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STUDIES ON PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL BY *ARBUSCULAR MYCORRHIZAL FUNGUS*

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Abstract

Associative and symbiotic AM fungi are common beneficial microbes of cereals plants oat (*Avena sativa*). It is frequently suggested that AM may improve nutrition, enhance HM uptake, improve disease resistance in their host plants or adaptation to various environmental stresses. AM fungi and thereby benefit plant development and growth. The mycorrhizal symbiosis becomes even more important in sustainable agricultural systems such as in heavy metals polluted soil. The principal objective of this work was to attempt using dual inoculation of AM fungi to induce heavy metal tolerance of oat plants grown under heavy metals stress. From a number of physiological indices measured in this study, microsymbiont significantly increased dry weight, root : shoot ratios, leaf number and area, plant length, leaf pigments, total carbohydrates, N and P content of infected plants as compared with non infected controls at all levels of heavy metal concentrations. Tolerance index of oat (*Avena sativa*) plants was increased in the presence of microsymbionts than in their absence in polluted soil. Microsymbionts dependencies of oat (*Avena sativa*) plants tended to be increased at higher levels of Zn and Cd in polluted soil. Metals accumulated by microsymbionts-infected oat (*Avena sativa*) plant were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance widely exists in them. This study provides evidence for benefits of AM fungi in the protection of host plants against the detrimental effects of heavy metals. If so, AM-oat (*Avena sativa*) symbiosis could be a new approach to increase the heavy metal tolerance of cereals plants under heavy metal polluted soil.

Keywords: - Arbuscular Mycorrhiza, oat (*Avena sativa*), heavy metals.

Introduction

Amongst a number of burning problems the modern world is facing today, one is the pollution of water bodies – heavy metals being one of the major pollutants. The metals which have specific weights more than five gram per cm^3 are termed as "heavy metals". These include Al, Co, Cd, Cu, Pb, Zn, Ni, Mn, Fe, Hg, Se, As, Cr and Sn. Phytoremediation (such as phytoextraction, phytostabilization and rhizofiltration) of soils contaminated by heavy metals has been widely accepted as a cost-effective and environmental-friendly cleanup technolology. However, the progress in this field is hindered by lack of understanding of complex interactions in the rhizosphere and plant -based mechanisms which allow metal translocation and accumulation in plant (Yu et al., 2004).

Complex interactions between roots, microorganisms and fauna in the rhizosphere have a fundamental effect on metal uptake and plant growth. The arbuscular mycorrhiza (AM) fungi are important rhizospheric microorganisms. They can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan and Angus, 2003), and consequently, they increase root and shoot biomass and improve plant growth. It has been indicated that AM fungi can colonize plant roots in metal contaminated soil (Vogel-Mikus et al., 2005), while their effects on metal uptake by plants are conflicting. In slightly metal contaminated soil, most studies show that AM fungi increased shoot uptake of metals (Weinstein et al., 1995), while in severely mm sieve,

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contaminated soil, AM fungi could reduce shoot metal concentration and protect plants against harmful effects of metals (Malcova et al., 2003). Thus the benefits of mycorrhizae may be associated with metal tolerance, and also with metal plant nutrition. Therefore, in degraded and contaminated soils, that are often poor in nutrients and with low water holding capacities, mycorrhizae formation would be of great importance.

A biotechnological goal is to use a combined inoculation of selected rhizosphere microorganisms to minimize toxic effects of pollutants and to maximize plant growth and nutrition. Selected combinations of microbial inocula enhanced the positive effect achieved by each microbial group, improving plant development and tolerance in polluted soils. The application of bioinoculants like AM fungi and one of the plant growth- promoting rhizobacteria such as *Azospirillum, Agrobacteria, Rhizobium, Pseudomonas*, several Gram positive *Bacillus* species is an environment-friendly, energy efficient and economically viable approach for reclaiming wastelands and increasing biomass production. The beneficial effects of these bacteria in combination with AM fungi have been reported by a number of workers (Tain et al., 2002; Domenech et al., 2004; Rabie and Almadini, 2005). It has been reported that these bacteria may affect AM fungi and their plant host through a variety of mechanisms that include (1) effects on the receptivity of the root; (2) effects on the root-fungus recognition; (3) effects on the fungal growth; (4) modification of the chemistry of the rhizospheric soil; and (5) effects on the germination of the fungal propagules. On the other hands, other reports stated that the presence of AM fungi is known to enhance nodulation and N fixation by legumes (Amora-Lazecano et al., 1998; Johansson et al., 2004). Moreover, AM fungi and N-fixing bacteria often act synergistically on infection rate, mineral nutrition and plant growth. Although the interaction between AM fungi and N-fixing bacteria was previously reported, less attention was paid to bacteria-AM-legume tripartite symbioses under heavy metal stress. Therefore, this article is considered a good attempt to increase the heavy metal tolerance of one of the most popular legume of the world, oat (*Avena sativa*), by using dual inoculation of the AM-fungi and nitrogen -fixing bacteria under heavy metal stress conditions.

MATERIALS AND METHODS

Soil

Sandy loam soil (1:1) was air dried, passed through 2 mixed thoroughly for homogencity and sterilized by autoclaving at 121°C for 20 min to kill soil microflora. The soil is non-saline, with pH 7.9, field capacity 653 ml/kg air dry soil and has 1.35 % organic matters. The total soluble salts were 1.17 %, with total nitrogen content of about 0.89 mg/kg and phosphorus content of 0.042 mg/kg.

Seeds

Oat (*Avena sativa*) seeds were obtained from local seed supplier-National Seed Corporation (NSC), Sottiganj, Meerut, U.P.

Mycorrhizal spores: The spores of AM fungi were extracted from their cultivation and propagation pots, planted by *Zea mays*, using wet sieving and decanting method (Daniels and Skipper, 1982). The spore suspension was diluted with water, so that each ml has 55 spores. For soil inoculation, the surface soil crushed and mixed thoroughly with 15 ml spore suspension and return back to its pot.

Growth conditions

The experiment was a complete for each heavy metal used. It was comprised of 6 metal concentrations and 2 inoculation treatments with 5 replicates for each treatment. The seeds sizes and weights were homogenous. They were surface sterilized (0.1% $HgCl_2$ + 0.2% HCl for 5 min), followed by repeated washes with sterile distilled water (Vincent, 1976). Seeds were planted in plastic pots (18 cm in diameter and 13 cm in depth) loaded with 2 kg air dried sterilized sandy loam soil. The pots were arranged in randomized block. It was carried out with followed treatments: non-inoculation control and inoculation with AM spores in paired inoculum. Pots received 60 ml weekly of Hoagland's solution, minus phosphate. Twenty seeds were planted in each pot at equal intervals. Field capacity of tap water was applied per pot for irrigation. The seeds were irrigated three times a week, until the plant seedlings emerge (about 5 cm height) and thinned to ten per pot.

Analytical methods

Soil characteristics: The pH of sandy loam soil (1:1) was recorded using pH-meter (Richards, 1954) and the moisture content was calculated using an oven at 105°C (Kramer, 1983). The organic matter was estimated at 550°C using a muffle (Peach and Tracey, 1956) and the total soluble salts were determined by weight after evaporation of soil filtrate at 105°C. The field capacity was calculated by weight (Premachandra *et al.*, 1992).

Growth parameters of oat (Avena sativa): The plant height (cm), root system length (cm), leaf number and leaf area (cm^2) were measured. The fresh

and dry weights of the root and shoot systems were also determined.

Total carbohydrates: Total carbohydrate amount was estimated by anthrone colorimetric method (Yemm and Willis, 1954).

Plant pigments: Chlorophyll *a, b* and carotenoids were estimated spectrophotometrically (Arnon's, 1949), after acetone extraction of the pigments from fresh leaves.

Total nitrogen content: Nitrogen content in leaves was determined according to the method developed by Snell and Snell (1954).

Table 1. Effect of different concentrations of Fe and Pb on some parameters of oat (*Avena sativa*) in the presence and absence of AM fungi after 3 weeks of planting.

Heavy metal conc. (mg/kg dry soil)	Micro-symbiont	Dry weight (g/plant)		Leaf		Plant length (cm)	
		Total	Root: shoot ratio	No./plant	Area (cm^2)	Shoot	Root
Control	N	0.44	0.18	21.6	10.6	53.3	14.7
0.0	I	o.55	0.21	27.4	11.4	64.8	16.2
Fe	N	0.47	0.19	22.7	10.6	54.4	15.4
50	I	0.58	0.24	29.3	12.1	67.5	18.5
100	N	0.52	0.18	25.3	11.4	59.6	17.4
200	I	0.63	0.26	31.6	13.9	74.7	19.5
200	N	0.46	0.18	24.6	11.5	61.8	19.7
500	I	0.57	0.27	28.4	13.2	84.9	20.4
500	N	0.35	0.14	19.7	7.7	47.5	14.2
1000	I	0.47	0.27	25.4	9.7	74.3	16.4
1000	N	0.29	0.17	16.3	5.8	37.3	10.6
L.S.D.at 5%	I	0.45	0.18	20.3	7.9	52.7	14.7
L.S.D.at 5%		0.18	-	3.45	1.29	9.38	1.64
Pb	N	0.48	0.23	21.5	10.2	54.9	14.2
5	I	0.56	0.23	27.6	12.2	66.4	16.5
10	N	0.41	0.20	20.1	10.6	53.4	13.7
20	I	0.53	0.25	26.0	11.6	65.2	16.4
20	N	0.31	0.23	19.8	8.4	53.6	13.3
50	I	0.41	0.25	25.4	11.6	63.8	15.6
50	N	0.27	0.31	13.2	5.4	47.5	13.3
100	I	0.38	0.25	18.7	7.3	59.7	15.6
100	N	0.18	0.35	9.7	3.2	30.2	11.3
L.S.D.at 5%	I	0.29	0.32	13.9	5.5	46.2	12.7
L.S.D.at 5%		0.12	-	2.47	1.13	6.25	2.32

I: Plants inoculated by AM spores. **N**: Non-inoculated plants.

Table 2. Effect of different concentrations of Fe and Pb on oat (*Avena sativa*) inoculated with or without AM spores after 3 weeks of planting.

Heavy metal conc. (mg/kg dry soil)	Micro-symbionts	Leaf pigments (mg/g)	Total carbohydrate (mg/g dry wt.)	Total N2 (mg/g dry wt.)	Total P (mg/g dry wt.)
Control	N	8.66	202.6	18.98	1.34
0.0	I	9.67	214.4	23.15	2.65
Fe	N	9.38	205.3	19.45	2.16
50	I	10.56	222.6	25.57	2.92
100	N	8.20	216.8	21.06	2.12
	I	10.14	244.3	28.75	3.07
200	N	7.47	207.7	21.34	2.09
	I	8.78	225.6	32.27	2.09
500	N	5.57	114.3	12.76	1.34
	I	6.94	187.5	18.79	2.67
1000	N	3.96	98.5	10.10	1.09
	I	6.09	144.2	13.53	1.69
L.S.D. at 5%		2.57	14.56	4.20	0.16
Pb	N	8.75	183.7	19.29	2.30
5	I	10.6	214.8	25.56	2.76
10	N	7.87	131.9	17.71	2.24
	I	9.69	202.5	23.96	2.63
20	N	7.54	114.4	16.98	2.03
	I	9.29	144.8	21.45	2.42
50	N	6.14	65.6	11.54	1.38
	I	7.87	118.8	14.78	1.84
100	N	5.70	35.6	7.65	0.98
	I	7.12	87.7	12.47	1.44
L.S.D. at 5%		1.76	21.25	3.03	0.28

I: Plants inoculated by AM spores. **N**: Non-inoculated plants.

RESULTS

The results in Table 1, showed that the effect of AM fungi on dry weight, root : shoot dry weight ratios, height and leaf number and area of oat (*Avena sativa*) plants under various levels of heavy metals, Fe and Pb. Fe level of 100 mg/kg soil was the best to attain higher values of tested growth parameters of oat (*Avena sativa*) (leaf number, leaf area and dry weight), either the soil has no microsymbiont of AM fungi or it is inoculated with them. In the presence of microsymbionts, root shoot dry weight ratios were slightly increased with increasing Fe concentrations up to 500 mg/kg dry soil, while it decreased at 1 g/kg dry soil. However, Fe concentration of 200 mg/kg air dry soil was optimum for stem and root lengths either with microsymbiont inoculation or none, and lower or higher concentrations were concomitant with lower stem and root lengths. Generally, it is safe to conclude that Fe at 100 to 200 mg/kg soil was necessary to attain the best growth parameters, under the tested condition by oat (*Avena sativa*) plant. However, higher levels of Fe are conducive to the tested parameters. The results fairly indicated that the presence of AM fungi noticeably improving the tolerance of oat (*Avena sativa*) plants against toxicity of Fe. Thus at the more toxic Fe concentration (1 g Zn/kg air dry soil) microsymbiont inoculation increases dry weights, leaf number and area as well as stem and root length by about 64, 28, 40, 40 and 38%, respectively, as compared to non-inoculated soils.

Inoculation of plants with AM fungi significantly increases Pb tolerance of oat (Avena sativa) plants at all concentrations used in this study. The maximum height of plants were obtained in plants inoculated with both AM fungi at corresponding levels of Pb concentrations (Table 1). It was also revealed that the effect of inoculation of AM fungi on dry weight and leaf number and area of oat (*Avena sativa*) plants grown in Pb -polluted soil follow similar trends of these inoculants on the height of oat (Avena sativa) plants under the same soil conditions. Thus, at the most toxic Pb level (0.1 g/kg soil) microsymbiont inoculated soils showed increases in plant growth parameters of dry weights, leaf number and area, as well as stem and root lengths reached to about 87, 38, 64, 49 and 9%, respectively, as compared to non-inoculated soils. On the other hand, root shoot dry weight ratios exhibited constant values with increasing Pb concentrations in the presence of AM fungi.

The data presented in Table 2 indicated that inoculation of AM fungi to soil, in presence of different concentrations of Fe improves oat (*Avena sativa*) growth and nutrient assimilation in compared with microsymbiont-free soil. Fe concentrations of more than 50 mg/kg air dry soil were conducive to pigments formation by oat (*Avena sativa*) leaves either in absence or presence of microsymbionts in the soil. However, at a high Fe concentration (1000 mg/kg) pigments production was inhibited by about 55 and 37% in the absence and presence of microsymbionts in the soil, respectively. The results in Table 2 also indicated that carbohydrates biosynthesis by oat (*Avena sativa*) stimulated by microsymbionts inoculation more than non-microsymbionts and Fe concentration of 100 mg/kg was necessary for maximal carbohydrates formation. Pb level of 5 mg/kg air dry soil (inoculated or non-inoculated) induced better pigments formation, total carbohydrates, nitrogen and phosphorus content than control treatment (0.0 Pb), i.e., oat (*Avena sativa*) needed Pb within 5 mg/kg soil to attain improved plant growth. On the other hand, pigments formation, total carbohydrates, nitrogen and phosphorus content of non-inoculated and inoculated plants were inhibited at Pb concentration more than 5 mg/kg soil and the inoculated plants still showed higher pigments formation, total carbohydrates nitrogen and phosphorus contents than those of corresponding non- inoculated plant (Table 2).

DISCUSSION

AM fungi are common beneficial microbes of cereals plants. It is frequently suggested that AM may improve P nutrition, enhance N uptake, improve disease resistance in their host plants or adaptation to various environmental stresses. The mycorrhizal symbiosis becomes even more important in sustainable agricultural systems such as in heavy metals polluted soil. The principal objective of this work was to attempt using AM fungi to induce heavy metal tolerance of oat plants grown under heavy metals stress.

This study (Table 1) indicates that AM fungi can increase the dry weight, root shoot ratio, leaf number and area as well as length of oat plants more than non-inoculated one at all Fe and Pb levels. In this connection, Andrad *et al.* (2004) and Rabie (2005) proved that VA-mycorrhizal fungi and N-fixing bacteria can play an important role in the establishment of plants in soil contaminated with heavy metals.

The data in Table 2 showed that physiological

indices, as expressed by some plant metabolites, accounted for plants infected with the two symbionts were significantly higher than that for non-infected one grown in heavy metal contaminated soil. This finding supported results from previous studies reporting that AM fungi has the ability to alleviate many anthropogenic stresses including effects of metals, polychlorinated aliphatic and phenolic compounds and polycyclic aromatic hydrocarbons (Entry et al., 2002; Yu et al., 2004; Johansson et al., 2004). These results indicated that AM Fungi effects on physiological growth of infected plants, probably improved plant development and indirectly minimized the stress caused by excess heavy metals in the soil. The agriculturally important symbiotic microorganisms play a remarkable role in nutrient (nitrogen, phosphorus, potassium and microelements) acquisition for plants. In pursuit of that goal, various workers (Chezhiyan et al., 1999; Cairney, 2000; AlKaraki et al., 2001; Rabie et al., 2005) have used AM fungi, as single inoculants and in combination with each others in various plants, regardless of the presence or the absence of anthropogenic stresses. These symbiotic organisms have high ability to increase N, P and K as well as other nutrients in inoculated plants (Patreze and Cordeiro, 2004). The present study (Table 2) indicates that microsymbionts inoculation increased the concentration of N and P in oat plant tissue compared with non- inoculated one. These results that agree with previous works suggest that the combined or single effect of microbial symbiosis may play a great role in nutrient acquisition for plants. In this connection, Jha et al. (1993), Johansson et al. (2004) and Rabie and Almadini (2005) showed that AM fungi can support both needs for N and P and increase the growth of host plant.

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