



Original Research Article

Prevalence of keratinophilic fungi in the garden soil samples in Western India and study of its clinical impact

Madhu Yadav¹, Neha Agrawal^{1,*}, Mukul Chaurasia¹, Vijaylatha Rastogi¹, Pushpanjali Verma¹, Abila Parashar¹, Govind Meena¹

¹Dept. of Microbiology, J.L.N. Medical College, Ajmer, Rajasthan, India



ARTICLE INFO

Article history:

Received 02-03-2021

Accepted 06-03-2021

Available online 02-04-2021

Keywords:

Dermatophytosis

Dermatophytic

Keratinophilic fungi

ABSTRACT

Background: Dermatophytosis has been a common contagious disease and remain an important public health problem among people worldwide and particularly in developing countries. This study was done in order to know prevalence of keratinophilic fungi in the soil samples of Ajmer region and its clinical correlation.

Materials and Methods: Five samples (5gm each) of garden soil were collected from each of the randomly selected 10 sites of Ajmer region in sterile polythene bags & pH of soil was recorded. All samples were processed in the laboratory using hair bait technique and incubated at 28 + 2°C for 3-4 weeks. Fungi grown were subcultured onto Sabourauds Dextrose Agar (SDA) with chloramphenicol & SDA with cycloheximide and identified using standard mycological procedures.

Results: Out of 50 soil samples cultured 28 (56%) samples were positive for keratinophilic fungi. 64.28% were dermatophytes and 12.8% were non-dermatophytic fungi. The predominant isolate among dermatophytes was *Trichophyton* spp. (64.28%) where as *Aspergillus* spp. (12.8%) was the commonest non-dermatophyte.

Conclusion: In our hospital, dermatophytic infection including Tinea corporis is one of the most common clinical presentations of skin infection. *Trichophyton* spp. is commonest causative agent of Tinea corporis. Interestingly *Trichophyton* spp. was the most commonly isolated dermatophyte in our study suggesting soil as a source of dermatophytic infections.

© This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1. Introduction

Dermatophytes are filamentous, septate molds closely related keratino-philic fungi which are capable of invading keratinized tissues of skin and its appendages like hair & nail (that are able to digest & obtain nutrients from keratin by unique enzymatic capacity). 'Dermatophytosis' commonly referred to as 'ringworm'. The name dermatophyte consists of two parts, namely "derm" which means skin and "phytes" which means plants. They possess the affinity for parasitizing keratin rich tissues like skin, hair and nails and produce dermal inflammatory response and intense itching in addition to a cosmetically poor appearance. The

causative fungi colonize only cornified layers of epidermis or suprafollicular portions of hair and do not penetrate into deeper anatomical sites. However, infections of deeper structures like subcutaneous soft tissue can occur under conducive condition. Dermatophytosis comprises of 30-40% of patients in India. The increase in prevalence of dermatophytosis in India is attributed to poor diagnosis, improper treatment and administrative negligence. During the last 40 years, studies of mycotic infections in humans and animals have increased significantly. The prevalence of superficial mycotic infection has risen at such a level that skin mycoses now affect more than 20-25% of the world's population, making them one of the most frequent forms of infections.¹ Recently fungal infections have

* Corresponding author.

E-mail address: agrnehadr@gmail.com (N. Agrawal).

gained the attraction of physicians and microbiologists which is mainly attributed to increase in number of immunodeficiency conditions like AIDS, patients receiving immunosuppressive drugs for malignancies and those under transplantation. So also the widespread use of broad spectrum antibiotics has contributed to the increase in the occurrence of fungal infection.² Dermatophytosis tends to be a chronic condition, disfiguring and is associated with social stigma. Moreover an epidemiological data regarding the prevalence and incidence of dermatophyte species actually causing these infections is not known because patients are mostly treated based on clinical diagnosis. The classical presentation of tinea infection is a lesion with central clearing surrounded by an advanced, red, scaly, elevated border. This presentation though very typical of ringworm infection is very often confused with the other skin disorders, making laboratory diagnosis and confirmation necessary. The prevalence of dermatophytosis is governed by environmental conditions, personal hygiene and individual's susceptibility from place to place.³ This study is carried out to know the profile of the dermatophytes in soil setting of our area Ajmer and its correlation with clinical samples.

2. Objective

To determine the prevalence of keratinophilic fungi in the garden soil samples of Ajmer region and study of its clinical impact.

3. Materials and Methods

It was a prospective ecological study done over a period of 3 months at Department of Microbiology, J.L.N. Medical College, Ajmer.

3.1. Sample collection

Soil samples were collected in sterile polythene bags by scooping up to a depth of 2-5 cm with the help of sterile disposable spoon. Five samples of garden soil (5gm each) were collected from each of the randomly selected 10 sites of Ajmer city & pH of soil was recorded. All samples were processed in the laboratory using hair bait technique and incubated at 28 + 2°C for 3-4 weeks.

3.2. Hair baiting technique^{4,5}

Moist chambers were prepared using sterile Petri dishes and blotting paper & sterile Petri dishes were half filled with soil samples. 2-3 cm short hair strands of sterilized baits were placed over soil surface and 10- 15 ml of sterile water was added to the soil to provide optimal condition for facilitate germination of fungal spores. Petri dishes were than incubated at 28 + 2°C for 3-4 weeks and plates were examined periodically for the development

of mycelium. For fungal examination, a small portion of the fungal growth was picked up and examined by normal saline or Lactophenol cotton blue wet mount preparation microscopically. After a preliminary examination of fungal growth on baits, the growth was subsequently subcultured onto the slants of Sabouraud's dextrose agar (SDA) with chloramphenicol (0.05 mg/ml) and cycloheximide (0.5 mg/ml) and also onto DTM to selectively isolate dermatophytes. 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. The fungi were examined for colony characters, color, texture, pigmentation on reverse side of colony and types of hyphae, hyphal characteristics, presence or absence of spores (macroconidia or microconidia). Further identification of the fungal isolates was on the basis of hair perforation test and urea hydrolysis. All materials and culture media were obtained from HiMedia laboratory Mumbai, India.



Fig. 1: LPCB mount showing macroconidia with few microconidia of *T. rubrum*

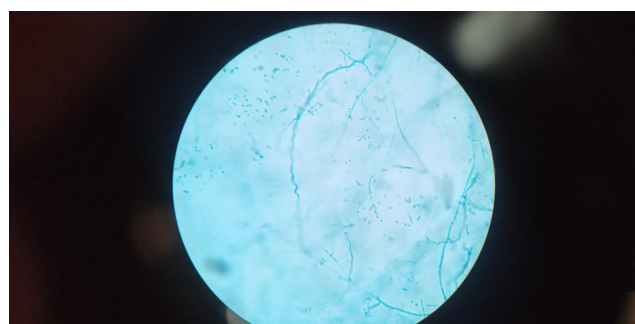


Fig. 2: LPCB mount showing spiral hyphae of *Trichophyton* spp.

3.3. Hair perforation test⁶

The hair perforation test distinguishes between *Trichophyton mentagrophytes* and *T. rubrum*. Hairs exposed to *Trichophyton mentagrophytes* show wedge shaped perforations perpendicular to the hair shaft (a

positive test result), whereas *T.rubrum* do not show these perforation structure.

Short strands of human hair were placed in Petri dishes and dishes were autoclaved at 121°C for 10 min; 25 ml of sterile distilled water and 2 or 3 drop of 10% sterilized yeast extract were added. These plates were inoculated with several fragments of the test fungus that has been grown on SDA. The plates were incubated at 25°C and examined microscopically for perforations by removing a few segments and placing them in a drop of LPCB mounting fluid.

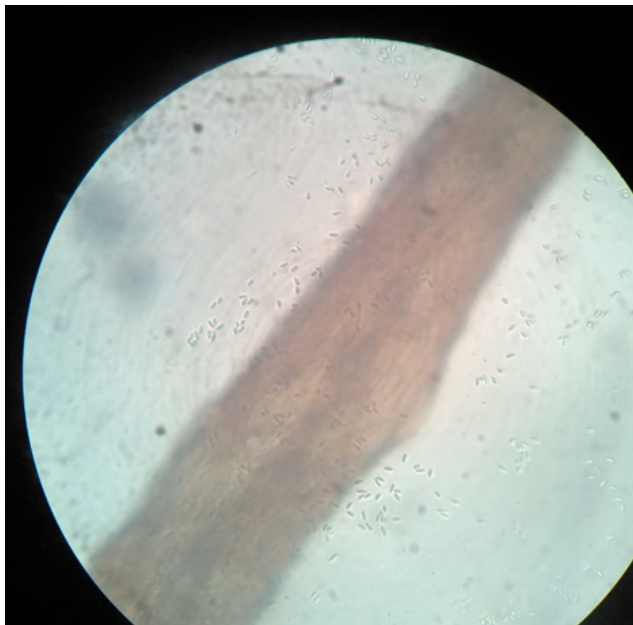


Fig. 3: Negative hair perforation test

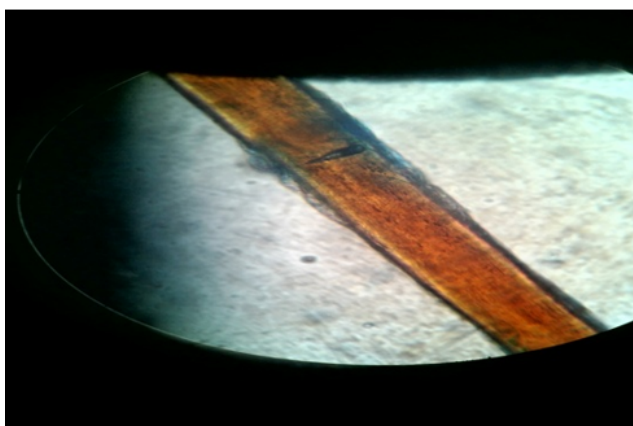


Fig. 4: Hair perforation test showing wedge shaped perforation

4. Results

A total of 50 soil samples were collected from different sites in Ajmer city. Out of 50 soil samples, 28 (56%) were positive for keratinophilic fungi by hair bait technique. Among 28 positive samples, 18(64.28%) were dermatophyte while 10 (35.71%) were non dermatophyte. Most common isolate among dermatophytes isolated was *T. rubrum*(53.71%) followed by *T. mentagrophytes* (10.71%). While among non dermatophyte isolate were *A. niger* (17.85%) and *A. flavus* (17.85%). The optimum pH range for the keratinophilic for was favourable 5.5- 6.8.

5. Discussion

In this study 50 soil samples were collected from different garden soil of Ajmer city. This study was done during the period from October to December 2019. Hair bait technique was found to be very useful in isolation of keratinophilic fungi. Soil sample rich in organic matter was more suitable for keratinophilic fungi. In our study the optimum pH range of the soil yielded growth of keratinophilic fungi was found to be 5.5-6.8 is consistent to the other study result of Ziegler.⁷ While some other studies reported alkaline pH optimum for growth of keratinophilic fungi.⁸ The soil having pH of more than or equal to 6 was found to be favourable for *T. rubrum*, *A.flavus*, *A.niger* while pH range of 5.5 – 6 (acidic) was favourable for *T. mentagrophyte*.

Out of 50 soil samples, 28 (56%) were positive for keratinophilic fungi by hair bait technique. Among 28 positive samples, 18(64.28%) were dermatophyte while 10 (35.71%) were non dermatophyte. Most common isolate among dermatophytes was *T. rubrum*.(53.71%) followed by *T. mentagrophytes* (10.71%). While among the non dermatophyte, isolates were *A.niger* (17.85%) and *A. flavus* (17.85%). In our study, then most common isolate was *T.rubrum* which is also the most common causative agent of *Tinea corporis*. In our hospital, dermatophytic infection including *Tinea corporis* is one of the most common clinical presentation of skin infection. Hence, soil may be a source of infection in case of *Tinea corporis*. Also *A.flavus* & *A.niger* from soil may be a source of human infection. Appropriate preventive measures need to be instituted along with education of the mass to prevent such infections.

6. Conclusion

In this study, the most common isolate was *Trichophyton rubrum* which is also the most common causative agent of *Tinea* infection in our clinical setting. This correlation between environmental and clinical isolates suggests the reason for the increasing dermatophytic infections in our area. The hot & humid climatic conditions of Ajmer were found to be favourable for the growth of dermatophytes which help in acquisition & maintenance of the dermatophytic infections. In association with favourable

Table 1: Distribution of keratinophilic fungi in garden soil samples of ajmer city

S. No.		No of samples	Ph of the soil	Culture status	Dermato-phytes	Non dermatophytes
1	Garden-1	5	6.5	All Positive	<i>Trichophyton</i>	-
2	Garden-2	5	6.8	All Negative	No growth	No growth
3	Garden-3	5	6	All Positive	<i>Trichophyton</i>	-
4	Garden-4	5	6	All Positive	-	<i>Aspergillus</i>
5	Garden-5	5	5.5	2/5 positive	Two sample <i>Trichophyton</i> , Three sample no growth	-
6	Garden-6	5	6	All Negative	No growth	No growth
7	Garden-7	5	5.5	1/5 positive	One sample <i>Trichophyton</i> & Four sample no growth	-
8	Garden-8	5	6	All Positive	-	<i>Aspergillus</i>
9	Garden-9	5	6.8	All Positive	<i>Trichophyton</i>	-
10	Garden-10	5	6.5	No growth	No growth	No growth

Table 2: Prevalence of dermatophytic & non-dermatophytic species in garden soil samples of Ajmer

S. No.	Category	Percentage	Name of spp	Percentage
1	Dermatophytes	18(64.28%)	(A) <i>T.rubrum</i> (B) <i>T. mentagrophytes</i>	15(53.57%) 3(10.71%)
2	Non-dermatophyte	10(35.71%)	(A) <i>A.flavus</i> (B) <i>A.niger</i>	5(17.85%) 5(17.85%)

Table 3: Frequency of occurrence of keratinophilic fungi in soil habitat

S.No.	Name of the fungus	Total culture positive	Percentage value
1	<i>A.niger</i>	5	17.85%
2	<i>A.flavus</i>	5	17.85%
3	<i>T.rubrum</i>	15	53.57%
4	<i>T. mentagrophytes</i>	3	10.71%
5	Total	28	100%

Table 4: Distribution of keratinophilic fungi in soil habitat at Ajmer city

S.No.	Sampling site	Name of species			
		<i>T.rubrum</i>	<i>T.mentagrophytes</i>	<i>A.niger</i>	<i>A.flavus</i>
1	Anasagar	+	-	-	-
2	Madar gate	-	-	-	-
3	Panchsheel	+	-	-	-
4	Jln hospital	-	-	+	-
5	Kundan nagar	-	+	-	-
6	Shastri nagar	-	-	-	-
7	Dhola bhata	-	+	-	-
8	Bus stand	-	-	-	+
9	Vaishali nagar	+	-	-	-
10	Civil lines	-	-	-	-

Table 5: Colony & conidial characteristics of isolated species of keratinophilic fungi

Fungus	Fungus	Colony characteristics	Conidial characteristics
Anamorph <i>T.rubrum</i>	Teleomorph -	Cottony to velvety, white to reddish surface, typically wine red reverse	Macroconidia seldom seen pencil shaped, microconidia drop shaped abundant
<i>T.mentagrophytes</i>	<i>A.benhamiae</i> <i>A.vanbreuseghemii</i>	Powdery to cottony, yellow cream to white surface, pale to red brown reverse	Macroconidia uncommon club shaped, smooth; microconidia drop shaped, abundant, produced mainly in dense tufts
<i>A.niger</i>	-	Colonies black, cottony type, reverse is white	Vesicle is globular- shaped Phialides in two rows Conidia arise from entire vesicle Conidia are black in color
<i>A.flavus</i>	-	Colonies yellow green, velvety, reverse is white	Vesicle is globular- shaped Phialides in one or two rows Conidia arise from entire vesicle Conidia are hyaline

climatic conditions, poor hygiene also plays an important role in the growth of dermatophytes. The diagnosis of this fungal disorder is necessary due to clinical consequence with respect to its contagious nature, cosmetic consequences, chronicity, recurrences, and therapeutic difficulties. Therefore, education of masses about personal hygiene, good sanitation and use of protective measures while working with soil are important measures in controlling these infections. However significant isolation of particular species from specific garden soil needs to be elucidating by further elaborated studies on larger sample size. The importance of this however needs epidemiological follow up of clinical cases with tracking of their location.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare that there is no conflict of interest.

References

- Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. *Mycoses*. 2008;51:2–15. doi:10.1111/j.1439-0507.2008.01606.x.
- Pakshir K, Hashemi J. Dermatophytosis in Karaj, Iran. *Indian J Dermatol*. 2006;51(4):262–4. doi:10.4103/0019-5154.30290.
- Ninet B, Jan I, Bontems O, Lechenne B, Jousson O, Panizzon R, et al. Identification of Dermatophyte Species by 28S Ribosomal DNA Sequencing with a Commercial Kit. *J Clin Microbiol*. 2003;41(2):826–30. doi:10.1128/jcm.41.2.826-830.2003.
- Sharma M, Sharma M. Incidence of dermatophytes and other keratinophilic fungi in the schools and college playground soils of Jaipur, India. *Afr J Microbiol*. 2010;4(24):2647–54.

- Rizwana H, Amal A, Hazzani AL, Siddiqui I. Prevalence of dermatophytes and other keratinophilic fungi from soils of public parks and playgrounds of Riyadh, Saudi Arabia. *J Anim Plant Sci*. 2012;22:948–53.
- Fire A, Ozcan K. Diagnostic value of morphological, physiological and biochemical tests in distinguishing *Trichophyton rubrum* from *Trichophyton mentagrophytes* complex. *Med Mycol*. 2008;46(8):811–22.
- Ziegler. Ectoenzymes of dermatomycetes. II pH dependence and substrate specificity. *Arch Clin Exp Dermatol*. 2006;226:282–99.
- Jain N, Sharma M. Distribution of dermatophytes and other related fungi in Jaipur city, with particular reference to soil pH. *Mycoses*. 2011;54(1):52–8.

Author biography

Madhu Yadav, MSc Student

Neha Agrawal, Senior Demonstrator

Mukul Chaurasia, Senior Demonstrator

Vijaylatha Rastogi, Professor and Head

Pushpanjali Verma, Senior Demonstrator

Abila Parashar, Senior Research Fellow

Govind Meena, MSc Student

Cite this article: Yadav M, Agrawal N, Chaurasia M, Rastogi V, Verma P, Parashar A, Meena G. Prevalence of keratinophilic fungi in the garden soil samples in Western India and study of its clinical impact. *Indian J Microbiol Res* 2021;8(1):15-19.