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Incidence of keratinophilic fungi in areas of Raipur City, Chhattisgarh region, India

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In an endeavor to search for some wild isolates of keratinophilic fungi from the Raipur City of Chhattisgarh, 24 soil samples from different regions of the city were collected and screened using hair baiting technique. A total of six species which belong to two genera were isolated. They are *Chrysosporium keratinophilum* (27.78%), *Chrysosporium pannicola* (5.56%), *Chrysosporium tropicum* (13.89%), *Chrysosporium* sp. (close to *Chrysosporium pannicola*) (11.11%), *Chrysosporium* sp. (16.67%) and *Microsporum gypseum* (25%). The *Chrysosporium* species prevalent in this region are able to tolerate temperature of about 40°C and all isolates belong to moderate individual risk group.

Key words: Keratinophilic fungi, Raipur, *Chrysosporium*, *Microsporum*.

INTRODUCTION

Soil is the main reservoir of different types of fungi and some of them are pathogenic to both humans and animals. Soil is also a good source of keratinophilic fungi and the probability of the incidence of such fungi increases manifold if it is rich in keratinous materials (Marchisio, 2000). Keratinophilic fungi, also known as dermatophytes, are mostly pathogens of humans and other animals but also have the ability to live saprotrophically. Every keratinophilic fungus has the potential to cause infection and tissue invasions (Simpanya, 2000).

In recent past, many investigations have been carried out to find the distribution pattern of the keratinophilic fungi in many parts of India (Jain and Sharma, 2011; Singh et al., 2009; Sharma et al., 2008; Anbu et al., 2004; Deshmukh, 2002, 2004; Ghosh and Bhatt, 2000; Kaul and Sumbali, 1997). Barring a few reports, this area of Chhattisgarh remained unexplored for the occurrence of keratinophilic fungi (Khanam et al., 2002). Hence, it was felt significant to screen soil of different areas of Raipur City, in order to evaluate the extent and presence of keratinophilic fungi in the environments. The study was undertaken at two potential sites viz. poultry farm and hair-dumping/garbage area and the results obtained are reported.

MATERIALS AND METHODS

Soil samples were collected from the superficial layer at a depth of 3-6 cm of four poultry farms and six hair-dumping/garbage sites from different areas in and around Raipur city. The soil samples were placed in sterile polythene bags, brought to the laboratory and stored overnight at 4°C. Approximately 18 to 20 g of soil from each sample were placed in 90 mm sterile Petri plates in five replicates. Short (1-2 cm length) sterilized defatted human hair fragments were scattered on the surface of soil for baiting. The plates were moistened with an antibiotic solution containing cycloheximide (0.5 mg/ml) and chloramphenicol (0.05 mg/ml). The plates were incubated at room temperature (26±2°C) for a period of 4 to 6 weeks and remoistened with sterile deionized water periodically. The plates were examined daily under a stereoscopic binocular microscope and if growth was observed then the baits were selected at random from each Petri plate and transferred to plates containing Sabouraud Dextrose Agar (SDA) medium supplemented with cycloheximide (0.5 mg/ml) and chloramphenicol (0.05 mg/ml). The SDA plates were incubated at room temperature (26±2°C) for

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Table 1. Distribution of soil sample examined.

Site	Name	Soil sample type		Positive		Positive (%)
		F	H/G	F	H/G	
I	Nandanvan	3	2	1	1	40
II	Krishak Nagar	4	3	1	1	42.86
III	Sarona	3	3	2	1	33.33
IV	Raipura	3	3	1	1	33.33
Overall total		13	11	5	4	37.5

F- Poultry farm; H/G- hair dumping/garbage site.

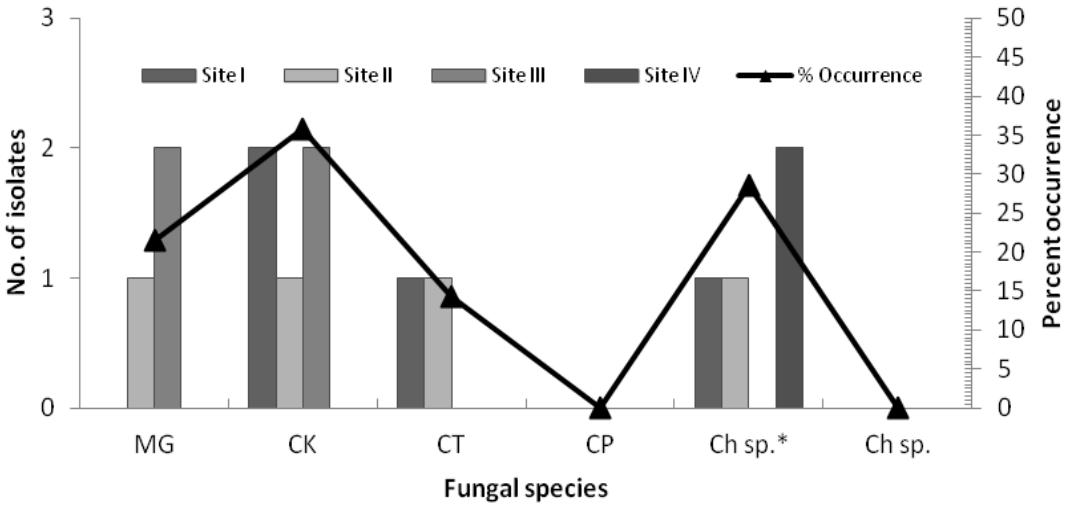


Figure 1. Distribution of fungi in poultry farm soil (MG- *M. gypseum*, CK- *C. keratinophylum*, CT- *C. tropicum*, CP- *C. pannicola,* Ch- *Chrysosporium*).

further examination.

Fresh developed colonies were examined and identified following the key proposed by Chabasse (1988), von Oorschot (1980) and Carmichael (1962). The identification of isolates was further confirmed by NFCCI (Pune) and IMTECH (Chandigarh).

RESULTS

A total of 24 soil samples were examined from poultry farm and hair-dumping/garbage sites. Out of them nine samples (37.5%) were positive for keratinophilic fungi (Table 1).

Distribution of keratinophilic fungi in poultry farm soil

Distribution of keratinophilic fungi in poultry farm soils around Raipur is presented in Figure 1. A total of 14 colonies of keratinophilic fungi were isolated from five soils samples. Four species belonging to two genera were observed in poultry farm soil. Only one dermatophyte, *Microsporum gypseum* (21.43%) was observed and among them closely related species *Chrysosporium keratinophilum, Chrysosporium tropicum* and *Chrysosporium* sp.* (close to *Chrysosporium pannicola*) were present. Occurrence of *C*. *keratinophilum* (35.71%) was highest followed by *Chrysosporium* sp.* (28.57%) and *C. tropicum* (14.29%) (Figure 1). Other than keratinophilic fungi, different species of the fungal genera viz., *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus* were also observed.

Distribution of keratinophilic fungi in hair-dumping/garbage soils

Occurrence of keratinophilic fungi from hair-dumping/-garbage sites is given in Figure 2. Out of four soil samples examined, 22 colonies of keratinophilic fungi were isolated. *Microsporum gypseum* (21.43%) the only dermatophyte and four other closely related species of *Chrysosporium* were isolated. Most commonly observed species was *Chrysosporium* sp. (27.27%) followed by *C.*

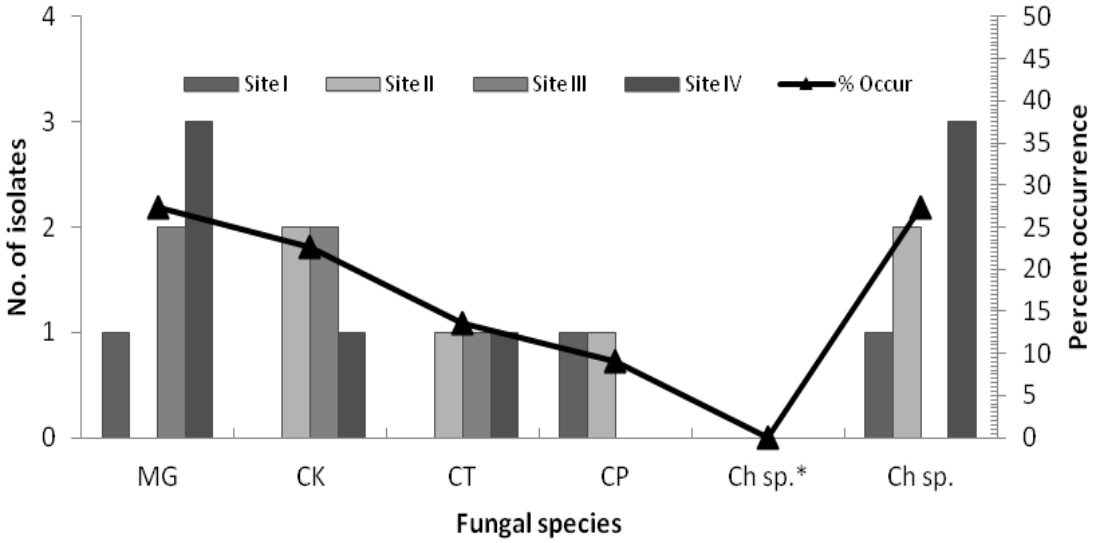


Figure 2. Distribution of fungi in hair dumping/garbage soil.

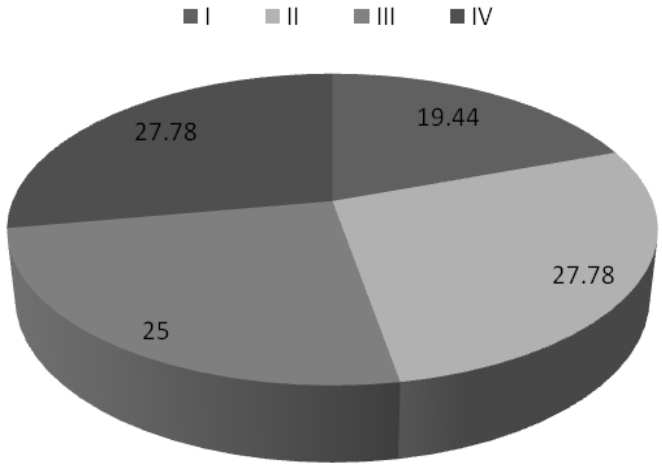


Figure 3. Site-wise percent distribution of keratinophilic fungi.

keratinophilum (22.73%), *C. tropicum* (13.64%) and *C. pannicola* (9.09%) (Figure 2). Other than keratinophilic fungi different species of the fungal genera viz. *Aspergillus*, *Penicillium* and *Fusarium* were observed.

Distribution of keratinophilic fungi at different areas

The percent distribution of keratinophilic fungi in different sites is depicted in Figure 3. Site II (27.78%) and site IV (27.78%) had the highest percentage of keratinophilic fungi, followed by sites III (25%) and I (19.44%), respectively. The species diversity was highest in sites I and II (6 sp./site, each) followed by sites IV (5 sp./site) and III (3 sp./site) respectively. Overall in this region *C. keratinophilum* (27.78%) was the most prevalent species among the areas studied followed by *M. gypseum* (25%), *Chrysosporium* sp. (16.67%), *C. tropicum* (13.89%), *Chrysosporium* sp.* (11.11%) and *C. pannicola* (5.56%) (Figure 4).

DISCUSSION

Keratinophilic fungi are very closely related to dermatophytes, having the ability to cause infection. They parasitize hard keratin tissues and can also invade and degrade them. Thus, they are keratinophilic and keratinolytic in nature. Soils enriched with keratin substrate like hairs, feathers, horn and hoofs, skin etc. are good

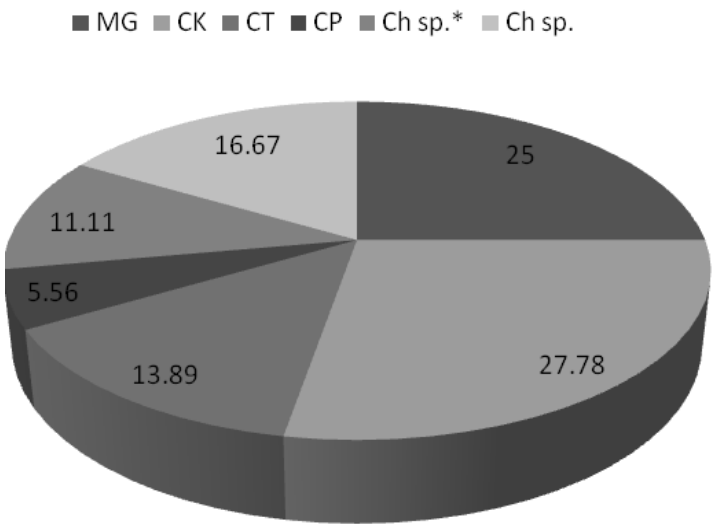


Figure 4. Percent distribution of keratinophilic fungi in areas of Raipur City.

reservoir of dermatophytes as well as keratinophilic fungi (Marchisio, 2000; Ajello, 1974). Dermatophytes are classified into three genera namely *Epidermophyton*, *Microsporum* and *Trichophyton* (Summerbell, 2000). Other than this, closely related species includes 100 genera like *Chrysosporium*, *Malbranchea*, *Geomyces*, *Scopulariopsis*, *Doratomyces* and many others having keratinolytic ability (Blyskal, 2009). Kushwaha (2000) reviewed the presence of 47 species, a few of them with uncertain positions, among the genus *Chrysosporium* throughout the globe.

In the present study, the only dermatophyte isolated was *M. gypseum* (25%), the most common species reported amongst the dermatophytes. It is one of the most prevalent soil borne species and a major causative agent of infection to human and animals (Simpanya, 2000). Among the closely related species the occurrence of *C. keratinophilum* (27.78%), *C. tropicum* (13.89%) and *C. pannicola* (5.56%) were noteworthy in this region. These species are thermotolerant and can grow considerably at 35 to 40°C; the exceptional one is *C. tropicum* which can withstand temperature upto 45°C (Ellis et al., 2007; van Oorschot, 1980). The high occurrence of keratinophilic fungi especially *Chrysosporium* sp. in the soil of Raipur region support the fact that these species are able to withstand the high temperature of this region which is about 45 ± 2°C in summer.

According to World Health Organization (Laboratory Biosafety Manual, 2004) infective microorganisms are classified into four risk groups viz. RG - 1 to 4. It is based on pathogenicity of the organism, modes of transmission and host range of the organism. RG-1 having no or low individual and community risk whereas RG-2 have the moderate individual risk and low community risk. RG-3 shows high individual risk but low community risk and RG-4 have high individual and community risk. Following the classification criteria of WHO, Australia, Canada, European Union (EU), USA and other nations has placed *Microsporum* sp. in RG-2 (American Biological Safety Association, 2013).

Other than *M. gypseum*, all isolated *Chrysosporium* sp. were geophilic and keratinolytic, often recovered from skin and nail scrapings of feet. Some of the *Chrysosporium* sp. closely resembles true deramatophytes like *Trichophyton* and *Microsporum* (van Oorschot, 1980). Based on pathogenicity, *Chrysosporium* sp. has been placed in RG-2 (Table 2) (Ellis et al., 2007).

The prevalence of fungal infections in the region as reported routinely in district hospital and Medical College of Raipur showed that 66.7% infective patients belong to urban areas and 33.3% from rural areas. Superficial fungal infection by various species of *Trichophyton* was reported to be highest (89%) followed by *Microsporum* sp. (11%). The reported disease types most prevalent in this area are tinea corporis (44.5%) followed by tinea pedis (22.3%).

Among other types of tineas, tinea capitis, tinea cruris and tinea manuum have also been reported occasionally in this region (Pradhan, 2012). In any region, tinea corporis and tinea pedis are the most common types of infection and all types of dermatophytes have the potential to cause tinea corporis. Tinea pedis is possibly caused by *Trichophyton rubrum*, *Trichophyton mentagrophytes* var. *interdigitale* and *Epidermophyton floccosum* (Degreef, 2008). Apart from these anthropophilic dermatophytes mentioned above the present study revealed that this part of India has good presence of geophilic and keratinophilic *Chrysosporium* and *Microsporium* species, which could add up to the increase in dermatophytic infection in this region.

Table 2. Species wise characteristics and pathogenicity.

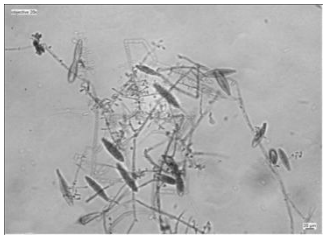
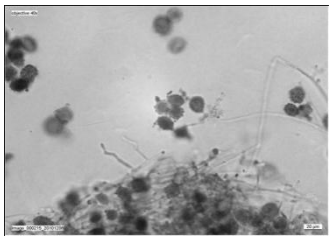
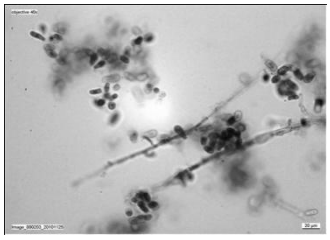
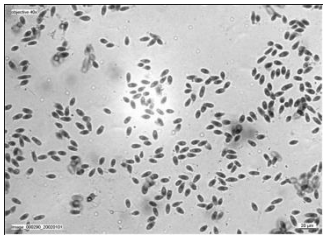
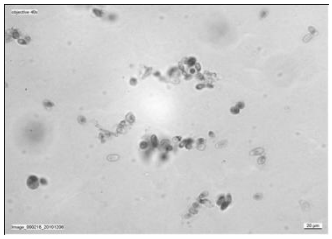
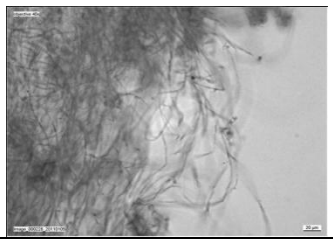
S/N	Species	Brief characteristics & Pathogenicity
1.	*Microsporum gypseum* 	Geophilic; Macroconidia abundant, symmetrical, thin-walled, ellipsoidal, verrucose, four- to six-celled, clavate-shaped Microconidia also present RG-2 organism
2.	*Chrysosporium keratinophilum* 	Geophilic; Conidia numerous, single-celled, smooth-walled or echinate, thick-walled, obovoid to clavate shaped RG-2 organism
3.	*Chrysosporium pannicola* 	Geophilic; Sub-hyaline, single-celled, fairly thick-walled, obovoid to clavate, initially smooth-walled, chinulate at maturity; Chlamydospores absent. RG-2 organism
4.	*Chrysosporium tropicum* 	Geophilic; Numerous conidia, hyaline, single-celled, slightly thick-walled, clavate to pyriform, smooth surface; Macroconidia absent RG-2 organism
5.	*Chrysosporium* sp. (close to *C. pannicola*) 	Geophilic; RG-2 organism

Table 2. Contd.

	Chrysosporium sp.	
6.		Geophilic; RG-2 organism

(Scale - 20 µm), Fungal photographs were taken in Leica DM 1000 microscope with microphotographic attachment.

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