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Effect of Pollution with Certain Heavy Metals on the Growth of the Nematophagous Fungus, *Arthrobotrys Oligospora,* Trap Formation, Root-knot Nematode Infection and Enzymes Production

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Abstract

In vitro studies revealed that there was an inversely correlation between metal concentration and mycelial growth. Cadmium on was the most toxic metals. ?However, zinc 10 ppm was the only metal that had stimulatory effect on mycelial growth. Results also showed that maximum number of traps produced by the fungus was performed with zinc 600 ppm and manganese 200 ppm. However, trap formation was greatly inhibited with all concentrations of cadmium except 0.1 ppm. Laboratory tests also revealed that the sensitivity of fungal growth to all tested metals was higher in broth than on solid media. Pot experiment showed that the four tested metals had stimulatory effect on shoot length and root weight of sunflower free of nematode infection. Single application of *A. oligospora* caused significant reduction in number of galls and egg masses produced by *M. incognita* besides a remarkable increase in enzymes activity. The effectiveness of such metals on protease activity was more pronounced with zinc or manganese. Application of zinc or manganese singly or in combination with *A. oligospora* caused significant reduction in number of galls as well as egg masses . Better suppression was performed in concomitant treatment. Moreover, fresh shoot and root weight of sunflower infected with *M. incognita* was greatly improved following the application of fungus plus either zinc 600 ppm or 1000 ppm with total plant fresh weight 18.50 and 16.58 respectively. On the other hand pots receiving fungus alone or in combination with cadmium or lead showed non significant difference in number of galls and egg masses. The inhibitory effect of the tested metal ions on enzyme production was less pronounced *in vivo* than *in vitro.* In addition application of such metals at higher concentrations showed no phytotoxicity in sunflower plant.

Introduction

Heavy metals are found naturally in the soil or can be introduced from such diverse sources as fossil fuel combustion, industrial processes, sewage treatment effluents, and applications of pesticides, fungicides and nematicides (Bohn, 1972 and Babich & Stotzky, 1979). Some metal ions are essential, *e.g.,* they are required for normal cellular growth, while others are non-essential and they fulfill no known cellular role (Nieboer and Richardson, 1990). So, the sensitivity of soil microorganisms to different heavy metals vary according to both individual metal ion and its relative concentrations (Zibilske & Wagner, 1982). Most studies of the toxicology of heavy metals have focused on human beings and plants, however little attention has been paid to the possibility that the presence of metals might be affecting the soil microorganisms life on which the majority of plants and animals directly or indirectly depend. The nematode trapping fungi are considered facultative parasites that produce traps only under special conditions *e.g.,* traps are induced by nematodes, partially decomposed organic matter, and microbial competition (Jaffee *et al.,* 1992). *Arthrobotrys oligospora* Fres. is one of the most common nematode-trapping species, capturing nematodes in traps of adhesive network type. During the infection process, the nematode cuticle, which mainly consists of proteins, is lysed at the point of contact and penetrated by a hypha. The nematode is immobilized, and the prey is finally invaded and digested by the fungus (Dijksterhuis *et al.,* 1994).

Previous researches have indicated that the nematophagous fungus, *Arthrobotrys oligospora* effectively controlled root-knot nematodes under laboratory and green house conditions (Matskevich *et al.,* 1991; Duponnois *et al.,* 1995; Dias & Ferraz, 1994; Arndt & Leuprecht, 1994 and Colombo *et al.,* 1995). The effect of certain heavy metals on the development, population densities and nematode reproduction has been studied by many investigators (Turlygina, 1979; Kobamota & Izumi, 1984; Mondal & Miah, 1984 and Mostafa & Amin, 1991). However, little attention has given to the effectiveness of heavy metals pollutants on the predaceous or parasitic activity of the nematophagous fungi and enzyme production (Rosenzweig & Pramer, 1980; Lopez-Llorca *et al.,* 1996; Parveen & Alam, 1997).

Therefore, the present study was conducted to determine 1) the effectiveness of the following metals; cadmium, lead, manganese, and zinc at different concentrations on the mycelial growth of the nematophagous fungus, *A. oligospora* as well as trap formation produced under laboratory conditions; 2) the influence of two selected concentrations of each metal on the fungus activity in broth; 3) the effectiveness of the previous metals at two selected concentrations, on the predaceous activity of *A. oligospora* and the resulting effect on the development of the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood as well as sunflower plant growth, 4) the

influence of heavy metals on chitinase and protease produced by *A. oligospora* in soil under greenhouse conditions

Materials and Methods

Laboratory experiment: Effect of heavy metals on mycelial growth and trap formation of A. oligospora on corn meal agar. An isolate of the nematophagous fungus, *A. oligospora*, (Amin & Budai, 1993) obtained from Szentes, Hungary was cultured and maintained on potato dextrose agar (PDA). The fungus was grown on PDA plates and after incubation for 7 days at 28°C, circular plugs (5 mm in diameter) made with a sterile metal cork borer were transferred (with the fungal growth up; growth side down; one plug per dish) to the center of Petri dishes (10 cm in diameter) containing fixed amount of quarter strength corn meal agar (CMA) unamended (as control) or amended with (μg/ml) Cd as $CdCl_2$ (0.1, 0.5, 1, 5, 10, 50, 100), Mn as $MnCl_2$ (50, 100, 200, 400, 800, 1000), Pb as $Pb(NO_3)_2$ (100, 200, 400, 600, 800, 1000) or Zn as ZnSO4 (10, 50, 100, 200, 400, 600, 800, 1000). The pH of the medium was adjusted, after addition of the heavy metals, to 6.0 with either 1N HCl or 1N NaOH. There were six replicates dishes for each concentration. In order to determine the influence of the above metals, the diameters of fungal colony (in four directions) were measured in millimeters after 7 days incubation at 28°C. Three petri dishes were used for each concentration of each metal and data are expressed as percentages of the control (free of heavy metal).

However, the other three replicate dishes containing *A. oligospora* (7 days old) were inoculated with approximately one hundred individuals of second stage juveniles (J2) of *M. incognita*. After 48 h, Petri dishes were examined for number of networks (traps) formed by *A. oligospora* under a light microscope. The experiments were repeated twice.

Effect of heavy metals on growth and enzymes production of *A. oligospora* in corn meal broth cultivation was made in 500 ml Erlenmeyer flasks each containing 100 ml of sterilized corn meal broth unamended or amended with selected concentrations of each heavy metal salt. Each flask was inoculated with spore suspension obtained from 7 -days old culture (10^6 spores/ml, final concentration), then incubated at 28°C. The fungal mats were harvested on the 7th day of growth by filtration, washed thoroughly with distilled water, and oven dried at 80°C to a constant weight. The centrifuged clear supernatant were used for enzyme assays.

Greenhouse experiment: Influence of heavy metals on growth of sunflower infected with root-knot nematode, *M. incognita,* predaceous activity and enzymes produced by *A. oligospora.* Seeds of sunflower, *Helianthus annus* L. cv. Vidoc -5were sown in plastic pots 10 cm diameter filled with steam sterilized sandy loam soil 1:1. Immediately after planting, *A. oligospora* inoculum (9 X 10^6 spores/ pot) and the tested four metal ions (cadmium, lead, manganese, and zinc) in their salt solutions were added separately to pots. Two weeks later, nematode inoculum consisted of 1000 second stage juveniles (J2) were added. Treatments were as follows; 1. Plant treated with metal alone; 2. Plant treated with fungus alone; 3. Plant treated with metal + fungus, 4. Plant treated with nematode, 5. Plant treated with nematode + metal, 6. Plant treated with nematode + fungus, 7. Plant treated with nematode + metal + fungus, and 8. Untreated plant. Two selected concentrations were used for each metal. Each treatment was replicated four times and plants were randomly arranged in a greenhouse bench. Plants were irrigated as needed. The experiment was terminated 45 days after nematode infestation. Plants were removed from pots and data dealing with shoot length and fresh shoot and root weight were recorded. The predaceous activity of *A. oligospora* was determined in terms of total number of galls and egg masses per root system. Data were subjected to analysis of variance (ANOVA). The enzyme activities were determined using 10 g of air-dried soil samples treated with 1.5ml toluene for 15 min.

Enzyme Assays: The enzyme activities whether in broth or soil were determined as follows:

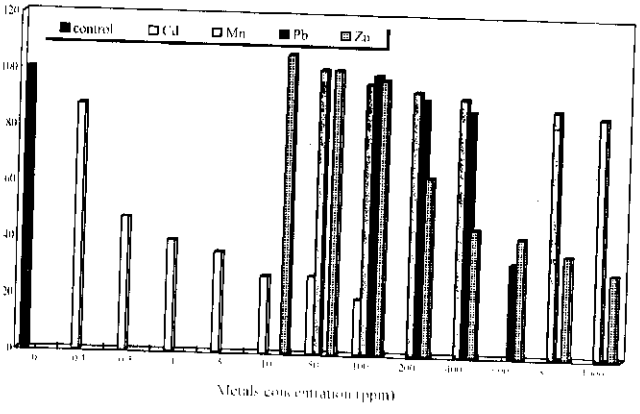
Chitinase activity: Chitinase activity was determined by incubating appropriate amount of enzyme source with 1% (w/v) colloidal chitin, prepared by solubilizing in concentrated HCl as described by Godoy *et al.* (1982), in 0.1 M acetate buffer, pH 5.0. The mixture was shaken and incubated at 35°C for 1 h. After incubation, the reaction mixture was diluted (soil samples) and centrifuged (20 min, 4000g). N-acetyl-glucosamine (NAGA) released was determined according to Aminoff *et al.,* (1952). One unit of chitinase activity (U) was expressed as the amount of enzyme liberated 1μmol of NAGA per ml of culture filtrate per hour.

Protease activity: Protease activity was measured by the method described by Chopra and Mathur (1983). Enzyme activity was determined by adding appropriate amount of enzyme source to 1% prepared casein (Hammerstein quality) in 0.1 M acetate buffer, pH 5.0. The mixture was shaken and incubated at 37°C for 18h. After incubation 10 ml of 0.4 M trichloroacetic acid (TCA) was added to each bottle to terminate the reaction and after standing for 30 min at room temperature, the solutions were filtered. The amount of TCA-soluble casein breakdown fragments was determined. One unit of protease activity (U) was defined as the amount of enzyme releasing TCA-soluble fragments giving a blue colour equivalent to 1μg tyrosine / 1ml / min.

Results

Influence of Cd, Mn, Pb, or Zn on mycelial growth of *A. oligospora:* Growth of nematophagous fungus, *A.*

oligospora was decreased as the metal concentration increased (Fig. 1). Except a remarkable stimulation in mycelial growth was noticed with the application of the lower concentration of Zn (10 ppm). Among different concentrations of tested metal ions, cadmium was the most toxic effect to growth of *A. oligospora* on CMA medium. As mycelial growth was partially reduced (87.7% of control) at 0.1 ppm Cd and the reduction was reached to 27 per cent of control at 10 ppm Cd (Fig. 1). Only a very little inhibition of *A. oligospora* growth was noticed at 100 ppm of either Mn, Pb, or Zn separately. Although lead was completely inhibited the mycelial growth at 800 ppm, as well as the reduction reached to 36% of control by zinc, the fungal growth was only slightly reduced to 88 per cent of control in the same concentration of manganese (Fig. 1).



g. 1: Influence of different concentrations of some heavy metals on *A. oligospora*

luence of Cd, Mn, Pb, or Zn on trap formation produced the nematophagous fungi, A. oligospora: Fig. 2 showed at after two days from root-knot nematode addition, *A. gospora* produced traps in most treatments containing MA plus metals with different concentrations. Abundant ps were observed when *A. oligospora* was grown on rn meal amended with either zinc or manganese. ximum number of traps as well as numerous trapped matodes was performed with zinc 600 ppm and nganese 200 ppm with percentage of control 321.0 and 7.0 respectively (Fig.2 & 3). However, lower number of ps was produced by *A. oligospora* grown on CMA dium amended with lead at both 100 ppm and 200 ppm h percentage of control 37.1, 52.9 respectively. On the er hand, neither mycelial growth nor trap formation was erved with lead either at 800 or 1000 ppm. With pect to cadmium, trap formation was greatly inhibited with all concentrations used except 0.1 ppm by which number of traps reached 82.19 per cent of control. Two selected concentrations of the four tested metal ions, that have a pronounced effect (inhibitory or stimulatory) on the fungal growth and trap formation (Fig. 1&2) were examined for enzyme activity in broth and greenhouse experiment.

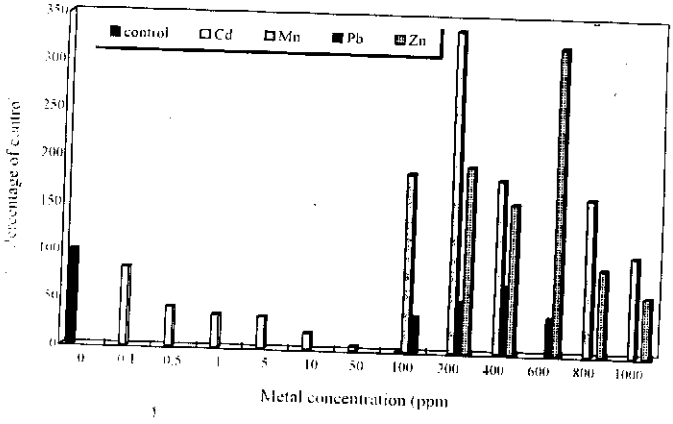


Fig. 2: Influence of different concentration of heavy metals on trap formation of *A. oligospora.*

The effect of Cd, Mn, Pb, or Zn on A. oligospora activity in broth: The nematophagous fungus, *A.oligospora* was grown in corn meal broth medium in the presence of Cd, Mn, Pb, or Zn at two selected concentrations. Data represented in Table 1 showed that the effect of studied metal ions on the mycelial dry weight of *A. oligospora* was in the same direction comparing to their effect on the fungal growth on solid medium. Moreover, the toxicity tested metal ions in broth was higher than on solid medium. For instance, the reduction of mycelial growth on CMA medium was 87.7; 91.9; 87.5 per cent of control (Fig. 1), however, the inhibitory effect reached to 76; 74.4; 66.7 per cent of control (Table 1) in corn meal broth medium amended with Cd (0.1pp); Mn (400 ppm); or Pb (400) respectively. The reduction of filtrates' pH value ranged from 0.57 to 1.23 in the presence of low concentration of Cd or Zn respectively. Data represented in Table 1 showed that *A. oligospora* produced extracellular protease and chitinase when grown in liquid culture (Tunlid & Janson, 1991). The concentration of cadmium (50 ppm) and lead (800 ppm) at which fungal growth reduction occurred, had strong inhibitory effect on protease (13.4 & 11.5 % of control respectively) and chitinase (30.3 & 18.2% of control respectively) production. However, manganese ions had slight inhibitory effect on both enzymes activity at 200 ppm by which was not toxic to the fungal activity. Protease and chitinase activity as well as fungal growth was moderately reduced in the presence of zinc.

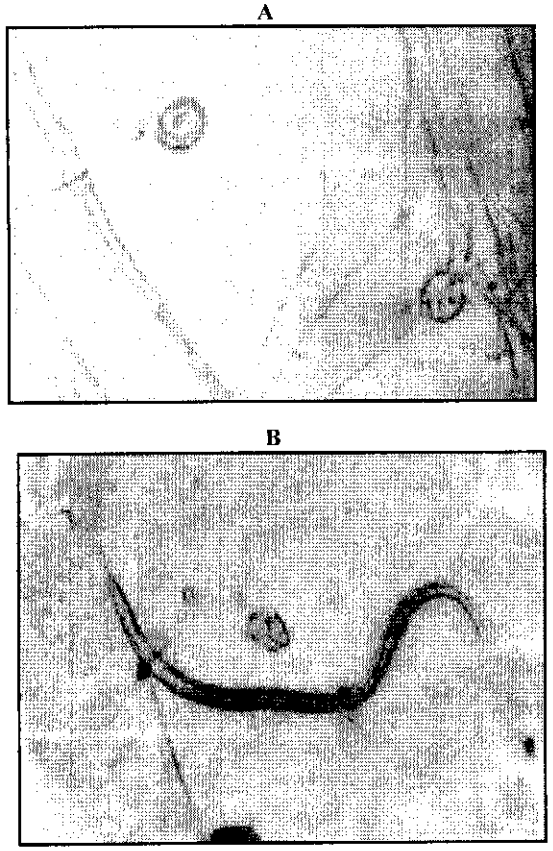


Fig. 3: Light microscopy graphs illustrating (A) vegetative mycelium of *A. oligospora* formed adhesive, networks (traps) on CMA medium, 48h followed initiation of infection, (B) Second –stage juvenile of *M. incognita* captured into fungal traps .

Influence of Cd, Pb, Mn, and Zn on growth of sunflower infected with root-knot nematode, M. incognita, predaceous activity and enzymes produced by *A. oligospora:* Data presented in Tables 2 and 3 indicated that both of cadmium and lead at two tested concentrations, caused an obvious improvement in both fresh shoot and root growth of sunflower plants free of nematode infestation with total plant fresh weight (13.96 & 14.04) and (14.68 & 13.77) respectively. Although the introduction of either lead or cadmium to plant infected with nematode caused a remarkable enhancement in shoot and root weight, number of galls as well as egg masses were not significantly reduced as compared to untreated plants. In general, data showed that the presence of *A. oligospora* caused significant reduction in number of galls and egg masses as compared with nematode alone. In addition protease and chitinase activity was more pronounced in pots receiving nematode and fungus than those receiving fungus only (Tables 2-5). However, the predaceous activity of *A. oligospora* in terms of number of galls or egg masses were not significantly affected by cadmium or lead at two tested concentrations under greenhouse conditions. Therefore, non-significant difference was noticed in pots receiving fungus alone or in combined with metal ion. The addition of either cadmium (50 ppm) or lead (800 ppm) had slightly inhibitory effect on the protease activity, however, moderately inhibition was performed in the presence of nematode. As for chitinase activity, both cadmium and lead had inhibitory effect (Tables 2 & 3).

From Table (4 & 5) it was evident that manganese or zinc element at two tested concentrations had stimulatory effect on shoot length and root weight of sunflower plants free of nematode infestation as compared with untreated plants. In Table 4 plants infected with nematodes showed better performance in fresh shoot length and weight when manganese was introduced to soil at 400 ppm singly or in combination with *A. oligospora* with total plant fresh weight 13.08 and 13.18 respectively. However, number of galls as well as number of egg masses was significantly decreased in all treatments receiving manganese or fungus alone or in combination. Better suppression was recorded in concomitant treatments.

Data presented in Table 5 pointed out that the addition of fungus with either zinc 600 or 1000 ppm to sunflower plants free of nematode infestation caused an obvious promotion in both shoot and root weight with total plant fresh weight 16.77, 15.76 respectively. The addition of zinc at two tested concentrations to sunflower plants infected with *M. incognita* showed significant increase in shoot length, as compared with nematode alone. On the other hand, both fresh shoot and root weight of sunflower infected with nematode were greatly enhanced following the concomitant treatment of fungus with either zinc 600 ppm or 1000 ppm with total plant fresh weight 18.50 and 16.58 respectively. Results also showed that gll formation was significantly decreased in pots receiving zinc or fungus alone or in combination (Table 5). Greater suppression was performed in pots receiving the combination of fungus and zinc followed by those receiving fungus then zinc. However, non-significant difference was noticed in pots receiving fungus alone or in combination with zinc. Similar trend was observed with number of egg masses. On the other hand, the effectiveness of either manganese or zinc on protease production were more pronounced in pots receiving fungus alone than those receiving fungus and nematode, however, chitinase activity was reduced in both

Discussion

The mycelial growth performed by the nematophagous fungus, *A. oligospora*, varies with different degree according to the type of metal and concentration used. In general, it was obvious that the rate of the fungal growth was directly correlated with metal concentration. The present results showed that among the tested metal ions cadmium has the most toxic effect on the mycelial growth of *A. oligospora*. These results are in accordance

Table 1: The influence of metal ions on *A. oligospora* growth and enzymes production in broth after 7 days.

Treatment	ppm	pH	Biomass mg D.W.	Enzyme activity (U)ppm	
				Protease	Chitinase
Control	0	5.51	90.1	14.28	0.33
Cd	0.1	5.93	68.6	10.48	0.11
	50	5.45	14.4	1.92	0.10
Mn	200	5.33	83.5	13.52	0.23
	400	5.30	67.0	10.12	0.21
Pb	400	5.72	60.1	9.24	0.02
	800	5.69	10.3	1.64	0.06
Zn	600	5.63	30.2	6.96	0.15
	1000	5.27	20.4	6.72	0.12

Table 2: Effect of cadmium ions on the growth of sunflower, *M. incognita* development and enzymes produced by *A. oligospora*

Treatments	Fresh shoot Length (cm)	Fresh shoot Weight (g)	Fresh root Weight (g)	Total plant fresh weight (g)	Number of Galls	Number of egg masses	Enzyme activity (U) Protease	Enzyme activity (U) Chitinase
Untreated	64.0	9.33	3.06	12.39				
Cd*	73.3	9.74	4.22	13.96			0.44	0.005
Cd**	75.5	8.64	5.40	14.04				
Fungus (F)	61.5	8.66	4.82	13.48				
Cd* + F	61.8	7.91	5.62	13.53			2.44	0.200
Cd** + F	58.5	6.93	4.46	11.39			2.44	0.080
Nematode (N)	55.5	7.23	4.35	11.58	43.0	36.0	2.28	0.070
Cd* + N	74.5	9.65	4.26	13.91	43.0	37.0		
Cd** + N	65.0	9.27	5.29	14.57	44.3	36.0		
F + N	62.6	7.91	4.78	12.69	17.5	8.0	3.64	0.250
Cd* + F + N	60.3	7.18	5.91	13.09	11.0	5.0	3.00	0.060
Cd** + F + N	54.6	7.31	4.21	11.52	10.0	8.0	2.24	0.060
$LSD_{0.05}$	NS	NS	NS		8.62	7.04		

*mention to 0.1 ppm Cd; **mention to 50 ppm Cd; Each number presented the mean of four replicates

Table 3: Effect of lead ions on the growth of sunflower, *M. incognita* development and enzymes produced by *A. oligospora*

Treatments	Fresh shoot Length (cm)	Fresh shoot Weight (g)	Fresh root Weight (g)	Total plant fresh weight (g)	Number of Galls	Number of egg masses	Enzyme activity (U) Protease	Enzyme activity (U) Chitinase
Untreated	64.0	9.33	3.06	12.39				
Pb*	77.0	10.50	4.18	14.68			0.44	0.005
Pb**	74.8	9.25	4.52	13.77				
Fungus (F)	61.5	8.66	4.82	13.48				
Pb* + F	60.5	8.50	3.36	11.86			2.44	0.20
Pb** + F	50.0	8.03	5.89	13.92			2.44	0.09
Nematode (N)	55.5	7.23	4.35	11.58	43.0	36.0	2.00	0.08
Pb* + N	71.0	11.90	6.87	18.77	49.8	49.0		
Pb** + N	64.0	10.30	5.54	15.84	45.5	44.0		
F + N	62.6	7.91	4.78	12.69	13.5	12.0	3.64	0.25
Pb* + F + N	51.0	6.19	4.68	10.87	20.0	10.0	2.64	0.06
Pb** + F + N	55.0	6.23	3.69	9.92	30.0	20.0	2.48	0.05
LSD	10.26	0.96	2.22		8.26	7.04		

*mention to Pb 400 ppm; ** mention to Pb 800 ppm; Each number presented the mean of four replicates

Table 4: Effect of manganese ions on the growth of sunflower, *M. incognita* development and enzymes produced by *A. oligospora*

Treatments	Fresh shoot Length (cm)	Fresh shoot Weight (g)	Fresh root Weight (g)	Total plant fresh weight (g)	Number of Galls	Number of egg masses	Enzyme activity (U) Protease	Enzyme activity (U) Chitinase
Untreated	64.00	9.33	3.06	12.39			0.44	0.005
Mn*	71.63	8.34	3.97	12.31				
Mn**	69.87	8.93	4.58	13.51				
Fungus (F)	61.5	8.66	4.82	13.48			2.44	0.200
Mn* + F	64.0	8.20	4.36	12.56			2.92	0.130
Mn** + F	63.5	7.88	4.72	12.60			3.56	0.130
Nematode (N)	55.5	7.23	4.35	11.58	43.0	36.00		
Mn* + N	67.6	8.16	3.95	12.11	30.5	18.00		
Mn** + N	61.0	9.30	3.78	13.08	25.0	27.00		
F + N	62.9	7.91	4.78	12.69	17.5	8.0	3.64	0.250
Mn* + F + N	61.5	7.44	4.56	12.00	5.0	1.0	2.48	0.140
Mn** + F + N	65.0	8.26	4.92	13.18	7.5	1.0	1.96	0.140
$LSD_{0.05}$	7.98	1.00	1.59		8.62	7.04		

* mention to Mn 200 ppm; ** mention to Mn 400 ppm; Each number presented the mean of four replicates

Table 5: Effect of zinc ions on the growth of sunflower, *M. incognita* development and enzymes produced by *A. oligospora*

Treatments	Fresh shoot Length (cm)	Fresh shoot Weight (g)	Fresh root Weight (g)	Total plant fresh weight (g)	Number of Galls	Number of egg masses	Enzyme activity (U) Protease	Enzyme activity (U) Chitinase
Untreated	64.0	9.33	3.06	12.39			0.44	0.005
Zn*	70.3	8.51	4.73	13.24				
Zn**	67.0	8.31	4.38	12.69				
Fungus (F)	61.5	8.66	4.82	13.48			2.44	0.200
Zn* + F	63.5	10.44	6.33	16.77			2.60	0.150
Zn** + F	64.5	8.51	7.25	15.76			4.24	0.150
Nematode (N)	55.5	7.23	4.35	11.58	43.0	36.00		
Zn* + N	66.5	8.69	3.43	12.12	22.5	11.00		
Zn** + N	70.1	8.42	4.70	13.12	14.0	9.00		
F + N	62.6	7.91	4.78	12.69	17.5	8.0	3.64	0.250
Zn* + F + N	66.0	9.27	9.23	18.50	7.5	5.0	2.68	0.140
Zn** + F + N	61.0	8.07	8.51	16.58	10.0	3.0	2.04	0.140
$LSD_{0.05}$	7.14	NS	NS		8.62	7.04		

* mention to Zn 600 ppm; ** mention to Zn 1000 ppm; Each number presented the mean of four replicates.

findings reported by Rosnzweig & Pramer (1980) and Lopez-Llorca *et al.* (1996). Cadmium had the most significant effect on reduction of the growth rate of the nematophagous fungus, *Verticillium suchlasporium* (Lopez-Llorca *et al.*, 1996). However, cadmium at a concentration of 1 ppm significantly inhibited growth of *A. oligospora* (Rosenweig & Pramer, 1980). In addition, Babich and Stotzky (1977) were found that *Botrytis allii, B. cinerea, Penicillium vermiculatum, Aspergillus fischeri, A. giganteus* were capable of growth in the presence of up to 10 ppm of Cd and inhibited by 100 ppm. On the other hand, slight inhibition of fungal growth was noticed at 100 ppm of lead. However, the mycelial growth of some phylloplane fungi, e.g. *Rhizoctonia solani, A. giganteus, Fusarium solani,* and *Trichoderma viridi* was inhibited by lead at comparable concentration (Babich and Stotzky, 1979). Stimulation in *A. oligospora* growth was noticed with zinc at10 ppm. As Zn is essential for the growth of several microorganisms including fungi, structure and function of many enzymes, nucleic acid metabolism, as well as cell division, and also the production of secondary metabolites (Failla and Niehaus, 1986). Moreover, the maximum used concentration of manganese was 1000 ppm by which the growth of *A. oligospora* partially reduced (86% of control). However, Metwally and Abou Zeid (1996) found that the growth of *A. ochraceus, A. niger* and *P. digitatum* was reduced (50%) at 400 ppm Mn, although still detectable even 1000 ppm Mn. From the present results, it could be concluded that the toxicity order of the used heavy metal ions was Cd > Pb > Zn > Mn forward *A. oligospora* growth on CMA medium.

Concerning trap formation, results showed that traps were abundantly performed with zinc (600 ppm) and manganese (200 ppm) with percentage of control 321 and 337

respectively. These results support the findings reported by Rosenweig and Pramer(1980) in respect to zinc. They mentioned that in most treatments the enhancement of growth of *A. oligospora* was directly correlated with the increase in the capacity of fungus to form traps and capture nematodes and vice versa. Although slight inhibition in mycelial growth was performed with lead at 100 and 200 ppm, lower number of traps produced by *A. oligospora* was noticed. This result is in accordance with that reported by Rosenweig & Pramer (1980) who found that in a few instances, trap formation was inhibited either more or less than was growth. For example, in the presence of 50 μg of zinc per ml, growth of *Monacrosporium eudermatum* was 96 + 1.4 per cent of the control, and trap formation was completely inhibited. It is worth to note that trapsformation was greatly inhibited with all concentrations of cadmium except 0.1 ppm by which number of traps reached 82.19 of control.

The sensitivity of the fungal growth of *A. oligospora*, to the used concentrations of all tested metal ions was higher in broth than on solid medium. It is well known that binding or complexation of metals to organic materials of microbiological media (e.g. agar, peptones, yeast extract) can markedly reduced toxicity of metals. But solidified agar media have proved useful in qualitative studies of morphogenetic effects of heavy metals on fungi (Gadd, 1983).

Pot experiment showed that although lead and cadmium at two tested concentrations caused a detectable improvement in sunflower plant growth, number of galls and egg masses were not significantly suppressed. This result led to the conclusion that both lead and cadmium caused plant tolerance to nematode infection. However, the single application of *A. oligospora* caused significant reduction in galls as well as egg masses and an obvious increase in enzyme activity. These results agree with Duponnois *et al.* (1995) who mentioned that the nematophagous fungus, *A. oligospora* reduced the population of *Meloidogyne* species and stimulated tomato seedling growth. These results also support the findings of Schenk *et al.* (1980) and Tunlid *et al.* (1991 & 1992) who reported that extracellular enzymes production by the nematophagous fungi is important for the infection of nematodes. Non-significant difference in number of galls as well as egg masses was noticed in pots receiving fungus alone or in combination with cadmium or lead. This result could be attributed to the encountering conditions as well as the presence of many factors govern both availability and relative toxicity of the metals such as the pH, clay minerals and organic matter content, and the nutritional status (Tuner, 1994).

Concerning manganese and zinc elements, number of galls as well as egg masses were significantly decreased in all pots receiving either metal ion or fungus alone or in combination. These results support the findings of Turlygina (1979) and Mostafa and Amin (1991) in respect to the application of metal alone. Better suppression was recorded in concomitant treatment that gave evidence to the importance of such metals for the growth of fungus and enzyme production. Moreover, an obvious promotion was recorded in both fresh shoot length and root weight of sunflower free of nematode infection. Pons (1996) reported that the average shoot length was greatly enhanced by zinc. In addition, the stimulatory effect of manganese and zinc for the protease production was reported by Premi and Cornfield (1969) who found that Mn (100 ppm) stimulated and Zinc (1000 ppm) unaffected the rate of ammonification in an alkaline sandy loam soil.

As a whole it could be concluded from pot experiment that the four tested metals (zinc, manganese, lead, and cadmium) had stimulatory effect on shoot length and root weight of sunflower free of nematode infection. However, application of such metals at higher concentrations showed no phytotoxicity in sunflower plants. Moreover, the predaceous activity of the nematophagous fungus, *A. oligospora* was not affected by the toxic metals in pot experiment as did in laboratory especially at higher concentrations. Subsequently, the inhibitory effect of ions on the enzyme production was less pronounced *in vivo*.. Finally the criteria used to assess whether levels of metals in soil are present in toxic amounts, are not universally defined and vary from different countries and land-use purposes.

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