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PREVALENCE OF DERMATOPHYTES AND OTHER KERATINOPHILIC FUNGI FROM SOILS OF PUBLIC PARKS AND PLAYGROUNDS OF RIYADH, SAUDI ARABIA

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ABSTRACT

In order to determine the incidence of dermatophytes and related keratinophilic fungi present in Riyadh soil, eighty soil samples were collected .These samples were screened using hair baiting techniques for isolation. Out of a total 80 samples, 69 (86%) were positive for growth of keratinophilic fungi. Eleven genera and 19 species were isolated and identified, of which *Chrysosporium indicum* (33.75%) was the most predominant species isolated followed by *C.tropicum* (26.25%), *Aspergillus flavus* (17.50%), *Microsporum gypseum* (13.75%) and *Trichophyton terrestre* (11.25%). Garden soils, followed by playground soils were found to be the most suitable for fungal growth. Some of the other fungi isolated were *C.zonatum , Alternaria alternata*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium moniliforme,F.solani ,Aphanoascus fulvescens* etc. To our knowledge, this appears to be the first report concerning the isolation of keratinolytic fungi *M. canis* and *Trichophyton terrestre* from soils of Riyadh by hair baiting technique

Key words: Playground, Garden, Keratinophilic fungi, Dermatophytes, Riyadh soils, Saudi Arabia.

INTRODUCTION

Keratinophilic fungi are important ecologically and play a significant role in natural degradation of keratinous substrates. Soils, that is rich in keratinous materials are most conducive for the growth and occurrence of keratinophilic fungi. These are a group of fungi that colonize various keratinous substrate and degrade them .Their distribution is variable with the environment and depends on different factors, such as human and or animal presence .Keratinous substances which are important natural material, occurring in nature mainly in the form of hairs, wools, feathers, horns, hooves, nails, skin and other cornified appendages constitutes natural baits for these fungi. (Khanam and Jain, 2002).

Several researchers have studied soil mycoflora with respect to keratinophilic fungi in the past few decades. (Garg *et al.,* (1985); Al – Musallam (1988); Soon (1991); Augt *et al.*, (1995); Ulfig and Korcz (1995); Caglar and Akgun (2006); Deshmukh *et al.*,(2008) and Min Jung Lee *et al.*,(2011).

Keratinophilic fungi include a variety of filamentous fungi, mainly comprising hyphomycetes and several other taxonomic groups. Hyphomycetes include dermatophytes and a great variety of non dermatophytic filamentous fungi.(Mukesh and Sharma, 2010).The majority of dermatophytes can live saprophytically and every keratinophilic fungi can be considered as a potential pathogen. Dermatophytes cause human and animal mycoses and thus have drawn the attention of medical and veterinary epidemiologists. (Marsella and Mercantini,1986). Keratinolytic fungi are a group of microorganisms that are able to decompose keratin remains in environment and are pathogenic to humans and animals. They occur in many natural and manmade habitats.These fungi 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). Based on their occurrence in natural habitat, kertainophilic fungi are divided into three categories: 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.

Soil serves as a natural reservoir for both pathogenic and saprophytic fungi. Factors influencing the distribution of keratinophilic fungi have been relatively well recognized in the soil environment. (Piontelli *et al*., 1990; Vollekova, 1992 and Neetu and Sharma, 2011). Keratinophilic microbes represent a huge biodiversity of form, habitat and substrates in soil. It is therefore, reasonable to anticipate soil as a huge reservoir of these keratinophilic fungi. Places like play grounds and public parks are often invaded by humans and animals. Soils which are contaminated with keratinaceous debris and mainly propugules of fungal pathogens, thereby cause infections in human beings and animals. Studies on the ecology and epidemiology of human dermatophytoses in the West Bank of Jordan by Ali Shtayeh and Arda (1989); show that about 36% of the dermatophytoses patients were school children in the age group of 6–14. Hence it will be significant to analyze and identify the mycoflora of school playgrounds, public parks and Zoo

in order to evaluate the presence of keratinophilic fungi and dermatophytes in these environments.

This paper, reports on the prevalence of keratinophilic fungi and its related dermatophytes in the soils of Riyadh, Saudi Arabia. This would help us know, the distribution and occurrence of dermatophytes and other keratinophilic fungi; it will also through light on the risk of human dermatophytosis in these regions.

MATERIALS AND METHODS

Collection of soil samples: A total of 80 soil samples were collected from different locations in Riyadh (Public parks, playgrounds, Zoological park- in and around animal and Bird enclosures). Soil samples were collected in sterile polyethene bags by scooping upto a depth of 2-5cms with the help of sterile disposable spoon. Each bag was tightly packed and labeled indicating the site of collection and date .These samples were brought to the laboratory and processed immediately and stored at 4^0C for further studies.

Isolation of keratinophilic fungi: Sterile Petri dishes were half filled with thoroughly homogenized soil samples. Hair baiting technique (Vanbreuseghem, 1952.) was used for isolating the fungi. The half filled soil samples were moistened with sterile distilled water; the amount of water added varied from sample to sample, depending on the moisture content of the sample. It was then baited with short strands of sterilized human hair. Strands of human hair were spread uniformly as baits on the surface of moistened soil samples. The plates were then incubated at 28±2^o C for a period of 3-4 weeks. Five replicates were set for each sample.

Isolation and Identification: The hair baited plates were regularly examined for any fungal growth. Samples of hair showing fungal growth were carefully and aseptically picked up and inoculated on Sabourauds dextrose agar (SDA) supplemented with chloramphenicol (0.05 mg/ml) and cycloheximide (0.5 mg/ml) to check the bacterial and saprophytic fungal growth, respectively. Prior to the inoculation on SDA, the baits were observed microscopically on a clean slide under a covered glass either containing a drop of sterilized distilled water or any other staining solution for any mixed growth. If it exhibited mixed growth, a dilute suspension of the material was transferred on SDA Petri dishes in triplicates. After an incubation of 24 hours, 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. (Mukesh. S and M. Sharma, 2010).These fungi were examined microscopically, identified according to their macro and micro morphological characteristics, following the manuals proposed by Rebell, G. and Taplin, D. (1974);Frey *et al*.,(1979); Van Oorschot (1980); Cano. J, Gurrao, (1990) and with the help of Fungal Culture Bank, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.

RESULTS

A Total of 80 soil samples were screened for the presence of dermatophytes and keratinophilic fungi. Of which, 69 soil samples (86%) were positive for fungal growth. It was observed that all the soil samples collected from garden of Zoo Park were positive followed by public Parks and play grounds. (Table 1).

One hundred and fifty isolates, belonging to 11 genera and 19 species, were isolated from various soils of Riyadh. Some soils samples however yielded mixed growth, while others represented single species. *Chrysosporium indicum*, *C. tropicum, Aspergillus flavus, Microsporum gypseum and Trichophyton terrestre* were isolated frequently. *M. canis and T. terrestre* were isolated for the first time from soils of Riyadh by hair baiting technique. Garden soils, followed by playground soils were found to be the most suitable for fungal growth. *C.indicum* (33.75%) was the most predominant species isolated. *C.tropicum* (26.25%) was the second highest, followed by *Aspergillus flavus* (17.50%).

In the present study, *M. gypseum* (13.75%), was the most common dermatophyte isolated followed by *T.terrestre* (11.25%).

However *M.canis* was isolated only from garden soils of Zoo Park. *C. zonatum, T. mentagrophytes, Alternaria alternata* and *Fusarium oxysporum* were also isolated besides other dermatophytes and keratinophilic fungi listed in (Table 2).Both the playgrounds (66 isolates) and garden soils (61 isolates) were found to be rich reservoirs of keratinophilic fungi and dermatophytes. Birds and animal enclosures (in and around of enclosures) also showed 17 and 6 isolates respectively.

Table 1. Percent prevalence of keratinophilic fungi and dermatophytes isolated from soil samples of various habitats.

Habitat	Zoo				School	Public Park		Total
	Playground	Garden	Birds	Animals	Playground	Garden	Playground	
No of soil samples studied	4	6	9	10	14	18	19	80
No of samples positive	3	6	7	7	13	17	16	69
Percentage of positive samples	75%	100%	78%	70%	93%	94%	84%	86%

Table 2. Frequency occurrence of keratinophilic fungi and dermatophytes from different soils of Riyadh (Saudi Arabai).

Fungi Isolated	Zoo				School	Public Park		Total	% value
	Playground	Garden	Birds	Animals	Playground	Garden	Playground		
Aphanoascus fulvescens	1	3	1	-	-	2	-	6	7.50%
Chrysosporium indicum	2	4	2	-	8	6	5	27	33.75%
C.tropicum	2	-	3	1	6	3	6	21	26.25%
C.zonatum	-	3	-	2	-	2	-	7	8.75%
Microsporum gypseum	1	2	1	1	1	3	2	11	13.75%
M.canis	-	2	-	-	-	-	-	2	2.50%
Trichophyton rubrum	1	1	-	-	-	-	1	3	3.75%
T.mentagrophytes	-	1	2	-	2	1	1	7	8.75%
T.terrestre	1	1	2	1	3	-	1	9	11.25%
Aspergillus niger	-	-	2	-	-	3	-	5	6.25%
A.flavus	1	3	-	-	2	3	5	14	17.50%
A.terreus	-	2	-	-	1	-	-	3	3.75%
Alternaria alternata	1	2	-	-	2	1	1	7	8.75%
Fusarium oxysporum	-	2	-	-	-	3	1	6	7.50%
F.solani	-	2	-	-	1	-	1	4	5.00%
Curvularia lunata	-	1	-	-	1	1	-	3	3.75%
Penicillium chrysogenum	-	1	2	-	-	-	2	5	6.25%
Rhizopus stolonifer	1	-	1	-	1	2	1	6	7.50%
Scopulariopsis brevicaulis	-	-	2	1	-	1	-	4	5.00%

DISCUSSION

The study clearly indicates the varied distribution of keratinophilic fungi and dermatophytes in soils of Riyadh. Keratinophilic fungi are important ecologically and 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. Diverse soil habitats have been screened from different countries e.g-Kuwait Pakistan,Iran, Brazil and India indicating that this group of fungi are distributed worldwide**.(**Al Musallam *et al*.,1995**;**Irshad *et al*., 2007; Fozia. I. *et al*., 2007; Ali and Majid, 2008; Da Silva. and Oliveira, 2008; Itisha and Kushwaha, 2010 and Deshmukh and S. Veerkar, 2012).

It's interesting to note that the maximum number of fungi was recorded from soils of gardens and play grounds. The most frequently isolated keratinophilic fungi in this study was *Chrysosporium indicum, C. tropicum, A flavus, M. gypseum and T. terrestre.* The high prevalence of these fungi from these soils explain that, hair of human and animals and feather from birds which come to the soil either as dead or dropped off , serves as substrates and are subjected to microbial decomposition. Keratinophilic fungi play a significant role in the natural degradation of keratinized residues. (Sharma and Rajak, 2003). Itisha. S and R. K. S Kushwaha (2010), obtained 641 isolates from 125 soil samples of parks from Uttar Pradesh indicating soils of park to be a rich source of many keratinophilic fungi and dermatophytes.

C. tropicum (2.50%) and *C. indicum* (2.50%) has also been reported from soils of Bahrain by Deshmukh *et al.,* (2008).Abdel and Zaki (2008), isolated *C. indicum* from fields,animal and birds enclosures, animal hairs and birds feathers. Ramesh and Hilda (1999) also reported large number of keratinophilic fungi from primary schools and public parks of Madras city indicating these soils to be a reservoir of diverse fungi. In our study *Aspergillus flavus* accounted for the third postion (17.50%).It was previously reported as second dominant species in soils of Gorgan (19.5%) and Gonbad –e Kavus (19%) areas in Iran by Moallaei *et al.* (2006).

Among the dermatophytes, *M. gypseum(13.75%)* was the most predominant fungi isolated from all the soils screened . This fungi is a common geophilic dermatophyte widely distributed in soils globally and causes ringworm of the scalp and glabrous skin in human and animals (Ali and Rana, 2000). Singh *et al.*, (2009) isolated *M. gypseum* from 13 hospital dust samples and it causes tinea corporis and tinea capitis in humans and is also reported from cats, dogs and rodents.

The other dermatophytes isolated were *Trichophyton terrestre*(11.25%), *T. mentagrophytes* (8.75%) and *T.rubrum* (2.50%) followed in decreasing order.*T. mentagrophytes* is reported as the causal agent of tinea pedis, tinea corporis, tinea cruris and onychomycosis. *T. mentagrophytes* has been isolated from soils of public park in Mumbai (Sunil and Veerkar, 2012) while both *T. terrestre* and *T. mentagrophytes* has been recorded from hospital dust of Kanpur (U.P) by Singh *et al*. (2009). *Chyrsoporium zonatum* and

Alternaria alternata accounted for the same prevalence in the present study. *Aphanoascus fulvescens* (7.50%) was isolated from garden soils .It has also been isolated from soils of Bahrain by Deshmukh *et al.,* (2008). Two *Fusarium* species were isolated from this study. Simpanya and Baxter (1996) recorded *Fusarium* species to be the most frequently isolated potential pathogens from soils collected in parks, cleared areas, paddocks, river and roadsides of New Zealand. *Scopulariopsis brevicaulis* was isolated from gardens, birds and animal enclosures. *Scopulariopsis sp*, *Curvularia lunata* and *Fusarium solani* has been reported from soils of gardens and school of Jhansi and Jaipur, (India). (Ganaie *et al*., 2010; Mukesh. and Sharma, 2010).

The fungi isolated in this study are reported to be either well known agents of mycosis or have been recovered from human and animal lesions such as *Microsporum gypseum, Trichophyton rubrum*, *Geotricum candidum*, *Aspergillus flavus, Fusarium oxysporum, Chrysosporium .sp* and others .The dermatophytes are transmitted by contact with infected hair, fomites (clippers, brushes) or from the environment (spores in soil).Dogs and cats harbor many saprophytic moulds and yeasts on there hair coats and skin. The most common of these fungi isolated were species of *Alternaria, Aspergillus, Cladosporium, Mucor, Penicillium* and *Rhizopus*. Most of these saprophytic isolates represent transient contamination by fungi in soil and airborne fungi and are potential pathogens causing mycosis. (Bernardo *et al*., 2005)

Avasn *et al.,(2008),* on screening a total of 2804 primary section pupils from Vishakapatnam ,for hair scalp infection reported 336 children to be positive for dermatophyte infection. The majority of the isolated dermatophytes were *Microsporum audouinii*, *Chrysosporium keratinophilum, Trichophyton mentagrophytes*, *Trichophyton terrestre* (3.33%). However *Fusarium moniliforme*, *Aspergillus flavus*, *Fusarium oxysporum* (5.55%) and *Penicillium funiculosum* were isolated from other skin mycoses infections. Childrens playgrounds, animal inhabited fields and infected domestic animals constituted the apparent source of infection for these children.

It is clear from our results that soils of Gardens and playgrounds are ideal environment for the growth of keratinophilic fungi and dermatophytes, and this could be attributed to the high organic debris and keratinous substrates like hair and feathers from birds and animals and plant litter present in these soils. However the isolation of fungi was not uniform as it depends on organic matter. Organic matter content of soils is one of the major factors affecting the presence of keratinophilic fungi in soils. (Chmel *et al*., 1972). Identifying both environments and fungi where people are exposed to them is of major health concern. (Madisen *et al.,* 2007). Thus these fungi which are human pathogens could be considered as bioindicators of environmental pollution with animal faeces, hair, plant debris and other keratinous substrate and can pose risk of human and animal mycoses.

However in conclusion, the investigated soils exhibit narrow diversity of keratinophilc fungi and dermatophytes which is illustrative to its hot and arid environment.

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