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Effect of *Piriformospora indica* on antioxidant enzymes activity of tomato (*Lycopersicon esculentum* Mill) under lead stress

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Abstract

The endomycorrhizal fungus *Piriformospora indica* is well known for inducing disease resistance, elevation of salt tolerance and increasing biomass in symbiotic plants. The aim of the present study is to evaluate the effect of *P. indica* on tomato (*Lycopersicon esculentum* Mill) under lead stress (Concentrations of 1 and 2 mM Pb $(NO_3)_2$). Lead (Pb) is not an essential nutrient for plants and is known as a hazardous pollutant in the environment which originates from various sources. In this study, the effect of lead toxicity and *P. indica* on antioxidant enzyme activities in both mycorrhizal (MR) and non-mycorrhizal (NMR) tomato plants were investigated. The experiment was performed by using six treatments (mycorrhizal and non-mycorrhizal with and without lead stress) and two concentrations of Pb (1and 2 mM Pb $(NO_3)_2$ solution) and then antioxidant enzyme activities (CAT, SOD and APX) in roots and stems of plants were determined. The results demonstrated that lead could affect the activity of antioxidant enzymes in treating plants. As well, in most cases, the rates of the enzyme activities were higher in roots than those of stems, but the impact Pb treatment on enzyme activities in P. *indica* colonized plants was not significant.

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Introduction

Tomato (*Lycopersicon esculentum* Mill) is a eudicotiledonous plant that belongs to the family Solanaceae together with other economically important crops such as pepper, eggplant and potato. It is the most important grown fresh market vegetable worldwide with more than 5 million hectares harvested in China, United States of America, India, Turkey and Egypt as the five first producers, respectively (Peralta *et al.*, 2008).

Plants are constantly exposed to a wide array of environmental stresses that cause major losses in productivity. Resistance and susceptibility to the biotic and abiotic stresses are complex phenomena, in part because stress may occur in multiple stages of plant development and often more than one stress simultaneously affects the plant. To handle with various environmental challenges, plants execute a number of physiological and metabolic responses (Bohnert *et al.*, 1995). On the other hand, the effects of various environmental stresses are known to change the antioxidant content of tomato (Dumas *et al.*, 2003).

With rapid development in industry all around the world since the twentieth century, the heavy metal concentrations in agricultural soil increased quickly in many areas around the world (Kabata and Pendias, 1984). Heavy metals are important environmental pollutants, and many of them are toxic even at very low concentrations. Lead (Pb) is not an essential nutrient for plants, and it is one of the hazardous heavy metal pollutants of the environment that originates from various sources like mining and smelting activities, burning of coal, effluents from storage battery industries, automobile exhausts, pesticides, and from additives in pigments and gasoline as well as from the disposal of municipal sewage sludge enriched with Pb (Eick *et al.*, 1999). Pb pollution of the environment is a major ecological concern due to its impact on human health through the food chain and its high persistence in the environment (Piechalak *et al.*, 2003). Toxic levels of heavy metal affect a variety of processes in plants. One of the major consequences is the enhanced production of reactive oxygen species (ROS) (Verma and Dubey 2003; Souguir *et al.,* 2011). ROS can cause oxidative damage to the biomolecules when produced in larger amounts leading to cell membrane peroxidation, loss of ions, protein hydrolysis, and even DNA fragmentation. Plants' defenses to metal toxicity may constitute different strategies. First is the avoidance of metal entry into the cell via exclusion or binding of metal in the cell wall. For lead, binding to cell wall is one of the major mechanisms of detoxification (Antosiewicz and Wierzbicka 1999). Second, plants have developed an anti-oxidative system, including low-molecular-mass antioxidants as well as anti-oxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbic acid peroxidase (APX) (Cakmak and Horst 1991).

The beneficial fungi play a crucial role in improving plant growth and yield, and also take part in stress tolerance, bioremediation, food safety and sustainable crop production (Amatussalam *et al.*, 2011; Borde *et al.,* 2009; Singh *et al.*, 2011). In general, an endophyte can be defined as a microorganism, typically bacterial or fungal, that lives symptomatically within a plant, causing no signs of harm to the host (Clay and Schardl, 2002; Porras-Alfaro and Bayman, 2011). The endophytic fungus *Piriformospora indica* interacts with many plant species. It colonizes the roots, grows inter- and intracellularly, forms pear-shaped spores within the cortex and extramatrically, and does not invade the endodermis and the aerial parts of the plants. The endophyte promotes nutrient uptake, allows plants to survive underwater, temperature and salt stress, confers (systemic) resistance to toxins, heavy metal ions and pathogenic organisms and stimulates growth and yield (Varma *et al.*, 2001). A symbiotic association of fungi leads to stress tolerance in plants which involves two mechanisms: (1) trigger-action of host stress responsive systems while exposed to a particular stress, directing the plants to either keep

away from or mitigate that stress; and (2) biosynthesis of anti-stress biochemical by endophytes (Singh *et al*., 2011).

The relevance of tomato in human nutrition is increasing, since it is generally considered as a healthy food because of the high content of lycopene and other health promoting natural compounds. A large part of this crop is grown in greenhouses, using special substrates and fertilization techniques involving utilization of water, therefore implying an increased risk of heavy metal concentration increases (Gil *et al*., 2004). Some research was done on the effects of mycorrhiza fungi including *Piriformospora indica* on plants growth and accumulation of heavy metals (Sun *et al*., 2010; Shahabivand *et al*., 2012) and lead stress effects on antioxidant enzyme activities in different plants (Verma and Dubey, 2003; Gupta *et al*., 2009; Wang *et al*., 2012) , which often reflects the positive impact of fungi on plant growth and increasing effect of lead on activities of antioxidant enzymes . However, no comprehensive study has been done on the simultaneous effects of lead stress and *Piriformospora indica* on antioxidant enzyme activities as an indicator of oxidative stress in the tomato plant in response to heavy metals (especially lead) as one of the most widely consumed crops and exposed to lead contamination in many parts of the world. Therefore, research in this area may be a new way for the diagnosis and management of heavy metal stress, with regard to changes in the antioxidant enzyme activities and the ability of the *Piriformospora indica* to reducing lead stress in tomato. Therefore, inspection of plants such as tomato mechanisms in response to Pb toxicity is worthy to known. Thus, the aim of this work is to evaluate antioxidant enzyme activities in roots and stems of tomato plant inoculated with *P. indica* under Pb stress.

Materials and methods

Preparation and planting of tomato seeds

Healthy and improved seeds of tomato (*Lycopersicon esculentum* Mill.) obtained from seed and Plant Improvement Institute, Karaj, Iran. To sterilize, the seeds placed in 50% ethanol for 1 minute, then immersed for 15 minutes in 1.5 % of the active chlorine solution. Treated seeds washed several times with sterilized distilled water. For germination, the disinfected seeds were sown on sterilized sand and perlite (50:50) substrate.

Preparation of P. indica culture and inoculation of tomato seedlings

The fungus culture was grown on a complex medium (CM) at the 27 ^{O}C for one month. The root of 2 weeks old seedlings immersed in 10^{6} ml^{-1} of *P. indica* chlamydospores suspension for 12 hours and shook. Infected seedlings transferred into pots containing sterilized mixture of sand and perlite substrate. After planting, the pots were transferred to growth chamber with 16 h light period and 22 to 24 ° C, 60% relative humidity, for 4 weeks. To detect *P. indica* root samples, stained with ink following by Vierheilig *et al.* (2005) method with minor changes. Molecular detection of *P. indica* in plants carried out using Tef specific primer pairs (accession no. AJ249911).

Treatments conditions

In this research, experiment was arranged in six treatments with three replicates. treatments were as follows: **A** (tomato inoculated with *p.indica*), **B** (tomato), **A1** (tomato inoculated with *p. indica* under lead stress (1 mm pb (no3)2), **B1** (tomato under lead stress (1 mm pb (no3)2), **A2** (tomato inoculated with *p. indica* under lead stress (2mm pb (no3)2), **B2** (tomato under lead stress (2 mm pb (no3)2)..

Antioxidant enzyme activities in tomato plants

In order to study the impact of colonization with *P. indica* on the antioxidant enzyme activity of the plant, the antioxidant enzyme activities were checked in the presence and absence of *P. indica*. For this purpose, all experiments were carried out 28 days after the colonization under the conditions employed. The antioxidant enzyme activities were measured in roots and stems of tomato plants.

Superoxide dismutase assay

The activity of SOD was analyzed by measuring its ability to inhibit the photochemical reduction of Nitro blue tetrazolium (NBT) using the method of Beauchamp and Fridovich (1971). Three milliliters of reaction mixture containing 50 mM phosphate buffer at pH 7.8, 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 1.0 mM EDTA and 20 µl enzyme extraction. Riboflavin was added last and the reaction was initiated by placing the tubes 30 cm below 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 10 min. Switching off the light stopped the reaction and the tubes were covered with black cloth. Non-illuminated tubes served as control. The absorbance at 560 nm was read. One unit of SOD is the amount of extracts that gives 50% inhibition of the rate of NBT reduction (El-Beltagi *et al.*, 2012).

Catalase activity assay

Catalase activity (CAT; EC 1.11.1.6) was determined by consumption of H_2O_2 using the method of Dhindsa *et al.* (1981). The reaction mixture (3 ml) contained 50 mM potassium phosphate buffer at pH 7.0, 15 mM H_2O_2 and 50 ml enzyme extraction. The reaction was initiated by adding the H_2O_2. The consumption of H_2O_2 was monitored spectrophotometrically at 240 nm (extinction coefficient 39.4 mM^{-1} cm^{-1}) for 3 min(El-Beltagi *et al.*, 2012).

Ascorbate peroxidase assay

Ascorbate peroxidase activity (APOX; E.C. 1.11. 1. 11) was estimated according to the method of Nakano and Asada (1981). Enzyme activity was determined by the decrease in absorbance of ascorbate at 290 nm. The reaction mixture consisted of enzymatic extract, 50 mM sodium phosphate buffer (cold), pH 7, 0.5 mM ascorbate, 0.5 mM H_2O_2 and 0.1 mM EDTA, in a 0.3 ml final volume. The reaction started after the hydrogen peroxide addition. The molar extinction coefficient 2.8 mM^{-1} cm^{-1} was used to calculate ascorbate peroxidase activity. Enzyme activity was expressed as units mg^{-1} protein. One unit of enzyme was the amount necessary to decompose 1µmol of substrate per minute at 25°C (El-Beltagi *et al.*, 2012).

Statistical analyses

Results of all experiments were expressed as means ± SE. Analysis of variance performed by ANOVA procedures using SPSS 17.0 and means was compared with Duncan test at the 0.05 level of confidence.

Results and discussion

Microscopic images show the proper growth of fungus *P. indica* in tomato plant root cells, and this phenomenon has a considerable importance in the study (Fig. 1). Then the visual appearance of plants in different treatments was studied, the results showed that the apparent plant growth rate and root growth in mycorrhiza plants relative to non-mycorrhiza plants was significantly higher. Heavy metals such as lead induce oxidative stress by generation of superoxide radical, hydrogen peroxide, hydroxyl radical and single oxygen that called ROS (Devi and Prasad, 1998). ROS can rapidly attack all types of biomolecules such as nucleic acids, proteins, lipids and amino acids (Luna *et al.*, 1994), leading to irreparable metabolism and cell death. To cope and repair the damage caused by ROS, plants have evolved complex antioxidant systems, such as antioxidant enzymes (CAT, SOD, APX, and POX). The results presented in Figures 2-7 reveal the antioxidant enzymes responses of tomato plants grown under different Pb concentrations (1 and 2 mM Pb $(NO_3)_2$) in the presence and in the absence of endomycorrizal fungus *P. indica*.

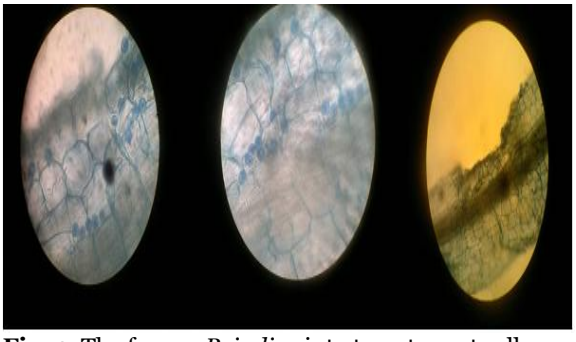

Fig. 1. The fungus *P. indica* into tomato root cells.

Superoxide dismutase (SOD) activity

In the case of roots, highest and lowest SOD activity belong to B2 (6.803 ± 0.612) and A (3.534 ± 0.537)

treatments respectively (Fig.2). Results showed significant effects of lead stress on SOD activity in roots of Pb treated plants as compared to that of untreated one ($P<0.05$). Moreover, SOD activity was increased with increasing lead concentration but there is not difference between two concentrations of Pb (1 and 2 mM). Also it was observed that tomato plants colonized with *P. indica* showed low SOD activity as compared to those of non-colonized plants, which was not statistically significant between treatments ($P> 0.05$) but was statistically significant between treatments (Fig. 2). These results showed that *P. indica*is have not significant effect on SOD activity in tomato plant roots.

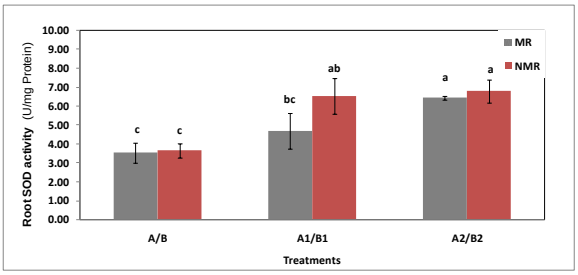

Fig. 2. SOD activity in roots of tomato exposed to lead. **A** (tomato inoculated with *P.indica*), **B** (tomato), **A1** (tomato inoculated with *P.indica* under lead stress (1 mM Pb $(NO_3)_2$, **B1** (tomato under lead stress (1 mM Pb $(NO_3)_2$), **A2** (tomato inoculated with *P.indica* under lead stress (2 mM Pb $(NO_3)_2$), **B2** (tomato under lead stress (2 mM Pb $(NO_3)_2$).**MR**: Mycorrhized tomato, **NMR**: Non Mycorrhized tomato. The means marked by different letters are significant differences according to the Duncan test ($P<0.05$).

In stems of tomato plants, highest and lowest SOD activity belong to B1 (5.747 ± 0.478) and A2 (1.189 ± 0.357) treatments respectively (Fig. 3). The results did not show significant effects of lead stress on SOD activity in stems of treatments ($P>0.05$). Moreover, SOD activity doesn't show the same trend with increasing of lead concentration between treatments. In the case of plants colonized with *P. indica* under high Pb concentration (2 mM), significance decreased activity was found for SOD as compared to non-colonized plants. These results showed that *P. indica* has no significant effect on SOD activity in roots, but Pb concentration, especially in high concentration (2 mM) has a significant effect on SOD activity in stems (Fig.3).

SOD is a metallo enzyme present in various cellular compartments, functioning at the first step of ROS generation, i.e., superoxide formation and superoxide radicals can act as a precursor to other ROS (Alscher *et al.,* 2002). SOD dismutates two superoxide radicals to H_2O_2 and oxygen, thus maintains superoxide radicals in a steady state level. In this study, increasing SOD activity at two concentrations of Pb (1 and 2 mM) compared to non-lead stress treatment (A and B) may be indicating that the oxygen scavenging function of SOD was not impaired or response of plants to this stress. Some authors suggested that increasing of SOD activity could possibly be the result of both a direct effect of heavy metal ions and an indirect effect mediated via increasing in levels of O_2 (Chongpraditnum *et al.*, 1992).

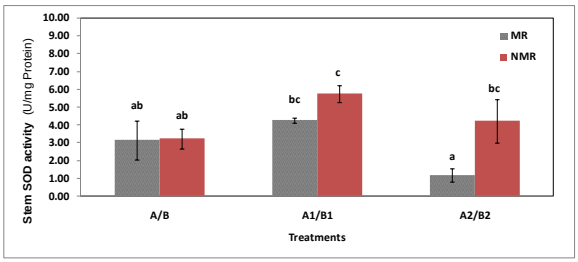

Fig. 3. SOD activity in stems of tomato exposed to lead. **A** (tomato inoculated with *P.indica*), **B** (tomato), **A1** (tomato inoculated with *P.indica* under lead stress (1 mM Pb $(NO_3)_2$), **B1** (tomato under lead stress (1 mM Pb $(NO_3)_2$), **A2** (tomato inoculated with *P.indica* under lead stress (2 mM Pb $(NO_3)_2$), **B2** (tomato under lead stress (2 mM Pb $(NO_3)_2$).**MR**: Mycorrhized tomato, **NMR**: Non Mycorrhized tomato. The means marked by different letters are significant differences according to the Duncan test ($P<0.05$).

Kumar *et al.* (2009) suggest that induction of SOD in maize root might be associated with recognition of *P. indica* and activation of the plant defense system. It has been reported that unusually strong induction of antioxidative enzymes during the colonization period results in detoxification of ROS (generated during colonization) and plays a protective role in the interaction between plants and fungi (Alguacil *et al.*,

2003). Increased activity of antioxidant enzymes minimizes the chances of oxidative burst (excessive ROS production), and therefore *P. indicia* might be protected by the oxidative defense system during colonization. Also, Induction of antioxidant enzymes observed in shoots of plants colonized with *P. indica*. These data suggest the systemic induction of antioxidative defense in the case of colonization by *P. indica* (Kumar *et al.,* 2009). Garg and Aggarwal (2012) reported that superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) increased in *Cajanuscajan* under cadmium and lead stress and Increased activities of SOD, CAT, POX as well as GR were observed in all mycorrhizal (arbuscular mycorrhizal fungi) stressed plants.

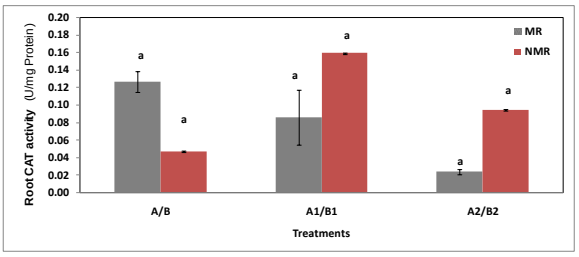

Fig. 4. CAT activity in roots of tomato exposed to lead. **A** (tomato inoculated with *P.indica*), **B** (tomato), **A1** (tomato inoculated with *P.indica* under lead stress (1 mM Pb $(NO_3)_2$), **B1** (tomato under lead stress (1 mM Pb $(NO_3)_2$), **A2** (tomato inoculated with *P.indica* under lead stress (2 mM Pb $(NO_3)_2$), **B2** (tomato under lead stress (2 mM Pb $(NO_3)_2$).**MR**: Mycorrhized tomato, **NMR**: Non Mycorrhized tomato. The means marked by different letters are significant differences according to the Duncan test (P<0.05).

Catalase (CAT) activity

Under similar conditions, the results showed that despite the changes in CAT activity in the roots have not observed significant differences between treatments (P>0.05). As seen in the Figure 4, the highest CAT activity was observed in treatment B1 (0.1589 ± 0.0677) and the lowest rate in treatment A2 (0.0233 ± 0.0030), but these differences were not significant (P>0.05). Moreover, in this research did not find a significant difference in CAT activities of root plants colonized with *P. indica* (MR) and non-colonized with *P. indica* (NMR) (Fig.3). Apparently, overall lead stress causes to increasing of CAT activity and in lead stress treatments, increasing the lead concentration led to reduced levels of CAT activity (2 mM to 1 mM) but not statistically significant to other treatments (P>0.05).

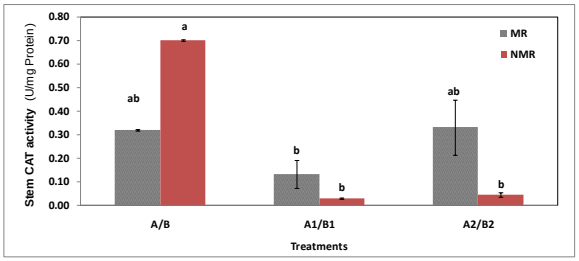

Fig. 5. CAT activity in stems of tomato exposed to lead. **A** (tomato inoculated with *P.indica*), **B** (tomato), **A1** (tomato inoculated with *P.indica* under lead stress (1 mM Pb $(NO_3)_2$), **B1** (tomato under lead stress (1 mM Pb $(NO_3)_2$), **A2** (tomato inoculated with *P.indica* under lead stress (2 mM Pb $(NO_3)_2$), **B2** (tomato under lead stress (2 mM Pb $(NO_3)_2$).**MR**: Mycorrhized tomato, **NMR**: Non Mycorrhized tomato. The means marked by different letters are significant differences according to the Duncan test (P<0.05).

In the case of stems, plants colonized with *P. indica* did not show a significant increase in CAT activity (Fig. 5), but it seems, lead stress, decreased effect on CAT activity in stems. As seen in the figure 4, the highest CAT activity was observed in treatment B (0.7013 ± 0.0021) and the lowest in B1 (0.0282 ± 0.0036), and this difference was significant (P<0.05). Moreover, in this study did not find a significant difference in CAT activities of stems plants colonized with *P. indica* (MR) and non- colonized with *P. indica* (NMR) in the treatment (Fig.5).The results showed that increasing the concentration of lead has no significant effect on CAT activity in stems.

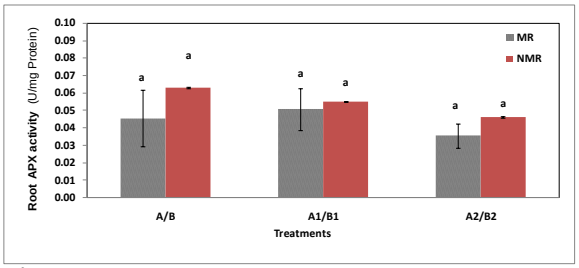

Fig. 6. APX activity in roots of tomato exposed to lead. **A** (tomato inoculated with *P.indica*), **B**

(tomato), **A1** (tomato inoculated with *P.indica* under lead stress (1 mM Pb $(NO_3)_2$), **B1** (tomato under lead stress (1 mM Pb $(NO_3)_2$), **A2** (tomato inoculated with *P.indica* under lead stress (2 mM Pb $(NO_3)_2$), **B2** (tomato under lead stress (2 mM Pb $(NO_3)_2$).**MR**: Mycorrhized tomato, **NMR**: Non Mycorrhized tomato. The means marked by different letters are significant differences according to the Duncan test (P<0.05).

CAT is a universally present oxidoreductase that decomposes H_2O_2 to water and molecular oxygen, and it is one of the key enzyme involved in the removal of toxic peroxides. The scavenging of H_2O_2 by CAT may be an efficient mechanism to attenuate the elicitation of plant defense responses, for example against heavy metal stress. In the present study, CAT activities in roots of non-inoculated tomatoes increased (but not significantly) at two Pb concentrations, while at inoculated tomatoes with lead stress decreased, however, not significant (P>0.05). Increase in CAT activity can be explained by an increase in its substrate, i.e., to maintain the level of H_2O_2 as an adaptive mechanism of the plants (Reddy *et al.*, 2005). Decline observed at higher concentration of Pb (2 mM) in roots, might be attributed to inactivation of enzyme by ROS, decrease in synthesis of enzyme, or change in the assembly of its subunits (Verma and Dubey, 2003).

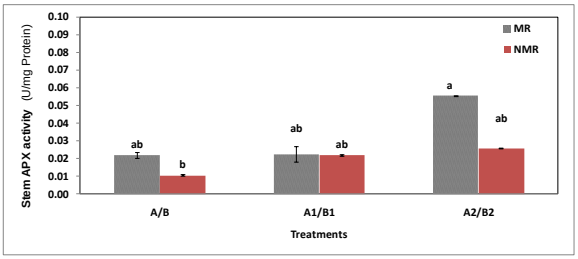

Fig. 7. APX activity in stems of tomato exposed to lead. **A** (tomato inoculated with *P.indica*), **B** (tomato), **A1** (tomato inoculated with *P.indica* under lead stress (1 mM Pb $(NO_3)_2$), **B1** (tomato under lead stress (1 mM Pb $(NO_3)_2$), **A2** (tomato inoculated with *P.indica* under lead stress (2 mM Pb $(NO_3)_2$), **B2** (tomato under lead stress (2 mM Pb $(NO_3)_2$).**MR**: Mycorrhized tomato, **NMR**: Non Mycorrhized tomato. The means marked by different letters are significant differences according to the Duncan test (P<0.05).

Ascorbate peroxidase (APX) activity

APX Activity results showed that despite the changes in APX activity in the roots and stems (Fig. 6 and Fig.7), but not observed significant difference between treatments (P>0.05). As seen in the figure 5, in roots, the highest APX activity was observed in treatment B (0.0630 ± 0.0091) and the lowest rate in treatment A2 (0.0353 ± 0.0069), and in stems, the highest APX activity was observed in treatment A2 (0.0553 ± 0.0002) and the lowest rate in treatment B (0.0105 ± 0.0003), but these differences were not significant (P>0.05). Moreover, in this research did not find a significant difference in APX activities of root or stems plants colonized with *P. indica* (MR) and non- colonized with *P. indica* (NMR) (Fig.6-7). Ekmekci *et al.* (2009) showed APX activity significantly decreased as the highly toxic Pb level (8 mM Pb $(NO_3)_2$. $4H_2O$) in the one cultivar of maize while a significant increase observed in other cultivars, therefore, some cultivar of plants are more tolerant to Pb toxicity compared to other cultivars. The results of the present study suggested that APX not involved in the plant response to lead stress.

Conclusions

Some authors suggested that all the antioxidant enzyme activities (SOD, CAT, POX, and GR) in case of mycorrhizal plants were significantly higher than those of non-mycorrhizal plants at all concentrations of fungal inoculation could improve the antioxidant enzyme systems to alleviate destructive stress. A positive role of fungi in defense mechanisms under individual metal treatments has been reported (Bowler *et al.,* 1992; Rivera-Becerril*et al.,* 2005). The results of our study showed that lead can affect the activity of antioxidant enzymes in tomato plant which most cases higher in roots compared to stems, but in this case, the impact of colonization by fungi was not significant and maybe additional researches are needed to understand effect of *P. indica* in plant protection under Pb stress.

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References

Alguacil MM, Caravaca F, Azcón R, Pera J, Díaz G, Roldán A. 2003. Improvements in soil quality and performance of mycorrhizal *Cistusalbidus* L. seedlings resulting from addition of microbially treated sugar beet residue to a degraded semiarid Mediterranean soil. Soil Use and Management **19,** 277-283. http://dx.doi.org/10.1111/j.14752743.2003.tb00316.x

Alscher RG, Erturk N, Heath LS. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Jounal of Experimental Botany **53,** 1331–1341. http://dx.doi.org/10.1093/jexbot/53.372.1331

Amatussalam A, Abubacker MN, Rajendran RB. 2011. In situ *Carica papaya* stem matrix and *Fusarium oxysporum* (NCBT-156) mediated bioremediation of chromium. Indian Journal of Experimental Biology **49 ,** 925-931.

Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry **44,** 276–287. http://dx.doi.org/10.1016/0003-2697(71)90370-8

Bohnert HJ, Nelson DE, Jensen RG. 1995. Adaptations to environmental stresses. The Plant Cell **7(7),** 1099–1111.

Borde M, Dudhane M, Jite PK. 2009. Role of bioinoculant (AM fungi) increasing in growth, flavor content and yield in *Allium sativum* L. under field condition. Notulae Botanicae Horti Agrobotanici Cluj-Napoca **37,** 124-128.

Bowler C, Van Montagu M, Inze D. 1992. Superoxide dismutase and stress tolerance. Annual Reviews Plant Physiology and Plant Molecular Biology **43,** 83–116. http://dx.doi.org/10.1146/annurev.pp.43.060192.000503

Cakmak I, Horst WJ. 1991.Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*). Physiologia Plantarum **83,** 463-469. http://dx.doi.org/10.1111/j.1399-3054.1991.tb00121.x

Chongpraditnum P, Mori S, Chino M. 1992. Excess copper induces a cytosolic Cu, Zn-superoxide dismutase in soybean root. Plant & Cell Physiology **33,** 239–244.

Clay K, Schardl CL. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. American Naturalist **160,** S99–S127. http://dx.doi.org/10.1086/342161

Devi SR, Prasad MNV. 1998. Copper toxicity in *Ceratophyllum demersum* L. (coontail), a free-floating macrophyte: response of antioxidant enzymes and antioxidants. Plant Science **138,** 157–165. http://dx.doi.org/10.1016/S0168-9452(98)00161-7

Dumas Y, Dadomo M, Di lucca G, Grolier P. 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. Journal of the Science of Food and Agriculture **83,** 369-382. http://dx.doi.org/.10.1002/jsfa.1370

Eick MJ, Peak JD , Brady PV and Pesek JD. 1999. Kinetics of lead absorption/desorption on

goethite: residence time effect. Soil Science **164,** 28–39.

Ekmekci Y, Tanyolac D, Ayhan B. 2009. A crop tolerating oxidative stress induced by excess lead: maize. Acta Physiology Plantarum **31,** 319–330. http://dx.doi.org/.10.1007/s11738-008-0238-3

El-BeltagiHossam S, Farahat Ahmed A, AlsayedAlsayed A, MahfoudNomer A. 2012. Response of Antioxidant Substances and Enzymes Activities as a Defense Mechanism against Root-Knot Nematode Infection. Notulae Botanicae Horti Agrobotanici Cluj-Napoca **40(1),** 132-142.

Garg N, Aggarwal N. 2012. Effect of mycorrhizal inoculations on heavy metal uptake and stress alleviation of *Cajanus cajan* (L.) Millsp. genotypes grown in cadmium and lead contaminated soils. Plant Growth Regulation **66,** 9–26
. http://dx.doi.org/10.1007/s10725-011-9624-8

Gil C, Boluda R, Ramos J. 2004. Determination and evaluation of cadmium lead and nickel in greenhouse soils of Almeria (Spain). Chemosphere **55,** 1027–1034.
http://dx.doi.org/10.1016/j.chemosphere.2004.01.013

Gupta DK, Nicolosoa FT, Schetingerb MRC, Rossatoa LV, Pereirab LB, Castroa GY, Srivastavac S, Tripathi RD. 2009. Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. Journal of Hazardous Materials **172,** 479–484.
http://dx.doi.org/10.1016/j.jhazmat.2009.06.141.

Kabata A, Pendias H. 1984. Trace elements in soils and plants. CRC Press, Florida, 218 p.

Kumar M, Yadav V, Tuteja N, Johri AK. 2009. Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. Microbiology **155,** 780–790.
http://dx.doi.org/10.1099/mic.0.019869-0

Luna CM, Gonzalez VS, Trippi VS. 1994. Oxidative damage caused by excess copper in oat leaves. Plant & Cell Physiology **35,** 11–15.

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant & Cell Physiology **22,** 867-880.

Peralta IE, Spooner DM, Knapp S. 2008. The Taxonomy of tomatoes: a revision of wild tomatoes (*Solanum* section *Lycopersicon*) and their outgroup relatives in sections *Juglandifolium* and *Lycopersicoides*. Systematic Botany Monographs **84,** 1-186.

Piechalak A, Tomaszewska B, Baralkiewicz D . 2003. Enhancing phytoremediative ability of *Pisum sativum* by EDTA application. Phytochemistry **64,** 1239–1251.
http://dx.doi.org/10.1016/S00319422(03)00515-6

Porras-Alfaro A, Bayman P. 2011. Hidden fungi, emergent properties: endophytes and microbiomes. Annual Review of Phytopathology **49,** 291-315. http://dx.doi.org/10.1146/annurev-phyto-080508-081831

Reddy AM, Kumar SG, Jyonthsnakumari G, Thimmanaik S, Sudhakar C. 2005. Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.). Chemosphere **60,** 97–104.
http://dx.doi.org/10.1016/j.chemosphere.2004.11.092

Rivera-Becerril F, Van Tuinen D, Martin-Laurent F, Metwally A, Dietz KJ, Gianinazzi S, Gianinazzi-Pearson V . 2005. Molecular changes in *Pisumsativum* L. roots during arbuscularmycorrhiza buffering of cadmium stress. Mycorrhiza **16,** 51–60.
http://dx.doi.org/10.1007/s00572-005-0016-7

Shahabivand S, ZareMaivan H, Mohammadi Goltapeh E, Sharifi M, Aliloo AA. 2012. The

effects of root endophyte and arbuscular mycorrhizal fungi on growth and cadmium accumulation in wheat under cadmium toxicity. Plant Physiology and Biochemistry **60,** 53-58.
http://dx.doi.org/10.1016/j.plaphy.2012.07.018

Singh LP, Gill SS, Tuteja N. 2011. Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signaling & Behaviour **6,** 175-191.
http://dx.doi.org/104161/psb.6.2.14146

Souguir D, Ferjani E, Ledoigt G, Pascale G. 2011. Sequential effects of cadmium on genotoxicity and lipoperoxidation in *Viciafaba* roots. Ecotoxicology **20,** 329–336.
http://dx.doi.org/10.1007/s10646-010-0582-0

Sun C, Johnson JM, Cai D, Sherameti I, Oelmuller R, Lou B. 2010. *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. Journal of Plant Physiology **167,** 1009-1017.
http://dx.doi.org/10.1016/j.jplph.2010.02.013

Varma A, Singh A, Sudha Sahay N, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Franken P, Hurek T,

Blechert O, Rexer K-H, Kost G, Hahn A, Hock B, Maier W, Walter M, Strack D, Kranner I. 2001. *Piriformospora indica*: a cultivable mycorrhiza-like endosymbiotic fungus. The Mycota IX **9,** 125–150.
http://dx.doi.org/10.1007/978-3-662-07334-6_8

Verma S, Dubey RS. 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science **164,** 645–655.
http://dx.doi.org/10.1016/S0168-9452(03)00022-0

Vierheilig H, Schweiger P, Brundrett M. 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. Physiologia Plantarum **125,** 393–404.
http://dx.doi.org/10.1111/j.1399-3054.2005.00564.x

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*. Ecotoxicology and Environmental Safety **78,** 28–34.
http://dx.doi.org/10.1016/j.ecoenv.2011.11.008