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Isolation and Identification of Keratinophilic fungal biota from different soil samples of Agricultural lands of Kota city of Rajasthan, India

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Abstract

Keratinophilic fungi are a group of ecologically important fungi that cycle one of the most abundant and highly stable animal proteins on earth. The biological function of keratinolytic fungi in the soil is the degradation of keratinized materials. Due to decomposition of keratin substances decay, these fungi are important in soil ecosystem. The study was conducted to analyse the isolation and identification of keratinophilic fungi from various soil samples. The soils samples were obtained from agricultural land of Kota (Rajasthan) which were rich in pathogenic keratinophilic fungi including dermatophytes. The samples were analysed in laboratory using a spread hair bait technique. The isolated 7 genera and 10 species of different fungi from twenty soil samples belongs to *Aspergillus niger, Aspergillus flavus, Trichophyton rubrum, Microsporum gypseum, Microsporum canis, Rhizopus arrhizus, Pencillium citrinium, Chrysosporium tropicum, Fusarium solani, Aspergillus fumigatus. Aspergillus flavus* was found to be the most predominant fungi in alkaline pH and salinity environment and *Trichophyton rubrum* was the second predominant fungi. The present study concluded that the agricultural soil that consists of keratin as substrate were very rich in the population of keratinophilic fungi. The fungi isolated in our study belongs to both keratinophilic and dermatophytes group. The study needed to be extended upto the enzymatic level in further assignments.

Keywords: *Keratinophilic Fungi, Hair bait technique, Dermatophytes, Fungal Isolates, Human hair.*

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Introduction

The soil constitutes one of the most complex of microbial habitats in which many fungi complete their entire life cycle [1]. Soil rich in keratinous materials are the most conductive medium for the growth and occurrence of keratinophilic fungus [2]. Keratinophilic fungi found in various sources 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 [1-2].

Keratin is a natural fibrous protein forming the outermost keratinized layer of humans and animals. Various keratinous substrates occur in nature in various forms such as hair, wool, feathers, nails, claws, quills, scales, horns, hooves, and tortoise shell and in the outer layer of skin. Keratinophilic fungi are natural colonizers of different keratinous substrates and degrade them to components of low molecular weights. These fungi are classified as (a) Keratinophilic fungi (grow on keratinic materials) (b) keratinolytic fungi (capable of decomposing keratin completely) and (c) Dermatophytes belong to the genera *Trichophyton, Microsporum, and Epidermophyton* [3]. All the dermatophytes are keratinolytic in nature. It creates inflammation, pruritus, and desquamation by invading the stratum corneum as well as the nails and the hair shaft. [4] However, globally they occur in countries with a hot and humid climate [5]. The best candidates for the growth of keratinolytic and saprophytic fungi are the forest, agricultural field, farmyard, park soils, as well as sediments of the rivers and oceans contained humus and organic material [6]. Therefore, the ecological and hygienic interests have led us to study the keratinophilic mycoflora of agricultural field where farmers, and animals spend a large proportion of their time and get exposed to pathogenic fungi [7]. This study helped us to know the distribution and occurrence of dermatophytes and other keratinophilic fungi in the selected areas. Also, the study emphasizes on the risk of human dermatophytosis in those regions, which could have a role in degradation of keratinous materials from industrial point of view [8].

These fungi play an important ecological role in decomposing α-keratins, the insoluble fibrous protein. Because of tight packing of hair polypeptide chains in α-helix structures and their linkages by disulphide bonds, they are poorly biodegradable [9]. The soil of agricultural field of Kota District has not been investigated for keratinophilic fungi previously, therefore the study sites chosen in this project are much suitable for the investigation of keratinophilic fungi

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and their distribution in the agricultural soils of Kota because of having a subtropical climate and geographical diversity leading to the area suitable for the habitat of these kind of fungi. The study has been chosen particularly to know the assortment of these keratinophilic fungi in the agricultural fields and the presence of dermatophytes among these fungi and the exposure of farmers towards these fungi.

Materials and Methods

Collection of soil samples

A total of 20 soil samples were collected from different Agricultural fields of Kota (Rajasthan) during the period from May-June 2022. The samples were collected in sterile polythene bags and brought to the laboratory for further microbiological analysis [10].

Physicochemical properties of soil samples

The collected soil samples were analyzed for physicochemical parameters such as pH, temperature, electronic conductivity, total dissolve solid (TDS), and Salinity using soil analysis kit. Other physicochemical parameters like dissolve oxygen (DO), Chemical oxygen demand (COD), and Chloride content were analyzed by Standard procedures [11].

Isolation and Identification of keratinophilic fungi from soil

The isolation was carried out as per the method of "hair bait technique'' given by Vanbreuseghem in 1952 [12-13]. The cultured fungi were identified on the basis of their cultural colony characteristics and microscopic observations (Appearances and Texture of mycelium, color, attachment, and size of conidia) [14,15].

The percentage frequency was defined as follows: [16]

$$\text{Percentage (\%) frequency} = \frac{\text{No. of isolates of a fungus}}{\text{Total no. of isolates}} \times 100$$

Results and Discussion

The soils samples were collected from different agricultural area of Kota and the fungal species were isolated and identified using cultural characterization on agar plates. The physico-chemical parameters analysis of soil samples was represented in table 1 and it was obtained that maximum numbers of fungi were present in the pH range of 7.10- 7.91 as

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keratinophilic fungi develop much better in alkaline pH. The salinity was found suitable for the growth and development of these fungi according to the results obtained. The high TDS values indicates the high pollution level of soil that automatically decreases the DO of that particular soil. The results of isolation and characterization of keratinophilic fungi were listed in table 2 and 3 and Fig. 1 and 2. The data reveals that the twenty soils samples contain seven genera and ten species of keratinophilic fungi from different agricultural fields contaminated with keratin rich substrates for these fungi. The numerous mycelium structures of hyphae and spores visualized under microscope confirms the presence of keratinophilic fungi. As per the analysis of Simpanya and Baxter (1996), several rich keratinophilic fungal flora were investigations from various parts of India during last few years [17].

Table.1 Physicochemical parameters soils samples collected from various agricultural land sites

S.NO.	Source Sites	pH	EC (mS)	TDS (mg/l)	Salinity (mg/l)	DO (mg/l)	SOC %
	Standard	7- 8.5	0.01-1.6 S m^{-1}	>450mg/l	4-18	Above 6.5-8 mg/l	3-6 %
1	Sanija bavadi Sultanpur	7.49±0.03	0.63±0.03	63.0±0.03	0.391±0.03	23.1±0.03	0.59±0.035
2	Valapura Kota	7.81±0.016	0.64±0.03	151.1±0.03	0.421±0.03	21.5±0.018	0.53±0.03
3	Kalyan pura village	7.51±0.08	0.77±0.03	143.4±0.03	04.31±0.03	22.6±0.03	0.60±0.06
4	Budadit village	7.55±0.10	1.00±0.03	51.2±0.016	0.435±±0.01	19.1±0.08	0.53±0.03
5	Kisan ganj	7.59±0.02	0.62±0.03	76.5±0.03	0.532±0.016	6.7±0.03	0.55±0.03
6	Khajali village	7.62±0.03	0.71±0.03	3.17±0.03	0.157±0.03	68.9±0.03	0.48±0.02
7	Sangdod	7.41±0.01	1.10±0.03	0.342±0.03	0.532±0.10	7.4±0.03	0.53±0.03
8	Kaliya Khedi	7.12±0.11	1.00±0.03	4.18±0.03	0.251±0.03	79.6±0.03	0.57±0.03
9	Kota ARS	7.75±0.03	0.32±0.03	5.16±0.03	0.981±0.01	8.2±0.01	0.66±0.01

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	Akhnera						
10	Teliya khedi near Ujad river	7.29±0.03	1.00±0.03	4.02±0.03	0.297±0.03	28.2±0.01	0.69±0.03
11	Ladpura	7.13±0.03	1.10±0.03	5.39±0.03	0.335±0.03	13.3±0.03	0.59±0.03
12	Nayagaun	7.10±0.03	1.30±0.05	58.1±0.01	0.153±0.001	14.9±0.01	0.57±0.05
13	Kheda	7.75±0.01	1.30±0.01	2.97±0.03	0.386±0.03	15.1±0.03	0.89±0.03
14	Alod	7.41±0.01	1.50±0.02	21.39±0.01	1.88±0.04	24.8±0.03	0.50±0.05
15	Arjunpura	7.57±0.03	0.67±0.01	24.55±0.03	0.268±0.04	12.7±0.01	0.64±0.03
16	Balupa	7.11±0.03	1.22±0.03	3.21±0.03	0.324±0.03	16.1±0.03	0.43±0.05
17	Aamli	7.31±0.05	0.42±0.01	8.33±0.03	0.514±0.07	67.2±0.02	0.56±0.03
18	Bhadana	7.42±0.01	1.38±0.08	43.16±0.03	0.236±0.03	35.1±0.03	0.76±0.02
19	Bhaura	7.91±0.03	0.64±0.02	38.27±0.07	0.159±0.03	46.5±0.03	0.73±0.03
20	Bhanwaria	7.13±0.02	0.71±0.01	2.18±0.03	0.489±0.07	15.9±0.04	0.87±0.01

EC=Electronic Conductivity, TDS= Total dissolve solids, DO= Dissolved oxygen, SOC= Soil organic carbon

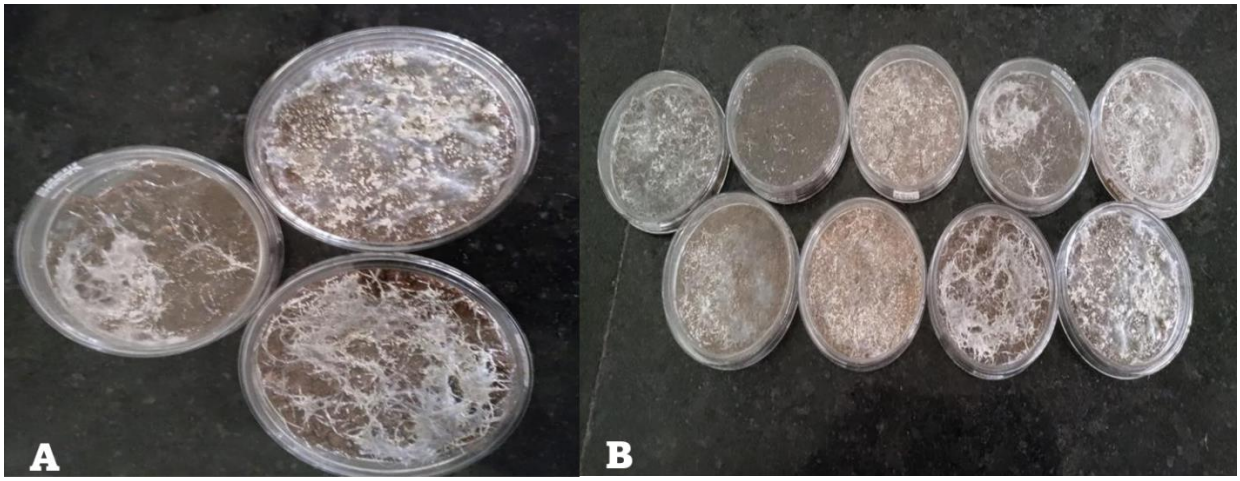

Figure 1 Keratinophilic fungi were isolated from different soils samples A. using 'Hair bait technique' and B. growth of fungi after 15 days

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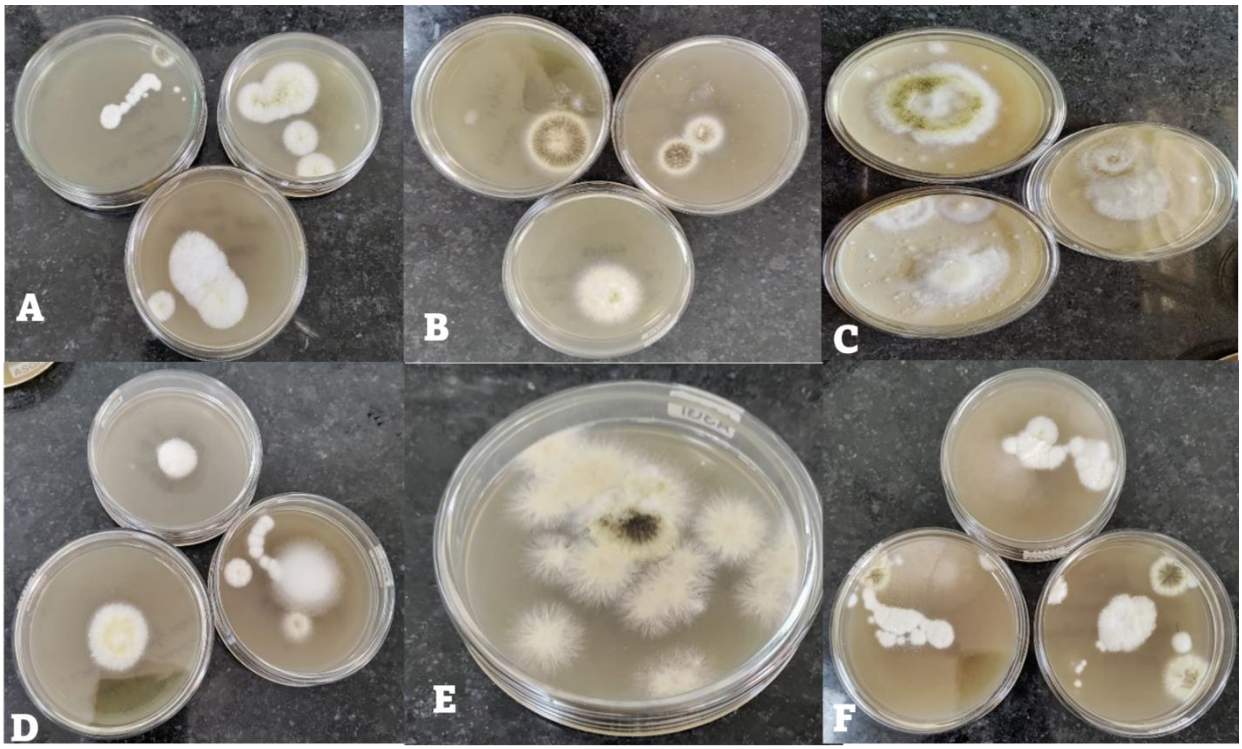

Figure-2 Isolated culture showing A. *Microsporum gypseum* and *Trichophyton rubrum*; B. *Aspergillus flavus* and *Fusarium solani*; C. *Aspergillus niger* and *Chrysosporium tropicum;* D. *Trichophyton rubrum* and *Aspergillus flavus;* E. *Rhizopus arrhizus* and *Microsporum gypseum;* F. *Rhizopus arrhizus* and *Penicillium citrinium*

In the present study most of the isolated keratinophilic fungi belongs to the following genera: *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Trichophyton rubrum, Microsporum canis, Microsporum gypseum, Chrysosporium tropicum, Penicillium citrinum, Fusarium solani, Rhizopus arrhizus* as identified by their characteristic colony morphology and microscopic view. The results for the characterization has been listed in Table-2. The keratinophilic fungi of these genera were found in agricultural field due to the activity of wild and domestic animals in these fields and the reservoirs for these organisms. *Aspergillus flavus, Trichophyton rubrum, Aspergillus niger* and *Microsporum canis* were found to be the most common keratinophilic fungi present in soils having slightly alkaline pH.

Table – 2. Morphological and Microscopic characterization of fungal isolates

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S. No.	Fungal isolates	Morphological Characterization		Microscopical Observations
		Surface	Reverse	
1	*Aspergillus flavus*	Olive to lime green	Cream or pale	Mycelium septate, Conidial head are mostly radiate, conidiophores are rough, conidia are round with smooth to finely roughed walls, appear in chain
2	*Trichophyton rubrum*	White to bright yellowish beige or red violet	Pale or yellowish brown	Slender clavate to pyriform Microconidia are irregular shape, macroconidia are absent
3	*Aspergillus niger*	White to black	Pale yellow	Septate, unbranched mycelium, conidial head are mostly radiant, small rough conidia.
4	*Microsporum canis*	White to yellowish	Deep yellow to yellow orange	Septate hyphae, macroconidia are spindle shaped with an asymmetrical apical knob, 6 to 15 celled long rough. Macroconidia are unicellular and clavate to pyriform in shape.
5	*Penicillium citrinium*	White to grey green	Pale, brown or deep yellow brown	Mycelium is septate and hyaline, smooth walled conidiophores stipes brush like cluster penicillin.
6	*Fusarium solani*	Cream to white	Cream reverse	Mycelium septate and hyaline, moderately curved, thick-walled macroconidia, microconidia are 1 to 3 celled, borne from long monophylies.
7	*Microsporum gypseum*	White to yellowish	Deep yellow or yellow orange	Septate hypha, Macroconidia are thin and rough contain 3to6 cells. Macroconidia are drop-shaped, pear and club shaped.
8	*Rhizopus arrhizus*	White to grey black	Pale white	Filamentous branching hyphae Long rare septa, rhizoids are present, black sporangia at the tip of sporangiophore.
9	*Chrysosporium tropicum*	White cream tan to pale brown	White to brown	Septate hyphae, conidia are broad based, one celled and occur terminally on pedicels along the sides of hyphae.
10	*Aspergillus fumigatus*	Smoky grey green	Slight yellow	Aseptate hyphae, spiked conidia present in chain, flask-shaped (phialides) conidiophores

Table - 3 Majority of fungal isolates obtained from various sample sites

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S. No.	**Species**	Sites of Isolation	**% Isolation**
1	*Aspergillus flavus*	Sanija bavadi Sultanpur, Valapura, Budadit village, Kisan ganj, Sangdod, Kota ARS Akhnera, Arjunpura, Aamli, Bhadana, Kheda	**50%**
2	*Trichophyton rubrum*	Valapura, Kisan ganj, KaliyaKhedi, ARS Akhnera, Kheda, Balupa, Bhaura	**35%**
3	*Aspergillus niger*	Kalyan pura village, Khajali village, Teliya khedi near Ujad river, Balupa, Bhaura	**30%**
4	*Microsporum canis*	Teliya khedi near Ujad river, Kisan ganj, Khajali village	**20%**
5	*Microsporum gypseum*	Sanija bavadi Sultanpur, Bhadana, Bhanwaria	**15%**
6	*Fusarium solani*	Budadit village, Sangdod	**10%**
7	*Penicillium citrinium*	Kalyan pura village, Aalod,	10%
8	*Rhizopus arrhizus*	ARS Akhnera	**5%**
9	*Chrysosporium tropicum*	Nayagaun	**5%**
10	*Aspergillus fumigatus*	Kaliya Khedi	**5%**

The results revealed that the 50% fungal isolates obtained from various agricultural fields belongs to *Aspergillus flavus* followed by *Trichophyton rubrum* (35%) and *Aspergillus niger* (30%) (Table-3). Similar results were observed in the study of Ramesh and Hilda, performed in 1999 [18]. The keratinophilic fungal flora isolated from Kota district was marked with particular references of soils pH. *Aspergillus* genera as the most predominant fungi and *Trichophyton* genera as the second predominant fungi were obtained from soils having pH range from 7.12 to 7.91. There is natural evaluation of keratin-utilizing soil saprophytes (geophilic species) that associate with and finally invade thickly cornfield substrates in living animals (zoophilic species) and human beings (anthropophilic species) [19]. Adaptation to parasitic existence has resulted in a reduced ability to produce spores, which are abundant in soil inhabiting species. *T. rubrum* is an anthropophilic species [20].

According to results, growth of *Microsporum gypseum*, *Rhizopus spp* and *Fusarium spp* were reported for the first time in Jaipur, Rajasthan and evident with several researchers' studies and documentation on distribution of keratinophilic fungi from soil of India [21-22]. This has also reported that most of the keratinophilic fungi grow in the soil with pH range 7.75-8.81 and humid temperature [23]. Similar to present study, several findings also supported the

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reporting of 154 isolates belonging to 16 genera and 31 species and a few had reported the abundance of *Chrysosporium tropicum* in the soil of Rajasthan and varieties of keratinophilic fungi from UP [24-26].

Conclusion

The present study concluded that the agricultural soil that consists of keratin as substrate were very rich in the population of keratinophilic fungi. The fungi isolated in our study belongs to both keratinophilic and dermatophytes group. These fungi could be considered as bioindicators of keratin pollution and used for bioconversion of keratinous waste (animal feed and fertilizers) into valuable products. The study also indicate that the farmers might be getting exposed to these dermatophytes leading the spread of severe cutaneous diseases among them. The study needed to be extended up to the enzymatic level in further assignments.

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