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EFFECT OF TRACE ELEMENTS ON THE GROWTH AND SPORULATION OF KERATINOPHILIC FUNGI

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Trace elements are utilized by all the fungi in different concentration. These elements play an important role on growth and nutrition of fungi. For the present study three trace elements viz. $ZnSO_4$, $MnSO_4$ and $FeSO_4$ with six different concentrations viz. 10, 20, 40, 60, 80 and 100 ppm were used to evaluate the effect on the growth and sporulation of the two keratinophilic fungi *Chrysosporium tropicum* and *Trichophyton mentagrophytes*. A control was also run along with the experiment. *Chrysosporium tropicum* showed excellent sporulation at 10-40, 20-60 and 10-40 ppm concentrations of $FeSO_4$, $ZnSO_4$ and $MnSO_4$ respectively. Almost similar results were observed with respect to another fungi *Trichophyton mentagrophytes* which showed best sporulation at 20-60, 20-80 and 10-60 ppm concentrations of $FeSO_4$, $ZnSO_4$ and $MnSO_4$ respectively. Thus it can be inferred that the average concentrations of trace elements as mentioned above is good whereas high concentration of the same is toxic for sporulation and growth of fungi.

Keywords: *Chrysosporium tropicum;* Keratinophilic fungi; Sporulation; Trace elements; *Trichophyton mentagrophytes.*

Introduction

Keratinophilic fungi are a group of fungi that colonize various karatinous substrates and degrade them to components of low molecular weight. These fungi are generally considered as soil saprophytes[1,2]. Most of the keratinophilic fungi are not dermatophytes but soil inhabitants. They occur on cornified debris in the soil and degrade keratin and other keratinous material. Keratinophilic fungi are generally reported as nature's keratin degrading machines[3]. Different climatic and physiological factors show immense role on growth and sporulation of keratinophilic and dermatophytic fungi. The climatic conditions favour the incidence of the fungi. Various physiological parameters help in excellent sporulation of keratinophilic fungi. Trace elements have also been found to be beneficial for good concentration as reported by some workers like Foster[4], Steinberg[5] and Chandra and Banerjee[6].

Trace elements also play an important role in the nutrition of fungi. It has been reported by various workers viz. Foster[4], Sadasivan and Subramanian[7] that growth and sporulation of many fungi is accelerated if the medium contains traces of zinc. Chandra & Banerjee[6] studied the effect of metal ion on *Trichophyton rubrum*. Peter[8] observed that these elements inhibit the mycelial growth of *T. mentagrophytes*. Rulcker *et al.*[9] studied the interactions between a soil fungus *Trichoderma harzianum* and IIb metals. This interaction shows adsorption to mycelium and production of complexing metabolites. The *in vitro* effects of three metabolic salts and soot were studied on the growth of five species of dermatophytes by Okafor and Ngwogu[10]. The result showed that *Trichophyton rubrum* and *Microsporum audouinii* appeared to be most sensitive to zinc chloride, calcium chloride, magnesium chloride and soot collected from kitchens compared to the other three species which include *T. mentagrophytes, T. tonsurans* and *M. gypseum*. Madan[11] studies the effect of fifteen trace elements on the growth of *Phellinus pachypholeus* Pat. The results showed that the concentration higher than the optimum were progressively inhibitory for its growth. Only little work has been performed on the trace element requirements.

Materials and Methods

Two keratinophilic fungi *Chrysosporium tropicum* and *Trichophyton mentagrophytes.* were selected for the present study. Both fungi were isolated from soil samples. Simple spore culture was raised and maintained on Sabouraud's Dextrose-Agar medium. For present study the experiment was performed on liquid medium and control was simultaneously maintained for comparison of growth (in terms of dry weight of the mycelium) and sporulation. Spore suspension of selected fungi was added to each flask, aseptically with the help of 1 ml. pipette. Garrett's agar disc method[12] was generally followed for inoculating the flasks, except where stated other wise. All the inoculated flasks were incubated at 28±2°C in a culture room. The incubation period in experiment was 15 days and growth and sporulation were examined on 16th day. The mycelial mats were harvested on the 16th day by filtering through previously dried and weighed Whatman 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. pH of the fresh media and culture filtrates after 16 days were determined at the end of each sampling by using pH meter. The estimation of fungal sporulation were counted by using Tuite[13] formula as follows :-

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

A total of 15 countings were made in each case and an average was calculated as suggested by Wilson and Knight[14]. Statistical analysis were made on computer.

Growth response of selected fungi *Chrysosporium tropicum* and *Trichophyton mentagrophytes*. were studied using different concentrations of trace elements viz., sulphates of zinc ($ZnSO_4$), iron ($FeSO_4$) and manganese ($MnSO_4$) in corporated in the basal medium. The basal medium was made free of traces of iron, manganese and zinc following the process of Steinberg (1935). The salts were added to the medium in flasks individually and separately at six concentrations of 10, 20, 40, 60, 80, and 100 ppm. The results were then compared with control.

Results and Discussion

These trace elements (Z, Fe, Mn) in low concentrations caused a pronounced effect on the sporulation of fungi as compared to their vegetative growth but in higher concentrations these trace elements produced toxic effect on the growth and sporulation of these fungi.

Chrysosporium tropicum showed excellent sporulation at 20-60 ppm concentrations of $ZnSO_4$. *Trichophyton mentagrophytes* required 20-80 ppm concentration for excellent sporulation. Higher concentration of zinc suppressed the growth and sporulation (Tables 1-2, Fig. 1-2,). In the presence of $FeSO_4$, *Chrysosporium tropicum* sporulates best at 10-40 ppm. concentrations. *Trichophyton mentagrophytes* required 20-60 ppm concentration for excellent sporulation. *Chrysosporium tropicum* sporulates excellent at 10-40 ppm concentrations of $MnSO_4$. In case of second fungi *Trichophyton mentagrophytes* excellent sporulation occurred at 10-60 ppm concentrations (Table 1-2, Fig. 1-2).

The results showed that the sporulation of both the keratinophilic fungi was poor to fair in their controls. Different concentrations of these three trace elements, however, caused a pronounced effect on their sporulation. The spore production increased with the addition of these trace elements upto certain concentrations which was different for individual fungus studied.

Steinberg[5] observed that with the addition of traces of iron to the medium, certain fungi gave 40-75 times more growth than that on iron free media. Chandra & Banerjee[6] studied effect of metal ions on *T. rubrum* and found that concentration of Fe^{++}, Zn^{++} and Mn^{++} upto 2.5 µg/ml, 0.5 µg/ml and 0.05 µg/ml respectively are suitable for this fungus. Further increase in concentration caused growth inhibition. Sharma[16] studied the effect of trace elements on growth and sporulation of five keratinophilic and dermatophytic fungi viz. *Gymnoascus reessii, Trichophyton simii, Microsporum gypseum, Cephaliophora irregularis, Chryososporium tropium.* All the above test fungi showed lesser sporulation af higher concentrations of trace elements and fair sporulation at lower to medium concentration.

Plaza *et al.*[17] investigated the effect of Cd on the mycelial growth of some potentially pathogenic soil fungi. The nonkeratinolytic fungi showed higher resistant to Cd than keratinolytic fungi.

Lulloff *et al.*[18] used zinc deprivation against reversing growth of six pathogenic fungi. Zinc was more effective than iron in reversing growth inhibition against all the six pathogenic fungi.

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Table 1a. Average dry weight, sporulation and final pH of *Chrysosporium tropicum* at different concentrations of $ZnSO_4$.

Concentration in ppm	Final pH	Average dry weight in gms	Sporulation
Control	8.2	0.267	++
10	8.2	0.225	++
20	7.3	0.428	++++
40	8.2	0.337	++++
60	7.9	0.353	++++
80	8.0	0.238	+++
100	8.2	0.146	+

Table 1b. Average dry weight, sporulation and final pH of *Chrysosporium tropicum* at different concentrations of $FeSO_4$.

Concentration in ppm	Final pH	Average dry weight in gms	Sporulation
Control	8.2	0.267	++
10	7.7	0.341	+++
20	7.7	0.358	+++
40	8.1	0.247	+++
60	8.3	0.251	++
80	7.9	0.255	++
100	8.5	0.112	+

Table 1c. Average dry weight, sporulation and final pH of *Chrysosporium tropicum* at different concentrations of $MnSO_4$.

Concentration in ppm	Final pH	Average dry weight in gms	Sporulation
Control	8.2	0.267	++
10	8.2	0.276	++++
20	8.1	0.277	++++
40	8.2	0.289	++++
60	7.9	0.280	+++
80	8.1	0.274	+++
100	8.1	0.265	++

Table 2a. Average dry weight, sporulation and final pH of *Trichophyton mentagrophytes* at different concentrations of $ZnSO_4$.

Concentration in ppm	Final pH	Average dry weight in gms	Sporulation
Control	6.0	0.142	++
10	6.3	0.240	+++
20	6.6	0.206	+++
40	6.6	0.256	+++
60	6.9	0.261	+++
80	6.1	0.246	+++
100	6.6	0.189	++

Table 2b. Average dry weight, sporulation and final pH of *Trichophyton mentagrophytes* at different concentrations of $FeSO_4$.

Concentration in ppm	Final pH	Average dry weight in gms	Sporulation
Control	6.3	0.143	++
10	5.4	0.258	++++
20	6.0	0.231	++++
40	5.8	0.253	++++
60	5.3	0.207	++++
80	7.0	0.163	++
100	5.4	0.145	+

Table 2c. Average dry weight, sporulation and final pH of *Trichophyton mentagrophytes* at different concentrations of $MnSO_4$.

Concentration in ppm	Final pH	Average dry weight in gms	Sporulation
Control	6.3	0.143	++
10	6.6	0.250	+++
20	6.5	0.167	+++
40	6.6	0.284	++++
60	6.1	0.251	++++
80	7.8	0.148	++
100	7.6	0.075	+

Fig. 1a : Effect of $ZnSO_4$ on growth and sporulation of *Chrysosporium tropicum*

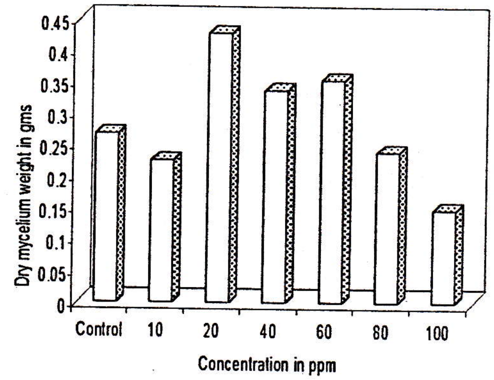


Fig. 1b : Effect of $FeSO_4$ on growth and sporulation of *Chrysosporium tropicum*

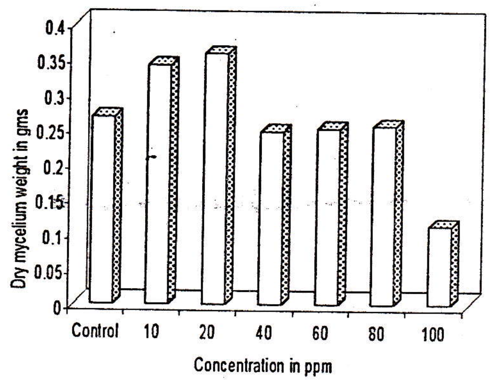


Fig. 1c : Effect of $MnSO_4$ on growth and sporulation of *Chrysosporium tropicum*

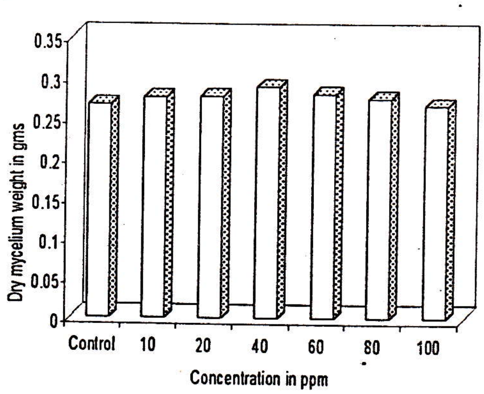


Fig. 2a : Effect of $ZnSO_4$ on growth and sporulation of *Trichophyton mentagrophytes*

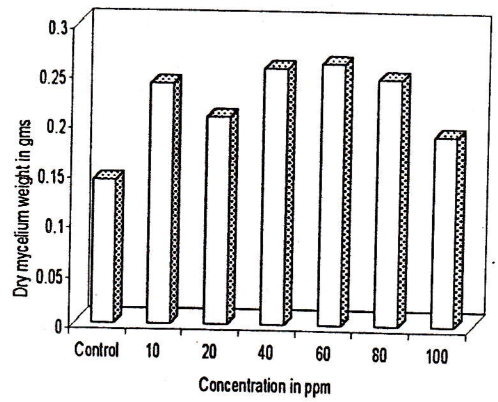


Fig. 2b : Effect of $FeSO_4$ on growth and sporulation of *Trichophyton mentagrophytes*

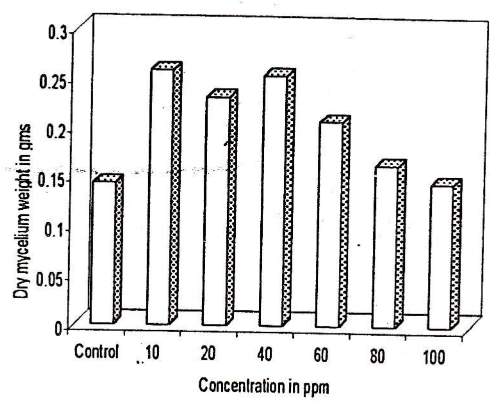
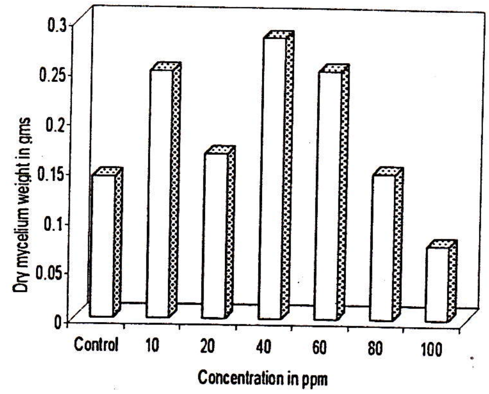


Fig. 2c : Effect of $MnSO_4$ on growth and sporulation of *Trichophyton mentagrophytes*



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