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Rapid Communication

Influence of Environmental Factors on the Growth And Sporulation of Geophilic Keratinophiles from Soil Samples of Public Park

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Abstract : Environmental factors play an important role in the growth and sporulation of 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. But in case of humidity the increase level of relative humidity shows excellent growth and decrease level of humidity shows poor growth and sporulation of fungi. In the present study various temperature regimes i.e. 0°, 5°,10°, 15°, 20°,25°,28°,30°,35°, 40°, 45°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 *Chrysosporium tropicum* and *Trichophyton mentagrophytes*. Both keratinophilic fungi isolated from public parks soil. These fungi showed their maximum growth at 28-30°C temperature and best sporulation at 25°C-35°C temperature. On the other hand *Chrysosporium tropicum* showed maximum growth at 75.00% relative humidity and best sporulation at 50-95% relative humidity. *Trichophyton mentagrophytes* showed maximum growth at 95.00% relative humidity and excellent sporulation at 62-95% relative humidity.

Key words : Environmental factors, Keratinophilic fungi, Temperature, Relative humidity.

Introduction

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). The soils represent the main reservoir of fungi. Soil that is rich in keratinous material is most conducive for the growth and occurrence of kerationophilic fungi. 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).

Study of the growth of an organism in different *in vitro* environmental conditions could reveal certain physiological characteristics of an organism which can be used for its laboratory identification. Chmel *et al.* (1972) investigated 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. Doohan *et al.* (2003) studied the influence of climatic factors on pathogenecity of *Fusarium* species on cereals. They found that climatic factors directly affect the pathogenicity of *Fusarium.* Keratinophilic fungi are generally reported as nature's keratin degrading machines (Sharma and Rajak, 2003).

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The metabolic activities and sporulation of fungi in culture and in the host are greatly influenced with variation temperature. 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

In the present study Sabourand's Dextrose Agar medium (SDA modified) was prepared and used to evaluate the growth and sporulation of keratinophilic fungi. All the experiments of physiological studies were performed in liquid medium. The pH of 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. The pH of medium after autoclaving was also determined because the autoclaving process is likely to change the initial pH of the media.

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 with the help of BOD incubator. The different relative humidity i.e. 11.05%, 22.45%, 33.00%, 50.00%, 62.00%, 75.00% and 95.00% were also maintained with the help of desiccators by using respective salt solutions and acids according to each humidity parameters.

The equal quantity of SDA broth media i.e. 25 ml was taken in every flask, the suspension of the test fungi, *Chrysosporium tropicum* and *Trichophyton mentagrophytes* was prepared aseptically in sterilized distilled water. Known quantity (0.2 ml) of each fungus under test was dropped in every flask aseptically by using the auto micropipette

For measurement of temperature effect, the all inoculated flasks were incubated at 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 28°C, 30°C, 35°C, 40°C and 45°C in BOD incubator, for 15 days. Likewise, the measurement of effect of relative humidity on growth of fungi the flasks were kept in desiccators for 15 days. The growth and sporulation were examined on 16th 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. Mats were pooled together in one filter paper and

the average dry weight calculated. The mycelial mat (in filter paper) was washed three times with distilled water, then dried in an incubator for 48 hours at 60-65°C temperature and then weighed under non-humid condition, along with the filter papers. 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).

Statistical Analysis of Data

Results given are mean ± standard error. Data obtained were statistically analyzed with one-way analysis of variance and the observations were considered significant when P-value of ANOVA F-test was 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 *Chrysosporium tropicum* showed maximum growth at 30°C temperature but the excellent sporulation was observed at 25°C to 35°C temperature. Below 20°C and above 35°C the growth and sporulation decreased sharply (Table 1). 62.00% and 75.00% relative humidity was excellent for fungal growth but best sporulation of *Chrysosporium tropicum* observed at 50.00% to 95.00% relative humidity. The fungal growth and sporulation were decreased with decrease in relative humidity (Table 2).

Table 1 : Average dry weight and sporulation of *Chrysosporium tropicum* and *Trichophyton mentagrophytes* at different temperature regimes (Initial pH 7.5)

Temperature °C	*Chrysosporium tropicum*			*Trichophyton mentagrophytes*		
	Final pH	Average dry weight of mycelium (gm)	Sporulation	Final pH	Average dry weight of mycelium (gm)	Sporulation
0	6.5	0.061±0.021	-	5.9	0.031±0.017	-
5	7.4	0.090±0.002	-	6.7	0.073±0.005	+
10	6.5	0.113±0.006	++	7.6	0.101±0.062	+
15	7.1	0.119±0.012	++	7.3	0.109±0.051	++
20	6.8	0.141±0.031	+++	7.7	0.139±0.007	+++
25	8.1	0.151±0.011	++++	7.9	0.171±0.008	++++
28	8	0.158±0.022	++++	7.8	0.207±0.018	++++
30	7.6	0.169±0.005	++++	7.8	0.174±0.012	++++
35	7.1	0.130±0.001	++++	6.8	0.160±0.001	++++
40	7.5	0.096±0.003	+	7.2	0.131±0.009	+
45	6.7	0.049± 0.041	-	6	0.069±0.015	-

Table 2 : Average dry weight and sporulation of *Chrysosporium tropicum* and *Trichophyton mentagrophytes* at different relative humidity (Initial pH 7.5)

Relative humidity (%)	*Chrysosporium tropicum*			*Trichophyton mentagrophytes*		
	Final pH	Average dry weight of mycelium (gm)	Sporulation	Final pH	Average dry weight of mycelium (gm)	Sporulation
11.05	8.2	0.129±0.003	++	7.6	0.108±0.051	+
22.45	8.2	0.136±0.009	++	7.9	0.140±0.016	++
33	7.9	0.195±0.018	++	8.2	0142±0.019	+++
50	8.2	0.217±0.015	++++	7.9	0.139±0.011	+++
62	8.1	0.226±0.007	++++	8	0.157±0.001	++++
75	8.2	0.228±0.019	++++	7.9	0.145±0.009	++++
95	7.9	0.221±0.031	++++	7.9	0.163± 0.026	++++

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)

Trichophyton mentagrophyes also showed the same results. Maximum growth occurred at 28°C temperature and best sporulation was achieved at 25°C to 35°C temperatures. Below 20°C and above 35°C temperature, the fungus showed poor sporulation and the dry mycelium weight decreased (Table 1). *Trichophyton mentagrophytes* showed maximum growth at 95.00% relative humidity and best sporulatoin at 62.00-95.00%. The sporulation and fungal growth of fungi were decreased with decrease in relative humidity (Table 2).

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. 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 found to be 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.

Ninomiya (2000) reported the effect of temperature, humidity and minor injury to the penetration of dermatophytes into human stratum corneum. The results showed that 35°C temperature with 95%-100% humidity were most suitable for penetration of dermatophytes. Morishita *et al*. (2003) investigated the effect of temperature, humidity, minor injury and washing on penetration of dermatophytes in human stratum corneum and observed that 35°C temperature with 95%-100% humidity were most suitable for penetration of dermatophytes into human stratum corneum.

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