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Effect of Temperature and pH variations on Growth Pattern of keratinophilic fungi from Jaipur, India

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ABSTRACTS

Earth has native milieu for fungi that cover individual kingdom since evolution. The Keratinophilic fungi are allied molds that produce the keratinase enzyme to degrade the keratinous materials in or on the soil. These saprophytic fungi also play a role as causative of superficial infections in the environment. In order to present study, three isolates of fungi were evaluated to determine the effect of physical conditions (temperature & pH) on fungal growth on keratinophilic species. They were grown in an inundated culture medium containing various parameters of temperature & pH variations. In the study, Among isolated species, all species Chrysosporium indicum showed the highest growth weight of dry mycelium at 15ºC to 35ºC temperature & pH 3. While another two remaining species show slime increased growth at 35°C & 7pH. In the present study, the best growth was recorded at 25°C to 35°C and 5-7 pH. Usually too alkaline and too acidic solutions or too high and too low temperature are not favorable for the growth of fungi. The leading phenomena of keratinophilic fungi in Jaipur are possibly due to the certain environmental conditions.

Keywords: *Arthoderma multifidium*; *Chrysosporium indicum; Fusarium solani;* Keratinophilic; filamentous fungi

INTRODUCTION

Fungi have an individual territory with an important role in the environment since evolution that is in the second population after bacteria in soil [1-2]. During the last decades, fungal infections are raised to 20–25% of the world's population [3]. In Major classification system of fungi, more than 100 species of fungi are generally recognized as pathogen found in soil [4]. They choose the forest, farmyard, park soils, as well as sediments of the rivers and oceans contained humus and organic material as the best candidate for growth. These fungi also cause infections i.e. known as dermatophytosis [5]. Environmental factors play an important role in the growth and sporulation of keratinophilic fungi [6-7]. Each fungus comprises a specific range of temperature & pH where they can grow and sporulates. Usually too alkaline and too acidic solutions or too high and too low temperature are not favorable for the growth of fungi. Typically the optimum temperature ranging from 15°C to 35°C and pH also ranging from 4.2–9.3 for the fungi growth [8-9]. The main aim of the present study is to evaluate the effect of physical conditions (temperature & pH) on fungal growth on keratinophilic species such as *Arthoderma multifidium* (KU578107), *Chrysosporium indicum* (KU578108) and *Fusarium solani***.**

MATERIALS AND METHODS

Soil collection: Soil samples were collected in black polybags after removal of surface litter to a depth of about 5cm from poultry farms, cattle yard, roadsides, barber's dump, Lake Sites and public parks in Jaipur city.

Isolation of keratinophilic fungi from soil: The keratinolytic fungi were isolated using 'hair baiting technique' where defatted keratin substrates were spread over the surface of the soil and incubated at room temperature 20-25ºC in dark for three to four weeks. The sterile distilled water was added in the keratinous substrates baited plates after every one week. The sterile distilled water provides the moistened conditions for the Keratinophilic fungal growth [10]. After A vigorous growth, the growth is transferred on the slants of Potato dextrose Agar (Hi-Media) with antibiotics for pure culture isolation, identification, and future analysis.

Identification of isolated fungi: After the preliminary examination, fungal growth the fungus was identified on the basis of Macroscopic, Microscopic, and 18S rRNA sequencing. Sequences are submitted to NCBI Genebank.

Screen out the Effect of physical factors on the growth of fungi: For the assessment of the growth of keratinophilic fungi on temperature & pH variations, modified Sabourand's Dextrose broth medium was used. For the same, the temperature was maintained at 5º, 15º, 25º, 35º, 45º, and 55ºC with shaking on rotation speed of 30 revolutions per minute whereas the pH of the medium was adjusted as 3, 4, 5, 5.6, 6, 7, 9, 11, 13 pH using 1N NaOH or 1N HCl. An equal quantity of SDA broth media i.e. 100 ml was taken in triplicates flask and known loop full quantity of 15 days old fungal cultures was inoculated & incubated for 15 days at variable conditions. On sixteen day of inoculation, the mycelium was harvested by filtering through dried and weighed Whatman's filter paper No. 1. Experiments were performed in triplicate and data analyzed are mean± SE subjected to one-way ANOVA with significant (P<0.05).

Results: Effect of different temperature & pH regimes on growth was analyzed from the dry mycelium weight using SDA modified broth medium in triplicates. Hydrogen ion concentration (pH) of the culture filtrates was also determined by calibrated pH meter at the end of each sampling. Almost all fungi grew in a wide range of temperature and relative pH. Both physiological factors were found to be different for different fungal growth. It study, expected results were found as *Chrysosporium indicum* showed highest growth weight of dry mycelium at 15ºC to 35ºC temperature & pH 3. There was a sharp decrease in growth with increasing the relative pH in acidic to alkaline manner. In follow up *Fusarium solani* also show a slime increased growth at 35°C & 7pH. The rate of growth decreased at acidic pH 5 and alkaline pH 11 as well as low and high temperature. Similarly, *Arthoderma multifidium* also show a growth pattern on 25ºC to 35ºC temperature with 6-7pH (Table 1&2). In the certain study, too alkaline and too acidic solutions or too high and too low temperature are not favorable for the growth of fungi. There was also recorded a slime changes in the pH of growth medium (Figure 1&2).

Table-1: - Effect of pH on the Growth of keratinophilic fungi.

Sr. No	Sample / Initial pH	*Chrysosporium indicum*	*Final pH*	*Arthoderma multifidium*	*Final pH*	*Fusarium solani*	*Final pH*
1.	**3**	1.24±0.17	3	0	2.7	0	3
2.	**4**	1.16±0.08	4.9	0.8±0.01	5	0.32±0.08	4.8
3.	**5**	1.10±0.03	6.8	1.02±0.03	5.16	0.51±0.09	5.6
4.	**6**	1.14±0.02	6.7	1.07±0.32	5.9	0.53±0.33	6.75
5.	**7**	1.16±0.02	8	1.25±0.029	5.9	0.56±0.12	7
6.	**9**	0.88±0.04	7	1.21±0.024	6.82	0.45±0.38	5.8
7.	**11**	0.90±1.02	7	1.11±0.106	6.93	0.41±0.30	6.8
8.	**13**	0	13	0	10.34	0.47±0.09	6.8
9.	**5.6**	0.93±0.12	6.8	1.55±0.057	5.33	0.36±0.27	5.6

Experiments were performed in triplicate and data analyzed are mean± SE subjected to one-way ANOVA with significant (P<0.05).

Table-2: - Effect of temperature on the Growth of keratinophilic fungi.

Sr. No	Sample / Initial temp	*Chrysosporium indicum*	*Final pH*	*Arthoderma multifidium*	*Final pH*	*Fusarium solani*	*Final pH*
1.	**5**	0.30±0.025	5.9	0	5.6	0.21±0.040	6.8
2.	**15**	0.86±0.081	8.2	0.25±0.105	5.7	0.28±0.045	6.8
3.	**25**	1.32±0.020	9.2	0.78±0.020	5.8	0.83±0.260	5.9
4.	**35**	1.29±0.070	8.6	1.19±0.210	5.8	1.20±0.055	5.7
5.	**45**	1.05±0.036	8.3	1.03±0.035	5.8	0.78±0.185	5.9
6.	**55**	0.72±0.050	7.5	0	5.4	0.50±0.138	5.9

Experiments were performed in triplicate and data analyzed are mean± SE subjected to one-way ANOVA with significant (P<0.05).

DISCUSSION

The Temperature & pH are the important ecological factors that affect the growth of microorganisms and their reproduction. Cochrane (1958) also detailed that there is no single temperature for optimum growth because growth is also dependent upon various other conditions which may, in fact, be limiting at the optimum temperature. They also observed that at low pH values, the enzyme systems may be disrupted and at high pH, metal solubility may be affected. Cochrane also suggested that many of the growth of fungi can raise or low the pH of an initially unfavorable medium containing different carbon and nitrogen sources [11]. De Maranon et al., 1999 found the maximum growth at 32°C of the dermatophytes because they can grow best in the culture at the lower temperature than human body [12]. The certain facts were also reported by Stockdale, 1953. Goldfarb and Herrman, 1956 also discovered the ability to change the pH of medium paralleled for Dermatophytes [13-14]. It the present study, the similarities were agreed with Chi et al., 1964's Studies indicated that *Fusarium solani* isolates grew well at a higher temperature of 28°C or a range amid 15°C -30°C [15]. According to Abdel-Fattah et al., 1982, the *Fusarium solani* species was recorded in soil samples from the Antarctic region having a temperature of -4°C to soil in many parts of Europe, Asia, America and East island, where the temperature range 10°C to 30°C [16]. Subsequently, Sharma, 1983 studied the in vitro growth pattern in dermatophytes viz., *Gymnoascus reesssii*, *Microsporum gypseum*, *Trichophyton simii*, *Cephaliophora irregularis* and *Chrysosporium tropicum* by using different combinations of temperature and pH [17].

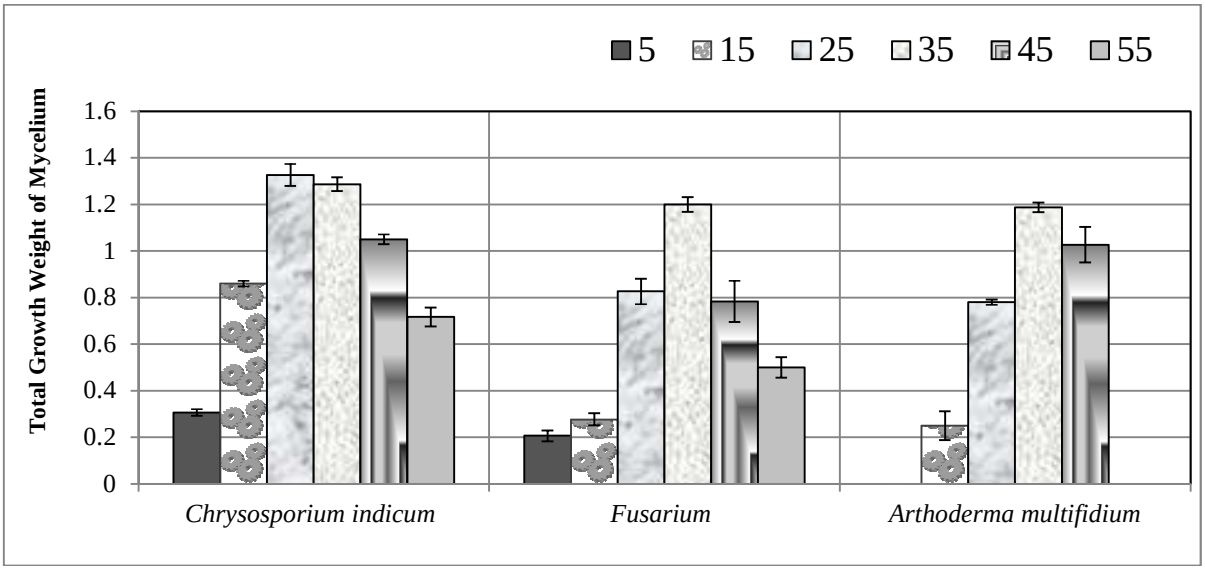

Figure-1: Effect of temperature on the Growth of test keratinophilic fungi.

Subsequently Khilare et al., 2012 conducted study to evaluate the effect of different pH and temperature levels on mycelial growth of *Fusarium oxysporum* and observed the most suitable pH level for growth of fungus was 6.0 and 6.5 with a temperature range from 15°C - 35°C with a maximum Growth at 30°C [18]. Llamas et al., 2007 also documented that the Mycelia growth of *Fusarium solani* was maximal at 25°C [19]. Gupta et al., 2010 also reported the optimum temperature and pH range at 28°C and 5.5 for the growth of *Fusarium spp* [20]. Data presented by Merlin et al., 2013 clearly reveals that pH 6.0 and 25±2°C temperature was found as best factors for the growth of *Fusarium solani* [21].

Subsequently, Sharma et al., 2011 also recorded that Fungus grows maximally at pH 6.8-7.2 and 28ºC-37ºC temperature combinations [22]. A similarity was related to findings of Hashimoto et al., 1972 who documented the optimal pH appeared to be between 6.0 and 6.5 for germination of *Trichophyton mentagrophytes'* microconidia. The best optimal temperature was also recorded at 37°C for micro conidial germination, 30°C for vegetative growth of fungus and 25°C for sporulation23. A similar approach was also achieved by Knight, 1976 who recorded the optimal temperature range as 27–33° C but reasonable growth was obtained between 24 and 36°C [23].

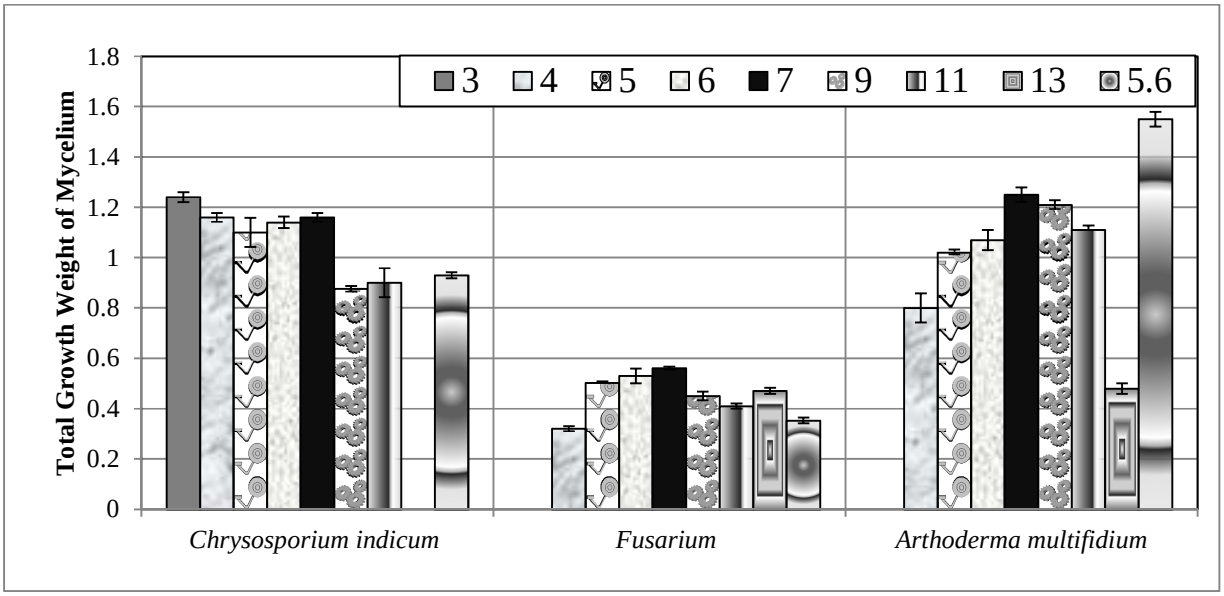

Figure-2: Effect of pH on the Growth of test keratinophilic fungi.

In the follow-up to all recently Sharma et al., 2012 also confirmed the excellent sporulation at an optimal temperature range 30°C–35°C and maximum growth at 30°C for keratinophytes [9]. Kadhima et al., 2015 reported the optimal conditions as 30°C, pH6 for the growth of Dermatophytes [25]. In the present investigation, the ranges of various environmental factors act as limiting factors for the growth of these pathogenic fungi and can be utilized in controlling its growth.

CONCLUSION

The present study states that the soils of Jaipur city, India may be major reservoirs in the presence of favorable pH and temperature for the growth and existence of keratinophilic fungi in the environment. According to the present study, each fungus comprises a specific range of temperature (25-35ºC) & pH (5-7 pH) that can be favorable for the growth of fungi.

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