the other hand, these included 15 genera and 24 species through the entrance zone, 11 genera and 20 species through the twilight zone, and 10 genera and 19 species from the dark zone. (Fig.6)

The results of the study have provided a comparative analysis between the diversity of fungi and propagule species found in cave entrances, as well as at twilight or under darkness zones. In the cave environment, natural agents, such as floods and air, may introduce fungal spore and mycelium into the cave through organic substances such as plant and animal remain that are transported into the cave by trogloxens. The fungus that gets into the cave starts to grow on suitable substrates like insect remains and bat guano. The total number of colonies of fungi/gram cave soil at the entrance zone was presumably higher when compared to twilight and dark zone. There were more varieties of fungus in the entrance and sunset zones. In addition to physical characteristics, the soil affects the presence of particular fungus species in each cave zone by influencing temperature, relative humidity, light availability, and other factors.

Soil samples collected from the entrance, twilight and dark zones of six caves revealed 35 species of sporulating mesophilous microfungi and seven types of unsporulated fungi. The variety of fungi in the domica cave system was also studied by Novakova (2009), who isolated 195 distinct fungal taxa from various cave substrates. .The number and diversity of microorganisms in caves decreased from the entrance through the deep zones, as reported by Mulec *et al.* (2012). Similarly, the present research indicates a reduction in the amount of fungal species that reach dark zones.

The species richness of the cave ecosystem's soil microfungi and the physical and chemical variables influencing the variety of fungus at particular locations are the subjects of the current study. The color, pH, and micro- and macronutrient content of the local soil vary .Due to frequent visitor traffic to the two distinct caves in the Chakrata region, which directly affects the fungal population, a significant number of fungal species were isolated in the current study. Soil fungi that were categorized as common or frequent, but not unusual, have a species distribution that is unlikely to disappear quickly from their current habitats (Chilias *et al*. 2009) claim that changes in the number and diversity of cave mycota are probably caused by human-

induced organic inputs including dander, hair, and lint, as well as the introduction of new spores. *Aspergillus* genus was the dominant species of both caves, with 5 types followed by Penicillium and Fusarium spp. The entrance zone of both caves has been found to have the highest diversity from various sources. The majority of the 24 species recorded were common, moderate and frequent, indicating that they will not disappear easily from the sites. The entrance to Pandav and Tiger caves, the Twilight zone of both caves was found to be most similar while the darkest part of these caves had been shown to be more dissimilar.This species was more evenly distributed in the Twilight zone of both caves. There was a very high chance that various species would be identified in this area because Simpson's index predictions were not near to 1. It is also possible to explain the low diversity index by pointing out that, as other research has previously shown, some other fungi that are closely linked to trees and other plants were not included in the laboratory isolation of cultivable soil fungus for this study (Guleri *et al.* 2016).

CONCLUSION

In conclusion, this study is a preliminary attempt to investigate fungal diversity of cave ecosystems in selected sites within the Chakrata region.As a result, the isolated species will greatly advance our knowledge of the distribution and diversity of cave ecosystems. Additionally, it will provide a platform for future research into the basic taxonomy of cave microfungi and the effects of fungal spores on human populations.It is clear that there is still much to learn about the kind, distribution, and ecology of fungi that grow in caves.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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