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EFFECT OF ECOLOGICAL FACTORS (pH AND TEMPERATURE) ON GROWTH AND SPORULATION OF KERATINOPHILIC FUNGI

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Abstract

Fungi are occurred almost in each ecological niche and their occurrence in niche depends upon ecological factors which vary from place to place. The ecological factors play a vital role in growth and sporulation of fungi. Keratinophilic fungi are the biggest group of fungi that have capability to degrade keratin residues. The present investigation was done to determine the in-vitro effect of physical factors (pH and Temperature) on growth and sporulation of keratinophilic fungi (*Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum gypseum* and *Chrysosporium tropicum).* All fungus isolated from soil samples collected from different soil habitats of Ajmer district. They were grown on different range of pH (5,6,7,7.5,8,8.5,9) and temperature (10,20,25,30 and 40ºC). Each fungus requires optimal pH and temperature for growth and sporulation. In the present study, the best growth was recorded at 25º to 35ºC and 5-8 pH.

Keyword: - Ecological factors, keratinophilic fungi, optimum pH and temperature.

1. INTRODUCTION

Fungi are eukaryotic organisms which are present almost everywhere and are the second largest group after insects.[1] Fungi lives as saprophytes in soil, their occurrence in soil depends upon the physio-chemical parameters of soil which vary from place to place. Soil are rich in keratinous residues is suitable for the growth and occurrence of keratinophilic fungi because it has capability to degrade keratin residues and has recently received a great deal of interest around the world.[2] Hair Baiting Technique' used for first discovery of keratinophilic fungi from soil habitat.[3] Keratinophilic fungi are the vital group of fungi and useful for natural degradation of keratin material. Environmental factors play the important role in growth and sporulation of keratinophilic fungi.[4]

Ecological conditions i.e., temperature, inorganic and organic matter and pH affecting the sexual reproduction in Keratinophilic fungi.[5] Every fungus comprises a specific range of pH and temperature where they can grow and sporulates. Temperature is the most important abiotic factor which determine the growth of fungi.[6] Fungi grow on the optimum temperature 15ºC to 35ºC but some fungi require a high range of temperature for optimum growth[7] and for each fungus there is a maximum, optimum and minimum temperature for growth and sporulation.[8] Usually too acidic and too alkaline conditions are not favourable for the growth of fungi. Fungi grow on the optimum pH range from 4.5-9.0.[9]

The present research was focused on the study of *in vivo* and *in vitro* effect of physical conditions (temperature and pH) on mycelium growth and sporulation of some keratinophilic species isolated from soil samples collected from various habitats of Ajmer district, Rajasthan.

2. MATERIALS AND METHODS

2.1 Collection of soil samples: Soil samples were collected from various habitats of Ajmer district, Rajasthan. Samples were collected in sterile polybags after removal of surface litter and 2 to 4cm depth from soil surface and maintained at room temperature for further processing.

2.2 Analysis of physical factors (temperature and pH) of soil samples

Soil temperature: Soil temperature was checked at the time of soil sample collection by using thermometer.[10]

Soil pH: 5 gm of soil samples were mixed in 10 ml distilled water. pH meter was calibrated with buffer solution of 4 to 9.2. for soil pH analysis. Then pH of soil was checked with glass electrode.[11]

2.3 Isolation and purification of keratinophilic fungi from the soil samples

Keratinophilic fungi were isolated by the hair baiting technique of Vanbreuseghem using autoclaved human nail, human hairs, and animal hairs as keratin bait.[12] For this, each soil samples were homogeneously mixed in it and 20 grams soil from each sample were placed in sterile Petri dishes and moistened with sterile distilled water were baited by burying sterile keratinous bait in the soil. The petri dishes were closed and incubated for 3-4 weeks at room temperature. After a vigorous growth, the growth is sub-cultured on the newly prepared SDA medium with antibiotics for purification, identification and future analysis.

2.4 Identification of isolated fungi: All the fungal isolates was identified on the basis of macro and micro morphological characters.[13,14,15] Out of total isolates obtained from various soil samples four keratinophilic fungi (*Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum gypseum* and *Chrysosporium tropicum*) were selected for the present study.

2.5 Effect of physical factors on mycelium growth of keratinophilic fungi

Modified Sabouraud's dextrose broth (SDB) medium was used for the study of the effect of various range of temperature and pH on mycelium growth and sporulation of selected keratinophilic fungi.

Temperature: Temperature is a significant factor for mycelial growth and sporulation of fungi. 100 ml of Sabouraud's Dextrose broth medium (SDB) was set up in various 250 ml conical flasks and incubated keratinophilic fungi in these conical flasks at 28±2.0^{o}C with different temperatures (10^{o}C to 40^{o}C at intervals of 5^{o}C) in the incubator. Dry weight of mycelium and sporulation were recorded after 21 days. The experiment was performed in triplicate and data was analyzed by mean ± SE.

pH: pH is also playing a significant role in growth and sporulation of keratinophilic fungi. 100 ml of Sabouraud's Dextrose broth medium (SDB) was prepared in 250 ml conical flasks and labelled with name of fungi and pH. The pH of media was adjusted with various pH range, from 5.0 to 9.0, by adding HCl or NaOH. Keratinophilic fungi were inoculated in the flasks and incubated at 25^{o}C for 21 days in the incubator. The experiment was replicated thrice and data was analyzed by mean ± SE.

2.6 Assessment of Mycelial dry Weight

The mycelial growth of the keratinophilic fungi was assessed by weighing the dry of mycelium after entire growth. Mycelial dry weight and sporulation was recorded after 21 days at 25^{o}C.[16] The collected mycelium was filtrate through Whatman filter paper no. 1. The filter papers were dried into a hot air oven. The weight of filter paper along with mycelium weight was noted for calculation of fungal biomass.[17] Tuite had given the formula to determine the degree of sporulation of keratinophilic fungi.[18]

3. RESULTS

The present investigation was done to determine the *in vitro* effect of various ranges of temperature and pH on the growth of fungus and sporulation of *Trichophyton mentagrophytes, T. rubrum, Microsporum gypseum, chrysosporium tropicum*. This test fungi were isolated from soil sample and maintained on Sabouraud's Dextrose broth medium (SDB).

3.1 Effect of Temperature

Temperature is one of the most important environmental factors which regulate the fungal growth and their sporulation. In the current study, the optimum temperature range for the growth of keratinophilic fungi had between 25-30^{0}C. In this study the rate and amount of growth of various temperature varied for different species.

The data of effect of various temperature range on the growth and sporulation of keratinophilic fungi were shown in **table 1-2** and **fig. 1.** The growth and sporulation of fungi were analyzed from average dry weight and spore count in triplicates. The greatest mycelial growth in all isolated keratinophilic fungi was reported at 30^{o}C. The highest weight of fungi at 30^{o}C was reported by *T. mentagrophytes* (1.58±0.02), *M. gypseum* (1.49±0.10), *T. rubrum* (1.26±0.05) *and Chrysosporium tropicum* (1.19±0.03).

In this study the maximum sporulation was recorded at 25ºC and 30ºC. The rate of growth and sporulation of keratinophilic fungi was varied for every fungus on different temperature range.

The fungi *Trichophyton mentagrophytes* showed highest mycelial growth at 25^0C and 30^0C temperature ranges. The sporulation of *T. rubrum* and *M. gypseum* were excellent at 25^0C 30^0C, and 40^0C. The fair sporulation was observed at 10^0C and 20^0C.

3.2 Effect of pH

pH is the most significant ecological component which plays a vital role in growth of keratinophilic fungi. pH requirement for fungal growth is varying for different fungi. The data of effect of various pH range on the growth and sporulation of keratinophilic fungi were shown in **table 3-4**, and **fig. 2**. In the present study, the most congruent pH for the growth and prevalence of keratinophilic fungi was observed to be 7.0 and 8.0 pH. Similarly, the best pH for sporulation of keratinophilic fungi was observed to be at pH 7.5 followed by pH 6and 8.5.

During present investigation it was observed that the maximum average dry weight of mycelium was reported by *Trichophyton mentagrophytes* at pH 7.5 (1.17±01gm), *Trichophyton rubrum* at pH 7.0 (1.85±05 gm), *Microsporum gypseum* was at pH 7.5 (1.73±0.07 gm) and C*hrysosporium tropicum* was at pH 8.0 (1.04±0.10 gm).

The highest sporulation was showed by *T. mentagrophytes* at pH 7.5, *T. rubrum* pH 7, *M. gypseum* at pH 7.5 and *C. tropicum* at pH 8.0. The pH ranges below at 6.0 and above at 8.5 showed poor sporulation of fungi.

In the current study, change was observed in the pH of culture (Sabouraud's Dextrose Agar) media, after incubation of 21 days. It was reported that the initial pH of the culture (SDA) media was floated from acidic towards neutralize range and near alkaline range. The maximum range of pH was found between pH 7.0 to 8.5. (**Table 5**).

Trichophyton rubrum, Trichophyton mentagrophytes and *Microsporum gypseum* could tolerate acidic as well as alkaline conditions but sporulation was excellent in alkaline condition only viz., 7.0-7.5. *Chrysosporium tropicum* showed preference for alkaline pH levels and excellent sporulation occurred at pH 8.0-8.5. It is clear from above data that all the four fungi studied showed the excellent growth and sporulation in alkaline media having pH value between 7.0-8.5.

Table 1: Average dry weight of dermatophytes at various range of Temperature.

Temp.	Average dry weight of mycelium (in gram)			
	T. mentagrophytes	***T. rubrum***	***M. gypseum***	***C. tropicum***
10^0C	0.22±0.11	0.45±0.07	0.38±0.07	0.48±0.02
20^0C	0.61±0.01	0.75±0.04	0.43±0.05	0.72±0.12
25^0C	1.18±0.07	1.09±0.07	0.72±0.15	1.12±0.07
30^0C	1.58±0.02	1.26±0.05	1.49±0.10	1.19±0.03
40^0C	0.54±0.02	1.11±0.03	1.21±0.07	1.13±0.06

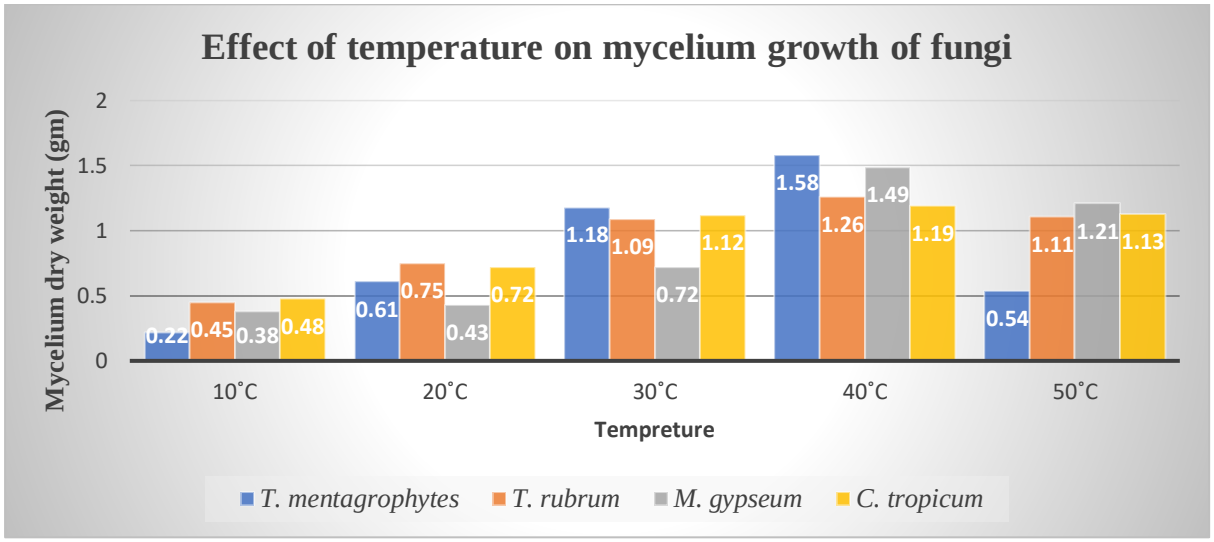

Fig.1: Effect of various range of temperature on mycelium dry weight of dermatophytes.

Table 2: Effect of various range of temperature on sporulation of dermatophytes.

pH	Average dry weight of mycelium (in gram)			
	T. mentagrophytes	***T. rubrum***	***M. gypseum***	***C. tropicum***
0^0C	-	-	-	-
10^0C	+ +	+ +	+ +	+ +
20^0C	+ +	+ + +	+ + +	+ + +
25^0C	+ + + +	+ + + +	+ + + +	+ + + +
30^0C	+ + + +	+ + + +	+ + + +	+ + + +
40^0C	+	+	+ +	+
45^0C	-	-	-	-

Sporulation grades: - - = Negative, + = Poor, ++ = fair, +++ = Good, ++++ = Excellent.

Table 3: Average dry weight of dermatophytes at various range of pH.

pH	Average dry weight of mycelium (in gram)			
	T. mentagrophytes	***T. rubrum***	***M. gypseum***	***C. tropicum***
5	0.50±0.04	0.58±0.03	0.32±0.02	0.26±0.02
6	0.72±0.06	0.74±0.05	0.45±0.02	0.33±0.01
7	0.68±0.03	1.85±0.05	0.92±0.06	0.36±0.04
7.5	1.17±0.01	1.84±0.06	1.73±0.07	0.42±0.03
8	0.65±0.02	1.27±0.03	1.31±0.04	1.04±0.10
8.5	0.41±0.02	0.89±0.04	0.66±0.05	0.38±0.01
9	0.38±0.01	0.72±0.03	0.56±0.06	0.28±0.04

Table 4: Effect of various range of pH on sporulation of dermatophytes.

pH	Average dry weight of mycelium (in gram)			
	T. mentagrophytes	***T. rubrum***	***M. gypseum***	***C. tropicum***
5	+ +	+	+	-
6	+ + +	+ + +	+ + +	+ +
7	+ + +	+ + +	+ + + +	+ + +
7.5	+ + + +	+ + + +	+ + + +	+ + + +
8	+ + +	+ + + +	+ + +	+ + + +
8.5	+ + +	+ + +	+ + +	+ + + +
9	+ +	+ +	+ + +	+ +

Sporulation grades: - - = Negative, + = Poor, ++ = fair, +++ = Good, ++++ = Excellent.

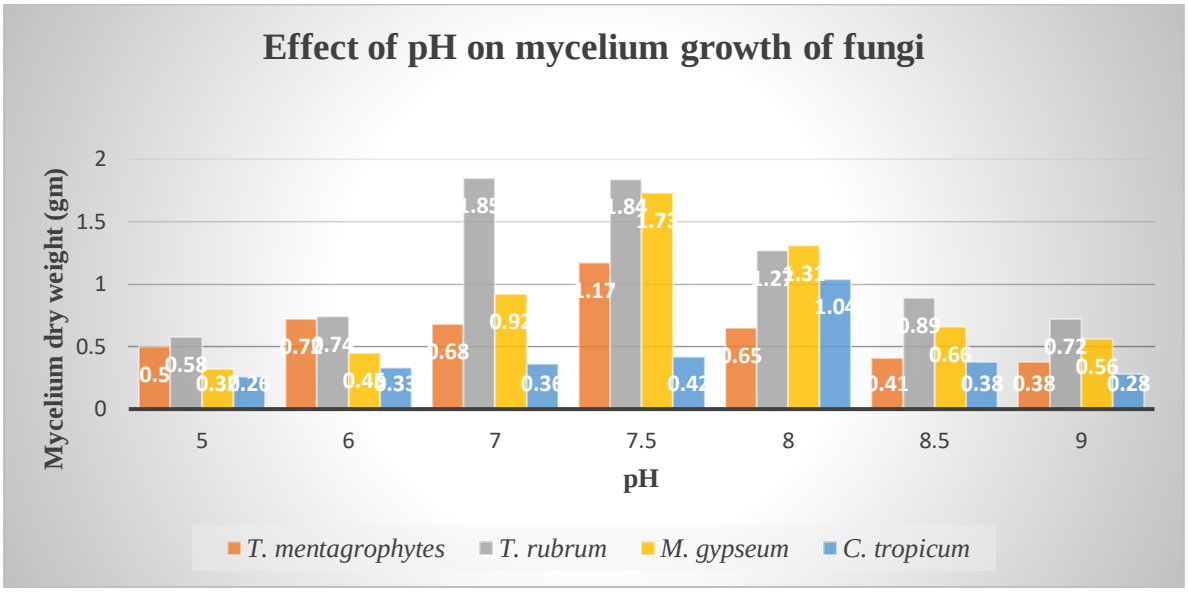

Fig. 2: Effect of various range of temperature on mycelium dry weight of dermatophytes.

Table 5: Final pH of culture filtrates of dermatophytes at different H-ion concentrations.

pH	Final pH of culture filtrate after 21 days			
	T. mentagrophytes	***T. rubrum***	***M. gypseum***	***C. tropicum***
5	5.0	5.0	5.2	5.0
6	6.0	6.0	6.0	6.0
7	7.2	7.0	7.2	7.0
7.5	7.5	7.2	7.0	7.2
8	8.0	7.6	8.0	7.5
8.5	8.0	7.8	8.5	8.5
9	8.5	8.5	8.8	8.5

4. DISCUSSION

Ecological factors are affected the growth of microorganisms. Each fungus has an optimum range of pH and temperature where fungus can grow and sporulates and sometimes it can adapt and grow under different culture conditions. The physical factors (temperature and pH) and culture media are important criteria for growth and sporulation of keratinophilic fungi.[19] Cochrane (1958) suggested that the growth of fungi affected at low and high pH because at low pH, the enzyme systems may be disrupted and at high, metal solubility may be affected.[20] Knight

(1976) recorded the optimal temperature range as 27-33°C for growth of dermatophytes.[21] Generally, pH range 4.2-9.3 and temperature range 15°C-35°C promote the growth of fungi.[22]

In the present study, the optimum pH for growth of keratinophilic fungi was found at pH 7.0-8.0 and maximum sporulation at 7.0-9.5. The results of present study coincide with the results of Sharma and Sharma (2008) who reported the growth and sporulation of *T. mentagrophytes* and *C. tropicum* at pH 7.5-9.0.[23]

The optimum temperature for growth of fungi was 30-35º C and maximum sporulation was recorded at 30ºC. Sharma (2011) observed the dry weight of mycelium at 33°C and 7.0 pH have optimum for growth of *T. rubrum* which consent with the present study. He also reported that *T. mentagrophytes* showed maximum growth at pH 8.0 and at 8.5. and excellent sporulation at pH 7.5-9.5. The excellent sporulation in *C. tropicum* was recorded pH 7.5-9.0 and best growth at pH 7.5.[24] Findings of Stockdale (1953) and Sharma (2012b) are in support of findings of the current study as temperature range of 25-35°C was found to be suitable for the growth of *Microsporum gypseum* in both the studies. [25,26]

5. Conclusion

Present study concluded that the effect of pH and temperature on growth and sporulation of keratinophilic fungi. According to present study, fungus grow at specific range of pH (5-8pH) and temperature (25-35ºC) that can be favorable for the growth of fungi. This study may help researchers for selecting suitable physical condition for growth of keratinophilic fungi.

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