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# EFFECT OF HYDROGEN ION CONCENTRATIONS ON THE GROWTH AND SPORULATION OF SOIL BORNE KERATINOPHILIC FUNGI *IN VITRO*

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Hydrogen ion concentration (pH) plays a major role in determining the growth of fungi and governs the metabolic activities of growing organisms both in nature and artificial cultures. Every fungus shows excellent growth at certain range of pH values of the medium and fails to grow at high and low pH. The optimum pH for best growth of each fungus differed due to the difference in their metabolic activities. For the present study various hydrogen ion concentrations viz. 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 12.0 were studied to evaluate the growth and sporulation of two keratinophilic fungi *Chrysosporium tropicum and Trichophyton mentagrophytes. C. tropicum* showed excellent sporulation at 7.5-9.0 pH values and best growth was achieved at 7.5 pH. *T. mentagrophytes* showed excellent sporulation from 7.5-9.5 pH values and best growth at 8.0 and 8.5 pH. From the above findings it can be concluded that hydrogen ion concentration is an essential requirement for the growth and sporulation of keratinophilic fungi.

**Keywords** : *Chrysosporium tropicum;* Hydrogen ion concentration; Keratinophilic fungi; *Trichophyton mentagrophytes.*

## Introduction

Physical and environmental studies of fungi or any other organism, is necessary for understanding their relationship with the substratum/host on which they grow. These studies include the effect of different media compositions, temperature, pH, humidity, moisture etc. on general physiology of the fungus. Monica[1] studied the growth of *Trichophyton rubrum* at different pH range and also studied nutritional requirements of the *Trichophyton rubrum*. Michael *et al.*[2] investigated the effect of ecological factors like pH, temperature and ionic strength on *Candida milleri*.

The history of physiological studies on keratinophilic fungi started long back in 1887 when Verujsky[3] observed that a neutral or a slightly acidic medium and an optimum temperature around 33°C were most favourable for the growth of *Trichophyton schoenleinii* and *T. tonsurans*. Hydrogen ion concentration of media plays an important role in determining the growth and sporulation of fungi. This concentration of media can be different for growth of each fungi. All fungi grow at optimum range of pH. Coehrane[4] observed that a fungus grows maximally over a certain range of pH values of the medium and fails to grow at high and low pH. Dermatophytes could also grow over a wide range of pH (4.0-10.0). It was confirmed by the observations of Ganderson[5] and Stockdale[6]. Uppal *et al.*[7] and Patel[8] reported that *Alternaria burnssi* was able to withstand a wide range of pH varying from 3 to 10, with an optimum of 6 to7.

Keratinophilic fungi are well known and well adapted group of fungi and are generally consider as a soil saprophytes[9,10]. From India, the first report of isolation of a keratinophilic fungi *Microsporum* from soil was achieved by Dey and Kakoti[11]. Soil provides an excellent habitat for survival of these fungi. Keratinophilic fungi degrade keratin and other keratinous material obtained from soil and other sources. Keratinophilic fungi are generally reported as nature's keratin degrading machines[12].

## Materials and Methods

In the present study two keratinophilic fungi *Chrysosporium tropicum* and *Trichophyton mentagrophytes* were selected. Both were isolated from soil samples. Single spore cultures of both these fungi were raised and maintained on Sabouraud's Dextrose Agar medium. Sabouraud's Dextrose Agar medium (SDA modified) was used to evaluate the growth and sporulation

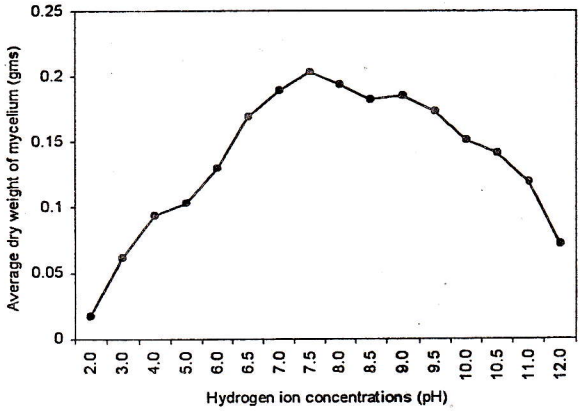


**Fig. 1.** Effect of Hydrogen Ion Concentrations (pH) on the growth and sporulation of *Chrysosporium tropicum.*

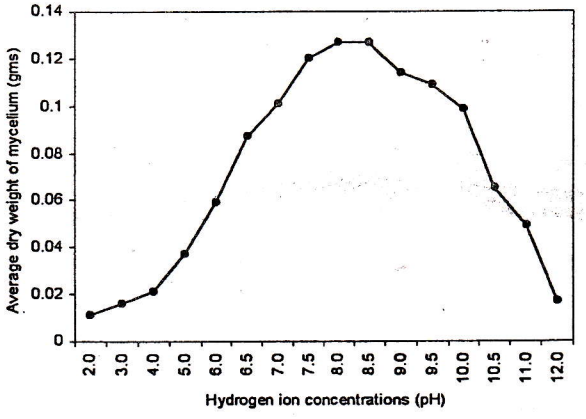


**Fig. 2.** Effect of Hydrogen Ion Concentrations (pH) on the growth and sporulation of *Trichophyton mentagrophytes.*

of both keratinophilic fungi. The present experiment was performed on liquid medium and control was simultaneously maintained for comparison of growth (in term of dry mycelium weight) and sporulation. The pH of nutrient medium was adjusted 7.5 with the help of a pH meter using N/5 NaOH or N/5 HCl before autoclaving or fractional sterilization. The pH of the medium was also determined after autoclaving because the autoclaving process is likely to change the initial pH of the medium.

During this investigation various pH levels i.e. 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 12.0 were adjusted as above mentioned method. The equal quantity of SDA broth medium i.e. 25 ml was taken in every flask. The suspension of *Chrysosporium tropicum* and *Trichophyton mentagrophytes* was prepared aseptically in sterilized distilled water. Known quantity (0.2 ml) of each test fungus was dropped in every flask aseptically by using the automicropipette. Garett's agar disc method[13] was generally followed for inoculating the flasks, except where stated otherwise. After the inoculation, all pH adjusted flasks were kept in culture room for 15 days at 28±2°C. The growth and sporulation were examined on 16th day and mycelium 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 mats (in filter paper) were 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. pH of the culture filtrates was also 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 estimation of fungal sporulation were counted by using Tuite[14] formula as follows :

No. of spores/ml = No. of spores counted × microscopic field × 1000

A total of 15 counting were made in each case and an average was calculated as suggested by Wilson and Knight[15].

**Results and Discussion**

Effect of different pH levels on the fungal growth was analysed from dry mycelium weight and spore count was determined by using SDA broth (modified) medium. Almost all fungi grew in a wide range of hydrogen ion concentrations of the medium but they could sporulate well only at certain range of pH. The mechanism of action differs at different concentration of hydrogen ion but majority of fungi flourish between 5 to 6.5 pH[16]. Some fungi grow in acidic conditions and some require alkaline while others prefer near of neutral conditions. Adeyefa[17] reported the optimum growth of zoophilic dermatophytes at pH ranging from 5.0-6.0.

On the basis of observation noted after incubation, it was found that *Crysosporium tropicum* showed excellent sporulation at 7.5 to 9.0 pH. The excellent growth of *C. tropicum* was observed at pH 7.5. Highly acidic medium (2.0-4.0 pH) were not found suitable for sporulation of *C. tropicum* and highly alkaline pH condition was less suitable for sporulation (Table 1 and Fig. 1).

*Trichophyton mentagrophytes* preferred alkaline pH conditions and showed excellent sporulation at 7.5-

**Table 1.** Average dry weight, sporulation and final pH of *Chrysosporium tropicum* at different hydrogen ion concentrations (pH).

| Initial pH | pH after autoclaving | Final pH after 15 days | Average dry weight of mycelium (gm) | Sporulation |
|---|---|---|---|---|
| 2.0 | 1.9 | 2.1 | 0.017 | - |
| 3.0 | 3.2 | 3.1 | 0.061 | - |
| 4.0 | 4.1 | 4.3 | 0.093 | - |
| 5.0 | 5.1 | 5.5 | 0.103 | +1 |
| 6.0 | 5.6 | 5.8 | 0.129 | +2 |
| 6.5 | 6.3 | 8.0 | 0.169 | +3 |
| 7.0 | 6.6 | 8.0 | 0.189 | +3 |
| 7.5 | 7.3 | 7.9 | 0.206 | +4 |
| 8.0 | 7.1 | 7.8 | 0.193 | +4 |
| 8.5 | 6.7 | 7.8 | 0.182 | +4 |
| 9.0 | 7.6 | 7.4 | 0.185 | +4 |
| 9.5 | 6.6 | 6.0 | 0.173 | +3 |
| 10.0 | 7.5 | 7.1 | 0.151 | +2 |
| 10.5 | 7.3 | 5.1 | 0.141 | +2 |
| 11.0 | 7.2 | 7.6 | 0.119 | +2 |
| 12.0 | 9.1 | 9.0 | 0.071 | +1 |

**Table 2.** Average dry weight, sporulation and final pH of *Trichophyton mentagrophytes* at different hydrogen ion concentrations (pH).

| Initial pH | pH after autoclaving | Final pH after 15 days | Average dry weight of mycelium (gm) | Sporulation |
|---|---|---|---|---|
| 2.0 | 1.9 | 2.0 | 0.011 | - |
| 3.0 | 3.2 | 3.0 | 0.016 | - |
| 4.0 | 4.1 | 4.2 | 0.021 | - |
| 5.0 | 5.1 | 5.9 | 0.037 | - |
| 6.0 | 5.6 | 6.1 | 0.059 | +1 |
| 6.5 | 6.3 | 6.8 | 0.087 | +2 |
| 7.0 | 6.6 | 6.9 | 0.101 | +3 |
| 7.5 | 7.3 | 7.3 | 0.120 | +4 |
| 8.0 | 7.1 | 6.6 | 0.127 | +4 |
| 8.5 | 6.7 | 6.2 | 0.127 | +4 |
| 9.0 | 7.6 | 6.5 | 0.114 | +4 |
| 9.5 | 6.6 | 6.0 | 0.109 | +4 |
| 10.0 | 7.5 | 6.7 | 0.099 | +3 |
| 10.5 | 7.3 | 6.8 | 0.065 | +2 |
| 11.0 | 7.2 | 7.1 | 0.049 | +2 |
| 12.0 | 9.1 | 9.0 | 0.017 | - |

9.5 pH. The maximum growth of *T. mentagrophytes* was observed at two pH levels i.e. 8.0 and 8.5 pH. A double maxima was recorded in this fungus. At 5.0 pH and below the sporulation did not occur. Highly alkaline pH condition (pH 12.0) was not suitable for sporulation (Table 2 and Fig. 2).

It is clear from the above result that both keratinophilic fungi did not show good growth and sporulation in highly acidic and strong alkaline range of pH. They however showed excellent growth and sporulation in alkaline media having pH value from 7.0-9.0. Sharma[18] studied the effect of hydrogen ion concentrations on the growth and sporulation of five keratinophilic and dermatophytic fungi. Her observations further corroborated the present findings.

Majority of fungi have been found to grow well at a pH range from 4.2 to 9.3. Generally too alkaline and too acidic solutions are not favourable for the growth of fungi. This can be due to the fact that in too acidic and too alkaline media proteins have a tendency to develop lesser viscosity and simultaneously, its colloidal behaviour changes in the sense that hydrolysis of the proteins to form simpler products resulting in the formation of colloidal particals of smaller dimensions. Under such circumstances the formation of complex protein molecules become difficult resulting in the retardation of growth[19].

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