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The functional roles of arbuscular mycorrhizal fungi in improving growth and tolerance of *Vicia faba* plants grown in wastewater contaminated soil

Salem M. Al-Amri

Department of Biological sciences, College of Science and Art, Shaqra University, Saudi Arabia.

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Wastewater contaminated soil poses serious environmental and health problems in Saudi Arabia and requires technological solutions for mitigating potential environmental risks. In spite the efforts of the Agriculture Ministry and agencies of water resources to overcome those problems, they still exist. In this concern, the effects of an arbuscular mycorrhizal (AM) fungus (*Glomus deserticola* Trappe and John) on growth, relative chlorophyll content and some mineral nutrients and heavy metal contents of broad bean (*Vicia faba*) plants grown in sterilized soil irrigated with different concentrations of wastewater were studied. Application of wastewater significantly reduced growth, chlorophyll content, nutrient contents, and levels of mycorrhizal colonization of bean plants comparing to control untreated plants, mainly at high concentrations. However, the rate of reduction was more pronounced in non-mycorrhizal treated plants. Mycorrhizal broad bean plants had significantly higher biomass, plant heights, leaf area, nutrients content (N, P, K), and relative chlorophyll content compared to those of non-mycorrhizal plants irrigated with or without sewage water. Under sewage water application, the AM colonization had greatly reduced the heavy metal contents (Zn, Co, Mn, Cu) in shoot and root tissues of the broad bean plants as compared to their equivalent non-mycorrhizal plants. This study indicates that growing broad bean plants with AM inoculum can minimize the heavy metals toxicity and increase growth and P uptake. In this regard, the AM fungi have a protective role to the host plants, and thus play important roles in soil contaminant immobilization processes. Therefore, the AM fungi are important in phytoremediation of heavy metals in wastewater contaminated soil.

Key words: Arbuscular mycorrhizal, heavy metals, growth responses, *Vicia faba*, nutrients content, phytoremediation, wastewater water contaminated soil.

INTRODUCTION

At present, water is becoming an increasingly scarce commodity in most plant production in the Kingdom of Saudi Arabia, and thus planners and decision makers are looking and searching precisely for additional water sources. Municipal wastewater reuses, reclamation and recycling are essential for water conserving and sustainable using, developing agricultural and environmental management policies. In arid and semi- arid regions of the world like in Saudi Arabia, wastewater reuses is needed for irrigating of some plants; mainly

E-mail: salem1618@hotmail.com. Tel.: +966 554433055; Fax: +966 1 4678467.

Abbreviations: AM, Arbuscular mycorrhizal; **EC,** electric conductivity; **G,** glomus; non-AM, non-arbuscular mycorrhizae; **MD,** mycorrhizal dependency; **Ti,** tolerance index; **DWT,** dry weight.

crop as another ensure alternate water source. Municipal wastewater (treated or untreated) used for irrigation of plants in economic plants could lead to accumulation of some heavy metals and organic pollutants in plants (Abdel-Fattah and Rabie, 1985; Ahmed et al., 2006; Lin et al., 2007). These pollutants can be strongly harmful and affect growth and yield of crop plants. The use of arbuscular mycorrhizal (AM) fungi as a potential biological agent to improve soil structure, and enhance plant resistance to various adverse stresses has received further attention (Ahmed et al., 2006; Gohre and Paszkowski, 2006; Repetto et al., 2007). In this connection, AM fungi may play a significant role in soil remediation by enhancing metal removal from the contaminated soil, protecting the host plant against metal toxicity, degradation of organic pollutants, retention and immobilization of metal in chitin or glomalin of the fungal wall (Abdel-Fattah and Rabie, 2005; Khan, 2005; Rabie, 2005; Audet and Charest, 2006).

Wastewater used for irrigation of cultivated soils in Saudi Arabia can lead in turn to accumulate of some elements like heavy metals in growing plants. These elements are strongly toxic to crops, horticulture and vegetable plants (Ahmed et al., 2006; Soares and Siqueira, 2008). Heavy metal contamination of soil poses serious environmental and health problems and requires technological solutions for mitigating potential environmental risks. Contrast to the traditional remediation methods for contaminated soils, bioremediation is an economically non-destructive approach (Tang et al., 2009). Bioremediation is defined as the use of biological systems to clean up contaminated environments. AM fungi are important components of soil phytoremediation in terms of increasing plant growth and nutrient uptake (Abdel-Fattah and Rabie, 1985; Gohre and pazkowski, 2006; Repetto et al., 2007; Rashid et al., 2009).

AM symbiosis can form potentially beneficial associations with roots of more than 90% of terrestrial plant species (Smith and Smith, 1997). AM fungi have proved to be able to improve soil structure and enhance plant resistance to environmental stresses (Weissenhorm et al., 1893; Auge, 2001; Abdel-Fattah et al., 2002; Asrar and Elhindi, 2011; Abdel-Fattah and Asrar, 2012; Asrar et al., 2012; Al-Amri, et al., 2013). In this concern, previous studies (Alguacil et al., 2006; Audet and Charest, 2006; Soares and Siqueria, 2008; Wang et al., 2008; Rashid et al., 2009) have reported that AM symbiosis in the context of soil bioremediation and concluded that AM fungi exude enzymes that participate in the immobilization process of the contaminated soil. In addition, AM fungi can improve plant growth in contaminated soils by enhancing mineral nutrition absorption and resisting various stresses.

In Saudi Arabia, broad bean *(Vicia faba* L.*)* is one of the most important crop plant. Raising broad bean through increasing the production per unit area as well as expanding the cultivated area in newly reclaimed lands is the major important national target. Increasing productivity per unit area, particularly in wastewater contaminated soil, could be achieved by cultivating high yielded cultivar along with importing agronomical practices. As a result, AM fungi are usually more tolerant to heavy metals in wastewater than plants without these symbionts (Gaur and Adholeya, 2004; Soares and Siqueira, 2008; Rashid et al., 2009), but mechanisms underlying this protections are largely unknown.

It has been suggested that the AM effects on host plant tolerance to heavy metals may depend on reduced metal uptake because of retention and immobilization in chitin or glomalin in the fungal wall (Khan et al., 2000) and reduced metal transfer from roots to shoots (Bi et al., 2003; Christie et al., 2004). Other protective effects may include metal dilution in plant tissue as a result of increased root or shoot growth, uptake exclusion by precipitation or chelation in the rhizosphere (Kaldorf et al., 1998). In addition, AM fungi exude enzymes that participate in the immobilization process of soil contamination in which case accumulation in plant is reduced (Weissenhorn et al., 1993; Audet and Charest, 2006).

In contrast to the traditional remediation methods of wastewater contaminated soils, the bioremediation is an economically non-destructive approach. Arbuscular mycorrhizal bioremediation is a promising and prospective technique for soil contaminated with heavy metals and organic pollutants of the wastewater. Therefore, the purpose of this project is aimed to evaluate the effects of an AM fungus, *Glomus deserticola* (isolated from wastewater contaminated soil) inoculation on the growth, mineral nutrients content, heavy metal tolerance of broad bean plants grown in soil contaminated with different concentrations of wastewater.

Furthermore, the effects of wastewater concentrations on the levels of mycorrhizal colonization of broad bean plants were also investigated.

MATERIALS AND METHODS

Sewage water

Wastewater used in this study was provided from drainage Station of King Khalid University Hospital, Riyadh, Saudi Arabia). The chemo-physical analysis of the polluted wastewater is listed in Table 1.

Mycorrhizal inoculum

G. deserticola Trappe and John was isolated from the rhizosphere soil of *V. faba* in Durab Experimental Station where the soil had been contaminated by sewage water. The single spore of *G. deserticola* was multiplied with sudangrass (*Sorghum halepense* L.) plants for 4 months using autoclaved polluted sand soil collected from the same site, in controlled greenhouse conditions (25°C day / 20°C night temperature, 65% relative humidity, 16 / 8 h light / dark period cycle with a photosynthetic photon flux density of 500 - 700 µmol. $m^{-2}s^{-1}$) at the Department of Botany and Microbiology, College of Sciences, King Saud University. The inoculum consisted

of rhizoshere soil, spores and mycelium of *G. deserticola*.

Growth conditions

The factorial block design (one plant sp. x two arbuscular mycorrhizal treatments x four wastewater concentrations) used in this experiment consisted of AM and non-AM plants grown in soil subjected to four levels of wastewater. The soil used in this study was collected from the top layer (0 to 20 cm) of the Garden Research station, college of Science, Riyadh region, Saudi Arabia. Soil characteristics were sandy loamy; pH, 7.65, an organic matter, 0.63%; an available phosphorus, 8.12 mg kg^{-1}; an available nitrogen, 26.8 mg kg^{-1} and EC, 0.61 dsm^{-1}. These eight treatments were replicated six times to give a total of 48 pots. Half of the pots (AM) were inoculated with *G. deserticola* containing 5 g of stock culture soil / pot (80 fungal spores / g soil), whereas an equivalent volume of control substrate (without propagules) was incorporated in the non-mycorrhizal pots (non-AM). Plants were distributed randomly and grown in a glasshouse of the Plant and Microbiology Department, College of Science, under natural day / night conditions (minimum / maximum temperature, relative humidity and day length, 25/17°C, 55/65% and 16/8 h, respectively). Four weeks after planting, each treatment was watered with an equal volume of the corresponding wastewater level (0, 20, 40 and 60% diluted with tap water, respectively) were provided from the drainage Station of King Khalid University Hospital, Riyadh, Saudi Arabia). All the plants were fertilized weekly (100 ml / pot) from week 6 to 10 using a modified Long-Ashton nutrient solution minus phosphorus.

Harvest

All the plants were harvested 10 weeks after planting. Shoot heights were measured. The fresh plant tissues were weighed separately as shoots and roots biomass. Leaf area of the plants for each treatment was measured with a leaf area meter (Li-Cor, Lincoln, Nebraska). Shoots and roots were oven dried at 80°C for 24 h and weighed. The relative content of chlorophyll was measured using SPAD-502 portable chlorophyll apparatus. Tolerance indices (Ti) of mycorrhizal and non-mycorrhizal plants to polluted sewage water were determined according to Shetty et al. (1995) as follow:

Ti = 100 X {shoot dry weight of plants at polluted level / shoot dry weight of plants at control level}

The dependence of plant on mycorrhiza (MD) was defined as the percentage of the plant growth that was subject to the adding of AM, and calculated with the following formula (Menge et al., 1978):

MD (%) = 100 X {leaf area of AM plants - leaf area of non-AM plants} / leaf area of non-AM plants

Part of roots for each treatment were washed gently with tap water, cleared 45 min in 7% KOH at 90°C, rewashed with tap water, acidified in 1% HCl and stained in 0.05% trypan blue in lactophenol. For the mycorrhizal colonization levels, the frequency of colonization (F%), the intensity of colonization (M%) and rate of arbuscular development (A%) of the stained roots were estimated by the method of Trouvelot et al. (1986). Total phosphorus in the dry tissue of shoot and root was determined by the vanadono-molybdophosphoric colorimetric method (Jackson, 1973). Total nitrogen was determined by the Kjeldahl method (Nelson and Sommers, 1973). Potassium was assayed directly by atomic absorption spectrophotometer Shimadzu AA-670 (Price, 1979). Oven-dried shoots and roots were milled and digested by 5 ml concentrated HNO_3 at 160°C using microwave accelerated reduc-reduction system (Mars 5, CEM Co. Ltd, USA). The dissolved samples were analyzed for Zn, Cu, Co and Mn concentrations and measured by inductively coupled plasma-optical emission spectroscopy using a Perkin Elmer Optima 2000 Dv (Pearson and Jakobsen, 1993).

Statistical analysis

The data were analyzed using two - factor analysis of variance (ANOVA) with AM inoculation and wastewater concentration as fixed factors. Means were separated by Duncan's multiple range tests at the 5% level using Costat software (Cohort, Berkeley, Calif.).

RESULTS

Plant growth

Shoot and root biomass and shoot height of both mycorrhizal and non-mycorrhizal plants grown in soil contaminated with wastewater were significantly lower than those plants grown in control (unpolluted) soil (Table 2). However, the reduction in the plant growth parameters was markedly distinct in non-mycorrhizal than mycorrhizal broad bean plants. AM plants had significantly higher tolerance index (Ti) than the non-AM plants, and the rate of Ti was significantly decreased with increasing sewage water level.

Leaf area, relative chlorophyll and magnesium contents

Leaf area, relative chlorophyll and magnesium contents of mycorrhizal and non-mycorrhizal plants increased at the concentration of 20% wastewater, and then reduced as wastewater concentration increased (Table 3). The relative chlorophyll content, leaf area and Mg content of the mycorrhizal plants was significantly higher than that of the non-inoculated plants grown in soil contaminated with different levels of wastewater stress, indicating that high concentration of polluted water might inhibit leaf area and Mg content of broad bean plants.

Mycorrhizal colonization levels

With the increase of the wastewater concentration in the soil, the frequency (F%) and intensity (M%) of mycorrhizal colonization and arbuscular development (A%) in broad bean roots gradually declined (Table 4), and this effect was markedly distinct with the highest wastewater levels. However, no significant differences were observed in the levels of mycorrhizal colonization between AM plants grown in unpolluted (control) and 20% wastewater contaminated soils. No signs of mycorrhizal colonization were observed in the non-inoculated plants.

Table 1. Analytical characteristics of the polluted wastewater used throughout this study.

Parameter	Value
Electrical conductivity (dS/m)	1.41
Ash (%)	15.8
pH (1:5)	5.98
Total organic carbon (%)	8.55
Cadmium (ppm)	9.1
Lead (ppm)	88.4
Zinc (ppm)	91.6
Copper (ppm)	55.6
Manganese (ppm)	31.5
Cobalt (ppm)	1.02
Nitrate (ppm)	155.0
Total P (ppm)	515
Total N (%)	0.46

Table 2. Effect of arbuscular mycorrhizal inoculation on growth responses of broad bean grown in wastewater contaminated soil.

Treatment	AMF status	Fresh matter (g / plant)		Dry matter (g / plant)		Shoot height (cm / plant)	Tolerance indices (TI)
Wastewater level (%)		Shoot	Root	Shoot	Root		
0.0 (control)	Non-AM	30.00^{c}*	2.18	1.75^{c}	0.35^{c}		
	AM	52.16^{b}	3.42^{b}	2.35^{b}	0.42^{b}	30.4^{b}	-
20	Non-AM	31.30^{cd}	2.25^{bc}	1.80^{c}	0.39^{bc}	29.2^{b}	1.02^{b}
	AM	86.65^{a}	4.33^{a}	4.15^{a}	0.60^{a}	33.9^{a}	1.77^{a}
40	Non-AM	26.87^{d}	2.18^{bc}	1.51^{cd}	0.32^{c}	26.9^{c}	0.86^{c}
	AM	43.85^{bc}	2.40^{b}	2.45^{b}	0.44^{b}	30.4^{b}	1.04^{b}
60	Non-AM	15.87^{e}	1.21^{d}	0.95^{d}	0.21^{d}	25.2^{c}	0.54^{d}
	AM	37.14^{c}	1.85^{c}	1.67^{cd}	0.30^{cd}	28.0^{bc}	0.71^{c}
LSD (0.05)		10.250	0.402	0.349	0.052	2.440	0.210

*Values in each column followed by the same letter(s) are not significantly different at P ≤ 0.05 (Duncan's multiple range test). TI, Sshoot dry weight of plants at polluted level / shoot dry weight of plants at control level

Table 3. Effect arbuscular mycorrhizal (AM) colonization on the content of chlorophyll, leaf area and leaf magnesium content and mycorrhizal dependence (MD) of broad bean plants grown in wastewater contaminated soil.

Wastewater level (%)	AMF status	Relative content of chlorophyll	Mg (mg g^{-1} dwt)	Leaf area (cm^2/plant)	MD (%)
0.0 (Control)	Non-AM	22.24^{c}*	1.73^{c}	128.7^{cd}	-
	AM	27.31^{b}	1.99^{b}	196.6^{b}	52.8^{b}
20	Non-AM	23.00^{c}	1.83^{c}	135.1^{c}	-
	AM	29.40^{a}	2.23^{a}	225.4^{a}	66.8^{b}
40	Non-AM	18.36^{d}	1.68^{e}	116.3	-
	AM	20.22^{cd}	1.98^{c}	185.9^{b}	59.8^{b}
60	Non-AM	13.58^{e}	1.36^{d}	77.8^{d}	-
	AM	17.13^{d}	1.69^{e}	143.2^{c}	84.1^{a}
LSD (0.05)		2.080	0.226	21.75	14

*Values in each column followed by the same letter(s) are not significantly different at *P* ≤ 0.05 (Duncan's multiple range test). Mycorrhizal dependency (MD) = 100 X {leaf area of AM plants - leaf area of non-AM plants} / leaf area of non-AM plants.

Table 4. Frequency (F%), intensity of mycorrhizal colonization (M%) and arbuscular frequency (A%) in the root tissues of mycorrhizal (AM) and non-mycorrhizal (Non-AM) broad bean plants grown in sewage water contaminated soil.

Treatment	AMF status	Levels of mycorrhizal colonization (%)		
Wastewater level (%)		F	M	A
0.0 (Control)	Non-AM	0.0^{d}*	0.0^{e}	0.0^{d}
	AM	93^{a}	77.5^{a}	61.7^{a}
20	Non-AM	0.0^{d}	0.0^{e}	0.0^{d}
	AM	89^{a}	79.5^{a}	63.0^{a}
40	Non-AM	0.0^{d}	0.0^{e}	0.0^{d}
	AM	83^{b}	60.5^{b}	55.6^{b}
60	Non-AM	0.0^{d}	0.0^{e}	0.0^{d}
	AM	72^{c}	46.0^{c}	39.4^{c}
LSD (0.05)		11.11	8.50	5.91

*Values in each column followed by the same letter(s) are not significantly different at P ≤ 0.05 (Duncan's multiple range test).

Table 5. Effect of arbuscular mycorrhizal (AM) colonization on mineral nutrient contents (mg g^{-1} dwt.) in both shoots and roots of broad bean plants grown in wastewater contaminated soil.

Wastewater level (%)	AMF status	Shoot			Root		
		N	P	K	N	P	K
0.0 (Control)	Non-AM	32.50^{ab}*	2.07^{b}	15.11^{c}	19.95^{b}	1.87^{d}	11.10^{c}
	AM	35.69^{a}	3.17^{a}	21.31^{b}	22.57^{a}	2.62^{b}	16.52^{b}
20	Non-AM	33.20^{b}	2.61^{b}	18.10^{bc}	19.42^{b}	2.46^{c}	13.03^{c}
	AM	37.11^{a}	3.20^{a}	24.21^{a}	22.82^{a}	3.12^{a}	18.20^{a}
40	Non-AM	23.06^{d}	1.50^{d}	16.95^{c}	16.03^{cd}	1.31^{f}	10.90^{d}
	AM	28.07^{c}	1.95^{c}	20.15^{b}	18.18^{c}	1.81^{e}	15.11^{b}
60	Non-AM	19.03^{e}	1.10^{e}	13.80^{d}	13.21^{d}	1.09^{g}	09.98^{e}
	AM	23.84^{d}	1.48^{d}	16.89^{c}	16.38^{cd}	1.45^{f}	13.25^{c}
LSD (0.05)		3.11	0.255	2.81	2.08	0.195	1.78

*Values in each column followed by the same letter(s) are not significantly different at *P* ≤ 0.05 (Duncan's multiple range test).

Nutrients content

The data in Table 5 shows that AM broad bean plants had higher shoot and root P, N and K contents than non-AM plants, regardless of wastewater treatments. However, both mycorrhizal and non-mycorrhizal plants grown in control (unpolluted) soil had higher P, N and K contents than sewage water stressed plants particularly at higher concentrations (40 and 60%). Reduction in shoot and root nutrient contents as a result of wastewater stress was more pronounced in non-mycorrhizal than in mycorrhizal broad bean plants.

Heavy metals content

Shoots and roots Zn, Cu, Co and Mn contents of mycorrhizal and non-mycorrhizal broad bean plants were increased by increasing wastewater in the soil (Table 6). Mycorrhizal colonization significantly reduced shoot and root metals content when compared to non-mycorrhizal broad bean plants grown in soil contaminated with different concentrations of wastewater. On the other hand, AM broad bean plants exhibited high shoots and roots Zn, Cu, Co and Mn contents when compared with non-AM plants grown in unpolluted (control) soils

DISCUSSION

The use of sewage sludge or wastewater in agriculture lands lead to accumulation of some toxic elements like heavy metals in soils and then changed the physical and chemical properties of the exposed soil. Current evidence indicates that the level of pollution in irrigated water can be considered as an important factor controlling nutritional or morphological characters of the growing plants (Shaw, 1989; Tordoff et al., 2000; Soares and Siqueira, 2008). Metals can be removed or immobilized in non-available forms by different techniques, which involve

Table 6. Effect of arbuscular mycorrhizal (AM) colonization on heavy metals concentrations (μg^{-1} dwt.) in both shoots and roots of broad bean plants grown in wastewater contaminated soil.

Wastewater level (%)	AMF status	Shoot				Root			
		Zn	Cu	Co	Mn	Zn	Cu	Co	Mn
0.0 (control)	Non-AM	115^{f}*	35.6^{e}	16.3^{c}	61^{e}	120^{g}	140^{f}	18.1^{e}	40^{f}
	AM	130^{ef}	19.1^{de}	18.1^{de}	67^{e}	210^{f}	150^{e}	19.3^{e}	45^{f}
20	Non-AM	180^{d}	25.6^{c}	25.6^{c}	110^{de}	180^{de}	180^{c}	32.1^{d}	69^{d}
	AM	142^{e}	20.0^{d}	20.0^{d}	95^{d}	150^{e}	165^{d}	30.3^{d}	60^{e}
40	Non-AM	242^{c}	34.1^{b}	30.0^{b}	150^{c}	313^{c}	215^{b}	45.4^{b}	130^{b}
	AM	198^{e}	26.8^{c}	25.8^{c}	100^{d}	207^{d}	188^{c}	38.3^{c}	95^{c}
60	Non-AM	352^{a}	47.0^{a}	45.0^{a}	180^{a}	510^{a}	295^{a}	56.8^{a}	155^{a}
	AM	290^{b}	31.3^{b}	32.3^{b}	120^{b}	385^{b}	218^{b}	40.1^{b}	132^{b}
LSD (0.05)		25.9	5.82	5.82	10.3	30.9	13.23	7.11	10.6

*Values in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ (Duncan's multiple range test).

a variety of procedures based on physio-chemical processes, including re-vegetation, phytoremediation and bioremediation leading to the development of a plant cover (Vangronsveld and Cunningham, 1998; Wang et al., 2005; Rashid et al., 2009). The effects of AM fungi (in the context of phytoremeditation) on plant growth and uptake of heavy metals are varied (Heggo et al., 1990; Khan et al., 2000; Ahmed et al., 2006; Shen et al., 2006., Gohre and Paszkowski, 2006; Repetto et al., 2007; Wang et al., 2008).

It appears from the present study that AM inoculation improved growth of broad bean plants grown in either unpolluted or wastewater contaminated soils compared to non-mycorrhizal plants. The rate of growth in response to mycorrhizal colonization was more pronounced at higher levels of sewage water in soil. These results are in agreement with those reported by Shen et al. (2006) who reported that mycorrhizal inoculation increased growth of maize plants with enhancement of P nutrition, perhaps increasing plant tolerance to Cd and Zn or by lowering the concentrations of soluble heavy metals in the soil dilution and / or by adsorption onto the extrametrical mycelium of mycorrhizal fungi. Enhanced growth of mycorrhizal plants are often related to improve P, N and K acquisition (Wang et al., 2005; Soares and Siqueira, 2008) who suggested that AM protecting effect against heavy metal toxicity could be mediated by the enhancement of P nutrition (Alguacil et al., 2006; Abdel-Fattah and Asrar, 2012).

The relative chlorophyll content of broad plants was reduced under sewage water stress. The polluted wastewater might affect the synthesis of chlorophyllase enzyme, thereby reducing the plants photosynthesis and inhibiting the growth of plants (Feng, 2006). Wang et al. (2005) found that the inoculation with AM could improve the chlorophyll synthesis in plants and increase the photosynthesis. Moreover, the results indicated that arbuscular mycorrhizae might increase the chlorophyll content, improve the synthesis capacity of maize plants (Rashid et al., 2009) and protect or slow the process of chlorophyll degradation (Tang et al., 2009).

The present study demonstrated that the levels of mycorrhizal colonization in broad bean roost decreased with increasing wastewater concentrations in the soil. The results obtained here are in agreement with the study of Tang et al. (2009) who demonstrated that the colonization rate of AMF on maize root decreased with the increase of diesel concentration in the soil. In other study, Gang et al. (2002) reported that the organic matter contamination did not affect the colonization of AM fungi on poplar. However, other studies have reported high levels of mycorrhizal colonization in agricultural soils contaminated with metals of different origins (Audet and Alguacil et al., 2006; Wang et al., 2008). The inconsistence of the results may be probably due to the origin of mycorrhizal fungus, plant species and the dose of heavy metal used (Khan et al., 2000; Gohre and Paszkowski, 2006; Repetto et al., 2007; Soares and Siqueira, 2008).

Of particular interest in this study, the concentrations of zinc, cobalt, copper and manganese in shoots and roots of mycorrhizal broad bean plants were significantly lower than that in non-mycorrhizal plants grown at higher levels of wastewater contaminated soils. These results corroborate those by Ahmed et al. (2006) and Soares and Siqueira (2008) who reported that mycorrhizal colonization reduced the shoot concentrations of Cd and Zn in field growing maize and grass when the soil had high available concentrations of both metals. A possible reason for such reduction may be that AM plants yielded higher biomass, which contributed to dilute metals in the shoot tissue (Cavagnaro, 2008; Soares and Siqueira, 2008) or that the AM mycelium retained the absorbed metals (Chen et al., 2007; Repetto et al., 2007). Metal immobilization in fungal tissues can occur as metal sequestration in fungal wall components such as the glycoproteins-glomalins, which have high affinity to metals (Gonzalez-Chavez et al., 2004).

Under soil contamination, AM inoculation decreased all metals (Zn, Co, Cu and Mn) content in shoots and roots when compared to non-mycorrhizal broad plants. These metal contents in the root tissues were much higher than in the shoot tissues. These results corroborate those by Lee and George (2005) who suggested that AM inoculation can restrict root metal translocation to shoots by the formation of less mobile metal-phosphate compounds, thus favoring plant growth.

Conclusion

The present study shows that mycorrhizal colonization enabled the plants to accumulate more P, N, K and increase chlorophyll content. In addition, mycorrhizal plant absorbed less metal than non-mycorrhizal plants from the polluted soil. In this connection, AMF exhibit reduced metal translocation and enhanced shoot growth, maintaining metal concentration at tolerable levels below toxicity-critical content. Besides plant nutrients improvement, arbuscular mycorrhizal fungi increased plant tolerance to metal via such mechanisms as dilution effects, increased tolerance to metals, decreased metal uptake and translocation from root to shoot. These mechanisms could be utilized in phytoremediation of heavy metals and protecting the host plant against metal toxicity.

REFERENCES

Abdel-Fattah GM, Asrar AA (2012). Arbusculr mycorrhizal fungal application to improve growth and tolerance of wheat (*Triticum aestivum* L.) plants grown in saline soil. Acta Physiol. Plant. 166:268-281.

Abdel-Fattah GM, Meghaed FF, Ibrahim AH (2002). Interactive effects of the endomycorrhizal fungus *G. etunicatum* and phosphorus fertilization on growth and metabolic activities of broad bean under drought stress. Pakis. J. Biol. Sci. 5:853-841.

Abdel-Fattah GM, Rabie GH (2005). The use of vesicular arbuscular mycorrhizal fungus (*Glomus fasiculatum*) to overcome the negative effects of the industrial effluents on the growth of cowpea. Pharmacy J. Zagazig Univ. 13:34-40.

Ahmed FR, Killham K, Alexander I (2006). Influences of arbuscular mycorrhizal fungi *G. mosseae* on growth and nutrition of lentil irrigated with arsenic contaminated water. Plant Soil 258: 33-41.

Al-Amri SM, Al-Waibi MH, Abdel-Fattah GM, Siddiqui MH (2013). Role of mycorrhizal fungi in tolerance of wheat genotypes to salt stress. African J. Microbiol. Res. 7:1286-1295.

Alguacil M, Caravaca F, Dias-Vivancos P, Hernandez JA, Roldan A (2006). Effect of arbuscular mycorrhizal and induced drought stress on antioxidant enzyme and nitrate reductase activities in *Juniperus oxycedrus* L. grown in a composted sewage sludge-amended semi-arid soil. Plant Soil 279:209-218.

Asrar AA, Abdel-Fattah GM, Elhindi KM (2012). Improving growth, flower yield and water relations of snapdragon (*Antirhinum majus* L.) plants grown under well-watered and water-stressed conditions using arbuscular mycorrhizal fungi. Photosynthetica 50:305-316.

Audet P, Charest C (2006). Effects of AM colonization on "wild tobacoo plants grown in zinc-contaminated soil. *Mycorrhiza* 16:227-283.

Auge RM (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3-42.

Bi YL, Li XL, Christie P (2003) Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus amended with zinc and phosphorus. Chemosphere 50:831-837.

Cavagnaro TR (2008). The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review". Plant Soil 304:315-325.

Chen BD, Zhu YG, Duan J, Xiao XY, Smith SE (2007). Effects of the arbuscular mycorrhizal fungus *G. mosseae* on growth and metal uptake by four plant species in copper mine tailings. Environmental Pollution 147:374-380.

Christie P, Li XL, Chen BD (2004). Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc. Plant Soil 261:209-217.

Feng G (2006). Effects of single pollution of the different density mercury, lead and chromium on physiological and biochemical indexes of mungbean. J. Shanxi Agric. Univ. 3:382-387.

Gang CN, Li PJ, Chen SH, Xiao BY, Han JY, Zhang CG (2002). Effects of different arbuscular mycorrhizal fungi on soil tolerance of *Trifolium subterraneum* L. *Chinese* J. Appl. Envir.Biol. 8:648-652.

Gaur A, Adholeya A (2004). Prospects of arbusular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr. Sci. India 86:538-534.

Gohre V, Paszkowski U (2006). Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115-1122.

Gonzalez-Chavez MC, Carrillo-Gonzalez R, Wright S.F, Nichols KA (2004). The role of glomalin a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ. Pollut. 130:317-323.

Heggo AM, Angle JS, Chaney RL (1990). Effects of vesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol. Biochem. 22:865-869.

Jackson ML (1973). Soil chemical analysis. Prentice Hall of India Ltd. New Delhi.

Kaldorf M, Kuhn AJ, Schroder WR, Hildebrandt U, Bothe H (1998). Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. J. Plant Physiol. 154:718-728.

Khan AG (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J. Trace Elements Med. Biol.18355-364.

Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197-207.

Lee YJ, George E (2005). Contribution of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply. Plant Soil 278:361-370.

Lin AJ, Zhang XH, Wong MH, Ye ZH, Lou LQ, Wang YS, Zhu YG (2007). Increase of multi-metal tolerance of three leguminous plants by arbuscular mycorrhizal fungi colonization. Environ. Geochem. Health 29:473-481.

Menge JA, Johnson ELV, Platt RG (1978). Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. New Phytologist 81:553-559.

Nelson DW, Sommers LE (1973). Determination of total nitrogen in plant material. Agron. J. 65:109-112.

Pearson JN, Jakobsen I (1993). The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants measured by dual labeling with ^{32}P and ^{33}P. New Phytologist 124:489-494.

Price NJ (1979). Spectrochemical analysis by atomic absorption: Pye Unicam Limited.

Rabie GH (2005). Role of arbuscular mycorrhizal fungi in phtoremediation of soil rhizosphere spiked with poly aromatic hydrocarbons. Mycobiology 33:41-5.

Rashid A, Ayub N, Ahmed T, Gul J Khan AG (2009). Phytoaccumulation prospects of cadmium and zinc by mycorrhizal plant species grown in industrially polluted soils. Environ Geochem. Health 31:91-98.

Repetto O, Massa N, Gianinazzi-Pearson V, Dumas-Gaudot E, Berta G (2007). Cadmium effects on populations of root nuclei in two pea genotypes inoculated or not with the arbuscular mycorrhizal fungus *G. mosseae*. Mycorrhiza 17:111-120.

Shaw AJ (1989). Heavy metal tolerance in plants: evolutionary aspects.

CRC, New York. p. 355.
Shen H, Christie P, Li X (2006) Uptake of zinc, cadmium and phosphorus by arbuscular mycorrhizal maize (*Zea mays* L.) from a low available phosphorus calcareous soil spiked with zinc and cadmium. Environ. Geochem. Health 28:111-119.
Shetty KG, Hetrick BAD, Schwab AP (1995). Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. Environmental Pollution 88:307-314.
Smith FA, Smith SE (1979). Structural diversity in vesicular-arbuscular mycorrhizal symbioses. New Phytologist 137:373-388.
Soares CRFS, Siqueira JQ (2008). Mycorrhiza and phosphate protection of tropical grass species against heavy metal toxicity in multi-contaminated soil. Biol. Fert. Soils 44:833-841.
Tang M, Chen H, Huang JC, Tian ZQ (2009). AM fungi effects on the growth and physiology of *Zea mays* seedlings under diesel stress. Soil Biol. Biochem. 41:936-940.
Tordoff GM, Baker AJM, Willis AJ (2000). Current approaches to the revegetation and reclamation of mettaliferous mine wastes. Chemosphere 41:219-228.
Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986). Mesure du taux de mycorrhization d'un system radiculaire recherché de methods d'estimation ayant une signification fonctionnelle.. In: Gininazzi-Pearson, V. and Giainazzi, S. (eds). Physiological and genetical aspects of mycorrhiza. INRA Publications, Paris. pp. 217-221.
Vangronsveld J, Cunningham SD (1998). Introduction to the concepts. In: Vangronsveld, J. and Cunningham, S.D (eds). Metal-contaminated soils; in situ inactivation and phyto-restoration. Springer, Dordrecht. pp. 1-15.
Wang FY, Lin XG, Yin R (2005|). Heavy metal uptake by arbusular mycorrhizas of *Elsholtzia splendens* and the potential for phytoremediation of contaminated soil. Plant Soil 269:225-232.
Wang ZH, Zhang JL, Christie P, Li XL (2008). Influence of inoculation with *G. mosseae* or *Acaulospora morrowiae* on arsenic uptake and translocation by maize. Plant Soil 311:235-244.
Weissenhorn I, Glashoff A, Leyval C, Berthelin J (1993). Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal polluted and unpolluted soils. Plant Soil 167:189-196.