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Research Article

INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON GROWTH AND SPORULATION OF SOME COMMON DERMATOPHYTES

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ABSTRACT
Spore morphologies are a major character in fungal taxonomy, although many isolates are not able to sporulate on common artificial media. Environmental factors play an important role in the growth and sporulation of dermatophytic and keratinophilic fungi. Fungi grow best at optimum temperature and related humidity. Both the factors govern metabolic activities of growing organism. The extremely high and very low temperature decreases the growth of keratinophilic fungi. The increased level of relative humidity shows excellent growth. In the present study various temperature regimes i.e. 0°, 5°, 10°, 15°, 20°, 25°, 30°, 35°, 40°, 45°, 50°C and different relative humidity i.e. 11.05%, 22.45%, 33.00%, 50.00%, 62.00%, 75.00%, 95.00% were used to evaluate the growth and sporulation of *Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis and Mycosporum gypseum.*

Key Words: *Fungal Taxonomy, Dermatophytic And Keratinophilic Fungi, Temperature, Relative Humidity*

INTRODUCTION
Soil has long been recognized as a natural habitat for certain fungi. The fungi are of second population after bacteria in soil. The forest, farmyard, park soils, as well as sediments of the rivers and oceans contained humus and organic material are the best candidate for growth of keratinolytic and saprophytic fungi. More then 100 species of fungi which are generally recognized as pathogen of man found in soil (Batia and Ichhpujani, 1994). They are scavengers and serve to break the complex organic compounds including the keratin containing material such as skin, nails, hair, hooves, fur, feathers etc by producing extracellular keratinase (Robert and Mackenzie, 1985; Heinz, 1988). Dermatophytes are a group of morphologically and physiologically related moulds, which cause well-defined infection called dermatophytosis. The enzymatic ability of ungi to decompose keratin has long been interpreted as a key innovation in the evolution of animal dermatology (Sharma *et al.,* 2012). Environmental factors play an important role in the growth and sporulation of keratinophilic fungi. Keratinophilic fungi are unique in the sense that they require and utilize keratin for growth. These are generally considered as soil saprophytes (Ajello, 1953, 1956). Studies of keratinophilic fungi are now of considerable significance for their important role in the breakdown of keratinous debris of man and animals in nature, and they have a worldwide distribution (AI-Doory, 1967; Sur and Ghosh, 1980; Karam EI-Din *et al.,* 1996). The influence of some ecological factors on keratinophilic fungi isolated from soil and reported that ecological factors play significant role on the growth of keratinophilic fungi (Chmel *et al.*, 1972).
Usually most of the fungi grow at temperature ranging from 15°C to 35°C; some of the fungi require a range of higher temperature for their optimum growth. Michael *et al.,* (1998) investigated the effect of the ecological factors like pH, temperature and ionic strength on *Candida milleri*. Fustier *et al.,* (1998) investigated the effect of inoculation techniques and relative humidity on the growth of molds on the

surfaces of yellow layer cakes. The combination of temperature and relative humidity create an environment in which fungi show good growth and excellent germination. Malik and Singh, (2004) studied the effect of temperature and relative humidity against fungi and reported that the temperature and relative humidity play important role in germination of spores. Kim and Xiao, (2005) also studied the influence of culture media and environmental factors on mycelial growth and pycnidial production of *Sphaeropsis pyriputrescens* and concluded that optimum range of environmental factors act as a growth limiting factors. However, the influence of environmental factors in Jaipur has never been investigated for keratinophilic fungi. Therefore, hygienic and ecological interests have led us to study the environmental factors on growth and sporulation of keratinophilic fungi from public parks soil, where human beings spend their time and may be exposed to pathogenic fungi. This would help us to know the immense role of environmental factors for controlling the growth of kertinophilic fungi and overcome the risk of human dermatophytosis by keratinophilic fungi in these regions.

MATERIALS AND METHODS

For the evaluation of growth and sporulation of keratinophilic and dermatophytic fungi Sabourand's Dextrose broth medium (SDA modified) was used. pH of the nutrient medium was adjusted to 7.5 for temperature and relative humidity studies using N/5 NaOH or N/5 HCl before autoclaving or fractional sterilization. During, the environmental factors study various temperature regimes i.e. 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 28°C, 30°C, 35°C, 40°C and 45°C were maintained in BOD incubator shaker. Different relative humidity i.e. 11.05%, 22.45%, 33.00%, 50.00%, 62.00%, 75.00% and 95.00% were maintained in desiccators using respective salt solutions and acids according to humidity parameters. The equal quantity of SDA broth media i.e. 25 ml was taken in each flask and known quantity(0.2 ml) of test fungi (*Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis, Microsporum gypseum*) which was prepared aseptically in sterilized distilled water was inoculated in every flask. The flasks were incubated for 15 days at variable environmental conditions of temperature, relative humidity and pH. The growth and sporulation were examined on 16^{th} day and mycelial mats were harvested by filtering through previously dried and weighed Whatman's filter paper No. 42, using three replicates of flasks for each treatment and average dry weight calculate after washing and drying of mycelial mat..

Hydrogen ion concentration (pH) of the culture filtrates was determined at the end of each sampling. For this the pooled filtrates of the three replicates of a single treatment were first made to their original volumes (i.e. 20ml × 3 = 60 ml) by adding double distilled water and then the pH of the filtrate was determined as before. The degree of sporulation was determined before harvesting the mycelial mats, using standard methods as recommended by Wilson and Knight, (1952) and Tuite, (1969). Results given are mean ± standard error. Data obtained were statistically analyzed with one-way analysis of variance and the observations were considered significant when standard deviation was found less than 0.05.

RESULTS

Effect of different temperature regimes and various relative humidity on the selected fungal growth was analysed from the dry mycelium weight and spore count using SDA modified broth medium in triplicates. Almost all fungi grew in a wide range of temperature and relative humidity but they could sporulate well only at certain temperature and relative humidity. Both physiological factors were found to be different for different fungal growth.

It was found that *Trichophyton mentagrophytes* showed maximum growth at 30°C but the excellent sporulation was observed at 30°C – 35°C temperature. There was a sharp decrease in the growth and sporulation of the culture, below 25°C and above 35°C temperature (Table1). There was a sharp increase in growth of *T. mentagrophytes* with increasing relative humidity and maximum sporulation was achieved in 62% to 95% relative humidity (Table3). The fungal growth and sporulation were decreased with decrease in relative humidity. *Trichophyton rubrum* showed maximum growth and sporulation at

20°C to 35°C. A sharp decrease in growth and sporulation was observed above and below this temperature. Maximum growth was obtained at 30°C and highest sporulation was obtained from 25°C to

Table1: Average dry weight and sporulation of *Trichophyton mentagrophytes* and *Trichophyton rubrum* at different temperature (Initial pH 7.5)

	Trichophyton mentagrophytes			***Trichophyton rubrum***		
Temp (°C)	**Final pH of the medium**	**Average dry weight of mycelium (gm)**	**Sporulation**	**Final pH of the medium**	**Average dry weight of mycelium (gm)**	**Sporulation**
0	7.5	0.00± 0.002	-	7.4	0.012±0.011	-
5	7.6	0.017±0.009	-	7.5	0.031±0.004	+
10	7.8	0.039±0.011	-	7.7	0.067±0.16	++
15	7.7	0.073±0.023	+	7.6	0.109±0.021	++
20	7.9	0.116±0.014	++	7.8	0.134±0.014	+++
25	7.9	0.179±0.027	+++	7.8	0.173±0.029	++++
30	8.0	0.219±0.037	++++	7.9	0.214±0.039	++++
35	8.1	0.206±0.041	++++	7.7	0.194±0.027	++++
40	7.8	0.165±0.036	+++	7.4	0.107±0.037	++
45	7.6	0.064±0.021	+	7.5	0.037±0.031	+
50	7.5	0.015±0.013	-	7.5	0.019±0.041	-

Note : Values are means ± standard errors (SE) of measurements taken in triplicates (n=3) and P<0.05 (- = No sporulation, + = Poor sporulation, ++ = Fair sporulation, +++ = Good sporulation, ++++ = Excellent sporulation)

Table2: Average dry weight and sporulation of *Microsporum canis* and *Microsporum gypseum* at different temperature (Initial pH 7.5)

	Microsporum canis			***Microsporum gypseum***		
Temp (°C)	**Final pH of the medium**	**Average dry weight of mycelium (gm)**	**Sporulation**	**Final pH of the medium**	**Average dry weight of mycelium (gm)**	**Sporulation**
0	7.5	0.017±0.014	-	7.5	0.011±0.029	-
5	7.5	0.034±0.037	+	7.3	0.043±0.009	+
10	7.6	0.057±0.042	+	7.7	0.068±0.17	+
15	7.6	0.099±0.19	++	7.7	0.086±0.023	++
20	7.8	0.139±0.024	+++	7.9	0.104±0.19	+++
25	7.9	0.184±0.039	++++	8.0	0.173±0.021	++++
30	7.9	0.221±0.026	++++	8.1	0.204±0.036	++++
35	7.8	0.194±0.032	++++	7.8	0.166±0.14	++++
40	7.6	0.141±0.023	+++	7.7	0.114±0.037	+++
45	7.5	0.073±0.007	+	7.5	0.041±0.19	+
50	7.5	0.027±0.013	-	7.5	0.019±0.011	-

Note: Values are means ± standard errors (SE) of measurements taken in triplicates (n=3) and P<0.05 (- = No sporulation, + = Poor sporulation, ++ = Fair sporulation, +++ = Good sporulation, ++++ = Excellent sporulation)

35°C (Table1). Higher degree of sporulation was achieved at 75 percent to 95 per cent relative humidity and growth was decreased sharply below 50 percent relative humidity (Table2). *Microsporum canis* has shown maximum growth at 30°C and high rate of sporulation was observed in a temperature range of 25°C to 35°C. The growth and sporulation decreased sharply below 20°C and above 40°C (Table2). The growth of *Microsporum canis* was found optimum above 50 percent relative humidity and best sporulation was found at 75 percent and above relative humidity (Table4). *Microsporum gypseum* showed higher sporulation from 25°C to 35°C temperature while the highest growth was obtained at 30°C (Table2). Less than 50 percent relative humidity adversely affected the sporulation and growth in *Microsporum gypseum* and maximum growth was obtained at 95 percent humid conditions (Table4).

Table 3. Average dry weight and sporulation of *Trichophyton mentagrophytes* and *Trichophyton rubrum* at different relative humidity (Initial pH 7.5)

Relative humidity (%)	*Trichophyton mentagrophytes*			*Trichophyton rubrum*		
	Final pH of the medium	Average dry weight of mycelium (gm)	Sporulation	Final pH of the medium	Average dry weight of mycelium (gm)	Sporulation
11.05	7.6	0.098±0.051	+	7.6	0.057±0.011	+
22.45	7.6	0.127±0.031	+++	7.66	0.074±0.16	+
33	7.8	0.133±0.027	+++	7.8	0.117±0.029	++
50	7.9	0.164±0.033	+++	7.8	0.129±0.037	+++
62	7.9	0.172±0.042	++++	7.9	0.170±0.024	+++
75	8.0	0.187±0.026	++++	8.0	0.194±0.017	++++
95	7.9	0.175±0.029	++++	8.0	0.196±0.043	++++

Note: Values are means ± standard errors (SE) of measurements taken in triplicates (n=3) and P<0.05 (- = No sporulation, + = Poor sporulation, ++ = Fair sporulation, +++ = Good sporulation, ++++ = Excellent sporulation)

Table 4. Average dry weight and sporulation of *Microsporum canis* and Microsporum gypseum at different relative humidity (Initial pH 7.5)

Relative humidity (%)	*Microsporum canis*			*Microsporum gypseum*		
	Final pH of the medium	Average dry weight of mycelium (gm)	Sporulation	Final pH of the medium	Average dry weight of mycelium (gm)	Sporulation
11.05	7.5	0.012±0.010	-	7.6	0.039±0.014	+
22.45	7.6	0.054±0.021	+	7.7	0.094±0.032	++
33	7.7	0.099±0.019	++	7.7	0.113±0.023	++
50	7.7	0.114±0.016	++	7.9	0.152±0.031	+++
62	7.9	0.137±0.028	+++	7.9	0.161±0.031	+++
75	8.0	0.173±0.031	++++	8.0	0.180±0.007	++++
95	8.0	0.183±0.022	++++	8.0	0.183±0.011	++++

Note: Values are means ± standard errors (SE) of measurements taken in triplicates (n=3) and P<0.05 (- = No sporulation, + = Poor sporulation, ++ = Fair sporulation, +++ = Good sporulation, ++++ = Excellent sporulation)

DISCUSSION

Temperature plays an important role in influencing the growth and sporulation of fungi (Cochrane, 1963). Sharma, (1983) studied the effect of different temperatures on the growth and sporulation of *Gymnoascus reessii, Microsporum gypseum, Trichophyton simii, Cephaliophora irregularis* and *Chrysosporium tropicum*. It was reported that these fungi grew well at temperature between 15°C to 30°C. Prevalence of keratinophilic fungi in semi-arid region, with particular reference to soil pH and temperature was performed by Sharma *et al.*, (2010). Abarca *et al.*, (1990) studied the effect of temperature on 17 strains of genus *Epidermophyton* and found that 28°C and 31°C temperature was found to be most suitable for optimum growth of most of the strains. Stockdale (1953a) recorded 25-30°C as optimum temperature range for *Microsporum gypseum* and *Trichophyton persicolar*. In the present work, 30-35°C temperature is the optimum temperature for *Microsporum gypseum*. According to Stockdale (1953b), the dermatophytes grow best in culture at temperature, lower than human body temperature. Mehra and Jaitly, (1995) found that 28°C temperature is suitable for optimum growth of some common fungi from city waste. Michael *et al.*, (1998) investigated the effect of the ecological factors like pH, temperature and ionic strength on *Candida milleri*. Relative humidity also plays huge role in fungal growth and sporulation along with optimum range of temperature. Knight, (1976) investigated the effect of temperature and humidity on the growth and sporulation of *Trychophyton mentagrophytes* on human stratum corneum in vitro and found that 24°C to 36°C temperature was the best for the growth along with 97% relative humidity. The present investigations are in agreement with the above mentioned reports in a way that dermatophytes were showing maximum growth and sporulation at 75% - 95% relative humidity and 25°C – 35°C temperature. Ninomiger, (2000) reported the effect of temperature, humidity and minor injury to the penetration of dermatophytes into human stratum cornium. The result showed that 35°C temperature with 95% - 100% humidity were most suitable for penetration of dermatophytes. Morishita *et al.*, (2003) investigated that 35°C temperature and 95% - 100% relative humidity were most suitable for penetration of dermatophytes into human stratum cornium.

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