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Incidence of dermatophytes and other keratinophilic fungi in the soil of Shivpuri in India

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Introduction

Keratinophilic fungi include a variety of filamentous fungi, mainly comprising hyphomycetes and several other taxonomic groups. Hyphomycetes include dermatophytes and a great variety of nondermatophytic filamentous fungi. Keratinophilic fungi are an ecologically important group of fungi that grow on keratinous substances. They are generally considered as soil saprophytes (Ajello, 1953, 1956). The species of the keratinophilic fungal group have been divided into three categories according to their natural habitats: Anthropophilic, when human beings are the natural hosts; Zoophilic, when a variety of animals act as natural hosts; Geophilic, when the soil is the natural habitat. Keratinolytic fungi occur in many natural and man-made habitats. Keratinophilic fungi are present in the environment with variable distribution patterns that depend on different factors, such as human or animal presence, which are of fundamental importance. Dermatophytes are the most important microorganisms. Several studies have shown that soil is a rich place where the keratinophilic and other related fungi are most conducive to the degradation of keratin substrates or substances (Hainer, 2003; Papini et al., 1898). The potentially pathogenic keratinophilic fungi and allied geophilic dermatophyte species are widespread throughout the world, and their distribution seems largely to depend on the amount of keratinous material deposited by humans and animals, (Dey and Kakoti, 1955; Balajee, et al., 1997; Ulfig, et al., 1998; Sharma, et al., 1997; Hasegawa, 1997; Ali-Shtayeh & Arda, 1989; Ali-Shtayeh & Asad Al-Sheikh, 1988; Mercantini, et al., 1986). Soil that is rich in keratinous material is most conducive to the growth and occurrence of keratinophilic fungi.

The distribution of keratinophilic fungi in Indian soils was given by various workers (Randhawa and Sandhu, 1965; Garg, 1966; Kushwaha and Agrawal, 1976; Jain and Agrawal, 1977; Sur & Ghosh, 1980; Verma et al., 1982; Deshmukh and Agrawal, 1983; Deshmukh and Agrawal, 1983; Jain, 1983; Singh and Agrawal, 1983; Deshmukh, 1985; Nigam and Kushwaha, 1990; Dixit, 1991; Jain et al., 1993;

Vanbreuseghem, 1952). Screening of various soil samples of different habitats, such as Schools/college, gardens, farmhouse, Gardens, roadsides, hostels, animal habitats, bird habitats, marriage gardens, temples, vegetable markets, and house dust, was previously carried out by Jain and Sharma (2009) for the presence of dermatophytes and related fungi. According to them, roadside and garden soils were found to be the most suitable sites for almost all keratinophilic fungi.

Shivpuri is one of the districts of Madhya Pradesh, located in the northern part of the state, has specialized geographical locations, climate, and census, and is famous for being rich in biodiversity & National Parks. The district mostly consists of small hills and forests. Shivpuri is a good candidate for this study because it has a subtropical climate and geographic diversity, leading to an area suitable for the wise distribution of these fungi. Hygienic and ecological interests also have led us to study the keratinophilic mycoflora. The present paper reports the prevalence of dermatophytes and related keratinophilic fungi in the various locations of Shivpuri.

Materials and methods

Shivpuri district is located in the central Indian state of Madhya Pradesh, India, and lies between 25° 43’ and 27° 52’ North latitude and 77° 65’ and 76° 50’ East longitude. It has an encompassing area of 10,278 km2 (3,968 sq mi) (Figure 1). A total of 60 soil samples were collected from Agriculture Fields, Public Parks, School/ College Campuses, and National Park from different parts of the Shivpuri district at randomly selected sites. Approximately 100 g of surface soil (depth not exceeding 2–3 cm) was collected with the help of a sterilized spoon in pre-sterilized plastic bags (10 x 20 cm). Each plastic bag was labeled, indicating the date and site of collection. These samples were then tightly closed to maintain the original moisture and kept in a culture room at a temperature of 28 ± 2°C till needed. The ‘hair bait technique’ (Vanbreuseghem, 1952) was used to isolate the keratinophilic fungi. Sterile Petri dishes were half-filled (approximately 50 gm) with soil samples and moistened with sterilized distilled water. Bits of sterilized human hair and nails were used as baits. The hair and nails were scattered uniformly only on wet soil. These dishes were incubated at 28 ± 2°C temperature and examined periodically for fungal growth and then daily until 4 weeks had passed. When growth was observed using a stereo-scopic binocular microscope, it was cultured on Sabouraud's dextrose agar medium supplemented with chloramphenicol (50 mg L-1) and cycloheximide (500 mg L-1) Hi Media. The colonies obtained were periodically sub-cultured onto slopes of Sabouraud’s dextrose agar in test tubes for maintenance. The count of each species is expressed as the frequency, and not the density of isolation at each site, i.e. each species is counted as one in every soil sample in which it occurred, even if recovered several times.

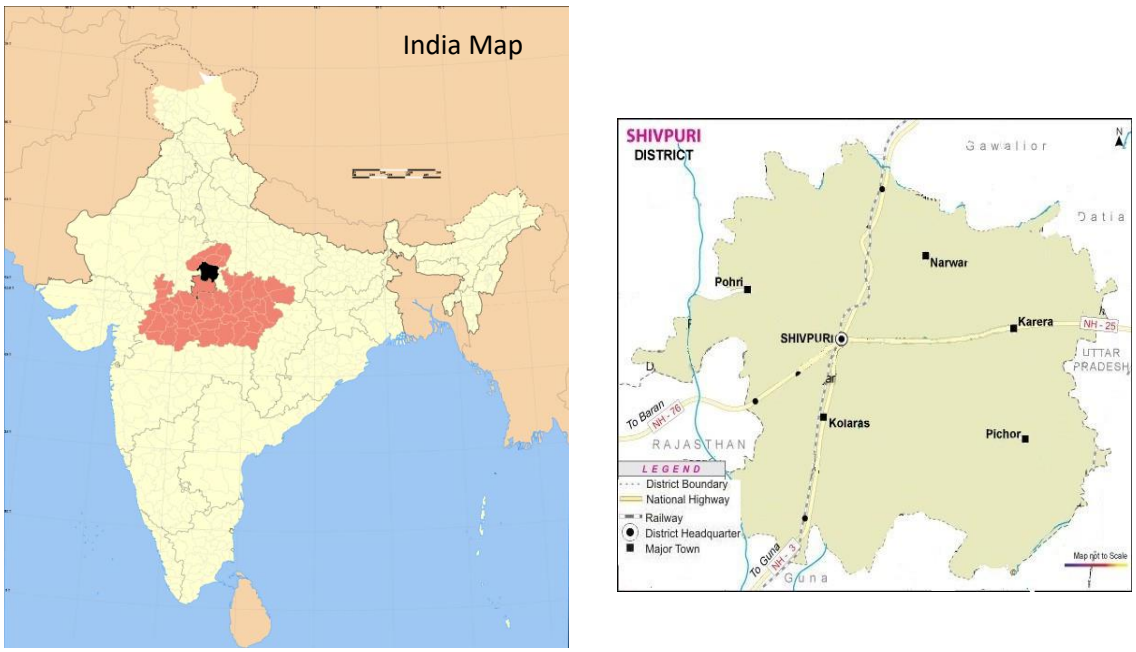

Figure-1 Location of the study area

Distribution of Keratinophilic fungi in different types of soil from Shivpuri district

Districts →	Shivpuri									
Source of sample →	**Agriculture Field**		**Public Park**		**School/College Camp.**		**National Park**		**Total**	**Distribution %**
No of the Samples examined	**18**		**18**		**12**		**12**		**60**	
No of the Samples positive	**9**		**16**		**9**		**8**		**42**	
Frequency %	**50%**		**89%**		**75%**		**67%**		**70%**	
Fungi recorded	n	%	n	%	N	%	n	%		
Alternaria alternate	**0**	0%	**2**	11%	**0**	0%	**0**	0%	**2**	**3**
Aspergillus fumigatus	**0**	0%	**3**	17%	**0**	0%	**0**	0%	**3**	**5**
Aspergillus niger	**2**	11%	**0**	0%	**0**	0%	**0**	0%	**2**	**3**
Chrysosporium indicum	**1**	6%	**2**	11%	**1**	8%	**1**	8%	**5**	**8**
Chrysosporium keratinophilum	**1**	6%	**1**	6%	**2**	17%	**0**	0%	**4**	**7**
Chrysosporium sp.	**1**	6%	**1**	6%	**0**	0%	**1**	8%	**3**	**5**
Chrysosporium tropicum	**1**	6%	**2**	11%	**5**	42%	**0**	0%	**8**	**13**
Chrysosporium state of Ctenomyces serratus	**1**	6%	**0**	0%	**0**	0%	**0**	0%	**1**	**2**
Microsporum audouinii	**0**	0%	**1**	6%	**0**	0%	**0**	0%	**1**	**2**
Microsporum canis	**1**	6%	**0**	0%	**0**	0%	**0**	0%	**1**	**2**
Microsporum gypseum	**0**	0%	**3**	17%	**1**	8%	**3**	25 %	**7**	**12**
Penecillium sp.	**1**	6%	**0**	0%	**0**	0%	**0**	0%	**1**	**2**
Trichophyton equinum	**0**	0%	**1**	6%	**0**	0%	**0**	0%	**1**	**2**
Trichophyton mentragrophytes	**0**	0%	**0**	0%	**0**	0%	**3**	25 %	**3**	**5**
Total	**9**	**50%**	**16**	**89%**	**9**	**75%**	**8**	**67 %**	**42**	**70**

Table: 2

in-vitro occurrence of keratinophilic fungi on keratin baits buried in the soil sample

Organism	*Alternaria alternate*	*Aspergillus fumigatus*	*Aspergillus niger*	*Chrysosporium indicum*	*Chrysosporium keratinophilum*	*Chrysosporium sp.*	*Chrysosporium tropicum*	*Chrysosporium state of Ctenomyces serratus*	*Microsporum audouinii*	*Microsporum canis*	*Microsporum gypseum*	*Penecillium Sp.*	*Trichophytom equinum*	*Trichophytom mentragrophyte*
keratin baits	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
Human Hair	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Human Nails				+	+		+	+		+	+			+

Results and discussion

A total of 42 isolates were recovered out of 60 analyzed soil samples using two human baits (hair and nails) for the presence of dermatophytes and related keratinophilic fungi. Table-1 shows the species of keratinophilic fungi isolated and the total number and frequency of their occurrence in different sites of agricultural fields, public parks, school/ college campuses, and national parks of the Shivpuri district. A total of 70 % of soil samples were found positive. The maximum number of isolates recovered from soil samples of public parks (89%). Next to public parks, school/college campuses (75%), national parks (67%), and 50% of keratinophilic fungi were isolated from soil samples of agricultural fields in Shivpuri district (M.P.). Similar results were obtained by Chemel *et al.*, (1972), who reported that the soil of gardens is a rich source of keratinophilic fungi due to their high organic debris. The maximum number of keratinophilic fungi in the soil of public parks and school/ college campuses may be attributed to the excessive presence of hair (keratin) in the soil of these sites.

42 isolates belonging to six genera with 14 species were recorded in this investigation. These were grouped into two categories, dermatophytes and non-dermatophytes. In the study, some of the soil samples yielded a single species and some samples yielded a mixed growth of two or more than two species of keratinophilic and dermatophytic fungi. In the mixed growth, *Chrysosporium tropicum*, *C. indicum* and *Trichophyton mentagrophytes* were observed more frequently.

The frequency of occurrence of these fungi in different soil samples was noticed as *Chrysosporium tropicum* was the most frequently isolated species and was recovered from 13% of the soil samples with the highest occurrence on the school/ college campus. *Micosporium gypseum*, a well-known geophilic dermatophyte, was recovered (12%) and was the next most frequently isolated species, followed by *Chrysosporium indicum* (8%), it was present in all sampling sites and *Chrysosporium keratinophilum* (7%). The other *Chrysosporium species* (5%) and *Chrysosporium state of Ctenomyces serratus* account for 2% and

are reported from diverse regions in India. (Garg, 1966; Randhawa and Sandhu,1965; Deshmukh and Agrawal, 1983; Deshmukh et al., 2000; Roy et al., 1972; Deshmukh, 1985; Ramesh and Hilda, 1998) reported *C. keratinophilum* from several regions of India. A total of 8 strains of *Chrysosporium tropicum* were isolated. Out of five strains were isolated from the soil of schools, two were from the soil of public parks, and one was from agricultural land. Overall, in the study of different public parks, they had the highest levels of keratinophilic fungi (89%), followed by school gardens and playgrounds (75%). *Chrysosporium indicum* dominates the Indian soil flora because it is adapted to warmer conditions in India. *Microsporum gypseum* has been reported to be in higher numbers than *Chrysosporium indicum* in other Indian places by various workers (Deshmukh et al., 2000). *Chrysosporium species* were also earlier reported from Indian soils (Deshmukh, 2004; Deshmukh and Agrawal, 1983; Kushwaha and Agrawal, 1976; Randhawa and Sandhu 1965; Sur and Ghosh, 1980). The other prominent species isolated was *Trichophyton mentagrophytes* (5%). It was also reported from the costal habitat of Goa (Deshmukh and Agrawal, 1983) and it has been found in many Indian parks.

In addition to well-known dermatophytes, were *Trichophyton equinum* (2%), and other species of the genus *Microsporium* which were found in less abundance, *M. canis* (2%) and *M. gypsium* (2%). Garg, (1966) also reported these fungi from Jaipur and Mount Abu. The Microsporum canis was isolated from Mysore by Deshmukh et al., (2000). These pathogenic isolates were observed in school and college playground soils but higher in college playgrounds. Hair fragments are highly utilized by this fungus. The other species of saprophytic fungi, genus *Aspergillus fumigatus* (5%), *A. niger* (2%), *Alternaria alterneta* (2%) were isolated in the present study which, in support of previous findings by Khanam and Jain (2002) from Damoh (MP, India).

Organic matter contents of soil are well known to support the transient or ongoing existence of keratinophilic fungi in soil and potential sources of infection for humans and animals (Chmel et al., 1972). The different keratinous residues present in the soil or dust are favorable for the growth of these fungi (Sharma et al., 1997; Hasegawa, 1997; Mercantini et al., 1986; Ali-Shtayeh, et al., 1988; Ali-Shtayeh, et al., 1989; Ranganathan, et al., 1998.; Fujihiro, 1997). The distribution and abundance of keratinophilic fungi concerning the presence of organic materials in soil and different soil factors have been well studied (Balajee et al.1997; Ulfig, 1998; Sharma et al., 1997, Hasegawa, 1997; Mercantini et al., 1986; Ali-Shtayeh, et al., 1988 Ali-Shtayeh, et al., 1989). It is also recognized that the distribution of keratinophilic fungi varies in the soil of different locations and the dominant species in any soil or dust may change with time (Balajee et al., 1997; Ulfig, 1998; Sharma et al.,1997; Hasegawa, 1997; Mercantini et al., 1986; Ali-Shtayeh et al., 1988; Ali-Shtayeh, et al., 1989; Ranganathan, et al.,1998, Filipello at el., 1991. Tuck Soon, 1991; Al-Musallam, 1989; Abdel-Fathah et al., 1982; Calvo et al., Feuermann et al., 1975).

The data in Table 2 reveals the growth of different keratinophilic and related dermatophytes on different baits. Hair fragments were found most suitable for the growth of fungi compared to nail baits. This differential degradation of the keratin substrates can be attributed to the hardness of the keratin source. The keratin present in hair bits is comparatively less hard and can be easily broken down by the extracellular enzymes of the fungi (Sharma and Sharma, 2010).

The isolation of keratinophilic from different sites (Table 1) is not uniform. This could be due to differences in the organic matter of the soil. The data obtained from this study adds information on the flora of keratinophilic fungi of Shivpuri district of Madhya Pradesh state, a part of central India. The findings of this study reveal that the keratinophilic flora of this region is different from that in other parts of India. This may be due to the high temperature in summer, humidity, and temperate climatic conditions prevailing in this area.

Thus, the present study reveals the presence of important and common dermatophytes and keratinophilic fungi in Shivpuri, which are significant from central India and their ecological and clinical point of view. Given the present study that the environment of elementary schools and public parks is contaminated with potentially pathogenic keratinophilic fungi, more hygienic attention should be paid to these places to protect this susceptible group of children from fungal infections.

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