Impact of endophyte fungus *Serendipita indica* on fungus-assisted phyto-stabilization and performance of *Carthamus tinctorius* in a lead polluted soil

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Abstract

The beneficial root endophytes play a crucial impact in improving plant performance, and participate in enhancing phytoremediation efficiency of host in metal-polluted sites. This experiment investigated the influence of root endophyte fungus *Serendipita indica* on lead (Pb) accumulation and translocation, some physiological attributes, and antioxidant system of leaf in *Carthamus tinctorius* L. (safflower) cv. Sina in soil contaminated with different levels Pb (0, 400, 800 and 1200 mg Pb/kg soil). By increasing Pb levels in soil, Pb uptake by root, and Pb accumulation in root and shoot were significantly elevated. A significant increase on Pb accumulation in root and a significant reduction on shoot Pb amount were observed under fungal symbiosis. Also, *S. indica*-inoculated plants had lower translocation factor (TF) and bioaccumulation factor of Pb in shoot (BFS), whereas higher bioaccumulation factor of Pb in root (BFR), in compare to non-inoculated ones. Presence of *S. indica* yielded in greater growth parameters, photosynthetic pigments, chlorophyll fluorescence parameters, and antioxidant enzymes activities of CAT, APX and SOD, under all levels Pb in soil. We suggest the consideration of this endophyte for fungus-assisted phyto-stabilization/immobilization of Pb in host roots. Also, we concluded that *S. indica* mediated different tolerance strategies to mitigate Pb toxicity and higher performance of safflower.

Keywords: Lead, *Serendipita indica*, *Carthamus tinctorius*, Heavy metal accumulation

Introduction

Human industrial activities have been caused serious hazards to environment and human health by pollutants. Lead (Pb), as a heavy metal element, is one of the toxic metals in ecosystems that derived mainly from anthropogenic sources such as pesticides, lead based paints, lead glazed ceramics, lead-based solder, lead ore mining, tailings and smelting [29]. Agricultural soils with higher level of Pb require an effective and economically viable solution, because Pb affects consumer health via the food chain [10]. The metal toxicity has also reported in plants with symptoms including leaf chlorosis, blackening of the root, and reduced growth and productivity caused because of Pb interference on their physiology, biochemistry and cellular ultrastructure [31]. Decreased chlorophyll pigments, damaged chloroplasts, inhibited photosynthetic electron transport and the net assimilation of CO_2, altered antioxidant enzyme system and redox balance, disturbed mineral nutrition and obstructed Calvin cycle enzymes activities are the main interrupted physiological processes by excess Pb in plants [13, 15, 19, 27]. Even though, Pb toxicity induces accumulation of reactive oxygen species (ROS) and raises the level of lipid peroxidation [49], the antioxidant defense system will efectively eliminate the plant excessive ROS to attenuate Pb toxicity in plants [16].

Different bio-approaches have been used to remediate the heavy metal polluted sites such as usage of plants, algae and microbes, but in most cases their tolerance to heavy metal is low. In the plants, some symbioses can effectively enhance their resistance to the pollutions that among them mycorrhizal and endophyte fungi, as major groups of plant mycobionts, contribute greatly in plant compatibility to stresses [47]. Root-colonizing endophytes play a crucial impact in improving plant performance, and participate in heavy metal stress tolerance, fungus-assisted phytoremediation, food safety and sustainable crop production by modulating the plant physiology and biochemistry under metal stressful environment [4, 22, 47]. A root endophytic fungus was isolated from the rhizosphere of woody shrubs growing in the Thar desert of Rajasthan, India, that was named *Serendipita indica* (formerly named *Piriformospora indica*) based on its characteristic pear shaped chlamydospores [46, 54]. This endophyte is related to the Hymenomycetes phylum of the Basidiomycota class and belongs to the order Sebacinales [37, 55]. It colonizes the roots of a wide range of hosts including various members of gymnosperms and angiosperms, and mimics the capabilities of typical arbuscular mycorrhizal fungi (AMF) [51]. The colonization of various plants root with this fungus stimulates growth and yield, promotes macro- and micronutrient uptake, and allows host to survive under biotic and abiotic stresses [34, 42, 51, 55]. *S. indica* confers heavy metal tolerance in tobacco and sunflower by increasing the chlorophyll content and leaf antioxidant enzymes activity, and improving the fluorescence parameters [25, 42].

Safflower (*Carthamus tinctorius* L.) is an annual herbaceous crop, highly branched and with a deep root system that belongs to family Compositae. In the recent decades, it is grown mainly for the oil that is extracted from the seeds, and therefore is well known as an oilseed crop. Safflower is tolerant to various metals and can be grown on polluted soils with heavy metals, hence it has potential to use in phytoremediation process [2]. This crop possesses interesting characteristics in terms of Cd accumulation so that it is capable of accumulating high levels of Cd in root and above-ground parts without showing symptoms of toxicity [44]. The metal phyto-stabilization process refers to minimize the transportation and leaching of metal in soil, allowing metal accumulation in plant root with only small amounts of metal being translocated to the above-ground parts.

By literature, data on *S. indica* colonization with safflower roots in Pb-contaminated sites is rare. Therefore, the main objective of this study was to evaluate the potential effects endosymbiosis fungus *S. indica* on improving phytoremediation efficiency, growth status, fluorescence parameters, and antioxidant capacity of safflower under increasing Pb levels in soil. We hypothesized that (1) *S. indica* would change the uptake, accumulation and translocation of Pb in the host organs, (2) root colonization by *S. indica* was an advantage that would promote the growth and biomass accumulation of safflower under Pb stress, and (3) the physiological and biochemical changes induced by *S. indica* would contribute to Pb tolerance of safflower at toxic levels of Pb in soil.

Materials and methods

Plant and fungal materials: A pot experiment was conducted under greenhouse conditions and consisted of a completely randomized factorial design (2 fungus inoculation treatments × 4 Pb levels) in 5 replicates. Healthy seeds of safflower (*Carthamus tinctorious* L.) cv. Sina obtained from the Dryland Agricultural Research Institute, Maragheh, Iran, were surface sterilized for 2 min in ethanol followed by dipping for 10 min in 1%

NaClO solution, then washed with distilled water six times and germinated on moist filter paper at 4 °C for 48 h in the dark. *S. indica* (gifted by Prof. Goltapeh, Department of Plant Pathology, Tarbiat Modares University, Tehran, Iran) was propagated in Petri dishes on a Hill & Käfer medium [24] and incubated at 29 ± 1 °C in the dark for two weeks.

Soil preparation: A sandy loam topsoil (0–25 cm) was taken from the surface horizon of Maragheh University Campus farm. The physico-chemical characteristics of the soil used in our study were as follows: 68% sand, 20% silt, 12% clay, 1.2% organic matter, 0.05% total N, 7 mg/kg available P, 35 mg/kg available K, 9.7 mg/kg total Pb, pH 7.2 and 1.28 dS/m EC. Fresh soil was air-dried and then passed through a 5-mm sieve and finally was steam-sterilized at 100 °C for 1 h (3 times in 3 consecutive days) to elimination of various microorganisms in the experimental soil. After that, soil samples were artificially contaminated with different Pb concentrations (0, 400, 800 and 1200 mg Pb/kg soil) using $Pb(NO_3)_2$ solution. After Pb contamination, soil samples were permitted to equilibrate for 4 weeks in order to Pb distribution into various fractions of soil.

Planting setup: The uniform and well-grown seedlings (two days after seed germination) were transplanted in plastic pots filled with 5 kg of sterilized soil that contained four added Pb levels. At sowing time, two fungal plugs (10 mm diameter) were placed at a distance of 1 cm below the safflower seedlings in the soil. *S. indica*-free treatments were mock-inoculated with autoclaved plugs. The experimental safflowers were grown in a greenhouse under a photoperiod of 14 h day at 28 ± 2 °C and 10 h dark at 18 ± 2 °C, with 60–70% average relative humidity. The irrigation was done once every three days to near field capacity using deionized water. Furthermore, 100 ml of Hoagland's nutrient solution was added to each pot every week for improvement plant growth. At harvesting time (45 days after sowing), safflowers were separated into shoots and roots, washed and then plant growth attributes including shoot length and root length were recorded. For determination of shoot and root dry matters and Pb content, the samples were oven dried at 75 °C until constant weight. The fresh plant samples were stored at −80 °C until biochemical analyses.

Determination of root colonization: The percentage of root colonization by *S. indica* was measured by the method of Oelmüller *et al.* [32] after root (1 g) cleaning in 10% KOH and staining with 0.1% Trypan blue [36]. In this method, *S. indica*-chlamydospores distribution within the roots of colonized plants was estimated as an index for root colonization.

Determination of Pb content: The oven-dried shoots and roots (0.1 g) were finely ground separately, and then digested in a mixture of concentrated HNO_3 and $HClO_4$ (7:1 ratio, v/v). The content of Pb in plant extracts was determined using an atomic absorption spectrophotometr (Shimadzu, Japan). The translocation factor (TF) of Pb was determined as the ratio of Pb concentration in shoot to Pb concentration in root. Also, bioaccumulation factor of shoot (BFS) and root (BFR) was computed as ratio of Pb content in shoot or root to Pb concentration in the soil

Determination of photosynthetic pigments: For measurement of chlorophyll (Chl) in the youngest fully expanded leaf, 0.1 g of leaf sample was homogenized with acetone 80% (v/v) and then filtrated through filter paper. The absorbance of filtrate was read at 663 and 645 nm for Chl *a* and Chl *b*, and the pigments contents were estimated according to Arnon [5].

Bioassay of antioxidant enzyme activities: Frozen fresh leaves (0.3 g) were

powdered in liquid nitrogen, homogenized with 3 ml of ice-cold potassium phosphate buffer (100 mM, pH 7.5, 0.5 mM EDTA for assay SOD and CAT activities, and soluble protein content; 1 mM ascorbic acid and 0.5 mM EDTA as reaction mixture for assay APX activity) and then centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was used to record the soluble protein and enzymatic activities. Total soluble protein was measured with bovine serum albumin as the standard by the method of Bradford [11]. The superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by measuring SOD ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) by superoxide radicals generated at 560 nm, according to Dhindsa and Matowe [18]. One SOD unit was considered as the amount of enzyme required to cause 50% inhibition of the photochemical reaction of NBT (using methionine and riboflavin). Catalase (CAT, EC 1.11.1.6) activity was assayed spectrophotometrically at 240 nm by Aebi method [1]. The hydrogen peroxide decomposition rate was monitored during 1 min at 25 °C. The activity of APX (EC 1.11.1.1) was assayed by Nakano and Asada method [31]. The reaction of ascorbic acid oxidation was started by addition of H_2O_2 and the decrease in absorbance was read at 290 nm.

Measurements of chlorophyll fluorescence: After darkening the leaf for 30 min, the chlorophyll fluorescence parameters including Fv/Fm (maximum quantum efficiency of PSII photochemistry) and ETR (the relative PSII electron transport rate), as physiological indicators, were measured on the new fully expanded leaf using a portable chlorophyll fluorometer (model Hansatech, Instruments LTD, UK).

Determination of malondialdehyde (MDA): The level of leaf MDA, a lipid peroxidation product, was analyzed by the thiobarbituric acid (TBA) reaction as described by Heath and Packer [23]. Absorbance was recorded at 600 nm and 532 nm (the blank was 1% thiobarbituric acid in 20% trichloroacetic acid). The level of leaf MDA was determined by an extinction coefficient of 155 mM^{-1} cm^{-1}.

Statistical analysis: Two-way analysis of variance (ANOVA) was performed on all experimental data using SAS 9.4 software version 2013. Comparisons between means were carried out using Duncan's multiple range tests at $P < 0.05$. The results were expressed as the means of five replicates ± standard deviation (SD).

Results

Pb accumulation in root and shoot: By increasing Pb concentration in the soil, the root and shoot Pb amounts were significantly increased in colonized and non-colonized plants (Table 1). According to Table 1, Pb contents in root were higher than soil Pb concentration, as well as shoot Pb contents at all levels of Pb in the soil. Translocation factor (TF) and bioaccumulation factor in root (BFR) and shoot (BFS) were reduced from 0 to 400 mg Pb (this reduction, in the case of TF was not significant, but in the case of BFR and BFS was significant), and then were approximately constant from 400 to 1200 mg in the soil (Table 1). On the other hand, fungal inoculation significantly elevated Pb content in the root, whereas significantly reduced Pb content in the shoot, in different soil Pb concentrations (except at 0 mg Pb), in comparison to non-inoculated treatments. The safflowers inoculated with *S. indica* showed increases of 36.3, 20.6 and 12% in root Pb content, and reductions of 39.7, 23.2 and 45.8% in shoot Pb content under 400, 800 and 1200 mg Pb/kg soil, respectively, compared to un-inoculated ones (Table 1). The TF value was also reduced by the inoculation of safflowers with the fungus, but this reduction was not

significant at all levels of Pb in soil (Table 1). In comparison with absence of the endophyte, presence of *S. indica* significantly increased BFS and BFR at the dose of 0 mg Pb soil, but had no significant influence on these values at the other levels of Pb in the soil, and mentioned parameters were approximately constant (Table 1).

Table 1. The effect of *S. indica* on Pb content in root and shoot, translocation factor (TF), bioaccumulation factor of root (BFR) and bioaccumulation factor of shoot (BFS) in safflower under Pb toxicity.

Fungus treatment	Pb treatment (mg/kg)	Root Pb (mg/kg)	Shoot Pb (mg/kg)	TF	BFR	BFS
-*S. indica*	0	28 ± 2 g	5.5 ± 0.7 g	0.22 ± 0.04 a	27.5 ± 3.4 b	6.2 ± 1.90 b
	400	933 ± 96 f	159 ± 28 e	0.17 ± 0.01 ab	2.3 ± 0.15 c	0.4 ± 0.04 c
	800	1567 ± 98 d	233 ± 10 b	0.14 ± 0.02 ab	1.9 ± 0.10 c	0.3 ± 0.02 c
	1200	2295 ± 149 b	365 ± 35 a	0.16 ± 0.02 ab	0.9 ± 0.10 c	0.3 ± 0.01 c
+*S. indica*	0	53 ± 5 g	6.5 ± 1 g	0.18 ± 0.09 ab	48.2 ± 7.8 a	8.2 ± 2.10 a
	400	1272 ± 55 e	96 ± 7 f	0.07 ± 0.01 b	3.2 ± 0.1 c	0.2 ± 0.02 c
	800	1890 ± 20 c	179 ± 5 d	0.09 ± 0.01 b	2.4 ± 0.1 c	0.2 ± 0.01 c
	1200	2571 ± 29 a	198 ± 4 c	0.07 ± 0.01 b	2.2 ± 0.1 c	0.2 ± 0.01 c

Values are mean ± SD, n = 5. The same letter within each column indicates no significant difference among treatments using Duncan's Multiple Range Test at $P \leq 0.05$.

Root colonization and growth: The results shown in Table 2 illustrate that *S. indica* was able to successfully colonize safflower roots. Root colonization was not observed in the roots of non-inoculated plants after harvesting. In inoculated roots, increasing soil Pb concentration significantly reduced root colonization so that highest level (52%) and lowest level (31%) in root colonization were observed under 0 mg and 1200 mg Pb/kg soil, respectively (Table 2). In colonized and non-colonized safflowers with *S. indica*, shoot and root lengths, and shoot and root dry weights were significantly decreased in response to increasing Pb levels in the soil (Table 2). In non-inoculated plants, maximum decrease in growth indicators was observed at 1200 mg Pb spiked soil so that reduction in shoot length by 34.2%, root length by 41.9%, shoot dry weight by 46.9% and root dry matter by 53.2% was obtained under 1200 mg Pb, as compared to control. However, inoculation with the endophytic fungus improved the growth parameters at all levels of metal stress. Under different levels of Pb in the soil, presence of *S. indica* enhanced the shoot length by 10.3-23%, root length by 9.3-18.1%, shoot dry weight by 23.9-39.1% and root dry weight by 27.1-30.1% compared to plants grown without inoculation (Table 2).

Chlorophyll content and chlorophyll fluorescence parameters: In this research, in both colonized and non-colonized plants with the endophyte, the levels of Chl *a* and Chl *b*, and Fv/Fm and ETR values were decreased when the Pb concentration was increased in soil (Table 3). Maximum level Pb in the soil (1200 mg) produced the minimum levels of photosynthetic pigments (Chl *a* and Chl *b*) and fluorescence parameters (Fv/Fm and ETR). As shown in Table 3, compared to the non-colonized plants, Chl *a* and *b* contents, and ETR value were significantly higher in colonized plants by *S. indica* at all Pb concentrations in the soil. Although, presence of the fungus increased Fv/Fm value in compare to absence of the fungus, but this increase was not significant at different Pb levels in soil (Table 3). *S. indica*-inoculated safflowers showed greater Chl *a* content (8.5-19.9%), Chl *b* content (18.4-25.7%), Fv/Fm (2.4-8.4%) and ETR (5.5-9.2%) than non-inoculated ones (Table 3).

Table 2. The effect of *S. indica* on root colonization (RC), shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW) in safflower under Pb toxicity.

Fungus treatment	Pb treatment (mg/kg)	RC (%)	SL (cm)	RL (cm)	SDW (g/plant)	RDW (g/plant)
-*S. indica*	0	0 ± 0 e	39.65 ± 2.33 b	18.15 ± 1.90 b	15.70 ± 0.84 c	6.30 ± 0.70 b
	400	0 ± 0 e	36.20 ± 2.45 c	14.90 ± 0.56 c	13.60 ± 0.56 d	4.70 ± 0.28 c
	800	0 ± 0 e	29.25 ± 1.20 d	12.15 ± 0.91 e	10.15 ± 0.49 e	3.70 ± 0.56 d
	1200	0 ± 0 e	26.10 ± 0.28 e	10.55 ± 1.34 f	08.35 ± 0.21 f	2.95 ± 0.21 e
+*S. indica*	0	52.0 ± 1.4 a	48.80 ± 0.28 a	19.85 ± 1.34 a	21.85 ± 0.07 a	8.20 ± 0.14 a
	400	44.0 ± 2.8 b	39.95 ± 0.07 b	17.60 ± 0.84 b	16.85 ± 0.49 b	6.05 ± 1.06 b
	800	36.5 ± 0.7 c	34.70 ± 0.42 c	13.35 ± 0.63 d	13.45 ± 0.63 d	4.80 ± 0.48 c
	1200	31.0 ± 0.0 d	29.75 ± 3.04 d	11.85 ± 0.35 e	10.45 ± 0.21 e	3.75 ± 0.49 d

Values are mean ± SD, n = 5. The same letter within each column indicates no significant difference among treatments using Duncan's Multiple Range Test at $P \leq 0.05$.

Table 3. The effect of *S. indica* on photosynthetic pigments (Chl *a* and *b*) and fluorescence parameters (Fv/Fm and ETR) in safflower under Pb toxicity.

Fungus treatment	Pb treatment (mg/kg)	Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Fv/Fm	ETR
-*S. indica*	0	3.10 ± 0.13 b	2.01 ± 0.09 b	0.81 ± 0.01 ab	119 ± 7 b
	400	2.56 ± 0.12 c	1.59 ± 0.12 c	0.71 ± 0.02 bcd	108 ± 3 d
	800	2.19 ± 0.09 d	1.31 ± 0.06 d	0.67 ± 0.03 cd	97 ± 1 e
	1200	1.87 ± 0.13 e	1.03 ± 0.08 e	0.59 ± 0.02 e	87 ± 2 f
+*S. indica*	0	3.46 ± 0.08 a	2.38 ± 0.08 a	0.83 ± 0.02 a	128 ± 1 a
	400	3.07 ± 0.17 b	2.00 ± 0.12 b	0.76 ± 0.01 abc	114 ± 4 c
	800	2.58 ± 0.14 c	1.61 ± 0.11 c	0.71 ± 0.01 bcd	106 ± 2 d
	1200	2.03 ± 0.19 d	1.27 ± 0.06 d	0.64 ± 0.01 de	94 ± 3 e

Values are mean ± SD, n = 5. The same letter within each column indicates no significant difference among treatments using Duncan's Multiple Range Test at $P \leq 0.05$.

MDA content and antioxidant enzymes activity: Based on the Table 4, in response to increasing Pb concentration in soil, in both colonized and non-colonized plants with the endophyte, MDA content and CAT activity were significantly elevated. Also, with and without *S. indica* inoculation, Pb treatment significantly increased APX and SOD activities from 0 to 800 mg Pb/kg soil. Compared to 800 mg Pb in soil, Pb exposure at the level of 1200 mg significantly reduced APX activity, but had no significant influence on SOD activity (Table 4). Presence of *S. indica* significantly reduced MDA content, whereas increased CAT, APX and SOD activities under different levels of Pb in the soil. In inoculated safflowers, the reductions in MDA contents were 21.7, 30.9, 21.1 and 15.4% under 0, 400, 800 and 1200 mg Pb/kg soil, respectively than those of un-inoculated ones. Also, in *S. indica*-inoculated plants, the enhancements for CAT activity were 10.8-41.1%, for APX activity were 3.7-16.9%, and for SOD activity were 7.9-15.1% at various concentrations of Pb in soil, as compared to non-inoculated ones (Table 4).

Table 4. The effect of *S. indica* on MDA content and antioxidant enzymes activity in safflower under Pb toxicity.

Fungus treatment	Pb treatment (mg/kg)	MDA (nmol/g FW)	CAT (U/mg protein. min)	APX (U/mg protein. min)	SOD (U/mg protein)
-*S. indica*	0	9.47 ± 0.79 g	2.09 ± 0.29 g	3.72 ± 0.29 e	55.27 ± 3.11 e
	400	18.62 ± 1.38 e	3.28 ± 0.27 e	4.52 ± 0.90 c	65.60 ± 3.41 d
	800	29.67 ± 2.09 c	4.15 ± 0.17 c	5.32 ± 0.15 b	77.97 ± 3.33 b
	1200	38.65 ± 2.44 a	4.65 ± 0.12 b	4.55 ± 0.12 c	77.40 ± 4.78 b
+*S. indica*	0	7.42 ± 0.85 h	2.95 ± 0.19 f	4.35 ± 0.17 d	63.62 ± 3.90 d
	400	12.87 ± 0.65 f	3.67 ± 0.09 d	5.20 ± 0.08 b	70.82 ± 2.28 c
	800	23.45 ± 1.64 d	4.60 ± 0.14 b	5.52 ± 0.12 a	84.52 ± 6.52 a
	1200	32.72 ± 1.89 b	5.22 ± 0.09 a	5.25 ± 0.13 b	85.40 ± 2.75 a

Values are mean ± SD, n = 5. The same letter within each column indicates no significant difference among treatments using Duncan's Multiple Range Test at $P \leq 0.05$.

Discussion

Data from our study (Table 1) showed that Pb amount in the roots of safflower was higher than soil Pb concentration, demonstrating that Pb uptake mechanism is an active process in the roots of safflower cv. Sina. Pb could possibly enter the roots through ionic channels/transporters and its uptake greatly depends on the functioning of an H^+/ATPase pump to maintain a strong negative potential in rhizoderm cells of the root [57]. Based on Table 1, the Pb levels in both roots and shoots illustrated positive and linear relationships with Pb levels in soil. Furthermore, root accumulated remarkably more Pb than shoot under different Pb concentrations in soil (Table 1), indicating a restriction on Pb transport upwards. It has been reported that Pb highly sequestered into roots than shoots in *Raphanus sativus* [58] and *Coronopus didymus* [45]. Zhivotovsky *et al.* [60] observed that Pb was mainly deposited in the root after formation of $PbCO_3$ and $Pb_3(PO_4)_2$. The root tissues act as barriers to apoplastic and symplastic Pb translocation, resulting in restricted transport of Pb to shoot [50]. Bioaccumulation factor (BF) is used to estimate plants ability to pump heavy metals from the substrate to plant organ, and an important indicator of metal accumulation capacity. In this research, bioaccumulation factor of root (BFR) was higher than bioaccumulation factor of shoot (BFS) under different soil Pb levels (Table 1), showing that safflower cv. Sina prevented Pb partitioning to shoots. Also, TF (translocation factor) was very low (below 0.22) for all tested levels of Pb in soil. Considering that root is the main accumulation place of Pb, as well as higher BFR than BFS, and TF below 1 (TF<1) in all used metal concentrations, it should be noted that safflower cv. Sina is a potential candidate to be used in phyto-stabilization process and not phyto-extraction. Accordingly, Al Chami *et al.* [2] reported that safflower cannot be considered as Pb accumulator as the concentration of shoot Pb was below 1000 mg Pb/kg dry weight.

More importantly, presence of *S. indica* changed the accumulation and translocation of Pb in safflower (Table 1). The infection with the endophyte notably increased root Pb in compare to non-infected plants (Table 1). It has been reported that some fungi can increase the solubility of heavy metals in soil, hence improve their uptake by the root through producing chelators, siderophores, organic acids and various degrading enzymes [2]. Under fungal symbiosis, Pb was mainly accumulated in the roots, and Pb content in shoot was reduced (Table 1). In fungus-treated safflowers in compare to

non-treated ones, the ratio of Pb content in shoot to that in root (TF) at four levels of Pb, as well as BFS values at 400, 800 and 1200 mg Pb were lower, but BFR values at all levels of Pb in soil were higher, indicating an attenuation in Pb toxicity of leaf as a physiologically most active organ in plants. Accordingly, Ban et al. [7] found that under symbiosis of maize plants with endophytic fungus *Gaeumannomyces cylindrosporus*, Pb was retained mainly in the root system and TF was significantly reduced. It seems that the larger surface area provided by *S. indica* in the safflower root, and retention of Pb by fungal mycelia as an important sink for heavy metal via chelation of Pb ions inside the fungus or adsorption of Pb to chitin in the fungal cell wall, could be involved in Pb accumulation in root and reduced translocation of Pb from the root to shoot. The fungal cell walls possess functional groups such as hydroxyl, carboxyl and phosphoryl that act as binding sites for the adsorption of heavy metals [26]. Higher BFR value, and lower BFS and TF values in inoculated plants indicated *S. indica* potential in immobilization of Pb in safflower root, thus minimizing the migration of Pb into surface- and groundwater and reduce the risk of Pb entry into the food chain.

The results obtained from this study showed that *S. indica* colonization occurred in all levels of Pb in the soil, indicating a heavy metal tolerance of this endophyte fungus. However, by increasing Pb stress, root colonization percent was significantly reduced (from 52% at 0 mg Pb to 31% at 1200 mg Pb/kg soil), but *S. indica* formed was still functional. Similar findings concerning reduced root colonization were also reported in our previous researches in wheat and sunflower under heavy metal stress [41, 42]. It seems that a direct influence of Pb toxicity on *S. indica* is the reduction of chlamidospore germination and hyphal development, which can inhibit the establishment of root colonization by the fungus. Furthermore, the toxic effect of Pb on photosynthesis rate (Table 3) and biomass accumulation (Table 2) in safflower can harmfully affect the endophyte fungus, resulting a decline in root colonization.

It has been widely studied that excess Pb in the soil can cause negative effects on morphological growth and biomass accumulation [19, 21, 27]. In agreement with previous studies, our study showed that safflowers grown under Pb stress had a significantly lower shoot length, root length, and shoot and root dry weights (Table 2), which suggested that the growth was limited by Pb stress in soil. Lower growth under Pb stress might be due to the disadvantage in activities of enzymes that involved in the photosynthetic Calvin cycle, and generation of ROS resulting in oxidative stress and damage to cell membrane [43, 53]. As shown in the Table 2, root colonization by *S. indica* significantly enhanced growth indicators at all levels of Pb soil contamination in comparison to non-colonized safflowers, indicating growth promotion activity of *S. indica*. These results are in accordance with Shahabivand *et al.* [42] in sunflower and Hui *et al.* [25] in tobacco under heavy metal stress. The modulation of phyto-hormones involved in plant growth, increased uptake of macro- and micronutrients, and the elevation in photosynthesis efficiency of host are *S. indica*-mediated mechanisms in growth enhancement of associated plants [37, 41, 58].

In this work, the levels of Chl *a* and Chl *b* were decreased when the soil Pb concentration was increased, with and without fungus treatments. This reduction in chlorophylls content may be attributed to reduced rate of chlorophyll production due to Pb induced oxidative stress, chlorophyll degradation by stimulated chlorophyllase activity, and minimizing Fe and Mg uptake by the plant under Pb stress [43].

Furthermore, the suppression of chlorophyll biosynthesis can be correlated with a reduction in activity of enzyme δ-aminolevulinic acid dehydratase (a key enzyme in the biosynthesis of tetrapyrrole in active core of Chl) in treated plants by excess Pb [41]. Based on Table 3, Pb treatment reduced Fv/Fm and ETR values in both inoculated and non-inoculated safflowers. A decline in Fv/Fm value has been shown in sunflower under heavy metal stress [42]. Also, Bezerril Fontenele *et al.* [9] reported that Pb stress reduced ETR in *Vigna unguiculata* cultivars. Pb inhibits oxygen-evolving complex (OEC) and electron transport from Q_A to Q_B [8]. It is known that Pb reduces the quantum yield of PSII and ETR via modifying the PSII proteins and altering their function [59]. Pb ion could replace Mg^{2+} in chlorophyll molecule and Ca^{2+} in the oxygen evolving complex, leading structural changes and causing inhibition of energy transfer in PSII [39]. On the other hand, *S. indica* inoculated safflowers had higher content of Chl *a* and Chl *b*, and Fv/Fm and ETR values than non-inoculated ones (Table 3). A significant enhancement in Chl *a* and *b* contents in the presence of *S. indica* was probably correlated with the increased mineral nutrition. Moreira *et al.* [30] and Ghaffari *et al.* [20] reported that *S. indica* symbiosis in barley and pineapple elevated Mg content, as a pivotal point in the chlorophyll structure. Pan *et al.* [35] found a remarkably elevation on ETR and a slight increase on Fv/Fm under inoculation by endophyte fungus in Chinese white poplar. Higher capability of fungal plants to use light energy, and increase in the density of photosynthetic units and electron transport rate was confirmed by higher levels of Fv/Fm and ETR in *Medicago truncatula* under heavy metal stress [3]. Based on these results along with the results obtained in promoting biomass accumulation (Table 1) and Chl *a* and Chl *b* contents, it was suggested that inoculated safflowers with *S. indica* had higher photosynthetic performance than non-inoculated ones, indicating positive impact of this fungal-plant interaction in the attenuation of host Pb toxicity.

Pb stress is known to induce higher levels ROS, leading to oxidative stress [43]. The overproduction of ROS by Pb stress degrades polyunsaturated lipids in cellular membranes (peroxidation of lipids that causes the loss of membrane integrity), as a result forming MDA which is used as a biomarker to evaluation of oxidative stress level and its magnitude [17]. As shown in Table 4, Pb treatment resulted in elevated levels MDA in colonized and non-colonized plants. Elevated MDA content under Pb-stressed conditions was also observed in different plant species including *Morus alba* [38] and *Acalypha indica* [52]. These results demonstrated that antioxidant enzymes and other non-enzymatic antioxidants for combating excessive generated ROS are not a sufficient defense system under higher Pb levels in soil. The presence of *S. indica* inhibited MDA accumulation in leaves of safflower at all levels of Pb in soil (Table 4), thus *S. indica* could partially counteract Pb toxicity. Hui *et al.* [25] observed that the quantity of MDA in *S. indica*-inoculated tobacco plants exposed to heavy metal was lower than non-inoculated ones. Based on these results, it can be stated that this endophyte could inhibit or retard the formation of MDA by preventing excess ROS generation via increasing in antioxidant enzyme activities (Table 4). According to Table 4, in response to Pb treatment in colonized and non-colonized plants, SOD, CAT and APX activities were notably enhanced. These findings are similar to those reported for *Vallisneria natans* [56] and *Najas indica* [48]. Also, Chehregani Rad *et al.* [14] and Padash *et al.* [33] reported a significant increase on catalase and peroxidase activities in petunia and basil plants under Pb stress. SOD plays

a pivotal role in $O.^{2-}$ removal and its convert to H_2O_2. H_2O_2 can be further converted into H_2O and O_2 by CAT and APX. Increased SOD, CAT and APX activity in this study under Pb stress might be ascribed to the increase of $O.^{2-}$ radical and H_2O_2 concentrations. According to Verma and Dubey [53], in this study, the decreased APX activity under higher Pb level in soil (1200 mg) may be caused by the enhanced oxidative stress, the decrease in enzyme synthesis or the change in assembly of enzyme subunits. From 800 to 1200 mg Pb in the soil, SOD activity illustrated no significant change (Table 4), indicating that safflower plants had reached the limits of their ability to scavenge $O.^{2-}$ using SOD. Data summarized in Table 4 showed that presence of *S. indica* conferred protection to safflowers and reduced the harmful influence of oxidative stress caused by Pb, as evidenced by enhanced activity of three enzymatic antioxidants SOD, CAT and APX. This increase in activity of antioxidants helps to safflower plants for maintain of ROS induced by Pb under controlled level. It is known that activation in antioxidant enzyme systems is a main target of *S. indica* in leaves [6]. A further enhancement in the activities of enzymatic antioxidants suggests the impact of *S. indica* in quick scavenging ROS induced by Pb stress, thus strengthening the plant's defense system. As regards to the elevated SOD activity could result in a potential increase in cyclic electron transport [12], thus the higher ETR value (Table 3) maintained in inoculated plants by *S. indica* may be associated with the increase of SOD activity. Further detailed studies will be needed for better understanding *S. indica*-mediated the biochemical and physiological mechanisms, especially under field conditions.

Conclusion

We here found that endophyte fungus *S. indica* was able to establish beneficial symbiosis with safflower cv. Sina roots under toxic levels of Pb in soil. Pb stress adversely affected the growth rate and physiology of safflower plants. However, inoculation with *S. indica* rescued host growth attributes, and improved plant tolerance to Pb stress by immobilizing Pb in the roots, enhancing enzymatic antioxidant activities, Chl contents and chlorophyll fluorescence indicators, and reducing MDA concentration. An elevation in BFR and the reduction in TF and BFS of Pb in *S. indica*-inoculated plants suggest a capacity of this endophyte in fungus-assisted phytoremediation and alleviating Pb toxicity in the host plant. Therefore, as a sustainable and affordable approach, *S. indica* can be a good candidate for Pb phyto-immobilization in safflower root under exposure to higher levels Pb in the soils.

References

1- Aebi, H. (1984). Catalase in vitro. Methods Enzymol. 105, 121-126.

2- Al Chami, Z., Amer, N., Al Bitar, L., Cavoski I. (2015). Potential use of *Sorghum bicolor* and *Carthamus tinctorius* in phytoremediation of nickel, lead and zinc. Int. J. Environ. Sci. Technol. 12, 3957–3970.

3- Aloui, A., Recorbet, G., Robert, F., Schoefs, B., Bertrand, M., Henry, C., Gianinazzi-Pearson, V., Dumas-Gaudot, E., Aschi-Smiti, S. (2011). Arbuscular mycorrhizal symbiosis elicits shoot proteome changes that are modified during cadmium stress alleviation in *Medicago truncatula*. BMC Plant Biol. 11(75), 1-17.

4- Amatussalam, A., Abubacker, M.N., Rajendran, R.B. (2011). In situ *Carica papaya* stem matrix and *Fusarium oxysporum* (NCBT-156) mediated bioremediation of chromium. Indian J. Exp. Biol. 49, 925-931.

5- Arnon A. (1967). Method of extraction of chlorophyll in the plants. Agron. J. 23, 112–121.

6- Baltruschat, H., Fodor, J., Harrach, B.D., Niemczyk, E., Barna, B., Gullner, G., Janeczko, A., Kogel, K.H., Schäfer, P., Schwarczinger, I.,

Zuccaro, A., Skoczowski, A. (2008). Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytol. 180, 501–510.

7- Ban, Y., Xu, Z., Yang, Y., Zhang, H., Chen, H., Tang, M. (2017). E□ect of dark septate endophytic fungus *Gaeumannomyces cylindrosporus* on plant growth, photosynthesis and Pb tolerance of maize (*Zea mays* L.). Pedosphere 27(2), 283–292.

8- Belatik, A., Hotchandani, S., Carpentier, R. (2013). Inhibition of the Water Oxidizing Complex of Photosystem II and the Reoxidation of the Quinone Acceptor Q_{A2} by Pb^{2+}. PLoS One 8.

9- Bezerril Fontenele, N.M., Otoch, M.L.O., Gomes-Rochette, N.F., Sobreira, A.C.M., Barreto, A.A.G.C., de Oliveira, F.D.B., Costa, J.H., Borges, S.D.S.S., do Nascimento, R.F., Fernandes de Melo, D. (2017). Effect of lead on physiological and antioxidant responses in two *Vigna unguiculata* cultivars differing in Pb-accumulation. Chemosphere 176, 397-404.

10- Boussen, S., Soubrand, M., Bril, H., Ouerfelli, K., Abdeljaouad, S. (2013). Transfer of lead, zinc and cadmium from mine tailings to wheat (*Triticum aestivum*) in carbonated Mediterranean (Northern Tunisia) soils. Geoderma 192, 227–236.

11- Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

12- Casano, L.M., Zapata, J.M., Martín, M., Sabater, B. (2000). Chlororespiration and poising of cyclic electron transport. Plastoquinone as electron transporter between thylakoid NADH dehydrogenase and peroxidase. J. Biol. Chem. 275, 942–948.

13- Chatterjee, C., Dube, B.K., Sinha, P., Srivastava, P. (2004). Detrimental effects of lead phytotoxicity on growth, yield, and metabolism of rice. Commun. Soil Sci. Plan. 35, 255–265.

14- Chehregani Rad, A.K., Farzan, S., shirkhani Z. (2017). Effect of lead treatment on some morphological and physiological parameters of *Petunia hybrida* L. J. Plant Res. 30(1), 226-243.

15- Chen, L., Gao, S., Zhu, P., Liu, Y., Hu, T., Zhang, J. (2014). Comparative study of metal resistance and accumulation of lead and zinc in two poplars. Physiol. Plantarum 151, 390–405.

16- Chen, M., Zhang, L.L., Xiao, J.L., He, X.J., Cai, J.C. (2015). Bioaccumulation and tolerance characteristics of a submerged plant (*Ceratophyllum demersum* L.) exposed to toxic metal lead. Ecotoxicol. Environ. Saf. 122, 313–321.

17- Del Rio, D., Stewart, A.J., Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr. Metab. Cardiovasc. Dis. 15, 316–328.

18- Dhindsa, R.S, Matowe, W. (1981). Drought tolerance in two mosses: correlated with enzymatic defense against lipid peroxidation. J. Exp. Bot. 32(1), 79–91.

19- Ekmekçi, Y., Tanyolaç, D., Ayhan, B. (2009). A crop tolerating oxidative stress induced by excess lead: maize. Acta Physiol. Plant. 31, 319–330.

20- Ghaffari, M.R., Ghabooli, M., Khatabi, B., Hajirezaei, M.R., Schweizer, P., Salekdeh, G.H. (2016). Metabolic and transcriptional response of central metabolism affected by root endophytic fungus *Piriformospora indica* under salinity in barley. Plant Mol. Biol. 90, 699-713.

21- Han, Y., Wang, L., Zhang, X., Korpelainen, H., Li, C. (2013). Sexual differences in photosynthetic activity, ultrastructure and phytoremediation potential of Populus cathayana exposed to lead and drought. Tree Physiol. 33, 1043–1060.

22- He, Y., Yang, Z., Li, M., Jiang, M., Zhan, F., Zu, Y., Li, T., Zhao, Z. (2017). Effects of a dark septate endophyte (DSE) on growth, cadmium content, and physiology in maize under cadmium stress. Environ. Sci. Pollut. Res. 24(22), 18494-18504.

23- Heath, R.L., Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125(1), 189–198.

24- Hill, T.W., Käfer, E. (2001). Improved protocols for Aspergillus minimal medium: trace element and minimal medium salt stock solutions. Fungal Genet. Newsl. 48, 20–21.

25- Hui, F., Liu, J., Gao, P., Lou, B. (2015). *Piriformospora indica* confers cadmium tolerance in *Nicotiana tabacum*. J. Environ. Sci. 37, 184–191.

26- Javanbakht, V., Alavi S.A., Zilouei H. (2014). Mechanisms of heavy metal removal using microorganisms as biosorbent. Water Sci. Technol. 69(9), 1775-1787.

27- Kabir, M., Iqbal, M.Z., Shafiq, M. (2009). Effects of lead on seedling growth of *Thespesia populnea* L. Plant Soil Environ. 3, 184–190.

28- Kopittke, P.M., Asher, C.J., Blamey, F.P.C., Menzies, N.W. (2007). Toxic effects of Pb on the growth and mineral nutrition of signal grass (*Brachiaria decumbens*) and Rhodes grass (*Chloris gayana*). Plant Soil 300, 127–136.

29- Kushwaha, A., Hans, N., Kumar, S., Rani, R. (2018). A critical review on speciation, mobilization and toxicity of lead in soil microbe-plant system and bioremediation strategies. Ecotoxicol. Environ. Saf. 147, 1035-1045.

30- Moreira, B.C., Mendes, F.C., Mendes, I.R., Paula, T.A., Prates Junior, P., Salomão, L.C.C., Stürmer, S.L., Otoni, W.C., Guarc¸oni, A.M., Kasuya, M.C.M. (2015). The interaction between arbuscular mycorrhizal fungi and *Piriformospora indica* improves the growth and nutrient uptake in micropropagation-derived pineapple plantlets. Sci. Hort. 197, 183-192.

31- Nakano, Y., Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.

32- Oelmüller, R., Sherameti, I., Tripathi, S., Varma, A. (2009). *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis 19, 1-19.

33- Padash, A., Ghanbari, A., Sirousmehr, A.R., Asgharipour M.R. (2018). Effect of salicylic acid on basil resistance against lead. J. Plant Res. 31(1), 68-79.

34- Padash, A., Shahabivand, S., Behtash, F., Aghaee, A. (2016). A practicable method for zinc enrichment in lettuce leaves by the endophyte fungus *Piriformospora indica* under increasing zinc supply. Sci. Hort. 213, 367-372.

35- Pan, X., Qin, Y., Yuan, Z. (2018). Potential of a halophyte-associated endophytic fungus for sustaining Chinese white poplar growth under salinity. Symbiosis, DOI: 10.1007/s13199-018-0541-8.

36- Phillips, J.M., Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161.

37- Qiang, X., Zechmann, B., Reitz, M.U., Kogel, K.H., and Schäfer, P. (2012). The mutualistic fungus *Piriformospora indica* colonizes Arabidopsis roots by inducing an endoplasmic reticulum stress-triggered caspase-dependent cell death. Plant Cell 24, 794–809.

38- Qin, F., Liu, G., Huang, G., Dong, T., Liao, Y., Xu, X. (2017). Zinc application alleviates the adverse effects of lead stress more in female Morus alba than in males. Environm. Exp. Bot. 146, 68-76.

39- Qufei, L., Fashui, H. (2009). Effects of Pb^{2+} on the structure and function of photosystem II of *Spirodela polyrrhiza*. Biol. Trace Elem. Res. 129, 251–60.

40- Rajkumar, M., Sandhya, S., Prasad, M.N., Freitas, H. (2012). Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol. Adv. 30, 1562-1574.

41- Rossato, L.V., Nicoloso, F.T., Farias, J.G., Cargnelluti, D., Tabaldi, L.A., Antes, F.G., Dressler, V.L., Morsch, V.M., Schetinger, M.R. (2012). Effects of lead on the growth, lead accumulation and physiological responses of *Pluchea sagittalis*. Ecotoxicology 21, 111–123.

42- Shahabivand, S., Parvaneh, A., Aliloo, A.A., 2017. Root endophytic fungus *Piriformospora indica* affected growth, cadmium partitioning and chlorophyll fluorescence of sunflower under cadmium toxicity. Ecotoxicol. Environ. Saf. 145, 496-502.

43- Sharma, P., Dubey, R.S. (2005). Lead toxicity in plants. Braz. J. Plant Physiol. 17 (1), 35-52.

44- Shi, G., Liu, C., Cai, Q., Liu, Q., Hou, C. (2010). Cadmium accumulation and tolerance of two safflower cultivars in relation to photosynthesis and antioxidative enzymes. Bull. Environ. Contam. Toxicol. 85(3), 256–263.

45- Sidhu, G.P.S., Singh, H.P., Batish, D.R., Kohli, R.K. (2016). Effect of lead on oxidative status, antioxidative response and metal accumulation in *Coronopus didymus*. Plant Physiol. Biochem. 105, 290–296.

46- Singh, A., Sharma, J., Rexer, K., Varma, A, (2000). Plant productivity determinants beyond minerals, water and light: *Piriformospora indica*–A revolutionary plant growth promoting fungus. Curr. Sci. 79(11), 1548-1554.

47- Singh, L.P., Gill, S.S., Tuteja, N. (2011). Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signal. Behav. 6, 175-191.

48- Singh, R., Tripathi, R.D., Dwivedi, S., Kumar, A., Trivedi, P.K., Chakrabarty, D. (2010). Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system. Biores. Technol. 101, 3025–3032.

49- Tauqeer, H.M., Ali, S., Rizwan, M., Ali, Q., Saeed, R., Iftikhar, U., Ahmad, R., Farid, M., Abbasi, G.H. (2015). Phytoremediation of heavy metals by *Alternanthera bettzickiana*: growth and physiological response. Ecotoxicol. Environ. Saf. 126, 138–146.

50- Trivedi, S., Erdei, L. (1992). Effects of cadmium and lead on the accumulation of Ca^{2+} and K^+ and on the influx and translocation of K^+ in wheat of low and high K^+ status, Physiol. Plant 84, 94–100.

51- Varma, A., Bakshi, M., Lou, B., Hartmann, A., Oelmüller, R. (2012). *Piriformospora indica*: A novel plant growth-promoting mycorrhizal fungus. Agric. Res. 1, 117-131.

52- Venkatachalam, P., Jayalakshmi, N., Geetha, N., Sahi, S.V., Sharma, N.C., Rene, E.R., Sarkar, S.K., Favas, P.J.C. (2017). Accumulation efficiency, genotoxicity and antioxidant defense mechanisms in medicinal plant *Acalypha indica* L. under lead stress. Chemosphere 171, 544-553.

53- Verma, S., Dubey, R.S. (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci. 164, 645-655.

54- Verma, S., Varma, A., Rexer, K.H., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Bütehorn, B., Franken, P. (1998). *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycologia 90, 898-905.

55- Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R., Neumann, C., von Wettstein, D., Franken, P., Kogel, K.H. (2005). The endophytic fungus *Piriformospora indica* reprograms barley to salt stress tolerance, disease resistance, and higher yield. Proc. Natl. Acad. Sci. USA, 102, 13386-13391.

56- Wang, P., Zhang, S., Wang, C., Lu, J. (2012). Effects of Pb on the oxidative stress and antioxidant response in a Pb bioaccumulator plant *Vallisneria natans*. Ecotoxicol. Environ. Saf. 78, 28–34.

57- Wang, H., Shan, X., Wen, B., Owens, G., Fang, J., Zhang, S. (2007). E□ect of indole-3-acetic acid on lead accumulation in maize (*Zea mays* L.) seedlings and the relevant antioxidant response. Environ. Exp. Bot. 61, 246–253.

58- Wang, Y., Shen, H., Xu, L., Zhu, X., Li, C., Zhang, W., Xie, Y., Gong, Y., Liu, L. (2015). Transport, ultrastructural localization, and distribution of chemical forms of lead in radish (*Raphanus sativus* L.). Front. Plant Sci. 6, 293.

59- Wioleta, W., Anna, D., Ilona, B., Kamila, K., Elżbieta, R. (2015). Lead induced changes in phosphorylation of PSII proteins in low light grown pea plants. BioMetals 28, 151-162.

60- Zhivotovsky, O.P., Kuzovkina, J.A., Schulthess, C.P., Morris, T., Pettinelli, D., Ge, M. (2011). Hydroponic screening of willows (Salix L.) for lead tolerance and accumulation. Int. J. Phytoremediat. 13, 75-9

نقش قارچ اندوفیت سرندیپیتا ایندیکا در تثبیت گیاهی وابسته به قارچ و عملکرد گیاه گلرنگ در خاک آلوده به سرب

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چکیده

قارچهای مفید اندوفیت ریشه یک نقش مهمی در بهبود عملکرد گیاه داشته و در افزایش کارایی گیاه پالایی میزبان در خاکهای آلوده به فلزات موثرند. در این آزمایش اثرقارچ اندوفیت سرندیپیتا ایندیکا بر تجمع و انتقال سرب، برخی ویژگیهای فیزیولوژیکی و سیستم آنتی اکسیدان برگ در گیاه گلرنگ واریته سینا، بررسی شد. با افزایش میزان سرب در خاک، جذب سرب در ریشه و تجمع آن در ریشه و اندام هوایی بیشتر شد. در گیاهان تلقیح شده با اندوفیت، یک افزایش معنی‌دار در تجمع سرب در ریشه و یک کاهش معنی‌دار در تجمع سرب در اندام هوایی مشاهده شد. همچنین گیاهان تلقیح شده با قارچ دارای شاخص انتقال (TF) و شاخص تجمع زیستی ریشه (BFR) بالاتر ولی شاخص تجمع زیستی اندام هوایی (BFS) پایین‌تر نسبت به گیاهان فاقد تیمار قارچی بودند. حضور قارچ باعث افزایش پارامترهای رشدی، رنگیزه‌های فتوسنتزی، شاخص‌های فلورسانس کلروفیل و آنزیم‌های آنتی‌اکسیدان کاتالاز، آسکوربات پراکسیداز و سوپراکسید دیسموتاز تحت همه غلظت‌های سرب در خاک شد. در نظر گرفتن این قارچ برای فرایند تثبیت/بی‌حرکت سازی فلز سرب وابسته به قارچ در ریشه گیاه میزبان پیشنهاد می‌شود. همچنین نتیجه‌گیری شد که قارچ اندوفیت راهبردهای متفاوتی را برای کاهش سمیت سرب و بالا بردن عملکرد گیاه گلرنگ میانجی‌گری می‌کند.

واژه های کلیدی: سرب، سرندیپیتا ایندیکا، گلرنگ، تجمع فلز سنگین