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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Isolation of keratinophilic fungi from soil in Isfahan province, Iran

Isolement de champignons kératinophiles du sol dans la province d'Isfahan, Iran

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KEYWORDS

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Summary

Objective. – To evaluate the epidemiology of keratophilic fungi in Isfahan province, Iran.

Material and methods. – The present research has been conducted on soil samples collected from 16 townships of Isfahan province. For isolate geophilic dermatophytes and keratinophilic fungi, the keratin baiting technique has been applied.

Results. – Of 800 soil samples examined, 588 (73.5%) keratinophilic fungi were isolated. The present studied recognized 727 isolates including 16 species of 11 genus, as follows: *Chrysosporium keratinophilum* (31.4%), *C. pannicola* (16.9%), *C. tropicum* (15.4%), *Microsporum gypseum* (12.4%), *Chrysosporium* spp. (9.9%), *C. indicum* (7%), *Sepedonium* spp. (3.3%), *Malbranchia* spp. (1%), *Trichophyton terrestre* (0.8%), *T. ajelloi* and *Paecilomyces lilacinus* (0.4%), *Engyodontium album* and *Acremonium* spp. (0.3%), *Curvularia* spp., *Fusarium* spp. and *Ulocladium* spp. (0.1%). In this study, *E. album* was isolated for the first time in this country (Iran). The frequency these keratinophilic fungi are discussed in relation to different agents such as soil pH.

Conclusion. – This study contributes to the knowledge of keratophilic fungi in Iran.

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MOTS CLÉS

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Sol ;
Iran

Résumé

Objectif. — Évaluer l'épidémiologie des champignons kératinophiles dans la province d'Isfahan, Iran.

Matériel et méthodes. — La présente recherche a été menée sur des échantillons de sol prélevés dans 16 cantons de la province d'Isfahan. Pour isoler les dermatophytes et les champignons géophiles et kératinophiles, la technique d'appâtage à la kératine a été appliquée.

Résultats. — Un total de 800 échantillons de sol a été examiné parmi lesquels 588 (73,5 %) avaient des champignons kératinophiles. La présente étude a permis d'isoler 727 champignons appartenant à 16 espèces de 11 genres différents comme suit : *Chrysosporium keratinophilum* (31,4 %), *C. pannicola* (16,9 %), *C. tropicum* (15,4 %), *Microsporium gypseum* (12,4 %), *Chrysosporium* sp. (9,9 %), *C. indicum* (7 %), *Sepedonium* sp. (3,3 %), *Malbranchia* sp. (1 %), *Trichophyton terrestre* (0,8 %), *T. ajelloi* et *Paecilomyces lilacinus* (0,4 %), *Engyodontium album* et *Acremonium* sp. (0,3 %), *Curvularia* sp., *Fusarium* sp. et *Ulocladium* sp. (0,1 %). Dans cette étude *E. album* a été isolé pour la première fois dans ce pays (Iran). La fréquence de ces champignons kératinophiles est discutée en relation avec différents agents tels que le pH du sol.

Conclusion. — Cette étude contribue à une meilleure connaissance de l'épidémiologie des champignons kératinophiles en Iran.

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Introduction

Keratinophilic fungi are a group of fungi that colonize various keratinous substrates and degrade them to components of low molecular weight. These include a host of filamentous fungi representing mainly hyalohyphomycetes and several other taxonomic groups [13].

Many keratinophilic fungi frequently live as a parasite on keratinous tissue as skin, nails and hair in man and animals; some of them share certain morphological features, constituting a special group called dermatophytes. Most species of dermatophytes are anthropophilic or zoophilic in their natural habitat, while some occur in soil as saprophytes and are termed geophilic dermatophytes [13,19,27].

Many surveys have been carried out in different parts of the world to study the distribution of these fungi in soil [1,7–9,11,22–24,26,33,34]. During recent years there are reports on the isolation of dermatophytes and related keratinophilic fungi from Iran soils [14,20]. However, our knowledge from keratinophilic fungi in Iran is still fragmentary. The province of Isfahan, with a land area of approximately 107,027 km², is located in the centre of the Iranian plateau. Constituting of 17 townships, this province had a population of 4,559,256 according to the 2006 census. The province experiences a moderate and dry climate on the whole, ranging between 40.6 °C and 10.6 °C on a cold day in the winter season. From 17 townships, the keratinophilic fungal flora of the Isfahan city only has been partially investigated [30]. But due to climate and vegetation diversity in Isfahan province, the present study carried out with the goals as follow:

- survey of frequency of keratinophilic fungi in studied regions;
- pH measurement from soil of the studied regions and associated with keratinophilic fungi;
- effect of climate types in the frequency of this group of fungi.

Material and methods

A total of 800 soil samples were collected from 800 different locations in 16 townships (including 16 cities and 16 villages) in Isfahan province. Fifty soil samples from each township (including 30 samples from central city and 20 samples from selected village) were collected (Table 1).

Sampling carried out in winter and work on the samples lasted for 18 months (2006–2008). Sampling locations in cities include squares, boulevard, street side, area offices, coastal and forest parks, riverine, waterfall along... and villages samples include corral livestock and domestic animals, nests, farm land, fruit gardens, garden houses... Soil samples to the amount of 200–300 g for each sample were collected from superficial layer with depth not exceeding 2–5 cm. The samples were placed in sterile polyethylene bags, transported immediately to the laboratory and were store at low temperature (4 °C) until tested. pH of the samples was measured immediately in a 1:5 soil/deionised water suspension (w/v) using a pH meter according to A1-Musallam [2].

Townships of studied base on air temperature and humidity that in the sampling time was measured, also with considering the geographical situation were divided to three groups including townships with cold and semi-wet climate, semi-nomadic climate, desert and brackish climate (Table 2).

Hair baiting technique was followed to isolate dermatophytes and related keratinophilic fungi from the soil samples. Seventy grams of soil from each sample were placed in 9 cm diameter sterilized petri dishes and baited with a mixture of sterile human child girls' hairs and horse to rate of 1 g for one petri dish.

Sterilization of hairs was performed with cut into pieces 1–2 cm, then were placed in 9 cm diameter glass petri dishes and covered with aluminium foil, then was autoclave.

The plates were then moistened with sterile distilled water and incubated at room temperature (25 °C) for a period of six weeks and were remoistened with sterile distilled water whenever necessary. The plates were observed periodically for the isolation and identification of keratinophilic

Table 1 Frequency of keratinophilic fungi isolated from soils of 16 townships in the province of Isfahan, Iran.
Fréquence des champignons kératinophiles isolés du sol dans 16 cantons de la province d'Isfahan, Iran.

	Townships																Total	% frequency
	Ard	Bor	Fer	Fla	Fsh	Gol	Kas	Kha	Kho	Len	Mob	Nai	Naj	Nat	Sem	Sha		
No.of samples ex.	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	800	
No.of positive	15	17	42	42	40	40	32	45	42	41	45	47	34	34	41	31	588	
Percentage of positive	30	34	84	84	80	80	64	90	84	82	90	94	68	68	82	62	73.5	
Species isolated																		
<i>Acremonium</i> spp.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2	0.27
<i>Chrysosporium indicum</i>	1	0	4	2	6	3	5	4	9	3	2	3	6	3	0	0	51	7
<i>C. keratinophilum</i>	5	5	19	13	14	17	14	22	15	9	16	20	12	21	14	13	229	31.45
<i>C. tropicum</i>	3	4	7	4	11	8	6	9	7	8	8	9	10	4	8	7	112	15.38
<i>C. pannicola</i>	4	2	8	7	9	12	14	10	11	7	7	9	4	6	5	8	123	16.89
<i>Chrysosporium</i> spp.	0	1	5	10	5	0	3	13	5	0	8	7	0	2	9	4	72	9.89
<i>Curvularia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.13
<i>Engiodontium album</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.27
<i>Fusarium</i> spp.												1					1	0.13
<i>Malbranchia</i> spp.			1						3						2	1	7	0.96
<i>M. gypseum</i>	0	8	8	8	5	4	1	6	3	16	6	5	4	1	13	2	90	12.36
<i>Paecilomyces lilacinus</i>	1							2									3	0.41
<i>Sepedonium</i> spp.	3	2					4	4		6			2	3			24	3.3
<i>Trichophyton ajelloi</i>			2	1												3	0.41	
<i>T. terrestre</i>	1		1		1	2				1							6	0.82
<i>Ulocladium</i> spp.											1						1	0.13
Unknown	1																1	0.13
Total	21	22	53	46	52	46	47	70	53	49	49	54	38	40	51	37	728	100

Ard: Ardestan; Bor: Borkharomeimea; Fer: Feraidan; Fla: Flavarjan; Fsh: Feraidoonshahr; Gol: Golpayegan; Kas: Kashan; Kha: Khansar; Kho: Khomainishahr; Len: Lenjan; Mob: Mobarekea; Nai; Naiin; Naj; Najafabad; Nat; Natanz; Sem; Semirom; Sha; Shahinshahr.

Table 2 Separation of studied townships according to the type of climate.

Répartition des cantons étudiés selon le climat.

Climate	Townships
Cold and semi wet	Khansar, Semirom, Feraidan, Feraidoonshahr, Golpayegan, Natanz
Semi nomadic	Borkharomeimeh, Shahreza, Flavarjan, Lenjan, Mobarekeh
Desert	Ardestan, Khomainishahr, Kashan, Naiin, Najafabad

fungi. Isolation were carried out by direct transfer of mycelium from the baits to sabouraud dextrose agar medium with cyclohexamide (1 mg/mL) and chloramphenicol (0.1 mg/mL), then incubated at room temperature for a period of two weeks. Identification of keratinophilic fungi was carried out according to standard procedures [15,27]. In this study, to confirm the existence of *Malbranchia* spp. and absence of its similar fungus (*Coccidioides immitis*), the fungus inoculated intraperitoneally to male mice.

Result

Among the 800 soil samples, 588 (73.5%) samples were positive for keratinophilic fungi (Table 1). In the present study, 727 isolates of keratinophilic fungi and one isolate of unknown aerial actinomycete were isolated. Keratinophilic fungi were belonging to 16 species of 11 genus as follows: *Chrysosporium keratinophilum* (31.4%), *C. pannicola* (16.9%), *C. tropicum* (15.4%), *Microsporium gypseum* (12.4%), *Chrysosporium* spp. (9.9%), *C. indicum* (7%), *Sepedonium* spp. (3.3%), *Malbranchia* spp. (1%), *Trichophyton terrestre* (0.8%), *T. ajelloi* and *Paecilomyces lilacinus* (0.4%), *Engyodontium album* and *Acremonium* spp. (0.3%), *Curvularia* spp., *Fusarium* spp. and *Ulocladium* spp. (0.1%) (Table 1).

In this study, keratinophilic fungi were isolated more in urban areas than in rural areas (Fig. 1).

The most keratinophilic fungi species was detected from region with cold and semi-wet climate (38.5%). The most

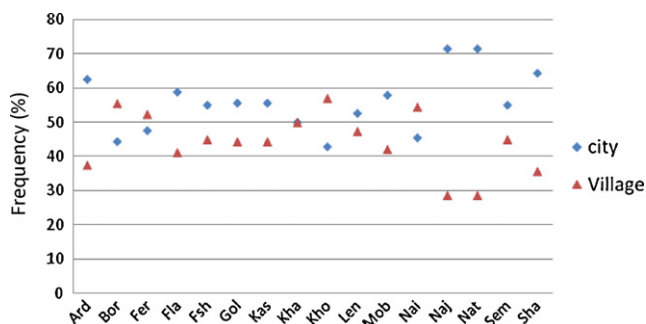


Figure 1 Frequency of keratinophilic fungi isolated from soils of urban and rural regions from 16 townships in the province of Isfahan, Iran.

Fréquence des champignons kératinophiles isolés du sol dans les zones urbaines et rurales de 16 cantons de la province d'Isfahan, Iran.

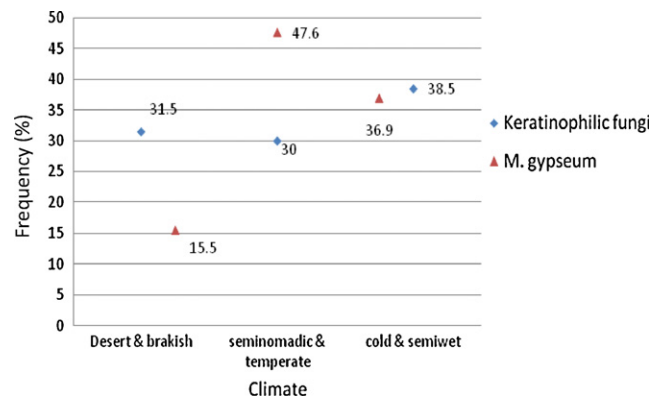


Figure 2 Frequency of keratinophilic fungi and *M. gypseum* isolated from soils of 16 townships in the province of Isfahan (Iran) at different climates.

Fréquence des champignons kératinophiles et de M. gypseum isolés du sol de 16 cantons de la province d'Isfahan, (Iran) à différents climats.

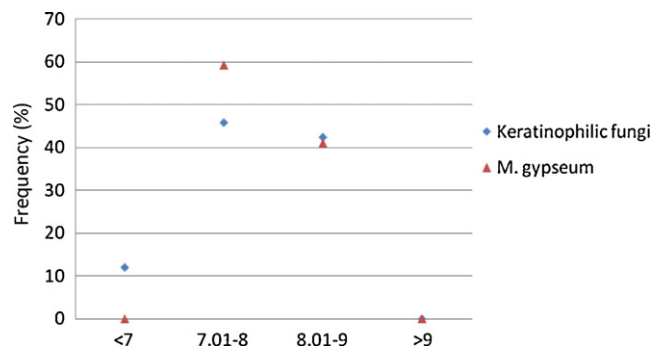


Figure 3 Frequency of keratinophilic fungi and *M. gypseum* isolated from soils of 16 townships in the province of Isfahan (Iran) at pH different values.

Fréquence des champignons kératinophiles et de M. gypseum isolés du sol de 16 cantons de la province d'Isfahan (Iran) à différents pH.

M. gypseum species was detected from region with semi-nomadic and temperate climate (47.6%) (Fig. 2). Fig. 3 shows the distribution of the keratinophilic fungi in relation to soil pH. We found 45.7% keratinophilic fungi from the soil samples with pH: 7.01–8, 42.3% from pH: 7.01–8 and 12% from pH: 6–7. In the present study, the most *M. gypseum* species (59.1%) were isolated from the soil samples with pH: 7.01–8.

Discussion

This study showed that the soil of studied areas was rich of keratinophilic fungi and geophilic dermatophytes, because these fungi were isolated from more than 70% of the total soil sample studied. *Chrysosporium* spp. (80.6%) were the most prevalent species of fungal isolates. They were isolated from more studied samples. Several studies show that *Chrysosporium* spp. frequently isolated from soil samples and a wide spread have in the soil [1,9,11,23,32].

In this study, *M. gypseum*, *Trichophyton ajelloi* and *Trichophyton terrestre* were isolated. Several researchers also inside of Iran, cases of this kind of dermatophytes have reported [1,2,14,20,23,26,30].

Cases of human infections caused by *M. gypseum* and *T. ajelloi* were reported, also *T. terrestre* as agent of dermatophytosis in humans and animals is known [3,16,18,21,25,28,29].

In the present study, *Engyodontium album* has been isolated for the first time from Iran soil. *E. album* is regarded as a keratinophilic fungus [15,17]. *E. album* as human infections such as keratitis, brain abscess, endocarditis and eczema vesiculosum have been reported [5].

In general, the soil of Isfahan province to several reasons were rich of keratinophilic fungi:

- sampling was performed from the soil surface and maximum depth of 3 cm that was according to Tuck soon [31];
- bait or substrate used in this study was mixture of the horse hair (due to high volume keratin) and the immature girl (due to being dry hair), according to the other researchers [1];
- the third factor is soil pH. The first time Bohme and Ziegler [6], effect of the pH on the soil keratinophilic fungi were studied, they reported that keratinophilic fungi are not seen in soils with low pH (3 to 4.5). Garg [12], also acidic soils with pH = 5.9 were introduced free of the keratinophilic fungi. Asahi et al. [4], as well as concluded that keratinolytic enzymes in pH = 6–9 and especially, the extracellular keratinase were active in pH = 9. Nasr-Esfahani [10] the best *M. gypseum* growth medium, soils of with pH weak alkaline was introduced. In this study, all 727 identified species of keratinophilic fungi were isolated from soil with the pH = 6–9. We found 45.7% keratinophilic fungi from the soil samples with pH: 7.01–8, 42.3% from pH: 7.01–8 and 12% from pH: 6–7. This confirmed the findings of others;
- according to findings of Garg [12] and Filipello Marchisio et al. [11], the most important factor in frequency of keratinophilic fungi is the existence of substrate and source of keratin in soil due to the activity of humans. The present study also confirmed this case. Because as you see in Fig. 1, in urban areas due to high population of humans and their activities, substrate and source of the keratin required for keratinophilic fungi is higher than the rural areas and therefore in this area the number of keratinophilic fungi were separated, more than the rural areas;
- according to research conducted by Garg [12] and Tuck Soon [31], soils rich in organic material, conditions for the spread of fungi in the soil are provided. In this study also, the most and the lowest keratinophilic fungi were isolated from Khansar township: 70 species (9.6%) due to increase of soil organic matter and humus and Ardestan township: 21 species (2.9%) due to decrease of soil organic matter, respectively;
- sixth factor is kind of the weather. In this study, the most species of keratinophilic fungi were isolated from soil samples of townships with cold and semi-wet climates. They were also isolated from more than 80% of the samples of these regions. However, soil samples of the townships with other climates had a high percentage of

frequency. For example, they were isolated from more than 70% of the samples of the townships with semi-nomadic climate and almost 68% of samples of with desert climates. In addition, most strains of *M. gypseum* and all of *T. ajelloi* species were isolated from townships with semi-nomadic climate. Thus, we can conclude that kind of weather is effective on frequency of keratinophilic fungi.

Previous studies in Iran were limited to the quantity and quality of this group of fungi in the soil, but the present study showed the influence of some ecological factors on keratinophilic fungi in the soil.

Disclosure of interest

The authors have not supplied their declaration of conflict of interest.

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