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Full Length Research Paper

Incidence of dermatophytes and other keratinophilic fungi in the schools and college playground soils of Jaipur, India

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A total of 50 soil samples collected from school and college playgrounds soil of Jaipur, India were examined for the prevalence of keratinophilic fungi and related dermatophytes. Two keratinous fragments, human hair and nails were used for the growth of fungi by hair-baiting technique. A total number of 25 species belonging to 16 genera were recovered, 8 of which were common to both the school and college playgrounds soil. Dermatophytes and closely related species were represented by 25 species; of which the following were the most commonly found species in soil: *Chrysosporium tropicum* (26%), *Chrysosporium indicum* (22%), *Trichophyton mentagrophytes* (16%), *Microsporum gypseum* (14%), etc. Some of the isolates of these related and dermatophytes like *Trichophyton verrucosum*, *Trichophyton tonsurans*, *Cladosporium* sp., *Paecilomyces* sp., *Aspergillus nidulans*, *Aspergillus versicolar*, *Alternaria* sp., *Fusarium moniliforme,* etc, were first isolated from playgrounds soil of Jaipur. The selection of certain keratinolytic isolates could become useful in managing polluted soil of playgrounds by students and people. To our knowledge, This appears to be the first report concerning the isolation of *T. tonsurans*, *A. versicolar*, *Cladosporium* sp., *Trichophyton verrucosum*, *Alternaria* sp., *Paecilomyces* sp., *F. moniliforme* and *A. nidulans* as keratinolytic fungi from soil samples of Jaipur (India).

Key words: Playgrounds soil, keratinophilic fungi, dermatophytes, Jaipur.

INTRODUCTION

Keratinophilic fungi are present in the environment with variable distribution patterns that depend on different factors, such as human and or animal presence, which are of fundamental importance. The potentially pathogenic keratinophilic fungi and allied geophilic dermatophytic species are widespread worldwide. 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. Keratinolytic fungi occur in many natural and manmade habitats. These microorganisms exist in communities together with keratinophilic fungi that have weaker affinity to keratin and utilize chiefly the products of its decomposition (Dominik and Majchrowicz, 1964). Most of the latter occur as saprophytes in soil, and some are plant pathogens. Soil is the main environment of fungal occurrence and activity. In the soil environment, the factors influencing keratinolytic fungi have been relatively well recognized (Garg et al., 1985; McAleer, 1980, Piontelli et al. 1990, Vollekova, 1992). Sharma (2010) isolated of soil mycoflora of katao near gangtok, India in low temperature regions. The biological function of keratinolytic fungi in the soil is the degradation of keratinized materials such as hides, furs, claws, nails and horns of dead animals.

Keratinophilic fungi are an ecologically important group of fungi that cycle one of the most abundant and highly stable animal proteins on earth. From India, the first

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report of isolation of keratinophilic fungi *Microsporum* from soil was by Dey and Kakoti (1955) and they isolated this fungus from a skin lesion in rabbit. Keratinophilic fungi are generally considered as soil saprophytes (Ajello, 1953, 1956). Soil that is rich in keratinous material is most conducive for the growth and occurrence of keratinophilic fungi. The species of 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. Dermatophytes are mycelial and keratinophylic fungi of the mold group, originally saprobial, but have adapted themselves to human and animal parasitism through evolution. Dermatophytes are the most important microorganisms, which cause superficial mycosis and the lesions characterized by circular disposition, desquamation, alopecia and erythema of the edges (Lacaz et al., 1991; Sabouraud, 1893). French dermatologists postulated that dermatophytes are primarily soil saprophytes. Soil is a rich place where the keratinophilic and other related fungi are most conducive for degradation of keratin substrates or substances. Papini et al. (1893) reported a survey of dermatophytes and related keratinophilic fungi isolated from soil in a City Park in Pisa, Italy. Screening of 217 soil samples of different habitats, such as PG study centre, garden, farmhouse, nursery, roadside, hostel, animal habitat, bird habitat, marriage garden, temple, vegetable market and house dust, was previously carried out by Jain and Sharma (2009) for the presence of dermatophytes and related fungi in relation to soil pH in Jaipur city. According to them, Roadside and garden soils were found to be the most suitable sites for almost all keratinophilic fungi.

This research reports the prevalence and distribution of geophilic keratinophiles and related dermatophytes in the playgrounds soil of Jaipur (India). The main objective of the research was to determine fungal contamination of school and college playgrounds soil for the first time in Jaipur, in order to promote the knowledge of students and people to observe health regulations to control and prevent fungal diseases.

MATERIALS AND METHODS

Collection of soil samples

Jaipur is one of the 32 districts in Rajasthan, India and lies between 26° 23' and 27° 52' North latitude and 74° 55' and 76° 50' East longitude. It has an encompassing area of 200.4 sq. km (Figure 1). 50 soil samples were collected from school and college playgrounds of Jaipur city in pre-sterilized plastic bags (10 x 20 cm). Surface soil (depth not exceeding 2 - 3 cm) was collected with the help of a sterilized spoon. 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 culture room at a temperature of 28 ± 2°C till needed. A part of the soil sample was used for baiting and for survival studies of the selected fungi.

Baiting of soil samples

Each soil sample was thoroughly homogenized and a sufficient amount of soil was taken in a separate sterilized Petri dishes from each sample. Hair baiting technique (Vanbreuseghem, 1952) was used for isolating the fungi (Figure 2). For that sterilized distilled water was added to provide moisture to the soil. Bits of sterilized human hair and nail were used as baits. The hair and nail were scattered uniformly only on wet soil. Each Petri dishes were separately labeled indicating the date, site of collection and type of bait, etc. Each Petri dishes was incubated at 28 ± 2°C for 3 to 4 weeks in the culture room. Fungal growth, if any, on the hair and nail bait was observed periodically.

Isolation, purification and identification of fungi

The baited cultures were examined after 3 - 4 weeks for the development of any fungal growth on the hair and nail bait. For fungal examination, a small portion of the fungal growth was picked up with the help of a sterilized needle, mounted on a slide under covered glass containing a drop of sterilized distilled water or any other staining solution, and examined under a microscope for the identification of the fungi. After a preliminary examination of fungal growth on baits, the fungus was subsequently transferred to the slants of Sabouraud's dextrose agar (SDA) fortified with chloramphenicol (0.05 mg/ml) and cycloheximide (0.5 mg/ml) to check the bacterial and saprophytic fungal growth, respectively. If mixed growth of fungi occurred, a dilute suspension of the material was transferred on SDA Petri dishes in triplicates. After an incubation of 24 h, the single germinating spores with initial hyphal growth were removed using a sterilized long needle and transferred to fresh slants of SDA medium. In this way, mixed cultures were made pure. Hyphal or spore measurements were recorded using an oculometer in the eyepieces of a microscope. Whenever essential, photomicrographs were also taken to get a clear picture about the microorganism. The measurements, shape, arrangements of spores and other structures were also taken. The color, texture, pigmentation on reverse side of colony and colony characters were recorded for fungal identification.

RESULTS

Out of 50 soil samples examined for school and college playgrounds of Jaipur using two human baits (hair and nails), 44 samples were found positive in fungal growth. A total of 78 isolates of keratinophilic and related fungi distributed in 16 genera and 25 species were isolated (Table 1). 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, *C. tropicum*, *C. indicum* and *T. mentagrophytes* were observed more frequently. It appears that, some keratinophilic fungi namely *Aspergillus nidulans*, *A. versicolar*, *Alternaria* sp., *Penicillum* sp., *Paecilomyces* sp., *Cladosporium* sp., *Scopulariopsis* sp. and *Fusarium moniliforme* reported in this study were isolated for the first time from the soil of Jaipur, India. Regarding the dermatophytes, two isolates viz. *T. verrucosum* and *T. tonsurans* were also isolated for the first time from the soil.

C. tropicum (26%) was the dominant ($P < 0.05$) and

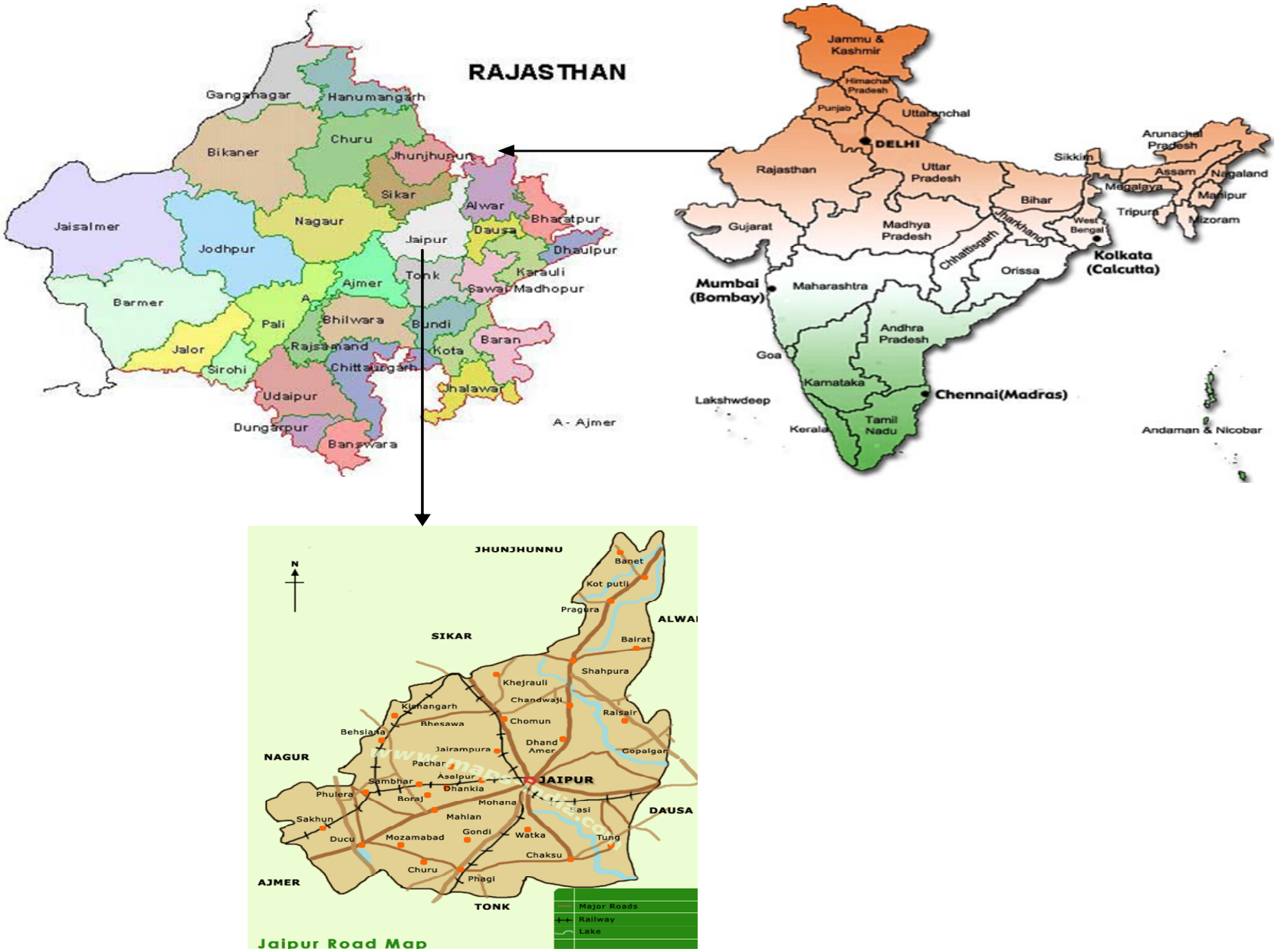

Figure 1. Location of the study area.

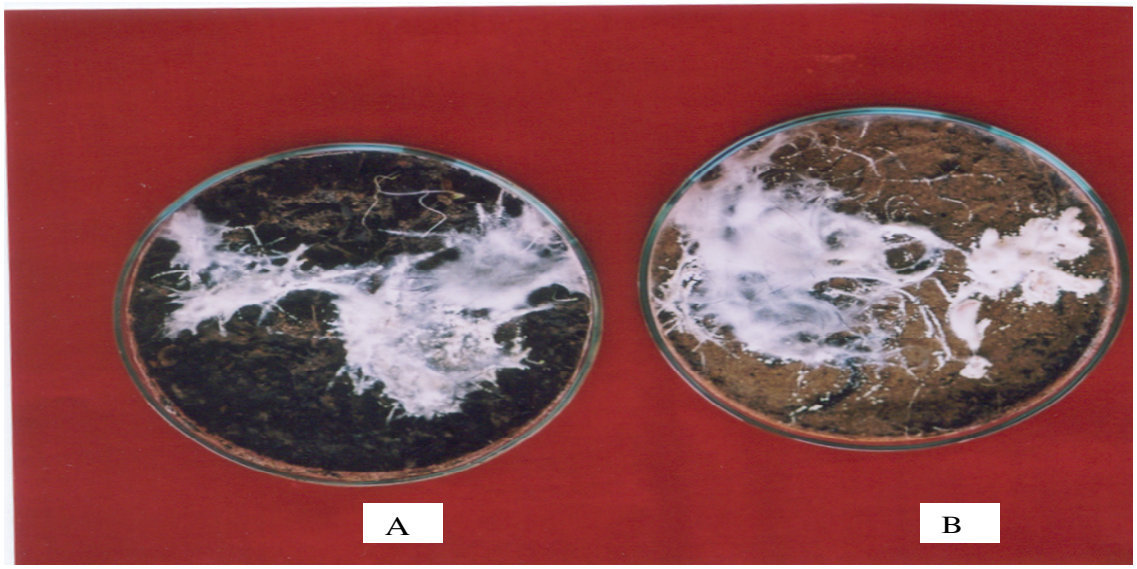

Figure 2. Hair baiting technique (Vanbreuseghem, 1952). A; Growth of fungus on hair baits, B; growth of fungus on hair and nail baits.

Table 1. Incidence and prevalence of keratinophilic fungi and related dermatophytes in school and college playgrounds soil of Jaipur, India.

Soil habitat (Playground)	Soil isolate of fungi																									
	C. tropicum	*C. indicum*	*T. rubrum*	*T. mentagrophytes*	*T. tonsurans*	*T. verrucosum*	*T. simii*	*T. terrestre*	*H. capsulatum*	*F. oxysporum*	*F. moniliforme*	*A. fumigatus*	*A. nidulans*	*A. versicolar*	*C. lunata*	*D. tetramera*	*Torula sp.*	*Penicillium sp.*	*Alternaria sp.*	*Cladosporium sp.*	*Paecilomyces sp.*	*Scopulariopsis sp.*	*G. reessii*	*M. gypseum*	Rhizopus sp.	Positive samples	Total
School	8 (32%)	7 (28%)	1 (4%)	3 (12%)	-	1 (4%)	1 (4%)	1 (4%)	1 (4%)	-	1 (4%)	2 (8%)	2 (8%)	1 (4%)	1 (4%)	-	1 (4%)	1 (4%)	-	-	-	2 (8%)	2 (8%)	5 (20%)	1 (4%)	23 (92%)	25
College	5 (20%)	4 (16%)	-	5 (20%)	1 (4%)	1 (4%)	-	3 (12%)	1 (4%)	2 (8%)	-	-	1 (4%)	1 (4%)	-	1 (4%)	-	2 (8%)	2 (8%)	2 (8%)	1 (4%)	2 (8%)	-	2 (8%)	-	21 (84%)	25
Total	13 (26%)	11 (22%)	1 (2%)	8 (16%)	1 (2%)	2 (4%)	1 (2%)	4 (8%)	2 (4%)	2 (4%)	1 (2%)	2 (4%)	3 (6%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)	3 (6%)	2 (4%)	2 (4%)	1 (2%)	4 (8%)	2 (4%)	7 (14%)	1 (2%)	44 (88%)	50

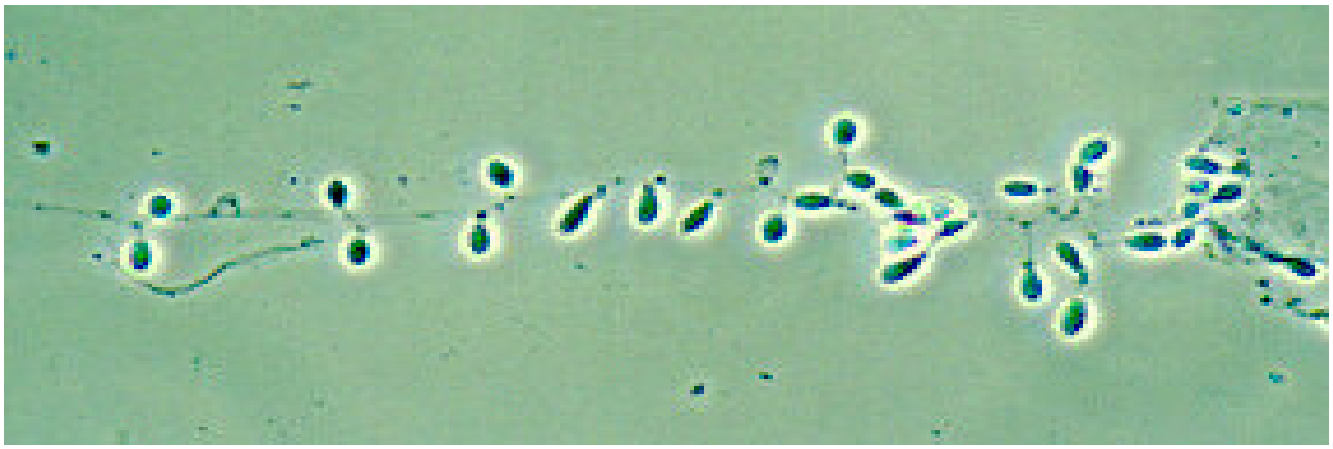

Figure 3. Pyriform to clavate shaped conidia of *Chrysosporium tropicum.*

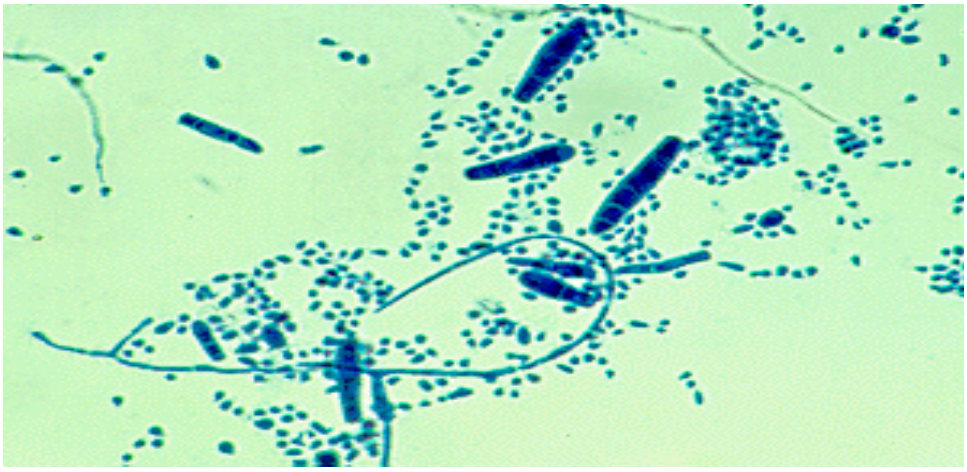

Figure 4. Macro and micro conidia of *Trichophyton mentagrophytes.*

most frequently isolated species of fungi (Figure 3). School playgrounds were found most suitable for its occurrence. *C. indicum* (22%) was the second most common fungus isolated from the playgrounds. Maximum isolates of *C. indicum* (28%) were recovered from school playgrounds. Nail baits were found to be more suitable for its growth.

Among the dermatophytic isolates, *T. mentagrophytes* (16%) was the most common species (Figure 4) followed by pathogenic *Microsporum gypseum* (14%). Both pathogenic isolates were observed in school and college playground soils but higher in college playgrounds. Hair fragments were highly utilized by this fungus. The other commonest observed species of dermatophytic and keratinophilic fungi were (in decreasing rank): *Trichophyton* terrestre> *Scopulariopsis* sp.>*A. nidulans*>*Penicillium* sp.

The data in Table 2 reveals the growth of different keratinophilic and related dermatophytes on different baits. Hair fragments were found most suitable for 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 extra cellular enzymes of the fungi.

DISCUSSION

Keratinophilic fungi are important ecologically and recently have attracted attention throughout the world. They play a significant role in the natural degradation of keratinized residues (Sharma and Rajak, 2003; Fillipello et al., 1994; Fillipello, 2000), have many properties in common with dermatophytes and some can probably cause human and animal infections (Connole, 1990; Ali-shatayeh et al., 1989; Filipello et al., 1996; Spiewak and Szostak, 2000; Spiewak, 1998; Restrepo and Deuribe, 1976; Cano et al., 1991). In recent years, the distribution of keratinophilic fungi and related dermatophytes in

Table 2. Keratinophilic and dermatophytic fungi on different baits.

Habitat (Playground)	Total number of sample	Growth on hair	Growth on nail	Growth on both bait	Total +ve samples	Percentage	Positive sample
School	25	9	5	9	23	92	2
College	25	10	3	8	21	84	4
Total	50	19	8	17	44	88	6

Indian soils were given by many workers (Randhawa and Sandhu, 1965; Garg, 1966; Roy et al., 1972; Deshmukh and Agrawal, 1983; Deshmukh, 1999; Deshmukh and Agrawal, 2000). However, there was no evidence of any study on mycoflora from playgrounds with particular interest on school and college ground soil. Therefore, this investigation was carried out for the detection of keratinophilic fungi from soil of 50 different school and college playgrounds. The use of these playgrounds by students may introduce keratinous danger. These keratinous wastes may serve as substrates for keratinophilic fungi in the soil of playgrounds. The most frequently isolated keratinophilic fungus in this study was *C. tropicum* (26%) followed by *C. indicum* (22%).

Similar results were observed by Ghosh and Biswas (1995) during the survey of Bharatpur Bird Sanctuary soil in Rajasthan and by Jain and Sharma (2009) during the survey of different habitats soil in Jaipur city, with particular reference to soil pH. Ramesh and Hilda (1999) also reported the same result during the survey of keratinophilic fungi of primary schools and public parks. Out of 45 soil samples studied, they reported 31 species belonging to 15 genera in which the incidence of *C. tropicum* was 62.2%. Rajak et al. (1991) reported *C. indicum* in the ground of a gelatin factory in Jabalpur. *T. mentagrophytes* and *M. gypseum* are dermatophytes in which *T. mentagrophytes* has been recorded as a human pathogen (Latkowski and Freedberg, 1999), frequently isolated from soil and *M. gypseum* is a common geophilic dermatophyte widely distributed in soils globally (Ali-Shtayeh and Jamous, 2000). *M. gypseum* causes ringworm of the scalp and glabrous skin in human and animals (Ali-Shtayeh and Jamous, 2000). In this study, both were isolated from playgrounds soil in third and fourth order of prevalence respectively. Marsella and Mercantini (1986) isolated *Microsporum* sp. in four (2.5%) samples out of 161 soil samples from different sites of the Abruzzo National Park, in Italy. Shome and Shome, (1965) Thammayya et al. (1967) and Knudtson and Robertstad (1970) previously recorded *T. terrestre* and *T. mentagrophytes* from Indian soil. In this study, *T. terrestrii* and *Scopulariopsis* sp. both showed same prevalence in college and school playgrounds soil. Likewise, *A. nidulans* and *Penicillium* sp. also showed same distribution in the studied soil samples. *A. nidulans* is a typical soil fungus with a worldwide distribution. Reis et al. (2003) isolated this species from soil of Poland, Brazil and studied on Xylanase production. Mercantini et al. (1993) isolated the *Scopulariopsis brevicaulis* and *Scopulariopsis* sp. from the dust of Antarctic. Kathiresan and Manivannan (2006) isolated *Penicillium fellutanum* from mangrove rhizosphere soil. *T. verrucosum*, *H. capsulatum*, *F. oxysporum*, *A. fumigatus*, *Alternaria* sp., *Cladosporium* sp. and *Gymnoascus reessii* reported equal frequency of order from playgrounds soil. Simpanya and Baxter (1996) isolated *Fusarium* sp. with the help of keratin bait technique from parks, cleared areas, paddocks, rivers and roadside soil samples. The variability of strains of *G. reessii* was investigated thoroughly by Orr et al. (1963) and Orr and Kuehn (1972). Kumari et al. (1998) isolated three species of *Aspergillus* and *Torula graminis* in association with bird feathers from the soil samples collected from Gaya (Bihar). Simpanya and Baxter (1996) recorded that *Paecilomyces* and *Fusarium* species were the most frequently isolated potential pathogens from soils collected in parks, cleared areas, paddocks, river and roadsides of New Zealand.

Emmons (1949) was the first to isolate *H. capsulatum* from the soil and bat Guano in the United States of America. Sanyal and Thammayya (1975) isolated *H. capsulatum* from the Gigantic Plain using the hair bait technique. Kumari et al. (1998) observed *H. capsulatum* in the soil of cremation grounds in Gaya (Bihar). In this investigation, *Trichophyton simii*, *T. tonsurans*, *T. rubrum*, *F. moniliforme, Curvularia lunata*, *A. versicolar*, *Drechslera tetramera*, *Torula* sp. *Paecilomyces* sp. and *Rhizopus* sp. were isolated from the soil in lesser numbers. Gugnani et al. (1967) had earlier reported *T. simii* from soil in India. Ogbonna and Pugh (1987) isolated *C. lunata* during a survey of keratinophilic fungi in Nigerian soil. Kumari et al. (1998) isolated *C. lunata* from soil on bird feather bait while *T. graminis* was observed in association with human nail, domestic cattle hair and bird feather baits. Larrando and Calvo (1989) isolated keratinophilic genera of *Penicillum*, *Cladosporium*, *Aspergillus*,

Acremonium, *Alternaria* and *Fusarium* from the sands of the Mediterranean coast. It can be concluded that the selection of certain keratinolytic isolates could become useful in managing polluted soil of playgrounds by students and people. Finally, this appears to be the first report concerning isolation of *T. tonsurans*, *A. versicolar*, *Cladosporium* sp., *T. verrucosum*, *Alternaria* sp., *Paecilomyces* sp., *F. moniliforme* and *A. nidulans* as keratinolytic fungi from soil samples of Jaipur (India).

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