Effect of environmental factors on isolation, mycelial growth and sporulation of keratinophilic fungi

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ABSTRACT

The environmental factors play an important role in the growth and sporulation of keratinophilic fungi. Each fungus requires optimal temperature and pH for their proliferation. The present study was undertaken to determine the effect of different temperatures (15, 20, 25,30,35,40 and 45^{0}C) and different pH (5.5, 6, 6.5, 7.0, 7.5, 8.0 and 8.5) on the mycelium growth (gm) of different keratinophilic (viz. Chaetomium seminis-citrulli, Penicillium guttulosum, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton eracenii and Microsporum nanum), isolated from soil samples collected from different habitats of Jaipur (Rajasthan), using baiting technique. The results of the present study reveals that the maximum mycelium growth and sporulation was recorded at 30^{0}C in all tested keratinophilic fungi. At pH 7.5 highest mycelium growth was recorded whereas the pH 6.5 exhibited the best sporulation in all test isolates. However, the lowest mycelial growth and spore formation was recorded at pH 5.5 and pH 8.5. Thus, from the results obtained in the present study it can be concluded that keratinophilic fungi isolated from soil samples collected from different habitats of Jaipur (Rajasthan), using baiting technique, have similar growth requirement range of pH and temperature in natural and in vitro conditions. The study of effect of different environmental factors can be utilized for in vitro development of culture conditions for growth of keratinophilic fungi.

Keywords: Environmental factors, keratinophilic fungi, mycelium growth and sporulation.

1. INTRODUCTION

Microbial world is vast and invisible to human eyes. The microbial world comprises of bacteria, fungi, algae, yeasts, protozoans and other micro fauna. Fungi are the second largest group of microorganisms after the insects which are capable to colonize in different habitats and environments [1,2]. Keratinophilic fungi are reported to be involved in the biodegradation of keratinaceous substrates and are found worldwide[3]. Keratinophilic fungi are ecologically important and recently have great concern throughout the world[4,5]. In the past two decades′ considerable evolution both of keratinophilic fungi and of the processes by which they degrade keratin and become associated with human and animal diseases have been reported[6]. Due to the keratinolytic nature, keratinophilic fungi are also known as nature′s gift[7,8]. The isolation of keratinophilic fungi was reported first time by Vanbreuseghem (1952) who employed " To. Ka.

Va. hair baiting technique" for isolation of keratinophilic fungi from soil[9]. This baiting technique has been used throughout the world for isolation of keratinophilic fungi using different baits including feathers, nail clippings, horns and hooves [10,11,12].

Since for the growth and sporulation all the fungus has a unique optimal requirement and ecological/environmental factors play a significant role in the growth and sporulation of keratinophilic fungi[13]. Different environmental conditions required by keratinophilic fungi are pH, temperature and culture media which play an important role in growth, sporulation, sexual reproduction and morphological characters[14]. The growth of keratinophilic fungi under different *in-vitro* environmental conditions could reveal some physiological characteristics.

Temperature is a major abiotic parameter which determine the growth of micro-organisms[15]. Most of the fungi show better growth at a temperatures range of 15°C to 35°C. But, growth and sporulation of fungi under laboratory conditions are greatly influenced by varying temperature range. The second envionmental condition is the pH of the culture media which considerably affects the growth of fungi by its action on the cell surfaces and on the availability of nutrients[16]. Earlier studies on pH have reported that fungi grow at neutral to the weak acidic environment, with the maximum production of dry mycelial weight[17]. Earlier studies have shown that pH 5.0-8.0 is the optimum pH range for conidial production and sporulation in liquid [18].

So, the present study was undertaken to study the *in vivo* and *in vitro* effect of temperature and pH on mycelium growth and sporulation of some keratinophilic fungi isolated from different soil samples collected from different habitats of Jaipur (Rajasthan) using baiting technique[9].

2. MATERIALS AND METHODS

2.1.Collection of soil samples

Soil samples were collected from the superficial layers of 2 to 3 cm from different habitats of Jaipur (Rajasthan) in sterile polythene bags and closed tightly to maintain the initial moisture of the soil. The samples were maintained at room temperature until processing.

2.2.Analysis of pH and temperature of soil samples

Soil temperature: Soil temperature was recorded using a thermometer by taking it into 2 cm depth of soil at the time of collection[19].

Soil pH: Five gm of soil samples were taken in 10 ml distilled water**.** For the analysis of soil pH, pH meter was firstly calibrated with buffer solution of 4, 7 and 9**.**2 and then the pH of soil was recorded with regular washing of glass electrode[20]**.**

2.3.Isolation and purification of keratinophilic fungi

Keratinophilic fungi were isolated using ″To. Ka.Va. hair baiting technique″. In this technique, soil samples were filled in sterile Petri-plates. Soil was moistened with autoclaved distilled water and

sterile human hair were spread evenly on the soil surface. The plates were closed and incubated for 21 days at room temperature. The baiting plates were observed for fungal colony and fungal mycelium. The growing colonies were periodically sub-cultured onto newly prepared (SDA) medium for further purification and identification[21]. All the fungal isolates were identified morphologically by macro and micro morphological features of isolates [22,23,24].

Keratinophilic fungi isolated from soil samples

Out of the total isolates obtained from different soil samples six fungal species viz. *Chaetomium seminis-citrulli, Penicillium guttulosum, Trichophyton mentagrophytes, Trichophyton rubrum*, *Trichophyton eracenii* and *Microsporum nanum* were selected for the present study on the basis of their morphological characters.

2.4.Effect of different temperatures on mycelial growth of fungi

Sabouraud′s dextrose broth (SDB) medium was used for the study of the effect of different temperatures on growth and sporulation of selected keratinophilic fungi. 100 ml of Sabouraud′s dextrose broth (SDB) media was prepared in 250 ml conical flasks for each selected fungal isolate. The selected fungal culture was inoculated in each experimental flask and incubated at different temperatures (from 15ºC to 45ºC at a regular interval of 5ºC) in the incubator. The fungal cultures were observed periodically for up to 14 days. Each treatment was replicated thrice and data was analyzed by mean ± SE.

2.5.Effect of various pH on mycelial growth of fungi

The influence of various pH on growth and sporulation of keratinophilic fungi was studied in Sabouraud′s dextrose broth (SDB) medium. 100 ml of culture media were prepared in 250 ml conical flasks for each selected fungi. The pH of media was adjusted to different pH, ranging from 4.0 to 8.5, by adding 1N HCl and 1N NaOH. The test fungi were inoculated in each experimental flask and incubated at 28±2ºC in the incubator. Mycelial growth and sporulation was recorded after 21 days[25]. For each pH condition, the treatment was replicated thrice and data was analyzed by mean ± SE.

2.6.**Assessment of dry weight of the fungus:** The growth of the fungi was assessed by weighing the mycelium weight after complete growth of 21 days**.** The mycelium was collected and filtered through Whatman filter paper no. 1. The filter papers along with mycelium were dried inside a hot air oven at 50±2ºC until dryness. The weight of filter paper along with mycelium weight was taken.

The actual dry weight of fungal mycelium was estimated using the following formula[26].

Weight of myceliym = (Weight of filter paper + Weight of Mycelium) – Weight of filter paper

The sporulation degree of keratinophilic fungi was determined by using the standard methods as mentioned by Tuite (1969)[27].

3. RESULTS

In the present study, soil samples were collected from the pH range of 6.29 to 8.82. Physical and chemical condition of soil plays an important role in growth and occurrence of fungi. A total of 48 isolates were recovered in this study including 6 isolates of *Chaetomium-seminis-citrulli*, 5 isolates of *Penicillium guttulosum*, 11 isolates of *Trichophyton rubrum*, 14 isolates of *Trichophyton mentagrophytes*, 3 isolates of *Trichophyton eracenii* and 9 isolates of *Microsporum nanum.*

In our study, the selected keratinophilic fungi i.e. *Chaetomium-seminis-citrulli* was isolated from soil with pH 7.5-8.0, *Penicillium guttulosum* from soil with pH 6.3-7.0, *Trichophyton rubrum* from pH 7.0-8.5, *Trichophyton mentagrophytes* from pH 6.5-7.5, *Trichophyton eracenii* from pH 7.0-8.0and *Microsporum nanum* from pH 6.5-8.0 *(*Figure 1*).*

However, the incidence of occurrence of keratinophilic fungi was low at pH <6.0 and pH> 8.50. The temperature range of 27ºC-31ºC was the most favorable temperature range for isolation of fungi. The fungi were also isolated from temperature range of 26ºC to 35ºC. *Chaetomium-seminis-citrulli* was isolated from soil temperature 32ºC-35ºC, *Penicillium guttulosum*from soil temperature 28ºC-31ºC, *Trichophyton rubrum* from temperature 29ºC-33ºC, *Trichophyton mentagrophytes* from temperature 26ºC-31ºC, *Trichophyton eracenii* from temperature 28ºC-32ºC and *Microsporum nanum* from temperature 28ºC-31ºC*.*

Table 1 and 2 shows the results of effect of different pH on the myclial growth and sporulation of keratinophilic fungi. In case of *in vitro* evaluation of the effect of different pH and temperature on mycelial growth and sporulation on the selected keratinophilic fungi, were analyzed from the dry mycelium weight and spore count in triplicates. In the present study, the most favorable pH for the growth of the keratinophilic fungi was found to be at pH 7.5. The fungi were found to grow well in the pH range 6.0 to 8.0. The pH 6.5 exhibited the best sporulation in all test isolates.

The maximum mycelium growth of *Chaetomium-seminis-citrulli* was at pH 7.5, *Penicillium guttulosum* at pH 6.0, *Trichophyton rubrum* at pH 7.5, *Trichophyton mentagrophytes* at pH 7.5, *Trichophyton eracenii* at pH 7.5 and *Microsporum nanum* at pH 6.5. The pH ranges at 5.5 and 8.5 showed poor growth and mycelium growth of keratinophilic fungi.

The excellent sporulation of *Chaetomium-seminis-citrulli* was found at pH 6.5, *Penicillium guttulosum* at pH 6.0 and 7.0, *Trichophyton rubrum* at pH 7.0 and pH 7.5, *Trichophyton mentagrophytes* at pH 6.0; 6.5; and 7.0, *Trichophyton eracenii* at pH 6.5; pH 8.0 and *Microsporum nanum* at pH 6.0; 6.5; 7.0*.* The pH ranges at 5.5 and 8.5 showed poor sporulation of keratinophilic fungi.

In the present study, that after incubation of 21 days, keratinophilic fungi changed the pH of culture media**.** It was found that the initial pH of the culture media floated from alkaline to acidic range and acidic range to near neutrality after 21 days of incubation**.** The change in pH of the medium may be owed to metabolic activity of keratinophilic fungi.

Table 1: Effect of various pH on mycelial growth (gm.) of keratinophilic fungi

pH	*Chaetomium seminis-citrulli*	*Penicillium guttulosum*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*	*Trichophyton eracenii*	*Microsporum nanum*
	Dry weight (gm.) of mycelium					
5.5	0.452±0.11	0.921±0.05	0.327±0.15	0.489±0.12	0.395±0.13	0.772±0.09
6.0	0.907±0.26	2.078±0.12	0.3541±0.24	0.561±0.13	0.435±0.27	1.732±0.23
6.5	1.131±0.36	1.129±0.04	1.141±0.16	0.942±0.11	1.175±0.54	2.071±0.29
7.0	1.241±0.12	1.148±0.12	1.322±0.10	1.265±0.21	1.239±0.14	1.673±0.16
7.5	1.861±0.33	0.940±0.24	1.621±0.23	1.447±0.18	1.575±0.23	1.541±0.14
8.0	1.429±0.22	1.790±0.15	0.336±0.05	0.523±0.09	0.978±0.19	1.964±0.27
8.5	0.357±0.06	0.781±0.02	0.296±0.08	0.349±0.15	0.189±0.16	1.167±0.22

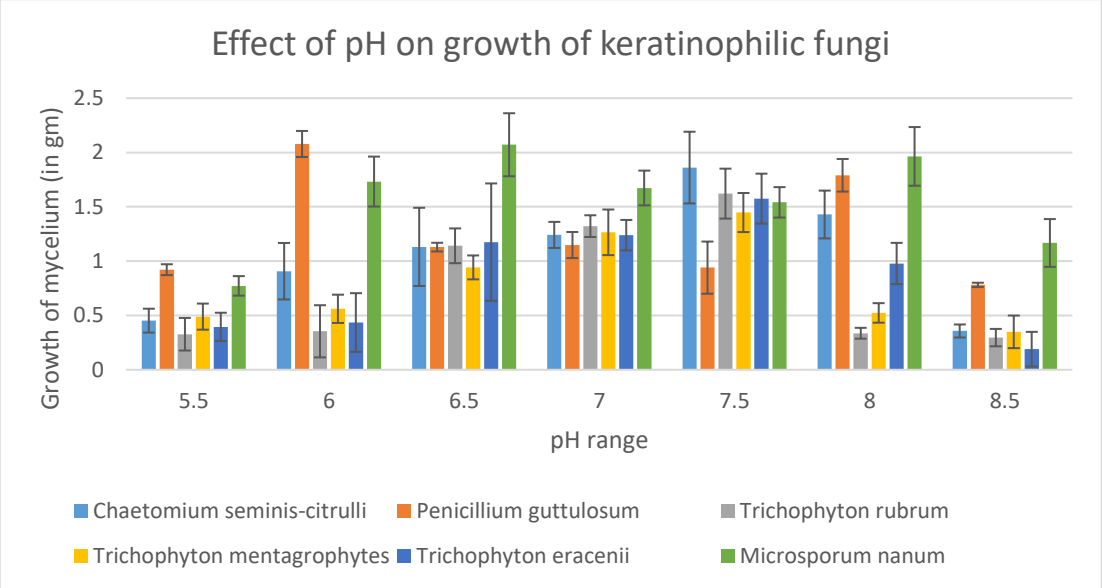

Figure 1: Effect of pH on growth of keratinophilic fungi in *in vitro* condition

Table 2: Influence of various pH on sporulation of keratinophilic fungi

pH	*Chaetomium seminis-citrulli*	*Penicillium guttulosum*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*	*Trichophyton eracenii*	*Microsporum nanum*
	Sporulation					
5.5	+	++	-	++	+	+++
6.0	+++	++++	++	++++	+++	++++
6.5	++++	++	+++	++++	++++	++++
7.0	+++	++++	+++	++++	+++	++++
7.5	+++	+++	+++**+**	+++	+++	+++
8.0	+++	+++	++	+++	+++**+**	+++
8.5	++	-	-	+	+**+**	++

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

The fungi *Microsporum nanum* showed maximum mycelium growth at all pH range and represented as best fungus for growth. *Chaetomium-seminis-citrulli* and *Penicillium guttulosum* also exhibited strong growth and sporulation after *Microsporum nanum. Trichophyton rubrum, Trichophyton mentagrophytes* and *Trichophyton eracenii* showed moderate growth rate and sporulation at different pH*.*

Table 3 and 4 shows the results of effect of different temperatures on the myclial growth and sporulation of keratinophilic fungi. Temperature is also an important environmental factors which influence the growth and sporulation of fungi. The rate of growth of fungi was different for each fungus on variable temperature range. The maximum mycelium growth of all isolated fungi was at 30ºC followed by 35ºC. The highest growth of fungi at 30ºC was reported as *Chaetomium-seminis-citrulli* (1.151±0.12 gm.), *Penicillium guttulosum* (1.713±0.18), *Trichophyton rubrum* (1.352±0.06 gm), *Trichophyton mentagrophytes* (1.455±0.14 gm), *Trichophyton eracenii* (1.141±0.31 gm) and *Microsporum nanum* (2.127±0.37 gm). The isolated fungi showed poor growth at 15ºC and 45ºC temperature (Figure 2).

Similarly, the best temperature range for sporulation of keratinophilic fungi was found to be at 30ºC followed by 35ºC and 25ºC. The temperature 15ºC, 20ºC and 45ºC showed poor or no sporulation rates.

Table 3: Mycelium growth (In gm.) of keratinophilic fungi at different temperatures

Temp. (ºC)	*Chaetomium seminis-citrulli*	*Penicillium guttulosum*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*	*Trichophyton eracenii*	*Microsporum nanum*
	Dry weight (gm.) of mycelium					
15	0.190±0.09	0.230±0.08	0.190±0.09	0.131±0.04	0.123±0.03	0.437±0.04
20	0.280±0.06	0.340±0.07	0.258±0.03	0.342±0.23	0.283±0.16	0.560±0.05

25	0.972±0.17	1.242±0.09	0.972±0.14	0.752±0.12	1.112±0.18	1.214±0.03
30	1.151±0.12	1.713±0.18	1.352±0.06	1.455±0.14	1.141±0.31	2.127±0.37
35	1.002±0.09	1.472±0.04	0.989±0.11	0.867±0.09	0.925±0.23	1.979±0.05
40	0.640±0.08	0.730±0.11	0.673±0.03	0.545±0.06	0.712±0.17	0.808±0.09
45	0.323±0.02	0.419±0.03	0.148±0.04	0.341±0.26	0.430±0.08	0.491±0.04

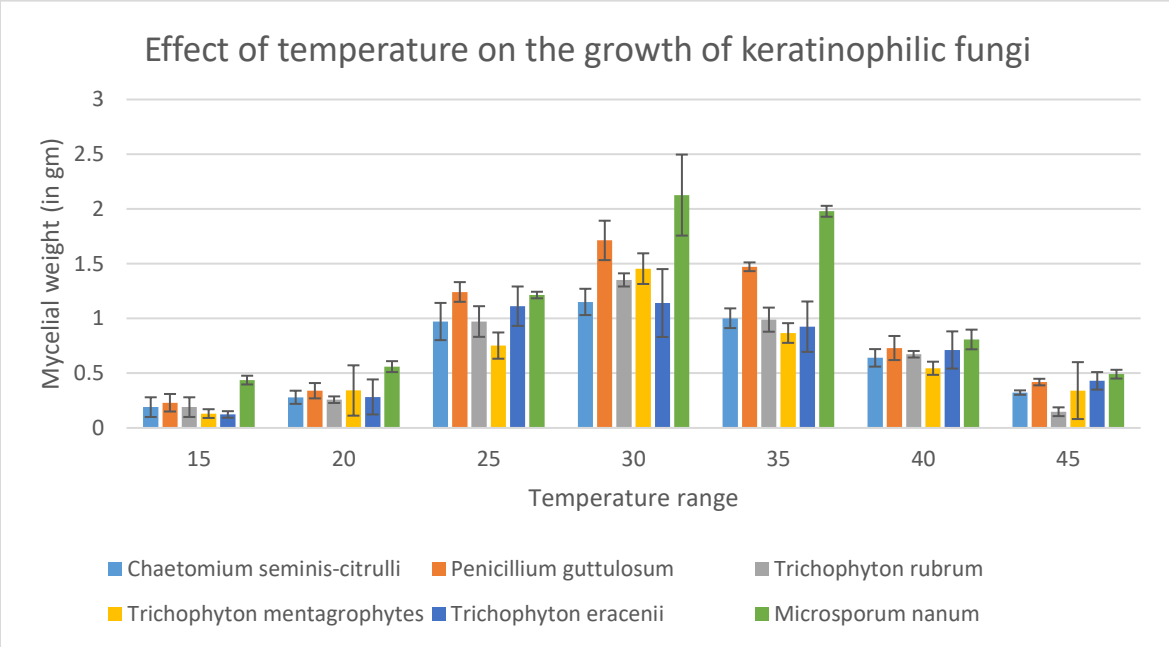

Figure 2: Effect of temperature on growth of keratinophilic fungi in *in vitro* condition

The fungi *Microsporum nanum* showed maximum mycelium growth at all temperature range and represented as best fungus for growth. *Penicillium guttulosum* also exhibited strong growth and sporulation after *Microsporum nanum*. *Chaetomium-seminis-citrulli*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Trichophyton eracenii* showed moderate growth rate and sporulation at various temperature.

The present study is an attempt to correlate the *in vivo* and *in vitro* environmental conditions for isolation and growth of keratinophilic fungi. The results obtained in the present study indicates that the keratinophilic fungi isolated have similar growth requirement range of pH and temperature *in vivo* and *in vitro* condition.

Table 4: Effect of various temperatures on sporulation of keratinophilic fungi

Temperature ºC	*Chaetomium seminis-citrulli*	*Penicillium guttulosum*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*	*Trichophyton eracenii*	*Microsporum nanum*
	Sporulation					
15	-	+	-	++	-	++
20	+	+	+	++	-	+

25	+++	+++	+++	++++	+++	++++
30	++++	++++	++++	++++	++++	++++
35	+++	++++	+++	++++	++++	++++
40	++	+++	++++	++++	+++	++++
45	+	++	++	+++	++	++

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

4. DISCUSSION

Every fungus has a definite range of optimum condition and sometimes it can adapt and grow under various culture conditions. The culture media and physiological parameters are important criteria for growth and sporulation of fungi[28]. Usually, high alkaline or acidic environments and high or low temperature are not favorable for the growth of fungi.

Temperature is the most important physical condition for regulating the growth and reproduction of keratinophilic fungi[29]. Generally, temperature range between 15°C to 35°C and 4.2– 9.3 pH promote the growth of fungi[30]. In our study, the optimal temperature for mycelial growth was 30ºC and 35ºC and maximum sporulation was recorded at 30ºC. The results of present study coincide with the results of Van Oorschot (1980) who reported 25ºC to 35 ºC as the optimum temperature for the growth of *Chrysosporium queenslandicum*[31]. Sharma and Sharma (2009) reported the growth and sporulation of *Chrysosporium tropicum* and *Trichophyton mentagrophytes* at 28°C-30°C and 25°C-35°C respectively[13]. Findings of the current study are in line with the findings of Sharma *et al.* (2012) with mycelium growth between 30°C–35°C[32].

Sharma and Sharma (2011) reported optimum growth of *T. rubrum* in terms of dry weight of mycelium at 33°C and pH 7 which is in agreement of the current study[33]. Similarly, Kadhima *et al.* (2015) reported 30°C temperature and pH 6.0 as the optimal condition for the growth of *Trichophyton rubrum*. Growth of *Microsporum nanum* was observed well between 25ºC to 35ºC temperature and optimum growth at 30ºC [34]. The present study supports the findings of Stockdale (1953) and Sharma *et al.* (2012) who also found the temperature range of 25-35°C for the growth of *Microsporum nanum*[32,35]. The findings of several researchers, including Chi *et al.*, 1964; Llamas *et al.*, 2007; Gupta *et al.*, 2010; khilare and Ahmed., 2012 are similar to the present study[36,37,38,39]. However, the results of this study are not in agreement with the results of Norries, (1999) who found that there was no effect of temperature on the growth of dermatophytes associated with the genus *Trichophyton*[40].

pH is also a significant environmental factor which influence the growth and sporulation of soil fungi. In the present study, the frequency of growth of fungi was different for each fungus on variable pH range. The optimum pH for growth of all isolated keratinophilic fungi was found at pH 7.5. The isolated keratinophilic fungi were found good growth at pH 6.0 to 8.0. The present study coincides with the findings of Bhadauria and Sharma, (2001) who observed that the best pH

range for the growth of dermatophytes lies between 6.5 to 7.5[41]. The result of pH effect is also in agreement with Abid-Ali, (2010) who concluded that the optimum pH for growth was 6.0[42]. But Perlman, (1965) reported that the length of hyphae of some fungi became shorter when pH concentration was increased from 6 to 8[43]. Saha, (2008) reported that the optimum pH for growth of *Lasiodiplodia theobromae* was at the range of pH 5.5-6.5and the results indicated that slightly acidic pH to neutral pH was optimum for the growth of the organism[18]. Singh and Chauhan, (2013) studied the effect of pH on the growth of *Aspergillus flavus* and *Penicillium chrysogenum in vitro*[44]*.* Maximum growth of *A. flavus* was observed at pH 6 (0.506±.030) and whereas pH 7 (0.356±.013) was most suitable for the growth of *P. chrysogenum*. Hasan and Al-Jubori, (2015) studied the effect of pH on radial growth of *T. mentagrophyte*, *T. rubrum*, and *M. canis* and concluded pH 6 as the most suitable pH for the growth of test fungi[45]. The different pH values affect the vital activities, which are necessary for fungal growth.

5. CONCLUSION

This study was concerned to investigate the effect of pH and temperature on isolation, mycelial growth and sporulation of keratinophilic fungi. The present study shows that isolates had optimal environmental condition including temperature range of 25^0C- to 35^0C and pH from 6.5 to 8.0. This study may help researchers for selecting the suitable incubation condition for keratinophilic fungi. The present study has a significant role to correlate the *in vivo* and *in vitro* environmental conditions requirement of keratinophilic fungi which can be used to provide the similar *in vivo* and *in vitro* conditions for growth of micro-organisms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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