Senhri Journal of Multidisciplinary Studies
A Journal of Pachhunga University College
(A Peer Reviewed Journal)
https://senhrijournal.ac.in
DOI: 10.36110/sjms.2019.04.01.005

ISSN: 2456-3757
Vol. 04, No. 01
Jan.-June, 2019
Open Access

STUDIES ON EFFECT OF COPPER (CU^{2+}) ON PHOSPHATASE ENZYME ACTIVITY AND BIOMASS OF *Aspergillus niger*

R. Lalfakzuala[1] & H. Lalruatsanga[2*]
[1]Department of Botany, Mizoram University, Aizawl, Mizoram
[2]Department of Botany, Pachhunga University College, Aizawl, Mizoram
*Corresponding Author: puia_rs@rediffmail.com
R. Lalfakzuala: https://orcid.org/0000-0003-2216-7680
H. Lalruatsanga: https://orcid.org/0000-0002-4622-8700

ABSTRACT

Although a large number of metals are essential for growth, some can be harmful for living cells. This is because of the fact that heavy metals can form complexes with protein molecules which render them inactive. The experiment was designed to carry out the effect of heavy metal (copper) on phosphatase enzyme activity and biomass of Aspergillus niger. We have observed that $CuSO_4$ had negative effect on the growth of Aspergillus niger, phosphatase activity and plant biomass. The effects of $CuSO_4$ upon the growth of fungal mycelium both in solid and liquid culture was observed after 3 days of incubation. The study revealed that control attained the highest growth rate, while application of varying concentrations of $CuSO_4$ hampered and retarded the growth of fungal mycelium correspondingly.

Keywords: *Aspergillus niger, Phosphatase, Mizoram University, Heavy Metal, $CuSO_4$.*

Introduction

The effects of metal on microbial ecosystems are primarily described in terms of the toxicity of heavy metals to microorganisms and their impact on microbial community, structure and function. Living organism requires certain metals for their growth and metabolism and they evolved an appropriate uptake mechanism for metals. It is particularly difficult to establish mutual relationship when soil is continuously contaminated with a variety of heavy metals. Under such conditions, the task of identifying which particular heavy metal predominantly destroys soil microbial properties become complicated, since the accumulated

influence of several metals may not necessarily be the sum of individual effects (Lane and Morel, 2000).

Assimilation of phosphate from organic compounds by plants and micro-organisms take place through the enzyme "phosphatases" which is present in wide variety of soil microorganisms. The principal mechanism for solubilization is the production of organic acids and acid phosphatases which play a major role in the mineralization organic phosphorous in soil.

It has been found that several cations inhibit soil phosphatase activity. Tyler (1976) has shown that Cu and Zn ions have a marked inhibitory effect on phosphatase activity. Acid phosphatase (orthophosphoric monoester hydrolases) are a group of enzymes that catalyze thehydrolyses of external phosphate esters (Joh *et al*., 1996). They played an important role in the mineralization of organic carbon, organic phosphate and low levels of free inorganic ions (Pi) (Straker and Mitchell 1986). They are secreted by various fungi, including *Aspergillus niger*, a commercially important fungus in the production of fungal enzymes.

Materials and methods:

Three different concentrations of $CuSO_4$ viz. 0.5mM, 1.0mM and 3.0mM were selected for this experiment. Serial dilution plate method (Waksman, 1992; Parkinson et al., 1971) was followed for isolation of *Aspergillus niger* form Mizoram University campus soil. Pikovskaya solid medium (with agar) and Pikovskaya broth medium (without agar) was used to confirm whether *Aspergillus niger* has a capacity to solubilize phosphate or not. *Aspergillus niger* is a haploid filamentous fungus which is used for waste management and biotransformation in addition to its industrial uses, such as production of citric acid and extracellular enzymes. The fungus also plays a role in the solubilization of heavy-metal sulfides.

1. **Enumeration, isolation and identification of fungal species:**

Rose Bengal Agar medium (Martin, 1950) was used for the growth of fungal population. 1ml of the soil dilution (1:1000) was transferred into petridishes containing Rose Bengal Agar medium and were incubated upside down at 25±1ºC for 7 days in a BOD incubator. Finally *Aspergillusniger* was selected for the experiment and they were again transferred into Potato Dextrose Broth Medium for maintaining stock culture medium.

2. **Estimation of Phosphatase (Tabatabai and Bremner, 1969):**

The culture filtrate was centrifuged at 5000 rpm for 10 minutes. 0.1ml of the filtrate was taken in each test tube maintaining three replicates for each treatment. 4ml of modified universal buffer (pH 6.5), 0.25ml of toluene and 1ml of 0.05M p-nitrophenyl phosphate (PNP) solution was added to the flask (Skyins, 1985). The flask is swirled for few seconds and then incubated at 37ºC for 1hr in an incubator. After incubation, 1ml of 0.5M $CaCl_2$ and 4ml of 0.5M NaOH is added to the mixture. The optical density was measured at 430nm in Spectrophotometer. Blank is maintained similarly without the filtrate.

3. Measurement of fungal growth and effect of heavy metal on rice:

Pikovskaya solid medium with different concentrations of Copper Sulphate was prepared and the pure culture was transferred into the hole in each three replicates of control, 0.5mM, 1.0mM and 3.0mM $CuSO_4$. The plates were incubated at 25±1ºC for about 3 days. After 3 days of incubation, colony diameter was measured. Rice seeds were taken and soaked in 5% mercury chloride for 10 minutes and washed with distilled water and then soaked overnight in the culture flasks that contain media supplemented with copper sulphate. After two weeks the height of the shoots, length of the roots and the biomass of rice plants was measured.

4. Statistical analysis:

Data obtained from the investigation will be analysed by using appropriate standard statistical methods and Statistica computer software.

Result and discussion:

1. *Aspergillus* colony in solid culture media:

The colony size of *Aspergillus* was measured from four different treated plates viz. CTRL, 0.5mM, 1.0mM and 3.0mM $CuSO_4$. Control plate showed maximum colony size followed by 0.5mM and 1.0mM and the smallest colony size was observed on 3.0mM $CuSO_4$ plate. The one way ANOVA results show a significant variation on *Aspergillus* colony size in different treatment.

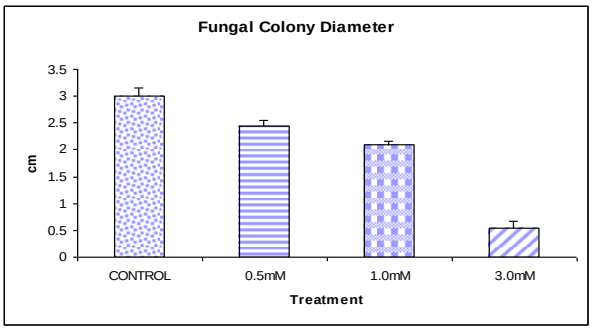

Fig.1: Fungal Colony Diameter

2. *Aspergillus* colony in liquid culture media:

Mycelium dry weight of *Aspergillus* was measured from four different treatments viz. Control, 0.5mM, 1.0mM and 3.0mM $CuSO_4$. It was noted that Control treatment showed maximum dry weight of mycelia and followed by 0.5mM, 1.0mM and 3.0mM $CuSO_4$.

Our experiments showed that control attained the highest growth rate, while application of varying concentrations of $CuSO_4$ hampered and retarded the growth of fungal mycelium correspondingly. This finding is in agreement with the result of Abu-Mejdad Najwa Mohammed Jameel (2013) using *Aspergillus niger*, *Candida albicans* and *Cryptococcus neoformans* for investigating the effect of heavy metals (Cu, Mg and Zn) on the growth in liquid and solid media under laboratory condition.

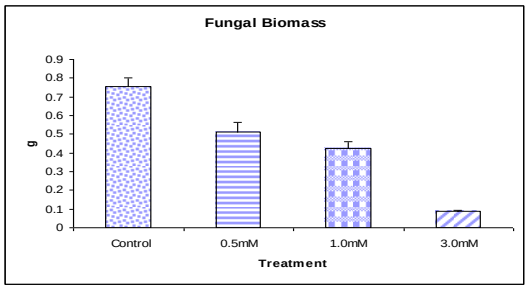

Fig.2: Fungal Biomass

3. Phosphatase:

Phosphatase enzyme activity was measured from four different treatments viz. Control, 0.5mM, 1.0mM and 3.0mM $CuSO_4$.It was noted that Control treatment showed maximum phosphatase activity followed by 0.5mM, 1.0mM and 3.0mM $CuSO_4$. The effects of $CuSO_4$ upon the growth of fungal mycelium both in solid and liquid culture was observed after 3 days of incubation. $CuSO_4$ had negative effect on phosphatase activity and this finding is in agreement with the result of Huang and Shindo (2000) that phosphatase decreased significantly with the increase of Cu concentration.

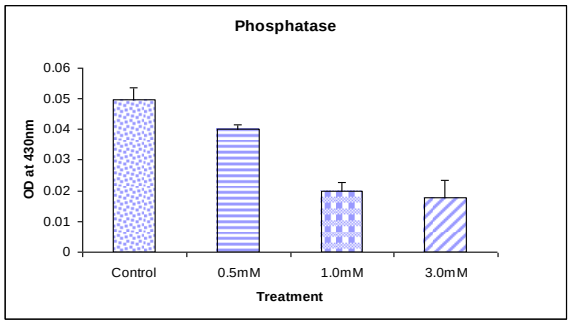

Fig.3: Phosphatase

4. Plant Biomass:

Plant biomass was measured from five different treatments viz. Control-1, Control-2, 0.5mM,1.0mM and 3.0mM $CuSO_4$. It was noted that Control-1 treatment showed maximum plant biomass and it goes on increasing from 0.5 to 3.0mM $CuSO_4$.

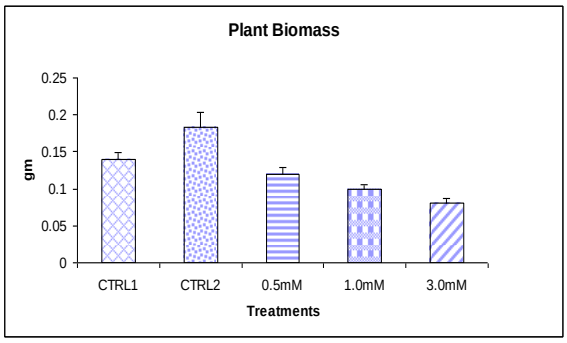

Fig.4: Plant Biomass

5. Height of rice shoots:

Height of rice shoots was measured from five different treatments viz. Control1, Control2, 0.5mM, 1.0mM and 3.0mM $CuSO_4$. It was noted that Control2 is higher than Control1 and it goes on decreasing from 0.5mM to 3.0mM $CuSO_4$.

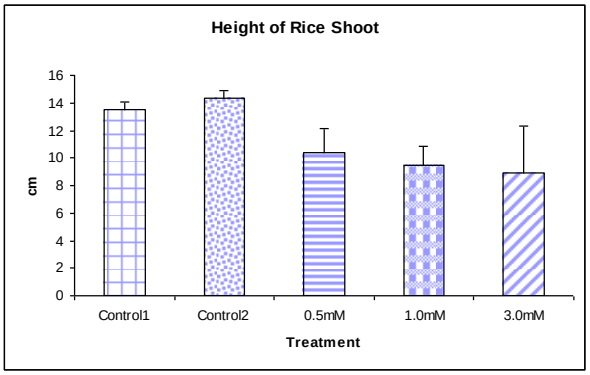

Fig. 5: Height of Rice shoots

It is shown that the biomass of rice plant, height of shoots and length of roots gradually decrease from control to 3.0mM $CuSO_4$. This finding is in agreement with Gupta *et al.* (2011) that the toxic effects of copper and cadmium on seed germination, seedling, root, shoot length and seedling dry biomass of maize was evaluated under laboratory conditions as compared to control values.

Conclusion:

The study revealed that $CuSO_4$ had negative effect on the growth of *Aspergillus niger*, phosphatase activity and plant biomass. Our results clearly showed that as the concentration of the metal increases, the degree of inhibition i.e. colony diameter and mycelium biomass in solid and liquid cultures increases correspondingly. This is in conformity with Ramya *et al.* (2011) findings upon effects of $CuSO_4$ on *A. niger*. Copper despite prevalent in our environment and was considered essential element, however is capable and in danger of showing toxic effects on plants and animals with their increased concentration in soil and water in our times because of heavy industrial establishments.

References

Abu-Mejdad, Najwa Mohammed & Jameel Ali (2013). Response of some fungal species to the effect of copper, magnesium and zinc under the laboratory condition, *European Journal of Experimental Biology*, 3(2), 535-540.

Gadd, G.M. & Griffiths, A.J. (1978). Microorganisms and heavy metals. *Microbiological Ecology*, 4, 303-317.

Gupta, Diwaker and Abdullah (2011). Toxicity of copper and cadmium on germination and seedling of maize. *Indian J.Sci.Res.*, 2(3), 67-70.

Huang, Q. &Shindo, H. (2000). Effects of copper on the activity and kinetics of free and immobilized acid phosphatase, *Soil Biology & Biochemistry*, 32,1885– 1892.

Joh, T., Malick, D.H., Yazaki, J., & Hayakawa, T. (1996). Purification and characterization of secreted acid phosphatase under phosphate deficient condition in Pholiotanameko. *Mycoscience*, 37, 65–70.

Lane, T.W. &Morel, F.M. (2000). A biological function for cadmium in marine diatoms, *Proc Natl Acad Sci U S A*, 25, 4627-31.

Martin, J.P. (1950). Use of acid, Rose Bengal and Streptomycin in the plate method for estimating soil fungi, *Soil Science*, 69, 215-232.

Parkinson,P.,Gray,T.R.G., & William, S.T.(1971). Methods for studying the ecology of soil microorganisms, *Blackwell Scientific publication Oxford* ,pp116.

Ramya, T., Jaquline, Chinna& Rani. I. (2011). A Study on the Effect of Heavy Metals on Select Fungal Isolates, *Adv Bio Tech.* 11(6), 07-09.

Straker, C.J. & Mitchell, D.T. (1986). The activity and characterization of acid phosphatases in endomycorrhizal fungi of the Ericaceae, *New Phytol* 104, 243–256

Straker C.J. & Mitchell D.T (1986). Plant productivity and P fertility indices, *Biol Fertil Soils*, 12, 189–194.

Tabatabai, M.A. &Bremner, J.M. (1969). Use of p-nitrophenylphosphate for assay of soil phosphatase activity, *Soil Biol.Biochem*. 1, 301 – 307.

Tyler, G. (1976). Heavy metal pollution, phosphatase activity, and

mineralization of organic phosphorus in forest soils, *Soil Biol. Biochem*, 8, 327 – 332.

Waksman, S.A.(1992). Microbial analysis of soil as index of fertility III. Influence of fertilization upon number of microorganisms in soil, *Soil Science*, 141, 321-346.