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# Effects of biocontrol agents and heavy metals in controlling soil-borne phytopathogens

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**Abstract**
In current scenario of agriculture soil borne diseases are of great concern and these are responsible for major crop loss. There is a worldwide need to control the diseases as well as to maintain the quality and abundance of food feed and fodder for the human beings as well as cattles. To overcome this problem different approaches are used to prevent, mitigate or control the plant diseases. Besides depending on synthetic chemicals researches are still going on to search the alternative inputs of biological control. Therefore, in the present study effect of heavy metals and *Trichoderma* spp. on the growth of microorganism was investigated. Four phytopathogens *Fusarium oxysporum, Alternaria solani, Helminthosporium oryzae and Aspergillus* species. Were used against Manganese chloride, Mercuric chloride, Copper sulfate, Zinc sulfate, Cadmium sulfate and Cobalt chloride. Morphological alternations and various degree of growth inhibition were reported during this study.

**Keywords:** Soil borne, phytopathogens, biocontrol, heavy metals, *Trichoderma* spp

## 1. Introduction
Loss of natural resources in agriculture is primarily cause by plant diseases. Soil-borne pathogens like fungi, bacteria etc. cause important losses, among which fungi being the most destructive. Due to change in the farming practices, distribution of some of the phytopathogenic fungi, such as *Pythium*, *Phytophthora*, *Botrytis*, *Rhizoctonia* and *Fusarium* spread widely showing with adverse effects on crops. Fungal infections destroy the stored food also [1]. Phytopathogens are capable of producing toxic metabolites that may be carcinogenic and therefore constitute a public health risk, whereas the growth of specific fungi results in nutritional and chemical changes and poor appearance and food flavor development [2]. Abiotic and biotic control measures are the prerequisite to control the plant pathogens.
In the first case the emphasis is on the heavy metals, which have antifungal nature and total fungal inhibition can be achieved by their use. These are having metallic properties (ductility, conductivity, stability as cations, ligand specificity etc.) and an atomic number > 20. The most common heavy metals to be used as abiotic factor are Cd, Cu, Hg, Pb etc. [2, 3]. Based on fungal physiology, metals can be divided into essential and non-essential for fungi. It has been reported that some heavy metals such as zinc or copper are essential for fungal growth but most of heavy metals are quite toxic. There is also some evidence for changes in fungal morphology and physiology caused by these substances [4]. Heavy metals leads the adverse effects on morphogenesis of the vegetative hyphae [5, 6], sporulation [8, 9, 10] and sexual and asexual reproduction [11, 12. 13] of some fungal groups. However, studies dealing with the morphogenesis of zoosporic fungi, especially Oomycetes responses to increased levels of heavy metals are less frequent [14, 15, 16].
In consideration to environmental aspects, eco-friendly methods play an important role for protecting the crops from pest and disease [17]. As compare to chemical methods biological control of plant disease especially soil borne plant pathogens and nematodes by microorganisms is the best alternative. Biological control not also reduces the damage [18, 19] caused by plant pathogens but also maintains the quality of food components of crops. Biological control consists different mechanisms such as antibiosis, competition, suppression, direct parasitism, induced resistance, hypo virulence and predation. The antagonistic activity has often associated with production of secondary metabolites [20]. Most biocontrol agents are from the species *T. harzianum*, *T. viride* and *T. hamatum*. The mycoparasite ability of *Trichoderma* species against some economically important aerial and soil borne plant pathogens [21, 22, 23, 24, 25] and nematodes [26, 27] leads to the development of different biotic control strategies.
Under natural conditions large number of *Trichoderma* species reduces the occurrence of soil borne plant pathogenic fungi [28, 29] however, physical, chemical and biological conditions of

soil are the important aspect for the efficacy of *Trichoderma*. The present paper deals with the effect of heavy metals and *Trichoderma* spp. on the growth of soil borne phytopathogens.

## 2. Materials and Methods

### 2.1 Test organism

Four phytopathogens *Aspergillus* spp., *Alternaria solani*, *Helminthosporium oryzae, Fusarium oxysporum* were used for the experiments. Potato Dextrose Agar (PDA) medium was used for the growth of fungus.

### 2.2 Chemicals used

The heavy metal salts such as Cadmium Sulphate, Zinc Sulphate, Cobalt chloride, Magnesium chloride, Mercuric Chloride, Copper Sulfate were used. Six concentrations (1 mm to 10 mm) were choosen (based on molecular weight of heavy metal salts). The heavy metals were added before autoclaving of the medium.

### 2.3 Isolation of microorganisms

*Aspergillus* spp., *Trichoderma* viridae and *Fusarium oxysporum* were isolated from chickpea rhizosphere by serial dilution method. *Alternaria solani* and *Helminthosporium oryzae* were isolated from brinzal and rice infected leaves respectively.

Diseased leaves were thoroughly washed in running tap water. Small piece of the leaves with leaf spot symptoms were cut from the sample, washed with sterile water, and surface sterilized using mercuric chloride solution (1:1000) for 30 seconds and again washing is done in several changes of sterile water and transferred aseptically on PDA petri plates and incubated at $28^0$C. Isolates were confirmed by studying the characters [30].

### 2.4 Antifungal activity of heavy metals

Pure culture of test fungi were grown on sterile PDA plates for 7d using single spore culture technique [31].

Using 5mm Cork borer, fungal agar blocks were punched from 7d old cultures grown on petri plates. The agar block inoculum was aspectically transferred to freshly prepared medium (PDA and PDA with different concentration of heavy metals) and incubated at $28^0$ C for 7 days. Inhibition was calculated and recorded in terms of % inhibition of mycelium growth. Experiments were repeated twice and each set contained three replicates.

### 2.5 Biocontrol of phyto pathogens by *Trichoderma viride*

For the effect of *Trichoderma viride* on the growth of tested organism, both tested organism and *Trichoderma viride* were seeded in the same dish at opposite side (dual culture) and their growth was recorded as above controls were performed seeding each fungus against itself.

## 3. Results and Discussion

In the present study four different phytopathogens *Aspergillus* spp. *Helminthosporium oryzae, Alternaria solani* and *Fusarium oxysporum* were selected.

*Aspergillus* spp., *Fusarium oxysporum* were isolated from chickpea rhizosphere and *Altrernaria solani, Helminthosporium oryzae* were isolated from rice and brinzal leaves respectively. Fungal growth is affected by many factors such as medium, temperature, humidity and age of inoculum. In this investigation we showed the effects of heavy metals and *Trichoderma* on the growth of tested organism.

The selective activity of tested heavy metals on all fungi is investigated. Cadmium sulphate is 100% toxic to the *Fusarium oxysporum* (1mm to 10mm), 1.5mm for *Helminthosporium oryzae*, 2.5mm for *Alternaria solani* and *Aspergillus* spp. (Fig. 1). Similar result for 1mm Cadmium showed the strongest inhibition towards isolates from the genera *Fusarium, Alternaria* and *Geotrichum* [32]. Fig. 2 indicates that Cobalt chloride 10mm concentration is 100% toxic to all four test pathogens and % of growth inhibition is in increasing order from 1mm to 10mm concentration.

Cobalt sulphate at 1mM concentration for *Fusarium moniliforme, Trichoderma harzianum* and *Aspergillus flavus* [2]. Under same experimental conditions only 2.5mm concentration of Mercuric chloride is toxic (100%) to all the organisms (Fig. 3).

It was reported that mercury is highly toxic to wood rotting basidiomycetes and caused a decrease of growth rate down to 8% [4]. In the presence of mercury, a 3 day lag phase was observed (Fig.4).

Copper is a co-factor in numerous enzymatic processes and represents the most abundant transition metal found in living organism [33]. The growth of fungi was decreased after addition of copper in comparison to zinc. The blue colour of the isolates mycelia on agar media amended with copper may be due to binding of copper ions to the fungal cell wall. The mycelia of *Trichoderma viride* appear blue on agar media at all concentrations of copper sulphate [34]. The MICs for copper sulphate was in the range of 15-20mM and 7.5-20mM for isolates belonging to the genera *Aspergillus* and *Penicillum* spp. respectively.

A similar observation is also reported. The concentrations 2.5 mM, 5 mM and 10 mM is 100% inhibitory for *Fusarium, Aspergillus, Helminthosporium* and *Alternaria* respectively. But zinc sulfate at concentration 10mM found inhibitory for *Helminthosporium* but not for the other test organisms. At maximum 10mM concentration it inhibits only 67.3% growth of *Fusarium oxysporum* (Fig.5).

Zinc is essential for all organisms, although, at high concentrations it can be toxic [35]. The majority of fungi tested were able to grow at the concentration of 12.5-20 mm and 12.5-15 mm for isolates of the genera *Aspergillus, Penicillum* and *Fusarium* respectively [36], while 100% inhibition was recorded by zinc sulphate on *Alternaria solani* (Fig. 6).

10 mm concentration of Manganese chloride is inhibitory only for *Aspergillus* and for other tested organisms. At maximum concentration 10mM it decreased only 94-97% inhibition of *Fusarium oxysporum, Helminthosporium oryzae* and *Alternaria solani.* Similar results were also observed in which most of organisms were resistant to much up to 10mm concentration [2].

High metal ion concentration causes reduction in growth and increased the length of the lag phase compared to the control. If the growth of fungi in a metal-free medium was observed after a day0+, metal prolonged the lag phase depending on the metal used and its concentration.

Fungal colonies growing in the proximity may effect significant changes in one another or may require intimate hyphal content changes include trophic stimulation, morphogenic alteration and various degree of growth inhibition. Fig.7 shows the effect of *Trichoderma* on the growth pattern of test organism (results are based on the distance between test organism and *Trichoderma viride).* *Trichoderma* also showed maximum inhibition of *Alternaria* (1.8 mm) which were also observed earlier [37].

### 3.1 Figures

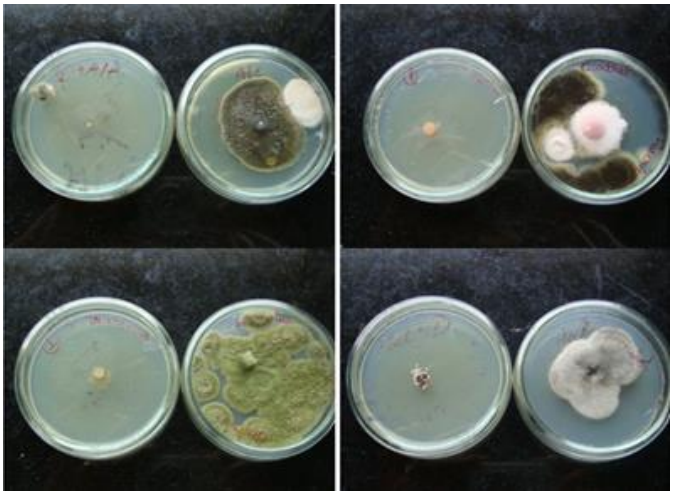

**Fig 1:** I, III, IV showing 100% inhibition by cadmium sulphate on *A. solani*, *H. oryzae* and *Aspergillus* spp. Photograph II showing 100% inhibition of Cadmium sulphate at 2.5 mm on *F. oxysporum* at 1 mm.

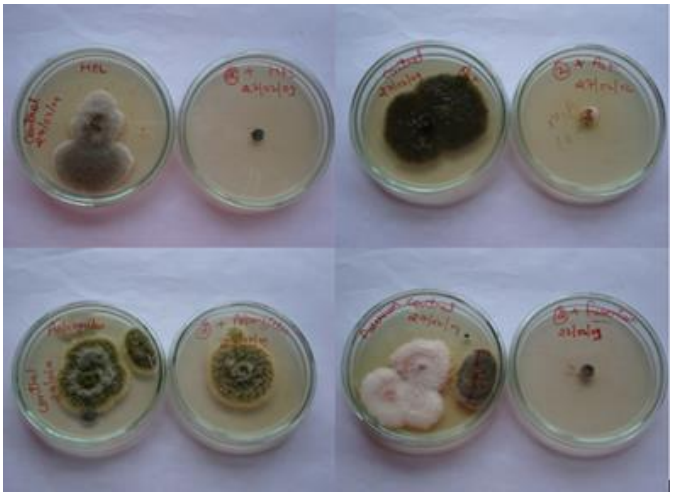

**Fig 2:** I, II, III and IV showing 100% inhibition by Cobalt chloride on *H. oryzae*, *A. solani*, *Aspergillus* spp. and *F. oxysporum* at 10mm.

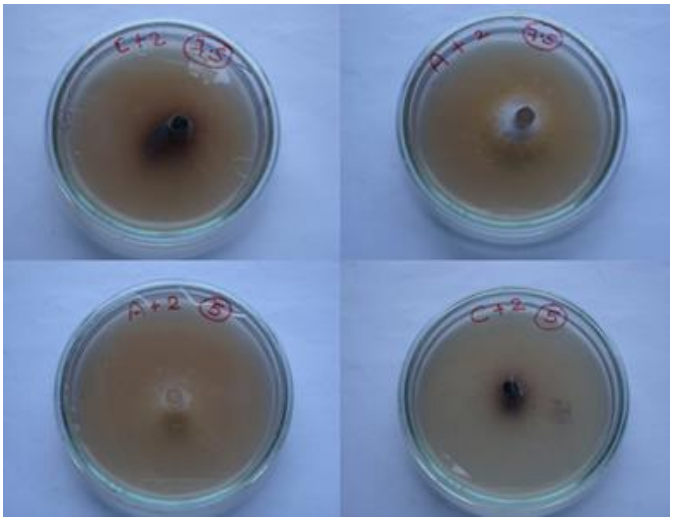

**Fig 3:** II and III showing 100% inhibition by Mercuric chloride on *F. oxysporum* at 7.5 and 5mm respectively. I and IV showing inhibition on *H. oryzae* at 7.5mm and 5mm respectively

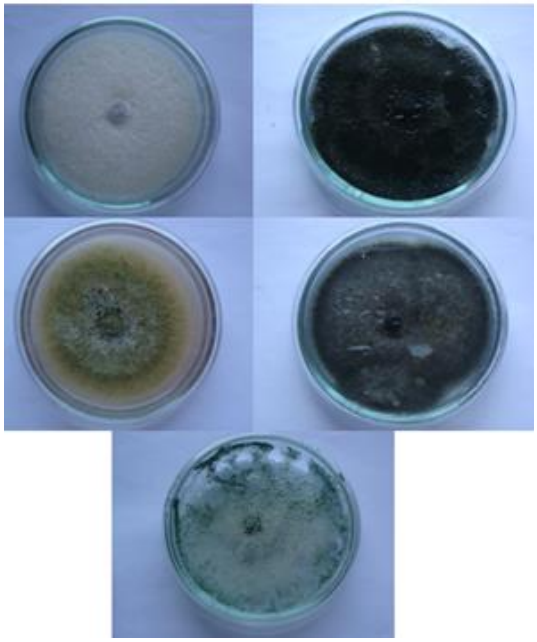

**Fig 4:** I, II, III, IV and V showing controls full growth of *F. oxysporum*, *A. solani*, *Aspergillus* spp., *H. oryzae* and *T. viride* grown for 5 days.

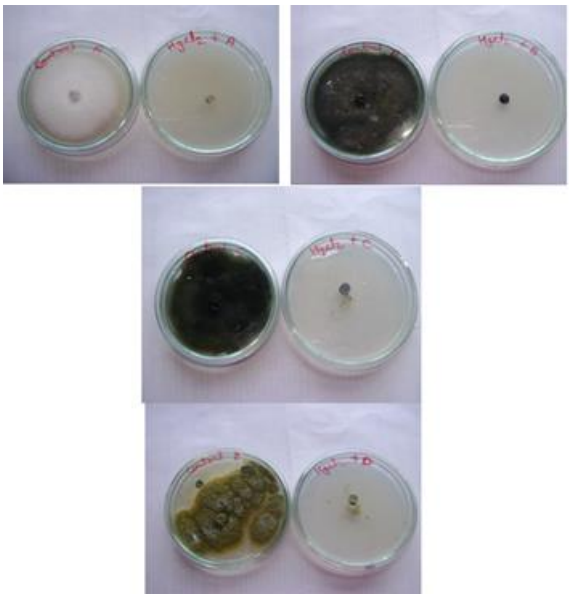

**Fig 5:** I,II,III and IV showing 100% inhibition by Zinc sulphate on *F. oxysporum*, *H. oryzae*, *A. solani* and *Aspergillus* spp. at 2.5 mm.

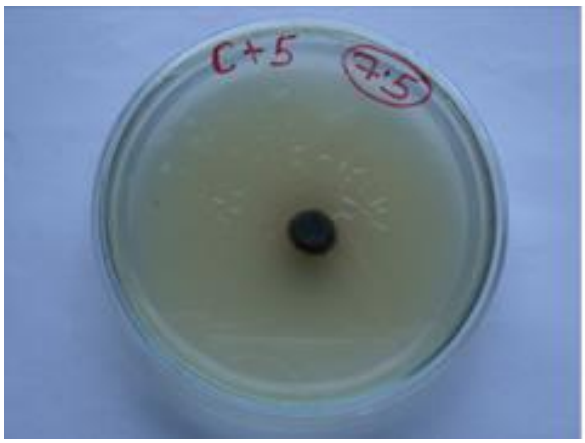


**Fig 6:** 100% inhibition by Zinc sulphate on *A. solani*

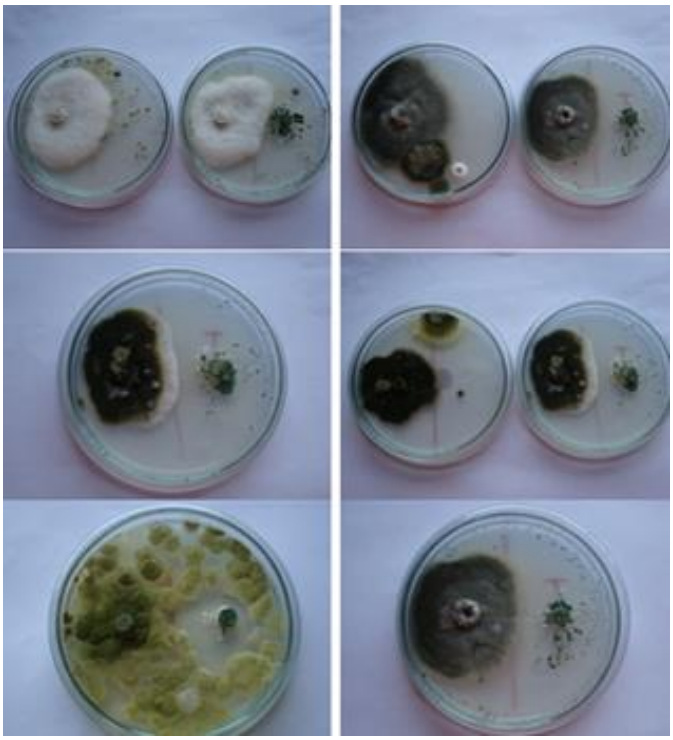

**Fig 7:** I showing antagonistic effect of *T. viride* on *F. oxysporum*, II and VI on *H. oryzae*, III and IV on *A. solani*, and V on *Aspergillus* spp.

## 4. Conclusion

Four phytopathogens *Fusarium oxysporum, Alternaria solani, Helminthosporium oryzae and Aspergillus* spp. were used against various heavy metals (Manganese chloride, Mercuric chloride, Copper sulfate, Zinc sulfate, Cadmium sulfate and Cobalt chloride) and inhibition was calculated in terms of % inhibition.

Dual cultures of *Fusarium oxysporum, Alternaria solani, Helminthosporium oryzae* and *Aspergillus* spp. were done with *Trichoderma* as a biocontrol agent and various morphological alternations and degree of growth inhibition was observed in terms of distance between *Trichoderma* spp. and test organisms. It has been observed that Biocontrol agents acts as cost effective, mitigate and eco-friendly measures against the soil borne pathogens. Further studies will be carried out to investigate the biological conditions under which these biocontrol measures will be successfully employed.

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