RESEARCH ARTICLE

Habitat and Season Dependent the Invasion of Micro-fungi in Decaying Wooden Flinders: a Case Study of the Dandak Cave of Chhattisgarh, India

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Study Area: Dandak cave, Bastar, Chhattisgarh, India

Coordinates: 18°51'30" N; 81°57'00" E

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Abstract

Subterranean caves represent itself always a sterile ecosystem as it usually has very bleak connections with the external world. Due to complete lack of light, the existence of autotrophs become out of question inside the cave and whatever the organic energy sources get available inside the cave are mostly transported from the external world. Thus, most of the micro-fungi found inside the caves are also generally transported inside from some external agencies. Further, the available habitats inside the cave either abolish them completely or accelerate them to flourish. In the present study, we have tried to isolate the micro-fungi responsible for decaying the wood log inside a cave, Dandak of Chhattisgarh, India. Samples were collected from two different habitats of the cave, one which remains under the influence of external world (outer chamber) the other which is almost isolated from it (inner chamber). We found that besides some other genera of micro-fungi, the genus *Aspergillus* is dominating the decaying process of wood in the outer chamber whereas the genus *Penicillium* is dominating the same for the inner chamber.

Introduction:

Deep parts of the caves are always representing itself as heterotrophic systems (Boling Jr. *et al.*, 1975). The rivers and/or rain and other water sources such as continuous seepage through fissures either from the roof or walls act as the major energy resources carrier for such subterranean environment (Simon *et al.*, 2007). Among such energy resources, the wood logs could only be considered as the long-lasting.

Deadwood is always considered as an important element to maintain healthy biodiversity in forest ecosystems. The species depends directly or indirectly on it for their nutrition are referred as saproxylic. The saproxylic species acts a crucial role in decomposing the wood log and maintaining proper nutrient cycling cum carbon storage (Harmon *et al.*, 2004). Further, most fungi are the dominated decomposers of wood in most of the terrestrial ecosystem (Boddy *et al.*, 2008; Stokland *et al.*, 2012). The decay process/rate of every wood log may vary depending on the species' characteristics (chemical composition of wood), dimensions and site conditions though; temperature and moisture are always the major deciding factors for their respective site conditions. Indeed the

warm, moist environments favour microbial and fungal growth and due to which the decay rates of the dead woods are also enhanced (Jordan, 1985; Yin, 1999).

The internal biosphere of every cave could be categorized into various ecological chambers depending on its architect and its respective connections with the external environmental conditions. The portion which receives light at the daytime is twilight chamber, the geophysical characteristics of such chamber vary as per its external environmental conditions. Further, the twilight chamber followed towards caves' inner chambers through the transient chamber which is characterized by perpetual darkness having varied geophysical characteristics. Finally, the final destination would be the dark chamber which is characterized by the perpetual darkness, almost constant geophysical characteristics, high humidity and carbon dioxide concentration, low energy input (organic matters) etc. In addition to such chambers/ecological habitats sometimes in some big caves, an additional chamber (ecological habitat) is also found, referred as a stagnant chamber. The life in such ecological habitat of a stagnant chamber always needs a very high degree of adaptation because, in spite of common characteristics of the deep

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chamber, the air movements are almost nil inside this chamber. In addition, this chamber is of almost lack of any energy resources (Biswas, 2009; 2010).

In the present study, we have collected season-wise the decaying wood flinders found somewhere in the floors inside the two different chambers (habitats) of the Dandak cave; twilight cum transient (outer chamber) and deep (inner chamber). Further, the species of fungi decomposing them with respect to the season and habitat effects have been tried to establish.

Materials and Method:

Study Area: Dandak cave is situated on the top of an isolated hillock exhibiting a few patches of exposed boulders. Despite a few side passages and downward sloping passages, the cave is mainly composed of two chambers which cover approximately 360 m in length. The two chambers are ecologically quite different from each other and are connected by a very narrow convoluted passage. Though the cave entrance exists in an upper segment of the first chamber, it illuminates the maximum part of the first chamber during the day hours. Thus, the most of the part of the first chamber could be referred as twilight chamber whether the remaining part of the same chamber could be the categorized as the twilight cum transient chamber. However, the second chamber perfectly represents itself as inner chamber having perpetual darkness, almost constant temperature and high humidity. The energy input in this chamber is too low (Biswas & Shrotriya, 2011).

Collection of samples: Wood logs usually enter the cave with rain stream and get settle in some nook and corner of the cave floor. It soon starts to decay under high humid and low air movement conditions of the cave. The flinders of such decaying wood, converting to soil forms complex sediment inside the cave (Fig.-1). Such complex sediments (flinders of rotten woods mixed with soil) were collected randomly from the two different chambers of the cave; outer and inner chamber during three different seasons; winter (December), Summer (May) and Rain (August).

The targeted sedimentary samples were collected very carefully with the help of sterilized scalpel and collected in small vials. At the time of sampling, various environmental factors were also recorded like humidity, light intensity, and temperature at the site where we collected the samples.

Figure-1: Wooden debris getting soil inside the cave.

Isolation of the Fungi: the collected soil samples were transferred to the laboratory within 12 hours for the further

investigation. Each sample was further air-dried and sieved through 2-mm mesh. One gram of soil from each sample was then used for further processing of inoculations and isolation of various sediment fungi. For isolation, we used Serial Dilution Plate Method (Waksman, 1916).

Identification of Cultures: the prepared pure cultures were observed from 5th -7th day of inoculation. Some colonies appeared even after 8-10 days of inoculation. For the identification of individual pure culture, numbers of characteristics were taken. On the basis of Visual fungal culture characteristics on plates such as topography of the colony, mycelial growth, outline of colony, texture of colony, pigmentation of the colony, exudance of substance by the colony, colour of colony on back and front side of Petri plate, changes in colour of the colony and medium, diameter of the colony etc. The diameter of the colonies was measured after five days of its appearance. The identification of different fungi was done with the help of direct mount from pure culture and its slide culture. A small piece of culture was picked from culture than mounted in the center of the slide; it was stained with cotton blue then mounted in lactophenol. The slides were sealed with nail polish and D.P.X. Different forms were identified up to generic and species level using monographs and relevant literature.

At end of the incubation period, the percentage frequency and percentage contribution of the fungal flora have been calculated.

Ecological Characteristics: to know the ecological characteristics which facilitate the growth of fungi, at the end of the sampling, isolation and incubation period of the fungi, percentage frequency (%F) is occurrence of a species in total no. of observation season wise, percentage contribution (%C) of particular mycoflora in total number of isolates, percentage Relative Frequency (% RF) of fungal flora was calculated.

Figure-2: Some of the common identified micro-fungi species

Results & Discussion :

The samples were collected during three different seasons, the geophysical characteristics of both the chambers and outer environment were tabulated as follows:

Table-1: Comparative geophysical status among three different habitats.

Habitat (Chamber)	Light intensity	Temp	Air movement m/sec.	Humidity
Winter				
First	0 to ~0.2 lx	26°C	0	100%
Second	0 lx	25°C	0	100%
Outer	6000 lx	23°C	0.8 -1.4	65%
Summer				
First	0 to ~0.5 lx	29°C	0- 0.2	80-95%
Second	0 lx	26.5°C	00	95%
Outer	10000 lx	40°C	1.0-2.0	30-35%
Rain				
First	0 lx	26°C	0	100%
Second	0 lx	26°C	0	100%
Outer	1100 lx	32°C	0.5-0.8	85%

Fungi isolated from various samples:

(Results have been tabulated in Table- 2 & Fig.-3)

As expected our result revealed, the types of myco-species associated with decomposing the wood logs/flinders were varying with respect to the two different ecological habitats and season.

Aspergillus spp. were the most occurred fungi isolated from the samples of decaying wooden flinders gathered from outer chamber, whereas only once a species of *Aspergillus niger* was isolated from a sample collected from the inner chamber during the summer season. It is well known that *Aspergillus* spp. are highly aerobic in nature and grows only in oxygen-rich environments (Bolu *et al.*, 2003; Chehri, 2013). On the other hand, it is mostly associated with plant and trees (Bennett *et al.*, 2010). Varieties of *Aspergillus* spp. were isolated from the samples collected from the outer chamber which is highly influenced by the ambient external environment is directly support the same fact. However, among them the species *Aspergillus niger* could grow safely in all such environment which is devoid of key nutrients. In addition to some other *Aspergillus* species, *A.niger* has been well documented from the various caves all over the world (Cunningham *et al.*, 1995; Nováková, 2009; Martin-Sanchez *et al.*, 2014; Taylor *et al.*, 2014; Zhang *et al.*, 2017). Even in wood logs inside the caves (Karkun *et al.*, 2012). Thus, the occurrence of the same in a sample gathered once from the inner chamber during summer is not at all surprising.

Nevertheless, irrespective of any specific season and/or habitat, we found frequently the species of *Penicillium*. Unfortunately, at the present status, we failed to identify most of them up to the species level at the present stage. *Penicillium* spp. always exist in the air and

Figure-3: Cumulative appearances of the isolates of myco-species /group of myco-species from the wooden flinders of both the chambers

dust of an enclosed environment. These are easily transportable from the outdoors, and flourish inside any house, public place, cave etc. where it even use its respective building material or accumulated soil to obtain nutrients for growth. Asper Fairs *et al.*, (2010) *Aspergillus*- and *Penicillium*-type spores are the most prevalent in the indoor air of residential properties, transported from the outdoor sources. However, *Penicillium* spp. are commonly found in various substrates inside the caves (Cunningham *et al.*, 1995; Lukešová & Nováková, 2007; Nováková, 2009; Karkun *et al.*, 2012; Martin-Sanchez *et al.*, 2014; Taylor *et al.*, 2014; Zhang *et al.*, 2017; Biswas & Biswas, 2017).

Besides *Aspergillus* and *Penicillium*, the species of *Phoma*, and even *Trichoderma* are also some of the micro-fungi commonly isolated from plant debris or decaying wood material Nováková (2009). In our present study, we obtained more varieties of fungi from decaying wooden flinders which were collected from the outer chambers. Among which few species remain unidentified whereas the other identified species are *Malbranchea Pulchella*, the taxa have already been reported from cave bat guano (Ulloa *et al.*, 2006) and other various sediments found inside the caves of Slovenia (Nováková, 2009), *Mucor* spp. (Lukešová & Nováková, 2007; Nováková, 2009; Taylor *et al.*, 2014; Zhang *et al.*, 2017), *Isaria* sp. (Lukešová & Nováková, 2007; Nováková, 2009; Zhang *et al.*, 2017;), *Histoplasma* sp. (Al-Doory & Rhoades, 1968; Carvajal, 1977a,b,c; Ashford *et al.*, 1999; Larcher *et al.*, 2003),

Table-2: Three Seasons dependent micro-fungi* isolated from the wooden flinders collected from 2 different habitats of Dandak cave

S. No.	Isolated Fungi	Summer Season		Rainy Season		Winter Season		Total 'N' Colonies	Percent Contribution	Percent Frequency
		Outer**	Inner**	Outer**	Inner**	Outer**	Inner**			
1	<i>Aspergillus fumigates</i>	1		2		2		5	6.25	50
2	<i>Aspergillus sps</i>	1						1	1.25	16.67
3	<i>Aspergillus niger</i>	3	3	4		3	1	14	17.5	83.33
4	<i>Aspergillus flavus</i>	2		1		1		4	5	50
5	<i>Aspergillus candidus gr.</i>			1		1		2	2.5	33.33
6	<i>Chaetomium sp.</i>						2	2	2.5	16.67
7	<i>Fusarium monaliforme</i>	1				1		2	2.5	33.33
8	<i>Histoplasma sp.</i>			2		1		3	3.75	33.33
9	<i>Isaria sp.</i>			1				1	1.25	16.67
10	<i>Malbranchea pulchella</i>	2		2				4	5	33.33
11	<i>Mucor haemalis</i>	1		2				3	3.75	33.33
12	<i>Paecilomyces lilacinus</i>			2		4		6	7.5	33.33
13	<i>Paecilomyces variotii</i>					1		1	1.25	16.67
14	<i>Penicillium sp.</i>	2			1			3	3.75	33.33
15	<i>Penicillium sp.</i>		2		1		1	4	5	50
16	<i>Penicillium sp.</i>		1		4		3	8	10	50
17	<i>Penicillium sp.</i>				3			3	3.75	16.67
18	<i>Penicillium glaucum</i>	1		1		1		3	3.75	50
19	<i>Penicillium sp.</i>						3	3	3.75	16.67
20	<i>Phoma sp.</i>		2					2	2.5	16.67
21	<i>Rhizopus stolonifera</i>	1				1		2	2.5	33.33
22	sterile mycelium				3			3	3.75	16.67
23	<i>Trichophyton rubrum</i>			1				1	1.25	16.67

*Mean; **chamber (habitat); N-number of

Total Colonies

80

100

Trichophyton sp. (Lurie & Borok, 1955; Nováková, 2009; Martin-Sanchez *et al.*, 2014;) and *Paecilomyces lilacinus*, where the anthropogenic pressure remains high (Saiz-Jimenez, 2015). The species *Trichophyton rubrum* (Rippon, 1988; Moore-Landekar, 1996) and *Rhizopus Stolonifera* (Fiorina *et al.*, 2000; Ogorek *et al.*, 2016; Vanderwolf *et al.*, 2013) isolated by us has also been reported earlier from some caves.

Conclusion:

As compared to the earlier reports on isolation of micro-fungi from the caves we got quite fewer numbers of fungi from the Dandak caves. It is because most of the earlier reports are from such caves which are either under high anthropogenic pressure or serving as big day-roosting sites for the bats. In both the conditions, the sterile environment of the caves gets contaminated due to the continued interference of the external/foreign resources. Fortunately, the Dandak cave has been tried to maintain an almost anthropogenic pressure free by the Forest Department and the internal architect of the cave is also not too favourable for holding bat colonies thus it could be suggested that the air current and the water sources (during rain) are only the major sources of bringing spores and its dispersal inside the cave. Further, periodic flushing of the cave sediments from rainwater during monsoon could be the possible reason of occurring the fewer numbers of micro-fungi during the rainy season.

Our study strengthened the earlier reports that some species of *Aspergillus* and *Penicillium* are major component participates in wood decaying even inside the cave. However, the *Aspergillus* is not found to be dominant in the inner chamber of the cave which is indeed highly isolated from the external environmental conditions and where proper air/ oxygen supply is also very bleak (Fig.-3). In spite of it, various other taxa also reported from both the chambers from the decaying wood flinders, but their role is still to be understood. Further, the roles of limited cave bats and some cave-inhabiting arthropods as the carrier and host for various species of fungi (Biswas & Shrotriya, 2011) could not be denied (Vanderwolf *et al.*, 2013).

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