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Research Paper

Microscopic examination of keratinophilic fungi isolated from soil samples of Saharanpur (U.P)

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Abstract

Fungal diversity and prevalence in each soil depends on environmental and nutritional conditions. Considering the importance of soil in transmission of diseases, this study aims at assessing the frequency of dermatophytes and related Keratinophilic fungi, potentially pathogenic fungi in various samples collected from animal's habitat and agricultural fields in Saharanpur Village. Twenty two samples were collected from different parts of the agricultural fields and animal's habitat. The fungal flora was analyzed in different samples for the presence of dermatophytes and Keratinophilic fungi by hair baiting technique. The samples were cultured on Sabouraud's Dextrose Agar and incubated at 28°C for 4-5 weeks aerobically. Isolates were identified by colony morphology, slide cultures, and differentiation tests. Most of the isolates, dermatophytes and Keratinophilic fungi were collected from the soil of agricultural fields. The most common keratinophilic fungal species were *T. tonsurans, T. mentagrophytes, T. rubrum, T.equinum, M. gypseum, M. canis, M. cookei, M. audouinii.* The results demonstrated that the fungal flora of Saharanpur area is different from other parts of animal habitats due to the different climatic conditions prevailing.

Keywords: Keratinophilic fungi, Morphology, Macroscopic, Microscopic

Introduction

Keratinophilic fungi are important ecologically and play a significant role in natural degradation of keratinous substrates. Soils that are 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 [1]. 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 [2]. 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 [3]. 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 [4]. Based on their occurrence in natural habitat, Kertainophilic fungi are divided into three categories anthropophilic when human beings are the natural hosts. Zoophilic when avariety 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[5-7].

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 et al. [8] 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 the prevalence of Keratinophilic fungi and related dermatophytes in the soils of animals habitat and agricultural fields in Saharanpur Village. This would help us to 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 66 Soils samples were collected from different areas of animals habitat and agricultural fields in Saharanpur Village July 2014 to march 2015. Different soils samples were collected in sterile polyethylene bags and brought to the laboratory for further microbiological analysis.

Isolation, Purification and identification of fungi

Hair baiting technique described by (9) was adopted. Each soil sample was thoroughly homogenised and 10mg portion of the Soil samples were placed in different Petri plates. Bits of sterilized human hairs, dog hairs, buffalo hairs, pig hairs, goat hairs, sheep hairs and horse hairs, were used as baits. These were scattered uniformly on the moistened soil samples. Each of the Petri plates were separately labelled indicating the date, site of collection and time of incubation. These Petri plates were then incubated at room temperature for 30 days but with regular checking at every 3 days interval, checking for fungal growth on the baited hairs. Samples with visible fungal growth were sub cultured to a fresh plate of SDA for purification and identification. The macroscopic identification different selective media were used. Sabouraud s Dextrose Agar (SDA), Trichophyton Agar, Dextrose Agar and Rice media were used for the growth and sporulation of different fungi. Morphological characteristics were examined under microscope. Slide culture technique was adopted for the identification of fungi with lacto phenol cotton blue.

Results and Discussion

The soil samples collected from different animal habitats and agricultural fields in Saharanpur Villages. The result of isolation of Keratinophilic fungi are presented in Table no 1and 2.Fig no 1and 2 The data revealed that out of sixty six soil samples collected, eight species of Keratinophilic fungi were isolated from different animals habitats and agricultural fields with different keratin substrates such as human hairs, buffalo hairs, dog hairs, pig hairs, and cow hairs tables 3 and4. These habitats contain also lots of keratin debries. In the present study most of the isolated Keratinophilic fungi viz *T. tonsurans, T. mentagrophytes, T. rubrum, T. equinum, M. gypseum, M. canis, M. cookei* and *M. audouinii.* The results are given in Table 2. In this study the maximum Keratinophilic fungi were found in agricultural fields due to the presence of wild and domestic animals in agricultural field, serving as a reservoir of organisms. In general the frequency of dermatophytes and Keratinophilic fungi in the agricultural fields was higher than animal habitats. In the present investigation, the fungal strains *Microsporum* and *Trichophyton sps* were frequently isolated Keratinophilic fungi and dermatophytes were also previously reported from India soils [10,11]. It is remarkable that the highest number of Keratinophilic fungi was observed from soils of agricultural fields in Saharanpur villages. The high prevalence of Keratinophilic fungi from these soils explain that buffalo hairs, human hairs and dog hairs which come to the soil either as dead or dropped of serves as substrates and are subjected to microbial decomposition. The present study clearly indicates the diverse existence of Keratinophilic fungi in soils of Saharanpur. Kertinophilic fungi are important ecologically and play major role in bioremediation in natural environment. Various workers also reported diverse soil habitats have been screened from different countries Brazil, Kuwait, Iran, and India indicating that these group of fungi are distributed worldwide[12,13]. *M. cookei* is a geophilic dermatophyte relatively frequently isolated from hairs lesions. Therefore this species is of special epidemiological importance. The waste water favored the growth of *M. cookie* on Keratinous substrate in a wide temperature range. A number of

studies have been performed in different part of Iran and demonstrated that a variety of Keratinophilic fungi exists in the soils of investigated region [14-17].

Tables 1: Culture Positive Fungal isolates from Animals habitats and Agricultural fields from Saharanpur villages

S. No.	Area	Total no of Samples	Total culture positive Isolates	Percentage of culture positive Isolate
1.	Animals habitat	25	18	72
2.	Agricultural field	41	26	63.41
	Total	66	44	66.6

Table 2: Collection of Various Soil Samples from different Animal habitat of Saharanpur (U.P.)

S. No.	Village Name	Total Number of samples collected	Number of fungal culture positive case	Percentage of culture positive case
1.	Nanauta	5	4	40%
2.	Ambheta	3	2	20%
3.	Nagal	4	3	30%
4.	Khudana	3	2	20%
5.	Luharli	4	3	30%
6.	Jagrolly	3	2	20%
7.	Nakur	3	2	20%

+ = Positive result (Growth observed), - = Negative result (No growth observed)

Table 3: Collection of Various Soil Samples from different Agricultural field of Saharanpur (U.P.)

S. No.	Village Name	Total Number of samples collected	Number of fungal culture positive case	Percentage of culture positive case
1	Sonaarjunpur	3	2	20%
2	Salempur	2	1	10%
3	Sadhalihariya	4	3	30%
4	Rampur	5	4	40%
5	Pilkhani	3	2	20%
6	Naurangpur	2	1	10%
7	Kanjoli	2	1	10%
8	Pahansu	3	2	20%
9	Jandhera	3	2	20%
10	Bahankla	2	1	10%
11	Dariypur	4	3	30%
12	Nandpur	3	2	20%
13	Tapri	1	0	0%
14	Charro	2	1	10%
15	Khatka heri	2	1	10%

+ = Positive result (Growth observed), - = Negative result (No growth observed)

Table 4: Incidence of Keratinophilic fungi Isolates of Animals habitats from Saharanpur (U.P)

S. No.	Fungal isolates	Incidence of fungal isolates	Percentage%
1.	*T. tonsurans*	3	16.66
2.	*T. mentagrophytes*	2	11.11
3.	*T. rubrum*	3	16.66
4.	*T. equinum*	2	11.11
5.	*M. canis*	2	11.11
6.	*M. cookei*	2	11.11
7.	*M. gypseum*	2	11.11
8.	*M. audouinii*	2	11.11

Table 5: Prevalence of fungal Isolates collected from different Soil Samples of Agricultural fields from Saharanpur villages

S. No.	Fungal isolates	prevalence of fungal isolates	Percentage%
1.	*T. tonsurans*	6	14.63
2.	*T. mentagrophytes*	6	14.63
3.	*T. rubrum*	4	9.75
4.	*T. equinum*	5	12.19
5.	*M. canis*	5	12.19
6.	*M. cookei*	4	9.75
7.	*M. gypseum*	6	14.63
8.	*M. audouinii*	5	12.19

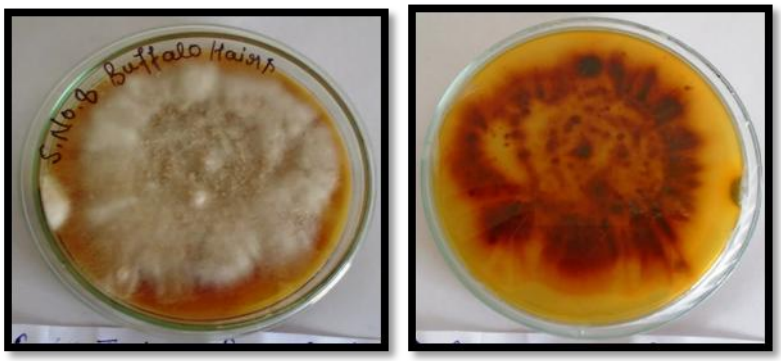
Table 6: Microscopic and Macroscopic Characteristic of Isolated Keratinophilic fungi

Macroscopic Identification	Microscopic identification	Identified species
White to creamy, powdery growth yellow to brown reverse pigmentation	Clavates shaped macroconidia are present	*T. mentagrophytes*
Creamy growth and wine red reverse pigmentation	Microconidia are Clavate to pyriform, macroconidia	*T. rubrum*
Thallus color cream to pale yellow deep to pyriform Pinkish or brownish in center	Macroconidia, rare club shaped macroconidia	T. equinum
Thallus color white to cream yellow rose Lemon yellow to brown may have spiral hyphae	Microconidia on stalks bollon froms	*T. tonsurans*
Flate, pale yellow, powdery growth dark yellowish brown pigmentation.	Micro conidia- elliptical and rough, Macroconidia-cylindrical, clavate.	*M. cookei*
White to cream with dense cottony growth, bright golden yellow reverse pigmentation	Pyriform to clavate shaped micro conidia. Macroconidia	*M. canis*
Creamy fluffy growth, yellow brown Clavate shaped and reverse pigmentation	Microconidia, symmetrical ellipsoidal shaped macroconidia are present	*M. gypseum*
Thallus color white to light tan rare	Club shaped microconidia, spindle shaped macroconidia	M. audouinii

Previous workers reported *M. gypsum* is a frequent geophilic dermatophyte commonly distributed in soil worldwide. *M. gypseum* was recovered in soil samples from Isfaha [14,16,18,19]. Studies have reported *T. mentagrophytes* was also found in several investigations in soils of different parts of Iran[14,16]. Occurrence of *Microsporum* sp. in different types of soil is important for pathogenic potential of fungus and was confirmed in several studies in different countries. *M. audouinii* was showed to cause systemic infection in a person with a chronic skin disease[20,21]. Several Keratinophilic fungi such as *T. tonsurans, T. equinum, M. gypseum* and *T. mentagrophytes* from the salt pans and coastal soils in India. In previous studies *T. rubrum* reported best producer of keratinase[22].

This possibly describes the recovery of fungus from the sterile hair bait. *T. rubrum* had been recognised as a strong producer of extracellular keratinase in medium including porcine nail as the source of nitrogen and carbon. Some strains of *T. mentagrophytes* were also described to be active in extracellular keratinoases after grown on agar including soluble keratin[22].

White to creamy, growth yellow to brown reverse pigmentation *T. mentagrophytes*



Creamy growth and wine red reverse pigmentation *T. rubrum*

Thallus color cream to pale yellow deep to pyriform Pinkish or brownish in center *T. equinum*

Thallus color white to cream yellow rose Lemon yellow to brown *T. tonsurans*

Flate, pale yellow, powdery growth dark yellowish brown pigmentation *M. cookie*

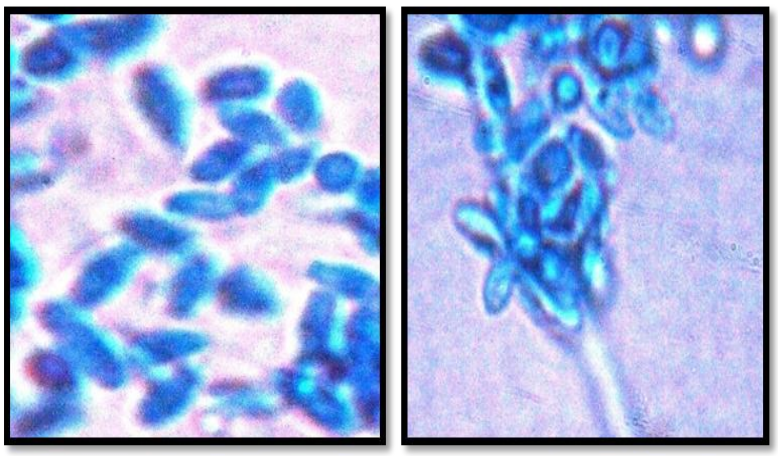
White to cream with dense cottony growth, bright golden yellow reverse pigmentation *M.canis* Depending on the seasonality animals habitats as a subtropical region, its ambient temperatures reach the peak of 40-50 ºC during June to August [23] believe that increasing temperature and decreasing humidity lead to providing a less favorable condition for the growth of *M. gypseum* in the soil. In the present study, three isolates of *T. mentagrophytes, T. rubrum* and *T. tonsurans* were isolated from animal habitats and agricultural fields' soil samples.

Creamy fluffy growth, yellow brown Clavate shaped and reverse pigmentation *M. gypseum*

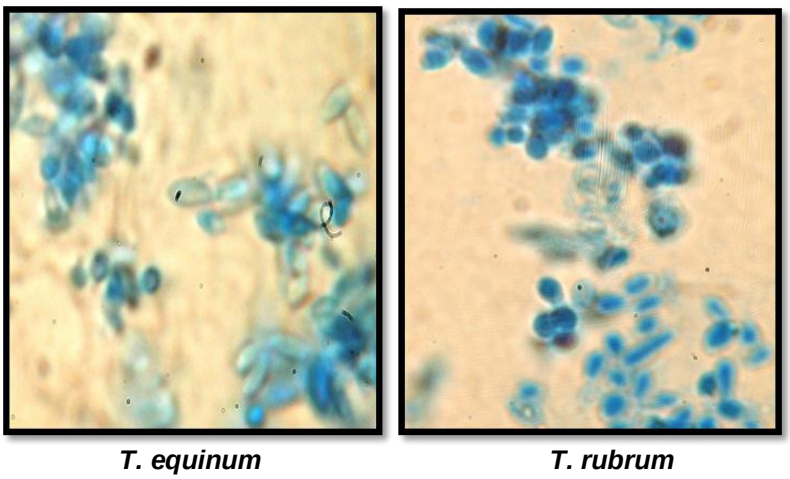
Thallus color white to light tan rare *M.audouinii*

Figure 1: Macroscopic Characteristic of Isolated Keratinophilic fungi

Both species are zoophilic and are usually associated with animals. *T. rubrum,* and *T. tonsurans* reported the most frequent isolate from public parks in Isfahan[14] Previous workers reported Healthy carriers can be important in the dissemination of the fungus and the occurrence of sporotrichosis cases when the conditions are favourable. Human infections have also been associated with insect stings, fish handling, and bites or scratches from birds, dogs, squirrels, horses, reptiles, parrots and rodents, even though clinical symptoms may not be present in these animals [24].



T. mentargrophytes ***T. tonsurans***



T. equinum ***T. rubrum***

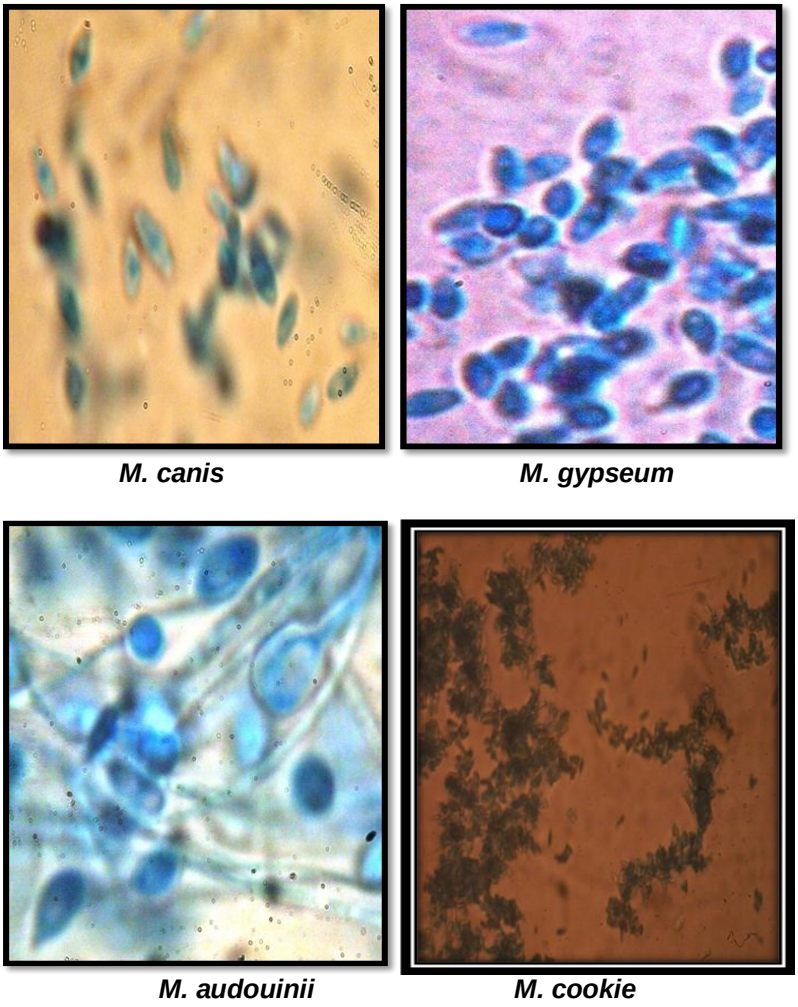


Figure 2: Microscopic photography of Isolated Keratinophilic fungi from Saharanpur (U.P.)

Conclusion

It is concluded from the present study that Keratinophilic and other fungi isolated from soil of agricultural fields and animals habitats of Saharanpur (U.P.) Agricultural field soil sample is somewhat different from other part of field soil samples due to the different climatic conditions and prevailing of wild and domestic animals. Finally, this study reveals that the soil is rich in dermatophytes and related fungi responsible for dermatophytosis or ringworm disease.

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