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COMPARISON OF SEVERAL ARBUSCULAR MYCORRHIZAL FUNGI AND SWEET MARJORAM (*ORIGANUM MAJORANA* L.) SYMBIOTIC ASSOCIATIONS IN HEAVY METAL POLLUTED SOIL

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Abstract

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Mycorrhizal fungi participated in plant nutrient assimilation, root development and metal absorption from the soil depending on the resistance and stress tolerance of the involved strain. The overview of several mycorrhizal and marjoram symbiotic associations represented the effect of the studied stress over different strains and their interactions with the plants. We tested four mycorrhizal isolates, derived from various rhizospheres: *Claroideoglomus claroideum* (Cc1), *Rhizophagus clarum*, *Claroideoglomus claroideum* (Cc2), *Funneliformis mosseae*. In order to get insight into the role of mycorrhizal symbiosis in protecting *Origanum majorana* L. against the excess of metals (Cd and Pb), we investigated growth, uptake and distribution of heavy metals in the plant parts, mycorrhizal colonization status, glomalin- related soil proteins and acid phosphatase activity (in root and soil). Pb and Cd partitioning in non-mycorrhizal plants were soil>shoots>root while in symbiotic associations variants changed to soil>root>shoots. Bio concentration factor (BCF) and translocation factor (TF) values confirmed by the less heavy metals shoot uptake in inoculated than control plants. The root dry weight increased in plants inoculated with *C. claroideum* strain (Cc1), which is isolated from industrially metal-contaminated sites. The highest shoot biomass correlated with the percentage of mycorrhization, relative mycorrhizal dependency, glomalin production and acid phosphatase activity was determined by *C. claroideum* (Cc2) and *Funneliformis mosseae*. Both strains derived from natural metalliferous sites. The findings in this study are essential to get the most benefits of mycorrhizal association in unfavorable conditions connected with plant development and herbal products free of harmful ingredients.

Key words: Arbuscular mycorrhizal fungi; *Origanum majorana* L.; heavy metals
Abbreviations: AMF – Arbuscular mycorrhizal fungi, HM – heavy metal, NM – non-mycorrhizal plants, APA – Acid phosphatase activity, Rc – *Rhizophagus clarum*, Cc – *Claroideoglomus claroideum*, Fm – *Funneliformis mosseae*

Introduction

Heavy metals in contaminated environments stand for a potential hazard to humans and animal health, and they have gradually become one of the major ecological concerns worldwide (Yoon et al., 2006). The contaminated soils characterized by negative properties such as poor nutrient availability, lack of structure, low organic content, high salinity and/or acid pH. Accumulated lead (Pb) and cadmium (Cd) in the growth' medium cause biological toxicity by affecting different physiological and biochemical processes in plants and soil microorganisms (Adriano, 2001).

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Arbuscular mycorrhizal symbioses occur in most habitats and about 95% of the terrestrial plants belong to characteristically mycorrhizal families (Liang et al., 2009). As for this ubiquitous symbiosis, AM provides a direct physical linkage between the soil and plant roots. It has been described that, in some extremely contaminated environments such as smelting and mining tailings containing abundant toxic heavy metals (HM), mycorrhiza can benefit host plants by facilitating water and nutrition uptake, improving the plant resistance to stressful environments and suppressing plant pathogens infection (Liang et al., 2009). Immobilization of metals in the fungal biomass is proposed as a mechanism by which they may increase plant tolerance to heavy metals (Siddiqui and Pichtel, 2008). Roots in symbiotic interactions with fungi may act as a barrier against HM move, reducing translocation and increasing Cd root/shoot proportion. This result is attributed to metal adsorption on hyphal walls since chitin has a significant binding capacity (Joner et al., 2000). It has been demonstrated that the glycoprotein produced by mycorrhizal fungi- glomalin, can chelate metal ions and, therefore, diminishing their availability for plants (Gonzalez-Chavez et al., 2004). Another possible tolerance mechanism includes dilution in plant tissues due to the promotion of plant growth by AM. The mycorrhizal associations with tolerant plants may redound to the HM storage in roots in a non-poison compound inside hyphal cell walls or complexes with phosphate materials inside the cells (Galli et al., 1995).

Information about the potential of medicinal and aromatic plants grown as alternatives crops in heavy metal polluted agricultural soils for phytoextraction was described in the literature (Zheljazkov et al., 2008; Doumett et al., 2008; Stancheva et al., 2010). Despite this fact data about the arbuscular mycorrhizal fungi (AMF) role on heavy metal stress reduction in medicinal plants is very limited. Among the herbs sweet marjoram (*Origanum majorana* L.) is of great economic and industrial importance (Werker et al., 1993). Marjoram oil distinguished with strong antioxidant potential and possesses antimicrobial, anti-inflammatory and anti-spasmolytic activities (Ezzeddine et al., 2001; Jun et al., 2001).

The importance of AMF in plant growth and nutrition has been well reported (Smith and Read, 2008). However, the impact of the symbiosis in the uptake of heavy metals can vary depending on the fungal strain isolate, the plant and the metal concerned (Orlowska et al., 2005).

To get insight into the AM association role in protecting *O. majorana* against the excess of metals (Cd and Pb), we investigated the parameters: growth responses; uptake and heavy metals' distribution in the organs; mycorrhizal colonization; glomalin- related soil protein and acid phosphatase activity in root and soil.

Materials and Methods

Biological materials and growth conditions

O. majorana L. plants starting from seeds in glasshouse conditions under natural sunlight for 120 days, from April to July. The temperature (night to day) was between 15°C and 30°C, and the relative humidity ranged from 40% to 65%. The plants were grown in 1.2 kg plastic pots (3 plants per pot) on the soil/sand substrate in the ratio 3:1 in four replicates for each variant.

The soil (type *Chromic Luvisols* (FAO, 1998), 30-40 cm depth) was collected from the field near a waste depository of a ferrous metallurgical plant and have the following agrochemical characteristics: pH = 7.8; 9.0 mg kg^{-1} soil total mobile nitrogen ($N\text{-}NO_3^-$ + $N\text{-}NH_4^+$), 26.0 mg kg^{-1} soil P_2O_5, 310 mg kg^{-1}soil K_2O; organic matter (3%), clay content (60%), sand content (13%) and silt content (26%). The concentrations of heavy metals (HMs) (mg kg^{-1}DW) in the start soil were measured: Cd – 6.7, Pb – 230 and Zn – 199.5. According to the Bulgarian legislation, the permissible limit concentrations (at pH = 7.8) are Cd < 3.0, Pb < 120 and Zn < 400 mg kg^{-1}DW).

The mycorrhizal isolates were kindly provided from the AMF collection of Estación Experimental del Zaidín (CSIC Granada, Spain):

1) *Claroideoglomus claroideum*, (N. C. Schenk & G. S. Sm.) Schüβler & Walker, 2010 (syn. *Glomus claroideum* N. C. Schenk & G. S. Sm.) isolated from the rhizosphere of *Zea mays* (Braunschweig, Germany), contaminated by an addition of sludge (ref. EEZ 35).

2) *Rhizophagus clarum* (T.H.Nicolson & N.C. Schenck) Schüβler & Walker, 2010 (syn. *Glomus clarum*, T.H.Nicolson & N.C. Schenck), isolated from the rhizosphere of *Zea mays* (Braunschweig, Germany), contaminated by the repeated addition of sludge's containing low amount of metals (ref. EEZ 37).

3) *Claroideoglomus claroideum*, a different isolate from the rhizosphere of *Lavandula stoechas* growing in a soil naturally enriched with heavy metals (Rio Tinto, Spain) (ref. EEZ 54).

4) *Funneliformis mosseae*, (Nicol. & Gerd.) Schüβler & Walker, 2010 (syn. *Glomus mosseae* (Nicol. & Gerd.) Gerd & Trappe), isolated from a soil naturally enriched with heavy metals (Rio Tinto, Spain) (ref. EEZ 55).

Mycorrhizal inoculation was done by placing the seeds over a thin layer of the AMF inoculum (2 g kg^{-1} soil substrate) following the layering method (Jackson et al., 1972). The inoculum consisted of colonized roots and soil from 4 months old oat pot cultures.

Five treatments were compared: 1 – control *non-mycorrhizal plants (NM)*, 2 – *O. majorana* L., inoculated with

strain *Claroideoglomus claroideum* EEZ 35 (Cc1); 3 – *Rhizophagus clarum* EEZ 37 (Rc) – *O. majorana* L. association; 4 – plants, inoculated with *Claroideoglomus claroideum* EEZ 54 (Cc2); 5 – *Funneliformis mosseae* EEZ 55 (Fm) – *O. majorana* L. association.

Determination of root colonization

The extent of mycorrhizal root colonization was determined using the gridline intersect method (Giovannetti and Mosse, 1980). To visualize the AMF colonization, roots were cleared in 10% KOH and staining with 0.05% Trypan blue in lactic acid (v/v), according to Phillips and Hayman (1970).

Acid phosphatase activity

Acid phosphatase activity (APA, EC 3.1.3.2) was measured according to the method of Schneider et al. (2000), based on the original one of Tabatabai and Bremner (1969). Root tissue was homogenized with 0.1 M sodium acetate buffer (pH 5.0). Following centrifugation, the supernatant was assayed for the enzyme activity by incubation in 5 mM p-nitrophenyl phosphate and 0.1 M sodium acetate buffer (pH 5.0). The reaction was stopped by the addition of 0.2 M NaOH, and absorbance measured at 405 nm. Soil phosphatase activity was assayed by colorimetric estimation of the *p*-nitrophenol released by phosphatase activity when the soil was incubated with buffered (pH 6.5) sodium *p*-nitrophenyl phosphate solution and toluene at 37°C for 1 h.

Easily- and total extracted glomalin

The extraction procedure followed the method reported by Wright and Upadhyaya (1996). The soil (2 g) was mixed with 8ml of 20mM sodium citrate at pH 7.0. The samples were autoclaved for 30 min (121°C) and immediately centrifuged at 5 000x *g* for 15 min. The supernatant represents the easily extracted glomalin-related soil proteins (EE-GRSP). The procedure for extracting total glomalin-related soil proteins (TE-GRSP) consisted of autoclaving 2 g soil in 8 ml of 50 mM sodium citrate at pH 8.0 for 60 min. Immediately, after autoclaving follows centrifugation at 5 000 *g* for 15 min, then the supernatant was poured off and stored at 4°C until analysis. For glomalin quantification (EE-GRSP and TE-GRSP) was used the Bradford assay (1976) based on measuring absorbance at 595 nm by using protein dye reagent and bovine serum albumin as standard.

Heavy metal content

Soil samples were air dried and ground using a mortar and pestle, and then were sieved through a 0.149 mm sieve. Both the plant and soil samples were digested in a solution containing 3:1 (v/v) HNO_3:$HClO_4$ solution. The samples were heated on a heating block at 200°C to evaporate the samples to dryness. The residue was taken up in 25 ml of 1N HCl (Doumett et al., 2008). Metal concentrations were determined on the inductively – coupled Plasma Mass Spectrometer (CCD Simultaneous ICP OES, Varian, Austria).

Statistics

Data are expressed as means ± standard error, where n varied between 3 and 10, depending on the type of analysis. Comparison of means was performed by the Fisher least significant difference (LSD) test at $P \leq 0.05$ following ANOVA. A statistical software package (StatGraphics Plus, version 5.1 for Windows, USA) was used.

Results and Discussion

Mycorrhizal fungi took part in plant nutrient assimilation, root development and metal absorption from the growth medium on the resistance and stress tolerance of the involved strain. Despite the elevated concentration of Cd and Pb above the critical rates (see section "2.1. Biological material and growth conditions") no visible phytotoxicity symptoms on plants were observed (pictures not presented). Rascio and Navari-Izzo (2011) described several hypotheses to explain while some plants developed during the evolution ability to accumulate high elemental concentrations without phytotoxicity signs, namely: metal tolerance/disposal, drought resistance, interference with neighboring plants, and defense against natural enemies.

The present study followed the changes in plant biomass; uptake and distribution of heavy metals in the plant parts; mycorrhizal colonization; glomalin- related soil protein and acid phosphatase activity in root and soil. Also, we compared the influence of different HM levels and AMF colonization by four strains (EEZ 35 (Cc1); EEZ 37 (Rc); EEZ 54 (Cc 2); EEZ 55 (Fm)).

The hyphal network functionally extends the root system of their hosts during its symbiotic interaction. So, AMF plants gain the ability to take up heavy metals from an increased soil volume and concentrate metals in the roots (Miransari, 2011). Our results showed that mycorrhizal plants took up less Cd and Pb than control plants in the shoots (Table 1), confirmed by the BCF and TF values found in our experiments (Table 2). Therefore, AMF seems to act as a barrier decreasing heavy metal uptake. The highest root content of Pb was detected in Rc followed by Cc1 plants where Cd concentration was above the other variants. Cc2 plants accumulated the lowest amounts of Pb both in roots and shoots (Table 1). Also, these plants didn't accumulate Cd in the roots as contrasted with the shoots, underline by TF, and the highest between the inoculated plants (Table 2). After Yoon et al. (2006) decreased TF and BCF values mean a limited ability of heavy metal accumulation and translocation by the plants.

Table 1
Pb and Cd concentrations (mg/kg) in soil and plant parts after harvest of *O. majorana* non-mycorrhizal (NM) plants and inoculated with four AMF strains: *Claroideoglomus claroideum* (Cc1, Cc2), *Rhizophagus clarum* (Rc) and *Funneliformis mosseae* (Fm)

Variants	Soil		Roots		Shoots	
	Pb mg kg^{-1} DW	Cd mg kg^{-1} DW	Pb mg kg^{-1} DW	Cd mg kg^{-1} DW	Pb mg kg^{-1} DW	Cd mg kg^{-1} DW
NM	124.276^{a}	3.133^{e}	24.28^{c}	0.678^{d}	60.93^{e}	1.541^{e}
Cc 1	162.351^{c}	1.5^{d}	26.14^{d}	0.704^{e}	15.06^{d}	0.2^{d}
Rc	178.139^{d}	1.278^{a}	35.96^{e}	0.617^{c}	8.94^{b}	0.094^{a}
Cc 2	129.4^{a}	1.380^{b}	12.4^{a}	0.288^{a}	8.021^{a}	0.173^{c}
Fm	140.301^{b}	1.420^{c}	13.5^{b}	0.540^{b}	10.125^{c}	0.150^{b}

* Letters in common within a graph indicate no significant differences assessed by Fisher LSD test (P≤0.05) after performing ANOVA (n = 6)

Table 2
Bio concentration (BCF) and translocation factor (TF) after harvest of *O. majorana* non-mycorrhizal (NM) plants and inoculated with four AMF strains: *Claroideoglomus claroideum* (Cc1, Cc2), *Rhizophagus clarum* (Rc) and *Funneliformis mosseae* (Fm)

Variants	BCF root*		BCF shoot		TF*	
	Pb	Cd	Pb	Cd	Pb	Cd
NM	0.106^{b}	0.101^{d}	0.265^{d}	0.230^{d}	2.509^{e}	2.273^{d}
Cc 1	0.114^{c}	0.105^{d}	0.065^{c}	0.030^{c}	0.576^{b}	0.284^{b}
Rc	0.156^{d}	0.092^{c}	0.039^{a}	0.014^{a}	0.249^{a}	0.152^{a}
Cc 2	0.054^{a}	0.043^{a}	0.035^{a}	0.026^{b}	0.647^{c}	0.601^{c}
Fm	0.059^{a}	0.081^{b}	0.044^{b}	0.022^{b}	0.750^{d}	0.278^{b}

*BCF = metal concentration ratio of plant tissues to soil and TF = metal concentration ratio of plant shoots to roots.

The uptake of heavy metals by plants affecting by AMF is unique as both increases and decreases were reported in the literature. The outcome probably depends on the selected plant also on the species and strain of the fungus used (Estaún et al., 2010). Rivera-Becerril et al. (2005) showed a Cd-stress buffering effect of mycorrhizal colonization on pea plantlets. The effect of AMF in decreasing heavy metal stress has been assigned to the selective immobilization of the toxic metal within the root tissues that are colonized by the fungus or to the high metal sorption capacity of the extraradical mycelium of the AMF (Joner et al., 2000).

Another point of view about the differences among the mycorrhizal fungi – marjoram symbioses could be the origin of the strains. *Claroideoglomus claroideum* varieties (EEZ 54 and EEZ 55) were isolated from soil, naturally enriched with metals, while EEZ 35 (*Funneliformis mosseae*) and EEZ 37 (*Rhizophagus clarum*) – from a place with industrial contamination. Both strains derived from native metalliferous sites are evolutionarily more fitted to manage with higher concentration and to reduce their uptake by plants. Ferrol et al. (2009) explained that isolation of indigenous and presumably adapted AM fungi are more suitable for phytostabilisation purposes than laboratory strains could be a potential biotechnological tool for successful restoration of degraded ecosystems.

Analyzes of dry weight biomass demonstrated an increasing trend in all of the plants inoculated with AMF strains in parallel to non-mycorrhizal plants (Table 3). DW root values

Table 3
Dry weight (roots and shoots), mycorrhization (%) and relative mycorrhizal dependency (RMD) of *O. majorana* non-mycorrhizal (NM) plants and inoculated with four AMF strains: *Claroideoglomus claroideum* (Cc1, Cc2), *Rhizophagus clarum* (Rc) and *Funneliformis mosseae* (Fm)

	Roots DW (g plant^{-1})	Shoots DW (g plant^{-1})	Mycorrhization (%)	RMD (%)
NM	0.210^{a}	0.485^{a}	-	-
Cc 1	0.426^{d}	0.607^{c}	32	30
Rc	0.328^{b}	0.586^{b}	35	24
Cc 2	0.385^{c}	0.667^{e}	42	34
Fm	0.364^{c}	0.620^{d}	33	30

Letters in common within a graph indicate no significant differences assessed by Fisher LSD test (P≤0.05) after performing ANOVA (n = 9)

of Cc1 plants were statistically higher compared to the other variants followed by Cc2.

While in shoot dry biomass the plants from Cc2 treatments were the leading. Among the mycorrhizal plants the lowest biomass was noticed in both parts- roots and shoots in Rc symbiotic system. The highest shoot DW in Cc2 plants positively correlates with the percentage of mycorrhization and relative mycorrhizal dependency (Table 3). The mycorrhizal colonization was almost equal to the rest of inoculated plant roots (Table 3). In parallel to the observed lower HM concentration in previous research (Hristozkova et al., 2015), the tendency was the same, but the rates of colonization slightly decreased when the HM concentration was higher.

Inoculation of marjoram plants with Cc2 strain altered the acid phosphatase activity (APA) in roots and soil (Figure 1). A negative trend was noticed in Rc plants expressed in lowest values in soil and relatively high root APA activity. About the root APA, NM variants were distinguished with minimal value, followed by Cc1, in conformity to the lowest mycorrhization. Plant roots, fungi and other microorganisms in the soil have different levels of APA (Abd-

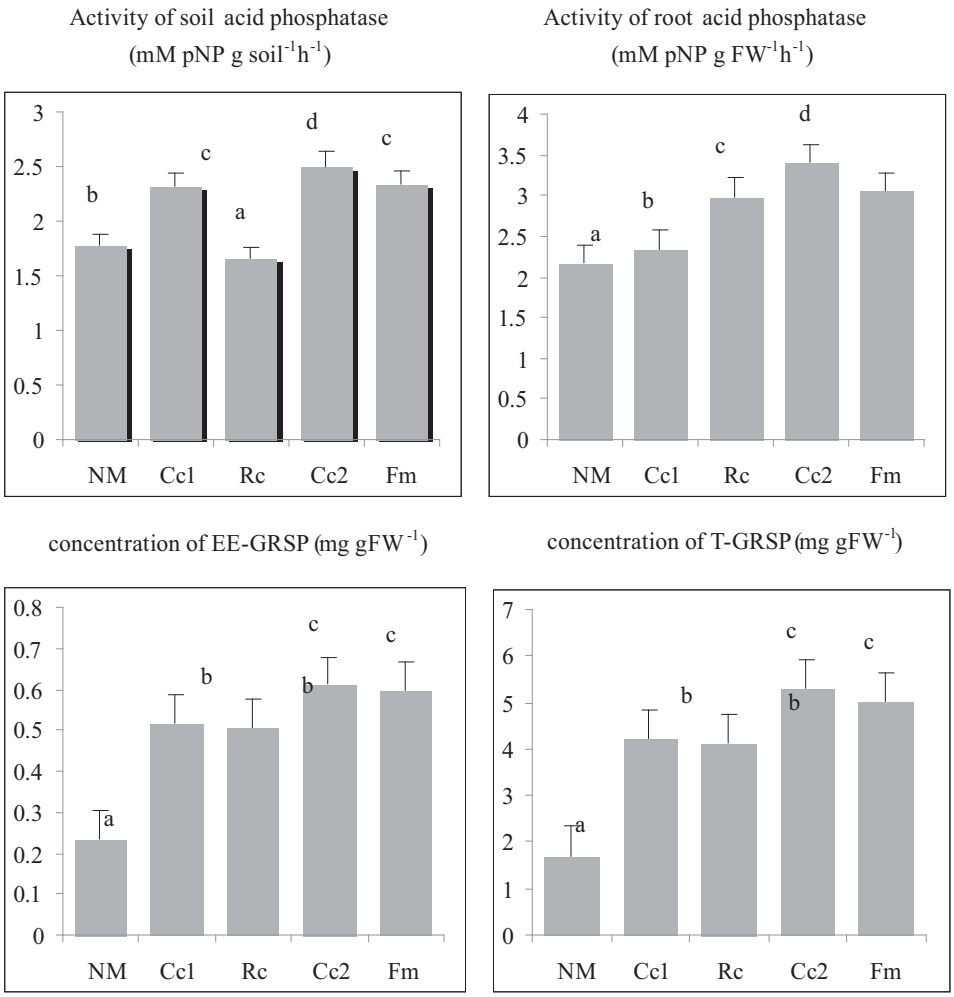

Fig. 1. Acid phosphatase activity (soil and roots), concentration of easily extracted glomalin- related soil proteins (EE-GRSP) and total extracted glomalin related soil proteins (TE-GRSP) of *O. majorana* non-mycorrhizal (NM) plants and inoculated with four AMF strains: *Claroideoglomus claroideum* (Cc1, Cc 2), *Rhizophagus clarum* (Rc) and *Funneliformis mosseae* (Fm)

Values are means ± SE; letters in common within a graph indicate no significant differences assessed by Fisher LSD test (P≤0.05) after performing ANOVA (n = 9)

Alla, 1994), and that is why sometimes a correspondence between soil and root phosphatase activities is missing. Greater enhancement of enzymatic acid phosphatase and alkaline phosphatase activity occurred with AMF roots in contrast to NM roots (Prasad et al., 2012). Results are supported by Garcia-Gomez et al. (2002) who reported that better biological compatibility between AM fungal strain and host plant species define higher activity in soluble and extractable root acid phosphatase. APA associated with the phosphorus acquisition in the rhizosphere, growth and development of the fungus within the host tissue and managed by an unspecified mechanism controlled by the plants (Prasad et al., 2012).

Glomalin production (EE-GRSP and TE-GRSP) showed an increase of the values in the Cc2 and Fm plants (Figure 1). The positive correlation between AM fungal root colonization and GRSP concentrations as in Cc2 plants, confirming the link between GRSP and AMF activity. There is evidence that different varieties can produce different amounts of GRSP (Wright and Upadhyaya, 1996). The difference in the GRSP as a consequence of the role of AMF has several important implications. There may be mycorrhizal species in a community which have high GRSP productivity. These particular species could be very useful in agro-ecosystem applications based on its primary functions to aggregate soil, increase water infiltration and improves nutrient cycling (Rillig et al., 2002).

Conclusions

The results in this paper clearly prove the importance of AMF for plant growth and development in high metal content soils. The overview of several mycorrhizal-marjoram symbiotic groups represented the effect of the studied stress over different strains and their interactions with the plants. AMF are active even in higher soil metal concentrations and even the positive effect is more distinctive, after the observation and comparison with earlier studies.

Sweet marjoram, grown under conditions of heavy metal pollution appeared to be a tolerant plant species, because of the absent of visible toxicity symptoms and significant plant biomass reduction.

Our results underline that the AMF strains depend on the genotype, have a different ability to determinate an effective mycorrhizal association with the host plant. Plants grown in symbiosis with a proper AMF are often more competitive and better able to tolerate different stresses than normally grown plants, potentially enhancing pollutant availability and plant tolerance. The findings in this study are essential to get the best benefits of mycorrhizal association in unfavorable conditions on plant development and herbal products free of harmful ingredients.

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References

Abd-Alla, M. H., 1994. Use of organic phosphorus by *Rhizobium leguminosarum* biovar Viceae phosphatases. *Biol Fertil Soil,* 18: 216-218.

Adriano, D. C., 2001. Trace elements in terrestrial environments. In: Biogeochemistry, Bioavailability and Risk of Metals, 2nd ed., *Springer*, 2001

Bradford, M. M., 1976. A rapid and sensitive method for the estimation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann Biochem*, 72: 248-254.

Doumett, S., L. Lamperi, L. Checchini, E. Azzarello, S. Mugnai, S. Mancuso, G. Petruzzelli and M. Del Bubba, 2008. Heavy metal distribution between contaminated soil and *Paulownia tomentosa* in a pilot-scale assisted phytoremediation study: Influence of different complexing agents. *Chemosphera*, 72: 1481-1490.

Estaún, V., A. Cortés, K. Velianos, A. Camprubí and C. Calvet, 2010. Effect of chromium contaminated soil on arbuscular mycorrhizal colonisation of roots and metal uptake by *Plantago S. J Agr Res*, **8** (S1): S109-S115.

Ferrol, N., M. González-Guerrero, A. Valderas, K. Benabdellah and C. Azcón-Aguilar, 2009. Survival strategies of arbuscular mycorrhizal fungi in Cu-polluted environments. *Photochem Rev*, 8: 551-559.

Galli, U., H. Schüepp and C. Brunold, 1995. Thiols of Cu-treated maize plants inoculated with the arbuscular mycorrhizal fungus *Glomus intraradic*es. *Physiol Plant*, 94: 247-253.

Garcia-Gomez, A., M. P. Bernal and A. Roig, 2002. Growth of ornamental plants in two composts prepared from agroindustrial wastes. *Biores Tech,* 83: 81-87.

Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist*, 84: 489-500.

Gonzalez-Chavez, M. C., R. Carrillo-Gonzalez, S. F. Wright and K. Nichols, 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. *Env Pollution*, 130 (3): 317-323.

Hristozkova, M., M. Geneva, I. Stancheva, M. Boychinova and E. Djonova, 2015. Aspects of mycorrhizal colonization in adaptation of sweet marjoram (*Origanum majorana* L.) grown on industrially polluted soil. *Turk J Biol*, 39: 461-468.

Jackson, N. E., R. E. Franklin and R. H. Miller, 1972. Effects of vesicular-arbuscular mycorrhizae on growth and phosphorus content of three agronomic crops. *Proceedings – Soil Sci Soc Am*, 36: 64-67.

Joner, E. J., R. Briones and C. Leyval, 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant Soil*, 226: 227-234.

Liang, C. C., T. Li, Y. P. Xiao, M. L. Liu, H. B. Zhang and Z. W. Zhao, 2009. Effects of inoculation with arbuscular mycorrhizal fungi on maize grown in multi-metal contaminated soils. *Int J Phytorem*, 11: 692-703.

Miransari, M., 2011. Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. *Biotech Adv*, 29: 645-653.

Orlowska, E., A. Jurkiewicz, T. Anielska, B. Godzic and K. Turnau, 2005. Influence of different arbuscular mycorrhizal fungal (AMF) strains on heavy metal uptake by *Plantago lanceolata* (Plantaginaceae). *Pol Bot Stud*, 19: 65-72.

Phillips, J. M. and D. S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55: 157-160.

Prasad, K., A. Aggarwal, K. Yadav and A. Tanwar, 2012. Impact of different levels of superphosphate using arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* on *Chrysanthemum indicum* L. *J Soil Sci Plant Nutr*, 12: 451-462.

Rascio, N. and F. Navari-Izzo, 2011. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Sci*, 180: 169-181.

Rillig, M. C., S. F. Wright and V. T. Eviner, 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant Soil*: 238: 325-333.

Rivera-Becerril, F., D. Van Tuinen, F. Martinlaurent, A. Metwally, K. J. Dietz, S. Gianinazzi and V. Gianinazzi-Pearson, 2005. Molecular changes in *Pisum sativum* L. roots during arbuscular mycorrhiza buffering of cadmium stress. *Mycorrhiza*: 16: 51-60.

Schneider, K., M. B. Turrion and J. F. Gallardo, 2000. Modified method for measuring acid phosphatase activities in forest soils with high organic matter content. *Comm Soil Sci Plant Anal*, 31: 3077-3088.

Schüßler, A. and C. Walker, 2010. The Glomeromycota: a Species List with New Families and New Genera. Gloucester, UK, pp. 1-58.

Siddiqui, Z. and J. Pichtel, 2008. Mycorrhizae: an overview. In: Siddiqui ZA et al. (Eds.), Mycorrhizae: Sustainable Agriculture and Forestry, *Springer*, pp. 99-134.

Smith, S. E. and D. J. Read, 2008. Mycorrhizal Symbiosis, 3rd ed. *Academic Press*, London.

Stancheva, I., M. Geneva, M. Hristozkova, Y. Markovska and I. Salamon, 2010. Antioxidant capacity of sage grown on heavy metals polluted soil. *Rus J Plant Phys*, 57: 799-805.

Tabatabai, M. A. and J. M. Bremner, 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol Biochem*, 1: 301-307.

Werker, E., E. Putievsky, U. Ravid, N. Dudai and I. Katzir, 1993. Glandular hairs and essential oil in developing leaves of *Ocimum basilicum* L. (Lamiaceae). *Ann Botany*, 71: 43-50.

Wright, S. F. and A. Upadhyaya, 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci*, 161: 575-586.

Yoon, J., X. Cao, Q. Zhou and L. Q. Ma, 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci Total Env*, 368: 456-464.

Zheljazkov, V., E. Jeliazkova, N. Kovacheva and A. Dzhurmanski, 2008. Metal uptake by medicinal plant species grown in soils contaminated by a smelter. *Env Exp Bot*, 64: 207-216.

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