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Isolation and Investigation of Keratinophilic Fungi in the Parks of Municipality Districts of Tehran

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Background: Keratinophilic fungi are a small but well-defined and important group of fungi that can degrade the hard keratin. So, keratinophilic fungi may be pathogenic with regard to keratinized tissues of human and animal.
Objectives: In this study we took samples from the soil of Tehran parks, to investigate the role of keratinophilic fungi in soil, because of the frequent contacts of human being with soil, especially children.
Materials and Methods: Two hundred soil samples from different parks were examined for the presence of geophilic keratinophilic fungi. Fungi were isolated from the samples by the method of hair baiting.
Results: A total of 222 isolates of keratinophilic fungi were obtained. The following species were isolated: Acremonium (17.5%), Chrysosporium (12.6%), Fusarium (11.3%), Cunninghamella (11.3%), Microsporum gypseum (10.4%), Gliocladium (9.9%), Ulocladium (6.7%), Penicillium (4.0%), Aspergillus niger (3.6%), Aspergillus (2.7%), Alternaria (2.2%), Scopulariopsis (1.8%), Verticillium (1.8%), Mucor (1.3%), Drechslera (0.9%), Rhizopus (0.9%), Trichophyton mentagrophytes (0.4%), Cladosporium (0.4%).
Conclusions: The soil represents the main reservoir of keratinophilic fungi including dermatophytes and non-dermatophytes. Therefore, soil can be a source of infection for humans and animals. From the results presented in this study, an association exists between keratinophilic fungi and the soil of parks in municipality districts of Tehran.

Keywords: Fungi; Soil; Keratinophilic

1. Background

Throughout the world great interest is shown by researchers in the soil mycoflora that can degrade keratinized residues. This is due to two factors, the extreme resistance of keratin to biological attack, and the pathogenic potential of every keratinolytic saprophytic species. Keratinolytic fungi are important ecologically, especially where human and animal populations exert strong selective pressures on the environment. In fact, the distribution of such fungi seems to depend largely on the amount of keratinic material available due to the presence of man and animals (1-3).

A soil rich in keratin residues constitutes a permanent or occasional reservoir for dermatophytes and other keratinophilic fungi and is a source of potential infection for human and animals. These keratinophilic fungi have attracted the attention of dermatologist and mycologist due to their association with human and animal mycoses. *Zoosporic true* fungi (sometimes called chytrids) are important agents responsible for deterioration of pollen in the tropical soil. The soil inhabiting keratinophilic fungi has a special affinity for keratinous substrates. The ability of these fungi to invade and live on cornified tissues is closely associated with the utilization of keratin by enzymatic digestion for their growth and nutrition. Therefore, it is significant to analyze and identify the Mycoflora of parks' soils in order to evaluate the presence of keratinophilic fungi in such environments (4). The present work reports the incidence of keratinophilic fungi and related dermatophytes in soils of Tehran's parks.

Dermatophytes are a closely interrelated group of keratinophilic fungi that cause infections of the skin, hair and nails known as dermatophytoses. Terms such as tinea, ringworm, trichophytia and athlete's foot are also sometimes used to refer to these infections. Of the dermatophytes that cause dermatophytoses, about 10 are common pathogens in humans. Dermatophytes have been divided into three categories according to their natural habitats: anthropophilic, when human beings are

Implication for health policy/practice/research/medical education:
In this study we investigated the prevalence of keratinophilic fungi in the soil of some of the most crowded parks of Tehran, considering the more frequent contacts of humans with soil especially children in such parks.

the natural hosts; zoophilic, when a variety of animals act as natural hosts; geophilic, when soil is their natural habitat. Most of the keratinophilic fungi are not dermatophyte but are soil inhabitants (5).

2. Objectives

In this study, we sought to investigate the prevalence of keratinophilic fungi in the soil samples taken from Tehran parks in different locales.

3. Materials and Methods

From March 2010 to July 2010, two hundred soil samples were collected from different parks in Tehran. In this regard, one park from each municipality district was chosen, so we had 22 parks. We tried to choose the largest and most crowded park in each municipality district. Before sampling, superficial vegetative materials and other debris were removed from the surface of the soil. Sterile stainless steel spoon was used to collect the specimen. Samples weighing approximately 500 g were taken from the surface layer of each site to the depth of 2-3 cm, poured and placed in sterile plastic bags. The majority of the keratinophilic fungi were found to be concentrated in the superficial layers of the soils to the depth of 2.5 cm (6). The time period between the collection of soils and the beginning of next step varied between 24 and 48 hours.

In this time soil samples were brought to the department of medical Mycology for processing. Isolation of keratinophilic fungi from the soil was carried out using Hair Baiting Technique (HBT) of Vanbreuseghem (To-Ka-Va). A sterile Petri dish was half filled with a portion of each soil sample. Then soil samples were moistened with sterile distilled water. Afterward, pieces of autoclaved horse hair, 1-2 cm long, were placed on the surface of the moistened soil samples and the plates were incubated at room temperature for six months with weekly examinations. Sterile distilled water was added from time to time to keep the soil moist. Soil cultures were examined every week and samples of hair that appeared to be invaded with fungal growth were stained with lactophenol cotton blue and examined microscopically.

Direct microscopic identification was achieved in some cases but in most instances invaded hair was inoculated into Sabouraud's glucose agar (S) and Sabouraud's glucose agar with Chloramphenicol and Cycloheximide (SCC). Fungal elements suspicious to dermatophytes were cultured into dermatophyte test medium (DTM). Finally, all the fungal colonies were slide cultured to determine their genera.

4. Results

A total of 222 isolates of keratinophilic fungi were obtained from 21 out of 22 parks with the exception of "Meead Park". Two species of dermatophytes were isolated: *Microsporum gypseum* and *Trichophyton mentagrophytes* . Among other soil keratinophilic fungi *Acremonium* was the most prevalent species. The 222 isolates comprised 17 different genera. Table 1 shows keratinophilic fungi isolated from park soils in Tehran. Distributions of soil samples examined are shown in Table 2.

Table 2. Distribution of Soil Samples Examined

Number of Districts	Site	No. of Samples Examined	No. of Samples Positive	Positive, %
1	Gheitarieh	7	3	42.85
2	Sattarkhan	2	1	50.00
3	Taleghani	19	11	57.89
4	Majeedieh	2	1	50.00
5	Meead	2	0	0.00
6	Laleh	19	13	68.42
7	Azadegan	4	2	50.00
8	Fadak	3	2	66.66
9	Almahdi	4	2	50.00
10	Tehrani	2	1	50.00
11	Razi	8	5	62.50
12	Shahr	14	8	57.14
13	Khayam	1	1	100.00
14	Baseej	8	3	37.50
15	F.islam	9	3	33.33
16	Bahman	12	5	41.66
17	Zamzam	2	1	50.00
18	Gheeam	20	11	55.00
19	Shariati	7	3	42.85
20	Modarres	3	2	66.66
21	Narges	2	1	50.00
22	Chitgar	50	32	64.00
Total		200	111	52.11

5. Discussion

The present study revealed the occurrence of 222 fungi belonging to 17 genera in the soil of public parks. Among dermatophytes isolated, *Microsporum gypseum* was recovered most frequently. This fungus was also reported as the first or second most common dermatophyte in soils in other studies. Similar results were found by Deshmukh, who examined soils of Kelara state (India) and recovered *Microsporum gypseum* with the highest frequency (5). He also isolated this fungus as the second highest frequency in examination of the vicinity of salt pan soils of Mumbai, India (7). Another study in India by Vidyasagar showed that *Microsporum gypseum* was predominant, followed by *Chrysosporium keratinophilum* (8).

Table 1. Keratinophilic Fungi Isolated from Park Soils in Tehran

Fungi	Name of The Parks																						
	Gheitarieh	Sattarkhan	Taleghani	Majeedieh	Meead	Laleh	Azadegan	Fadak	Almahdi	Tehrani	Razi	Shahr	Khayam	Baseej	F.islam	Bahman	Zamzam	Gheeam	Shariati	Modarres	Narges	Chitgar	Sum
Acremonium	1	-	-	-	-	8	1	1	2	1	2	2	-	-	4	2	1	4	4	1	1	4	39
Chrysosporium	2	-	-	-	-	2	-	-	-	1	2	2	-	2	1	2	1	4	3	1	1	4	28
Fusarium	-	1	1	-	-	4	-	-	2	1	-	4	-	2	1	-	2	1	2	-	-	4	25
Cunninghamella	1	1	-	-	-	4	-	1	1	-	2	2	1	-	3	3	-	3	1	-	-	2	25
M.gypseum	2	-	2	-	-	3	-	-	1	-	1	2	1	-	1	2	-	3	1	1	-	3	23
Gliocladium	1	1	-	-	-	2	-	-	-	-	3	2	1	1	1	-	-	4	1	-	1	4	22
Ulocladium	-	1	-	1	-	-	-	-	-	-	-	2	-	-	-	1	-	2	2	-	1	5	15
Aspergillus	-	-	-	-	-	1	1	-	-	-	1	4	-	-	1	1	-	2	-	-	-	3	14
Penicillium	1	-	1	-	-	2	-	-	1	-	-	-	-	1	1	-	-	1	1	-	-	-	9
Alternaria	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2	5
Scopulariopsis	-	-	-	1	-	-	-	-	1	-	-	1	-	-	-	-	-	1	-	-	-	-	4
Verticillium	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	4
Mucor	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3
Drechslera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	2
Rhizopus	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	2
T. mentagrophytes	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Cladosporium	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Sum	8	4	5	2	-	30	2	3	8	3	11	23	3	6	13	12	5	26	16	3	4	35	222

Periasamy Anbu et al. also reported this dermatophyte as one of the predominant fungi isolated from poultry farm and feather dumping soil in Tamil nadu, India (9). Deshmukh et al. again reported similar result of investigation from soils of Jammu, India (1). However Zaki et al. did not isolated *Microsporum gypseum* from muddy soil in Cairo vicinities, Egypt. They reported *Chrysosporium zonatum* as the most prevalent species that represented 42.5% of the total number of isolates (10). In Iran, Shadzi et al. isolated *Chrysosporium* as the most frequent keratinophilic fungus from elementary schools and public parks in Isfahan, Iran (4).

Hedayati et al. demonstrated the presence of *Microsporum gypseum* as dermatophyte and *Penicillium spp* followed by *Acremonium spp* as non-dermatophyte fungi in soil samples of potted plants from Sari hospitals, Iran (11). A total of 1568 patients with suspected Tinea capitis were examined for causative fungal agents between 1994 and 2001 in Tehran and the rate of *Microsporum gypseum* was found 1% (12), however in another study in Karaj this rate was 3.2% (13). The results obtained in our study provide data that are consistent with previously conducted surveys in the same geographical area.

The number of species isolated from different parks varied, which may indicate a selective effect of the characters of certain biotopes on the distribution of keratinophilic fungi in soil, such as the presence of direct sunlight, soil pH, geologic constitution, locality, season and other environmental factors. For example, *M. gypseum* has been found universally in humus-rich soil and in surroundings with intense animal populations, while *T. ajelloi* is more frequently found in places with humus-rich soils whereas the *chrysosporium* spp have been found universally in soil enriched with animal hair and bird feathers. Although it is possible to observe a certain distinction between the kind of keratinic baits used and the keratinophilic fungi isolated, but results suggest that the kind of keratinic baits is not the main factor responsible for the isolation of different keratinophilic species (14).

From the results presented in this study, an association may exist between keratinophilic fungi and the soil of parks in municipality districts of Tehran. The keratinophilic fungi occurring more commonly in the soil were *Acremonium, Chrysosporium, Fusarium, Cunninghamella* and *M. gypseum*. Regardless of dermatophytoses, there are some other pathogens that have been isolated from soil such as the causative agents of nocardiosis (*Nocardia* spp.), tinea nigra (*Phaeoannellomyces werneckii*), white piedra (*Trichosporon beigelii*), sporotrichosis (*Sporothrix schenckii*), entomophthoromycosis (*Conidiobolus coronatus*), pseudallescheriasis (*Pseudallescheria boydii*), mycetoma and chromoblastomycosis. Also, the agents of systemic mycosis were isolated from soil: histoplasmosis (*Histoplasma capsulatum*), coccidioidomycosis (*Coccidioides immitis*), paracoccidioidomycosis (*Paracoccidioides brasiliensis*). Therefore, it is important to keep the soil well-drained to avoid fungal infection.

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Authors' Contribution

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