

Research Article

***In vitro* Effect of Potassium iodide on Growth of *Sporothrix schenckii* in Mycelial Form**

Rajesh Bareja*¹, Prem Singh Grover², Sudhir Kumar Mehra³

¹Assistant Professor, Department of Microbiology, S.R.M.S. Institute of Medical Sciences, Bhojipura, Bareilly, India – 243202

²Professor, Department of Microbiology, M.M. Institute of Medical Sciences and Research, Mullana, Ambala, India – 134007

³Professor, Department of Microbiology, Pacific Medical College & Hospital, Udaipur, Rajasthan, India – 313001

***Corresponding author**

Rajesh Bareja

Email: rajeshbareja@gmail.com

Abstract: This study describes the *in vitro* effect of potassium iodide (KI) on the growth of filamentous form of *Sporothrix schenckii* (*S. schenckii*) in broth by turbidity method. A growth curve was determined using a standard strain of *S. schenckii* (ATCC 14284). Yeast nitrogen base medium (YNB) was used to study the different phases of growth in the mycelial form of *S. schenckii*. After doing the sub-culture, the optical density (OD) was recorded and then tube was incubated at 25°C, thereafter, daily readings of OD were taken to obtain a growth curve upto 34 days. After knowing the normal growth pattern of *S. schenckii*, the effect of increasing concentrations of KI on growth of *S. schenckii* (mycelial phase) was observed. YNB medium was prepared and potassium iodide was added into the medium in increasing concentrations in such a way so as to have final concentrations of 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 gram percent of the medium. These tubes were inoculated with standard inoculum and incubated at 25°C upto 34 days. Daily record of growth was maintained by recording the optical density of the tubes. With increasing KI concentration a significant decrease in optical densities as compared to control were observed (F=16.198; p<0.001). It reveals that KI has inhibitory effect on the growth of *S. schenckii*.

Keywords: Growth curve, *Sporothrix schenckii*, Potassium iodide, Optical density.

INTRODUCTION

Sporotrichosis is chronic, pyogranulomatous fungal infection of cutaneous or subcutaneous and is often associated with lymphangitis with or without lymph node enlargement. It is caused by the dimorphic fungus *Sporothrix schenckii* (*S. schenckii*) [1, 2]. A number of drugs and other therapies have been recommended for the treatment of sporotrichosis. These include potassium iodide (KI), itraconazole, fluconazole, terbinafine, amphotericin B and thermotherapy [3]. KI has been traditionally used in the treatment of sporotrichosis since the early 20th century, with satisfactory results. However, the exact mechanism of action remains unknown [3]. The immunological mechanisms involved in prevention and control of *S. schenckii* infections are still not very well understood. However, they probably include both humoral and cellular responses that appear to be triggered by distinct antigens [4-6].

There is paucity of studies that show the effect of KI on the growth of *S. schenckii*. Therefore, it was decided

to know the effect of KI on growth of *S. schenckii* in mycelial form using turbidity method.

MATERIALS AND METHODS

This experimental study was conducted in the department of Microbiology in a tertiary care hospital. A standard strain of *S. schenckii* (ATCC 14284 / MTCC 1359) was procured from Institute of Microbial Technology, Sector 39 A, Chandigarh, India. Yeast nitrogen base medium (YNB) from HiMedia Laboratories, India, was used for further growth character of *S. schenckii*.

A master culture was prepared by doing the subculture of *S. schenckii* from slope of Sabouraud's dextrose agar (SDA) in 50 mL of YNB medium in a screw-capped bottle and was incubated at 25°C. On the seventh day the suspension of YNB medium with *S. schenckii* was adjusted to 90% transmission at 540 nm on the photo-colorimeter. This was done by first withdrawing 5.0 mL of suspension from the respective bottle with sterilized syringe in a test tube, finding its

transmission and adding further amount of sterilized YNB medium till 90% transmission was obtained. Thus knowing the ratio of YNB to be added, an appropriate amount of YNB was added to the bottle containing the suspension of YNB with *S. schenckii* [7]. Master culture thus prepared was used for subsequent analysis.

A normal growth curve was obtained by the method described by Bareja *et al.* [1]. Along with the normal growth pattern of *S. schenckii*, the effect of increasing concentrations of KI on growth of *S. schenckii* (mycelial form) was observed. YNB medium was prepared and dispensed 5.0 mL each into 10 test tubes. Potassium iodide was added into the YNB medium in increasing concentrations in such a way so as to have final concentrations of 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 gram percent of the medium. One tube of YNB without KI was served as a control (normal growth). The pH (5.4) was corrected after the addition of KI. The tubes were sterilized in autoclave and then refrigerated till used. A 0.1 mL of suspension from the master culture was inoculated into each test tube. These tubes were incubated at 25°C upto 34 days. Daily record of growth was maintained by recording the optical density (OD) of the tubes at 540 nm.

RESULTS

A normal growth curve of *S. schenckii* was prepared by taking the daily readings of OD at 540 nm of wavelength on photo colorimeter upto 34 days using YNB medium. At day 0 the optical density was 0.01, at day 8 it was 0.17 and peak density at day 14 with OD value of 0.26, however, at day 34, the optical density was 0.17, thus indicating a steady rise and slower decline (Table 1). A growth curve was plotted against time and observations (Fig. 1).

On the other hand, with increasing KI concentration a significant decrease in optical density as compared to control was observed ($F=16.198$; $p<0.001$) whereas with passage of time a hyperbolic curve was observed with achievement of peak value at day 16 and a declining trend thereafter (Table 1 & Fig. 1). The ascending slope of the hyperbola was sharp and inclining whereas the descending slope was somewhat straight and declining, thus indicating that the decline was slower as compared to the incline. Statistically, a significant association between time and optical density values was observed ($F=6.957$; $p<0.001$).

DISCUSSION

Potassium iodide (KI), a drug useful in all forms of sporotrichosis has been used since 19th century but its mode of action is not understood exactly so far. Various hypotheses have been postulated in this regard [8]. This study describes the *in vitro* effect of KI on the growth of the filamentous form of *S. schenckii*.

In the present study, growth of *S. schenckii* was measured by turbidity method using photoelectric colorimeter at 540 nm of wavelength. On day of inoculation i.e. on day 0 and day 1 the OD was 0.01 but it had initiated rising from day 2 (Table 1 & Fig. 1). The lag phase was considered from the period of inoculation to the measurable growth, where cells were adjusting to new growth conditions. After adjusting under the new conditions, the culture entered the log phase where cells had approximately the same generation time [1,9]. In this study, a slight incline was observed after day 2, but after day 6 there was sharp incline was observed and it reached at peak on day 14 (Table 1 & Fig. 1). This phase was considered as the log phase. From day 14 to day 16 there was no incline in growth and the constant OD (0.26) was observed. This period was considered as stationary phase because there was no measurable increase in cell growth. On day 17, a slight decline in the growth was observed and it was continued till day 34 (Table 1 & Fig. 1). Therefore, this period was considered as a death phase. In the mycelial phase of *S. schenckii*, a moderate correlation between time and optical density was observed which was significant statistically ($r=0.56$; $p<0.001$).

Along with the normal growth pattern, effect of KI on the growth curve of *S. schenckii* (mycelial phase) was determined. In general with increasing KI concentration a significant decrease in optical density was observed ($F=16.198$; $p<0.001$). At the concentration KI 0.05, 0.10, 1.6, 3.2 and 6.4, the peak of log phase was observed on day 16, while it was day 14 in control (normal). It may be the inhibitory effect of KI that prolonged the log phase. However, a significant decrease in ODs was observed with increasing KI concentration through out all the days (Table 1). At the concentration KI 1.6, a significant inhibition in growth curve was observed as compare to the control. The same observations were noticed in higher concentrations also. At the concentration KI 12.8, a total inhibition of growth was observed (Table 1). After day 6, there was sharp incline was observed and it reached at peak on day 14 in control but no sharp incline was observed in different graded concentrations of KI. However, the decline was slower as compared to the incline for all the test concentrations and control (Fig. 1). Statistically, a significant association between time and optical density values was observed ($F=6.957$; $p<0.001$).

Table 1: Effect of KI on the normal growth of *S. schenckii* (mycelial form)

Conc. of KI	Optical Density at day																		Average OD during 34 days	
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	Mean	SD
0.00 control	0.01	0.02	0.07	0.10	0.17	0.19	0.22	0.26	0.26	0.24	0.23	0.22	0.21	0.20	0.19	0.18	0.18	0.17	0.17	0.08
0.05	0.01	0.03	0.05	0.10	0.12	0.15	0.16	0.23	0.25	0.25	0.23	0.20	0.20	0.18	0.17	0.17	0.17	0.17	0.16	0.07
0.10	0.01	0.03	0.03	0.08	0.12	0.15	0.18	0.25	0.26	0.25	0.23	0.20	0.18	0.18	0.18	0.17	0.16	0.16	0.16	0.08
0.20	0.01	0.03	0.03	0.12	0.12	0.16	0.19	0.22	0.22	0.21	0.22	0.21	0.21	0.17	0.17	0.16	0.16	0.16	0.15	0.07
0.40	0.01	0.04	0.05	0.07	0.14	0.18	0.20	0.22	0.22	0.21	0.20	0.20	0.20	0.18	0.18	0.18	0.17	0.16	0.16	0.07
0.80	0.01	0.03	0.04	0.11	0.14	0.16	0.17	0.21	0.21	0.21	0.21	0.20	0.15	0.14	0.14	0.14	0.14	0.13	0.14	0.06
1.60	0.01	0.01	0.02	0.07	0.08	0.10	0.11	0.18	0.19	0.18	0.18	0.18	0.15	0.13	0.13	0.13	0.13	0.13	0.12	0.06
3.20	0.01	0.01	0.01	0.04	0.05	0.08	0.11	0.13	0.15	0.17	0.18	0.17	0.17	0.16	0.15	0.14	0.14	0.13	0.11	0.06
6.40	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01
12.8	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
Mean	0.01	0.022	0.032	0.071	0.096	0.12	0.137	0.174	0.181	0.177	0.173	0.162	0.151	0.138	0.135	0.131	0.129	0.125	F=16.198; p<0.001 (ANOVA)	
SD	0.00	0.01	0.02	0.04	0.06	0.06	0.07	0.09	0.09	0.08	0.08	0.08	0.07	0.07	0.06	0.06	0.06	0.06		
F	6.957																			
"p"	<0.001																			

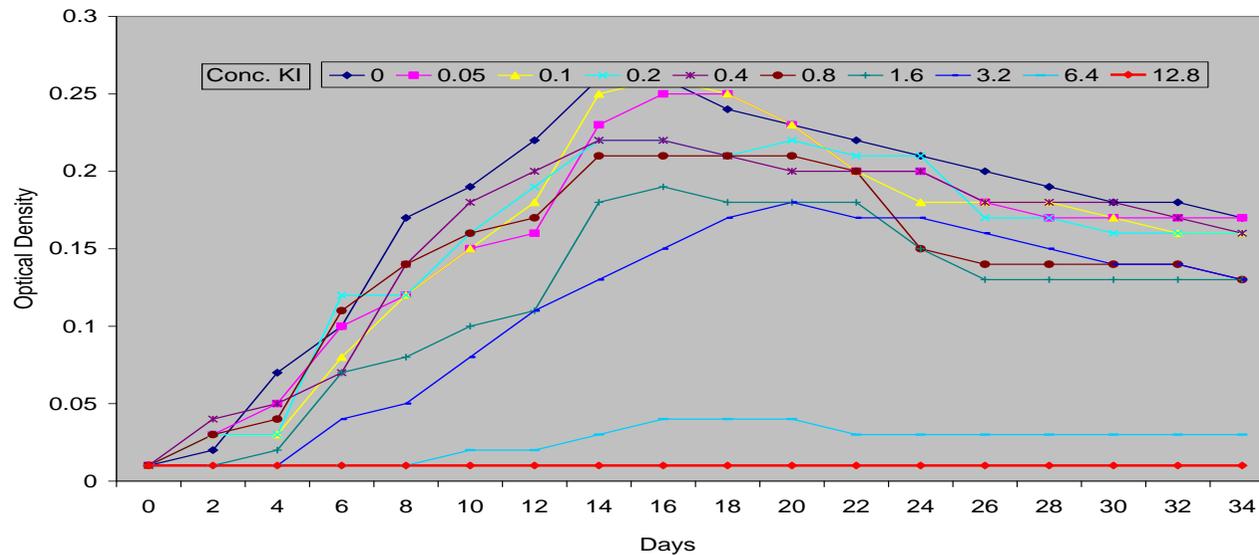


Fig. 1: Effect of increasing concentration of KI on normal growth of *S. schenckii* (mycelial phase)

CONCLUSION

There was sharp incline in the mean growth level of control and test specimens between day 6 (0.071 ± 0.04) and day 16 (0.181 ± 0.09), thereafter a gradual decline was observed upto day 34 (0.125 ± 0.06). It indicates that KI has inhibitory effect and this has led to diminution in the growth of *S. schenckii* (mycelial phase).

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