### Study of Keratinophilic Fungi Isolated from Nagaur (Rajasthan, India) Soil

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### ABSTRACT

This study investigates the presence of keratinophilic fungi in soil of Nagaur district of Rajasthan, India. Most of the people of Nagaur district of Rajasthan are involved in animal husbandry and farming, which make them prone to fungal infection due to soil contact. Rising incidents of skin-related fungal infections necessitate the study of the presence of keratinophilic fungi in the Nagaur district. In the present study, keratinophilic fungi were isolated using hair bait technique. In this technique, different hair baits were used such as pigeon feather, hen feather, human hair and goat hair. A total of 75 soil samples were collected from different areas of Nagaur district. Out of 75 soil samples, 52 samples were found positive for keratinophilic fungi in the soil of Nagaur district which can pose risk to public health.

Keywords: Keratinophilic fungi, Pathogenic, Dermatophytes, Soil sample, pH.

### **INTRODUCTION**

Throughout worldwide, soil is the main habitat for fungi and as such, the main reservoir for occurrence and activity of keratinophilic fungi. The keratinolytic activity of soil keratinophilic fungi has attracted the attention of many researchers and the keratinophilic fungi play an important role in the natural degradation of keratinized residues in the soil (Pakshir et al., 2013). Nagaur, a district in Rajasthan, India is characterized by extensive animal husbandry and farming practices, which generate large quantities of keratin-rich residues. The district also has environmental conditions favourable for the growth of fungi. The distribution of keratinophilic fungi and bacteria in the soil largely depends on the existence of a relatively high quality of keratinic material of either human or animal origin or both in the soil. Keratin is widely found in hair, nail, feather, hoof, horn and skin (English, 1969). Consequently, soil enriched with keratinous materials is highly conducive to the growth and survival of these fungi (Otcenasek, 1978; Mercantini, et al., 1993). Vanbreuseghem (1952) was the first to report the isolation of keratinophilic fungi using the "To. Ka. Va. hair baiting technique," which has been widely adopted for this purpose. In this technique, different baits are used, including feathers, hair, nail clippings, hooves. Environmental horns, and factors

responsible for growth, sporulation, sexual reproduction and morphological characteristics of keratinophilic fungi include pH, temperature and culture media (Singh and Sharma, 2014). The specific ecological conditions in Nagaur, including its arid climate and farming practices, may offer unique insights into the ecological role, behaviour, and distribution of these fungi. The present study was aimed to fill the research gap by examining the occurrence and distribution of keratinophilic fungi in the soils of Nagaur district, which is crucial for assessing soil health and nutrient cycling, particularly in arid ecosystems where organic matter is limited.

### MATERIALS AND METHODS

### Sample sites and collection of soil samples

Rajasthan is the largest state of India constituting 10.4% of total geographical area. Nagaur, a district in Rajasthan, experiences desert-like climatic conditions with extreme temperatures ranging from  $40^{\circ}$ C to  $49^{\circ}$ C during summer and as low as  $2^{\circ}$ C during winter. These environmental factors may influence the microbiota, soil including keratinophilic fungi. In the present study, 75 soil samples were collected from various sites of Nagaur district during August 2021 to February 2022. The soil samples were collected from animal habitats, hospitals, roadsides, municipal dump area,

near water bodies and the dump area of barber shops, as these selected sites are known to be rich in keratinous substrates, making them ideal environments for keratinophilic fungi. The soil samples were collected from the surface, ensuring a depth not exceeding 4–5 cm, using a sterile spatula. Each sample, weighing approximately 500 g of soil was placed into sterile polyethylene bags. These bags were then securely sealed, labelled with the location name, and transported to the laboratory for the isolation of keratinophilic fungi. In the laboratory, four replicates of each soil sample were prepared for analysis. This sample size was determined based on the need to collect a representative amount of soil from diverse environments to isolate the variety of keratinophilic fungi present.

### Measurement of soil pH

Model-LT-10 Microprocessor pH meter was used to determine the pH of soil, the device was calibrated with buffer solutions of pH 7.00 (neutral), 4.00, and 9.2. Calibration was started with the neutral buffer, followed by acidic and basic buffers. Soil samples (5 g) were mixed with 10 mL distilled water to prepare the soil suspension, and the pH was measured by inserting the electrode into the solution. For accuracy, 3 replicates were taken for each sample. The average of replicate readings was taken as the final pH. Controls included temperature compensation to adjust for Temperature effects and Slope Adjustment, ensuring that the slope remained between 95% and 105% for accurate results.

## Keratin substrate

Keratin substrates including human hair (from salons), animal hair (from farms and veterinary clinics), pigeon feathers (from birdhouses) and hen feathers (from poultry farms) were collected. These substrates represent diverse keratin types commonly found in environments where keratinolytic fungi thrive. Their relevance lies in mimicking natural keratin sources, aiding in studying fungal degradation. After collection, the substrates were defatted with chloroform:methanol, washed with deionized water, oven-dried at 40°C, cut into small pieces (2-3 cm) and sterilized by autoclaving.

### **Processing of samples**

Soil samples were shade-dried in a clean, covered area to prevent contamination. Sterilized tools and gloves were used and the surfaces were disinfected regularly. The drying environment was kept dustfree and protected from direct sunlight and sieved for the hair baiting technique (Vanbreuseghem, 1952). Shade-drying helps maintain the soil's integrity while preventing overheating and preserving its microbial community. In this technique, soil samples (60 g) were filled in sterile Petri dishes (90mm) and moistened with autoclaved distilled water. Small pieces of autoclaved keratin substances including human hair, animal hair, pigeon feathers, and chicken feathers were aseptically spread on top of the soil sample. The plates were incubated at  $27 \pm 2^{\circ}$ C under low light conditions for 2-3 weeks.

# Isolation and identification of keratinophilic fungi

Fungal mycelia were transferred to Sabouraud Dextrose (SDA) slants Agar containing streptomycin (100 mg/L) and chloramphenicol (50 mg/L) to prevent bacterial growth. The cultures were then incubated at 27±2°C for two weeks. For species identification, a compound microscope (Olympus BX53) with magnifications of 10x and 40x was used. Lactophenol cotton blue stain was applied when necessary to enhance the visibility of fungal structures. The cellophane tape method was used to lift delicate fungal structures from colonies for direct observation under the microscope. Key morphological traits such as growth time, colony colour of both the upper surface and reverse side of the colony, conidia attachment including size, shape, arrangement (whether singly attached or in chains/clusters) and reverse pigmentation were observed. These characteristics were compared to reference descriptions of Ellis (1971, 1976) and Walsh et al. (2018) for species identification. The percentage frequency was determined as follows (Nigam and Kushwaha, 1990).

# $\frac{Percentage \quad of \quad Frequency = \frac{No.of \ isolates \ of \ a \ fungi}{Total \ No.of \ iolates} \ X100$

This metric helps to evaluate the ecological dominance of specific keratinophilic fungi across different environmental niches. By determining the frequency of each fungus, identification of species which are more prevalent or adapted to particular conditions can be find out and it helps in the understanding of fungal distribution and ecological roles in keratin degradation.

### RESULTS

The results of the isolations are presented in **Tables 1** and **2** showing the prevalence and distribution of keratinophilic fungal biota in Nagaur district, Rajasthan. From the 75 soil samples collected, a total of 133 colonies of keratinophilic fungi were isolated, belonging to the following 13 genera: *Trichophyton* (33.83%), *Fusarium* (24.81%), *Aspergillus* (11.27%), *Keratinophyton* (8.27%), *Scedosporium* (5.26%), *Epidermophyton* (3.75%), *Scopulariopsis* (3.75%), *Chrysosporium* (3.75%), *Histoplasma* (2.25%), *Cladosporium* (0.75%), *Penicillium* (0.75%), *Rhizopus* (0.75%), and *Trichoderma* (0.75%) (**Table 1**). Among these, *Trichophyton* (33.83%) was the most predominant genus, followed by *Fusarium* (24.81%) and *Aspergillus* (11.27%).

<b>Table 1:</b> Keratinophilic fungi isolated from soil samples.	
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Name of Fungal Genus	Number	Percentage
Fusarium	33	25%
Trichophyton	45	34%
Epidermatophyton	5	3%
Trichoderma	1	1%
Histoplasma	3	2%
Chrysosporium	5	4%
Scedosporium	7	5%
Aspergillus	15	11%
Scopuloriopsis	5	4%
Keratinophyton	11	8%
Cladosporum	1	1%
Rhizopus	1	1%
Penicillium	1	1%
Total	133	100%

From the 75 soil samples, 25 species of keratinophilic fungi were isolated. *Fusarium solani* (11.2%) was the most common keratinophilic fungus found across all sites, followed by *Fusarium moniliforme* (9.7%) and *Trichophyton terrestre* 

(8.27%). The barber dump soils were found to be the most suitable habitat for the growth of keratinophilic fungi (27.06%) followed by animal habitat (25.56%) (**Table 2**).

 Table 2: Percentage frequency (%) of keratinophilic fungi isolated from Nagaur.

Isolated Fungi	Source of soil samples							Frequency %
	Animal Habitat	Road side	Barber dump	Hospital area	Municipal dump	Water bodies		
Aspergillus flavus	0	1	0	1	0	1	3	2.25
Aspergillus fumigatus	1	0	0	0	0	0	1	0.8
Aspergillus nidulans	0	1	1	1	0	1	4	3
Aspergillus niger	3	1	0	1	1	0	6	4.51
Aspergillus terreus	0	0	1	0	0	0	1	0.8
Chrysosporium indicum	0	0	1	0	1	0	2	1.5

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Isolated Fungi	Source of soil samples							Frequency %
	Animal Habitat	Road side	Barber dump	Hospital area	Municipal dump	Water bodies		
Chrysosporium tropicum	0	0	1	1	1	0	3	2.25
Cladosporum sp.	1	0	0	0	0	0	1	0.8
Epidermophyton floccosum	1	0	1	2	1	0	5	3.7
Fusarium moniliforme	6	3	0	0	0	4	13	9.7
Fusarium oxysporum	1	2	0	0	0	2	5	3.75
Fusarium solani	5	0	7	0	0	3	15	11.2
Histoplasma capsulatum	2	0	0	0	1	0	3	2.25
Keratinophyton terrum	2	0	1	0	3	0	6	4.51
Keratinophyton clavisporum	1	0	2	0	2	0	5	3.75
Penicillium sp.	0	1	0	0	0	0	1	0.8
Mucor sp.	0	0	0	0	0	1	1	0.8
Scedosporium satunence	0	0	2	3	2	0	7	5.26
Scopulariopsis brevicaulis	3	0	2	0	1	0	5	3.75
Trichoderma sp.	0	0	0	0	1	0	1	0.8
Trichophyton equinum	1	0	5	2	2	0	10	7.51
Trichophyton simii	1	1	3	1	2	0	8	6.01
Trichophyton terrestre	4	1	2	1	3	0	11	8.27
Trichophyton verrucosum	1	0	3	0	2	0	7	5.26
Trichophyton violaceum	1	2	4	1	1	0	9	6.76
Total isolates	34	13	36	14	24	12	133	100%
Percentage %	25.56	9.77	27.06	10.5	18.04	9.02	100	

The soil pH ranges from 6.00 to 10.00, with most fungi isolated from soil samples having a pH range between 7.00 and 7.99 (54.04%) followed by pH range of 8.00-8.99 (39.76%). Predominant genera in pH range 7.00-8.99 were *Trichophyton*, *Fusarium* and *Aspergillus*. *Scopulariopsis brevicaulis* (2.25%), *Fusarium moniliforme* (1.5%) and *Chrysosporium tropicum* (0.75%) were reported in soils with a pH of 6.00 to 6.99, while *Chrysosporium indicum* (0.75%) and *Penicillium sp.* (0.75%) were found in soils with a pH range 8.00- 8.99 (**Table 3**). Prevalence of keratinophilic fungal flora on various baits is presented in **Table 4**. Pigeon feather harboured maximum fungal species (49), followed by hen feature (40), goat hair (23) and human hair (21). The major incidence ratio of keratin degradation using the baiting technique was observed to be maximum on pigeon feathers (36.84 %), followed by hen feathers (30.07%), goat hair (17.29%) and human hair (15.78%), indicating a descending order of growth preference.

# **Table 3:** Keratinophilic fungi isolated from soils with different pH.

Isolated fungi	Soil pH							
	6.0	6.00-6.99 7.00-7.99			8.0	0-8.99	9.00-9.99	
	n	%	n	%	n	%	n	%
Aspergillus flavus	0	0.00%	1	0.75%	4	3.00%	0	0.00%
Aspergillus fumigatus	0	0.00%	5	3.75%	0	0.00%	0	0.00%
Aspergillus nidulans	0	0.00%	1	0.75%	3	2.25%	0	0.00%
Aspergillus niger	0	0.00%	6	4.51%	2	1.50%	0	0.00%
Aspergillus terreus	0	0.00%	1	0.75%	1	0.75%	0	0.00%
Chrysosporium indicum	0	0.00%	2	1.50%	7	5.26%	1	0.75%
Chrysosporium tropicum	1	0.75%	5	3.75%	2	1.50%	0	0.00%
Scedosporum satuense	0	0.00%	2	1.50%	4	3.00%	0	0.00%
Cadosporum sp.	0	0.00%	1	0.75%	0	0.00%	0	0.00%
Epidermophyton floccosum	0	0.00%	7	5.26%	3	2.25%	0	0.00%
Fusarium moniliforme	2	1.50%	0	0.00%	1	0.75%	0	0.00%
Fusarium oxysporum	0	0.00%	5	3.75%	2	1.50%	0	0.00%
Fusarium solani	0	0.00%	2	1.50%	0	0.00%	0	0.00%
Keratinophyton terrum	0	0.00%	5	3.75%	2	1.50%	0	0.00%
Keratinophyton sp.	0	0.00%	1	0.75%	5	3.75%	0	0.00%
Penicillium sp.	0	0.00%	0	0.00%	0	0.00%	1	0.75%
Rhizopus sp.	0	0.00%	1	0.75%	1	0.75%	0	0.00%
Scopulariopsis brevicaulis	3	2.25%	5	3.75%	1	0.75%	0	0.00%
Trichoderma sp.	0	0.00%	1	0.75%	0	0.00%	0	0.00%
Trichophyton equinum	0	0.00%	1	0.75%	2	1.50%	0	0.00%
Trichophyton simii	0	0.00%	6	4.51%	4	3%	0	0.00%
Trichophyton terrestre	0	0.00%	4	3.00%	5	3.75%	0	0.00%
Trichophyton verrucosum	0	0.00%	7	5.26%	3	2.25%	0	0.00%
Trichophyton violaceum	0	0.00%	3	2.25%	1	0.75%	0	0.00%
Total	6	4.50%	72	54.04%	53	39.76%	2	1.50%

**Table 4:** Prevalence of keratinophilic fungal flora on various baits.

Isolated fungi	Various baits							
	Human hair	Goat hair	Pigeon feather	Hen feather	Total plates	%		
Fusarium	4	5	20	4	33	24.81		
Trichophyton	7	8	14	16	45	33.83		
Epidermatophyton	0	1	3	1	5	3.75		
Trichoderma	0	0	1	0	1	0.75		
Histoplasma	2	1	0	0	3	2.25		
Chrysosporium	0	1	1	3	5	3.75		
Scedosporium	0	1	5	1	7	5.26		
Aspergillus	5	6	4	0	15	11.27		
Keratinophyton	3	0	1	7	11	8.27		
Cladosporum	0	0	0	1	1	0.75		
Mucor	0	0	0	1	1	0.75		
Penicillium	0	0	0	1	1	0.75		
Total	21	23	49	40	133	100%		

### DISCUSSION

Numerous investigations conducted in various parts of Rajasthan over the past decades have revealed a rich diversity of keratinophilic fungi in the soils of these areas. However, no studies have specifically focused on the mycoflora of the soil in Nagaur district. Therefore, this current study was aimed to detect prevalence and distribution of keratinophilic fungi in the soil of Nagaur district. The extensive farming and cattle rearing activity, along with the rising numbers of poultry farms, are likely to introduces keratinous matter. These keratinous wastes can serve as substrates for keratinophilic fungi.

In the present study, 75 soil samples were collected and a total of 133 colonies of keratinophilic fungi were isolated, belonging to 13 genera. Trichophyton (33.83%), *Fusarium* (24.81%), Aspergillus (11.27%), Keratinophyton (8.27%) were the predominant genera observed in this study which are in agreement with Kumawat et al. (2019) who collected 50 soil samples from Jaipur, Ajmer, Alwar, and Sikar in Rajasthan, India. From these samples, 154 isolates were obtained, identifying 31 keratinophilic fungal species across 16 genera. Sharma and Choudhary (2015) recorded a rich source of pathogenic keratinophilic fungi including some species of dermatophytes from the soil of agricultural fields in the Saharanpur Village (Uttar Pradesh). Randhawa and Sandhu (1964) described Keratinophyton terreum as gen. nov., sp. nov. from India. In the present study, Keratinophyton terreum was isolated for the first time from Rajasthan.

Sharma et al. (2020) collected 50 soil samples from various sites of Jaipur district, Rajasthan to study the occurrence of keratinophilic fungi. Out of 162 isolates, they reported 24 keratinophilic fungal species belong to 13 genera, including Trichophyton sp.(17.90%), Aspergillus sp.( 17.90%), Chrysosporium sp. (14.81%). The present study revealed that Fusarium solani (11.2%) was the most common keratinophilic fungus found across all sites, followed by Fusarium moniliforme (9.7%) and Trichophyton terrestre (8.27%). A comparative analysis of fungal incidence at various habitat showed that barber dump soils were found to be the most suitable habitat for the growth of keratinophilic fungi (27.06%) followed by animal habitat (25.56%). Members of Fusarium genus are causative agents of keratitis (Eghttedari and Pakshir, 2006). Moallaei et al. (2006) also reported that Fusarium was the most prevalent saprophyte in South and Razavi Khorasan Provinces, Iran. Sharma et al. (2023) observed that Aspergillus and Trichophyton genera was most predominant fungi in agricultural land of Kota (Rajasthan). Trichophyton terrestre was the third dominant species in the present study. This is in agreement with Nardoni and Mancinati (2021). The other geophiles belonging to Trichophyton genus were Trichophyton equinum (7.51%),Trichophyton violaceum (6.76%), Trichophyton simii (6.01%) and Trichophyton verrucosum (5.26%).

The current study explored the relationship between the frequency of fungi and soil pH. Fungal growth is heavily influenced by pH, as fungi optimize their metabolic processes within specific pH ranges. Enzyme activity, nutrient absorption, and membrane integrity are all pH-dependent. According to Bhadauria and Sharma (2001), several edaphic factors affect the distribution of keratinophilic fungi in soil samples. In the present study, all the 133 isolates of keratinophilic fungi were from soils with pH levels between 6.00 and 9.00. The majority of fungi were found in soil samples with a pH between 7.00 and 7.99 (55.54%), while 38.26% were isolated from soils with a pH of 8.00-8.99. Only 4.50% of keratinophilic fungi were present in soils with pH levels between 6.00 and 6.99. These findings align with previous research of Jain and Sharma (2022) who reported that most of the keratinophilic fungi grow in the soil with pH range 7.75-8.81 and humid temperature. The major incidence ratio of keratin degradation using the baiting technique was observed as maximum on pigeon feathers (36.84 %), followed by hen feathers (30.07%), goat hair (17.29%) and human hair (15.78%).

### CONCLUSION

The findings of present study indicated that soils from locations such as animal habitats, barber shop dump, roadsides, hospitals and Municipal dumps and areas near water bodies provide an ideal environment for the growth and presence of keratinophilic fungi, including geophilic dermatophytes. These environments support fungal proliferation due to the abundance of keratin substrates such as hair and feathers, as well as organic debris present in the soil. The presence of organic matter and keratin substrates significantly influences the occurrence of keratinophilic fungi in these soils. Additionally, the present study demonstrated that ecological factors, particularly soil pH, play a crucial role in the distribution and growth of keratinophilic fungi in the soil. This poses a potential public health risk, as these fungi can cause infections such as ringworm in humans and animals through frequent contact with contaminated soil. Mitigating exposure in such environments is crucial to reducing the risk of infection.

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