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Research Article

Growth and biochemicals assessment of some landscape turfgrasses under Cd toxicity through bio-inoculation system

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Abstract

Application of strategies inducing a heavy metal tolerant turfgrass is necessary for cultivation management. Water and soil contamination with heavy metals is an increasing concern for the human and environment health. This study was conducted to evaluate the mitigation of environmental Cd toxicity through landscape turfgrasses as affected by two arbuscular mycorrhizal fungi (AMF) species and to monitor some physiological and biochemical properties of the plants in various Cd concentrations. Plants were inoculated with *Rhizophagus intraradices* and *Glomus mosseae* and without AMF, with the addition of different Cd concentration (0, 200, and 300 µg/L). AMF could colonize with the roots of turfgrasses in order as follows: *Agropyron elongatum* > *Festuca aurandinace* > *F. ovina* > *Lolium perenne*. The highest AMF colonization (~70%), Cd concentration in shoot (250 mg/Kg dry weight) and aerial and underground biomass (about 3 and 1.2 g/pot, respectively) as well as growth rate were displayed in *Agropyron elongatum* when inoculated with *G. mosseae* under 200 µg/L Cd solution. Both AMF species reduced H_2O_2 production and lipid peroxidation and enhanced catalase, peroxidase and superoxide dismutase activity. *Lolium perenne* accumulated higher Cd in its roots as compared to the other turfgrasses under non-AMF. Although *A. elongatum* and *Festuca aurandinace* had a translocation factor (TF)>1, they could produce considerable biomass and grow well through AMF inoculation. It is suggested that the two latter species could be used under highly Cd-contaminated soil/water if AMF is prepared.

Keywords: Antioxidative system, Heavy metal, Mycorrhizal colonization, Phytotoxicity, Turfgrass

Introduction

Heavy metal contaminated lands are progressively increasing due to different anthropogenic activity including mining, agricultural runoffs and industrial effluents. Cadmium (Cd) is considered as one of the most phytotoxic heavy metals to different plant species due to its high solubility in water, resulting in a prompted uptake by plant roots. It can interfere with many physiological and biochemical processes such as respiration, photosynthesis, nutrient uptake, protein and nitrogen metabolism (Zhang *et al*., 2009; Zhu *et al*., 2018). Cd could also adversely affect plants germination and growth, plant biomass, the uptake and translocation of mineral nutrients, shoot and root height, leading to death in some severe cases (Kupper *et al*., 2007; Fard *et al*., 2016).

Phytoremediation is an environmental health and cost efficient approach used as an alternative to earlier remediation procedures. It involves use of plants to entirely or partially scavenge certain contaminants in contaminated surface water, waste water, soil, sediment and sludge (Vishnoi and Srivastava, 2007). Meanwhile, the cultivation of the most tolerant/resistant plant species is considered as a crucial strategy to cope with the heavy metal-contaminated environment. The great variation has been found among plant species in relation to heavy metal tolerance and accumulation (Broadley *et al*., 2001). Therefore, identifying of Cd tolerant genotypes by monitoring their growth and developmental characteristics would be one of the promising ways to decline the harmful impacts of Cd existed in soils and water. Furthermore, plants grown in heavy metal-contaminated soils have shown nutrient deficiency and a low biomass (Shahabivand *et al*., 2012). During the last decades, low input cropping systems and innovation of resource management have been considered as the most important objectives of sustainable agriculture, so applying of bio-substrates and resultantly reduction of inputs application is one step forward to sustainability (Sharma *et al*., 2021). The use of various biological tools has been applied as an efficient strategy against heavy metal contamination

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sites. Different mycorrhizal fungi have been reported to mitigate the effects of heavy metals on various plant species. The effect of mycorrhizal symbiosis on physiological leaf parameters changed as the intensity of environmental stress changed (Gunathilakae *et al*., 2018). The microorganisms have been shown to play a key role in availability of macro and micronutrients, improving soil rhizosphere, and enhancing of plant tolerance (Jacob *et al*., 2018). Moreover, different mycorrhizal fungi species even within the same genus have different effects on plant response to environmental stress (Zardak *et al*., 2018).

Turfgrass species are major crops used in urban landscapes and industrial areas in many countries in the various climates of Europe, Asia and America (Curk *et al*., 2017). They show a wide range of adaptations and sensitivity/resistance to several adverse abiotic and biotic conditions. Moreover, some turfgrasses growing in heavy metal contaminated soil have exhibited phytoextraction ability (Hu *et al*., 2013). Although there are several types of mycorrhizal fungi formed mycorrhizae with plants, arbuscular mycorrhizae as the largest group form with the most turfgrass species. Mycorrhizal fungi exist in soil as hyphae, spores, or as colonized roots (Torres *et al*., 2011). Significant increase in total biomass, shoot length and heavy metal tolerance was reported when sorghum was inoculated with mycorrhizae (Duponnois *et al*., 2006). Also, the application of mycorrhizae in nutrient poor and heavy metal contaminated soil have increased metal tolerance and growth in maize (Li and Ramakrishna, 2011).

The need for Cd resistance and phytoremediation capability in turfgrass species is progressing because of the increased application of industrial effluent and other low-quality water for irrigation of turfgrasses. In our previous study, we screened the sensitivity and tolerance of different cool-season turfgrass species to Cd (Fard *et al*., 2016). Although most turfgrasses form an arbuscular mycorrhizal fungi (AMF) symbiosis, to the best of our knowledge, to date, the effects of AMF on four turfgrass species including *Festuca aurandiance, Festuca ovina, Lolium perenne,* and *Agropyron elongatum* have not been scrutinized in Cd-contaminated environment. The hypothesis of present research is that the application of AMF enhances the tolerance of landscape turfgrasses to Cd toxicity and promotes their growth. Here, we have used two mycorrhizae species, *Rhizophagus intraradices* and *Glomus mosseae*, to investigate (1) the phytoremedial potential of the turfgrasses and (2) the effects of AM fungal inoculation on turfgrass performance and Cd uptake under Cd contamination conditions. This study introduces a turfgrass species which could be as a favorable candidate for application in urban landscapes suffering from heavy metal polluted soil and water.

Materials and methods

Plant materials and treatments: The seeds of grasses were purchased from Pakan Bazr Company, Isfahan, Iran. They were sterilized with 0.5% NaClO, washed with distilled water and then sowed into polyvinyl chloride pots (16 cm in diameter and 60 cm in depth). Plants were grown in a glasshouse at temperature 23/20 °C (day/night), under illumination of 250 µmol m^{-2} s^{1}, and a 14/10 h light/dark period. Each plant species were treated with three Cd $(NO_3)_2$ concentrations (0, 200 and 300 µg/L) as irrigation water. Irrigation was done three times in a week. Plants in each Cd treatment were inoculated with *R. intraradices* and *G. mosseae* separately. The control plants inoculated with no AM fungi. Each pot contained 5 kg of medium material (perlite to cocopeat ratio of 3:1) and each AM inoculated treatment was mixed with 200 g of mycorrhizal inoculum. Non-mycorrhizal plants grew in a medium free from AM samples. Plants were irrigated by a full strength of Hoagland nutrient solution (Hoagland and Arnon, 1950) to maintain soil moisture at about 50% of the water-holding capacity. To do this, firstly, we fully irrigated a pot sample by a certain volume of water and then we used a half of the volume for irrigation to obtain 50% capacity. The water used for preparing the nutrient solution and irrigation was distilled water. Plant samples were clipped at 2 cm above the base of the crown about 3 months after sowing.

AM fungal colonization: Subsamples of fresh roots were taken to assess mycorrhizal colonization. Mycorrhizal colonization was determined by histological detection of mycorrhizal structures (hyphae, arbuscules and vesicles) after root staining (Phillips and Hayman, 1970). Briefly, a sample of the fresh root was cleared with KOH (10% w/v, 10 min, 90 °C), acidified in HCl (1% v/v, 5 min) and then stained with trypan blue (0.05% w/v, 10 min, 90 °C) in acid glycerol. The root length percentage of containing AMF colonization were estimated by microscopic (Olympus CH2, Tokyo, Japan) examination for 100 random root intersections for each plant (Kormanic and McGraw, 1982).

Biomass quantification: For biomass measurement, roots and shoots were separated at harvest time. After rinsing with tap water and then deionized water, the fresh weights of the organs were measured. They were again weighed after oven drying at 60 °C for 72 h and then ground to <0.25 mm in a stainless mill. The difference between the water content in remaining tissues (roots and shoots) and the total tissue fresh weight were used to estimate total tissue dry weight. Furthermore, the height of largest shoot and root was separately measured for each treatment.

Malondialdehyde (MDA) content: The lipid peroxidation level was measured through estimating MDA. Briefly, in 3 mL of 50 mM phosphate buffer, the plant tissues were homogenized. The homogenate was centrifuged for 15 min at 15,000 g. To 1.0 mL of the supernatant, 2.0 mL of 0.5 % thiobarbituric acid (TBA) in 20 % trichloroacetic acid (TCA) was added. The mixture was exposed to water bath for heating and then cooled in an ice bath. After centrifugation at 10,000 g

for 10 min, the absorbance of the supernatant was recorded at 532 nm by an UV/VIS spectrophotometer (Shimadzu UV-1800, Japan). Also, the value for nonspecific absorption at 600 nm for each sample was recorded and subtracted from the absorbance recorded at 532 nm. The MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer, 1968).

H_2O_2 content: Using 5 mL of 0.1% (w/v) TCA, H_2O_2 was extracted from tissue samples on an ice bath. Crude extract was then centrifuged for 15 min at 12,000 g. Subsequently, 0.5 mL of the supernatant was transferred to a 15 mL tube. The supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0), 1 mL of 1 M potassium iodide and vortexed. The absorbance of mixture was measured at 390 nm. A mixture with no supernatant was used as a blank (Sergiev *et al*., 1997).

Cadmium quantification: Plants grown in each treatment were uprooted, separated into stem and root, dried in oven for 60 h at 70 °C, and then thoroughly powdered and used for analysis. After weighting 1 g dry powder of each sample, 10 mL concentrated HNO_3 was added. The mixture was boiled for about 40 min at a constant temperature and then cooled. After wards, 5 mL of $HClO_4$ (70%) was added and the mixture was again boiled until the dense white fumes were released. After cooling, distilled water (20 mL) was added and heated until obtaining a clear solution. The mixture was filtered at room temperature using Whatman no. 44 filter paper and quantitatively transferred to a 50 mL volumetric flask via adding double-distilled and de-ionized water. By Atomic Absorption Spectro photometer, samples were analyzed. The unit of Cd concentration in plant tissues was calculated as mg/Kg of dry weight (Shah *et al*., 2017).

Meanwhile, the translocation factor (TF), called shoot-root quotient, was calculated as the ratio of metal concentration in shoot to root tissues.

Assay of antioxidant enzymes: The leaves of the turfgrasses (200 mg) were homogenized in 1 mL ice-cold extraction buffer containing 50 mM phosphate buffer (pH 7.0), 1 mM EDTA and 1.5% (w/v) of PVP. The homogenate was centrifuged at 9000 g for 15 min. The supernatant was used for determination of enzyme activities (Zhang and Nan, 2007).

The SOD activity was measured spectro photometrically according to the method of Beyer and Fridovich (1987). The reaction solution (1 mL) contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 12 mM riboflavin, 13 mM methionine, 7 mM nitro blue tetrazolium (NBT) and 10 µL of extracted enzyme solution. A solution with no enzyme was used as the control. Test tubes were exposed to fluorescent lights at 100 µmol m^{-2} s^{-1} for 20 min. The absorbance of each solution was read at 560 nm. One unit of enzyme activity was described as the value of enzyme that would prevent 50% of NBT photo reduction.

The catalase (CAT) activity was assayed as reported by Brouwer and Brouwer (1998). The reaction solution (0.5 mL) contained 10 mM H_2O_2, 25 mM phosphate buffer (pH 7.0) and 10 µL of extracted enzyme solution. After adding the enzyme solution, the reaction was initiated. Alterations in absorbance at 240 nm were read every 10s for 60s by a spectrophotometer. The absorbance change of 0.01 units per min was defined as one unit of CAT activity.

The peroxidase (POD) activity was determined by the method of Chance and Maehly (1955).

The POD activity in leaves was obtained by the oxidation of guaiacol in the exposure to H_2O_2. The increase in absorbance was recorded at 470 nm. The reaction mixture contained 100 µL of crude enzyme extract, 500 µL of 28 mM guaiacol, 500 µL of 5 mM H_2O_2, and 1900 µL of 50 mM potassium phosphate buffer (pH 7.0). The POD activity of the extract was expressed as U/g FW min.

Statistical analysis was performed using SAS software (Version 9.0). Data were analyzed by GLM (general linear model) and the separation of means was carried out by Duncan's multiple range test (DMRT) at $P<0.05$ significance level. Values reported here are means of four replicates.

Results

Percentage of fungal colonization: The results of the present study revealed that AMF-inoculated turfgrasses revealed significantly high percentage of colonization. Among the inoculated turfgrasses, *A. elongatum* exhibited significantly the highest AMF colonization (~70%), while *L. perenne* did the lowest one (40%) (Fig. 1). However, Cd concentration of 200 and 300 µg had no significant effect on colonization percentage in any turfgrasses, as compared to non-Cd treated plants. The order of AMF colonization percentage was as follows: *A. elongatum* > *F. aurandinace* > *F. ovina* > *L. perenne*. Although there was no significant difference between the colonization ability of the two applied fungi, *G. mosseae* could be colonized better than *R. intraradices* on the roots of the turfgrasses.

Cd uptake and biomass analyses: In the present study, the uptake of Cd by the four turfgrasses was directly proportional to the metal treatments in soil. As shown in Figure 2 and 3, the amount of Cd uptake was different among different parts (shoot and root) of turfgrasses. Some of the studied plant species accumulated Cd in their shoots more than that in roots, and vice versa. Under non-AMF condition and 300 µg/L Cd in irrigation water, among the plants, *F. ovina* had the lowest amount of Cd uptake, about 10 mg/Kg, while *F. aurandinace* had the highest one, about 170 mg/Kg Cd in their aerial organs (Fig. 2). Meanwhile, *L. perenne* accumulated higher Cd (about 150 mg/Kg) in its roots as compared to the other turfgrasses under non-AMF and 300 µg/L Cd in irrigation water (Fig. 3). Overall, AMF treatment significantly enhanced Cd uptake in all turfgrasses.

However, *G. mosseae* had a higher effect on Cd

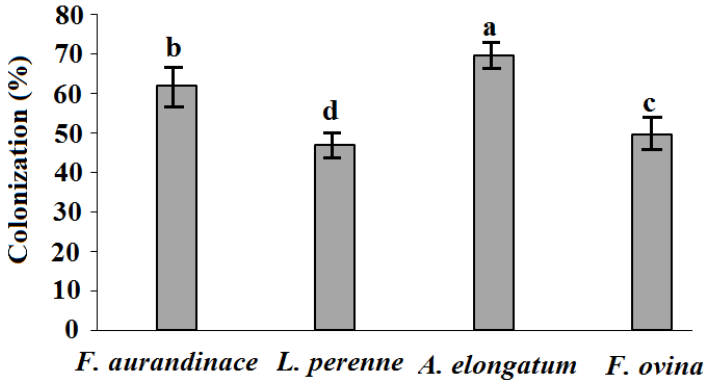

Fig. 1. Percentage Arbuscular mycorrhizal fungi (AMF) colonization in the four covering plants roots. Values represent means ± standard errors of 4 replications. Means with different letters are significantly different at 5% level using Duncan test.

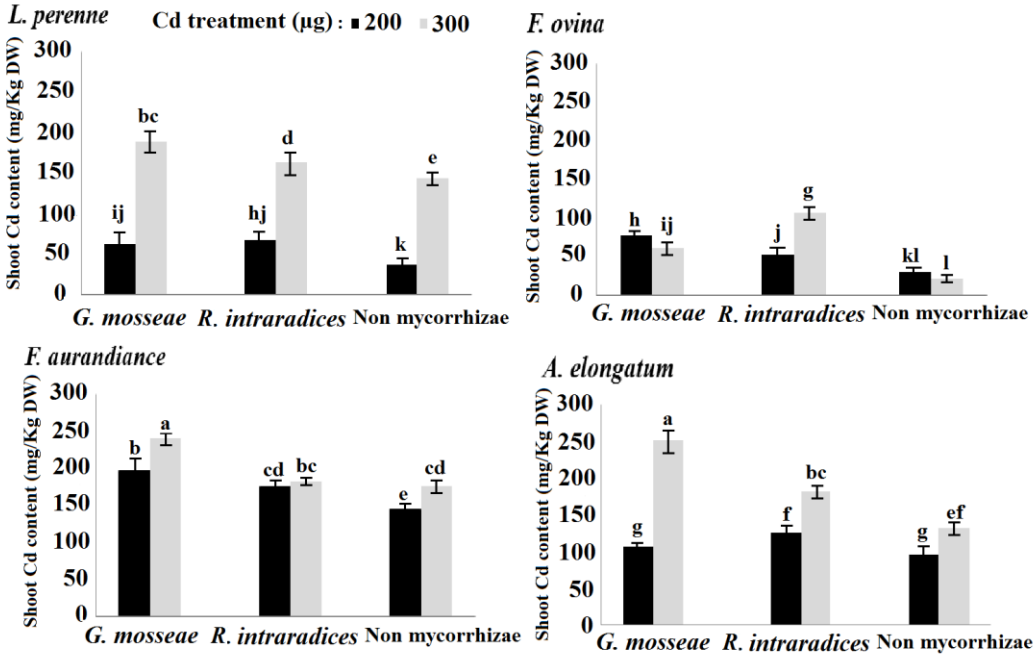

Fig. 2. Accumulation of Cd in the shoots of four covering plants inoculated with two AMF, *G. mosseae* and *R. intraradices*, and non- inoculated with AMF in added Cd levels treatments, 0, 200 and 300 µg. Values represent means ± standard errors of 4 replications. Means with different letters are significantly different at 5% level using Duncan test.

accumulation in both root and shoot of turfgrasses than *R. intraradices* did (Fig. 2 and 3). Under 300 µg Cd, *G. mosseae* led to 170, 250, 240, and 50 mg/Kg Cd accumulation in shoots of *L. perenne*, *F. aurandinace*, *A. elongatum*, and *F. ovina*, respectively, as compared to non-inoculated plants, while, respectively, *R. intraradices* caused Cd accumulation of 160, 170, 170, and 110 mg/Kg. Generally, the effects of the two AMF inoculations on Cd accumulation by the turfgrasses were much higher under 300 µg than 200 µg Cd application.

As Fig. 4 shows, the highest translocation factor (TF), 5.6 and 3.7, were observed in *F. aurandinace* and *A. elangatum* respectively, when treated with no AMF. In the other words, they had a capability to transfer the considerable amount of metal from their roots to shoots. AMF inoculation had no significant effect on TF in *L. perenne* and *F. ovina*. Exposure of the turfgrasses to different Cd concentrations and AMF inoculations altered the biomass production. The highest shoot and root weight, about 3 and 1.2 g/pot, respectively, was observed in *A. elongatum* inoculated with *G. mosseae* under 200 µg Cd, and the lowest one, about 0.9 and 0.12 g/pot, was in *F. ovina* inoculated with no AMF under 300 µg of Cd treatment (Fig. 5 and 6). The symbiosis with AMF ramped up biomass production in all the turfgrass species much higher than that in non-AMF inoculated species. The most root extension and shoot growth (about 65 cm and 38 cm) was observed in *A. elongatum* under inoculation of *G. mosseae* and non-Cd condition (Fig. 7). In all of the studied turfgrasses, at Cd concentration of 300 µg under no AMF inoculation, root

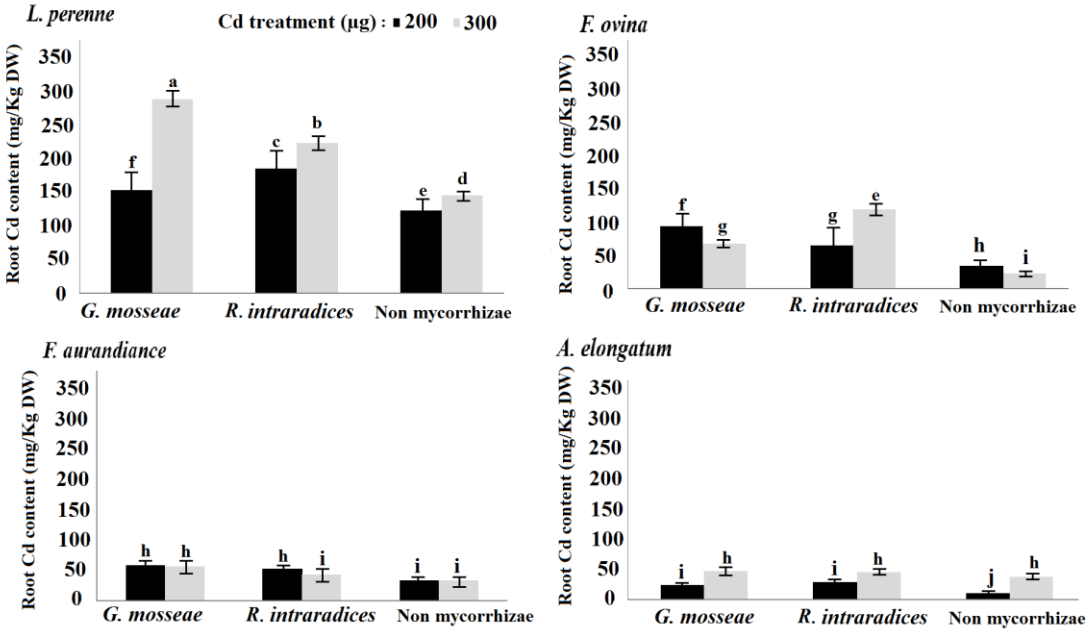

Fig. 3. Accumulation of Cd in the roots of four covering plants inoculated with two AMF, *G. mosseae* and *R. intraradices*, and non- inoculated with AMF in added Cd levels treatments, 0, 200 and 300 µg. Values represent means ± standard errors of 4 replications. Means with different letters are significantly different at 5% level using Duncan test.

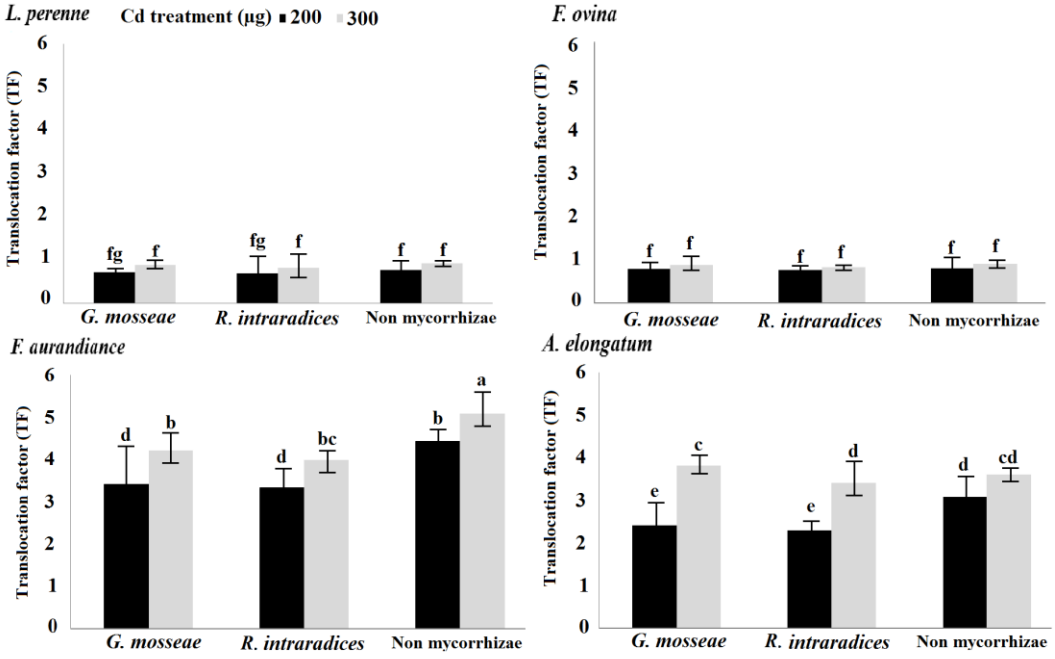

Fig. 4. Cd translocation factor (TF) of four covering plants as inoculated with two AMF species, *G. mosseae* and *R. intraradices*, in added Cd levels treatments, 0, 200 and 300 µg. Values represent means ± standard errors of 4 replications. Means with different letters are significantly different at 5% level using Duncan test.

growth was strongly reduced, so that *F. ovina* and *L. perenne* could only extend their roots by about 6 cm and 25 cm. As compared to the non-AMF inoculation, mycorrhizal species of *G. mosseae* increased root size by 95, 430, 10, and 8% and shoot size by 12, 38, 16, and 30% in *F. ovina*, *L. perenne*, *A. elongatum*, and *F. aurandinace*, respectively, at 300 µg Cd treatment.

Biochemical analyses: Biochemical analyses were conducted to monitor, in details, the role of AMF and its interaction with Cd in the turfgrasses. Results showed that exposure to Cd enhanced H_2O_2 and MDA production in all of the studied plant species. Among the plants, *L. perenne* showed the highest amount of H_2O_2, 2 µM, under non-AMF inoculation. However, as Cd concentration increased, more H_2O_2 and MDA were produced in the plants. AMF inoculation reduced both MDA and H_2O_2 production in the all Cd-treated turfgrasses. Meanwhile, among the plants, *A. elongatum*

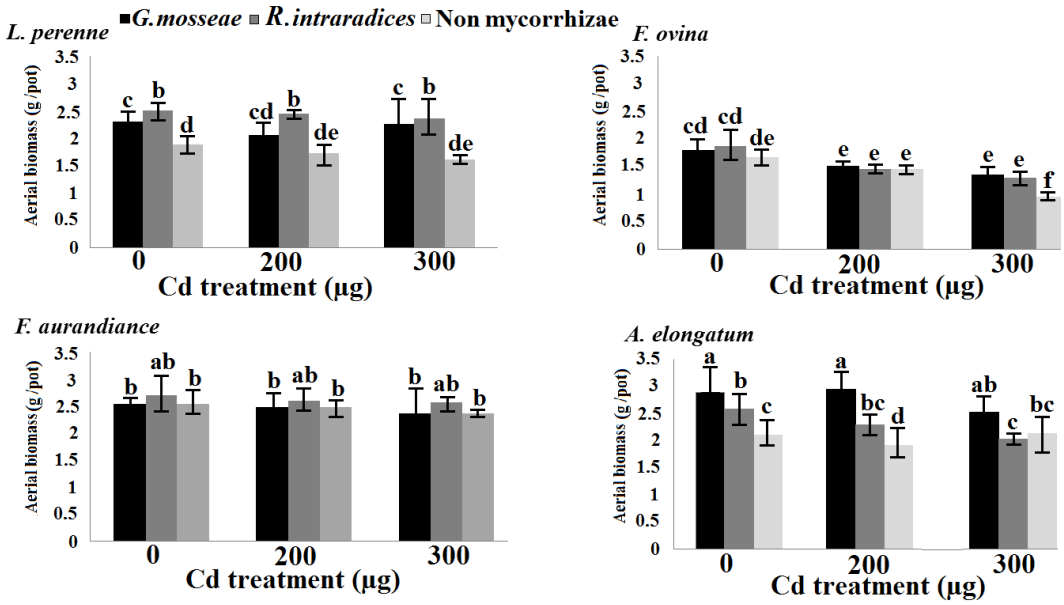

Fig. 5. The aerial dry biomass of four covering plants as inoculated with two AMF species, *G. mosseae* and *R. intraradices*, in added Cd levels treatments, 0, 200 and 300 µg. Values represent means ± standard errors of 4 replications. Means with different letters are significantly different at 5% level using Duncan test.

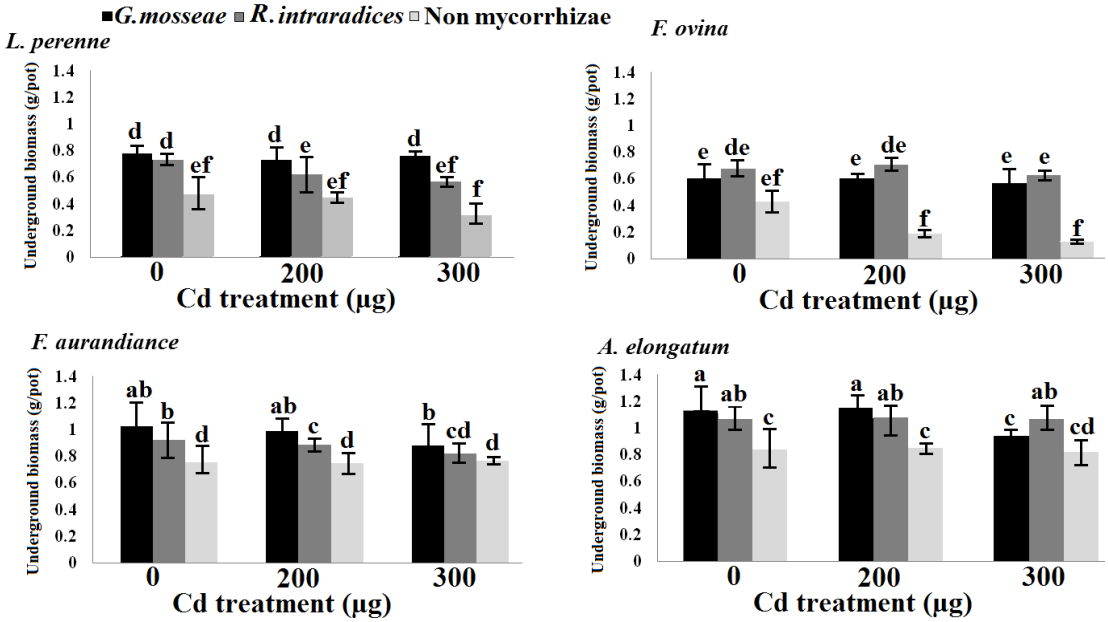

Fig. 6. The underground dry biomass of four covering plants as inoculated with two AMF species, *G. mosseae* and *R. intraradices*, in added Cd levels treatments, 0, 200 and 300 µg. Values represent means ± standard errors of 4 replications. Means with different letters are significantly different at 5% level using Duncan test.

was more affected by AMF inoculation concerning to H_2O_2 and MDA production. For example, under 300 µg Cd, *R. intraradices* led to 67, 41, 30 and 20% reduction and *G. mosseae* resulted in 80, 65, 20, and 12% reduction of H_2O_2 production in *A. elongatum*, *F. aurandinace*, *F. ovina* and *L. perenne*, respectively, as compared to their controls (non-inoculated plants) (Fig. 8). Moreover, the concentration of MDA ramped up as Cd concentration increased. However, similar to H_2O_2, MDA production was decreased in the plants inoculated with AMF. Under 300 µg Cd, *G. mosseae* and *R. intraradices* led to 25, 22.09, 8.93 and 4.48, % and 23.76, 19.22, 17.31, 6.06% reduction of MDA production in *A. elongatum*, *F. aurandinace*, *F. ovina* and *L. perenne*, respectively, as compared to their controls (Fig. 9). Our results indicated that, in most cases, Cd exposure caused an increase in oxidative stress (H_2O_2 and MDA), while AMF decreased the stress.

Antioxidant potential was altered in the turfgrasses as treated with different levels of Cd. As shown in Table 1, antioxidant enzymes activity increased in all

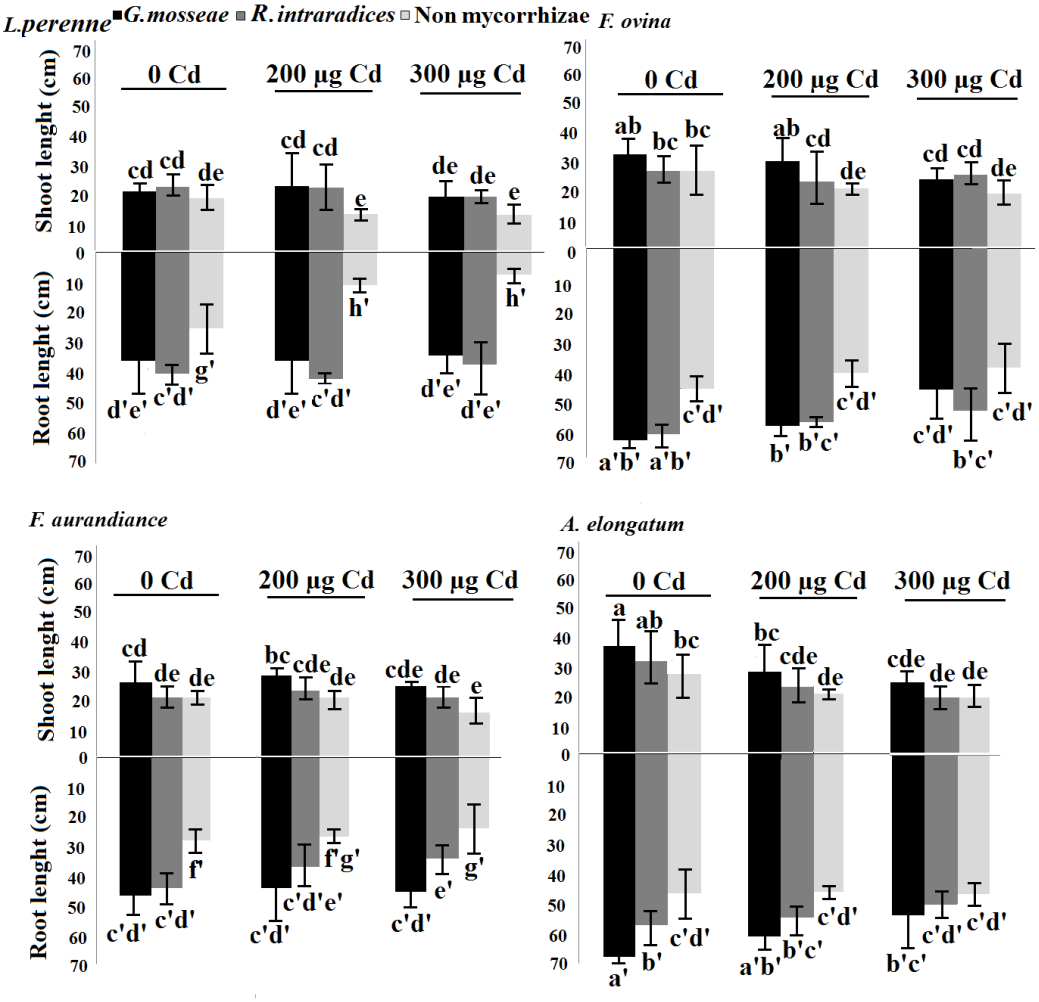

Fig. 7. The root and shoot length of four covering plants as inoculated with two AMF, *G. mosseae* and *R. intraradices*, and non- inoculated with AMF under added Cd levels treatments, 0, 200 and 300 µg. Values represent means ± standard errors of 4 replications. Means with different letters are significantly different at 5% level using Duncan test. The letters with apostrophe (a`, b`, etc.) and without it (a, b, etc.) were separately used to determine significance in root and shoot, respectively

turfgrasses after exposure to 200 µg Cd. However, under non-AMF inoculation, in contrary to *A. elongatum* and *F. aurandinace*, *F. ovina* and *L. perenne* showed low POD, SOD and CAT activities when treated with higher Cd concentration, 300 µg. Application of AMF significantly increased antioxidant enzymes activity in the four turfgrasses. The antioxidant potential in *A. elongatum* was more enhanced by AMF as compared to that in the other turfgrasses. The highest SOD (260 U/g FW min) and POD (1.12 U/g FW min) was observed in *A. elongatum*, and the highest CAT (1.12 U/g FW min) was found in *F.aurandinace* under both *R. intraradices* inoculation and Cd concentration of 300 µg.

Discussion

In the present study, four cool-season turfgrass species, *A. elongatum*, *F. aurandinace*, *F. ovina* and *L. perenne* were selected among the six turfgrasses based on our previous research (Fard *et al*., 2016). In that research, we had exposed six cool-season turfgrasses to various Cd concentrations to monitor their germination indices and incipient seedling growth. Two of them included *Festuca rubra* and *Poa pratensis* were too sensitive to germinate in an appropriate rate and to grow. The other four species showed relatively a considerable germination rate and had grown to a moderate extent under moderate Cd concentrations. Here, for enhancement of Cd-tolerance and phytoremediation ability in the four tourfgrasses, we applied *R. intraradices* and *G. mosseae* as AMF species. We evaluated and compared colonization rate, Cd uptake, biomass production, some factors regarding cell injuries as well as antioxidant defense system.

AMF colonization occurred in different rate among the studied turfgrasses. So that, *A. elongatum* had the highest AMF colonization. However, Cd concentration of 200 µg did not show any significant effect on

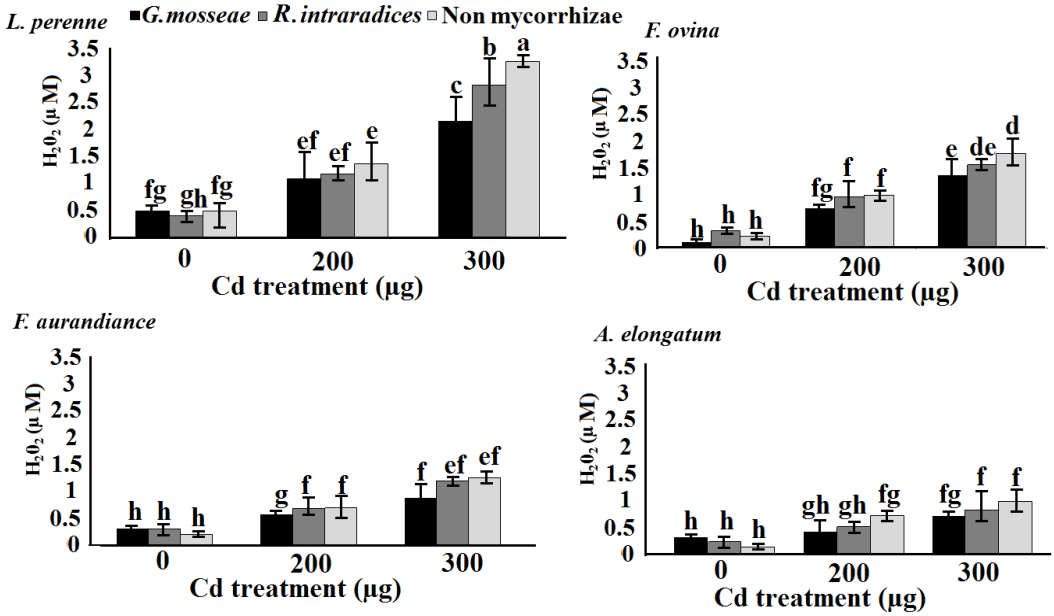

Fig. 8. The H_2O_2 content of four covering plants as inoculated with two AMF, *G. mosseae* and *R. intraradices*, and non-inoculated with AMF under added Cd levels treatments, 0, 200 and 300 µg. Values represent means ± standard errors of 4 replications. Means with different letters are significantly different at 5% level using Duncan test.

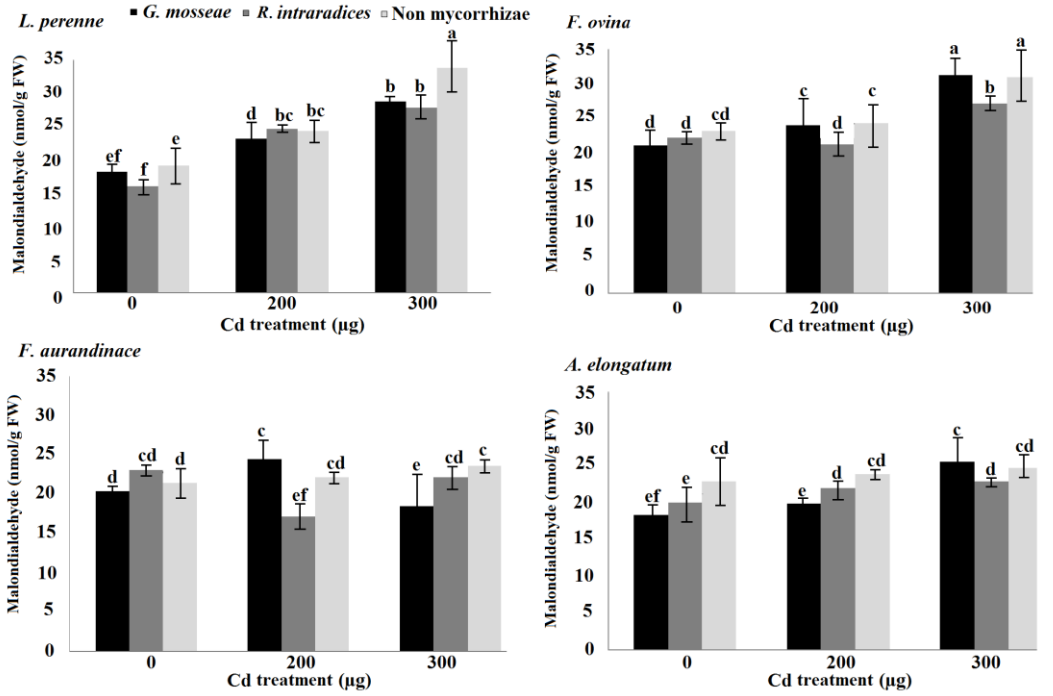

Fig. 9. The malondialdehyde (MDA) content of four covering plants as affected by inoculation with two AMF, *G. mosseae* and *R. intraradices*, and non- inoculated with AMF under added Cd levels treatments, 0, 200 and 300 µg. Values represent means ± standard errors of 4 replications. Means with different letters are significantly different at 5% level using Duncan test.

colonization percentage. It is in accordance with the findings of previous researches (Kanwal *et al*., 2015; Liu *et al*., 2015). It has been reported that the addition of Cd into the water/soil environment has not prevented from the formation of external hyphae and mycorrhizal colonization of plant roots (Bhaduri and Fulekar, 2012; Gunathilakae *et al*., 2018). Meanwhile, higher Cd levels (300 µg) significantly reduced root mycorrhizal colonization. According to Chen *et al*. (2004), the amount of colonization occurred by *Funneliformis mosseae* on the roots of *Zea mays* was not influenced with the Cd contaminations of 0 to 100 µg g^{-1} soil. Also, AMF inoculation enhanced Cd uptake by the turfgrasses. The uptake of Cd by the plants in our study suggested clearly that the plants functioned better in AMF as compared to the non-AMF under high concentration of Cd. Similarly, considerable influence of AMF on metal uptake has been also reported by

Bhaduri and Fulekar (2012).

The capability of a plant to transfer metal from its roots to shoots is defined as translocation factor (TF). It has been demonstrated that the plant species with TF>1 are suitable for using in phytoextraction process. Inversely, the plants with higher capability of metal accumulation in their roots, higher biomass production, and TF<1 are suitable for phytostabilization purposes (Mendez and Maier, 2008). However, the amount of TF<1 in *L. perenne* and *F. ovina* is in parallel with results from Kashem *et al*. (2008), that in *Ipomoea aquatica*, 88% of the absorbed Cd get accumulated in the roots. The highest proportion of Cd in the roots of the turfgrasses may be attributed to immobilization of Cd through adsorption and/or precipitation on the root surface and within the root cells symplasm as well. It also may be due to Cd sequestration by phytochelatins in the vacuoles of root cells (Shute and Macfie, 2006).

The production of biomass referred to relative growth altered after exposing the turfgrasses to different Cd concentrations and AMF inoculations. Biomass production in all the turfgrass species increased much higher by the symbiosis with AMF than non-AMF. Some studies indicated that AMF could immobilize heavy metals in the root and the mycorhizosphere of the plant and prevent from their transfer to the shoot (Garg and Chandel, 2015; Liu *et al*., 2015). The reduction in shoot Cd concentration compared to roots in AMF inoculated turfgrasses can be attributed to the compartmentalization of Cd by AMF hyphae in roots through Cd binding and adsorbing (Meier *et al*., 2012). Based on Nielsen and Jensen (1983), the reduction in shoot Cd concentration may be resulted from "dilution effect", in which as plant biomass enhances the concentration of metal decreases in a certain amount of tissue.

Tolerant response to a certain heavy metal is controlled through a complicated inter-related cascade of biochemical, physiological and genetic mechanisms. The ions of heavy metal bind to the different functional groups and are able to substitute with specific cations in binding sites, leading to the production of reactive oxygen species (ROS) and inactivation of many enzymes. In the other words, heavy metals cause oxidative stress in plants by inducing ROS production (Maleki *et al*., 2017). It indicates that the capability of AMF in reducing ROS amount, like H_2O_2, can protect the plant tissues even under high metal uptake. MDA is a direct product of cell membrane collapse (membrane lipid peroxidation) referring to the presence of higher cell degradation agents, including ROS (Salehi *et al*., 2012). Both AMF species, *G. mosseae* and *R. intraradices*, improved antioxidant potential in the plants. Furthermore, the turfgrasses with the higher AMF colonization showed the higher antioxidant potential. So that, *A. elongatum* had the highest AMF colonization and the highest activity of POD, SOD and CAT as well. The increase in heavy metal uptake was in parallel with the increase of colonization. According to our study, it seems that the application of AMF can be considered as an appropriate candidate for reinforcement of phytoremediation by enhancing heavy metal accumulation in turfgrasses, particularly in *A. elaongatum*, and increasing the antioxidant defense system in the plants. Although AMF treatment enhanced Cd uptake in all turfgrasses, the plants with the higher Cd accumulation continued to grow and remained healthy. The present study reveals that *A. elongatum* can be considered as a hyperaccumulator of cadmium, and it is more effective with AMF. AMF responsiveness depending on the plant-fungal genotype combination and on environmental conditions. *A. elongatum* and *F.* aurandinace had TF>1, they could produce considerable biomass and grow well. The two species may be suggested to use under highly Cd-contaminated soil/water if AMF is prepared. It also seems that *L. perenne* had the properties deserving it to be considered as an appropriate turfgrass for phytostabilization purposes.

Conclusion

In conclusion, our results indicate that the colonization percentage of AMF with the roots of the tested turfgrasses varies depending on the plant species. The highest AMF colonization, Cd accumulation in shoot, aerial and underground biomass and growth rate were observed in *A. elongatum* when inoculated with *G. mosseae*. AMF species, *G. mosseae* and *R. intraradices* strengthen antioxidant potential and prevents plant cells from damaging by H_2O_2 production. *L. perenne* accumulated higher Cd in its roots as compared to the other turfgrasses under non-AMF. There is a positive relationship between AMF colonization and Cd accumulation in the plants. According to our study, *A. elongatum* and *F. aurandinace* could be used under highly Cd-contaminated soil/water if AMF is prepared. However, more researches are needed to decipher the mechanisms involved in AMF-induced heavy metal tolerance of plants.

References

Beyer, W. F. and Fridovich, I. (1987) Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Analytical Biochemistry 161: 559-566.

Bhaduri, A. M. and Fulekar, M. H. (2012) Assessment of arbuscular mycorrhizal fungi on the phytoremediation potential of *Ipomoea aquatica* on cadmium uptake. Biotechnology 2: 193-198.

Broadley, M. R., Willey, N. J., Wilkins, J. C., Baker, A. J. M., Mead, A. and White, P. J. (2001) Phylogenetic variation in heavy metal accumulation in angiosperms. New Phytologist 152: 9-27.

Brouwer, M. and Brouwer, T. H. (1998) Biochemical defense mechanisms against copper-induced oxidative damage in the blue crab, *Callinectes sapidus*. Archives of Biochemistry and Biophysics 351: 257-264.

Chance, B. and Maehly, A. C. (1955) Assay of catalases and peroxidases. Methods of Enzymology 11: 764-775.

Chen, Y., Li, X. and Shen, Z. (2004) Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process. Chemosphere 57: 187-196.

Curk, M., Vidrih, M., Laznik, Z. and Trdan, S. (2017) Turfgrass maintenance and management in soccer fields in Slovenia. Urban Forestry and Urban Greening 26: 191-197.

Duponnois, R., Kisa, M., Assigbetse, K., Prin, Y., Thioulouse, J., Issartel, M., Moulin, P. and Lepage, M. (2006) Fluorescent pseudomonads occuring in *Macrotermes subhyalinus* mound structures decrease Cd toxicity and improve its accumulation in sorghum plants. Science of the Total Environment 370: 391-400.

Fard, K. G., Zakizadeh, H., Ghasemnezhad, M., Kafi, M. and Rejali, F. (2016) Response of six turf species to cadmium stress: Displaying the most tolerant cultivar. Vegetos 29.

Garg, N. and Chandel, S. (2015) Role of arbuscular mycorrhiza in arresting reactive oxygen species (ROS) and strengthening antioxidant defense in *Cajanus cajan* (L.) Millsp. nodules under salinity (NaCl) and cadmium (Cd) stress. Plant Growth Regulation 75: 521-534.

Gunathilakae, N., Yapa, N. and Hettiarachchi, R. (2018) Effect of arbuscular mycorrhizal fungi on the cadmium phytoremediation potential of *Eichhornia crassipes* (Mart.) Solms. Groundwater for Sustainable Development 7: 477-482.

Heath, R. L. and Packer, L. (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives in Biochemistry and Biophysics 125: 189-198.

Hoagland, D. R. and Arnon, D. I. (1950) The Water-Culture Method for Growing Plants Without Soil. 2nd Ed. Circular. California Agricultural Experiment Station 347.

Hu, J. L., Wu, S. C. and Wu, F. Y. (2013) Arbuscular mycorrhizal fungi enhance both absorption and stabilization of Cd by Alfred stonecrop (*Sedum alfredii* Hance) and perennial ryegrass (*Lolium perenne* L.) in a Cd-contaminated acidic soil. Chemosphere 93: 1359-1365.

Jacob, J. M., Karthik, C., Saratale, R. G., Kumar, S. S., Prabakar, D., Kadirvelu, K. and Pugazhendhi, A. (2018) Biological approaches to tackle heavy metal pollution: A survey of literature. Journal of Environmental Management 217: 56-70.

Kanwal, S., Bano, A. and Malik, R. N. (2015) Effects of arbuscular mycorrhizal fungi on metals uptake, physiological and biochemical response of *Medicago Sativa* L. with increasing Zn and Cd concentrations in soil. American Journal of Plant Sciences 6: 2906-2923.

Kashem, M. A., Singh, B. R., Huq, S. M. I. and Kawai, S. (2008) Cadmium phytoextraction efficiency of arum (*Colocasia antiquorum*), radish (*Raphanus sativus* L) and water spinach (*Ipomoea aquatica*) grown in hydroponics. Water, Air, and Soil Pollution 192: 273-279.

Kormanic, P. P. and McGraw, A. C. (1982) Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Methods and Principles of Mycorrhizal Research. (ed. Schenck, N. C.) Pp. 37-45. American Phytopathological Society, St. Paul.

Kupper, H., Parameswaran, A., Leitenmaier, B., Trtilek, M. and Setlik, I. (2007) Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. New Phytologist 175: 655-674.

Li, K. and Ramakrishna, W. (2011) Effect of multiple metal resistant bacteria from contaminated lake sediments on metal accumulation and plant growth. Journal of Hazardous Materials 189: 531-539.

Liu, H., Yuan, M., Tan, S., Yang, X., Lan, Z., Jiang, Q., Ye, Z. and Jing, Y. (2015) Enhancement of arbuscular mycorrhizal fungus (*Glomus versiforme*) on the growth and Cd uptake by Cd-hyperaccumulator *Solanum nigrum*. Applied Soil Ecology 89: 44-49.

Maleki, M., Ghorbanpour, M. and Kariman, K. (2017) Physiological and antioxidative responses of medicinal plants exposed to heavy metals stress. Plant Gene 11: 247-254.

Meier, S., Borie, F., Bolan, N. and Cornejo, P. (2012) Phytoremediation of metal-polluted soils by arbuscular mycorrhizal fungi. Critical Reviews in Environmental Science And Technology 42: 741-775.

Mendez, M. O. and Maier, R. M. (2008) Phytostabilization of mine tailings in arid and semiarid environments-an emerging remediation technology. Environmental Health Perspectives 116: 278-283.

Nielsen, J. D. and Jensen, A. (1983) Influence of vesicular-arbuscular mycorrhiza fungi on growth and uptake of various nutrients as well as uptake ratio of fertilizer P for lucerne (*Medicago sativa*). Plant and Soil 70: 165-172.

Phillips, J. M. and Hayman, D. S. (1970) Improved procedures for clearing and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158-161.

Salehi, M., Fotouhi-Ghazvini, R. and Jafarian, V. (2012) Evaluation of exogenous ascorbic acid application as a protective agent against simulated acid rain in Persian maple (*Acer velutinum* Boiss). Environmental Science 7: 413-420.

Sergiev, L., Alexieva, E. and Karanov, E. (1997) Effect of spermine, atrazine and combination between them on some endogenous protective systems and markers in plants. Comptes Rendus de l'Academie Bulgare des Sciences 51: 121-124.

Shah, K., Mankad, A. U. and Reddy, M. N. (2017) Cadmium accumulation and its effects on growth and biochemical parameters in *Tagetes erecta* L. Journal of Pharmacognosy and Phytochemistry 6: 111-115.

Shahabivand, S., Maivan, H. Z., Goltapeh, E. M., Sharifi, M. and Aliloo, A. A. (2012) The effects of root endophyte and arbuscular mycorrhizal fungi on growth and cadmium accumulation in wheat under cadmium toxicity. Plant Physiology and Biochemistry 60: 53-58.

Sharma, P., Kumar, T., Yada, M., Gill, S. S. and Chauhan, N. S. (2021) Plant-microbe interactions for the sustainable agriculture and food security. Plant Gene 28: 100325.

Shute, T. and Macfie, S. M. (2006) Cadmium and zinc accumulation in soybean: a threat to food safety. Science of the Total Environment 371: 63-73.

Torres, Y. A., Busso, C., Montenegro, O., Ithurrart, L., Giorgetti, H., Rodriguez, G., Bentivegna, D., Brevedan, R., Fernandez, O., de la Merced Mujica, M. and Baioni, S. (2011) Defoliation effects on the arbuscular mycorrhizas of ten perennial grass genotypes in arid Patagonia, Argentina. Applied Soil Ecology 49: 208-214.

Vishnoi, S. R. and Srivastava, P. N. (2007) Phytoremediation-green for environmental clean. Proceedings of Taal 2007. The 12th World Lake Conference.

Zardak, S., Movahhedi, M., Salehi, A. and Gholamhoseini, M. (2018) Effects of using arbuscular mycorrhizal fungi to alleviate drought stress on the physiological traits and essential oil yield of fennel. Rhizosphere 6: 31-38.

Zhang, F., Zhang, H., Wang, G., Xu, L. and Shen, Z. (2009) Cadmium-induced accumulation of hydrogen peroxide in the leaf apoplast of *Phaseolus aureus* and *Vicia sativa* and the roles of different antioxidant enzymes. Journal of Hazardous Materials 168: 76-84.

Zhang, Y. P. and Nan, Z. B. (2007) Growth and anti-oxidative systems changes in *elymus dahuricus* is affected by *neotyphodium* endophyte under contrasting water availability. Journal of Agronomy and Crop Science 193: 377-386.

Zhu, G., Xiao, H., Guo, Q., Zhang, Z., Zhao, J. and Yang, D. (2018) Effects of cadmium stress on growth and amino acid metabolism in two Compositae plants. Ecotoxicology and Environmental Safety 158: 300-308.