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Studies on the effect of heavy metals on soil fungi *Trichoderma* sp.

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Abstract

Development of plants is closely related with both the soil quality and the community of soil microorganisms, therefore plants are influenced by heavy metals directly transferring from the soil and indirectly via heavy metal impact on the microorganisms. Copper and Zinc are essential micronutrient, but above certain threshold concentration are toxic to both microbes and humans. Many heavy metals had been used to study the effects on several types of fungi. In this experiment, two heavy metals *viz.* $ZnCl_2$ and $CuSO_4$ of different concentrations (0.25 mM, 0.5 mM, 1 mM, 2.5 mM and 5 mM) were used to study their effect on selected fungi. The result from this experiment reveals that $ZnCl_2$ and $CuSO_4$ have negative effect on *Trichoderma* sp. in their growth rate and reduce the production of spores with the increasing concentration of the heavy metals. In the present study, $CuSO_4$ showed greater inhibitory effect on the growth of *Trichoderma* sp. than the concentration of $ZnCl_2$.

Key words: Heavy metals, hydrocarbons, mycoparasitism, *Trichoderma*.

Introduction

Trichoderma (teleomorph *Hypocrea*), family Hypocreaceae, is a genus of asexual fungi found in the soils of all climatic zones. It is a secondary opportunistic invader, a fast-growing fungus, a strong spore producer, a source of cell wall degrading enzymes (CWDEs: cellulases, chitinases, glucanases, etc.), and an important antibiotic producer. Numerous strains of this genus are 'rhizosphere competent' and are able to degrade hydrocarbons, chlorophenolic compounds, polysaccharides and the main biocontrol mechanisms that *Trichoderma* utilizes in direct confrontation with fungal pathogens are mycoparasitism,[1,2] and antibiosis.[3]

On the other hand, "heavy metals" are chemical elements with a specific gravity that is

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at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546.[4] In small quantities, certain heavy metals are nutritionally essential for a healthy life. Some of these are referred to as the trace elements (e.g. iron, copper, manganese, and zinc).

Both copper and zinc are a micronutrient and are essential for maximal growth of microbes and other cells. On the contrary, elevated concentration of copper and zinc may be inhibitory or toxic to cellular activities and growth. They have been shown to inhibit respirations of fungi and germination of fungal spores. A concentration of both copper and zinc ions reduce the mycelia growth of fungi but the complex anionic copper sulphate (CuSo4) and zinc chloride ($ZnCl_2$) exerted greater toxicities.[5]

It has been suggested that chemical transformations of heavy metals in heavy metal-amended soils may occur over short or long periods.[6] In the case of field surveys, studies were carried out only in extremely highly contaminated areas.[7,8] It has been pointed out that it is difficult to estimate the effects of low concentrations of heavy metals on the soil microbial population since soil microorganisms may be more strongly affected by other physical, chemical, and biological factors, such as soil water content, organic matter content, fertilizer application, and cropping. In addition, some of the effects, even in the highly contaminated areas, include indirect effects of heavy metal pollution.[7,9] The present study aims on the effects of heavy metals ($CuSO_4$ and $ZnCl_2$) on growth rate and fungal biomass of *Trichoderma* sp.

Materials and Methods

The experiment was designed to carry out the effects of heavy metals ($CuSO_4$ and $ZnCl_2$) on growth rate and fungal biomass of *Trichoderma* sp. $ZnCl_2$ concentration ranging from 0.25 mM, 0.5 mM, 1 mM, 2.5 mM and 5 mM and concentrations of $CuSo_4$ ranging from 0.25 mM, 0.5 mM, and 1 mM, 2.5 mM and 5 mM were used for this experiment. Serial dilution plate method[10] was followed for the isolation of *Trichoderma* sp. in a solid medium of Rose Bengal and Czapek Dox. The Czapek Dox broth medium was used for stock solution.

In order to prepare the metals treatment of varying concentrations, the following formula was used:

Molarity = moles of solute /litre of a solution

Where, the molecular mass of $ZnCl_2$=136.28 g/mol and CuSo4=249.68 g/mol

The metals were prepared by dissolving different grams of both $ZnCl_2$ and $CuSo_4$ in 100 ml of distilled water. For $ZnCl_2$, 0.003 g, 0.006 g, 0.03 g and 0.06 g were added to 100 ml distilled water to get 0.25 mM, 0.5 mM, 1 mM, 2.5 mM and 5 mM respectively. For $CuSo_4$ 0.006 g, 0.012g 0.24 g, 0.06 g and 0.124 g were dissolved in 100 ml of distilled H_2O to get 0.25 mM, 0.5 mM, 1 mM, 2. 5mM and 5 mM respectively. The amount of gram required for the different concentration was calculated by multiplying the desired molarity with the molecular mass of the metal compound which is then divided by the desired volume of solution. The protocols given below were then followed in a stepwise manner:

- ⊕ Enumeration of fungal population
- ⊕ Isolation and identification of the fungal species
- ⊕ Treatment of medium with Heavy metals
- ⊕ Measurement of fungal biomass
- ⊕ Determination of fungal growth rate
- ⊕ Statistical analysis

Results

Growth rate of Trichoderma

The colony size of *Trichoderma* sp. was meas-

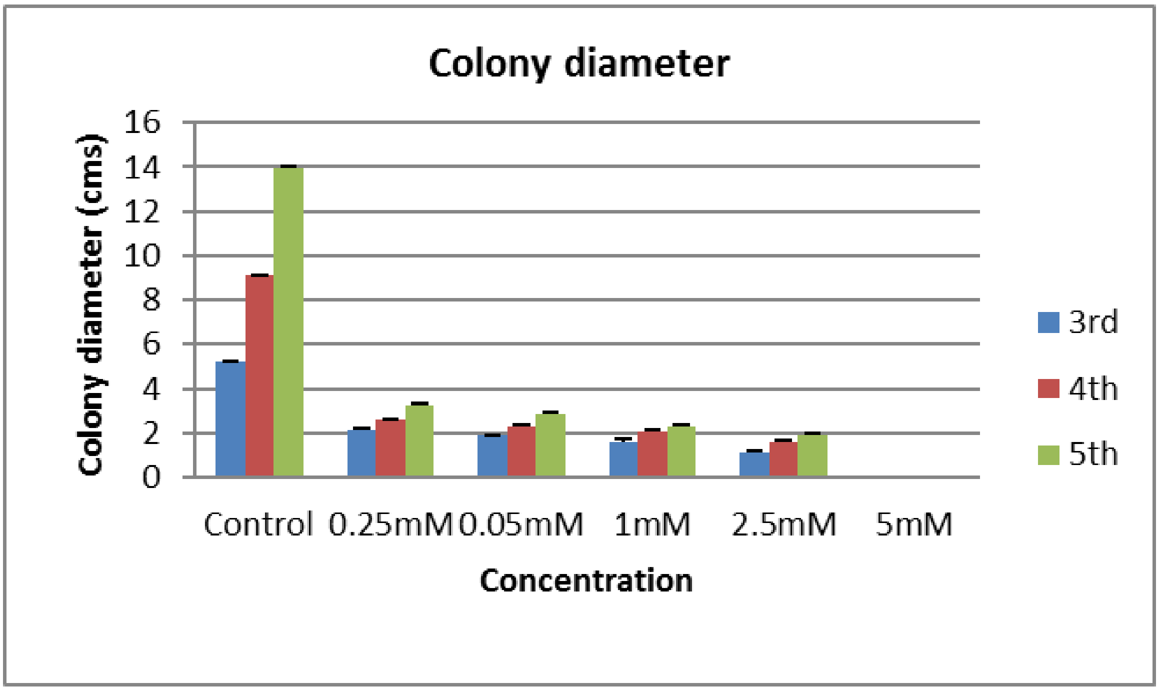

Figure 1. *Trichoderma* colony diameter treated with $CuSO_4$ of different concentration (3rd, 4th and 5th day of incubation).

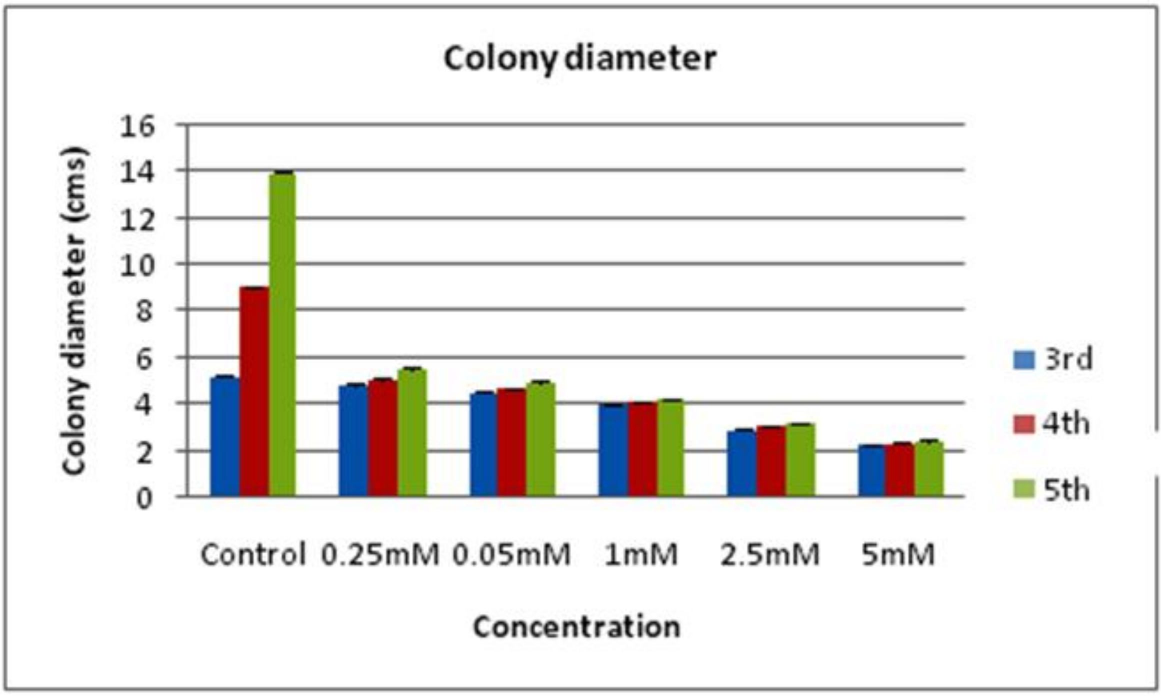

Figure 2. *Trichoderma* colony diameter treated with $Zncl_2$ of different concentration (3rd, 4th and 5th day of incubation).

ured from the different treated plate's *viz*. Control, 0.25 mM, 0.5 mM, 1 mM, 2.5 mM and 5 mM concentrations of $CuSO_4$ and $ZnCl_2$ on the 3rd, 4th and 5th day from incubation. Control plate shows maximum colony size of 5.22±0.035 & 5.19±0.035 cms after 3 days, 9.07±0.075 & 9.07±0.029 cms after 4 days, and 13.97±0.036 & 13.89±0.012 cms after 5 days of incubation in $CuSO_4$ and $ZnCl_2$ respectively. At the same time, minimum growth was observed in the 5mM concentration i.e. 0±0 & 2.28±0.055 cms after 3 days, 0±0 & 2.35±0.020 cms after 4 days, and 0±0 & 2.45±0.021 cms after 5 days of incubation in $CuSO_4$ and $ZnCl_2$ respectively. The size of the colony decrease with the decrease in the concentration of the heavy metals. The one-way ANOVA result shows a significant variation in the different size of the colony as shown in Fig. 1 & 2.

Dry weight biomass

Mycelium dry weight of *Trichoderma* sp. was measured from six different treatment *viz*. Control, 0.25 mM, 0.5 mM, 1 mM, 2.5 mM and 5 mM concentrations of $CuSO_4$ and $ZnCl_2$ respectively. Control plate showed maximum colony size (0.388±0.009 g in $CuSO_4$ & 0.389±0.009 in $ZnCl_2$. However, minimum growth was observed in the 5 mM concentration (0.023±0.001 g $CuSO_4$ & 0.018±0.006 g in $ZnCl_2$). Size of the colony correspondingly decreases with increasing concentration of the heavy metals. The one-way ANOVA results show a significant variation in the different size of the colony as shown in Fig. 3.

Discussion

Some heavy metals, although necessary for the growth of fungi at a low concentration, shows adverse effect on the growth and reproduction on some fungi *viz. Trichoderma* sp., with the increase in the heavy metal concentration. It has also been observed that the increase in the concentration of heavy metals decreased the growth of the fungi correspondingly.

Many heavy metals had been used to study

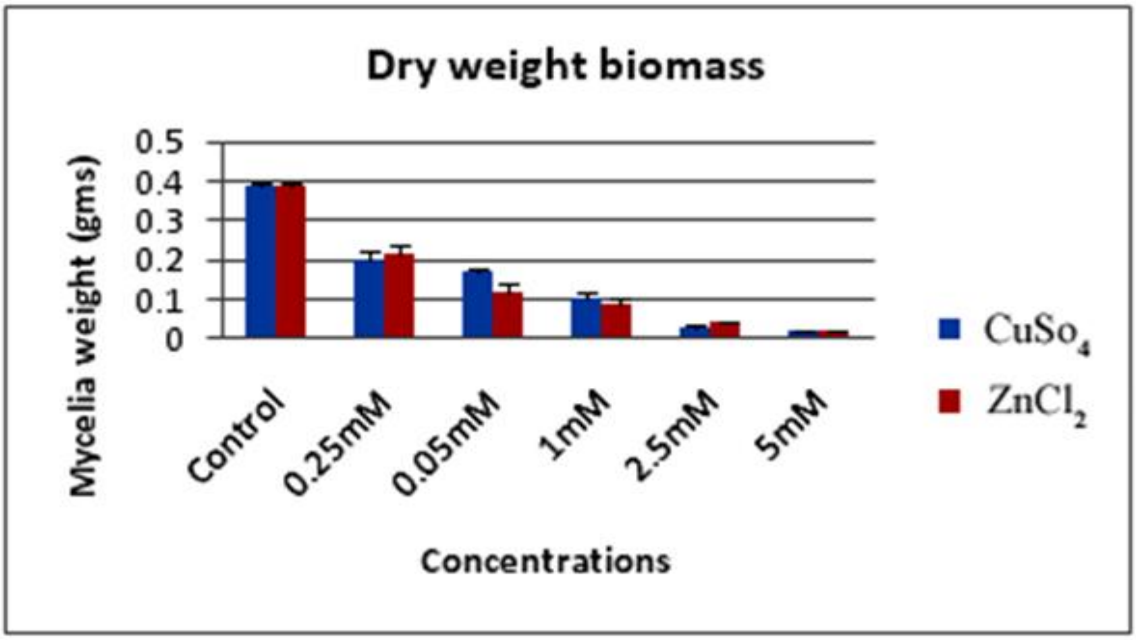

Figure 3. Dry weight biomass upon treatment with solutions of $CuSO_4$ and $ZnCl_2$.

the effects on several types of fungi. In this experiment, two heavy metals *viz*. $ZnCl_2$ and $CuSO_4$ of different concentrations (0.25 mM, 0.5 mM, 1 mM, 2.5 mM and5 mM) were used to study their effect on selected fungi. The present study reveals that $ZnCl_2$ and $CuSO_4$ have negative effect on the growth rate of *Trichoderma* sp. and correspondingly reduce production of spores with the increasing concentration of the heavy metals.

Copper is a co-factor in numerous enzymatic processes and represents the third most abundant transition metal found in living organisms.[11] It has been reported that number of fungi was relatively higher in heavy-metal polluted soils than in non-polluted soils[12] and was also established that the concentration of both Cu and Zn ions reduce the mycelia growth of fungi, however, the complex anionic copper sulphate and zinc chloride exerted greater toxicity.[5] The present study observed that the control attained the highest growth rate, and the treatment of different concentrations with heavy metal hampered and retards the growth of the fungal mycelium. Solid medium was used to study the effects of these ions on the growth rate of fungal mycelium and liquid medium was used to determine the effect in biomass of the fungi. The fungal growth rate and biomass are reduced with the increase in the concentration of heavy metals. Many authors have also reported that the microbial population is strongly affected by heavy metals.[12-14]

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