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Effect of Different Environmental Factors on the Growth of *Microsporum gypseum*

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ABSTRACT

Keratins are high molecular weight proteins and are classified as structural fibrous proteins, commonly called as scleroproteins. These keratins resist digestion by different proteolytic enzymes like pepsin and trypsin which are capable of digesting most common proteins and are insoluble in different solvents viz., dilute acids, alkalies, water, organic solvents etc. Keratinophilic fungi are a group of highly specialized fungi that degrade most non -degradable keratins and use this protein for their growth and metabolism. The study of the growth of keratinophilic fungi is focussed on different environmental factors like pH, temperature, humidity etc. The present study is taken up to study the impact of pH, temperature and relative humidity on the growth of one of the widely occurring keratinophilic fungus, *Microsporum gypseum*. *Microsporum gypseum* was cultured in Sabouraud Dextrose Agar (SDA) comprising of EN ISO 11133 which was used for examination of microbes. It has been observed in the study that the present isolate of *Microsporum gypseum* is mesophilic in nature and prefers high relative humidity and slightly acidic soils.

Key words: Environmental factors, Keratinophilic fungus, *Microsporum gypseum*, pH, Relative humidity, Temperature

INTRODUCTION

Fungi that are capable of colonizing and decomposing animal remains were found to be rich in keratin, i.e., proteins containing higher levels of nitrogen and sulphur (Bohacz *et al*., 2022). It is a well-known that the surface and deep soil layers contains keratin matter (hair of small mammals (especially rodents), feathers of birds, and few other keratinized remains of animals (Gungnani *et al*., 2012; Deshmukh *et al*., 2017; 2021). Ali-Shtayeh *et al*. (2002) reported that nearly fifty keratinophilic fungal species were recovered from the aquatic habitats studied, of which 42 were recovered from stream sites and 22 from swimming pools which included dermatophytes (*Microsporum gypseum*, and *Trichophyton mentagrophytes*) or dermatophyte related species (*Chrysosporium merdarium*, *Chrysosporium. tropicum*, *Chrysosporium keratinophilum* and *Trichophyton terrestre*).

These keratinaceous proteins are not easily digested by the proteolytic enzymes like pepsin and trypsin. There are certain group of fungi called as keratinophilic fungi which can grow on hair, feathers, nails, and other such related habitats (Korniłłowicz-Kowalska *et al*., 2022). They need very specialized ecological niches (occupy diverse niches and provide important ecosystems like decomposition of organic matter, mineralization and nutrient immobilization etc.) and certain environmental conditions (pH, oxygen, darkness, warmth, moisture etc.) for growth and development. In addition to the availability of keratinous substrates, the occurrence and survival of keratinophilic fungi in the soils is found to be influenced by various factors such as chemical composition and quantity of soil organic matter, humidity, pH, temperature, depth of soil, soil texture and structure.

Keratinophilic fungi are morphologically and physiologically allied molds which can produce the enzyme known as keratinase that is capable of degrading keratin materials in fifty soil samples collected from various locations including Jaipur, Ajmer, Alwar and Sikar in Rajasthan, India (Kumawat *et al*., 2020). Out of 154 isolates, a total of 31 keratinophilic fungal species were recovered consisting of *Chrysosporium tropicum* (11.04%),

Chrysosporium indicum (9.09%), *Trichophyton mentagrophytes* (8.44%), *Fusarium solani* (7.79%), *Trichophyton rubrum* (7.14%), *Microsporum canis* (5.84%), and *Aspergillus terreus* (4.19%) etc. as reported by Kumawat *et al*. (2020).

The important role of pH and temperature on the dermatophytes and other keratinophilic fungi has been well studied (Reetha *et al*., 2014). The low pH of the skin has been suggested as an important factor in protection against ringworm infection. The dermatophytes are not exact in their pH requirements (Sharma and Swathi, 2014). A pH range of 4-10 is compatible with the growth of most species, the optimum being slightly lower than neutrality. The pigmentation of certain species of *Trichophyton* is controlled by the pH of the medium. Under alkaline conditions, *T. rubrum* had a red pigmentation, whereas at acid conditions, has a yellow pigmentation (Blechert *et al*., 2019). Correlation between soil pH and distribution pattern of keratinophilic fungi was first emphasized by Marples (1965) and later by Ziegler (1966). Bohme and Ziegler (1969) found their frequency to be more in weak acidic (pH of 6-7) to weak alkaline soils (pH of 7-8). Based on pH requirements Hubalek (1974) categorized keratinophilic fungi into three groups viz., acidophilic, neutrophilic and alkalophilic.

Further, research studies also showed that the growth and development of the keratinophilic fungi were regulated by different environmental factors (Singh and Chauhan, 2013). Generally, Keratinophilic fungi are considered to be mesophilic but some strains are thermos-tolerant also (Singh and Kushwaha, 2010). In the present study an attempt has been made to study the effect of different environmental factors like pH, temperature and relatively humidity on the growth of widely occurring keratinophilic fungus *Microsporum gypseum.* The present research study revealed that the keratinophilic fungus *Microsporum gypseum* showed best growth at a pH of 6.5, 84.5% of relative humidity and 30^0C of temperature.

MATERIALS AND METHODS

Preparation of Sabouraud Dextrose Agar (SDA)

Sabouraud Dextrose Agar (SDA) is a non-selective isolation medium usually employed for the growth and maintenance of *Microsporum gypseum*. This medium usually consists of EN ISO 11133 which is used for examination of microbes. The medium comprises of dextrose, peptone and agar. 65 g of SGA media is suspended in 1 litre of distilled or deionized water.

The contents were mixed well, pH was adjusted to 5.6 and boiled for one to two minutes shaking until completely dissolved and autoclaved at 121°C for 15 minutes. The autoclaved media were then cooled to 45 to 50°C and used for culturing of *Microsporum gypseum.*

The inoculum comprised of a conidial suspension from the surface of 6 days old single spore cultures. The conidial suspension was obtained from culture tubes by brushing conidia in 5 ml of sterilized distilled water, and 2 ml of conidial suspension (300 conidia per ml) was added to each flask containing basal liquid medium. Each 100ml Erlenmeyer flask received 250mg of the sample. The cultures were incubated in stationary conditions at 28 ± 2^0C.

The effect of different pH levels 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5 on the growth of *Microsporum gypseum* was studied. Three replicates were maintained. The cultures were filtered on 7^{th} day through previously weighed Whatman No.1 filter paper. The filter papers were dried at 70^0C in an electric oven for three days and dry weights were obtained.

The effect of different percent relative humidities 44.5, 52.0, 76.5, 84.5 and 95.0 was studied. Humidity of the incubation chambers was maintained as suggested by Winston and Bates (1960). The growth of *Microsporum gypseum* was measured on 3^{rd}, 5^{th} and 7^{th} days respectively. Sabouraud's glucose agar medium was used. *Microsporum gypseum* was grown under four different temperatures 10 °C, 30 °C, 37 °C and 55 °C and the results were noted. Sabouraud's glucose agar was poured into sterile petriplates and then inoculated with 5mm diameter mycelia disc taken from the margins of 8–10-day old colonies raised on SGA. The plates were incubated for one week in different temperatures. The growth in the form of diameter of the colony was measured on 3^{rd}, 5^{th} and 7^{th} days.

RESULTS

The perusal of **Table 1** gives a picture of the effect of different pH levels on the growth of *Microsporum gypseum*. Varying results were obtained. It is evident from the results that the growth of the fungus is favoured by weak acidic

(pH of 6 - 7) to weak alkaline soils (pH of 7- 8) conditions. Highest dry weight of the mycelium was obtained from the medium with pH 6.5 followed by 7.5, 8.5, 9.5, 5.5 and 4.5.

Table 1: Effect of different pH levels on the growth (in g)* of *Microsporum gypseum*

pH	Weight in Grams (g)
4.5	0.03706
5.5	0.05115
6.5	1.2561
7.5	1.061
8.5	1.0122
9.5	0.5092

D-7days; * Values are mean ±S.E

The perusal of the results presented in the **Table 2** gives an effect of different relative humidities on the growth of keratinophilic fungus *Microsporum gypseum*. Maximum fungal growth was recorded at relative humidity of 84.5%. This was followed by 44.5%, 76.5%, 95% and 52%.

Table 2: Effect of different relative humidities on the growth (in cm)* of *Microsporum gypseum*

Relative humidity %	Days of incubation		
	D1	D2	D3
44.5	1.856	4.73	6.96
52			1.17
76.5		1.28	1.88
84.5	1.58	3.8	7.35
95			1.45

D1=3 days; D2 = 5 days; D3 = 7 days; *Values are mean ±S.E

The radial growth of the fungus incubated at different temperatures viz., 10 °C, 30 °C, 37 °C and 55 °C was recorded on 3rd, 5th and 7th days. The results are presented in the **Table 3**. It has been observed that of all the temperatures maximum growth of the fungus was recorded at 30 °C followed by 37 °C. However, no fungal growth was detected at 10 °C and 55 °C.

Table 3: Effect of different temperatures on the growth (in cm)* of *Microsporum gypseum*

Temperature	Days of incubation		
	D1	D2	D3
10^0C			
30^0C	1.85	2.8	4.43
37^0C	0.85	1.38	1.8
55^0C			

D1=3 days; D2 = 5 days; D3 = 7 days; *Values are mean ±S.E

The results were statistically analysed and were expressed in terms of standard error [Mean ± S. E (n=3)].

DISCUSSION

The hydrogen ion concentration (pH) and temperature are considered as important factors affecting the growth of fungi (Deshmukh, 2004; Jain and Sharma, 2011). The studies conducted by Khilare *et al*. (2012), Sharma *et al*. (2012) and Abubakar *et al*. (2013) have revealed that a pH range of 6.0-8.0 is optimal for the growth of keratinophilic fungi. Pahare *et al*. (2018) observed that dermatophytes are mostly found in temperate conditions however the hot and humid climate, with a temperature 22 - 35°C, the acidic pH of the

soils, seems to be more conducive in wet season rather than dry and hot summer season of low-land area in Chhattisgarh state. Furthermore, the distribution of keratinophilic fungi found that *Trichphyon ajelloi* is commonly found in colder climates but found sporadic in hot climates (Samuel *et al*., 2011). Further, Bohacz *et al*. (2022) reported that the frequency of keratinophilic fungi was based on the pH and higher growth was reflected in loamy, chernozem and rendzina soils (soils with higher content of silt and clay rather than sand).

In the present study different ranges of temperature has been tested on the growth of *Microsporum gypseum* using Sabouraud's glucose agar. The results indicated that the present isolate has shown better growth at 30^0C and 37^0C and no growth was recorded at 10^0C and 55^0C. The maximum growth at 30^0C indicates mesophilic nature of the present isolate of *Microsporum gypseum*. The present observations in confirmation with the findings of Shukla *et al.* (1984) who recorded maximum fungal growth and keratin degradation at 30 °C temperature. Moubasher *et al*. (1992) have analysed the soil samples collected from Nile valley and delta, desert, and sand marshes for keratinophilic fungi at three incubation temperatures 27 °C, 37 °C and 45 °C. At 27 °C, 44 spp. belonging to 21 genera were isolated and at 37 °C, 42 spp. belonging to 22 genera were found.

The effect of relative humidity has been studied on the growth of *Microsporum gypseum* on Sabouraud's glucose agar medium. Maximum fungal growth was recorded at 84.5% relative humidity. Sharma et al. (2012) reported that temperature and relative humidity influence the growth and sporulation of some common dermatophytes. In vitro studies conducted on the effect of moisture on the growth of *Aspergillus flavus* and *Penicillium chrysogenum* showed that 90% was best for the growth wherein different relative humidity percentages i.e. 30%, 45%, 60%, 75% and 90% were maintained with the help of desiccators by using respective salt solutions and acids according to each humidity parameters (Singh and Chauhan, 2012). Morishita *et al*. (2003) reported that application of *Trichophyton mentagrophytes* on the surface of stratum corneum obtained from a healthy human heel, samples incubated under designated conditions of temperature and humidity and the penetration of fungal elements was much faster at 35°C than 27°C even though the latter is an optimal temperature for fungal growth. Further, at 35 degrees C and 100% humidity, the minimum time required for penetration was one day compared to 80%. There is not much research carried out on the role of humidity in relation to the growth of keratinophilic fungi, *Microsporum gypseum.* Therefore, the present observation appears to be in tune with earlier research about the impact of relative humidity on the growth of keratinophilic fungi, *Microsporum gypseum*.

CONCLUSION

The present research study revealed that the keratinophilic fungus *Microsporum gypseum* exhibited best growth at a pH of 6.5, 84.5% of relative humidity and 30^0C of temperature.

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