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Research Article

Trichoderma and spermidine improve cadmium tolerance and phytoremediation potential in purslane (*Portulaca oleracea* L.) plant

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Abstract

In order to understand, the physiological and biochemical mechanisms of *Trichoderma longibrachiatum* (TL) and spermidine (SPD) polyamine treatment on cadmium (Cd) tolerance phytoremediation in purslane (*Portulaca oleracea*) plant and the activity of anti-oxidants enzyme (CAT, APX, POX, SOD), hydrogen peroxide and proline content as well as determination of cadmium accumulation in shoots, roots, soil and their ratio to each other, a factorial experiment was performed in a completely randomized design with three replications and three treatments. In the current study, mitigative roles of SPD and TL were assessed in Cd stressed *Portulaca oleracea* plants. SPD (1, 0.5, and 1 mM) was applied after 20 days of sowing on the branches and leaves of plants inoculated or without TL inoculating in the presence of Cd (0, 30, 60 and 90 mg.kg $^{-1}$). Cd stress and coexistence with TL increased the activity of antioxidant enzymes and leaf soluble protein in purslane plants. Also, the application of SPD, especially at 0.5 mM, resulted in a higher increase in leaf protein under cadmium stress in inoculated plants. Proline parameter responds differently to TL. SPD application reduced the severity of these changes. The amount of H_2O_2 was significantly reduced in plants when treated by both TL and SPD. Significant differences were observed between 0.5 and 1 mM of SPD in terms of the Cd uptake in the TL inoculated purslane shoots. Inoculated purslane plants treated by either 0.5 or 1 mM of SPD had lower Cd uptake and greater BF. In general, the results showed a synergetic effect between TL fungi and SPD application on improving the Cd phytoremediation in the purslane plant.

Keywords: Antioxidant enzymes, Cadmium toxicity, Phytoremediation, Purslane, Spermidine, *Trichoderma*

Introduction

The natural weathering of minerals, along with recent human activities, bring large quantities of heavy metals into the environment (Zhang *et al*., 2018). Heavy metals have become a serious threat to human health due to their direct and indirect toxic effects through accumulation in the food chain (Ghaderian and Ghotbi Ravandi, 2012). Cd is listed as the sixth most dangerous element in the US Agency for the study of toxic substances (Kumar Rai, 2008). Since some plants such as corn, canola, and purslane (Hechmi *et al*., 2013; Yaghoubian *et al*., 2016; Belouchrani *et al*., 2016) can absorb and remove metal contaminants such as cadmium from the contaminated environment, phytoremediation could introduce as an environmentally friendly, low-cost and new technology that uses plants to reduce the concentration of organic and metal pollutants (Luo *et al*., 2016). One of the aspects of phytoremediation is risk control through the stabilization of pollutants by the roots of plants in the soil.

Cd leads to increased production ROS (Shahid *et al*., 2014), and in return ROS rapidly damages existing macromolecules including membrane lipids, proteins, nucleic acids, and pigments, resulting in irreversible metabolic defects (Maldonado-Magna *et al*., 2011). To minimize the deleterious effects of ROS, plant cells have developed a complex network of enzymatic and non-enzymatic antioxidant systems to protect plant cells from oxidative disadvantage caused by different types of ROS (Fahr *et al*., 2013; Afzal *et al*., 2014). SOD, CAT, APX and GPX are ROS-scavenging enzymes that maintain ROS content within a specified range (Gupta *et al*., 2009). The key role of CAT, SOD, and APX as three enzymes involved in controlling Cd-induced H_2O_2 content has recently been described (Guo *et al*., 2019). It has been reported that annually a lot of cadmium from, industrial and municipal wastewater, along with unnecessary consumption of insecticides and high levels

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of chemical fertilizers, especially phosphate, are released into the environment (Chiapello *et al*., 2015). Therefore as sedentary plants, they need to have defense mechanisms for acclimating to change environmental conditions. Numerous studies indicate that Cd signals can be transmitted by polyamines and causes increased production of ROS (Arasimowicz-Jelonek and Floryszak-Wieczorek, 2012). Polyamines include Put, SPD and spermidine are known as a group of natural compounds with aliphatic nitrogen structure. Polyamines play an important role in the physiological mechanisms of plants and improve tolerance to abiotic stress (Tiburcio *et al*., 2014; Cai *et al*., 2015). Polyamines should not only be considered as protective molecules but should also be regarded as compounds involved in a complex signaling system that plays in regulating stress tolerance (Kasukabe *et al*., 2004). For example, Yang *et al*. (2013) reported enhanced salt and heavy metal enhanced tolerance in spermidine-treated *Hydrocharis dubia*, and *Salvinia natans* (Xu *et al*., 2008) and sunflower (Mutlu and Bozcuk, 2005).

Phytoremediation is an emerging, low-cost technology that uses plants to clean and remove pollutants from the environment, meanwhile only some plants (the metallophytes) can survive in adverse environmental conditions (soil contamination with heavy metals), so they can help clean up the contaminated environment (Yadav, 2010; Ghaderian and Ghotbi Ravandi, 2012). Some plants (the metallophytes) can survive in adverse environmental conditions (soil contamination with heavy metals), so they can help clean up the contaminated environment.

Fungal strains grouped in the genus *Trichoderma* species are known to alter the response of plants to abiotic stresses and a widespread free-living with different species that can benefit plants by promoting root extension and plant growth, succor the remediate soil and water pollution (Akladious and Abbas, 2012; Ghorbanpour *et al*., 2018; Yaghoubian *et al*., 2019). Recently, phytoremediation as a diverse eco-friendly remediation option, has been explored to restore the contaminated environments. This process combined two methods of phytoremediation and bioremediation simultaneously. Since purslane is a well-known plant for its antioxidant properties (Cai *et al*., 2004) along with its well-documented and valuable potential to phytoremediation the heavy metals, the purpose of this study was to investigate the effect of fungi symbiosis and polyamine on the improvement of phytoremediation by purslane.

Materials and methods

Plant materials and growth conditions: The experiment was designed as a factorial with completely randomized design with three replications. Treatments consisted of *Trichoderma longibrachiatum* (TL) fungi, three spermidine (SPD) levels: 0, 0.5 and 1 and four cadmium (Cd) levels: 0, 30, 60 and 90 $mg.kg^{-1}$ Cd in the soil. The level of cadmium studied in this experiment was selected based on the initial method for measuring the growth and tolerance of purslane (*Portulaca oleracea* L.) in Cd-contaminated soils at 11 levels of cadmium concentration. The soil was collected at 0-30 cm depth at the site of the research farm of Sari Agricultural Sciences and Natural Resources University (SANRU), Mazandaran province, Iran (N 36°39′40.1″ E 53°04′16.6″). The soil texture was sandy loam mixed with the river sand (2/1: v/v). Soil Cd initial concentration was measured prior to the experiment. To reduce microorganism's side effects, we autoclaved the soil in autoclavable bags at 120 °C for 30 min. For each treatment, the appropriate amount of Cd (from the source $CdCl_2$) was dissolved in 1 liter of double-distilled water and sprayed on the soil with thin layers. To ensure the uniformity of the Cd distribution, this procedure was repeated several times until the entire solution was applied. Contaminated soils were kept in the greenhouse for four weeks in order to perform the interactions between Cd and soil at a relative humidity of 53% to ensure the necessary balance between Cd and soil (Huang *et al*., 2009).

TL specie was prepared from the laboratory of mycology, Department of Plant Pathology at SANRU cultured in medium contained PDA (Potato-Dextrose Agar, Merck) and incubated for 7 days at 25 °C (Lopez Errasquin and Vazquez, 2003). After the incubation, 15 ml of double-distilled water was added to each medium. The resulting solution combined with Tween 20 (biological detergent) was used to inoculate germinated seeds. Therefore, a fungus suspension with 10^9 $conidia.ml^{-1}$ was used for the inoculum in the trial (Hjeljord and Tronsmo, 2003). Purslane seeds obtained from Karaj Seed Pakan Center, Iran then were surface-sterilized for 15 min in 1% (W/V) sodium hypochlorite and thereafter thoroughly washed with distilled water. After 24 h of germination, the seedlings were inoculated with TL spores as described below. Then inoculated seedlings were planted into 20 cm wide plastic pots filled with 5 kg of air-dried soil, then placed in a greenhouse at 26/17 °C (day/night) temperature with a 16 h photoperiod. SPD was purchased from Sigma company. After the acclimation period of at least two months, seedlings purslane was sprayed at the two-week interval (in the tenth leaf stage) with 0, 0.5 and 1 millimolar (mM) of SPD, until both sides of all the leaves were completely wet. At each level, spermidine was dissolved in water. Nonyl soap was added as a surfactant to both SPD solutions and also to the control treatments. The Cd and SPD concentrations were optimized and selected in a preliminary study. After 60 days of the experiment (before the emergence of flowers stage), various analyses were performed.

Enzyme extraction and assays: Antioxidant enzymes were extracted at 4 °C using 0.5 g tissue from the fresh samples of purslane seedling leaves after 14 days of SPD treatment. The samples were homogenized with liquid nitrogen, followed by adding 5 ml extraction buffer consisting of 0.1 M phosphate buffer with pH

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7.5, including 0.5 mM EDTA in case of CAT and POX, or 0.1 M phosphate buffer, pH 7.5, 0.5 mM EDTA, 2 mM ASA and 5% PVP in case of APX at 5 °C (Nakano and Asada, 1981). CAT activity was measured according to the method of Aebi (1984) in 240 nm. APX activity was measured by the ascorbate oxidation rate at 290 nm (E = 2.8 m/M/cm) according to Yoshimura *et al*. (2000). POX activity was measured according to Tang and Newton's method (2005) in 470 nm. Superoxide dismutase (SOD) activity was determined by measuring the inhibition in photo reduction of nitro blue tetrazolium (NBT) by SOD enzyme (Kumar *et al*., 2012). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L^{-1} methionine, 50 µM NBT, 10 µM riboflavin and 100 µL of crude extract in a final volume of 3.0 mL. A control reaction was performed without crude extract. The SOD reaction was carried out by exposing the reaction mixture to white light for 15 min at room temperature. After 15 min incubation, absorbance was recorded at 560 nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT.

Determination of hydrogen peroxide: First, 0.5 g of leaf sample was homogenized in 5 ml of 0.1% TCA solution (w/v) and then centrifuged at 12000 rpm for 15 min. Then, the reaction complex containing 0.5 ml of the supernatant, 0.5 ml potassium phosphate buffer 10 Mm (pH = 7), and 1 ml KI 1 M. was prepared, and their adsorption rate was measured at 390 nm (Sergiev *et al*., 1997).

Proline determination: Proline measurement was performed using the method of Bates *et al*. (1973). In this method, 0.5 g of fresh leaves of *Portulaca oleracea* was homogenized and after transfering to the tube and adding 10 ml of sulfosalicylic acid 3% (w/v) for 10 to 15 minutes at 4 °C, it was centrifuged in 15000 rpm. Then, we added 2 ml acid ninhydrin and 2 ml glacial acetic acid to 2 ml of filtered extract and incubated in a boiling water bath for 1 h. The reaction was finished in an ice bath, finally, 4 ml of toluene was added to each test tube and Vertex was performed for 20 seconds. The concentration of soluble proline in toluene was determined using a spectrophotometer at a wavelength of 520 nm, and finally, the amount of proline in the plant was determined according to the standard proline curve.

Determination of Cd accumulation: An atomic absorption device (AAS-6300, Shimadzu, Kyoto, Japan) was used to measure the concentration of cadmium in soil and aerial and terrestrial portions of purslane (Helrich, 1990). Also, the BCF and the TF, assessed to determine the relative translocation of Cd from belowground parts to the aboveground shoot of the plant. BCF and TF calculated as follows (Ghosh and Singh, 2005; Gupta *et al*., 2008):

BCF = concentration of a metal in roots / concentration of metal in the medium.

TF = concentration of a metal in the shoot / concentration of metal in the roots.

The experiment was conducted in a completely randomized design with three replicates. Mean comparisons were made by LSD (alpha = 0.05) using SAS version 9.1. The normality of data was tested using the one-sample Kolmogorov-Smirnov test (Massey, 1952).

Results and discussion

In the present study, the activities of four antioxidant enzymes SOD, APX, GPX, CAT were evaluated to assess the function of TL and SPD in regulating plant antioxidant against cadmium stress (Fig. 1 to 4). Our results indicated that SOD (Fig. 1A) showed a positive response to Cd concentration gradients. SOD activity increased by about 5, 14, and 14% at 30, 60, and 90 $mg.kg^{-1}$ of Cd concentrations, respectively as compared to the control. This result was consistent with the results of Guo *et al*. (2019) who found that Cd concentration increased the activities of SOD in different wheat varieties in different growth stages. Application of 0.5 mM SPD increased SOD activity at low levels of cadmium toxicity (0 and 30 $mg.kg^{-1}$ Cd) by more than 17.84 and 13.52% compared to the control, respectively. On the other hand, the use of spermidine in higher concentrations of cadmium had no significant effect on the SOD activity. These results are similar to some studies showing SPD was a collector of ROS (e.g. Satish *et al*., 2018). Most cases, during abiotic stress in plants, a close relationship has been reported between plant protection at the time of SPD application and increased antioxidant levels (Satish *et al*., 2018). Also, we demonstrated here that TL induced SOD expression in chloroplasts and significantly improved SOD activity (12.19%) in plant as compared to the uninoculated control (Fig. 1B). Decreased SOD activity in Cd toxicity had been previously reported in both wheat and bean plants (Cardinals *et al*., 1984; Milone *et al*., 2003), although it increased in sunflower, rice, and soybean (Kuo and Cao, 2004; Sobkowiak *et al*., 2004; Laspina *et al*., 2005).

The results of the APX are shown in Figure 2A. APX activity negatively responds to Cd concentrations (not always). However, a marked increase in APX activity was monitored after spraying 1 mM of SPD (Fig. 2C) which was more pronounced than the Cd treatment alone

SPD is a messenger regulator in stress signaling pathways leading to changes in the expression of different genes associated with stress in plants (Kasukabe *et al*., 2004) and may alter the antioxidant content in the plant (Radhakrishnan and Lee, 2014). APX activity was significantly increased in the presence of Cd, particularly in the inoculated plants as compared to the control. CAT, along with POX and APX, converts H_2O_2 to water and oxygen (Ahmad *et al*., 2010).

Significant results were obtained in CAT activity in

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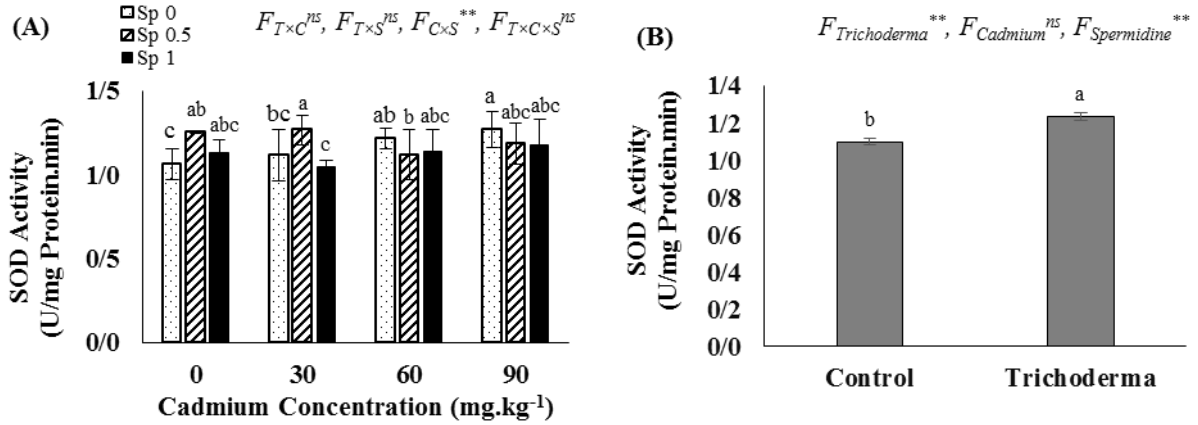

Fig. 1- Effect of exogenous SPD (0, 0.5, 1 mM) under four Cd concentrations (0, 30, 60, 90 mg.kg^{-1}) (A) and *Trichoderma* (B) on the activities of SOD in purslane seedlings. Different letters indicate significant differences (P≤0.05).

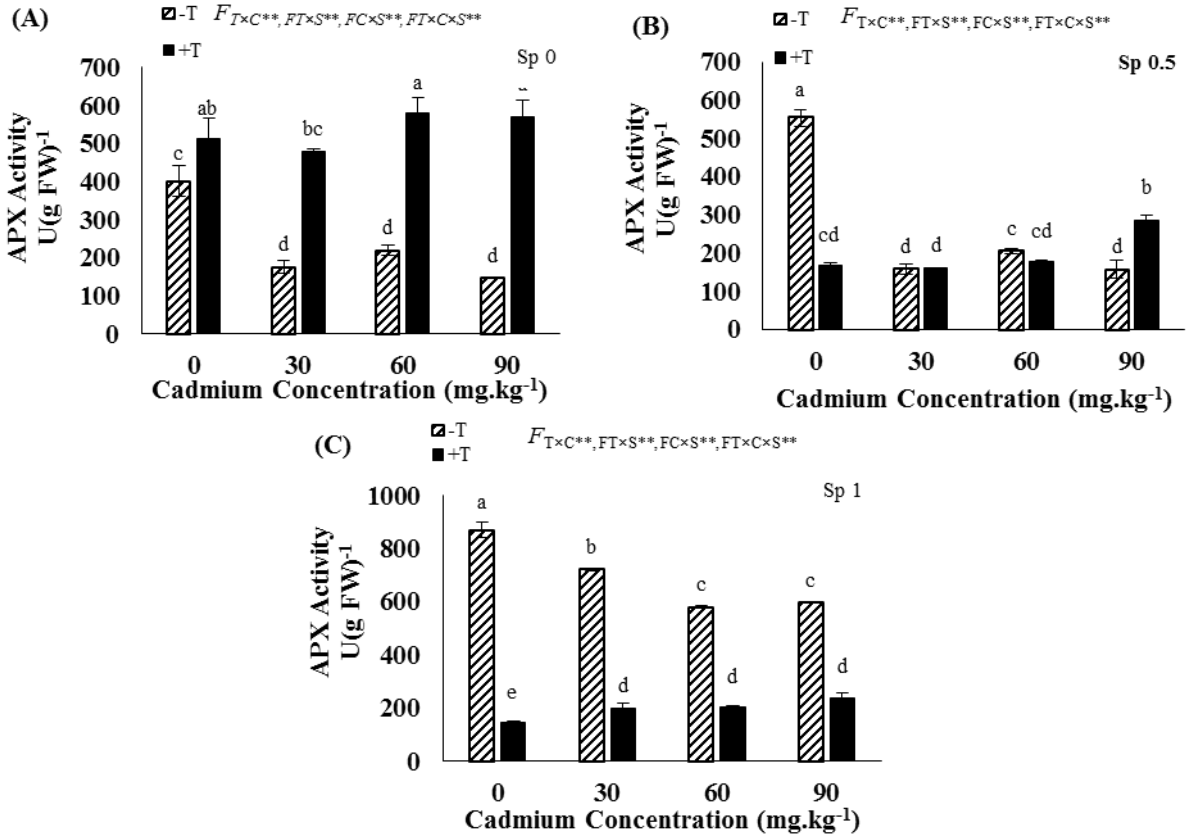

Fig. 2- Effects of exogenous SPD (0, 0.5, 1 mM) and *Trichoderma* on APX in purslane seedlings under four Cd concentrations (0, 30, 60, 90 mg.kg^{-1}). Different letters indicate significant differences (P≤0.05).

purslane under Cd stress at different doses of spermidine. Most of the changes occurred at lower levels of cadmium toxicity, with the difference that a spray of 0.5 mM of this polyamine solution increased the activity of this enzyme in plants inoculated with fungi (Fig. 3B), While the highest activity of this enzyme was observed during foliar application of 1 mM spermidine in plants not inoculated with fungi (Fig. 3C). In the first place, this effect was due to the exogenous Spd contribution to the improvement of the antioxidant Halliwell-Asada path way enzyme functioning (Kubis, 2001), and secondly, resulted from the fact that PAs are able to affect the other hydrogen peroxide scavenging enzyme activities – catalase and guaiacol peroxidase (Kubis, 2003). Shen *et al*. (2000) reported that SPD can counteract the chill-induced activation of NADPH oxidase, and by this way, diminishes ROS generation in cucumber cultivars.

By contrast, GPX activity was greater at higher concentrations of Cd, especially in inoculated plants (Fig. 3D). There was a synergistic effect between TL fungi and SPD. However, spraying of SPD at the rate of 0.5 Mm had a significant and positive effect on GPX activity in the uninoculated plants (Fig. 3E). The

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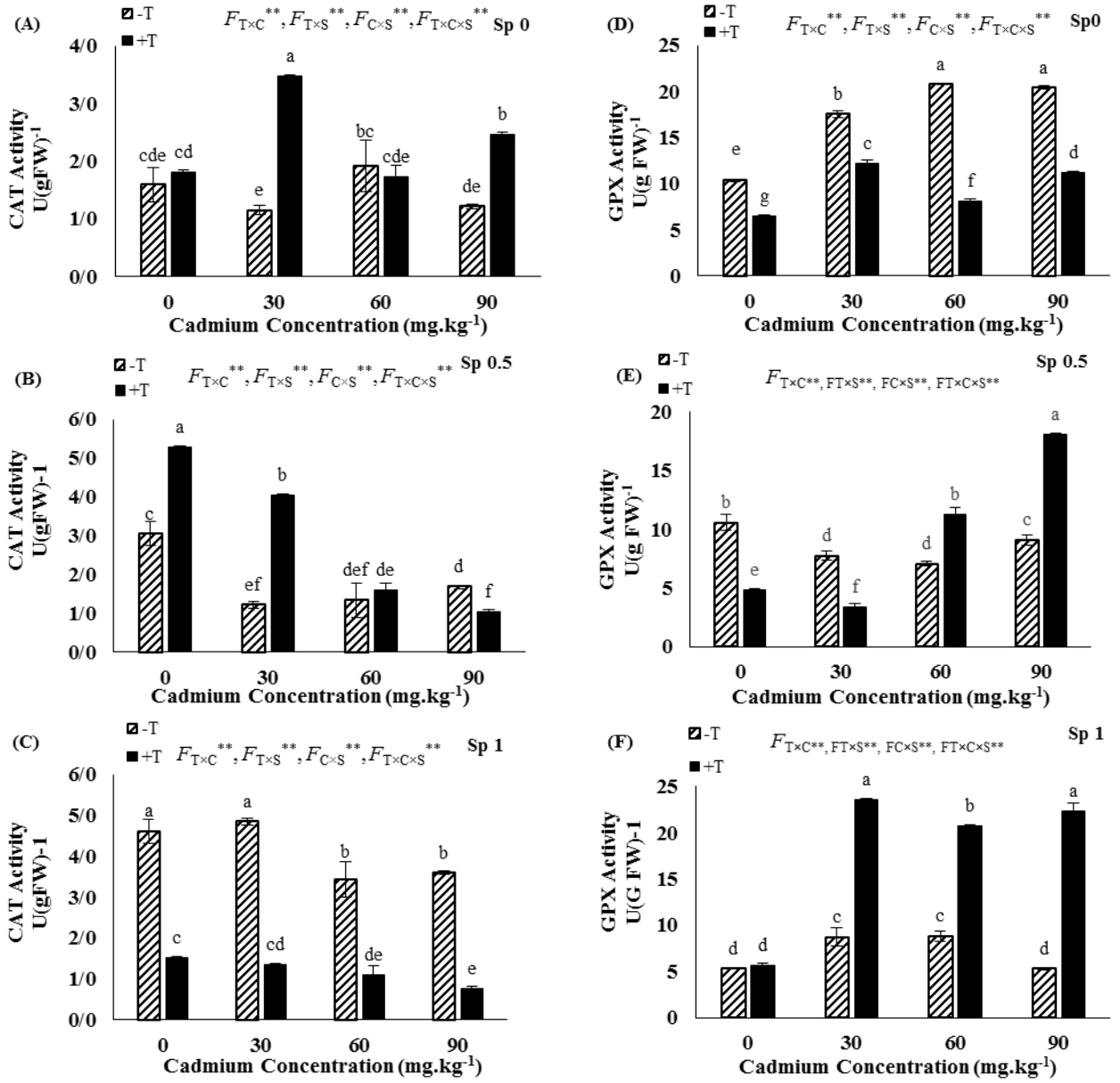

Fig. 3- Effects of exogenous SPD (0, 0.5, 1 mM) and *Trichoderma* on CAT (A, B, C) and GPX (D, E, F) in purslane seedlings under four concentrations (0, 30, 60, 90 mg.kg^{-1}) $CdCl_2$ stress. Different letters indicate significant differences (P≤0.05).

differences observed in antioxidant activities in different plant species under Cd toxicity probably depended on the amount of metal concentration and the duration of exposure to stress and the type of plant. (Gallego and Tomaro, 2005: Jawad Hassan *et al*., 2020). Our results could be indicative of cell adaptation to metal after prolonged exposure to the plant. Abiotic stresses such as heavy metals by ROS production lead to molecular damage to plant cells (Zhang *et al.*, 2005). Although Cd does not generate ROS directly, it generates oxidative stress by interrupting the antioxidant defense system (Toppi and Gabrielli, 1999). Antioxidant enzymes such as GPX, APX, and CAT balance the production and destruction of ROS. Meanwhile Cd inhibits the coenzyme by inhibiting the Calvin cycle enzymes, preventing the electron from receiving PSI (Vassilev *et al*., 2004).

Protein: As shown in Figure 4A, TL pretreatment caused a great induction of soluble protein content in the leaves of purslane plant (P<0.05), particularly in the 0.5 mM of SPD. Alternatively, SPD spraying (particularly in the 0.5 mM concentration), showed a significant increase in soluble protein content as compared to the control. SPD showed an ameliorating effect on the soluble protein content in purslane under Cd contamination (Fig. 4B). Cd toxicity caused a slight decrease in soluble protein content while SPD spraying increased soluble protein content in purslane leaves. On the other hand, Cd, at higher concentrations, exhibited a strong suppression on insoluble protein synthesis. The soluble protein content reached its maximum level (about 2.4 times over the control level) when purslane

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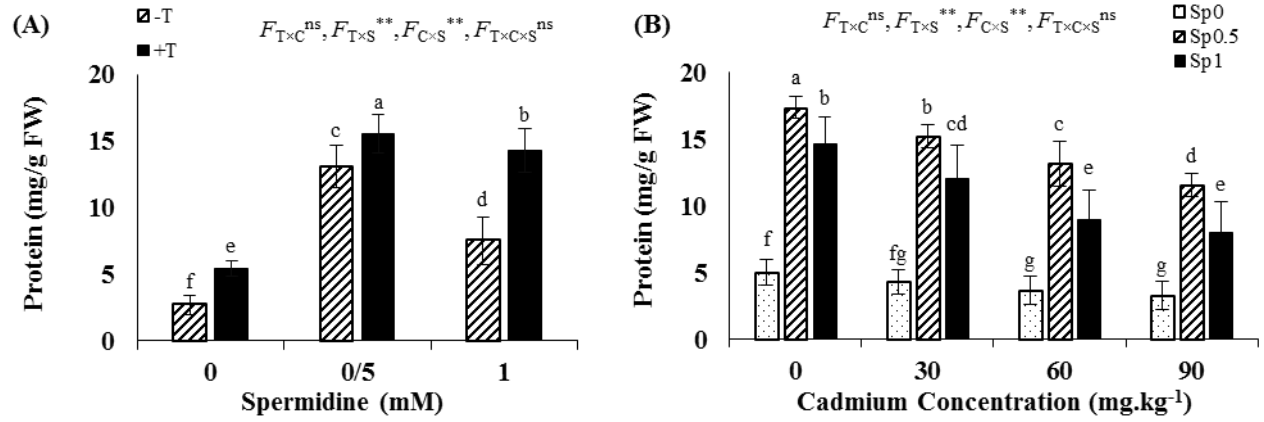

Fig. 4- Effects of exogenous SPD (0, 0.5, 1 mM) with *Trichoderma* (A), on proteine content free in purslane seedlings under four concentrations (0, 30, 60, 90 mg.kg-1) $CdCl_2$ stress (B). Different letters indicate significant differences (P≤0.05).

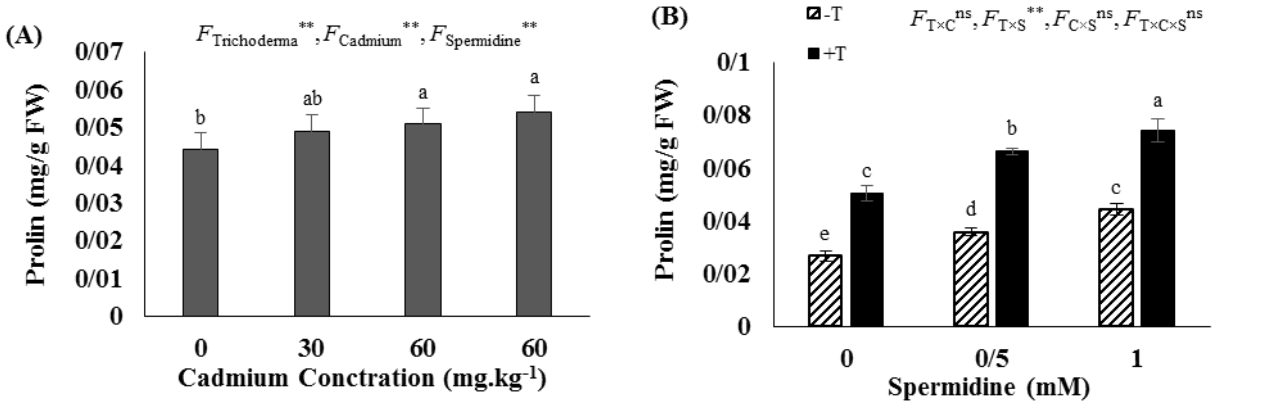

Fig. 5- Effects of four concentrations (0, 30, 60, 90 mg.kg^{-1}) $CdCl_2$ stress (A) and exogenous spermidine (0, 0.5, 1 mM) with *Trichoderma* (B) on prolin content in purslane seedlings. Different letters indicate significant differences (P≤0.05).

plants sprayed with 0.5 mM of SPD spraying. High levels of Cd accumulation in different parts of the plant may interfere with plant growth by reducing the activity of antioxidant enzymes, closing the stomata, and inhibiting nutrient uptake (Parrotta *et al*., 2015; Paunov *et al*., 2018; Guo *et al*., 2019). The amount of leaf protein was greatly affected by Cd treatments. When purslane plants were grown at 30, 60, and 90 mg.kg^{-1} of Cd, root protein content was declined by 14.5 27.3 and 34.5%, respectively (Fig. 4B).

The decrease in protein content may be due to increased protein degradation or reduced synthesis (Balestrass *et al*., 2003). The results indicated that the toxic effects of high levels of Cd on the mechanisms of protein synthesis resulted from decreased protease activity (Lee *et al*., 1976).

Proline activity: The results of the present experiment indicated an increase in proline content in response to an increase in cadmium concentration (Fig. 5A). Also, SPD increased the proline content, particularly in inoculated plants with fungi (Fig. 5B). Proline acts as an osmolyte in stress conditions and may increase antioxidant enzyme activity and thereby minimize the side effects of oxidative stress (Malhotra *et al*., 2017; Khamsuk *et al*., 2018).

In another finding, Islam *et al*. (2009) reported that proline acted as a growth regulator and maintains osmotic in plants, also the ability to protect cells from the accumulation of ROS. Proline is thought to be able to reduce the negative effects of cadmium toxicity on plant growth. Proline accumulates in plant tissues in response to exposure to heavy metals stress (Teklic *et al*., 2008; Radic *et al*., 2010). Despite the very low percentage of proline from the total free amino acid in the plant, it has been regarded as one of the important osmotic and common metabolites that are found in the cellular system exposed to stress (Matysik *et al*., 2002; Liang *et al*., 2013). Pal *et al*. (2015) and Szalai *et al*. (2017) have reported an increase in proline content by Put treatment. Sharma *et al*. (1998) supposed that proline may act as a metal Celator.

Hydrogen peroxide (H_2O_2): The results showed that the amount of H_2O_2 production in plants inoculated with TL was affected at all levels of Cd (Fig. 6A). However, this change was less obvious in seedlings treated with SPD. On the other hand, H_2O_2 production was higher at all cadmium levels in non-inoculated plants (Fig. 6B, C). Also, H_2O_2 production decreased significantly with increasing SPD concentration in all the studied plants. Also, it has been previously reported that *Trichoderma* enhances antioxidant enzyme activities and reduces reactive oxygen species

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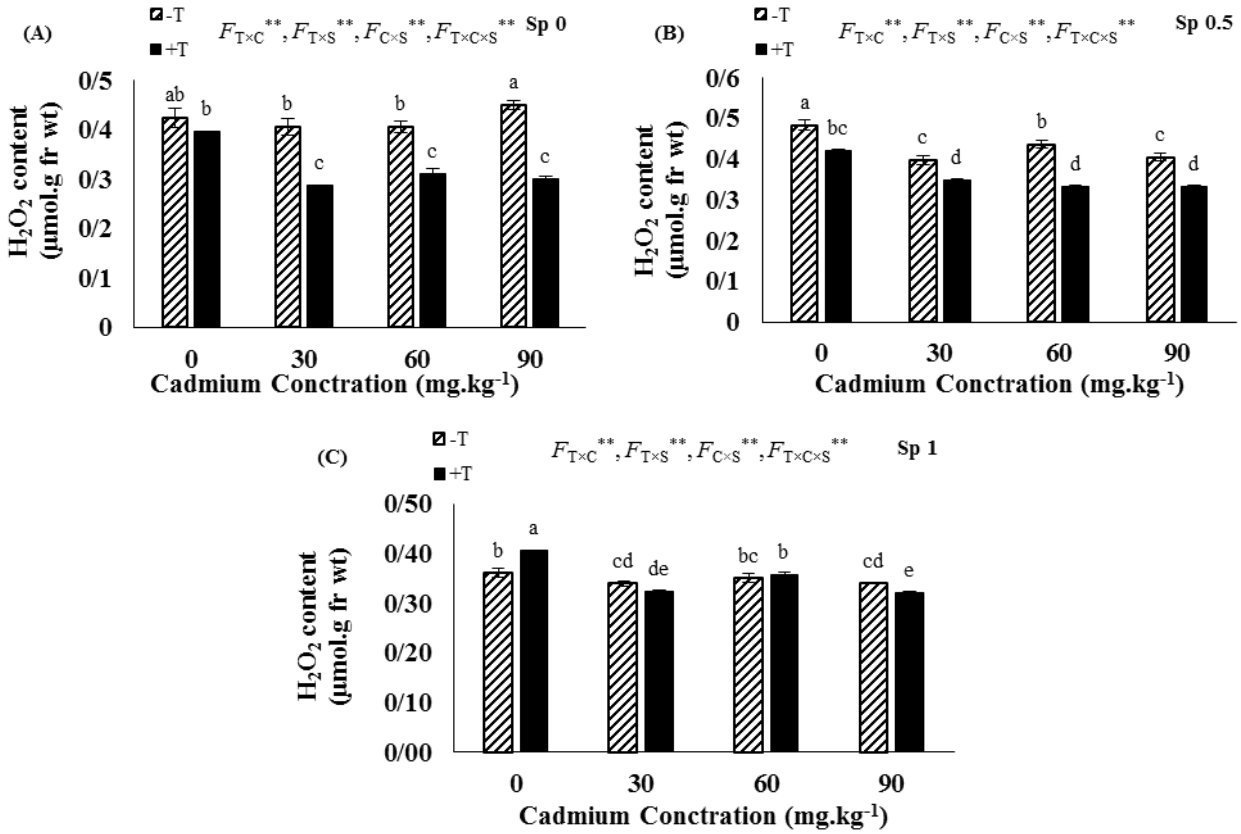

Fig. 6- Effects of exogenous SPD (0, 0.5, 1 mM) and *Trichoderma* on hydrogen peroxide in purslane seedlings under four concentrations (0, 30, 60, 90 mg/kg) $CdCl_2$ stress. Different letters indicate significant differences (P≤0.05).

production in plants (Alqarawi *et al*., 2014).

Application of Cd alone produces no change in the H_2O_2 content, contrasting with the results observed by Tang *et al.* (2005) in maize. Polyamines are likely to be effective in protecting plant tissues from oxidative damage by increasing the activity of antioxidant enzymes (Groppa *et al*., 2007, 2008; Liu *et al.*, 2015).

Bioaccumulation of heavy metals: The interaction effects between TL and SPD on uptake and translocation of Cd in purslane are shown in Figure 7. Cd was accumulated in both plant shoots and the roots at all Cd concentrations. This result clearly shows the Cd ability to accumulate and transport in all parts of the purslane (Fig. 7). On the other hand, the highest levels of Cd accumulation were obtained in purslane are in the shoots contrary to the pattern of accumulation heavy metal by other plants. Similarly, Linger *et al*. (2005) has reported Cd accumulation in *Cannabis sativa* and *Hibiscus cannabinus* plants. This abundance amount of Cd accumulation in the aerial part cannot be explained solely by the adsorption of active ions. Immobilization, by attaching to cell walls, may play a minor role (Chakravarty and Banerjee, 2012). Although, plants inoculated with *Trichoderma* had no significant difference in Cd values in the aerial and terrestrial part of the plant, spraying of 0.5 and 1 mM of SPD in leaf tissues caused a significant difference in the amount of Cd in those organs. In fact, under the application of spermidine, the amount of cadmium accumulation in the aerial and terrestrial portions of *Portulaca oleracea* decreased in both non-coexistent and coexisting plants with TL.

The highest Cd accumulation in the shoot (318.5 $mg.kg^{-1}$ of DW) was measured when purslane control plants were grown at 90 $mg.kg^{-1}$ of Cd with no SPD spraying. The effects of TL fungi and SPD on Cd concentration in shoot, root and soil were shown in Figure 7. Inoculation of plants with TL fungi showed varied changes in Cd concentration in the soil. The Cd content in shoot increased at 30 $mg.kg^{-1}$ of Cd while decreased at 90 $mg.kg^{-1}$ of Cd as compared to the control. The concentration of Cd in shoot and root significantly increased with increasing of Cd toxicity, however, SPD spraying particularly in the Low-Cd concentration significantly decreased Cd accumulation. These results were similar to those obtained by Hsu and Kao (2007), which observed a decrease in cadmium uptake by rice leaves treated with SPD. Also, these results were similar to those obtained by Hsu and Kao (2007), which observed a decrease in Cd uptake by rice leaves treated with SPD. It seemed that SPD was able to protect Cd-induced oxidative damage and this protection was related to the reduction of H_2O_2 generation and Cd uptake.

The highest Cd accumulation was obtained in the shoot of the plants grown on soil contaminated with 60 and 90 mg/kg Cd in the absence of spermidine foliar application (Fig. 7A). The Cd concentration in the aerial

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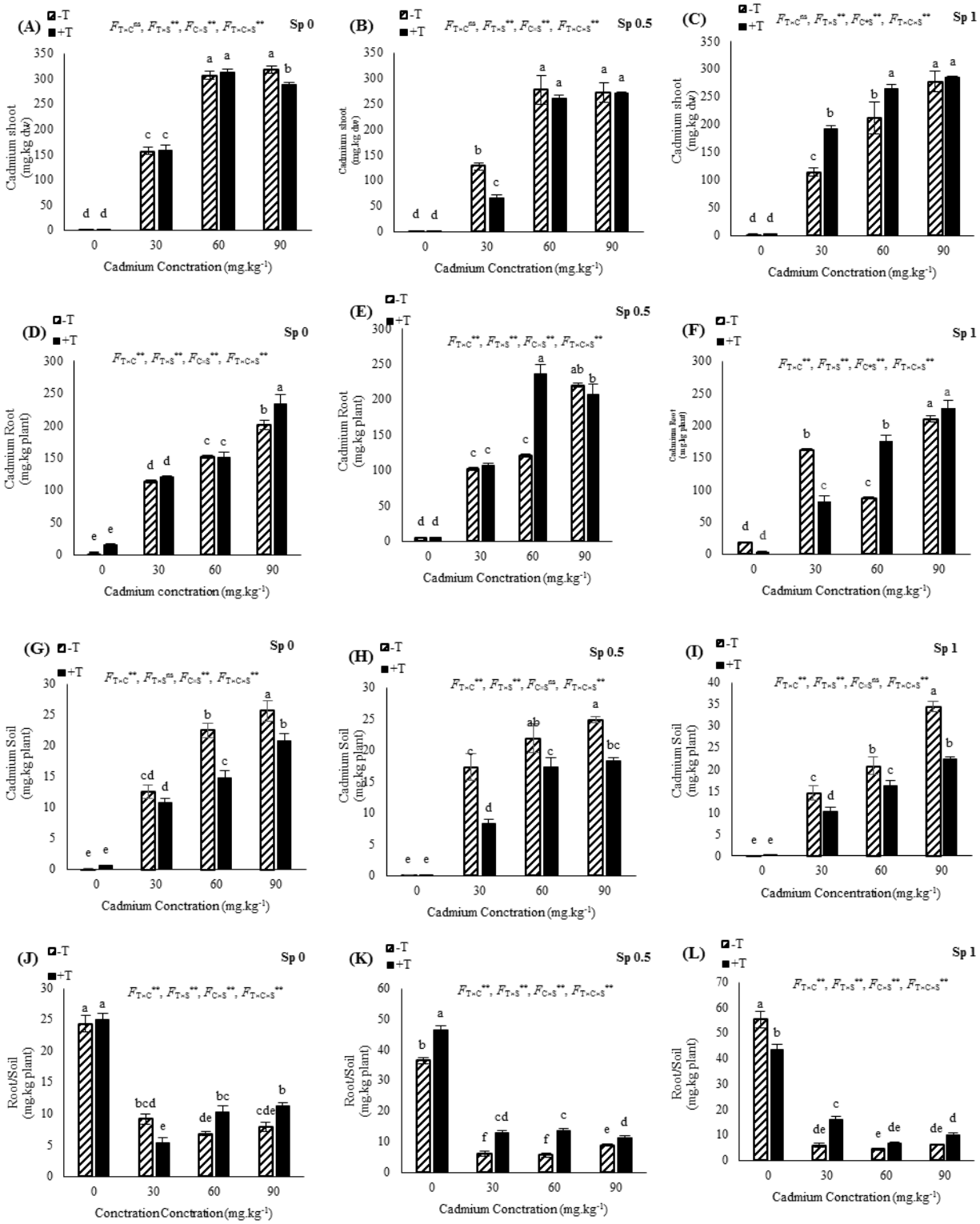

Fig. 7- Effects of exogenous SPD (0, 0.5, 1 mM) and *Trichoderma* on distribution of absorbed cadmium in the shoot (A-C), root (D-F), soil (G-I) and bioaccumulation factor (J-L) in purslane seedlings under four concentrations (0, 30, 60, 90 mg.kg^{-1}) $CdCl_2$ stress. Similar letters indicate the absence of significant differences (P≤0.05).

(shoot) and terrestrial (root) parts, and the shoot/root ratio are shown in Table 1. Plants grown under both levels of SPD had lower Cd in the shoot than the control.

A significant interaction effect was obtained (P≤0.05) between Cd and SPD in terms of Cd concentration in the shoot and root parts. The decrease of the shoot Cd concentrations triggered by elevated levels of SPD was more substantial in 30 mg.kg^{-1} of Cd concentration with 1 mM of SPD (Fig. 7). Our results

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Table 1- The shoot/root cadmium concentration ratio±standard error (MS/MR) in the treatment Trichoderma and levels of the SPD

Spermidin	Pretreatment	M_S/M_R			
		Cd0	Cd30	Cd60	Cd90
SPD 0	Control	0.63±0.04 ^{hi}	1.13±0.05 ^{g}	2.03±0.06 ^{c}	1.59±0.04 ^{d}
	Trichoderma	0.10±0.02 ^{k}	2.79±0.26 ^{a}	2.04±0.08^{c}	1.25±0.09 ^{fg}
SPD 0.5	Control	0.45±0.04 ^{ij}	1.54±0.07 ^{de}	2.29±0.10 ^{b}	1.23±0.04 ^{fg}
	Trichoderma	0.31±0.04 ^{jk}	0.62±0.02^{hi}	1.12±0.08 ^{g}	1.31±0.04 ^{efg}
SPD 1	Control	0.52±0.11 ^{hij}	1.39±0.04 ^{def}	2.41±0.12 ^{b}	1.32±0.03 ^{efg}
	Trichoderma	0.73±0.12 ^{h}	1.19±0.11 ^{fg}	2.39±0.09 ^{b}	1.25±0.04 ^{fg}

Means with common letters in the LSD test are not significantly different from each other (P<0/05)

confirmed that purslane possesses a high ability to accumulate Cd in its tissues, as in previous studies reported by Coakley *et al*. (2019) on plants such as *Impatiens glandulifera* in the environment contaminated with Cd.

On the other hand, *Trichoderma* inoculation improved purslane phytoremediation efficiency. Cd concentration ratios of shoots to roots are shown in Table 1. This value was greater than one. Our results showed that purslane especially when inoculated with TL and were treated with spermidine could be recommended as a suitable species for phytoremediation of Cd contaminated soils.

Conclusions

In the present study, we examined the effects of *Trichoderma* fungi coexistence and the application of polyamine spermidine on soil purification from cadmium contamination in the purslane plant. Our results showed that the Purslane plant could be recommended as a suitable species for soil purification from heavy metal such as cadmium due to the proper accumulation of Cd in its branches, especially when inoculated with TL. In other words, it increased the *Portulaca oleracea* phytoremediation in cadmium-contaminated soil. Also, SPD suppressed H_2O_2 production and improved proline content in plants under Cd stress. In general, the synergistic effect between the TL fungi and SPD could be an appropriate strategy to improve Cd phytoremediation efficiency in purslane plants.

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References

Aebi, H. (1984) Catalase in vitro. Methods in Enzymology 105: 121-126.

Afzal, M., Khan, Q. M. and Sessitsch, A. (2014) Endophytic bacteria: Prospects and applications for the phytoremediation of organic pollutants. Chemosphere 117: 232-242.

Ahmad, P., Jaleel, C. A., Salem, M. A., Nabi, G. and Sharma, S. (2010) Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Critical Reviews in Biotechnology 30: 161-175.

Akladious, S. A. and Abbas, S. M. (2012) Application of *Trichoderma harzianum* T22 as a biofertilizer supporting maize growth. African Journal of Biotechnology 11: 8672-8683.

Alqarawi, A. A., Hashem, A. and Abd-Allah, E. F. (2014) Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. Journal of Plant Interactions 9: 802-810.

Arasimowicz-Jelonek, M. and Floryszak-Wieczorek, J. (2011) Understanding the fate of peroxynitrite in plant cells - from physiology to pathophysiology. Phytochemistry 72: 681-688.

Balestrass, K. B., Benavides, M. P., Gallego, S. M. and Tomaro, M. L. (2003) Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. Functional Plant Biology 30: 57-64.

Bates, L. S., Waldren, R. P. and Teare, I. D. (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205-207.

Belouchrani, S., Mameri, N., Abdi, N., Grib, H., Lounici, H. and Drouiche, N. (2016) Phytoremediation of soil contaminated with Zn using Canola (*Brassica napus* L.). Ecological Engineering 95: 43-49.

Cai, Y., Agmon, N., Choi, W. J., Ubide, A., Stracquadanio, G., Caravelli, K., Hao, H., Bader, J. S. and Boeke, J. D. (2015) Intrinsic biocontainment: multiplex genome safeguards combine transcriptional and recombinational control of essential yeast genes. Proceedings of the National Academy of Sciences of the United States of America 112: 1803-1808.

Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences 74: 2157-2184.

Cardinals, C., Put, C., Van Asche, F. and Clijsters, H. (1984) The superoxide dismutase as a biochemical indicator, discriminating between zinc and cadmium toxicity. Biologia Plantarum 40: 131-135.

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[DOR: 20.1001.1.23222727.1400.10.43.3.7]

Chakravarty, R. and Banerjee, P. C. (2012) Mechanism of cadmium binding on the cell wall of an acidophilic bacterium. Bioresource Technology 108: 176-183.

Chiapello, H., Mallet, L., Guerin, C., Aguilera, G., Amselem, J., Kroj, T., Ortega-Abboud, E., Lebrun, M. H., Henrissat, B., Gendrault, A., Rodolphe, F, Thoreau, D. and Fournier, E. (2015) Deciphering genome content and evolutionary relationships of isolates from the fungus *Magnaporthe oryzae* attacking different host plants. Genome Biology and Evolution 7: 2896-2912.

Coakley, S., Cahill, G., Enright, A. M., O'Rourke, B. and Petti, C. (2019) Cadmium hyperaccumulation and translocation in *Impatiens glandulifera*: From foe to friend? Sustainability 11: 1-17.

Fahr, M., Laplaze, L., Bendaou, N., Hocher, V., El Mzibri, M., Bogusz, D. and Smouni, A. (2013) Effect of lead on root growth. Frontiers in Plant Science 4: 1-7.

Ghaderian, S. M. and Ghotbi Ravandi, A. A. (2012) Accumulation of copper and other heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. Journal of Geochemical Exploration 123: 25-32.

Ghorbanpour, A., Salimi, A, Ghanbary, M. A. T., Pirdashti, H. and Dehestani, A. (2018) The effect of *Trichoderma harzianum* in mitigating low temperature stress in tomato (*Solanum lycopersicum* L.) plants. Scientia Horticulturae 230: 134-141.

Ghosh, M. and Singh, S. P. (2005) A comparative study of cadmium phytoextraction by accumulator and weed species. Environmental Pollution 133: 365-371.

Groppa, M. D., Ianuzzo, M. P., Tomaro, M. L. and Benavides, M. P. (2007) Polyamine metabolism in sunflower plants under long-term cadmium or copper stress. Amino Acids 32: 265-275.

Groppa, M. D., Rosales, E. P., Iannone, M. F. and Benavides, M. P. (2008) Nitric oxide, polyamines and Cd-induced phytotoxicity in wheat roots. Phytochemistry 69: 2609-2615.

Guo, J., Qin, S., Rengel, Z., Gao, W., Nie, Z., Liu, H., Li, C. and Zhao, P. (2019) Cadmium stress increases antioxidant enzyme activities and decreases endogenous hormone concentrations more in Cd-tolerant than Cd-sensitive wheat varieties. Ecotoxicology and Environmental Safety 172: 380-387.

Gupta, D. K., Nicoloso, F. T., Schetinger, M. R. C., Rossato, L. V., Pereira, L. B., Castro, G. Y., Srivastava, S. and Tripathi, R. D. (2009) Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. Journal of Hazardous Materials 172: 479-484.

Gupta, S., Nayek, S., Saha, R. N. and Satpati, S. (2008) Assessment of heavy metal accumulation in macrophyte, agricultural soil and crop plants adjacent to discharge zone of sponge iron factory. Environmental Geology 55: 731-739.

Hechmi, N., Ben Aissa, N., Hassen, A. and Jedidi, N. (2013) Phytoremediation potential of maize (*Zea mays* L.) in co-contaminated soils with pentachlorophenol and cadmium. International Journal of Phytoremediation 15: 703-713.

Helrich, K. (1990) Method 988.05. Official Methods of Analysis. 15th Ed. The Association of Official Analytical Chemists Inc., Arlington.

Hjeljord, L. G. and Tronsmo, A. (2003) Effect of germination initiation on competitive capacity of *Trichoderma atroviride* P1 Conidia. Biological Control 93: 1593-1598.

Hsu, Y. T. and Kao, C. H. (2007) Cadmium-induced oxidative damage in rice leaves is reduced by polyamines. Plant and Soil 291: 27-37.

Huang, Y. Z., Hu, Y. and Liu, Y. X. (2009) Combined toxicity of copper and cadmium to six rice genotypes (*Oryza sativa* L.). International Journal of Environmental Science and Technology 21: 647-653.

Islam, M. M., Hoque, M. D. A., Okuma, E., Banu, M. N. A., Shimoishi, Y., Nakamura, Y. and Murata, Y. (2009) Exogenous proline and glycine betaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. Journal of Plant Physiology 166: 1587-1597.

Jawad Hassan, M., Ali Raza, M., Ur Rehman, S., Ansar, M., Gitari, H., Khan, I., Wajid, M., Ahmed, M., Abbas Shah, G., Peng, Y. and Li, Z. (2020) Effect of cadmium toxicity on growth, oxidative damage, antioxidant defense system and cadmium accumulation in two sorghum cultivars. Plants 9: 1575.

Kasukabe, Y., He, L., Nada, K., Misawa, S., Ihara, I. and Tachibana, S. (2004) Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant and Cell Physiology 45: 712-722.

Khamsuk, O., Sonjaroon, W., Suwanwong, S., Jutamanee, K. and Suksamrarn, A. (2018) Effects of 24-epibrassinolide and the synthetic brassinosteroid mimic on chili pepper under drought. Acta Physiologiae Plantarum 40: 1-12.

Kubis, J. (2003) Polyamines and"scavenging system": In fluence of exogenous spermidine on catalase and guaiacol peroxidase activities, and free polyamines level in bar ley leaves under water deficit. Acta Physiologiae Plantarum 25: 337-343.

Kumar Rai, P. (2008) Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: An ecosustainable approach. International Journal of Phytoremediation 10: 133-160.

Kumar, A., Dutt, S., Bagler, G., Ahuja, P. S. and Kumar, S. (2012) Engineering a thermo-stable superoxide dismutase functional at subzero to >50 °C, which also tolerates autoclaving. Scientific Reports 2: 387.

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[DOR: 20.1001.1.23222727.1400.10.43.3.7]

Kuo, M. C. and Kao, C. H. (2004) 'Antioxidant enzyme activities are upregulated in response to cadmium in sensitive, but not in tolerant, rice (*Oryza sativa* L.) seedlings. Botanical Bulletin of Academia Sinica (Taiwan) 45: 291-299.
Laspina, N. V., Groppa, M. D., Tomaro, M. L. and Benavides, M. P. (2005) Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. Plant Science 169: 323-330.
Lee, K. C., Cunningham, B. A., Poulsen, G. M., Liang, J. M. and Moore, R. B. (1976) Effects of cadmium on respiration rate and activities of several enzymes in soybean seedlings. Physiologia Plantarum 36: 4-6.
Liang, X., Zhang, L., Natarajan, S. K. and Becker, D. F. (2013) Proline mechanisms of stress survival. Antioxidants and Redox Signaling 19: 998-1011.
Linger, P., Ostwald, A. and Haensler, J. (2005) *Cannabis sativa* L. growing on heavy metal contaminated soil: Growth, cadmium uptake and photosynthesis. Biologia Plantarum 49: 567-576.
Liu, M., Chu, M., Ding, Y., Wang, Sh., Liu, Z., Tang, S., Ding, C. and Li, G. (2015) Exogenous spermidine alleviates oxidative damage and reduce yield loss in rice submerged at tillering stage. Frontiers in Plant Science 6: 1-11.
Lopez Errasquin, E. and Vazquez, C. (2003) Tolerance and uptake of heavy metals by *Trichoderma atroviride* isolated from sludge. Chemosphere 50: 137-43.
Luo, J., Qi, S., Peng, L. and Wang, J. (2016) Phytoremediation efficiency of Cd by *Eucalyptus globulus* transplanted from polluted and unpolluted sites. International Journal of Phytoremediation 18: 308-314.
Maldonado-Magana, A., Favela-Torres, E., Rivera-Cabrera, F. and Volke-Sepulveda, T. (2011) Lead bioaccumulation in *Acacia farnesiana* and its effect on lipid peroxidation and glutathione production. Plant and Soil 339: 377-389.
Malhotra, C., Kapoor, R. T., Ganjewala, D. and Singh, N. (2017) Sodium silicate mediated response of antioxidative defense system in *Lycopersicon esculentum* Under water stress. International Journal of Phytomedicine 9: 364-378.
Massey, F. J. (1952) Distribution table for the deviation between two sample cumulatives. Annals of Mathematical Statistics 23: 435-441.
Matysik, J., Alai Bhalu, B. and Mohanty, P. (2002) Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Current Science 82: 525-532.
Milone, M. T., Sgherri, C., Clijsters, H. and Navarri-Izzo, F. (2003) Antioxidant responses of wheat treated with realistic concentration of cadmium. Environmental and Experimental Botany 50: 265-276.
Mutlu, F. and Bozcuk, S. (2005) Effects of salinity on the contents of polyamines and some other compounds in sunflower plants differing in salt tolerance1. Russian Journal of Plant Physiology 52: 29-34.
Nakano, Y. and Asada, K. (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22: 867-880.
Pal, M., Szalai, G. and Janda, T. (2015) Speculation: Polyamines are important in abiotic stress signaling. Plant Science 237: 16-23.
Parrotta, L., Guerriero, G., Sergeant, K., Cai, G. and Hausman, J. F. (2015) Target or barrier? The cell wall of early- and later-diverging plants vs cadmium toxicity: Differences in the response mechanisms. Frontiers in Plant Science 6: 1-16.
Paunov, M., Koleva, L., Vassilev, A., Vangronsveld, J. and Goltsev, V. (2018) Effects of different metals on photosynthesis: Cadmium and zinc affect chlorophyll fluorescence in durum wheat. International Journal of Molecular Science 19: 1-13.
Radhakrishnan, R. and Lee, I. J. (2014) Endogenous antioxidants and phytohormonal regulation induced by spermidine improve cucumber plant growth. Pakistan Journal of Botany 46: 2151-2156.
Radic, S., Babic, M., Skobic, D., Roje, V. and Pevalek-Kozlina, B. (2010) Ecotoxicological effects of aluminum and zinc on growth and antioxidants in *Lemna minor* L. Ecotoxicology and Environmental Safety 73: 336-342.
Satish, L., Rency, A. S. and Ramesh, M. (2018) Spermidine sprays alleviate the water deficit-induced oxidative stress in finger millet (*Eleusine coracana* L. Gaertn.) plants. 3 Biotech 8: 1-11.
Sergiev, I., Alexieva, V. and Karanov, E. (1997) Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. Comptes Rendus de l' Academie Bulgare des Sciences 51: 121-124.
Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M. and Pinelli, E. (2014) Heavy-metal-induced reactive oxygen species: Phytotoxicity and physicochemical changes in plants. Archives of Environmental Contamination and Toxicology. Cham: Springer International Publishing 1-44.
Sharma, S. S., Schat, H. and Vooijs, R. (1998) In vitro alleviation of heavy metal-induced enzyme inhibition by proline. Phytochemistry 49: 1531-1535
Shen, W., Nada, K. and Tachibana, S. (2000) In volve ment of polyamines in the chilling tol er ance of cu cumber cultivars. Plant Physiology 124: 431-439.
Sobkowiak, R., Rymer, K., Rucinska, R. and Deckert, J. (2004) Cadmium induced changes in antioxidant enzymes in suspension culture of soybean cells. Acta Biochimica Polonica 51: 219-222.
Szalai, G., Janda, K., Darko, E., Janda, T., Peeva, V. and Pal, M. (2017) Comparative analysis of polyamine metabolism in wheat and maize plants. Plant Physiology and Biochemistry 112: 239-250.

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[DOR: 20.1001.1.23222727.1400.10.43.3.7]

Tang, W. and Newton, R. J. (2005) Peroxidase and catalase activities are involved in direct adventitious shoot formation induced by thidiazuron in eastern white pine (*Pinus strobus* L.) zygotic embryos. Plant Physiology and Biochemistry 43: 760-769.

Tang, W. H., Zhang, Z. X., Zou, X. L. and Zheng, Y. L. (2005) Functional genomics of maize submergence tolerance and cloning of the related gene Sicyp 51. Science in China Series B-Chemistry, Life Sciences and Earth Sciences 48: 337-345.

Teklic, T., Hancock, J. T., Engler, M., Paradicovic, N., Cesar, V., Lepedus, H., Stolfa, I. and Beslo, D. (2008) Antioxidative responses in radish (*Raphanus sativus* L.) plants stressed by copper and lead in nutrient solution and soil. Acta Biologica Cracoviensia Series Botanica 50: 79-86.

Tiburcio, A. F., Altabella, T., Bitrian, M. and Alcazar, R. (2014) The roles of polyamines during the lifespan of plants: From development to stress. Planta 240: 1-18.

Toppi, S. and Gabrielli, R. (1999) Response to cadmium in higher plants. Environmental and Experimental Botany 41: 105-130.

Vassilev, A., Schwitzguebel, J. P., Thewys, T., Van Der Lelie, D. and Vangronsveld, J. (2004) The use of plants for remediation of metal-contaminated soils. The Scientific World Journal 4: 9-34.

Xu, H., Fairman, J. W., Wijerathna, S. R., Kreischer, N. R., LaMacchia, J., Helmbrecht, E., Cooperman, B. S. and Dealwis, C. (2008) The structural basis for peptidomimetic inhibition of eukaryotic ribonucleotide reductase: A conformationally flexible pharmacophore. Journal of Medicinal Chemistry 51: 4653-4659.

Yadav, S. (2010) Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African Journal of Botany 76: 167-179.

Yaghoubian, Y., Siadat Mohammad, S. A., Moradi Telavat, M. R. and Pirdashti, H. (2016) Quantify the response of purslane plant growth, photosynthesis pigments and photosystem ii photochemistry to cadmium concentration gradients in the soil. Russian Journal of Plant Physiology 63: 77-84.

Yaghoubian, Y., Siadat Mohammad, S. A., Moradi Telavat, M. R., Pirdashti, H. and Yaghoubian, I. (2019) Bio-removal of cadmium from aqueous solutions by filamentous fungi: *Trichoderma* spp. and *Piriformospora indica*. Environmental Science and Pollution Research 26: 7863-7872.

Yang, Y. H., Morand, E. and Leech, M. (2013) Annexin A1: Potential for glucocorticoid sparing in RA. Nature Reviews Rheumatology 9: 595-603.

Yoshimura, K., Yabuta, Y., Ishikawa, T. and Shigeoka, S. (2000) Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. Journal of Plant Physiology 123: 223-233.

Zhang, H., Jiang, Y., He, Z. and Ma, M. (2005) Cadmium accumulation and oxidative burst in garlic (*Allium sativum*). Journal of Plant Physiology 162: 977-84.

Zhang, K., Chai, F., Zheng, Z., Yang, Q., Zhong, X., Fomba, K. W. and Zhou, G. (2018) Size distribution and source of heavy metals in particulate matter on the lead and zinc smelting affected area. Journal of Environmental Sciences 71: 188-196.

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[DOR: 20.1001.1.23222727.1400.10.43.3.7]