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Full Length Research Paper

Effect of some metal-containing compounds and fertilizers on mycoparasite *Trichoderma* species mycelia growth response

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Response of three biocontrol agents including *Trichoderma harzianum* T969, *Trichoderma hamatum* T614 and *Trichoderma virens* T525 in the presence of 0, 50, 91.2, 166, 302, 550 and 1000 mg/l concentrations of $MgSO_4.7H_2O$, $ZnSO_4.7H_2O$, $CuSO_4$, $FeSO_4.7H_2O$, $MnSO_4.H_2O$, $CoCl_2.6H_2O$, $CaCl_2.2H_2O$ and $(NH_4)_2SO_4$ via poisoned medium method, were studied. Radial growth of the *Trichoderma* isolates were recorded 48 and 72 h after inoculating. For each experimental set, experimental design used was completely randomized design (CRD) through factorial experiment with 3 factors. Also, inhibition percentage of the fungus mycelia growth due to metal containing compound at the inhibitory effect exhibited compound was calculated. The experiments indicated that *Trichoderma* species mycelia response to the compound differed noticeably in their sensitivities to the metal containing compounds and fertilizers exposure. $ZnSO_4.7H_2O$, $CuSO_4$, $FeSO_4.7H_2O$, $MnSO_4.H_2O$ and $CoCl_2.6H_2O$ compounds were effective as radial growth inhibitors, though $(NH_4)_2SO_4$, $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ were found to be non-toxic to the *Trichoderma* species. Meanwhile, $CuSO_4$ indicated the strongest inhibition towards the *in vitro* mycelia expansion of the fungi.

Key words: Metal containing compound, fertilizer, biocontrol agent, mycelia growth, EC_{50}, inhibition.

INTRODUCTION

Trichoderma spp. are among the most frequently isolated soil imperfect filamentous fungi, well known for their biocontrol ability against a wide range of plant pathogenic fungi (Howell, 2003), induction localized, systemic defense response in the plant (Hansen and Howell, 2004; Yedidia et al., 1999) and plant growth enhancement (Hoyos-Carvajal et al., 2009; Harman et al., 2004; Baker, 1988). Also, they have great role in ecology as they take part in decomposition of plant residues as well as biodegradation of man made chemicals and bioaccumulation of high amount of different metals from waste water and soil (Anand et al., 2006; Ezzi and Lynch, 2005). Metal-containing pollutant are increasingly being released into the soil from industrial waste water as well as from wastes derived from chemical fertilizers and pesticides as agricultural applications (Ting and Choong, 2009; Errasquim and Vazquez, 2003). Some metal-containing pollutants are not biodegradable and travel up the food chain via bioaccumulation (Errasquin and Vazquez, 2003). Some minute amount of metals except for non-essential biological functions metals such as mercury, lead and cadmium, as essential micronutrient are influencing vital metabolic processes and are required by all forms of life; however, they may to be toxic at higher concentration than the nutritional requirement. Evidence suggests that *Trichoderma* spp. exhibited considerable tolerance against metals and accumulates high amounts of the metals from polluted habitants (Anand et al., 2006; Errasquin and Vazquez, 2003). Therefore, metal tolerant *Trichoderma* species may become dominant organisms in some polluted soil and may play an important role in environmental-friendly metal-removal technology (Ting and Choong, 2009). Also, metal ions in soil may influence growth, sporulation and enzymatic activities of *Trichoderma* spp. (Jaworska and Dluzniewska, 2007) which can cause changes in the quantities of extra cellular enzymes produced and metabolites (Kredics et al., 2001a, b) as well as biological activities against plant pathogenic fungi and plant growth stimulated effect. However, the environmental requirement and the response of *Trichoderma*

spp. to exposure of each metal-containing compound may differ depending on the type of metal and *Trichoderma* isolates. It is necessary to collect data about the effect of metal-containing compounds on *Trichoderma* isolate from the point of the view of sustainable agricultural system and integrated pest management due to the application of *Trichoderma* spp. in combination with metal-containing fungicides and chemical fertilizers.

The aim of this study is to assess the capability of three biocontrol agents including *Trichoderma harzianum* T969, *Trichoderma hamatum* T614 and *Trichoderma virens* T525 in the presence of different soluble metal-containing compounds.

MATERIALS AND METHODS

The experiment was carried out at the Biology laboratory of the Department of Plant Production, Moghan Junior college of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran during 2009. The *Trichoderma* isolates selected for this study were obtained from a collection of *Trichoderma* spp. in the Plant Pest and Disease Institute, Tehran, Iran, including *T. harzianum* isolate T969, *T. hamatum* isolate T614 and *T. virens* isolate T525. The isolates were grown on potato dextrose agar (PDA, BDH Ltd, UK 39 g/l) medium, maintained on PDA medium and stored at 4°C for further use.

In this experiment, the mycelia growth capability of the *Trichoderma* isolates against some metal-containing compounds and fertilizers, consist of $MgSO_4.7H_2O$ (containing 9.87% Mg), $ZnSO_4.7H_2O$ (containing 22.73% Zn), $CuSO_4$ (containing 39.81% Cu), $FeSO_4.7H_2O$ (containing 20.09% Fe), $MnSO_4.H_2O$(containing 32.50% Mn), $CoCl_2.6H_2O$ (containing 24.79% Co) and $CaCl_2.2H_2O$ (containing 27.17% Ca) as well as $(NH_4)_2SO_4$, were evaluated via poisoned medium method. The medium was amended with progressively increasing concentration of the compounds including 0, 50, 91.2, 166, 302, 550 and 1000 mg/l (ppm). Supplemented medium were prepared with the addition of each concentration of compounds to PDA (BDH Ltd, UK 39 g/l) then sterilized (autoclaved at 121°C for 18 min) before being divided onto sterile Petri-dishes in the laminar air flow cabinet. 5 mm-diameter of mycelia agar plug was removed from the margin of 7-day old of each isolate colony by No.3 cork borer and placed on the center of petri-dishes containing PDA consisting of the tested concentration of each compounds. In control plates, there were no concentrations of mentioned compounds existing. The Petri dishes were then sealed with parafilm and incubated in a growth chamber at 25±1°C. Radial growth of the *Trichoderma* isolates (hyphae extension) were recorded at 48 and 72 h after inoculating.

For each experimental set, experimental design used was completely randomized design (CRD) through factorial experiment with 3 factors - (a) the *Trichoderma* isolates, (b) the mycelia growth recording time and (c) the compound concentration in three replicates for each treatment. The means were analyzed by analysis of variance (ANOVA) and were compared by Least Significant Difference (LSD) test at 1% significant levels with SAS software (SAS (1985) Institute Inc., Cary, NC, USA). Also inhibition percentage of the fungus mycelia growth due to metal-containing compounds and the inhibitory effect of exhibited compound was calculated in relation to the control using Abotte's formula:

$$I = [(C - T)/C] \times 100$$

Where, I is the percentage inhibition of radial mycelia growth (%); C is the radial growth measurement of the fungus in control (mm) and T is the radial growth of the fungus in treated plates (mm) (Edington et al, 1971). The average mean inhibition percentage was applied to determine the EC_{50} values (concentration of compound causing 50% inhibition of mycelia growth in relation to control growth) in the inhibitory effect exhibited metal-containing compound by subjecting the data to probit analysis (Statistical software SPSS 7.0 Inc Chicago, IL).

RESULTS

To assess direct *Trichoderma* spp. response to the metal-containing compounds and fertilizers stress, linear mycelia extension of *Trichoderma* spp. were separately measured 48 and 72 h after inoculating in solid enriched medium in the presence of seven concentrations. *Trichoderma* spp. mycelia response to the compound differed noticeably in their sensitivities to the metal-containing compounds and fertilizers exposure. Linear extension of *T. virens* were decreased markedly ($\rho \leq 0.01$) by the incorporation of $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, CuSO4 and $FeSO_4.7H_2O$. Whereas addition of $ZnSO_4.7H_2O$, $MnSO_4.H_2O$, $CoCl_2.6H_2O$ and $CaCl_2.2H_2O$ significantly ($\rho \leq 0.01$) reduced the mycelia development of *T. harzianum* T969 (Table 1).

As shown in Table 2, radial growth of *Trichoderma* mycelia were significantly ($\rho \leq 0.01$) increased 72 h after inoculation and exposure to all metal-containing compounds and fertilizers (Table 2). According to the evidence shown in Table 3, the response of *Trichoderma* to elevated concentrations of the compound differed noticeably (Table 3). In spite of increasing $(NH_4)_2SO_4$, $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ concentrations from 50 to 1000 mg/l, there were no significant ($\rho \geq 0.01$) differences in mycelia radial growth of each *Trichoderma* spp. when compared to their controls (Figures 1, 2 and 3).

In the case of $ZnSO_4.7H_2O$, *T. harzianum* growth rate decreased statistically ($\rho \leq 0.01$) when the medium was supplemented with $ZnSO_4.7H_2O$ from 50 to 550 mg/l content However, linear growth rate of *T. hamatum* insignificantly ($\rho \geq 0.01$) increased at 91.2 mg/l content of $ZnSO_4.7H_2O$; *T. hamatum* mycelia extension also decreased markedly ($\rho \leq 0.01$) when the compound concentration raised from 91.2 to 550 mg/l, and no growth was detected at 1000 mg/l concentration. Mean-while, *T. virens* linear growth expansion decreased slower than the other isolates and significant ($\rho \leq 0.01$) decrease in the fungus linear growth was observed from 302 mg/l concentration of $ZnSO_4.7H_2O$ (Figure 4). It was capable to survive even at 1000 mg/l of $ZnSO_4.7H_2O$. For $ZnSO_4.7H_2O$, the highest EC_{50} concentration was found for *T. virens* (Table4). Therefore, *T. virens* exhibited the highest resistance to Zn^{2+} when compared to the *Trichoderma* isolates.

In response to $CuSO_4$, the compound exhibited the strongest inhibition of mycelia extension in comparison to the effect of other tested compounds. There was no significant ($\rho \geq 0.01$) difference observed in linear growth of all tested species. In contrast, increasing $CuSO_4$ content from 91.2 to 166 consistently ($\rho \leq 0.01$) inhibited

Table 1. Linear extension of *Trichoderma* species (mm) grown on PDA amended with various metal-containing compounds and fertilizers (the main effect of *Trichoderma* species).

Trichoderma Species	Metal-containing compounds and fertilizers							
	$(NH_4)_2SO_4$	$MgSO_4.7H_2O$	$ZnSO_4.7H_2O$	$CuSO_4$	$FeSO_4.7H_2O$	$MnSO_4.H_2O$	$CoCl_2.6H_2O$	$CaCl_2.2H_2O$
T. harzianum T969	38.88(±2.043)b	52.71(±2.49)b	27.14(±3.59)c	23.19(±3.94)a	40(±3.44)b	34.12(±3.96)c	28.05(±4.12)c	24.08(±3.82)c
T. hamatum T614	52.60(±2.42)a	60.14(±2.63)a	31.48(±3.92)b	21.43(±4.22)b	43.74(±3.37)a	40.97(±3.55)b	29.81(±4.34)b	27.17(±4.01)b
T. virens T525	37.07(±1.36)c	44.69(±1.47)c	34.14(±2.46)a	16.86(±3.14)c	36.55(±2.65)c	47.24(±1.82)a	38.29(±2.92)a	34.07(±2.69)a
SEM	0.374	0.212	0.330	0.194	0.284	0.190	0.126	0.126
LSD	1.4027	0.7918	1.2283	0.7243	1.0602	0.7098	0.4693	0.4708

Values with the same letter within the column are not significantly different (P≤0.01) according to Fischer's protected LSD test results are means of three replicates for each treatment. The value in parentheses is the standard error of the mean. SEM: standard error of the mean

Table 2. Linear extension of *Trichoderma* species(mm) grown on PDA amended with various metal-containing compounds and fertilizers 48 and 72 h after inoculation (the main effect of time).

Time (h) after inoculation	Metal-containing compounds and fertilizers							
	$(NH_4)_2SO_4$	$MgSO_4.7H_2O$	$ZnSO_4.7H_2O$	$CuSO_4$	$FeSO_4.7H_2O$	$MnSO_4.H_2O$	$CoCl_2.6H_2O$	$CaCl_2.2H_2O$
48	30.78(±0.69)b	38.79(±0.52)b	20.97(±1.71)b	15.75(±2.32)b	27.43(±1.36)b	30.95(±1.71)b	20.94(±2.15)b	18(±1.88)b
72	54.92(±1.26)a	66.24(±1.29)a	40.87(±3.04)a	25.18(±3.62)a	52.26(±2.56)a	50.97(±2.93)a	43.16(±3.4)a	39.37(±3.17)a
SEM	0.307	0.173	0.269	0.158	0.232	0.155	0.102	0.103
LSD	1.1453	0.6465	1.0029	0.5914	0.8657	0.5795	0.3831	0.3844

Values with the same letter within the column are not significantly different (P≤0.01) according to Fischer's protected LSD test results are means of three replicates for each treatment. The value in parentheses is the standard error of the mean. SEM: standard error of the mean

Table 3. Linear extension of *Trichoderma* species(mm) grown on PDA amended with various concentration of metal containing compounds and fertilizers (The main effect of concentration).

Concentration (ppm)	metal-containing compounds and fertilizers							
	$(NH_4)_2SO_4$	$MgSO_4.7H_2O$	$ZnSO_4.7H_2O$	$CuSO_4$	$FeSO_4.7H_2O$	$MnSO_4.H_2O$	$CoCl_2.6H_2O$	$CaCl_2.2H_2O$
0	42.39(±3.35)bc	47.66(±3.55)e	48.11(±3.75)a	53.56(±3.45)a	42.28(±3.77)b	56.11(±3.68)a	51.11(±3.71)a	46.56(±3.69)a
50	41.56(±3.38)c	50.22(±3.44)d	46.33(±3.86)a	51.78(±3.35)b	47.67(±3.70)a	56.61(±3.92)a	50.6(±3.92)1a	46.61(3.92)b
91.2	43.22(±2.23)abc	53.89(±3.86)bc	47.56(3.80)a	32.05(±2.18)c	48.06(±3.96)a	52.89(±3.42)b	4811(±3.45)b	43.11(±3.45)c
166	41.67(±3.74)c	52.72(±3.82)c	39.33(±3.03)b	5.45(±1.28)d	48.50(±3.90)a	44.28(±2.99)c	39.44(±3.00)c	34.44(3.00)d
302	41.83(±3.51)c	54.55(±4.04)ab	20.89(±3.10)c	0.3889(±0.22)e	49.06(±3.83)a	34.22(±3.77)d	17.50(±5.11)d	15.17(±4.65)e
550	45.22(±3.69)a	55.33(±3.88)a	9.33(±2.42)d	0.00(±0.00)e	37.17(±2.94)c	21.78(±4.62)e	8.83(4.79)e	8.00(±4.34)f
1000	44.05(±3.53)ab	53.22(±3.81)c	4.38(±1.75)e	0.00(±0.00)e	4.94(±0.79)d	20.56(±4.53)f	8.72(±4.73)e	7.89(±4.28)f
SEM	0.574	0.324	0.503	0.296	0.434	0.291	0.192	0.193
LSD	2.1427	1.2094	1.6763	1.1063	1.6195	1.0842	0.7168	0.7192

- Values with the same letter within the column are not significantly different (P≤0.01) according to Fischer's protected LSD test results are means of three replicates for each treatment.
-The value in parentheses is the standard error of the mean. - SEM: standard error of the mean

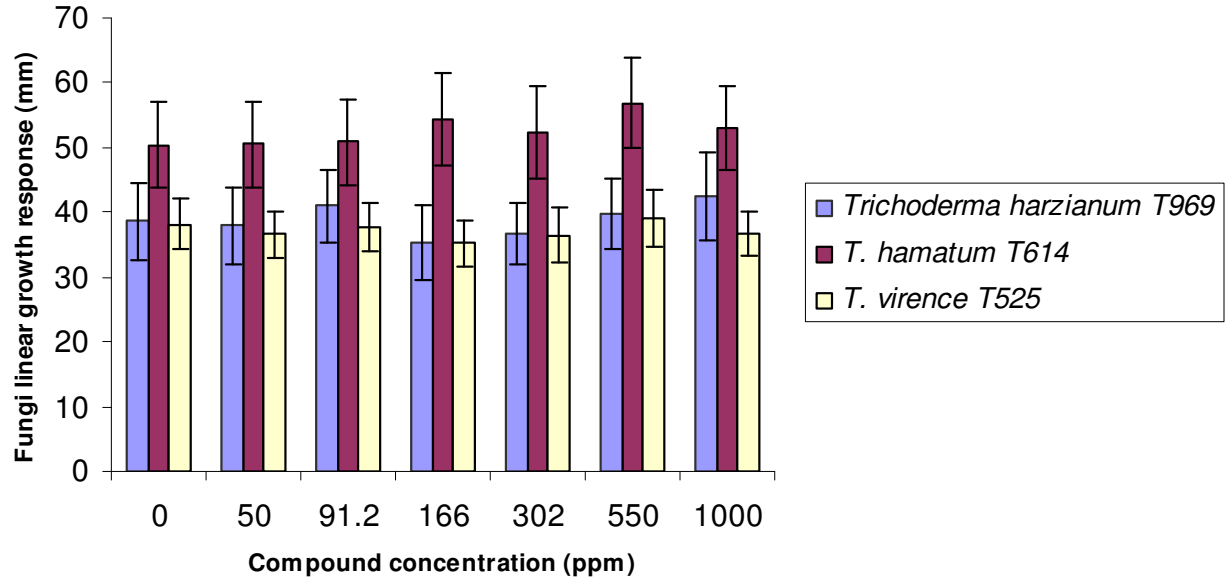

Figure 1. Radial growth response of *T. harzianum* T969, *T. hamatum* T614 and *T. virens* T525 on potato dextrose agar (PDA) medium amended with 0, 50, 91.2, 166, 302, 550 and 1000 mg/l (ppm) of $(NH_4)_2SO_4$. Bars represent standard errors (SE).

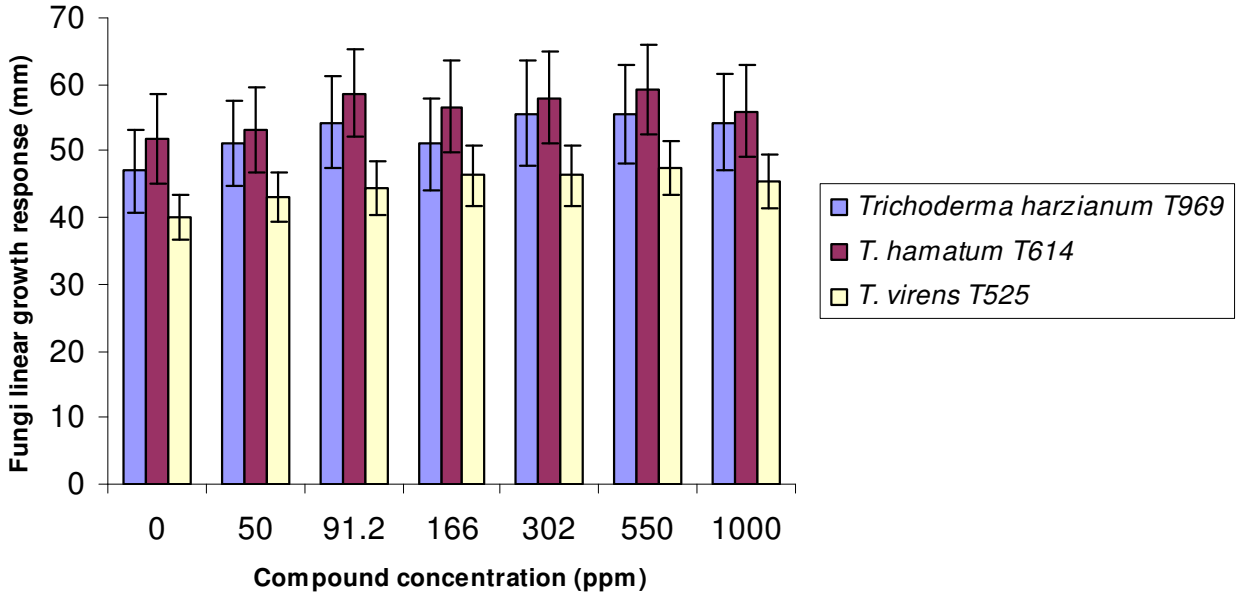

Figure 2. Radial growth response of *T. harzianum* T969, *T. hamatum* T614 and *T. virens* T525 on potato dextrose agar (PDA) medium amended with 0, 50, 91.2, 166, 302, 550 and 1000 mg/l (ppm) of $MgSO_4.7H_2O$. Bars represent standard errors (SE).

mycelia growth of all the *Trichoderma* isolates (Figure 5). Meanwhile, no mycelia extension were detected at 302, 550 and 1000 mg/l content of $CuSO_4$ by the tested fungi except for *T. harzianum* that had slight mycelia growth in 302 mg/l concentration of the compound (Figure 5). The EC_{50} concentration was found at 118.65, 86.68 and 96.3 mg/l for *T. harzianum*, *T. hamatum* and *T. virens*, respectively, indicating the strongest inhibition towards the *in vitro* mycelia expansion of the fungi.

The presence of $FeSO_4.7H_2O$ in growth medium from 50 - 302 mg/l concentration did not statistically ($\rho \geq 0.01$) affect the colony growth rate of each tested isolate (Figure 6). Increasing $FeSO_4.7H_2O$ concentration from 550 to 1000 mg/l caused a dramatic increase in the inhibition of mycelia growth from up to 13.19, 15.54 and 28.19% inhibition in 550 mg/l concentration to 91.96,

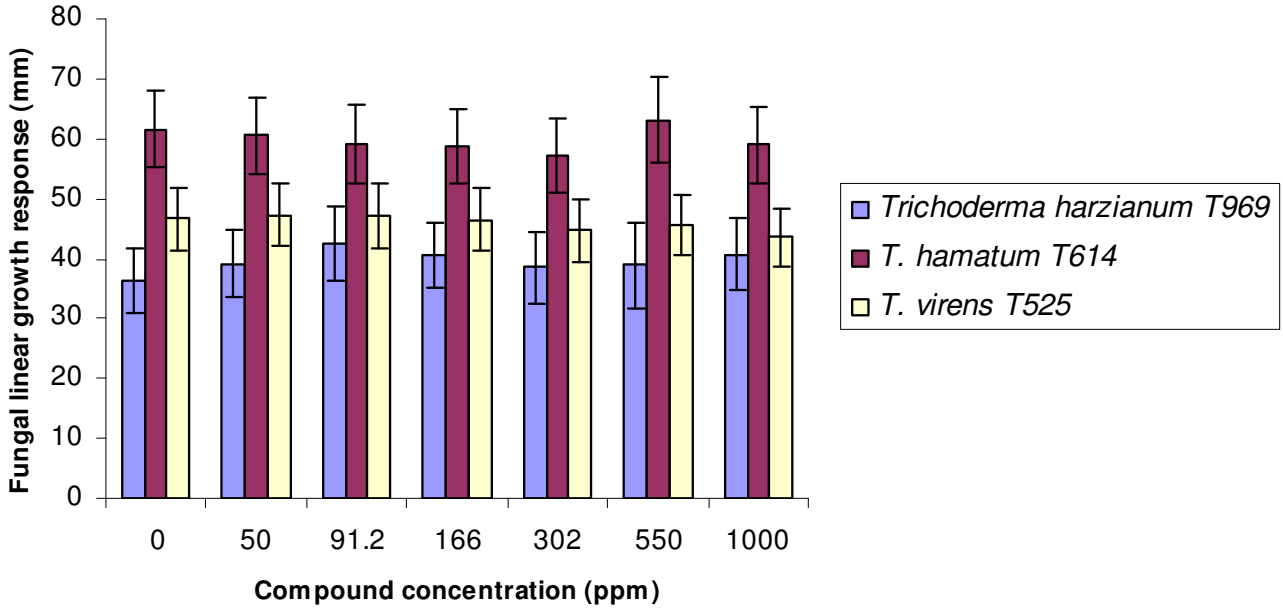

Figure 3. Radial growth response of *T. harzianum* T969, *T. hamatum* T614 and *T. virens* T525 on potato dextrose agar (PDA) medium amended with 0, 50, 91.2, 166, 302, 550 and 1000 mg/l (ppm) of $CaCl_2.2H_2O$. Bars represent standard errors (SE).

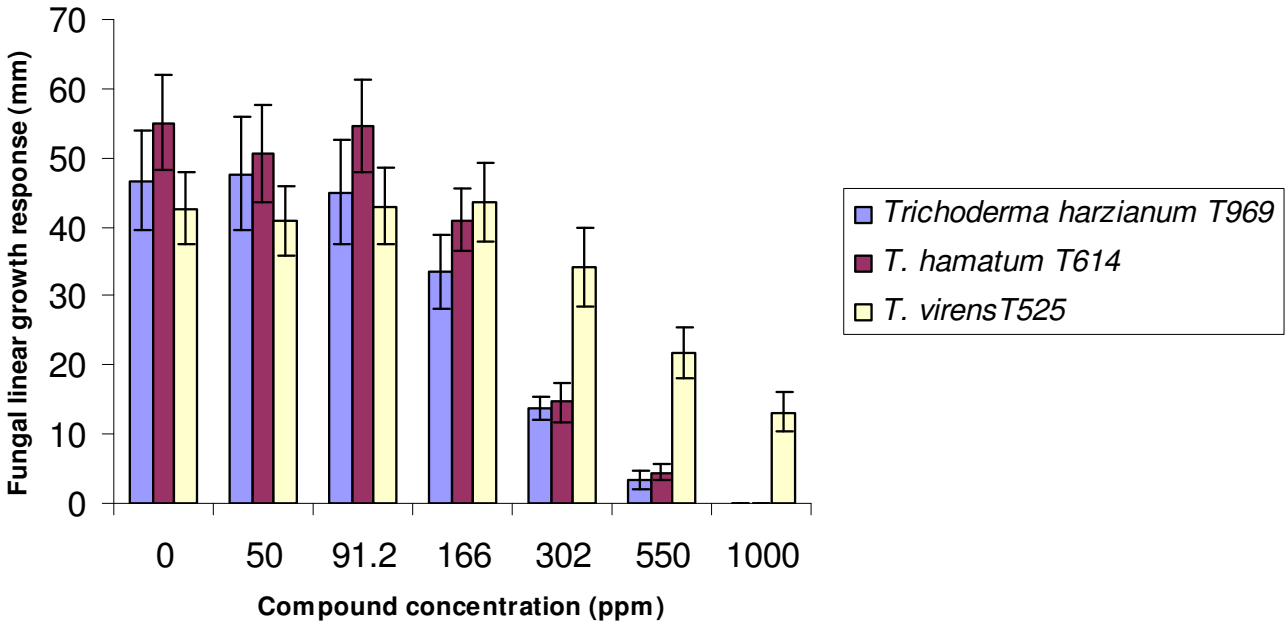

Figure 4. Radial growth response of *T. harzianum* T969, *T. hamatum* T614 and *T. virens* T525 on potato dextrose agar (PDA) medium amended with 0, 50, 91.2, 166, 302, 550 and 1000 mg/l (ppm) of $ZnSO_4.7H_2O$. Bars represent standard errors (SE).

89.65 and 85.31% inhibition in 1000 mg/l concentration for *T. harzianum*, *T. hamatum* and *T. virens*, respectively (Figure 6).

The inhibitory effect of $MnSO_4 .H_2O$ on the mycelia growth of *T. harzianum* and *T. hamatum* were observed from 166 mg/l concentration of the component, with a 50% reduction in radial growth rate at 219.25 and 343.46 mg/l concentration of $MnSO_4.H_2O$ for *T. harzianum* and *T. hamatum*, respectively. A different scenario was observed in *T. virens* response when grown in $MnSO_4.H_2O$ supplemented medium without any problem, and exhibited tolerance to Mn^{2+} (Figure 7).

For $CoCl_2.6H_2O$, sharp ($\rho \leq 0.01$) decreases were recorded at 91.2, 166, and 302 mg/l concentration of

Table 4; EC_{50}% (mg/l) value of metal-containing compounds on the fungi

Treatment	Metal contain compounds			
	$CuSO_4$	$ZnSO_4 .7H_2O$	$MnSO_4.H_2O$	$CoCl_2.6H_2O$
***Trichoderma harzianum* T969**	118.65 (110.10-127.82)*	229.12 (204.33-252.86)	219.25 (198.39-242.03)	134.5
***T. hamatum* T614**	87.68 (81.71-94.02)	267.47	343.46 (243.15-497.32)	154.16 (134.6-178.08)
***T. virens* T525**	96.3 (90.28-102.8)	609.65 (532.49-707.65)	-	258.16 (189.31-366.75)

Number in the parentheses indicates 95%confidence limits determined by probit analysis.

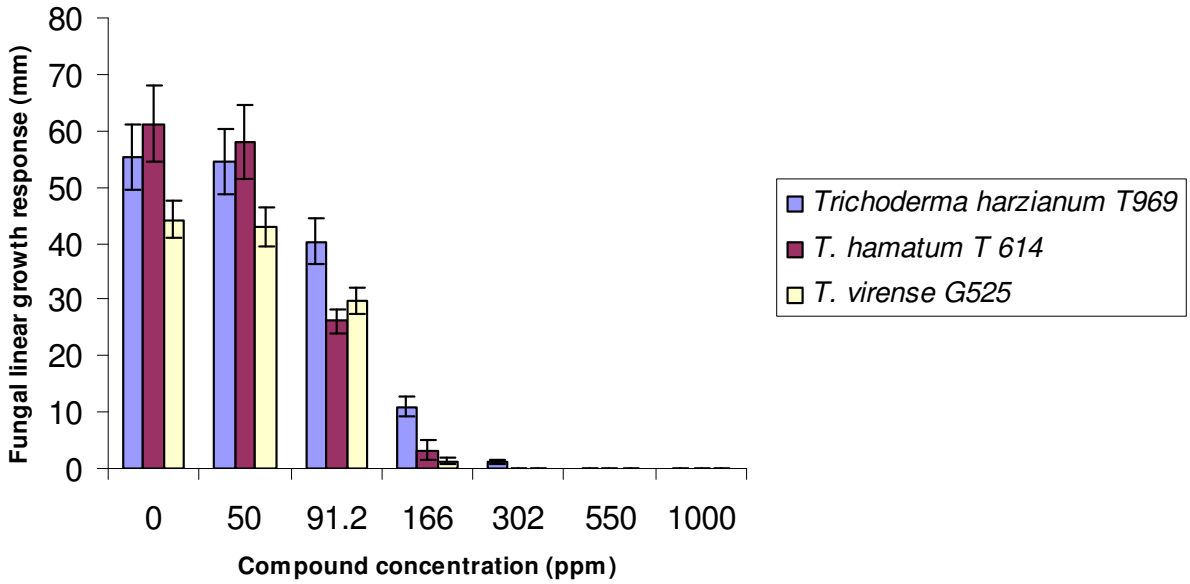

Figure 5. Radial growth response of *T. harzianum* T969, *T. hamatum* T614 and *T. virens* T525 on potato dextrose agar (PDA) medium amended with 0, 50, 91.2, 166, 302, 550 and 1000 mg/l (ppm) of $CuSO_4$. Bars represent standard errors (SE).

$CoCl_2.6H_2O$ among all tested species. *T. hamatum* did not grow at 302 mg/l concentration of $CoCl_2.6H_2O$ as well as *T. hamatum* did not proliferate at 550 and 1000 mg/l of $CoCl_2.6H_2O$. In contrast *T. virens* grew even at 1000 mg/l content of the compound (Figure 8). *T. harzianum* was more sensitive than *T. hamatum* (134.5 mg/l recorded EC_{50} for *T. harzianum* rather than 154.16 mg/l recorded for *T. hamatum*). *T. virens* was more tolerant than the other two isolates to $CoCl_2.6H_2O$.

DISSCUSION

In agricultural systems, metal-containing compounds are increasingly released from pesticides and chemical fertilizers as well as wastewater mixed industrial effluents into the soil. However, some minerals have important roles in organisms' growth and development, they may lead to toxicity at higher concentration via blocking, denaturing and inactivation of some biologically important molecules such as proteins and enzymes as well as disrupting cellular membrane intensity, especially for non-biological function metals such as mercury (Ochiai, 1987), because metals play important physiological and structural roles in metalloenzymes and in increasing membrane stability (Errasquin and Vazquez, 2003). Also, some minerals play major roles in plant growth and development as well as plant defense response against a wide range of biotic and abiotic stress at optimal concentration. Growing awareness of metal pollution in the soil has raised the need to study alternatives such as *Trichoderma* spp. as bio-pesticide, bio-fertilizer and bio-sorbent as well as bioremediation/bioaccumulation agent especially in agricultural soil on industrial sites. On the other hand, application of *Trichoderma* with some metal-containing pesticides and/or chemical fertilizer raises the

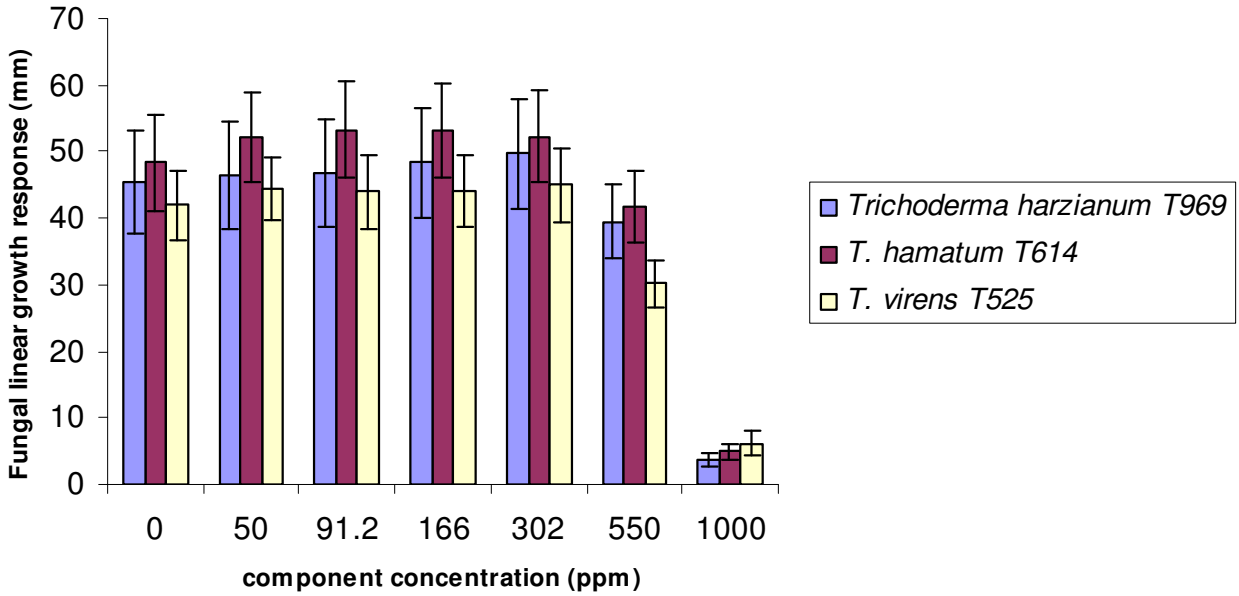

Figure 6. Radial growth response of *T. harzianum* T969, *T. hamatum* T614 and *T. virens* T525 on potato dextrose agar (PDA) medium amended with 0, 50, 91.2, 166, 302, 550 and 1000 mg/l (ppm) of $FeSO_4.7H_2O$. Bars represent standard errors (SE).

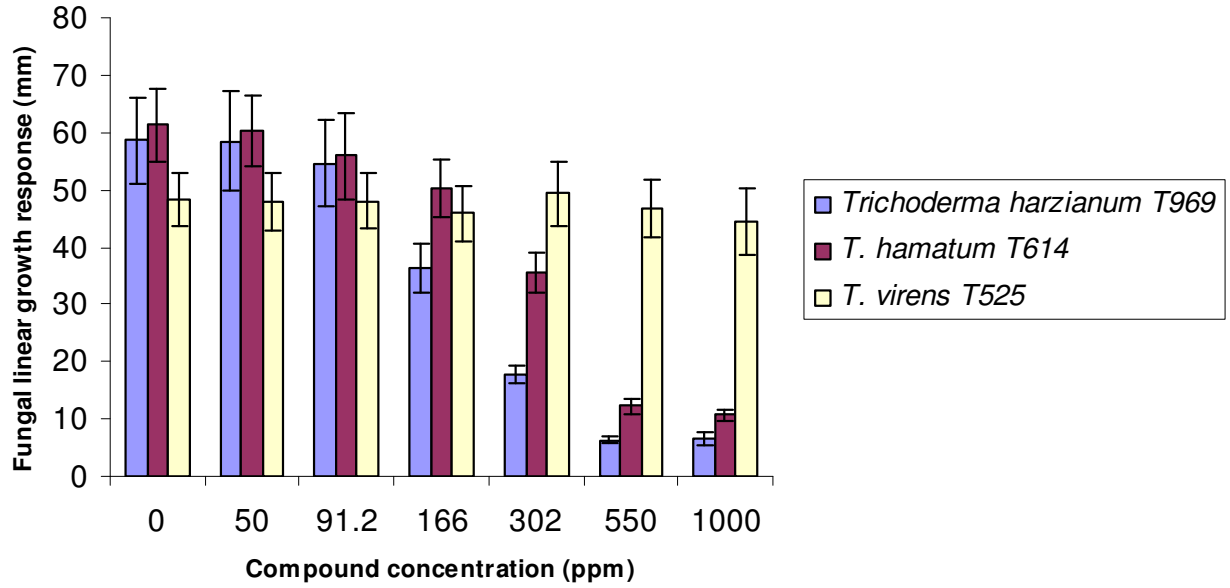

Figure 7. Radial growth response of *T. harzianum* T969, *T. hamatum* T614 and *T. virens* T525 on potato dextrose agar (PDA) medium amended with 0, 50, 91.2, 166, 302, 550 and 1000 mg/l (ppm) of $MnSO_4.H_2O$. Bars represent standard errors (SE).

need for detailed studies.

According to the obtained results, linear extension of isolates in plate culture revealed that the effect of metal-containing compounds on linear growth of the fungus depended on the compound and its concentration as well as the fungus species/isolates. The most toxic metal-containing compound against mycelia growth of the isolates was $CuSO_4$ with the least concentration for 50% reduction in radial growth of the species. The inhibitory effect of $CuSO_4$ on the growth of *Trichoderma* in general, is in agreement with Kucuk et al. (2008).

The species shows high tolerance to $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $CoCl_2.6H_2O$ and $CaCl_2.2H2O$. In spite of the *T. harzianum* and *T. hamatum*, *T. virens* was able to survive at higher concentration of $ZnSO_4.7H_2O$ and $CoCl_2.6H_2O$. *T. virens* expanded in $MnSO_4.H_2O$ supple-

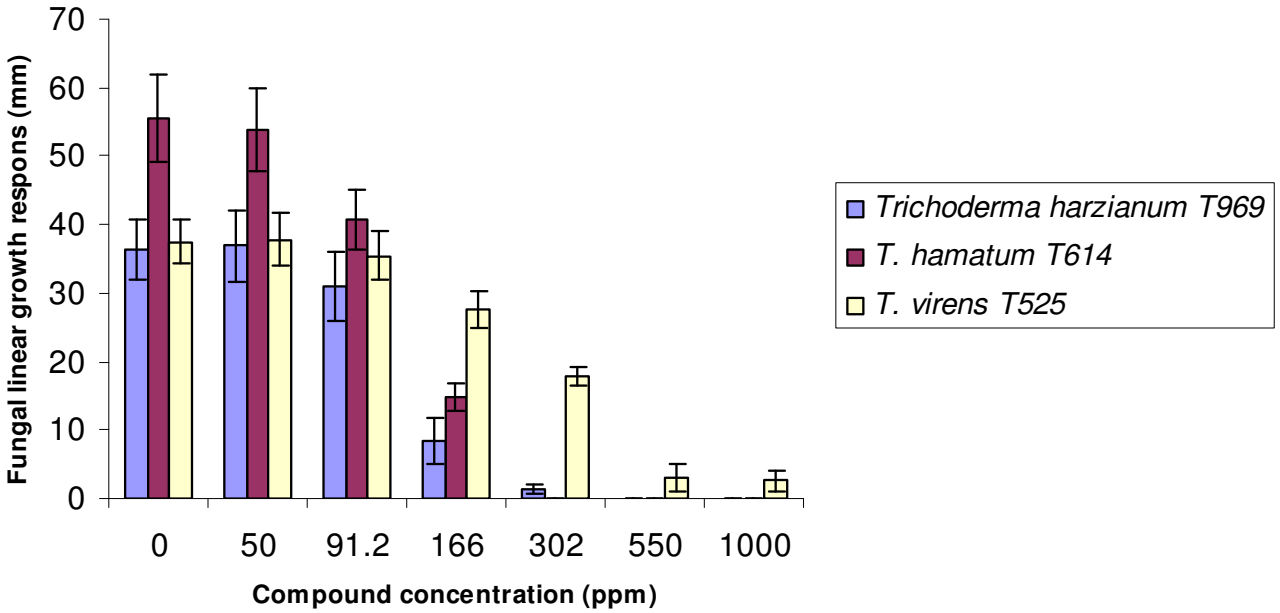

Figure 8. Radial growth response of *T. harzianum* T969, *T. hamatum* T614 and *T. virens* T525 on potato dextrose agar (PDA) medium amended with 0, 50, 91.2, 166, 302, 550 and 1000 mg/l (ppm) of $CoCl_2.6H_2O$. Bars represent standard errors (SE).

mented medium without any problem and exhibited a tolerance to Mn^{2+}. Jaworska and Dluzniewska (2007) similarly reported that Mn^{2+} unaffected *T. viride* mycelia growth at 800 mg/l whereas *T. harzianum* and *T. pseudokoningii* mycelia growth rate significantly decreased with increasing Mn^{2+} content from 200 to 600 mg/l.

In this study, the effects of metal-containing compound on the isolates growth response were studied individually. Therefore, it is difficult to extrapolate from *in vitro* observation to the soil environment. In the natural ecosystem, the fungi may encounter not one but more soluble and/or non-soluble metal-containing compound and the response of species/isolates in exposure to these multiple compounds due to additive, additional synergism or antagonistic interaction, may however be different (Kucuk et al., 2008; Errasquin and Vazquez, 2003). In this regard, Errasquin and Vazquez (2003) demonstrated that the toxicity of zinc combined with cadmium, on *T. atroviride* was greater than the sum of the inhibition effect of the individual metals. Evidence also suggested that the presence of metals except mercury that inhibit strongly all enzyme activities, did not influence enzymes especially those involved in mycoparasitism (Kredics et al., 2001a, b), therefore metal resistant *Trichoderma* strains with biocontrol activity my be effective against soil borne phytopathogenic fungi even in metal-containing soil (Kredics et al., 2003). However, the growth response of biocontrol agents and their biocontrol capability nature is dependent not only on the results of chemical condition, but also on more factors as the environmental physical and biological factors. In the meantime, further experiments should be performed to better understand the effect of environmental factors on growth response and development of biocontrol isolates in natural, even where biocontrol agents were applied combined with metal-containing pesticides and chemical fertilizers in the frame of a complex integrated pest management and sustainable agricultural system.

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