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EFFECT OF SALT PRIMING ON *IN VITRO* GROWTH OF SOME SOIL FUNGI

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Abstract

The present study was conducted to evaluate the effect of three salts of copper viz., Copper sulfate ($CuSO_4$), Copper chloride ($CuCl_2$) and Copper oxychloride [$CuCl_2.3Cu(OH)_2$] on *In vitro* growth of three soil fungi viz., *Aspergillus oryzae, A. niger* and *Drechslera tetramera.* The data recorded at the end of incubation period revealed a remarkable decrease in fresh and dry biomass of these fungi by the action of all salts of Copper. *Aspergillus oryzae* showed maximum suppression in growth in response to these treatments especially to Copper sulfate while *Aspergillus niger* proved highly resistant.

Introduction

Our country has been bestowed with the best of agricultural land and the economy is alargely based on the production of crops. About 70% of the population is involved in growing various crops on these fertile lands, however the yield per acre is unfortunately not up to the mark. There are a number of factors which may be responsible for this low yield. One of the major factors among them is loss of grains by various pests (Shah, 2000). To improve production, latest technologies are being applied in the field (Ludwing, 1990; Shukla & Srivastiva, 1992; Carbajo, 1994).

Drechslera species are typically known to inhabit plant and sandy soils. Some species are plant pathogens. *Aspergillus* is in general the most commonly isolated one from invasive infections. Generally cultural, chemical and biological methods are applied to control pests. Chemical control involves the use of different chemicals to inhibit the growth of these organisms. The fungicidal activity of copper sulfate was first recognized by Prevost (1807). Even today, copper fungicides are used widely in many countries. The Copper fungicides have been used for the protection of many vegetables, fruits and flowering plants for many plant diseases. They have also been commonly used in plantation crops like tea, rubber and coffee (Nene & Thapliyal, 1996).

Since late 1940s over 2 million metric tons of pesticides are usually applied annually in the world, 30% in North America, 45% in Western and Eastern Europe and 21% elsewhere. This is more than double the amount applied a couple of decades ago (Hill, 2003).

Copper sulfate is a naturally occurring inorganic salt, also known as basic Copper sulfate, blue stone and blue vitrol. It is used to control a variety of bacterial and fungal diseases of fruits, nuts, vegetables and field crops (Nene & Thapliyal, 1996). In combination with lime and water, Copper sulphate forms a protective fungicide (Bordeaux mixture) for leaf application and seed treatment. Its further uses are as herbicide, algaecide and molluscicide (McEwen & Stephenson, 1979; Hayes, 1982; Hartley & Kidd, 1983, Anon, 1986).

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Copper chloride, whose principle use is in petroleum industry for catalytic oxidation, is also known as a toxic substance having potential to kill pests. It is sparingly soluble in water and is effective to kill or inhibit the growth of fungi and Microbes (Hartley & Kidd, 1983). Copper oxychloride, a low soluble copper fungicide, is much less injurious but has not been found to be as effective as Bordeaux mixture, because the latter has more natural tenacity. However, it is widely used in many home gardens to control diseases on vegetables, tree crops and on ornamental plants (Nene & Thapliyal, 1996).

In the present study two objectives were addressed; firstly it was attempted to evaluate the response of three common soil fungi towards salt priming treatment of three different inorganic copper salts and secondly it was focused to analyze the response of these fungi in *In vitro* conditions towards various concentration of copper salts in terms of fresh and dry weight of the test fungus.

Materials and Methods

Present experiments were conducted to check the effect of three salts of copper on growth of three fungal species viz., *Aspergillus oryzae, A. niger* and *D. tetramera*. Sub-culturing and mass multiplication of these fungal isolates was done regularly during the course of experiment.

The glassware used in the experiment was autoclaved and sterilized in order to prepare pure stock solutions of salts of copper (100 mgL^{-1}). Then dilutions of these stock solutions were made in order to get the concentrations of 25, 50, 75 and 100 mgL^{-1}. Flame sterilized distilled water was used as control. Liquid malt extract medium (2%) was prepared and autoclaved at 121°C at 15lb/inch^2 for 75 minutes. The solutions of all the test substances were added in the media under aseptic conditions and saline media were again autoclaved.

Three of the treatment discs of 1cm in diameter of each fungus were added in the media flasks with different concentrations of salts. Each treatment was maintained in triplicates and incubated at 25±3°C. Harvests were taken after 7 days. The growth stress induced by these chemicals in fungi was assessed in terms of dry and fresh biomass produced.

Statistical analysis of all the data was carried out by using “ANOVA TEST’ and ‘DUNCUN’S NEW MULTIPLE RANGE TEST’ (Steel & Torrie, 1980).

Results

Treatment with any of three salts of copper showed a remarkable suppression in growth of test fungi. The data for the fresh and dry biomass showed a statistically significant reduction in growth of fungi growing in salt solution as compared to control ($p<0.05$).

Data showed that a significant loss resulted in fresh and dry biomass of *A. oryzae* at 100 mgL^{-1} of Copper sulfate at the end of incubation period in comparison to control. At this concentration the percentage losses in fresh and dry weights were 61.29% and 75% respectively. At more diluted concentrations reduction computed 34-46% as compared to control (Fig. 1A&B). The dosage effect on *A. niger* followed the same general pattern as in case of *A. oryzae* at the higher concentrations. Here the percentage losses in fresh and dry biomass productivity recorded 44.6% and 9.8% respectively. The data obtained for lower concentrations of salt reflected a non-significant decrease in fresh and dry biomass (Fig. 1A&B). Under the action of Copper sulfate, *D. tetramera* also showed the same response as other two. An inverse relationship between the biomass and dosage concentration was evidenced at highest concentrations. The loss in fresh weight was 79.49% and in dry weigh it was 40.6% (Fig. 1A&B).

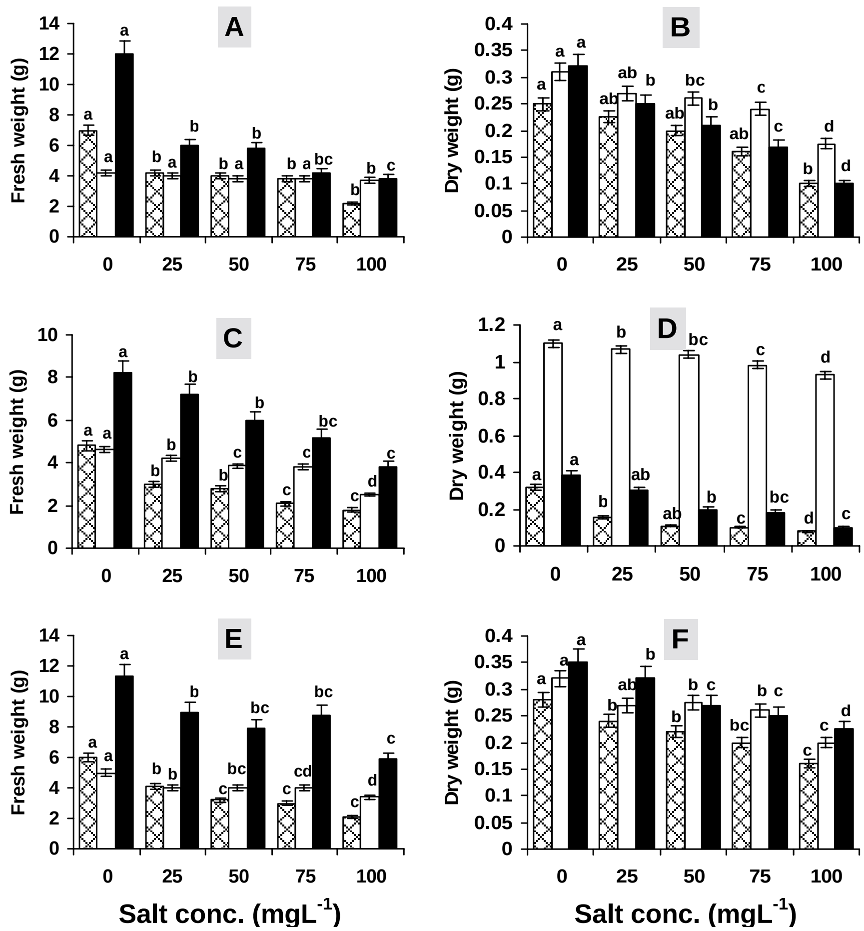

Fig. 1. Effect of salts of copper, Copper sulfate (A&B), Copper chloride (C&D) and Copper oxychloride (E&F), on fresh and dry weight of *Aspergillus oryzae* (shaded bars), *A. niger* (empty bars) and *Drechslera tetramera* (filled bars). Lines on data bars indicate S.E. of the mean while different alphabets on the bars show that data is significantly different at p=0.05 according to DNMRT.

Data regarding fresh and dry biomass productivity responses of *A. oryzae* to Copper chloride showed that the percentage losses at the lowest concentrations of 25 mgL^{-1} was 36.1% (for fresh weight) and 12% (for dry biomass). At highest concentration (100mgL^{-1}) a significant suppression in fungal growth i.e., 67.5% and 56% for fresh and dry weights respectively was noticed (Fig. 1C&D).

Same trend of growth was observed in *A. niger* and its growth rate was also inversely proportional to increase in concentration and a maximum biomass productivity was in control and maximum loss in biomass productivity was recorded to be 25% (fresh weight) and 35.4% (dry weight) at highest concentration (i.e., 100 mgL^{-1}) (Fig. 1C&D).

Results obtained when *D. tetramera* was given the treatment of Copper chloride, a maximum growth observed in unadded media and minimum fresh and dry mass was observed @ 100mgL^{-1} concentration. Here the losses in fresh and dry biomass in terms of percentage were 71.25% and 59.3% respectively (Fig. 1C&D).

All the three fungal species showed a sharp decline in growth in terms of fresh and dry biomass in response to the treatment of Copper oxychloride. At the highest dose of salt reduction in biomass of *A. oryzae* was 54.6% and 41.3% respectively (Fig.1E&F). The negative effects of Copper oxychloride were also maximized at the concentration of 100 mgL^{-1} in case of *A. niger*. Here the net percentage decrease in fresh and dry masses was 27% and 34% respectively (Fig. 1E&F).

Similarly, the dosage effect on *D. tetramera* was also significant at 100 mgL^{-1}, the resultant decrease in fresh and dry weights was 47.3% and 36.1% respectively. The growth in this case was significantly low as compared to control at $p=0.05$ (Fig. 1E&F).

Discussion

In the present experiment three common soil fungi were exposed to inorganic salts of copper. The results have shown that all the three treatments (Copper sulfate, Copper chloride and Copper oxychloride) reduced the fresh and dry biomass productivity of all the three test fungi viz., *A. oryzae, A. niger* and *D. tetramera.*

Earlier workers (Hallos & Cooney, 1981) have reported that heavy metals are generally toxic to microorganisms especially if these metals are present at high concentrations (Gadd & Griffth, 1978; Wood & Wang, 1985; Ehrlich, 1986). Copper which is one of the 16 essential elements also have toxic potential if present in higher quantities (Anon., 1986).

The treatment of three fungal species with Copper sulfate yielded a decrease in biomass productivity which inversely related with the increase in its concentration. Similar results have been reported for *Cladophora glomerata* where it proved to be a good algaecide (Sarim & Ali, 1986). Similar results of growth suppression were shown by Hoisiaslousma, 1976) for *Natrium*, and Khan & Saifullah, (1984) on *Oscillatoria*.

Treatment of three soil fungi with Copper chloride produced worthwhile results highlighting the fungicidal properties of these salts. Treatments of the fungal species with Copper oxychloride produced significant results and gradual reduction in biomass production was observed as compared to untreated fungal cultures. The observations made in the present study showed that *A. niger* was most resistant to all these compounds and this resistance may be due to its highly pathogenic potential (Alexopoulos, 1996). The results obtained by the application of salts of copper showing gradual decline in growth of fungal species may be due to hypertoxicity of these compounds on cellular metabolism of the fungi as suggested by Alexopoulos, (1996).

The growth reducing affects of the inorganic salts used in a fairly dilute concentration on the test fungi clearly indicates the biocidle potential of these salts as fungicides. Using these salts blindly would not only degrade the physical environment but would also be a hazard for an array of potentially useful organisms in the biological world.

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