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Evaluation of the effect of *Funneliformis mosseae* on enhancing the tolerance of *Capsicum annuum* L. to cadmium and lead stress

Sarah Jamal Jalil
* Corresponding author. Iraq Natural History Research Center and Museum, University of Baghdad, Baghdad, Iraq. E-mail: sarah.g@nhm.uobaghdad.edu.iq

Thamer A. A. Muhsen
Department of Biology, College of Education for Pure Science, Ibn Al- Haitham, University of Baghdad, Baghdad, Iraq. E-mail: thamer.a.m@ihcoedu.uobaghdad.edu.iq

Shaymaa A. H. Neamah
Department of Anatomy; Faculty of Medicine, Jabir Ibn Hayyan University, Baghdad, Iraq. E-mail: shaymaa.a.neamah@jmu.edu.iq

Abstract

Objective

Heavy metal pollution harms plants, so using biological methods to mitigate it is important. Arbuscular mycorrhizal fungi have been known to enhance mineral nutrition to plants, particularly uptake of comparatively immobile nutrients like phosphorus (P), zinc (Zn) and copper (Cu), and increase nitrogen uptake and water use efficiency. This study aimed to evaluate the effectiveness of *Funneliformis mosseae* inoculation in enhancing the growth and tolerance of *Capsicum annuum* L. under cadmium (Cd) and lead (Pb) stress conditions.

Materials and methods

The experiment was conducted over a period of 160 days using areplicated experimental design to evaluate the effects of *Funneliformis mosseae* under lead and cadmium stress. A pot experiment was conducted under the greenhouse conditions at the University of Baghdad. The experiment utilized a completely randomized design (CRD) featuring six replicates for each treatment. These treatment groups were established to assess the growth of *Capsicum annuum* plants in clay soil. Plant growth was assessed by measuring fresh and dry weights and stem and root while Pb and Cd concentrations were determined in root tissues and soil using Atomic Absorption Spectrophotometry (AAS). The statistical analysis of the experimental data was performed using the Statistical Analysis System (SAS) software (Version 9.4)

Results

Statistical analysis using two-way ANOVA indicated significant differences ($P \leq 0.05$) among the experimental treatments. Inoculation with *Funneliformis mosseae* improved the growth of hot pepper plants, as reflected by higher fresh and dry biomass together with increased shoot and root lengths under lead and cadmium stress. Furthermore, AAS analysis revealed lower Pb and Cd concentrations in the roots of mycorrhiza-inoculated plants, suggesting that mycorrhizal inoculation effectively reduced heavy metal stress.

Conclusion

This study provides evidence that mycorrhizal inoculation can be integrated into sustainable management strategies for crops grown in heavy metal-contaminated soils. The use of *Funneliformis mosseae* offers a practical biological approach to improving plant performance while minimizing the impact of lead and cadmium on agricultural systems.

Keywords*: Capsicum annuum* L., clay soil, *Funneliformis moseae*, heavy metals, mycorrhiza

Paper Type: Research Paper.

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Introduction

Fungi are eukaryotic, saprophytic, mutualistic and parasitic organism, some of which are plant, animal and human pathogenic (Stamatiades et al., 2018; Mohammed et al., 2020). One of the most useful beneficial fungi is arbuscular mycorrhizal fungi (AMF). They create mutualistic associations with roots of most terrestrial plants (Al-Ashbal et al., 2025; Jalil et al., 2025). In this symbiosis, vegetation provides photosynthetically-based carbon to the fungi and AMF enhance nutrient and water availability in soil by large extra-radical networks of hyphae. Due to these multifunctional advantages, AMF have been gaining more and more attention in sustainable agriculture, stress tolerance, and environmental remediation. Arbuscular mycorrhizal fungi have been known to enhance mineral nutrition to plants, particularly uptake of comparatively immobile nutrients like phosphorus (P), zinc (Zn) and copper (Cu), and increase nitrogen uptake and water use efficiency (Zou et al., 2015; Nacoon et al., 2020; Yasir et al., 2024). Their hyphae grow beyond the nutrient depletion zone around roots and thus they increase the effective absorptive surface area of plants. Recently, a meta-analysis study across the globe revealed that AMF inoculation significantly enhanced plant biomass, nitrogen concentration, phosphorus concentration, and the entire plant nutrient uptake in numerous crop species. Root system architecture may also be altered by AMF colonization. Previous research showed that mycorrhizal roots tend to have more branching and better root hair than non-mycorrhizal controls (Zou et al., 2017). This kind of structural plasticity enhances soil exploration and nutrient apprehension. Furthermore, AMF improve plant drought tolerance via osmotic adjustment, stimulation of antioxidant defense, enhanced stomatal control, and enhanced soil aggregation around the rhizosphere (Zou et al., 2015). These advantages are especially worthwhile in soils of low nutrient status or degraded soils that have low conventional fertilizer efficiency. Edaphic factors like soil moisture, pH, salinity and competing microbial populations may however affect the effectiveness of AMF. High water levels can lower AMF activity by promoting other fungi or by reducing the oxygen concentration in the rhizosphere (Randhawa & Amitabha, 2004). However, AMF are still valuable biological agents under the majority of field conditions to ensure reduced fertilizer dependence and enhanced crop productivity. Natural sources of heavy metals in soil are the natural weathering of mineral and anthropogenic activities, which involve mining, industrial effluent, sewage sludge, fertilizers, batteries, paints and ex-metal containers (Noaman & Al-Mayaly, 2023). It needs low doses of some of its micronutrients including iron, manganese, zinc and copper, but a number of other metals, including cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As) and nickel (Ni) are toxic when present in large doses. These pollutants destroy the growth of plants, prevent the actions of microbes and pose a risk to the safety of food. AMF have significant roles in immobilization or detoxification of heavy metals in polluted soils. They have functional groups in their hyphae and cell walls that can bind metal ions, thus limiting metal mobility and bioavailability. AMF prevent the translocation of metals between roots and shoots in the root to shoot of many host plants by trapping fungal structures or root cortical tissues (Chen et al., 2015). It has been demonstrated that AMF reduce the negative impact of heavy metal phytotoxicity by biosorption, chelation, compartmentalization, antioxidant enhancement, and transporter gene regulation. The other significant process is glomalin-associated soil proteins that are synthesized by AMF and lead to the aggregation of soil and the ability to trap toxic metals thus stabilizing the contamination in the rhizosphere. AMF also enhance plant vigor by improving nutrition, which has a dilution effect where the concentration of metals per unit biomass decreases. Protection however differs depending on the AMF species, host plant, soil characteristics and the nature of the contaminant. Thus, it is usually suggested to use native or metal-tolerant AMF strains in remediation programs. There are three main mechanisms proposed in previous studies that AMF help alleviate metal toxicity: (i) the metals in the rhizosphere and fungal structures are immobilized, (ii) the uptake of nutrients and water is improved and can counter-act the effects of stress, and (iii) the antioxidant and physiological defense mechanisms in host plants are stimulated. The size of these benefits however, is highly dependent on plant species and fungal identity as well as soil conditions (Ma et al., 2022; Zhang et al., 2018). Phytoremediation is an ecologically friendly process that involves the use of plants and related microorganisms to eliminate, stabilize or convert pollutants in the soil and water (Al-Maliki & Al-Mousawi, 2015). Of particular concern are vegetables grown in these contaminated soils whereby the edible tissues can accumulate toxic metals and pass them down to the human food chain. Phytoremediation aided by AMF has thus become a potential alternative towards safer production of vegetables. AMF is able to reduce the absorption of the heavy metals into the food organs and has been shown to ensure biomass production when combined with vegetable crops, such as tomato, lettuce, spinach, pepper, and *Brassica* species. AMF may be utilized to control phytoextraction (increased uptake in harvestable biomass) or phytostabilization (metals in roots and soil) depending on the crop and remediation objectives. Today, an investigation reported that AMF enhance the level of remediation through enhancing the growth of plants, raising their ability to withstand stress, and directly engaging in the immobilization or transformation of contaminants. AMF can also be useful in vegetable systems since they enhance soil fertility and diversity of microbes, thus facilitating long term restoration of the land. Native AMF spores in soil or air can be naturally dispersed and colonize crop root and re-establish useful symbioses (Al-Salman et al., 2021). The use of AMF inoculants combined with organic amendments, biochar, compost and proper choice of crops can also achieve greater improvements in remediation. AMF, therefore, are a viable biological tool to use in sustainable production of vegetables on marginal or moderately polluted soils with reduced environmental and human health hazards. There is still little information on the effectiveness of *Funneliformis mosseae* in increasing the tolerance of chili pepper (*Capsicum annuum* L.) in clay-rich soils with lead (Pb) and cadmium (Cd), and especially in conditions that reflect those of agricultural soils in Iraq. Clay soils have a high adsorption and have limited aeration that can change the bioavailability of metals and the effectiveness of AMF colonization. Thus, this research was conducted to analyze whether the inoculation with *F. mosseae* increased the growth, biomass production and metal tolerance of *C. annuum* grown in Pb- and Cd-contaminated clay soil. It was hypothesized that AMF inoculation would decrease metal stress and improve the performance of the plant significantly, compared to no inoculation.

Materials and Methods

Experimental site: The pot experiment was carried out using clay soil collected from an agricultural field in Al-Aziziyah District, Wasit Province, Iraq as a growth medium. The soil was air-dried, hand-crushed and screened through a 2-mm screen to remove stones, coarse particles and plant debris before use. The experiment was carried out to investigate the effect of heavy metal stress on sweet pepper plants under different experimental treatments (Rodríguez et al., 2016).

Source of fungal isolates: The arbuscular mycorrhizal fungal inoculum (*Funneliformis mosseae*) used in this study was obtained from Horticulture Administration Office, Ministry of Agriculture, Iraq. The inoculum was applied at the rate of 30 g per pot by placing it directly into the planting hole at the time of transplanting next to the root system.

Pot experimental design: A pot experiment was conducted under the greenhouse conditions at the University of Baghdad. Prepared agricultural soil samples were distributed into plastic pots, each containing 3 kg of soil. Healthy seeds of chili pepper were grown in the plastic pots filled with the agricultural soil. Ten seedlings of *Capsicum annuum* plants were placed in each pot. The experiment utilized a completely randomized design (CRD) featuring six replicates for each treatment. Six treatment groups were established to assess the growth of *Capsicum annuum* plants in clay soil for 160 days. Each treatment consisted of one specific amendment to create six groups. Group1 (Control group [Normal]: clay soil without any supplementation); Group 2 (Cadmium group [Cd]: clay soil supplemented with cadmium); Group 3 (Lead group [Pb]: clay soil supplemented with lead); Group 4 (*Funneliformis mosseae* group [FM]: clay soil inoculated with beneficial *Funneliformis mosseae)*; Group 5: *Funneliformis mosseae* + Lead group (FM + Pb): clay soil with both *Funneliformis mosseae* and lead); and Group 6: *Funneliformis mosseae* + Cadmium group (FM + Cd): clay soil with both *Funneliformis mosseae* and cadmium). Soil contamination was carried out by adding aqueous solutions of lead (2ml/kg dry soil) and cadmium (2ml/kg dry soil), followed by thorough mixing to ensure uniform distribution. The inoculum of *Funneliformis mosseae* was applied at 30 g pot 3 kg soil for each pot. Inoculum was placed directly into the planting hole in close contact with the root system at the time of transplanting to ensure effective root colonization of the mycorrhizal fungus. A natural photoperiod (about 12 h light/12 h dark) was used and temperature was kept at 25 $\pm$ 3^{o}C daytime and 18 $\pm$ 2^{o}C at night. The pot irrigation was done after every two days to ensure that field capacity was at about 70%. The establishment of ten seedlings per pot was initially done to achieve similar stand density and counteract the initial loss of seedlings to achieve similar competition among treatments. Growth parameters and physiological responses were monitored to evaluate the effects of treatments, including stem length, root length, soft weight of the plant and dry weight, biomass accumulation, and heavy metal uptake. During the 160-day experiment, one plant was randomly sampled from each replicate every three weeks. The shoot length, root length and fresh weight of the sampled plants were determined. The plant Samples were oven-dried to constant weight for dry weight determination. after 160 days of experimental period, soil and root samples were collected from the remaining plants and sent to the laboratories of the Iraqi Authority for Science and Technology for the determination of cadmium (Cd) and lead (Pb) concentrations using Atomic Absorption Spectrophotometer (AAS, AA-7000, SHIMADZU Corporation, Japan) according to the standard analytical procedures that are adopted by the laboratory.

Graphite furnace atomic absorption spectrophotometer (AAS): Flameless Atomic absorption spectrophotometer method by the technique of Graphite Furnace (GFAAS) was used to determine trace elements. SHIMADZU AA7000 Atomic Absorption Spectrophotometer was used for determination of these elements. GFAAS is one of the most important of the five techniques of the atomic absorption spectrometry in which has the higher sensitivity which can be reach to the low detection limits (in ppb units).

Digestion of soil samples for heavy metal determination: Wet digestion method was used for digestion of the soil samples. A 0.5 g portion of each air-dried, ground, and sieved soil sample was accurately weighted into a digestion tube. Then, 6 ml aqua regia and 1.5 ml H_2O_2 were measured and added into the digestive tube and swirled gently to mix the sample properly. The digestion tubes were then placed on a digestive furnace and heated at a temperature of 180℃ for 3 h. All the digests were cooled and filtered through Whatman No. 42 filter paper and diluted to 50 ml with double distilled water. Each sample was digested in replicates of five and transferred to acid-washed stoppered glass bottle, labeled and kept for metal analysis (Addis & Abebaw, 2017).

Plant sample preparation for heavy metal determination: Approximately 2.0 grams of each dried (at 105°C) and ground plant sample were weighed into clean, labeled conical flasks for heavy metal determination (Addis & Abebaw, 2017).

Determination of heavy metals in experimental samples: To determine the amount of cadmium in experimental samples, five standard solutions of cadmium were prepared (2.5, 5, 10, 15, 20 ng/ml) for the calibration curve. The concentrations of Cd in samples were determined directly and continuously, by reference to the calibration curve, rather than solely through the measurement of standard solutions. The analytical conditions were set as follows: lamp current, 8 mA; wavelength, 228.8 nm; slit width, 0.5 nm; lighting mode, BGC-D2; sample volume, 20 µl; and the analysis was performed in triplicate with three replicates (Schuh et al., 2022). The concentration of lead in the experimental samples was determined by initially preparing four concentrations (5, 10, 15, 20 ng/ml) of lead standard solutions. The concentration was measured directly and continuously by comparing sample absorbance to that of standard solutions, using the calibration curve. The analytical conditions were set as follows: lamp current, 10 mA; wavelength, 217.0 nm; slit width, 0.5 nm; lighting mode, BGC-D2; sample volume, 20 µl; and the analysis was performed in triplicate with three replicates (Schuh et al., 2022).

Statistical analysis: The statistical analysis of the experimental data was performed using the Statistical Analysis System (SAS) software (Version 9.4). Prior to analysis, data were tested for normality and homogeneity of variance. The effects of treatment, sampling time, and their interaction on all measured parameters were analysed by a two-way analysis of variance (Two-way ANOVA). If significant differences were found, the treatment means were separated using the Least Significant Difference (LSD) test at $P \leq 0.05$. Results are expressed as mean ± standard deviation (Mean ± SD).

Results and discussion

Calibration curve for (cd) and (pb) determination: The concentrations (expressed in parts per billion; ppb) of cadmium (Cd) and lead (Pb) in both the soil and root tissues of *Capsicum annuum* were determined using the standard calibration curves (Figures 1 and 2). Mycorrhizae are a symbiotic relationship that benefits the plant and may protect it from pathogens. In addition to these benefits, the relationship may also reduce the effect of heavy metals (Weng et al., 2022). Heavy metals may naturally occur in soil as inherent constituents or be introduced from external sources, such as atmospheric deposition (via rainfall or precipitation) and hydrological processes (including groundwater and surface water movement). The amount of cadmium and lead in the soil and root tissues of *Capsicum annuum* are presented in Table 1 and 2. The concentration of lead is relatively high, and this observation is due to several reasons, including car exhausts, pesticide usage, and catalysts used in agriculture, geological factors, dyes and building materials. This high contamination was first present in the soil and then transferred to the plant. In addition, lead and cadmium are similar in their composition to some basic elements such as calcium, iron, and zinc (Cook & Ni, 2007; Yu et al., 2022).

Figure 1. Standard curve for determining cadmium concentrations in experimental samples

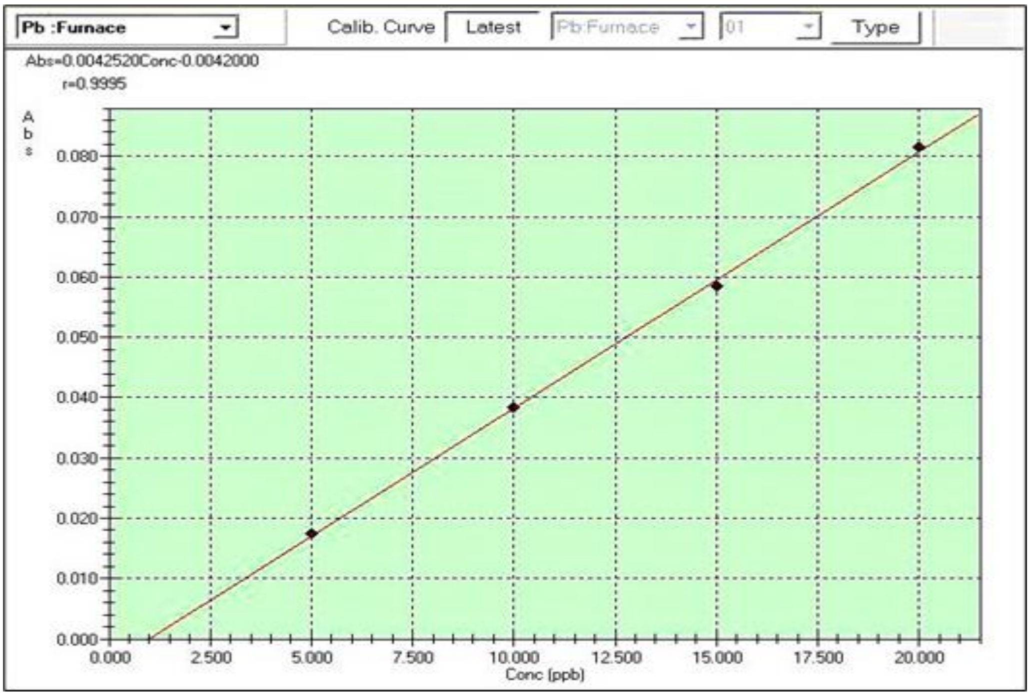

Figure 2. Standard curve for determining the concentration of lead in experimental samples

Effect of experimental treatments on plant growth parameters: The frequency of AMF colonization rose gradually with cultivation period, and peaked at 160 days, which showed that *F. mosseae* can be established successfully even in the presence of Pb and Cd stress. The same trend was observed in biomass responses whereby inoculated plants were found to have high fresh and dry weights as compared to the uninoculated plants. Treatments with metals lowered root and shoot elongation, and AMF co-application partially recovered growth, indicating alleviation of toxicity by enhancing nutrient acquisition and minimization of translocation of metals. As shown in Table 3 the treatment, cultivation period and their interaction significantly influenced the mycorrhizal colonization frequency (F%) ($P \leq 0.05$).

Table 1. Concentrations of cadmium and lead in the root tissues of *Capsicum annuum*

Root	Cd (ppb)	Pb (ppb)
Pb-Clay soil	11	2.7
Cd-Clay soil	98	0.75
FM-Cd-Clay soil	41	1.34
FM-Pb- Clay soil	BDL	1.36

BDL: Below detectable limit

Table 2. Concentrations of cadmium and lead in the soil samples

Soil	Cd (ppb)	Pb (ppb)
Pb- Clay soil	33	103
Cd- Clay soil	37	58
FM-Cd-Clay soil	166	157
FM-Pb- Clay soil	186	535

The highest colonization frequency (93.39 % after 160 days) was obtained in the plants inoculated with *Funneliformis mosseae* alone. Cadmium and lead significantly reduced the fungal colonization, with cadmium having the most inhibitory effect. colonization increased with progressive time of cultivation in all treatments showing the gradual establishment of mycorrhizal symbiosis. The increased inhibition may be due to higher toxicity of cadmium to fungal hyphae and root tissues. In particular, the lower bioavailability of lead in clay soil may have alleviated the effect. These results agree with recent reports that arbuscular mycorrhizal fungi enhance plant tolerance to heavy metal stress, but cadmium is more harmful to mycorrhizal colonization than lead. This is consistent with the results of Jia et al. (2026).

Table 3. Effect of the mycorrhizal frequency (F%) of *Funneliformis mosseae* on the roots of Sweet Pepper plants in Clay soil during 160 days of cultivation

Treatments	60 days	80 days	100 days	130 days	160 days	
Mycorrhiza+ Cadmium	14.46	22.86	29.28	41.31	55.06	32.59±6.74
mycorrhiza+ lead	15.15	26.59	37.34	53.66	67.24	40.00 ±9.81
mycorrhiza	24.24	46.52	65.37	81.64	93.39	62.23±7.93
Mean:Period	17.95±0.83	31.99±3.48	43.99±6.25	58.87±9.58	71.89±9.94	
LSD (0.05)	Treat:4.457*		Period:7.386*		Treat. ×Period: 10.646 *	

Results presented in Table 4 showed that root dry weight was significantly ($P \leq 0.05$) affected by treatment, the cultivation period and their interaction. *Funneliformis mosseae* inoculation alone resulted in the highest root dry weight during the experiment, increasing from 0.25 g plant-1 at 60 days to 0.99 g plant-1 at 160 days (average 0.63 ± 0.07 g plant-1). On the other hand, plants exposed to cadmium had the lowest mean value (0.29 ± 0.03 g plant-1) and plants treated with lead had an intermediate response (0.35 ± 0.04 g plant-1). Moreover, root dry weight increased significantly during the cultivation period with the highest mean value (0.69 ± 0.08 g plant-1) after 160 days. This indicates that the longer growth period favored the accumulation of root biomass. Inoculation with *F. mosseae* resulted in an increase in root dry weight which may be due to establishment of an effective arbuscular mycorrhizal association that improves phosphorus uptake, water absorption and uptake of important mineral nutrients through the extensive extraradical hyphal network. Therefore, plants will develop a stronger root system and will allow higher biomass production These results are consistent with what has been concluded by Gao et al. (2023). However, cadmium and lead strongly inhibited the root growth probably due to nutrients uptake deficiencies, inhibition of cell division and elongation, oxidative injury and impairment of root metabolic activities. However, mycorrhizal inoculation may alleviate heavy-metal toxicity by limiting the translocation of metals, enhancing antioxidant defense systems and maintaining the homeostasis of nutrients, thus partially restoring root development under stress conditions.

Table 4. Effect of the weight of mycorrhizal dry roots (g. plant-1) of *Funneliformis mosseae* on the roots of Sweet Pepper plants in Clay soil during 160 days of cultivation

Treatments	60 days	80 days	100 days	130 days	160 days	
Mycorrhiza+ Cadmium	0.12	0.18	0.26	0.41	0.49	0.29±0.03
mycorrhiza+ lead	0.15	0.23	0.34	0.46	0.58	0.35 ±0.04
mycorrhiza	0.25	0.42	0.67	0.84	0.99	0.63±0.07
Mean:Period	0.17±0.01	0.28±0.02	0.42±0.04	0.57±0.06	0.69±0.08	
LSD (0.05)	Treat:0.007*		Period:0.009*		Treat. ×Period: 0.046 *	

The results presented in Table 5 The treatment, growing period and their interaction had significant effect ($P \leq 0.05$) on mycorrhizal dependency (MD%) of sweet pepper plants. The MD% increased progressively in all treatments during the cultivation period and reached its maximum after 160 days. The maximum mean MD% was observed in plants inoculated with *Funneliformis mosseae* in response to cadmium stress (143.69%) followed by plants treated with lead (122.93%). The plants inoculated with mycorrhiza alone showed the minimum mean value (103.88%). These results suggested that the dependence of sweet pepper plants on the mycorrhizal symbiosis increased under heavy metal stress, especially cadmium. The increased dependence under cadmium stress indicates that the fungus increased plant tolerance by promoting nutrient uptake, lowering metal toxicity and enhancing physiological adaptation. Similar results have been reported in recent studies showing that arbuscular mycorrhizal fungi enhance plant dependence under heavy metal stress by improving mineral nutrition, antioxidant defense, and metal immobilization in the rhizosphere (Begum et al., 2019).

Table 5. Effect of the mycorrhizal dependency MD% of *Funneliformis mosseae* on the roots of Sweet Pepper plants in Clay soil during 160 days of cultivation

Treatments	60 days	80 days	100 days	130 days	160 days	
Mycorrhiza+ Cadmium	98.32	121.25	143.41	166.68	188.79	143.69±8.22
mycorrhiza+ *lead*	92.19	107.29	121.55	138.46	155.18	122.93±6.48
mycorrhiza	80.33	92.36	104.22	115.80	126.71	103.88±5.39
Mean:Period	90.28±3.19	106.97±5.62	123.06±6.67	140.31±8.84	156.89±9.24	
LSD (0.05)	Treat:4.857*		Period:3.725*		Treat. ×Period: 5.484 *	

The results of Table 6 showed that the presence of *Funneliformis mosseae* significantly decreased the accumulation of cadmium and lead in sweet pepper roots ($P \leq 0.05$). The non-mycorrhizal plants accumulated cadmium and lead up to 535 and 335 ppb, respectively, while mycorrhizal inoculation reduced their accumulation to 153 and 98 ppb, i.e., about 80.7% and 70.7% reduction, respectively. The higher accumulation of cadmium than lead suggests that cadmium is more mobile and bioavailable in plant tissues. *F. mosseae* plays a protective role, fixing metals in the rhizosphere and in fungal structures, reducing their movement to roots and enhancing the selective absorption of nutrients, as evidenced by the marked reduction in heavy metal accumulation after mycorrhizal inoculation. These results are consistent with recent studies reporting that arbuscular mycorrhizal fungi are alleviating heavy metal toxicity by reducing metal uptake, promoting plant growth under metal stress. These findings are consistent with the results of Jia et al. (2026).

Table 6. Effect of the heavy metals (ppb) and *Funneliformis mosseae* on the roots of Sweet Pepper plants in Clay soil

Treatments	Control (without heavy metals)	Cadmium	Lead
Clay soil	0	37	27
Roots without mycorrhiza	0	535	335
Roots with mycorrhiza	0	103	98
LSD (0.05)		9.453*	7.276*

The results presented in Table 7 showed significant effects of treatment, growing period and their interaction on stem length of sweet pepper plants ($P \leq 0.05$). All treatments resulted in gradual increase of the stem length during experimental period but heavy metal stress significantly inhibited plant growth. Cadmium resulted in the highest reduction of stem length with mean value 17.60 cm followed by lead (19.32 cm) as compared to control (24.54 cm). On the other hand, *Funneliformis mosseae* inoculation significantly increased stem growth, with the maximum mean stem length of 33.82 cm, while mycorrhizal inoculation under stress of cadmium and lead increased the mean stem length to 25.30 and 26.74 cm respectively. These results indicated that *F. mosseae* can effectively relieve the inhibition of plant growth by heavy metals. The increase in stem length is probably associated with increased nutrient and water absorption, increased photosynthetic efficiency, enhanced antioxidant defense, and decreased heavy metal absorption leading to enhanced plant development under stress conditions. These results are in line with recent studies reporting that arbuscular mycorrhizal fungi enhance vegetative growth and ameliorate heavy metal toxicity through improved plant nutrition, regulation of oxidative stress and limiting metal translocation to plant tissues (Alamri et al., 2022).

Table 7. Effect of fungus *Funneliformis mosseae* and heavy metals on length of stem (cm) on Sweet Pepper plants in Clay soil during 160 days of cultivation

Treatments	60 days	80 days	100 days	130 days	160 days	Mean:Treat
control	13.30	17.50	24.00	31.40	36.50	24.54±3.53
Cadmium	11.10	14.10	18.50	21.20	23.10	17.60±2.41
Lead	12.00	15.40	20.80	23.30	25.10	19.32±2.89
Mycorrhiza+ Cadmium	14.30	19.80	26.10	31.30	35.00	25.30±3.96
mycorrhiza+ *lead*	15.10	20.50	27.30	33.60	37.20	26.74±4.09
mycorrhiza	17.20	24.50	32.50	41.60	53.30	33.82±5.79
Mean:Period	13.83±0.79	18.63±0.98	24.87±1.51	30.40±1.74	35.03±1.81	
LSD (0.05)	Treat:1.571*		Period:2.438*		Treat. ×Period: 3.521 *	

Results of Table 8 indicated that treatment, cultivation period and their interaction had significant effects on root length ($P \leq 0.05$). During the cultivation period, the root length significantly increased in all treatments, but root growth was significantly inhibited by cadmium and lead compared with the control. Cadmium had the highest reduction in mean root length (10.92 cm) followed by lead (12.34 cm) and mycorrhizal inoculation greatly improved the root development, with the highest mean value (23.04 cm). Moreover, root length was significantly improved by inoculation of *Funneliformis mosseae* under cadmium and lead stress in comparison with non-mycorrhizal plants under the same stress. These results indicated that the fungus effectively alleviated heavy metal toxicity by increasing root growth, enhancing mineral and water uptake and retaining root physiological activity. Similar findings have been reported in recent studies demonstrating that arbuscular mycorrhizal fungi enhance root architecture and boost plant tolerance to heavy metal stress through enhanced nutrient uptake, heightened antioxidant activity, and diminished metal translocation into plant tissues. The data in Table 9 showed that the treatment, period of cultivation and their interaction had a significant effect on fresh weight of sweet pepper plants ($P \leq 0.05$). The fresh weight gradually increased with cultivation time in all the treatments. However, the plant biomass was significantly reduced under heavy metal stress. Cadmium caused the highest reduction with a mean fresh weight of 3.94 g followed by lead (4.58 g) in comparison to control (4.96 g).

Table 8. Effect of fungus *Funneliformis mosseae* and heavy metals on length of roots (cm) on Sweet Pepper plants in Clay soil during 160 days of cultivation

Treatments	60 days	80 days	100 days	130 days	160 days	Mean:Treat
(control)	8.50	11.10	14.80	19.00	22.50	15.18±2.07
Cadmium	7.00	8.80	10.80	13.00	15.00	10.92±0.76
Lead	7.40	9.50	12.00	15.00	17.80	12.34±1.89
Mycorrhiza+ Cadmium	9.50	11.70	15.00	19.60	23.20	15.80±2.26
mycorrhiza+ *lead*	10.20	12.70	16.60	21.80	25.20	17.30±2.09
Mycorrhiza	12.10	16.20	22.40	29.70	34.80	23.04±3.79
Mean:Period	9.12±0.85	11.67±0.48	15.27±1.42	19.68±1.38	23.08±2.31	
LSD (0.05)	Treat:1.694*		Period:2.018*		Treat. ×Period: 3.121 *	

In contrast, inoculation with *Funneliformis mosseae* significantly increased fresh weight (12.04 g) and had the highest mean value. Moreover, mycorrhizal inoculation significantly increased the fresh weight under cadmium and lead stress compared to non-mycorrhizal plants. The results suggest that *F. mosseae* can effectively reduce the toxicity of heavy metals by enhancing the uptake of nutrients and water, and by improving the efficiency of photosynthesis, activating the antioxidant defense systems and reducing heavy metal accumulation in the plant tissues. Thus, mycorrhizal plants showed higher biomass production under heavy metal stress. These findings are consistent with recent studies which have reported that arbuscular mycorrhizal fungi enhance plant biomass and ameliorate heavy metal toxicity by improving physiological performance and reducing metal uptake.

Table 9. Effect of fungus *Funneliformis mosseae* and heavy metals on fresh weight (gm) on Sweet Pepper plants in Clay soil during 160 days of cultivation

Treatments	60 days	80 days	100 days	130 days	160 days	Mean:Treat
(control)	1.99	3.56	5.61	8.31	10.35	5.96±0.74
Cadmium	1.05	2.19	3.40	5.75	7.30	3.94±0.41
Lead	1.20	2.45	4.06	6.95	8.25	4.58±0.69
Mycorrhiza+ Cadmium	2.24	4.09	6.89	10.95	13.59	7.55±1.26
mycorrhiza+ *lead*	2.44	4.50	7.37	11.09	14.55	7.99±1.49
Mycorrhiza	3.42	6.34	11.17	17.50	21.75	12.04±2.19
Mean:Period	2.06±0.09	3.86±0.11	6.42±0.31	10.09±1.02	12.63±1.41	
LSD (0.05)	Treat:0.004*		Period:0.008*		Treat. ×Period: 0.034 *	

The results presented in Table 10 demonstrated that treatment, cultivation period, and their interaction significantly affected the dry weight of sweet pepper plants ($P \leq 0.05$). Dry weight increased progressively with cultivation time in all treatments, whereas cadmium and lead significantly reduced biomass accumulation compared with the control. Cadmium exerted the greatest inhibitory effect, recording the lowest mean dry weight (0.36 g), followed by lead (0.44 g), indicating that cadmium imposed more severe physiological stress on plant growth. In contrast, inoculation with *Funneliformis mosseae* significantly enhanced dry matter accumulation, producing the highest mean dry weight (1.73 g). Furthermore, mycorrhizal inoculation markedly improved dry weight under both cadmium and lead stress compared with non-mycorrhizal plants. The increased dry biomass can be attributed to improved mineral nutrition, enhanced water absorption, maintenance of photosynthetic activity, stimulation of antioxidant defense mechanisms, and reduced uptake and translocation of heavy metals. These findings are consistent with recent studies reporting that arbuscular mycorrhizal fungi enhance dry matter production and improve plant tolerance to heavy metal stress by regulating nutrient acquisition and reducing metal toxicity (Lorenzo-Gutiérrez et al., 2019).

Table 10. Effect of fungus *Funneliformis mosseae* and heavy metals on dray weight (gm) on Sweet Pepper plants in Clay soil during 160 days of cultivation

Treatments	60 days	80 days	100 days	130 days	160 days	Mean:Treat
(control)	0.20	0.39	0.59	0.95	1.20	0.67±0.09
Cadmium	0.10	0.20	0.33	0.50	0.65	0.36±0.04
Lead	0.14	0.25	0.38	0.61	0.80	0.44±0.06
Mycorrhiza+ Cadmium	0.24	0.46	0.70	1.10	1.41	0.78±0.10
mycorrhiza+ *lead*	0.35	0.53	0.78	1.20	1.50	0.87±0.16
Mycorrhiza	0.53	0.92	1.62	2.59	3.01	1.73±0.29
Mean:Period	0.26±0.03	0.46±0.04	0.73±0.04	1.16±0.08	1.43±0.11	
LSD (0.05)	Treat:0.005*		Period:0.008*		Treat. ×Period: 0.028 *	

Fungal cellular defense against excessive concentrations of toxic metals in the environment includes intracellular metal sequestration, metal binding to cell walls, chemical transformations, and intracellular metal stabilization (Gajewska et al., 2022). AMF can form mutualistic associations with most terrestrial plants, creating a direct physical link between plant roots and the soil. This explains why plants grow better in the presence of fungi. AMF may enhance metal extraction from plants when soil metal concentrations are low. They also help plants accumulate a significant portion of toxic metals in the roots, preventing their translocation to the aerial parts when soil metal concentrations are high (Ma et al., 2022). Singh et al. (2019) studied the effect of inoculation of four AMF species on the removal of chromium, cadmium, nickel, and lead from tannery sludge. They found that all AMF species enhanced metal accumulation in roots but reduced it in shoots. The metal translocation factor was significantly lower compared to uninoculated plants. These findings provide important evidence of AMF's ability to enhance metal fixation in plants. Similarly, Yang et al. (2015) evaluated the effects of two AMF species, *F. mosseae* and R. intraradices, on plant growth, lead accumulation, photosynthesis, and antioxidant enzyme activity. The increased biomass, photosynthetic pigments, gas exchange capacity, and various enzyme activities in inoculated plants indicated that both AMF species protected plants from cellular damage by scavenging reactive oxygen species under lead stress. The low concentration of lead in plant leaves indicates that these two types of AMF have the ability to increase mineral fixation in the plant. This explains the increase in vegetative growth in some results, with a slight change in the root system, in a comparative study conducted by Muhsen (2018) on the role of mycorrhizae in reducing the effect of heavy metals on *Capsicum annuum* L. plants, the results were consistent, as it was concluded that mycorrhizae play a major role in helping the plant get rid of the effect of heavy metals.

Conclusions: The present study demonstrated that inoculation with *Funneliformis mosseae* established an effective symbiotic association with sweet pepper roots in clay soil, resulting in increased mycorrhizal colonization frequency and mycorrhizal dependency throughout the cultivation period. Mycorrhizal inoculation significantly reduced the accumulation of cadmium and lead in plant roots, indicating its important role in limiting heavy metal uptake and translocation. Consequently, plants inoculated with *F. mosseae* exhibited significant improvements in vegetative growth, including stem length, root length, fresh weight, and dry weight, even under heavy metal stress. Among the tested metals, cadmium exerted greater toxicity than lead, causing more pronounced reductions in mycorrhizal colonization and plant growth. Overall, *Funneliformis mosseae* proved to be an efficient and environmentally sustainable biofertilizer capable of mitigating heavy metal toxicity and enhancing the growth and performance of sweet pepper plants cultivated in contaminated clay soils. These findings support the potential application of arbuscular mycorrhizal fungi as an eco-friendly strategy for sustainable agriculture in heavy metal-polluted environments.

Author Contribution

The author was equally responsible for study conception and design, experimental work, data collection, data analysis, and manuscript preparation.

Data Availability Statement

Not applicable.

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Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this study.

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ارزیابی اثر *Funneliformis mosseae* در افزایش تحمل *Capsicum annuum* L. به تنش کادمیوم و سرب

ساره جمال جلیل

* نویسنده مسئول. مرکز تحقیقات و موزه تاریخ طبیعی عراق، دانشگاه بغداد، بغداد، عراق. ایمیل: sarah.g@nhm.uobaghdad.edu.iq

ثامر ا. ا. محسن

گروه زیست‌شناسی، دانشکده آموزش علوم پایه، ابن‌الهیثم، دانشگاه بغداد، بغداد، عراق. ایمیل: thamer.a.m@ihcoedu.uobaghdad.edu.iq

شیماء ا. هــ نعمه

گروه کالبدشناسی، دانشکده پزشکی، دانشگاه جابر بن حیان، بغداد، عراق. ایمیل: shaymaa.a.neamah@jmu.edu.iq

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چکیده

هدف: آلودگی فلزات سنگین اثرات زیان‌باری بر رشد و عملکرد گیاهان دارد؛ ازاین‌رو، استفاده از روش‌های زیستی برای کاهش آثار این آلودگی از اهمیت ویژه‌ای برخوردار است. قارچ‌های میکوریز آربوسکولار به دلیل افزایش جذب عناصر معدنی، به‌ویژه عناصر نسبتاً کم‌تحرکی مانند فسفر، روی و مس، همچنین بهبود جذب نیتروژن و افزایش کارایی مصرف آب، نقش مهمی در ارتقای رشد گیاهان ایفا می کنند. هدف این مطالعه، ارزیابی کارایی تلقیح قارچ *Funneliformis mosseae* در بهبود رشد و افزایش تحمل گیاه فلفل (*Capsicum annuum* L.) تحت تنش ناشی از کادمیوم (Cd) و سرب (Pb) بود.

مواد و روش‌ها: این آزمایش طی مدت ۱۶۰ روز و با استفاده از یک طرح آزمایشی تکراردار به‌منظور بررسی اثرات قارچ *Funneliformis mosseae* تحت تنش سرب و کادمیوم انجام شد. آزمایش گلدانی در شرایط گلخانه‌ای دانشگاه بغداد و بر پایه طرح کاملاً تصادفی (CRD) با شش تکرار برای هر تیمار اجرا گردید. برای ارزیابی رشد گیاهان *Capsicum annuum* در خاک رسی، این تیمارهای آزمایشی در نظر گرفته شد. رشد گیاه با اندازه‌گیری وزن تر و خشک، طول ساقه و ریشه ارزیابی شد. همچنین، غلظت سرب و کادمیوم در بافت ریشه و خاک با استفاده از دستگاه طیف‌سنجی جذب اتمی (Atomic Absorption

Spectrophotometry) اندازه‌گیری گردید. تجزیه‌وتحلیل آماری داده‌ها با استفاده از نرم‌افزار Statistical Analysis System (SAS) نسخه 9.4 انجام شد.

نتایج: نتایج تحلیل آماری با استفاده از آزمون تحلیل واریانس دوطرفه (Two-way ANOVA) اختلاف معنی‌داری ($P \leq 0.05$) را بین تیمارهای آزمایشی نشان داد. تلقیح با قارچ Funneliformis mosseae موجب بهبود رشد گیاهان فلفل شد؛ به‌گونه‌ای که وزن تر و خشک گیاه، همچنین طول اندام هوایی و ریشه، در شرایط تنش سرب و کادمیوم افزایش یافت. علاوه بر این، نتایج آنالیز جذب اتمی (AAS) نشان داد که غلظت سرب و کادمیوم در ریشه گیاهان تلقیح‌شده با میکوریزا کمتر از گیاهان شاهد بود. این یافته بیانگر آن است که تلقیح میکوریزی توانسته است به‌طور مؤثری شدت تنش ناشی از فلزات سنگین را کاهش دهد.

نتیجه‌گیری: نتایج این پژوهش شواهدی را ارائه می‌دهد که تلقیح با قارچ‌های میکوریزی می‌تواند به‌عنوان بخشی از راهبردهای مدیریت پایدار برای کشت گیاهان در خاک‌های آلوده به فلزات سنگین مورد استفاده قرار گیرد. استفاده از *Funneliformis mosseae* یک راهکار زیستی، عملی و سازگار با محیط زیست برای بهبود رشد گیاه و کاهش اثرات زیان‌بار سرب و کادمیوم بر سامانه‌های کشاورزی فراهم می‌آورد.

کلمات کلیدی: خاک رسی، فلزات سنگین، میکوریزا، *Capsicum annuum* L.، *Funneliformis mosseae*

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