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ORIGINAL PAPER

Heavy metals and arbuscular mycorrhizal (AM) fungi can alter the yield and chemical composition of volatile oil of sweet basil (*Ocimum basilicum* L.)

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Abstract The effects of increasing levels of metals (10 and 20 mg of Cr kg^{-1} and 25 and 50 mg of Cd, Pb, and Ni kg^{-1} soil) and arbuscular mycorrhizal (AM) fungi *Glomus intraradices* on the yield, chemical composition of volatile oil, and metal accumulation in sweet basil (*Ocimum basilicum* L.) were investigated in a pot experiment. The shoot yield, content of essential oil, and root yield of sweet basil were increased by the application of low dose of Cd, Pb, and Ni as compared to control. The application of high level of metals had deleterious effect on the yield. In soil with low dose of metal applied, AM fungi inoculation significantly enhanced the metal concentration in shoots and had adverse effect on the yield, whereas in soil with high dose of metal applied, AM fungal inoculation reduced the metal concentration in shoot and had beneficial effect on the yield. The content of linalool in basil oil was decreased and that of methyl chavicol was increased by the application of Cr, Cd, and Pb in soil as compared to control. Similarly, the level of linalool and methyl chavicol was decreased and that of methyl eugenol was increased by the application of Ni as compared to control. However, AM fungal inoculation led to maintain the content of linalool, methyl chavicol, and methyl eugenol in volatile oil, which were either increased or decreased by the application of metals. We conclude that the AM–sweet basil symbiosis could be used as a novel approach to enhance the yield and maintain the quality of volatile oil of sweet basil under metal-contaminated soils.

Keywords Metals · Arbuscular mycorrhizal fungus · Sweet basil · Yield · Volatile oil · Metal accumulation

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Introduction

The contamination of agricultural lands and irrigation water with metals poses an environmental risk to human and animal health. Higher metal concentration in the environment may come from the natural origins like weathering of mineralized ores or from anthropogenic activities like burning of fossils and fuels, mining and smelting of metalliferous ores, application of municipal wastes, fertilizers and pesticides, sewage sludge amendments and the use of pigments and batteries (Leyval et al. 1997). Some of the metals are micronutrient necessary for plant growth such as Fe, Mn, Zn, Cu, Ni, and Co (Marschner 1995), while others have no known biological function such as Cr, Cd, Pb, As, and Hg. Many of these metals are strongly retained in soils and do not readily leach, and when their bioavailability becomes high, toxicity can result. These negative effects can occur in soil microbes, soil fauna, higher animals, plants, and humans (McGrath et al. 2002). In metal polluted areas, agricultural products can be contaminated by toxic levels of metals, which can enter the nutrition chain and subsequently the human diet. The selection of metal-tolerant crops with not contaminated products can be an alternative for solving the problem.

The effects of metal application on the yield of food and non-food crops were extensively studied. Adhikari and Singh (2007) observed phytotoxicity of Cr in maize and spinach which was alleviated by the application of compost. Kopittke et al. (2007) reported that the growth of signal grass (*Bracharia decumbens*) and rhodes grass (*Chloris gayana*) decreased with the activity of Pb^{2+} in nutrient solution, and signal grass was considerably more tolerant to Pb^{2+} than rhodes grass. Chattarjee et al. (2004) observed the deleterious effects of excessive Pb on rice (*Oryza sativa*) and reduction of the dry matter and grain yield. Murch et al. (2003)

demonstrated that the presence of Ni in the culture environment can decrease the concentration of secondary metabolites (pseudohypericin and hypericin) in a medicinal plant St. John's wort (*Hypericum perforatumi* L.). Pandey et al. (2007) demonstrated that the application of Cr (10–40 mg kg^{-1} soil) increased the herb yield and bacoside content in Brahmi (*Bacopa monnieri*).

Plant roots interact with a wide variety of soil microorganisms, including bacteria and fungi, which greatly affect the metal uptake and plant growth. The arbuscular mycorrhizal (AM) fungi are important soil microorganisms. Soil pH, mineral weathering, pollutant precipitation with plant-excreted organic acids all may have a key role in constitutive and adaptive tolerance of mycorrhizal associations present on contaminated sites (Meharg 2003). AM fungi can contribute plant growth, particularly in disturbed or metal polluted sites, by increasing plant access to relatively immobile minerals such as P, Zn, and Cu (Ryan and Angus 2003; Christie and Chen 2004); in addition, they can improve soil structure by binding soil particles into stable aggregates that resist wind and water erosion and by binding metals into roots they restrict their translocation into shoot tissues (Gaur and Adholeya 2004). Metals have been shown to reduce or completely inhibit AM colonization in soils artificially polluted with metals (salts or sludge) (Gildon and Tinker 1983; McGee 1987). On the other hand, there are reports of higher levels of mycorrihizal cononization in agricultural soils contaminated with metals of different origins, including atmospheric deposition from a smelters and sludge amendments (Weissenhorn et al. 1995a, b). The effect of AM fungi on plant uptake of metals is not always clear. At high metals concentration, high uptake of metals by mycorrhizal plants has been observed (Gildon and Tinker 1983; Weissenhorn and Leyval 1995), with reduced concentration in shoots (Leyval et al. 1991; Weissenhorn et al. 1995c), or even no uptake (Guo et al. 1996). In a meta-analytical survey, Audet and Charest (2007) have focused on the dynamic roles of the AM symbiosis in metal phytoremediation as characterized by the "enhanced uptake" and "metal-Binding" hypotheses, the latter being associated with an enhanced metal tolerance in AM plants via stress avoidance at high soil–metal levels. Colonization of plant roots by symbiotic AM has also been shown to induce the accumulation of secondary metabolites in the medicinal and aromatic crops such as annual worm wood (*Artemisia annua* L.) (Kapoor et al. 2007) and purple coneflower (*Echinacea purpurea* L.) (Araim et al. 2009).

Sweet basil (*Ocimum basilicum* L.), belonging to the Lamiaceae family, is an economically important plant. Its essential oils are widely used in cosmetic, pharmaceutical, food, and flavoring industries (Copetta et al. 2006). The essential oil of *O. basilicum* L. consists mostly of monoterpenes, sesquiterpenes, and phenylpropanoids metabolites. Methyl chavicol and linalool are the major compounds present in the essential oil of *O. basilicum* L. Environmental conditions and agricultural practices can significantly alter yield and chemical composition of sweet basil (Sifola and Barbieri 2006). Ocimum species can be grown on a variety of soils, but well-drained sandy loam to loam soils are considered ideal for its cultivation (Hussain et al. 1988). *O. basilicum* L. is tolerant to higher concentration of Cu and Zn and is sensitive to Co and Ni (Hussain et al. 1988). Additions of municipal solid waste compost with concentration of Cu 311 mg kg^{-1}, Pb 223 mg kg^{-1}, Mo 17 mg kg^{-1}, and Zn 767 mg kg^{-1} to soil altered the chemical composition of basil oil, but the basil oil was free from metals (Zheljazkov and Warman 2004). Mycorrihization of sweet basil plants can modulate the essential oil yield (Copetta et al. 2006) and enhance the production of antioxidants (rosmarinic and caffeic acids) (Toussaint et al. 2007). To our knowledge, no study has been carried out on the interaction effects of metals and AM fungus on the yield and chemical composition of volatile oil of sweet basil. Hence, in this paper, we report the interactive effects of metals (Cr, Cd, Pb, and Ni) and AM fungi *Glomus intraradices* on the yield, chemical composition of volatile oil and mineral element accumulation in sweet basil (*O. basilicum* L.).

Materials and methods

Experimental design

A pot experiment was conducted in the glasshouse at the Central Institute of Medicinal and Aromatic Plants, Lucknow, India. The 9×2 factorial experiment was designed with nine metal treatments such as control, two levels each of Cr, Cd, Pb, and Ni, and two mycorrhizal conditions viz. non-inoculated and inoculated with *G. intraradices*. The treatments combinations were arranged in completely randomized design with three replicates.

Soil

The soil used in the study was collected from the experimental farm of the Central Institute of Medicinal and Aromatic Plants, Lucknow, India. The soil was air-dried and ground to pass through a 2-mm sieve. The soil had the following physicochemical properties: sandy loam texture, pH (1:2.5 soil water suspension) 8.0, electrical conductivity (EC) 0.08 dSm^{-1}, organic C 0.25%, and available N (alkaline $KMnO_4$-extractable), P (0.5 M $NaHCO_3$-extractable), and K (1 N neutral NH_4OAC-extractable) 77.0, 7.8, and 90.0 mg kg^{-1} soil, respectively. The native DTPA extractable Cr, Cd, Pb, and Ni were 0.002, 0.01, 0.12, and 0.031 mg kg^{-1}, respectively.

AM inoculum

The AM endophyte *G. intraradices* was obtained from the Division of Plant Protection and Microbial Technology, Central Institute of Medicinal and Aromatic Plants, Lucknow. The inoculum consisted of chopped root segments and soil from a 6-month-old pot culture of *G. intraradices* grown on palmarosa (*Cymbopogon martinii*, variety motia) in a sterile sandy loam soils. The inoculum contained about 90–100 propagules 10 g^{-1} soil.

Plant growth and measurements

Air-dried and sieved (<2 mm) soil (7 kg) was placed into each earthen pots. A basal dose of 30.0 mg of N and 25.0 mg K kg^{-1} of soil was applied to each pot by adding urea and KCl, respectively. The treatments (10 and 20 mg kg^{-1} soil of Cr as potassium chromate; 25 and 50 mg kg^{-1} soil of Cd, Pb, and Ni as cadmium sulfate, lead acetate, and nickel sulfate, respectively) were applied by adding aqueous solution of the required amount of metallic salts to soil in each pot. After carefully mixing the metals solution with soil, this was allowed to equilibrate for 21 days at field capacity soil moisture content. Sweet basil (*O. basilicum* L.; variety CIM-Somaya) seeds were surface-sterilized (gently soaking in a 1.0% NaOCl solution for 3 min, followed by repeated washing with sterile distilled water) and were sown in a plastic container (40 cm×30 cm×10 cm depth), loaded with 11.0 kg of air-dried autoclaved (20 min at 121°C and 15 psi) soil. The seeds were irrigated three times in a week and allowed to grow. At 35 days after seeding, two uniform seedlings of sweet basil were transplanted into each pot containing metal-treated soils on August 27, 2008. Sweet basil plants under each metal treatments were either left non-inoculated or inoculated with AM fungi *G. intraradices*. To inoculate the basil plants with AM fungi, 10.0 g of mycorrhizal inoculum *G. intraradices* was placed into planting hole in each pot before transplantation. Basil plants not inoculated with AM fungi received the same amount of the autoclaved inoculum. The soil water content in each pot was maintained near field capacity throughout the experiment. The pots were maintained under natural light and temperature conditions.

At the end of experimental trial (November 12, 2008), the plants were harvested, weighed, and sampled for mineral element analyses. The plant root samples were collected by uprooting the plants carefully, and roots were separated and sampled to evaluate the root colonization and to determine the root biomass. The dry weights of shoots and roots were determined after drying at 65°C to a constant weight. The essential oil content in the fresh shoot was determined by hydro-distillation for 2 h in a Clevenger-type apparatus. Oil yield was computed by multiplying the dry shoot yield by the oil content (ml 100 g^{-1} dry shoot). The essential oil thus obtained was dried over anhydrous sodium sulfate and stored in a sealed glass vials at 4–5°C prior to determination of chemical components.

Determination of root colonization

The fresh root samples were cleared with 10% KOH and stained with Trypan blue (0.1%) in lactophenol (Phillips and Hayman 1970). Mycorrhizal colonization was estimated by determining the percentage of the length of root segments containing AM fungal structures (arbuscules, vesicles, spores) according to Biermann and Linderman (1981).

Analytical methods

The chemical composition of the essential oil was determined using a Perkin-Elmer gas chromatograph Model Auto XL, equipped with FID and capillary column PE-5 (50 m×0.32 mm i.d., 0.25 μm film thickness), using a temperature program of 100 to 280°C at 3°C/min; carrier gas hydrogen at 10 p.s.i. inlet pressure; injector and detector temperatures, 220 and 280°C, respectively. Totalchrom software was used for peak percentage calculation. Identification of compounds was based on comparison of their retention indices and mass spectra with those obtained from authentic samples, the NIST version 2.1, and Wiley libraries registry of mass spectral data 7th edition, and the literature (Adams 1995).

Plant samples were thoroughly washed with tap water, distilled water and oven-dried at 65°C to a constant weight. After recording dry matter yield, the samples were ground in a stainless steel Wiley mill to pass 0.5-mm screen and stored in paper bags for chemical analysis. The finely ground plant samples were digested in the mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$; 10:4 v/v). The content of mineral elements, such as Cr, Cd, Pb, and Ni, in the digest were determined by inductively coupled plasma optical emission spectrometry (Perkin-Elmer, Optical Emission Spectrometer, Optima 5300 V).

Data analysis

Differences in the root colonization and herbage and root and oil yield were statistically analyzed for significance by analysis of variance using factorial randomized design (Panse and Sukhatme 1978), and treatments means were separated using the least significant differences (LSD) at 0.05 probability level.

Results

Mycorrhizal colonization

In the final harvest, the AM root colonization ranged from 6.0 to 60.0% (Fig. 1). The percent root colonization was significantly ($p<0.01$) higher in the basil plants inoculated with AM fungi *G. intraradices* than in the non-inoculated plants. The percent root colonization in the non-inoculated basil plants was not significantly affected by the application of Cr, Cd, and Ni, but it was significantly ($p<0.01$) declined by the application of the highest level of Pb (50.0 mg kg^{-1} soil) as compared to control. In AM-inoculated basil plants, the percent root colonization was significantly ($p<0.01$) increased by the application of lower level of Cr (10.0 mg kg^{-1}) and Pb (25.0 mg kg^{-1} soil) and further increase in the level of Cr and Pb decreased the root colonization. The percent root colonization in AM-inoculated basil plants was significantly ($p<0.01$) enhanced by the application of high level of Cd and Ni.

Plant biomass and essential oil yield

The shoot yield, content of essential oil, and roots yield of non-inoculated sweet basil were not significantly affected by the application of 10.0 mg Cr kg^{-1} soil, but further increase in the level of Cr decreased the shoot yield, content of essential oil, and root yield as compared to control (Table 1). The shoot yield, content of essential oil, and root yield of non-inoculated sweet basil were significantly ($p<0.05$) increased by the application of low dose (25.0 mg kg^{-1} soil) of Cr, Pb, and Ni as compared to control, and further increase in the level of Cr, Pb, and Ni decreased the shoot yield, content of essential oil, and root yield. When no metals were added (control soil), AM fungal inoculation significantly ($p<0.05$) increased the shoot yield, content of essential oil, and root yield of sweet basil, as compared to non-inoculated plants. In soils with low dose of metals (10.0 mg Cr, 25.0 mg Cd, 25.0 mg Pb and 25.0 mg Ni kg^{-1} soil) applied, AM fungal inoculation significantly ($p<0.05$) decreased the shoot yield, content of essential oil, and root yield of sweet basil, whereas, in soils with high dose of metal applied (20.0 mg Cr, 50.0 mg Cd, 50.0 mg Pb and 50.0 mg Ni kg^{-1} soil), it significantly ($p<0.05$) enhanced the shoot yield, content of essential oil, and root yield as compared to non-inoculated plants.

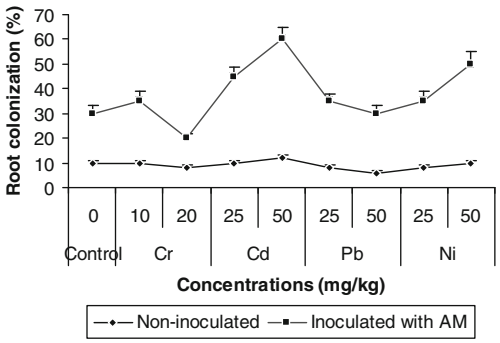

Fig. 1 Effect of metals on AM colonization of sweet basil roots. *Vertical bar* represents standard deviation of the mean value

Essential oil composition

The chemical composition of essential oil of sweet basil was significantly ($p<0.01$) altered by the application of metals and inoculation with AM fungi (Fig. 2). The content of linalool in volatile oil of non-inoculated sweet basil was significantly ($p<0.01$) decreased by the application of metals except in soils with low dose of Cr (10.0 mg kg^{-1}) and Pb (25.0 mg kg^{-1}). The content of linalool in volatile oil was significantly ($p<0.01$) enhanced by the inoculation of basil plants with AM fungi as compared to non-inoculated plants. The level of methyl chavicol in volatile oil of non-inoculated sweet basil was significantly ($p<0.01$) increased by the application of Cr, Cd, and Pb, but it was significantly ($p<0.01$) decreased by the application of Ni as compared to control plants. In control soil or soil supplemented with Ni, AM fungal inoculation significantly ($p<0.01$) increased the content of methyl chavicol in sweet basil oil as compared to non-inoculated plants, whereas in soils supplemented with Cr, Cd, and Pb, AM fungal inoculation significantly ($p<0.01$) decreased the content of methyl chavicol in sweet basil oil as compared to non-inoculated plants. The content of methyl eugenol in volatile oil of basil was significantly ($p<0.01$) increased by the application Cr, Cd, Pb, and Ni as compared to control, with the highest increase by application of Ni. Arbuscular mucorrhizal fungal inoculation significantly ($p<0.01$) increased the content of methyl eugenol in volatile oil of basil in soils with Cr, Cd, and Pb addition, but it decreased the methyl eugenol content in volatile oil of basil in soils with Ni addition.

Metals in plants

Metal accumulation in shoot and root tissues of sweet basil was significantly ($p<0.01$) affected by its application to soil and by AM inoculation (Fig. 3). The lead was accumulated in very low concentration in roots and shoot tissues of sweet basil as compared to accumulation of Cr, Cd, and Ni. In control soil (without metal addition) or in soil with low dose of metals (10 mg Cr, 25.0 mg of Cd, Pb, and Ni kg^{-1} soil) applied, AM fungal inoculation significantly ($p<0.01$) increased the concentration of Cr, Cd, Pb, and Ni in shoot tissues of basil plants, whereas in soil with higher dose of metals (20.0 g Cr, 50.0 mg of Cd, Pb, and Ni kg^{-1} soil) applied, AM fungal inoculation significantly ($p<0.01$)

Table 1 Effects of metals and AM fungi on the shoot, root, and essential oil yield of sweet basil

Elements	Level (mg kg^{-1})	Dry weight of shoot (g pot^{-1})		Oil yield (ml pot^{-1})		Dry weight of root (g pot^{-1})	
		Non-inoculated	Inoculated with AM	Non-inoculated	Inoculated with AM	Non-inoculated	Inoculated with AM
Control	0.0	15.3	22.7	0.132	0.142	1.30	1.98
Cr	10.0	15.3	7.2	0.133	0.060	1.18	0.82
	20.0	5.8	7.8	0.048	0.065	0.82	0.88
Cd	25.0	29.5	15.5	0.184	0.124	1.52	0.77
	50.0	25.3	26.9	0.152	0.162	1.30	1.44
Pb	25.0	19.0	7.2	0.158	0.043	1.55	0.84
	50.0	18.2	24.3	0.124	0.151	1.21	1.39
Ni	25.0	18.4	7.9	0159	0.069	1.93	1.29
	50.0	10.5	27.3	0.084	0.210	0.74	3.13
LSD (p=0.05)		Heavy metal (HM) =1.8, Inoculums (AM)=0.8, HM×AM=2.5		Heavy metal (HM) =0.012, Inoculums (AM)=0.005, HM×AM =0.017		Heavy metal (AM)=0.171, Inoculums (AM)=0.081, HM×AM =0.242	

decreased the concentration of Cr, Cd, Pb, and Ni in shoot tissues of sweet basil plants as compared to non-inoculated plant. The concentration of Cr, Cd, Pb, and Ni was significantly (p<0.01) higher in root tissues than in shoot tissues of sweet basil plants. When no metals were added (control soil), AM fungal inoculation significantly (p<0.01) decreased the concentration of Cr, Cd, Pb, and Ni in root tissues of sweet basil plant as compared to non-inoculated plants, whereas with the addition of Cr, Cd, Pb, and Ni in soils, AM fungal inoculation significantly (p<0.01) enhanced the concentration of those metals in root tissues of basil plants as compared to non-inoculated plants.

Discussion

We have demonstrated that the root colonization, shoot and root biomass, essential oil yield, chemical composition of essential oil, and metals accumulation in shoot and root tissues of sweet basil were significantly affected by the application of metals (Cr, Cd, Pb, and Ni) and inoculation with AM fungi *G. intraradices*. Non-inoculated sweet basil plants showed >10.0% root colonization due to native mycorrhizae, and the percent root colonization was significantly (p<0.01) enhanced by the inoculation of sweet basil with AM fungi. Metals have been reported to reduce or eliminate AM infection at high concentration in soils (Koomen et al. 1990). We have showed that the percent root colonization in inoculated sweet basil plants was significantly (p<0.01) reduced by the application of high level (20.0 mg kg^{-1}) of Cr, but it was significantly (p<0.01) enhanced by the application of Cd, Ni, and a low dose of Cr and Pb (10.0 and 25.0 mg kg^{-1}, respectively). The effect of metal application on the increase in root colonization was more pronounced with the application of higher levels of Cd and Ni than the application of low dose of Cr and Pb. Mechanisms underlying such striking differences in the mycorrhizal root colonization between plants grown at different levels of metals treatments are unknown; however, it is clear that the outcome of the plant–AM fungal association is metal-specific and depends on bioavailability of metals in soil and on both plant and AM species (Sudova and Vosatka 2007). High levels of mycorrhizal colonization were observed in agricultural soils contaminated with metals of different origins (Leyval et al. 1997).

The effect of metals on the crop yield depends upon the crop species, soil characteristics, and concentration of metals in soil. We have shown that the shoot yield, content of essential oil, and root yield of sweet basil were not significantly affected by the application of Cr at 10.0 mg kg^{-1} soil. Whereas they were significantly increased by low amount (25.0 mg kg^{-1} soil) application of Cd, Pb, and Ni to soil. Although Cr is not recognized as an essential plant nutrient, the stimulating effect of small amount of Cr on plant growth have been observed (Zayed and Terry 2003). The beneficial effect of Cd at low concentration on the growth of tomato seedlings has been reported by Dong et al. (2005). The same result has been shown with Pb on plant growth (Kabata-Pendis and Pandis 1984). Nickel can improve plant growth by stimulating urease activity (Witte et al. 2002). The negative effects of high level of Cr, Cd, Pb, and Ni on shoot yield, composition of essential oil, and root yield of sweet basil may be due to the accumulation of toxic level of these metals in plant tissues, which may decrease the chlorophyll content, photosynthetic rate, and root growth

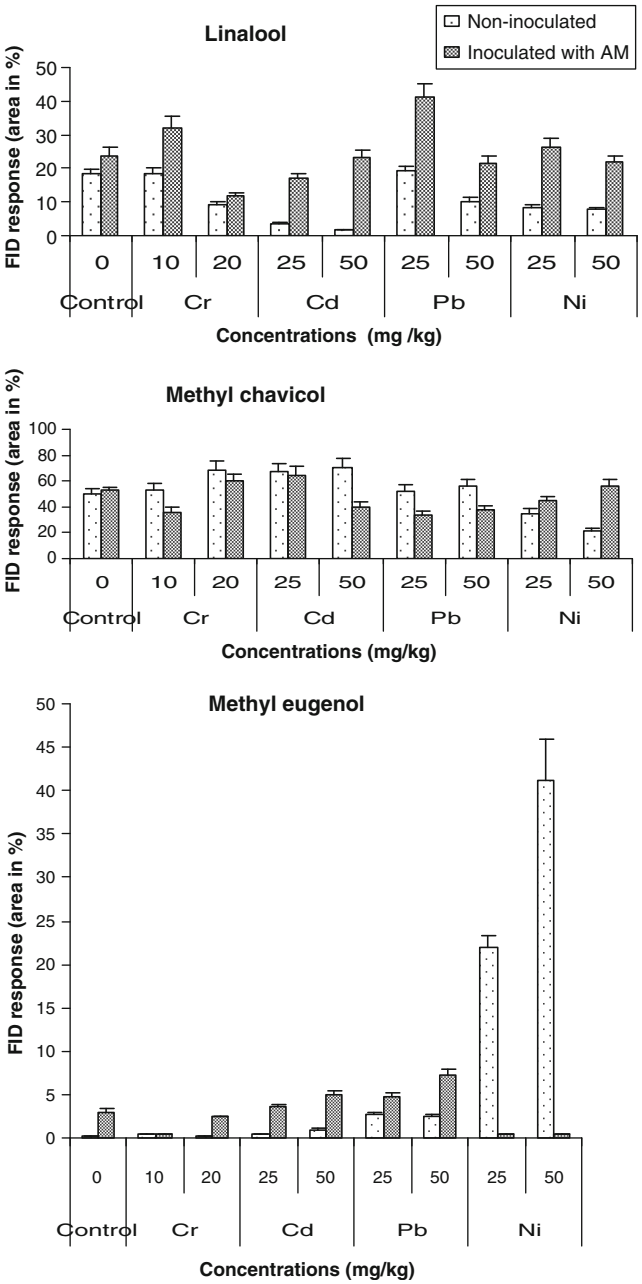

Fig. 2 Effects of metals and AM fungi on the chemical composition of volatile oil of sweet basil. *Vertical bar* represents standard deviation of the mean value

and thus affect uptake of plant nutrients (Adhikari and Singh 2007; Dong et al. 2005; Chattarjee et al. 2004, Sheoran et al. 1990).

AM fungal inoculation increased shoot yield, content of essential oil, and root yield of sweet basil probably due to the increase in the nutrient uptake (Marschner and Dell 1994). We have observed a significant interaction between metals (Cr, Cd, Pb, and Ni) and AM fungi. At low dose of metals addition, AM fungal inoculation decreased the shoot yield, the content of essential oil, and root yield of sweet basil, whereas at elevated levels of metal in soil, AM fungal inoculation showed an opposite behavior. In the former case, metals probably accumulated in shoot tissues, whereas the opposite occurred at high level of metals. It has been reported that metal uptake by mycorrhizal plants increases in soils with low metal concentration, but it can also decrease in soils with high levels of metals (Diaz et al. 1996).We have demonstrated that AM species could be potentially effective in protecting sweet basil exposed to high levels of metals. Mycorrhizal fungi are reported to protect plants from the toxic effects of high external concentration of several metals, possibly by binding the metals in their hyphae or by reducing the translocation of metals to the plant tops (Mozafar et al. 2002).

We have demonstrated that the content of monotepenoids (linalool) and phenylpropanoids (methyl chavicol and methyl eugenol) in sweet basil oil was altered by the application of metals and AM fungal inoculation. The content of linalool in sweet basil oil was decreased by the application of metals, but it was increased by the inoculation with AM fungi. A decrease in the linalool and an increase in 1–8 cineole content in the essential oil of sweet basil by the application of high levels of Cu, Cd, Mo, and Zn through municipal solid waste compost were also demonstrated by Zheljazkov and Warman (2004). We have observed a remarkable increase in content of methyl eugenol in volatile oil sweet basil with the exposure of Ni treatment. A 15- to 20-fold decrease in the concentration of pseudohypericin and hypericin was recorded in St. John's wort (*H. perforatum* L.) by the application of Ni in culture medium (Murch et al. 2003). The AM fungal inoculation maintained the level of linalool, methyl chavicol, and methyl eugenol in sweet basil oil, which were either increased or decreased by the application of metals. The mechanisms by which the various constituents in the volatile oil of sweet basil was increased or decreased by the application of metals and AM species are unknown, but it could be related to the uptake of essential and nonessential/phytotoxic metals. AM fungi enhanced the uptake of P and divalent metallic cations such as Cu, Zn and Fe in plant tissues (Marschner and Dell 1994). In some enzymes, metals are essential for the catalytic function by forming enzyme–substrate–metal complexes (Marschner 1995); metal enzymes play an important role in synthesis of monoterpene classes, which can produce several compounds (Harrewijin et al. 2001). Differences in the content of mono- and sesqui-terpens in mycorrhizal and non-mycorrhizal *Artemisia annua* have been reported by Rapparini et al. (2008) and probably were due to AM-specific regulation of the different enzymes used in terpene synthesis. An investigation on the effect of different AM fungal species on phytochemicals in sweet basil showed that the concentration of rosamarinic and caffeic acids

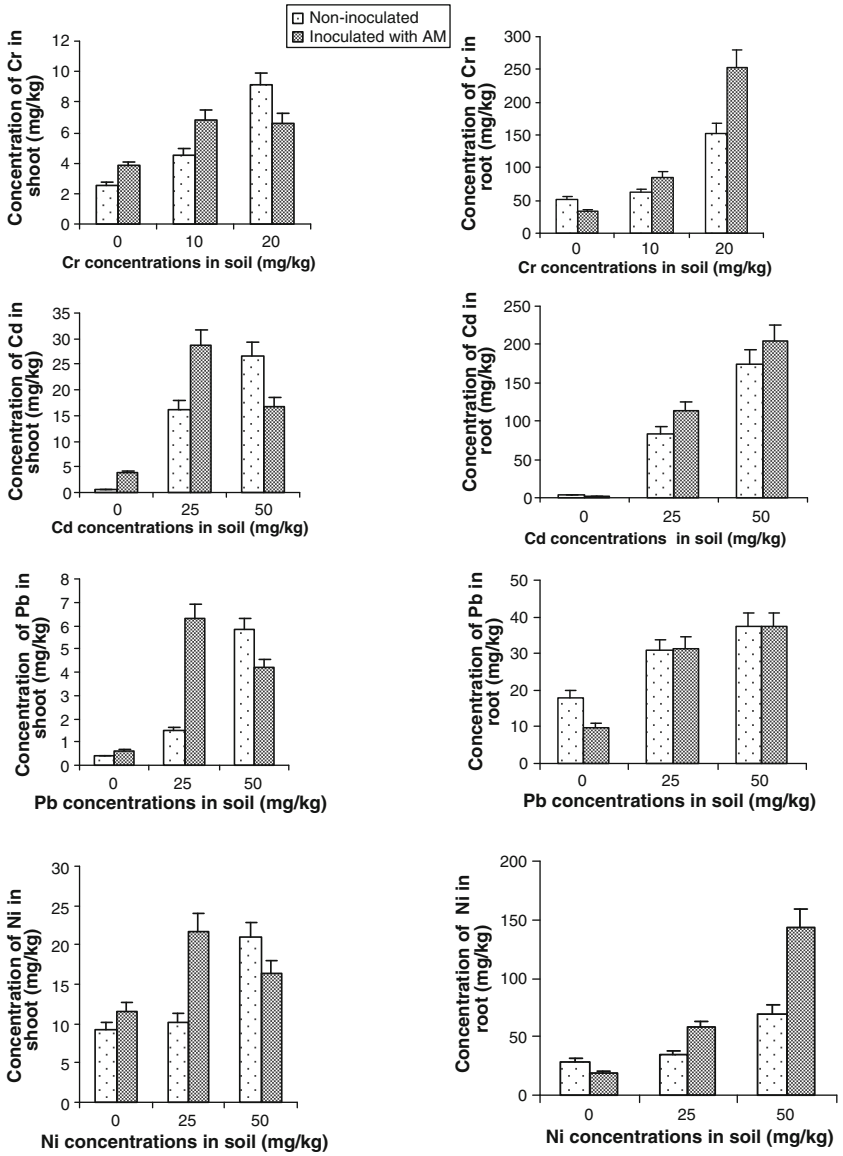

Fig. 3 Effect of metals and AM fungi on the accumulation of Cr, Cd, Pb, and Ni in sweet basil. *Vertical bar* represents standard deviation of the mean value

increases with *G. caledonium* and that of caffeic acid with *G. mossae* (Toussaint et al. 2007).

The metal concentration in plant is a function of metal content in the environment, because plants growing on metal-contaminated soils can take up the metal via root system (Kabata-Pendis and Pandis 1984). We have showed that there were increases in the concentration of metals in shoot and root tissues of non-inoculated sweet basil with the increases in the concentration of metals in soil. Among Cr, Cd, Pb, and Ni, Pb was relatively accumulated with the lowest concentration in shoot and root tissues of sweet basil. This might be attributed to the very low solubility of Pb in soil. Pb is known to bind to clay and organic matter, and it can be included in insoluble precipitates, making Pb unavailable for root uptake (Angelova et al. 2008). Metals are preferentially accumulated in the roots and partially translocated to the shoot. In the present study, the higher concentration of Cr, Cd, Pb, and Ni in the root tissues than in the shoot tissues indicates that the sweet basil possesses a regulatory mechanism that prevents the translocation of these metals from root to shoot. Zyed et al. (1998) demonstrated that Cr is poorly translocated from roots to shoots. Angelova et al. (2008) reported that the actively growing roots promote a barrier that restricts the movement of Pb to the aboveground parts of the plant. We have shown higher concentration of metals in roots of mycorrhized than non-mycorrhized sweet basil roots in soils amended with heavy metals. The higher AM–plant metal content of roots could be attributed to fungal metal binding and sequestration in intraradical hyphae, and these metal forms are not bioavailable to plants (Christie and Chen 2004). A hyper-accumulator plant takes up large quantities of contaminants

from soil and sequesters them in the aboveground biomass. The sweet basil is probably a non-hyperaccumulator of Cr, Cd, Pb, and Ni.

AM fungi may protect plants from the potential toxicity caused by metals, although this depends on plant growth conditions, AM fungus, and the metal (Weissenhorn et al. 1995c). Metals uptake by mycorrhizal plants increases in soils with low metal concentration, but it can decrease in soils with a high level of metals (Diaz et al. 1996). These results support our findings that AM fungal inoculation enhanced the concentration of metals in shoot tissues of sweet basil in soil with low metal concentration and reduced the concentration of metals in shoot tissues of basil in soils with high metal concentration. The retention of heavy metals by the fungal mycelium probably involves adsorption to cell walls, fixation by polyphosphate granules (Galli et al. 1994), and indirect effects of mycorrhizae on plant mineral nutrition, especially P nutrition (Christie and Chen 2004).

Conclusion

The growth and yield of sweet basil (*O. basilicum* L.) was restricted by the application of high level of metals such as Cr, Cd, Pb, and Ni. The deleterious effects of Cr, Cd, Pb, and Ni on the growth and yield of sweet basil were due to the toxicity of metals and/or inhibition in root growth. The significant changes in the content of linalool, methyl chavicol, and methyl eugenol in the volatile oil of sweet basil by the application of metals and AM fungi inoculation indicate that the metal and AM species had significant impact on the quality of oil produced. In soils with high level of metals applied, AM fungal inoculations can immobilize metals in the roots and limit their translocation to shoots; consequently, AM can have beneficial effects on the growth and yield of sweet basil. Thus, the AM–sweet basil symbiosis could be used as plant-based strategies for revegetation and phytostabilization of Cr-, Cd-, Pb-, and Ni-contaminated soils.

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