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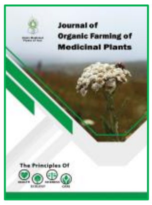

Original Article

Cadmium stress mitigation in lemon balm (*Melissa officinalis* L.) using mycorrhiza symbiosis: Nutrient uptake, dry weight, and morphological traits

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ABSTRACT

Mycorrhizal symbiosis is a sustainable way to empower plants to tolerate heavy metal stress, especially cadmium (Cd). Considering the medicinal importance of lemon balm, this study aimed to evaluate the effect of mycorrhizal fungi on lemon balm tolerance to Cd toxicity in the research greenhouse of the Faculty of Agriculture, Yasouj University, Iran, in 2022. The experiment was a factorial based on a completely randomized design with three replications. The first factor included Cd concentration (0, 5, 10, 15, 20 and 25, mg/kg soil, as $Cd(NO_3)_2$) and the second factor included no application and application of arbuscular mycorrhizal fungus, *Funneliformis moseae*. The results showed that 25 mg/kg soil Cd reduced the shoot nitrogen and potassium content by 64.2% and 45.3% and the root dry weight by 70% compared to the control. The mycorrhizal fungus also had a positive effect on the plant height (10%) and lateral branches (10.1%) compared to the non-application by reducing the root Cd content by 17.2%. The highest shoot dry weight were recorded from the unstressed + mycorrhizal application at 3.4 g/plant, which was lower than the maximum concentration of Cd + no mycorrhizal inoculation, showed a decrease of 79.7%. Also, at 25 mg Cd/kg soil, mycorrhiza improved the Cd tolerance index by 11.1%. Based on our results, although the dry matter yield of lemon balm was reduced by increasing the Cd concentration, the application of mycorrhiza fungi, especially at higher stress levels, partially prevented the adverse effects of stress on plant performance by reducing the Cd transfer factor from roots to shoots.

1. Introduction

As an ongoing industry, medicinal plant production and processing has an important contribution to the global economy and trade (Parvin et al., 2023). The climatic conditions and the existence of various climates in Iran have made the country's capacity to cultivate medicinal plants significant. Lemon balm, *Melissa officinalis* (L.), is a perennial medicinal plant of the Labiatea family of Mediterranean origin (Akbar, 2020), which is mainly used for its petiole-free leaves containing essential oil; including the monoterpenoid compounds citronellal and citral (Chrysargyris et al., 2022). Other secondary metabolites such as eugenol, actinone, citral hexenol, and haramine contribute to the powerful activity of lemon balm essential oil (Hajlaoui et al., 2021). Heavy metals (HM) are important sources of non-point source environmental pollution, and their presence in soil biota threatens soil quality, plant growth, and animal health (Tang et al., 2020). The harmful effects of these elements on plants inhibit the basic processes of cellular respiration and photosynthesis (Genthe et al., 2018).

Cadmium (Cd) is one of the most toxic pollutant HMs, which has been classified as a carcinogen with a biological life span of about 30 years (Genchi et al., 2020). Although the natural amount of this element in uncontaminated soils is less than 1 mg/kg and its critical concentration is stated to be 1.5 to 2.5 mg/kg, however, the standard measurements showed that Cd in contaminated Iranian soils is near 5 mg/kg of soil (Rezaei et al., 2022). Therefore, the consequences of oxidative stress it caused are twofold. Chlorosis, twisting, and shrinking of leaves are obvious symptoms of Cd toxicity in plants, which occurs following the absorption and transfer of this metal from the roots to the aerial parts and as a result, affects the availability of essential nutrients in the plant (Irfan et al., 2014).

The negative effect of Cd stress on the absorption of nitrogen, phosphorus, and potassium has also been reported by researchers in spinach (*Spinacia oleracea* L.) (Syed et al., 2022), wheat (*Triticum aestivum* L.) (Rahman et al., 2021), and purslane (*Portulaca oleracea* L.) (Pishkar et al., 2022). Following the increase in the concentration of HMs such as Cd in the soil solution, nutrient deficiency in plants occurs, preventing chlorophyll synthesis and consequently reducing and delaying plant growth. In a study on two mint species under Cd stress, the fresh biomass of shoots and roots of *Mentha piperita* decreased by 16.9% and 26.7%, respectively, and the fresh biomass of shoots and roots of *M. spicata* decreased by 22.1% and 20% (Jiang et al., 2022). In another study on peppermint (*Mentha piperita* L.), the highest concentration of Cd (15 mg/L) resulted in a decrease in root (46%) and shoot (53%) dry matter (Amirmoradi et al. 2017).

To face the consequences of HM stress and to benefit from a sustainable agricultural system, it is necessary to use inputs that, in addition to meeting plant needs and reducing environmental risks, improve the ecological aspects of the system (Nasiri et al., 2020). Mycorrhizal symbiosis, especially in humus-deprived and nutrient-poor soils, increases the absorption of phosphorus and other macroelements by the roots of the host plant via producing compounds that stimulate plant growth and the expansion of colonization and growth of hyphae (Brundrett and Tedersoo, 2018), and consequently improves soil structure and increases resistance to environmental stresses (Begum et al., 2019).

In this regard, the response mechanisms of mycorrhizal fungi to modulate HM toxicity include increased water and nutrient uptake, antioxidant response, stimulation of protein synthesis genes, alocation of HMs in the ecto-root mycelium, and chelation of these metals by binding them to glycoprotein-metal complexes through secreted glomalin (Dhalaria et al., 2020). The results of researchers in hemp (Sun et al., 2023) and pigeonpea (Aditi and Neera, 2022) indicate the positive effect of mycorrhiza in improving the quantitative and qualitative characteristics of these plants under Cd stress; however, the effectiveness of mycorrhiza varies depending on the concentration of Cd in the soil, the type of plant species, and the symbiotic fungus.

The increasing demand for medicinal plants has made it more important to find effective methods to develop the cultivation and production of these plants. Soil contamination with HMs has also increased in most developing countries - including Iran - due to intensive agriculture. Considering the effect of mycorrhizal fungi on the modulation of HM accumulation in medicinal plant performance, this study aimed to investigate the effect of mycorrhizal fungi on nutrient uptake and some morphological characteristics of lemon balm under the influence of different levels of soil Cd concentration.

2. Materials and Methods

2.1. Design and treatment framework

This study was conducted in 2022 in the Research Greenhouse at Yasouj University, which is located at an altitude of 1870 m above sea level and with coordinates 30° 40´ N and 51° 36´ E. The greenhouse temperature was 25 ± 2°C. The experiment was designed as a factorial based on a completely randomized design with three replications. The first factor included six Cd concentrations (0, 5, 10, 15, 20, and 25 mg/kg Cd from $Cd(NO_3)_2$ (Nourbakhsh Rezaei et al., 2019; Mousavi and Razavizadeh, 2021)) and the second factor included the arbuscular mycorrhizal fungus, *Funneliformis moseae*, at two levels (non-application and application). Each experimental unit consisted of two pots, and the experiment was conducted with 72 pots.

2.2. Planting operation

One-month-old lemon balm seedlings (Shiraz local accession) at the three-leaf stage were obtained from Zargyah Firuzabad Company and the arbuscular mycorrhizal fungus (*F. moseae*) was obtained from the Plant Protection Clinic of Hamadan. Plastic pots measuring 20×13×10 cm were filled such that the soil surface was 5 cm below the pot opening. The soil mixture consisted of a two-to-one ratio of field soil to soft sand. The pots were maintained in controlled greenhouse conditions at the university's research facility to stabilize the physical and chemical properties of the soil. Sampling was performed to evaluate the physicochemical properties of the soil and the results are presented in Table 1. For Cd contamination, a $Cd(NO_3)_2$ salt solution was prepared and sprayed on the soil and then sterilized in an autoclave for 1 to 2 hours (distilled water was used in the control pots). The pots were irrigated alternately for 45 days so that Cd and soil reached equilibrium as the soil dried and moistened (Shuhe et al., 2010).

In the transfer stage, 50 g of soil containing the fungus was applied to the root growth zone of the pots designated for mycorrhiza treatment, and then three seedlings were transferred to each pot. Three weeks after transfer, lemon balm plants were topped at a height of 10 cm to stimulate vegetative growth and branching, and two months later, before flowering (Torfi et al., 2023), they were harvested.

Table 1. Physical and chemical characteristics of the soil where the experiment was conducted.

Indicators	Value
phosphorus (mg/kg)	3.8
potassium (mg/kg)	164
Total nitrogen (%)	0.02
Cd (mg/kg)	0.5
pH	7.87
Organic matter (%)	0.43
Electrical conductivity	0.59
Lime (%)	42.8
Sand (%)	45.2
Silt (%)	43.4
Clay (%)	11.4

2.3. Root colonization

After being removed from the potting soil, the roots were carefully washed with water and 50 one-centimeter thin pieces were transferred to test tubes containing 10% KOH. Then, the tubes were placed in a water bath at 95°C for 30 minutes. When the roots became completely transparent and glassy, they were washed with water and diluted vinegar to remove the KOH effect. The transparent roots were boiled in a mixture of 3% blue dye and 5% acetic acid for three minutes. In the next step, the samples were decolorized with pure vinegar (Vierheilig et al., 1998). By preparing thin sections of the roots, the percentage of root colonization was calculated using a microscope (Biermann and Linderman, 1981).

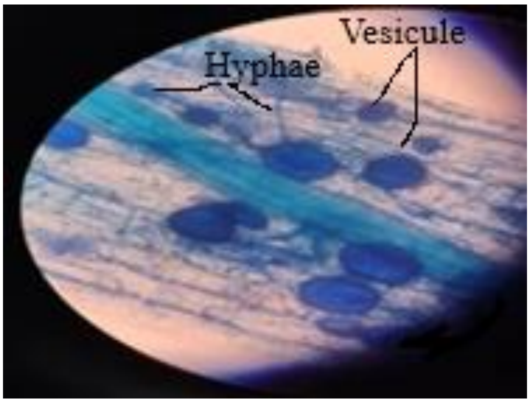

Figure 1. The extra- and intra-root organs of lemon balm inoculated with arbuscular mycorrhizal fungi observed using a microscope (model: Olympus CX21) at 40x magnification.

2.4. Measurement of shoot macroelements content

The extract required for measuring the content of phosphorus (P) and potassium (K) in the shoot was prepared by dry digestion. For this purpose, 0.5 g of the dried sample was ashed in an oven at 500°C for 2 hours. The desired ash was placed on a heater by adding 5 ml of 2N hydrochloric acid. When boiling began, the resulting solution was passed through filter paper and the volume of the samples was brought to 50 ml with double-distilled water. P was measured by calorimetry (molybdate-vanadate yellow color) using a spectrophotometer (Lambda EZ 210) at a wavelength of 420 nm (Chapman and Pratt, 1961) and K was measured with a flame photometer (Skoog and West, 1980). The nitrogen (N) content of the shoot was also obtained separately using a Kjeldahl apparatus through three stages digestion, distillation, and titration (Jones, 2001).

2.5. Cd measurement in roots and shoots

Root and shoot samples were dried in an oven at 80°C for 48 hours. Then, they were powdered using a porcelain mortar and passed through a 2 mm sieve. One gram of powder from each sample was weighed then poured into a crucible and transferred to a furnace at 550°C for 4 hours. Then, 10 ml of 2N hydrochloric acid was added to the samples at 95°C, and the solution was then passed through filter paper. The resulting extract was collected in a 50 ml volumetric flask and the Cd concentration was measured using a Hitachi Z 2300 atomic absorption spectrometer (Westerman, 1990).

2.6. Measurement of morphological traits

The height of the main stem of three plants in each pot was measured with a caliper and their average was recorded. Also, the number of lateral branches in the plant for each pot was counted and their average was calculated. Then, the roots and aerial parts of three plants in the pot were separated and after washing with distilled water and drying in an oven at 75°C for 24 hours, They were weighed with a digital scale (accuracy = 0.001 g), and their average was recorded.

The root-to-shoot Cd transfer factor was calculated using equation 1 (Jolly et al., 2013).

TF=Root Cd/Shoot Cd
[Eq 1]

Where, TF is the root-to-shoot transfer factor, Root Cd and Shoot Cd represent Cd concentration in roots and shoots, respectively. The Cd tolerance index was obtained using equation (2) (Lasat, 2002).

CTI= DW of aerial parts in contaminated soil/ DW of aerial parts of the plant in uncontaminated soil
[Eqation 2]

Where CTI is Cd tolerance index.

2.7. Statistical analysis

Data analysis was performed using SAS 9.1 software. Means comparison for significant interactions or main effects was performed using the LSD test ($p \leq 5\%$).

3. Results and Discussion

The interaction of Cd×mycorrhizal fungi on root colonization, shoot phosphorus, shoot Cd, Cd transfer factor, shoot DW, and Cd tolerance index was significant. Other traits were affected only by the main effect of Cd and mycorrhizal fungi (Table 2).

3.1. Root colonization

The results indicated that with the application of Cd together with mycorrhiza, the percentage of root colonization at the level of 5 mg Cd/kg soil increased by 3.24% compared to the without Cd application, and then at the levels of 10, 15, 20 and 25 mg Cd/kg soil, it decreased by 11.88, 22.15, 28.64 and 80% compared to the control, respectively (Figure 1). Considering that the roots were disinfected before transferring the seedlings to the pot, no colonization occurred without mycorrhiza inoculation.

Root colonization is an important indicator of the symbiosis of crop plants with arbuscular mycorrhizal fungi. In this experiment, the symbiosis was expanded by the mycorrhizal fungus up to a certain threshold of Cd oncentration and then limited. At moderate levels (such as 5 mg/kg), Cd may induce oxidative stress in plants. In response, plants often engage in symbiotic relationships with mycorrhizal fungi, which help to absorb and tolerate heavy metals in exchange for nutrients. This stress response could promote mycorrhizae inoculation, as the plant may rely on the fungi for enhanced metal uptake or detoxification mechanisms. On the other hand, at very high concentrations of Cd, the metal may become toxic to both the plants and the mycorrhizal fungi, thereby reducing the inoculation or effectiveness of the symbiotic relationship (Hasan et al., 2009). The increase in the root colonization percentage with increasing Cd concentration indicates an increase in the dependence and preference of the plant to establish symbiosis under heavy metal stress conditions (Mubeen., 2023).

Rask et al., (2019) reported that in four crop species (*Hordeum vulgare*, *Linum usitatissimum*, *Sorghum bicolor*, and *Matricaria recutita*) inoculated with arbuscular mycorrhizal fungi, root colonization increased with increasing Cd levels up to a certain concentration and then decreased. Colonization at higher concentrations of Cd was associated with a decrease in spore germination and inhibition of the growth of external mycelium and the spread of hyphae in plant tissue and soil due to the toxicity and contamination effect on fungal spores (Liu et al., 2018). At the highest Cd concentration, root colonization decreases, due to a decrease in the photoassimilate partitioning to the fungus symbiosis and, on the other hand, to protect the host plant from the consequences of severe stress. The development of the symbiosis depends on the growth conditions of the host plant and under unfavorable conditions, colonization is affected and limited (Lenoir et al., 2016).

3.2. Shoots nitrogen content

With increasing Cd level, shoot nitrogen content decreased, such that the treatment of 25 mg Cd/kg soil reduced the content of this trait by 64.2% compared to the control. Mycorrhizal inoculation also had a positive effect on nitrogen content and caused an increase of 32.24% compared to the absence of fungus application (Table 3).

Cd can have negative effects on plant nitrogen uptake by damaging the soil microbial population and disrupting the nitrogen mineralization process. Cd disrupts the nitrate metabolism process by inhibiting the activity of glutamine synthase, glutamate synthase, and nitrate reductase enzymes (Khan et al., 2016). In a study by Syed et al., (2022), on spinach, Cd was shown to reduce nitrogen concentration. The use of mycorrhizal fungi increases the availability of nutrients in the soil and their absorption through root colonization. Therefore, it will lead to a significant improvement in the nitrogen content in the shoot. The results have shown that mycorrhizal fungi have a profound effect on the physiology of the plant root and cause the activation of glutamine synthetase, arginase, and urease. As a result, they increase the nitrogen concentration in the host plant; because arginase and urease are key enzymes in the transfer of nitrogen from the mycelium into the root of the host plant (during the symbiotic process). Glutamine synthetase also converts nitrogen, which is absorbed by external mycelium in the form of nitrate or ammonium, into organic compounds (Makarov, 2019). The results of this study also showed that there is a positive and significant correlation between the percentage of root colonization and the shoot nitrogen content (r=0.65**) (Table 4). In this regard, Chang et al., (2018) stated in a study on corn that mycorrhizal plants had higher nutrient uptake (including nitrogen) under Cd toxicity compared to non-mycorrhizal plants because they have an extensive hyphal network between the soil and the roots and therefore absorb nutrients better with extensive root branching.

3.3. Shoot phosphorus content

With increasing soil Cd levels, the shoot P content decreased significantly. The highest phosphorus content was obtained from the unstressed level (with an average of 0.156 mg/g) and the lowest value (with an average of 0.0061 mg/g) was obtained at the level of 25 mg Cd/kg soil (Figure 2). At each of the Cd levels, the mycorrhiza inoculation caused an increase in phosphorus content. The highest value was observed in the control treatment of Cd, and the lowest value was observed in the level of 25 mg Cd/kg soil (Figure 2).

Phosphorus is involved in providing the energy necessary for the biosynthesis of glutathione, which is a prerequisite for phytochelatin synthesis, and the role of phytoclatins in sequestering Cd inside vacuoles through the formation of a Cd-phytochelatin complex is known (Shahid et al., 2017). Cd is an effective factor in reducing the rate of phosphorus absorption by plants due to nutritional disorders. Cd toxicity may cause phosphorus deficiency or problems related to phosphorus transport within the plant. Therefore, this HM can reduce the activity of enzymes related to phosphorus absorption in plants by affecting the activity of roots and their function in absorbing nutrients (Wang et al., 2019).

In accordance with the present findings, Rahman et al., (2021) showed that Cd caused a decrease in the concentration of phosphorus in the wheat shoots.

Table 2. Analysis of variance (mean square) for the effect of Cd and mycorrhizal fungus on nutrients content and some morphological traits of lemon balm

S.O.V	df	Root colonization	Shoot N	Shoot P	Shoot K	Root Cd	Shoot Cd	Cd transfer factor	Plant Height	Lateral Branch	Root DW	Shoot DW	Cd tolerance index
Cd (Cd)	5	650**	0.9**	0.03**	117**	21576**	4.9**	0.00023**	15.5**	17.2**	3.4**	3.3**	0.3**
Mycorrhiza (M)	1	21121**	1.1**	0.003**	37.9**	1982**	2.3**	0.00004**	14.7**	8.6**	3.3**	14.7**	0.6**
Cd × M	5	650**	0.01^{ns}	0.0001*	2.2^{ns}	118^{ns}	0.2**	0.00002**	0.6^{ns}	0.5^{ns}	0.05^{ns}	0.4*	0.06**
Error	24	3.8	0.02	0.00005	2.8	94.2	0.02	0.000004	0.4	0.5	0.07	0.1	0.02
CV (%)	-	8.1	14.5	9.4	8.3	10.1	9.8	17.3	4.8	6.6	13.1	15	13

ns, * and ** indicate nonsignificant and significant at 5% and 1% probability level, respectively; DW= Dry Weight.

Table 3. Means comparison of the main effect of Cd stress and mycorrhizal fungi levels for some traits in lemon balm

Treatment levels		Shoot nitrogrn (%)	Shoot potassium (mg/g)	Root Cd (mg/kg)	Plant height (cm)	Lateral branches (per plant)	Root DW (g/plant)
Cd (mg Cd/kg soil)	0	1.662^{a}	27.31^{a}	1.58^{f}	14.47^{a}	12.61^{a}	3.013^{a}
	5	1.079^{b}	22.91^{b}	51.67^{e}	12.62^{b}	11.17^{b}	2.731^{b}
	10	1.066^{b}	20.78^{c}	97.17^{d}	11.81^{c}	10.37^{c}	2.125^{c}
	15	0.969^{c}	18.58^{d}	120.17^{c}	11.52^{d}	10.07^{d}	1.952^{c}
	20	0.794^{d}	17.11^{e}	128.83^{b}	10.64^{e}	9.78^{e}	1.674^{d}
	25	0.595^{e}	14.94^{f}	165.00^{a}	9.86^{f}	7.55^{f}	0.905^{e}
Mycorrhiza	control	0.830^{b}	19.25^{b}	101.50^{a}	11.12^{b}	9.71^{b}	1.762^{b}
	inoculated	1.225^{a}	21.34^{a}	86.64^{b}	12.23^{a}	10.69^{a}	2.375^{a}

DW= Dry Weight; In each column, means with at least one common letter do not have a significant difference (p≤ 0.05; LSD).

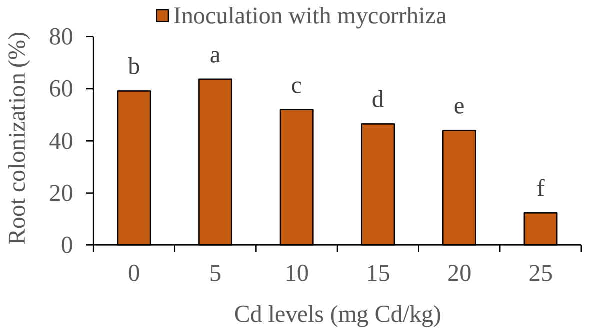

Fig.2. Means comparison of the interaction of arbuscular mycorrhiza and Cd for the percentage of root colonization in lemon balm. Different letter indicates statistical difference between the means based on the LSD test (*P* ≤0.05).

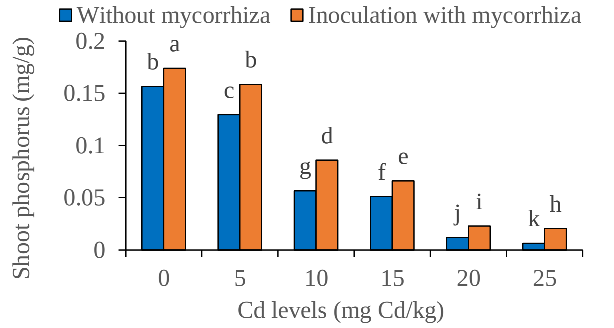

Fig.3. Means comparison of the interaction of arbuscular mycorrhiza and Cd for shoot phosphorus content in lemon balm. Different letter indicates statistical difference between the means based on the LSD test (*P* ≤0.05).

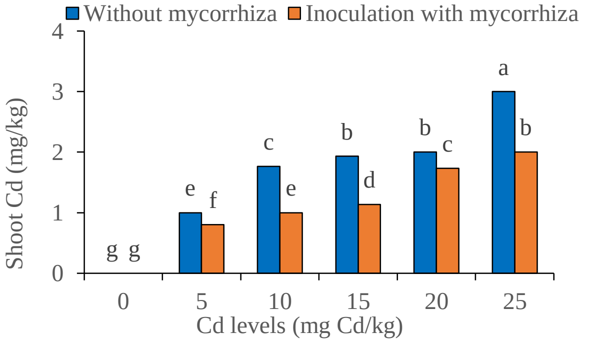

Fig.4. Means comparison of arbuscular mycorrhiza and Cd interaction for shoot Cd content in lemon balm. Different letter indicates statistical difference between the means based on the LSD test (*P* ≤0.05).

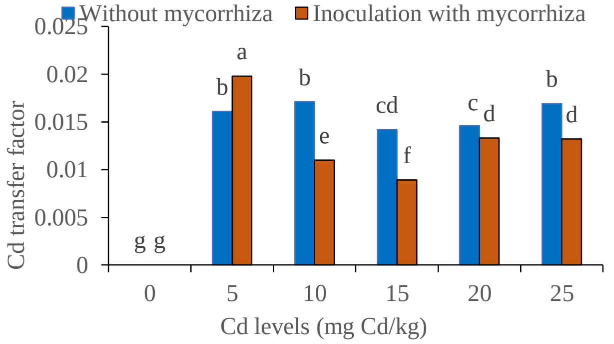

Fig.5. Means comparison of arbuscular mycorrhiza and Cd interaction for the Cd transport factor in lemon balm. Different letter indicates statistical difference between the means based on the LSD test (*P* ≤0.05).

The increase in phosphorus concentration in fungal treatments has been mentioned for various reasons, including the production of mineral acids (carbonic acid and sulfuric acid), organic acids (oxalic acid, citric acid, and lactic acid), the production of phosphatase enzymes, and, as a result, the dissolution of organic and inorganic phosphates (Saito and Ezawa, 2016). In the medicinal plant sage, it was found that the use of mycorrhizal fungi could develop extra-root hyphae and increase the absorption surface of the rhizosphere zone and resulted in higher phosphorus absorption in soil poor in phosphorus (Tarraf et al., 2017). In line with their results, in this study, a positive and significant correlation (r=0.40*) was recorded between the colonization percentage and the phosphorus content of the shoot of lemon balm (Table 4).

3.4. Shoot potassium content

Increasing Cd concentrations were associated with a decrease in shoot potassium content. The highest applied Cd (25 mg/kg soil) reduced potassium content by 45.29% compared to the control. Also, mycorrhizal application significantly (with an average of 19.25 mg/g) increased shoot potassium by 10.85% compared to no application (Table 3). High Cd mobility is one of the main reasons for the reduction of potassium uptake by roots. Cd reduces potassium uptake by reducing the activity of ATPase and ferric reductase enzymes. Cd also appears to compete with some nutrients, including potassium, for uptake through membrane transporters (Genchi et al., 2020). Potassium uptake by plants is selective and highly correlated with plant metabolic activities. Cd toxicity in the root environment and disruption of plant metabolism cause changes in the activity of enzymes that are somehow related to potassium. A study on purslane found that Cd stress reduced the potassium concentration in the shoot (Pishkar et al., 2022).

Mycorrhizal biofertilizers, by reducing soil pH, contribute to soil acidification, which in turn enhances soil organic matter. As a result, they improve plant nutrition, including increasing potassium concentration in plants. Chang et al. (2018) stated that mycorrhiza can increase the amount of potassium in corn, and the higher the fertilization amount, the greater the increase in this element. The results showed a positive and significant correlation between the colonization percentage and shoot potassium (r=0.42**) (Table 4). This well explains the relationship between the efficiency of potassium absorption and the percentage of root colonization.

3.5. Root Cd content

The increase in root Cd content was associated with the increase in Cd content in lemon balm, and the application of arbuscular mycorrhiza caused a decrease in root Cd uptake (Table 3). The highest amount of Cd uptake through the roots was obtained with an average of 165 mg/kg root DW at a concentration of 25 mg Cd/kg soil, which in this case showed a 10-fold increase compared to the control. Mycorrhizal fungi also reduced root Cd uptake with a positive effect of 17.15% compared to no application (Table 3).

In most plants, the accumulation of Cd in the roots is higher than in the aerial parts. Some researchers believe that due to the toxicity of Cd to the cytosol in its free form, plant cells try to stabilize this element in the roots by using strategies such as binding it to the cell wall, storing it in the vacuole, and chelating it with phytochelatin, thus reducing its toxicity (Li et al., 2023). In addition to increasing water and nutrient absorption, arbuscular mycorrhizal fungi can affect the bioavailability and forms of metals in the soil by developing external hyphae in the soil tissue and by increasing the surface area of metal ions (for storage) or releasing compounds such as organic acids and glomalin (for precipitation). Mycorrhizal symbiosis improves survival and adaptation in heavy metal-contaminated soils by reducing metal toxicity in plants and affecting their absorption and transport (Zhan et al., 2018; Liu et al, 2018). In the study by Zhao et al., (2021), Cd concentration in the roots of inoculated chicory plants was reduced as a result of inoculation with mycorrhizal fungi compared to uninoculated plants.

3.6. Shoots Cd content

Increasing HM stress, increased shoots Cd content, and at each of the Cd concentration levels, the application of biofertilizer caused a decrease in shoot Cd content (Figure 3). The highest Cd content of shoots (3 mg/kg) was obtained in the treatment of 25 mg Cd/kg soil + no mycorrhiza application. At the same treatment level, mycorrhiza caused a 33.33% decrease in Cd content. At the zero Cd level (with and without mycorrhiza), no Cd was observed (Figure 3).

The potential toxicity of heavy metals in the environment depends on their concentration in the soil solution. The higher the concentration of the metal in the solution phase, the greater its absorption by the plant and its transfer to the aerial organs. Therefore, the greater accumulation of Cd in the root tissue, as a positive point, is an obstacle to its further transfer to the aerial and functional organs. Among the reasons for the reduction in the transfer of Cd to the aerial parts of lemon balm is the entry of this element into the root apoplastic space, which easily penetrates deep into the root tissue near the endoderm layer along with the nutrient solution and without encountering the Casparian ring barrier, and is not removed by surface washing (Sheikhzadeh et al., 2021).

Mycorrhizal fungi can also disrupt the uptake and accumulation of Cd in plant organs by affecting the ion transport process. In plants, the transport of ions through the cell membrane is primarily mediated by proteins called transporters. These transporters transport a specific ion and act in a specific manner. Most of these ions are physically adsorbed to the cell wall. However, metal uptake in mycorrhizal plants depends on many factors such as the type of host plant, the species of arbuscular mycorrhiza, the concentration and type of metals, and soil conditions (Muthukrishnan et al., 2018). Researchers have shown that mycorrhizal symbiosis can reduce the concentration of heavy metals such as Cd in the shoots of rice (Li et al., 2023).

3.7. Cd transfer factor

Mycorrhiza symbiosis with the plant caused a decrease in Cd transfer from roots to shoots compared to non-mycorrhizal plants (Figure 4). The highest Cd transfer factor with an average of 0.02 was obtained from the treatment of 5 mg Cd/kg soil + mycorrhiza application, which showed a significant difference with other treatments (Figure 4). At the level of 25 mg Cd/kg soil, the plant's resistance to Cd was broken and the transfer factor increased compared to lower levels. Mycorrhiza at this level also caused a lower transfer of Cd to shoots with an effect of 21.89% (Figure 4).

It seems that the increase in the transfer factor at a concentration of 5 mg/kg soil Cd nitrate was due to the stimulating effect of nitrate on the fungus and the plant, the high symbiosis of mycorrhiza at this concentration, and also the mobility of Cd. With increasing stress levels, mycorrhiza had a positive effect on reducing the entry of Cd into the shoot. The transfer factor of less than one and its decrease with increasing Cd concentration compared to the control indicates that the mechanism of Cd accumulation in the roots occurred more than its transfer to the shoots. The immobilization of toxic metals occurs by rhizosphere exudation, deposition in polyphosphate granules, vacuoles, and vesicles, surface adsorption in the cell wall of fungal organs through the presence of chitin, melanin, or chelation in fungal organs by glomalin (Jan et al., 2019).

Given that mycorrhizal symbiosis improved the absorption of nitrogen and phosphorus nutrients and also reduced the Cd content of the shoot; therefore, it is a confirmation of the claim that the transfer factor is reduced under mycorrhizal biofertilizer. In a study by Li et al., (2023) on rice, they stated that in Cd-contaminated soil, the lowest level of the transfer factor belongs to the symbiotic treatment with the fungus *Glomus versiforme*. As they suggested, the transfer of this heavy metal to the grain can be inhibited by enriching the rice root with Cd.

3.8. Plant height

The highest plant height (14.47 cm) was achieved in the absence of Cd stress, and the highest stress treatment (25 mg Cd/kg soil) had the lowest plant height (9.86 cm), which showed a difference of 31.86% (Table 3). Also, inoculation of mycorrhiza biofertilizer increased plant height by 9.98% compared to un-inoculated control (Table 3).

The presence of Cd in the soil has negative effects on plant vegetative properties, and due to the presence of Cd, cell expansion is destroyed due to the toxic effect on the tonoplast water channels, which results in a decrease in plant shoot growth. Timori et al. (2023) reported that due to the presence of Cd in the aerial parts of mung bean, the metabolism of the cells in this part is disrupted, and therefore, the plant height is reduced. In a study on summer savory, the effects of Cd concentration on the length of the aerial parts also led to a significant reduction in the stem length (Azizollahi et al., 2019). The effects of two strains of mycorrhizal fungi *Rhizophagus mosseae* and *R. intraradices* on black cumin (*Nigella sativa* L.) showed that both strains caused a significant increase in plant height compared to the control treatment, and the reason for this was due to the greater absorption and solubility of phosphorus under the application of mycorrhiza (Abadi et al., 2015).In another study, the effects of applying biofertilizers, especially mycorrhiza, on black cumin were investigated and it was observed that inoculating black cumin seeds with mycorrhiza increased plant height (Darakeh et al., 2021). These researchers also attributed the improvement in black cumin growth conditions to the solubility and greater absorption of mineral elements (especially phosphorus and nitrogen). In accordance with these findings, in the present experiment, mycorrhiza biofertilizer also improved nutrient absorption, and plant height showed a positive and significant correlation with shoot nitrogen (r=0.77**), phosphorus (r=0.83**), and potassium (r=0.88**) (Table 4). In addition, it has been stated that arbuscular mycorrhiza fertilizers affect the growth and development process by producing plant growth-stimulating hormones and increasing growth indices, including plant height (Miransari et al., 2014).

3.9. Number of lateral branches

This trait decreased with increasing stress levels applied to lemon balm. The highest number of lateral branches was obtained at the level of no Cd application, and the lowest (with an average of 7.55 per plant) was obtained from the treatment of 25 mg Cd/ kg soil, which decreased by 40.13% (Table 3). The mean comparison of mycorrhiza treatments revealed that the inoculation with the highest lateral branches number (12.69 branches/plant) resulted in a 10.09% increase in this trait compared to the control (Table 3).

The limitation in shoot growth is most likely due to the reallocation of carbohydrates in favor of root growth or a decrease in the efficiency of photosynthesis (by reducing the absorption of structural chlorophyll elements) (Israel et al., 2022). As a result, Cd stress, by reducing the length of the plant growth period and also accelerating the entry into the reproductive phase, inhibits the growth of lateral buds and affects the number of lateral branches. Part of the increase in the number of lateral branches in the application of biofertilizers can be due to the ease of plant access to high amounts of nutrients, especially phosphorus, and nitrogen, which ultimately leads to an increase in the number of lateral branches through an increase in the rate of photosynthesis and the production of more dry matter.

The use of mycorrhiza by spreading mycelium and forming an additional absorption system makes it possible to utilize moisture and nutrients in a larger volume of soil that the feeding roots do not have access to, and this can increase growth in different parts of the plant, including lateral branches. The results of the study by Lamian et al., (2017) on tarragon (*Artemisia dracunculus*) indicated a significant main effect of mycorrhiza fungi on the number of secondary stems, such that the lowest and highest number of this trait belonged to the treatments of no use and use of mycorrhiza fungi.

3.10. Root dry weight

With increasing soil Cd, root DW decreased; so the highest root DW was obtained at the level of no Cd application and the lowest was obtained from the treatment of 25 mg Cd/kg soil, which showed a decrease of 69.96% (Table 3). The use of mycorrhizal fungi had a significant difference compared to no use and showed an increase of 34.79% (Table 3).

The decrease in root DW at high Cd concentrations was more pronounced in non-mycorrhizal plants than in mycorrhizal ones. Evidence suggests that toxicity occurs either directly due to cellular oxidative stress caused by metal uptake or indirectly due to an imbalance of nutrients in the rhizosphere (Yamin et al., 2023). In this regard, the colonization percentage showed a negative correlation with root Cd content (r = -0.34*). However, mycorrhizal colonization resulted in a direct association with increased uptake of N, K (Table 3), and P (Figure 3), ultimately leading to improved root DW under cadmium toxicity. The present study was consistent with the research of Amirmoradi et al., (2017) on peppermint plants. They stated that root DW decreased with increasing Cd concentration and decreased by 46% under the influence of 15 mg/L Cd concentration compared to the control.

3.11. Shoot dry weight

The results showed that Cd stress reduced shoot DW in lemon balm and the application of mycorrhizal fungi had a positive effect on it. The highest shoot DW with an average of 3.95 g per plant was obtained from the treatment of no Cd application + application of mycorrhizal fungi and the lowest was recorded from the treatment of 25 mg Cd/kg soil + no application of mycorrhizal fungi with an average of 0.80 g/plant (Figure 6).

Cd enters the plant through apoplastic or symplastic pathways and then in the infected tissues, it causes changes in cellular structures, and physiological and metabolic processes, leading to growth inhibition (Sheikhzadeh et al., 2021). Mycorrhiza also improves growth indices by positively affecting the absorption, transport, and stabilization of macronutrients and micronutrients (Hashem et al., 2018) and increasing the content of phytohormones auxin (Sieh et al., 2013), gibberellin (Miransari et al., 2014) and cytokinin (Yurkov et al., 2017).

In this regard, researchers stated that the increase in dry matter of black cumin plants inoculated with mycorrhizal fungi is probably due to the extensive hyphal network and the increase in the surface area to volume ratio of the root, which is the cause of better absorption and transport of substances in plant organs and increased photosynthesis (Darakeh et al., 2021). In this experiment, the positive and significant effect of mycorrhiza on improving the absorption of nutrients by increasing the DW of the lemon balm plant, as well as the effect of Cd stress on inhibiting growth and development by reducing the DW of the plant, is confirmed (Table 4). The negative effect of Cd on the dry biomass of lemon balm (Adamczyk-Szabela et al., 2019) and two species of mint (Jiang, et al., 2022) and the effect of two strains of mycorrhizal fungi (*R. mosseae* and *R. intraradices*) on the biological yield of black cumin (Abadi et al., 2015) have been reported.

3.12. Cd tolerance index

With increasing Cd levels, the tolerance index decreased, and with the application of mycorrhiza, the plant tolerance index improved compared to the non-application treatments (Figure 7). The lowest tolerance index with an average of 0.226 was obtained from the level of 25 mg Cd/kg soil + non-application of mycorrhiza, and the highest with an average of 1.0 was obtained from the level of non-application of Cd together with mycorrhiza, which shows a decrease of 77.4% (Figure 7).

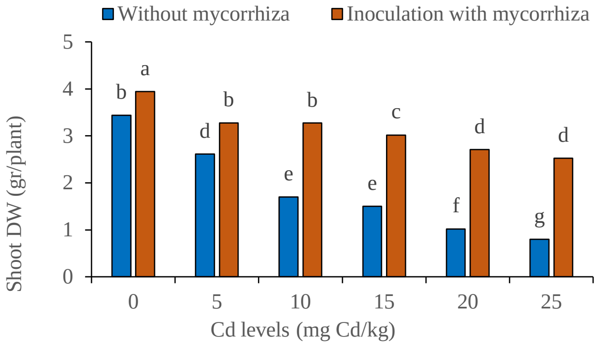

Fig.6. Means comparison of arbuscular mycorrhiza and Cd interaction for shoot DW in lemon balm. Different letter indicates statistical difference between the means based on the LSD test ($P \leq 0.05$).

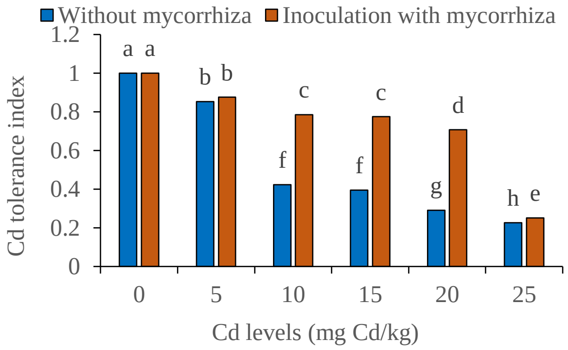

Fig.7. Means comparison of arbuscular mycorrhiza and Cd interaction for Cd tolerance index in lemon balm. Different letter indicates statistical difference between the means based on the LSD test ($P \leq 0.05$).

According to most researchers, the best index for selecting plants that are more tolerant to environmental stresses is the stress tolerance index (STI), because it can identify plants that have a favorable performance under environmental stress conditions. According to the mean comparison of the interaction, it can be seen that the plant has much less tolerance at the level of 25 mg Cd/kg soil than at other treatment levels, and even with the use of mycorrhizal fungi at this level, there was no significant change in the plant tolerance (although it showed a positive statistical difference), but with the use of mycorrhizal fungi at other treatment levels, the Cd tolerance index significantly increased compared to levels without mycorrhiza inoculation. The degree of plant tolerance to stress conditions is divided into three categories: high tolerance (greater than 0.6), medium tolerance (0.6-0.35), and sensitive (less than 0.35) (Lux et al., 2004). Accordingly, since the tolerance index of lemon balm in this experiment (except for the highest stress concentration) under mycorrhiza application is always higher than 0.6, this plant is considered a very tolerant plant. In *Neptunia oleracea*, similar to the present study, a decrease in the tolerance index from 91% to 37% at concentrations of 0.5 to 20 mg/L of Cd has been reported (Abdul et al., 2014).

Table 4- Correlation between some morphophysiological traits in lemon balm under mycorrhiza application and Cd toxicity (n=36)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Colonization percentage (1)	1							
Shoot Nitrogen content (2)	0.65**	1						
Shoot Phosphorus content (3)	0.40*	0.77**	1					
Shoot Potassium content (4)	0.42**	- 0.88**	- 0.90**	1				
Root Cd content (5)	- 0.34*	- 0.79**	- 0.92**	0.67**	1			
Shoot Cd content (6)	- 0.47**	- 0.84**	- 0.89**	0.52**	0.93**	1		
Plant Height (7)	0.50**	0.77**	0.83**	0.88**	- 0.82**	- 0.87**	1	
Root DW (8)	0.45**	0.73**	0.82**	0.42**	- 0.82**	- 0.89**	0.78**	1

* and ** indicate significant at 5% and 1% probability level, respectively; DW= Dry Weight

4. Conclusion

Exposure to high concentrations of heavy metals affects plant growth and development. In this experiment, the negative effect of Cd treatment on the studied traits in lemon balm was evident, as it was determined that with increasing Cd stress, the absorption of macro elements in the plant decreased and the Cd content in the shoot and root increased. High concentrations of Cd also reduced the traits of DW of the shoot, DW of the root, plant height, and number of lateral branches. In contrast, applying mycorrhiza fungi showed a positive effect compared to non-use. Inoculation with mycorrhiza fungi, by increasing the percentage of root colonization and consequently increasing the absorption of nutrients from the soil, improved the soot and root DW of the inoculated plants compared to the control. Considering that the goal of lemon balm cultivation is to increase its shoot yield, the use of mycorrhiza fungi can reduce the adverse effects of Cd stress and maintain the shoot yield at a desirable level. Therefore, applying mycorrhizal fungi to soil is a useful and economical solution to improve soil quality and, as a result, increase crop production under conditions of Cd stress. However, this does not prevent us from considering the potential risks of growing medicinal plants (including lemon balm) in contaminated soils. It is recommended that the use of mycorrhiza be combined with other soil management strategies (such as soil amendment with organic matter or continuous monitoring of contaminants) to prevent the entry of heavy metals into medicinal products and ultimately to consumers.

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