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Evaluation of heavy metals toxicity against soil-borne fusarial pathogens causing wilt in vegetable crops

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The heavy metals (cobalt nitrate, copper sulphate, mercuric chloride) were tested @ 100, 200 and 400 ppm for their efficacy against soil borne fusarial pathogens causing diseases in tomato and brinjal crops. Significant decrease in per cent inhibition of mycelial growth and wilt incidence was observed with the increase in concentration and incubation period. The metals mercury chloride, copper sulphate and cobalt nitrate @ 100 ppm (88.12, 60.00, 45.50%), @ 200 ppm (88.75, 69.37 , 55.62%) and @ 400 ppm (100, 75.00 , 77.50%) inhibited the mycelial growth against *Fusarium solani* and @ 100 ppm (89.16, 62.50, 39.37%), 200 ppm (100, 71.37, 43.12%) and 400 ppm (100, 87.50, 54.37%) against *Fusarium oxysporum* f. sp. *lycopersici* on 7th day of incubation. However, on 10th day the same metals against *F. solani* showed per cent inhibition of mycelial growth at 100 ppm (85.88, 49.64, 8.23%), 200 ppm (88.23, 60.20, 16.08%) and 400 ppm (100, 66.00, 59.26%) and of *F. oxysporum* f. sp. *lycopersici* at 100 ppm (82.94, 42.29, 5.88%), 200 ppm (89.41, 58.04, 17.64%) and 400 ppm (100, 78.82, 23.52%) as compared to control. It was also observed that mercuric chloride (Hg_2Cl_2) was most effective as it gave 100% inhibition of mycelial growth at 200 and 400 ppm of both the test fusaria.

Key words: *Fusarium solani, Fusarium oxysporum* f. sp. *lycopersici*, heavy metals, wilt disease, antifungal activity

INTRODUCTION

Industrial modernization and the use of heavy metals (Fe, Co, Cu, Ni, Zn, Cd, and Pb) can cause environmental pollution. On the other hand, some of these metals are present in traces are essential elements for biological systems. In order to understand the role of these metals in biological systems, structural studies of the biological compounds and their metal complexes are extremely important (Emen *et al.*, 2005; Binzet *et al.,* 2006). The heavy metal complexes display a wide range of biological activity including antibacterial, antifungal, antitubercular, antithroid, antihelmintic, rodenticidal, insecticidal, herbicidal, analgesic, sedative, antipyretic, antiinflammatory agents and plant-growth regulator properties (Eweis *et al.*, 2006; Borkow and Gabbay, 2009; Keter and Darkwa, 2012).

According to Walters *et al.* (2001) the heavy metal compounds are known to be increased as a result of the plant defence mechanism and is found that three spermidine conjugates are shown to reduce mycelial growth of *Pyrenophora avenae,* powdery mildew infection of barley seedlings and reduce *Verticillium* wilt of peanuts but enhance *Fusarium* wilt of tomato.

Metal complexes have been proved to be more fungitoxic compared to the basic organic com-

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pound by Balasubrahmanyan *et al.,* (2000). Dithocarbamate, morpholine dithiocarbamates and diphenyl dithiocarbamates complexes of Zn (II) Mn (II), Fe (II) and Cu (II) have been found to be very active against certain fungi, namely *Helminthosporium goffybii*, *Fusarium* and *Alternaria solanae*. Copper (II) sulphate and copper (II) oxychlorides are very effective against species of *Aspergillus, Penicillium, Fusarium* and *Gliocladium*, somewhat more on *Alternaria, Chaetomium* and *Trichothecium* (Peciulyte and Lugauskas, 2000). Copper complexes of thiophene oligomers have proved to be excellent fungicidal and also possessing other antimicrobial property against *Aspergillus niger*. These effects were attributed to a slow release of Cu_2 into the culture medium, thereby causing inhibition of fungus (Rathore *et al.,* 2009).

The screening of biological activities of ligand and its complexes against the fungi *Alternaria brassicae*, *Aspergillus niger* and *Fusarium oxysporum* and the pathogenic bacteria *Xanthomonas compestris* and *Pseudomonas aeruginosa* indicate that the complexes show the enhanced activity in comparison to free ligand (Chandra *et al.*, 2009). Coordination of these heavy metals, e.g. copper, nickel, cobalt and iron, often enhance their activities, as reported for pathogenic fungi (Singh *et al.*, 2000).

In view of the above, the aim of present study is to evaluate the antifungal properties of Co(II), Cu(II) and Hg(II) heavy metals against several soil- borne pathogenic fungi.

MATERIALS AND METHODS

Cultures

Fusarium solani and *Fusarium oxysporum* f. sp. *lycopersici* cultures were maintained in laboratory at room temperature by repeated sub-culturing on Czapek's Dox Agar medium from their respective hosts and soil collected from the Vegetable farm of Chandra Shekhar Agad Agriculture and Technical University, Kanpur. The cultures of the fungi were purified by the single spore isolation technique (Chauhan *et al.*, 2002).

Metal salts and Medium

Aqueous solutions of each metal salt, $CuSO_4.5\ H_2O$, Hg_2Cl_2 and $Co(NO_3)_2\ 6H_2O$ were individually prepared by dissolving the required amounts in distilled water and making concentrations of 100, 200 and 400 ppm. Czapek's Dox Agar medium was prepared and streptomycin (100 mg) was added to inhibit the bacterial growth.

Inoculation

The sterile medium (90 ml) and the stock solution (10 ml of 1000 ppm) of Mercury (II) chloride were mixed under sterile conditions to obtain the effective concentration of Mercury (II) chloride in the medium as 100 ppm. Similarly 20 ml of 1000 ppm solution of Mercury (II) chloride and 80 ml of medium were mixed in other Petri dishes so that the effective concentration was 200 ppm. In the same way 40 ml of 1000 ppm solution of Mercury (II) chloride and 60 ml of the medium were mixed in Petri dishes to get an effective concentration of 400 ppm. The same procedure was adopted in the case of the other two heavy metal complexs. Finally the control was prepared by pouring same amount of distilled water in the sterile medium into a separate Petri dish. After the medium solidified, each Petri dish was inoculated with a 6.0 mm actively growing mycelial disc of *F. solani* and *F. oxysporum* f. sp. *lycopersici* and incubated at 25±2 ^{0}C for 7 and 10 days. After inoculation, the per cent reduction in the radial growth diameter over the control was calculated (Tzatzarakis *et al.,* 2000). Data were statistically analyzed by the two-way ANOVA with replication method.

RESULTS AND DISCUSSION

The data of heavy metal salts ($CuSO_4.5\ H_2O$, Hg_2Cl_2 and $Co(NO_3)_2\ 6H_2O$) with respect to soil–borne pathogenic Fusarium (*F. solani* and *F. oxysporum* f. sp. *lycopersici*) at three different 100, 200 and 400 ppm concentration have been presented in Table 1 and 2. The antifungal activity of pathogens generally increased with the increase in the concentration of the heavy metal compounds. It was evident from the experimental data that Hg_2Cl_2 showed 100% activity against FS at 400 ppm and against FOL at 200 and 400 ppm, on 7th day of inoculation. On 10th day of inoculation, both the pathogenic Fusarium at 400 ppm of Hg_2Cl_2 showed 100% antifungal activity. Mercuric chloride, copper sulphate and cobalt nitrate against *F. solani* at 100 ppm (88.12, 60.00 and 45.50%), 200 ppm (88.75, 69.37 and 55.62%) and 400 ppm (100, 75 and 77.5%) and *Fusarium oxysporum* f. sp. *lycopersici* at 100 ppm (89.16, 62.5 and 39.37%),

200 ppm (100, 71.37 and 43.12%) and 400 ppm (100, 87.5 and 54.37%) on 7th day (Fig. 1). But on 10th day of inoculation, all the heavy metal com-

Table 1 : Effect of heavy metals against *F. solani* (FS) and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) at different ppm on 7th day of inoculation

Heavy metals	Concentration (ppm)	Pathogens/ Mean colony dia (mm)	
		FS	FOL
Mercuric (II) chloride	100	9.50±0.00	8.66±0.29
	200	9.00±0.00	0.00±0.00al
	400	0.00±0.00al	0.00±0.00al
Copper (II) sulphate	100	32.00±0.00	30.00±0.00
	200	24.67±0.29	22.90±0.36
	400	20.00±0.00	10.00±0.00
Cobalt (II) nitrate	100	43.57±0.11	48.50±0.87
	200	35.33±0.29	45.50±0.50
	400	18.00±0.50	36.33±0.58
Control		80.00±0.00	80.00±0.00

Values shown are the mean ± SE of 3 replicates, significant at $p \leq 0.05$

Table 2 : Effect of heavy metals against *F. solani* (FS) and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) at different ppm on 10th day of inoculation

Heavy metals	Concentration (ppm)	Pathogens/ Mean colony dia (mm)	
		FS	FOL
Mercuric (II) chloride	100	2.00±0.00	14.50±0.46
	200	10.00±0.00	9.00±0.00
	400	0.00±0.00al	0.00±0.00al
Copper (II) sulphate	100	42.80±1.89	44.80±0.29
	200	33.83±0.29	35.66±0.57
	400	28.90±0.85	18.00±0.00
Cobalt (II) nitrate	100	78.00±0.00	80.00±0.00
	200	71.33±0.29	70.00±0.00
	400	34.63±6.56	65.00±0.00
Control		85.00±0.00	85.00±0.00

Values shown are the mean± SE of 3 replicates, significant at pd"0.05

plexes at 100 ppm (85.88, 49.64 and 8.23%), 200 ppm (88.23, 60.20 and 16.08%) and 400 ppm (100, 66 and 59.26%) against *F. solani* and at 100 ppm (82.94, 42.29 and 5.88%), 200 ppm (89.41, 58.04 and 17.64%) and 400 ppm (100, 78.82 and 23.52%) concentration against *F. oxysporum* f. sp. *lycopersici* (Fig. 2) were very effective. The toxicity depends on the nature of the ligands, the nature of the metal ion, and the combined effect of the metal and the ligand besides other factors. The complexes varied in their antifungal activity in the following order of metal ions: Hg > Cu > Co (Fig. 3, 4 and 5).

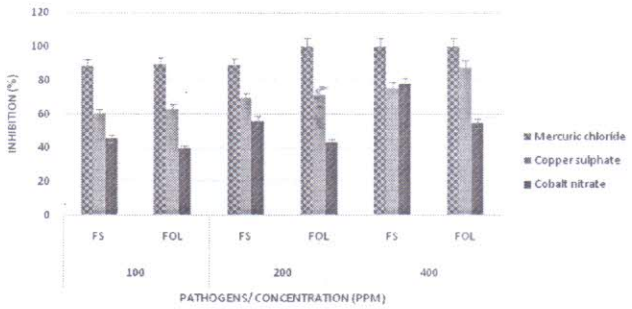


Fig. 1 : Percentage inhibition of *F. solani* and *F. oxysporum* f. sp. *lycopersici* at different ppm on 7th day of inoculation

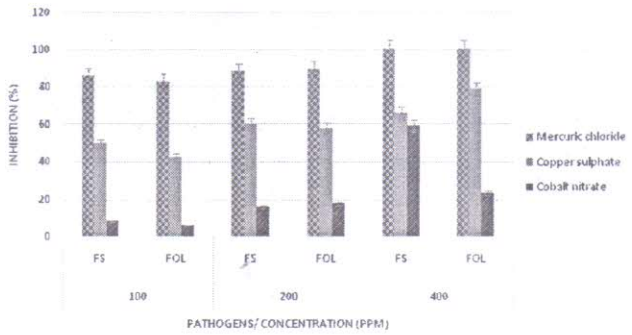
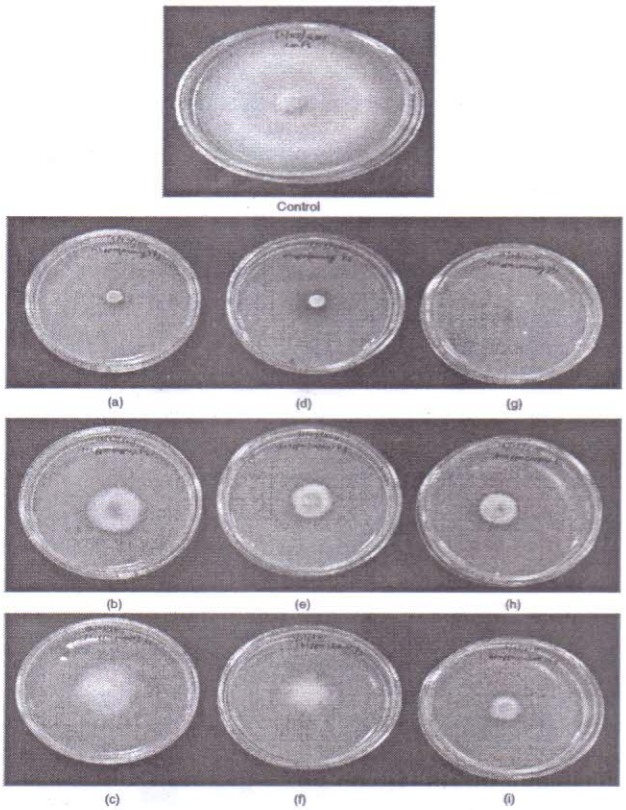


Fig. 2 : Percentage inhibition of *F. solani* and *F. oxysporum* f. sp. *lycopersici* at different ppm on 10th day of inoculation

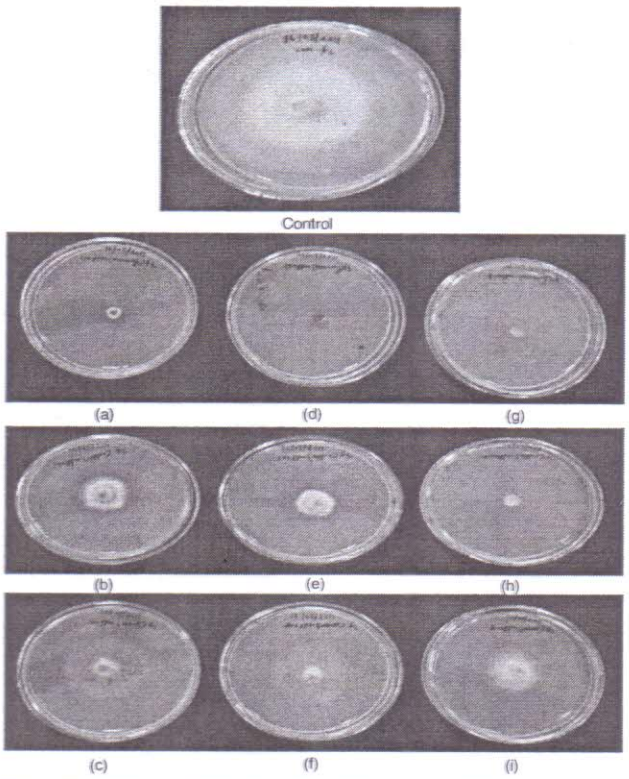
The antifungal activity indicated that the complexes showed an appreciable activity against *Aspergillus niger* and *Fusarium oxysporum* at 1 mg/ml concentration. Ligand has shown a lesser activity as compared to the metal complexes. The copper complex showed the highest activity against *F. oxysporum* and then in *A. niger* (Mounika *et al.*, 2010).

According to Pandey and Narang (2005) the metal (II) and ligands mixed in the ratio of 1:2 showed an excellent synergistic effect against *Curvularia lunata* and *Alternaria triticina*. At higher concentration, the effect of individual compounds was not significant against *C. lunata* and *A. triticina*, whereas when the two compounds were mixed even at the lowest concentration there was significant reduction in spore germination. Similar results were also obtained when *Alternaria triticina, Alternaria brassicae and Fusarium udurn* were taken for their mycelial growth on potato dextrose broth supplemented with the chemicals. The spores



FS 100 ppm (a) mercury, (b) copper (c) Cobalt
FS 200 ppm (d) mercury, (e) copper (f) cobalt
FS 400 ppm (g) mercury (h) copper, (i) cobalt

Fig. 3 : Antifungal activity of heavy metals against *F. solani* at different ppm after 7 days of inoculation



FOL 100 ppm (a) mercury, (b) copper (c) cobalt
FOL 200 ppm (d) mercury, (e) copper (f) cobalt
FOL 400 ppm (g) mercury (h) copper (i) cobalt

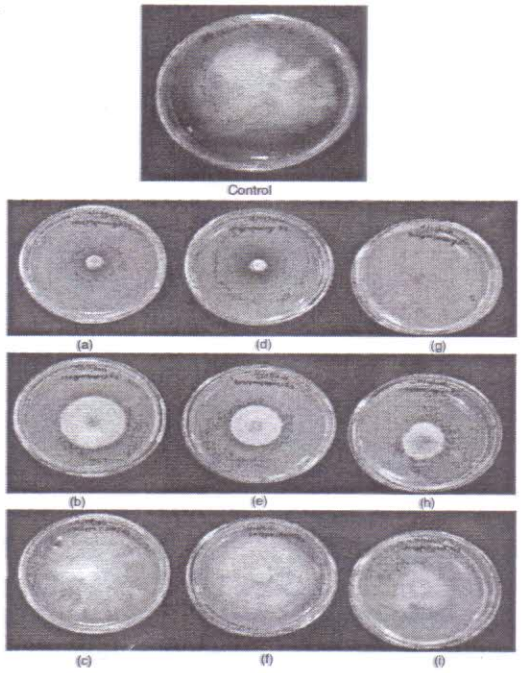
Fig. 4 : Antifungal activity of heavy metals against *F. oxysporum* f. sp. *lycopersici* at different ppm after 7 days of inoculation

which showed sensitivity against the chemicals also showed a similar trend in the production of mycelial dry weight. Out of the tested fungi, *Alternaria triticina* showed maximum sensitivity when the chemicals were mixed, followed by *Alternaria brassicae* and *Fusarium udurn.*

The antimicrobial screening studies revealed that the metal chelates have higher activity than the corresponding free ligand and control against the *A. niger* and *R. bataicola* under identical experimental conditions (Raman *et al.*, 2005).

The heavy metal salts of Zn (II) and Cu (II) exhibited a strong antimicrobial activity and Mn(II), Co(II), and Ni(II) showed a moderate antimicrobial activity against *A. niger* and *C. albicans* and also *S. aureus* (Mokhles *et al.*, 2012).

According to (Kalagouda *et al.*, (2006) the heavy metals, copper (II), nickel (II) and cobalt (II) complexes showed better antifungal activity as compared to the ligand and the corresponding metal



FS 100 ppm (a) mercury, (b) copper, (c) cobalt
FS 200 ppm (d) mercury, (e) copper, (f) cobalt
FS 400 ppm (g) mercury, (h) copper, (i) cobalt

Fig. 5 : Antifungal activity of heavy metals against *F. solani* at different ppm after 10 days of inoculation

salts. The antimicrobial activity of Hg (L)Cl_2 complex have showed the highest activities against most Gram positive and Gram negative bacteria and as well as yeast cultures (Aghatabay *et al.*, 2008).

The proposed study revealed that a mercury (II) complex was most effective antifungal agent followed by copper (II) and cobalt (II) complexes against both the test fusaria.

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