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Priming with L-arginine reduces oxidative damages in *Carthamus tinctorius* seedlings under the toxic levels of lead

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Abstract
Lead (Pb) stress adversely affects plant nutrient homeostasis and metabolism when present at an elevated concentrations in the surrounding media. In this research, the effects of 1mM $Pb(NO_3)_2$ on 14-day-old *Carthamus tinctorius* seedlings pretreated with arginine (Arg) as nitric oxide (NO) precursor, methylene blue (MB), a nitric oxide scavenger and N^{ω}-nitro-L-Arg-methyl ester (LNAME) and a nitric oxide biosynthetic inhibitor, were investigated in the greenhouse of the Department of Biology, Shahid Bahonar University of Kerman, Iran. Pb exposure caused oxidative stress, reduced root and shoot growth and elevated malondialdehyde (MDA) content of the seedlings. Pb stress also increased the ascorbate peroxidase activity while decreasing the activity of the catalase (CAT) enzyme. Arg pretreatment decreased the harmful effects of Pb stress by increasing the root and shoot length and reducing the MDA content. Additionally, Pb-induced reduction of CAT enzyme activity in roots was reversed by the Arg pretreatment of the plants. In many characteristics which we measured, the effects of Arg pretreatment on alleviation of Pb-induced oxidative stress were reversed by LNAME and methylene blue pretreatments. Therefore, it seems that Arg induces a positive effect through NO production. Data showed that in the presence of Arg, the uptake and translocation of Pb declined and the application of Arg with LNAME or MB reversed these positive effects of Arg. It seems that Arg can alleviate lead toxicity in plants through the prevention of Pb uptake and promoting the direct scavenging of reactive oxygen species or activating antioxidant enzymes. Also, results from the use of LNAME and MB indicated that the positive effect of Arg is probably related to its role in NO production.

Keywords: Antioxidant enzymes; Heavy metals; Methylene blue; Nitric oxide; Safflower; Translocation Factor

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Introduction

Lead (Pb) is one of the most widespread heavy metals (HM) which is toxic to plants, animals and humans (Wang *et al.* 2013). Toxic levels of Pb have shown harmful effects on the crop biomass and yield (Kobylinska *et al.* 2017). Heavy metal concentrations in the soil are usually very low, but using phosphate fertilizer for a long period can result in hazardously high levels of these pollutants even though it is also used for remediation of Pb toxicity (Sonmez and Pierzynski 2005; Yan *et al.* 2015).

Lead does not have any biological function in plants and is very toxic even at low concentrations. Lead is released into the environment by industrial human activity (Yoon *et al.* 2006). Lead can cause a broad range of negative symptoms in plants. For example, it can inhibit photosynthesis, antioxidant system and root elongation, and delay growth (Milone *et al.* 2003; Bandehagh 2013). A high level of Pb affects a widespread of different metabolic pathway enzymes and can induce oxidative stress

via ROS generation (Shahid *et al*. 2014; Javed *et al*. 2018; Zanganeh and Rashid Jamei 2020).

Amino acids have a specific role in the responses of plants to various stresses. The particular role of some amino acids in the responses of plants to heavy-metal stress has been reported (Deng *et al*. 2010; Muranaka *et al*. 2013; Sun *et al*. 2014; Colak *et al*. 2019). After uptake of the HM, plants detoxify them by the low amounts of some metabolites including amino acids (proline, histidine), peptides (phytochelatins, glutathione) and amines (spermine, spermidine, putrescine, nicotianamine) (Sharma and Dietz 2006). Trace metals bound with amino acids, especially those that are rich in carboxyl, amino, thiol and phenolic groups. These compounds are involved in the synthesis of glutathione and phytochelatin, which can form complex metal cations decreasing their reactivity with other molecules at the cellular level and serving as long-distance metal-chelating compounds (Zhu *et al*. 2018).

Among the amino acids, arginine is one of the most functionally diverse modulators in a variety of physiological and developmental processes in plants (Chen *et al*. 2004). One of the major storage and transport forms of nitrogen in plants is arginine. It plays an important role in protein synthesis and is known as a precursor for polyamines and nitric oxide (NO) (Liu *et al*. 2006). Nitric oxide is regarded as a signaling molecule in the plant stress response. NO is an important secondary messenger produced in the cell under normal and stress conditions. Arginine hydrolyzes to NO and citrulline by nitric oxide synthase (NOS), while final production of arginase and arginine decarboxylase are mostly polyamines and proline (Chen *et al*. 2004). In plants, the activity of NOS depends on Arginine and NOS inhibitors such as N-nitro-L-arginine methyl ester (LNAME) or N-nitro-L-arginine (LNNA) (Cueto *et al*. 1996; Durner and Klessig 1999; Corpas *et al*. 2004). However, compared with other stress conditions, our knowledge regarding the molecular and physiological mechanism of NO in alleviating the heavy-metal toxicity is not sufficient and needs further research (Xiong *et al*. 2010). There is some information regarding the functional role of endogenously-produced NO in the plants challenged with HM (Arasimowicz-Jelonek *et al*. 2011). There is some evidence about the role of sodium nitroprusside (SNP) as a NO donor in reducing heavy metal toxicity in plants by preventing oxidative stress development. For example, pretreatment with SNP ameliorated toxic effects of Cd on yellow lupins (Kopyra and Gwozdz 2003), rice (Hsu and Kao 2004), sunflower (Laspina *et al*. 2005), soybean (Kopyra *et al*. 2006) and wheat (Singh *et al*. 2008). Under the Cu stress conditions, using NO exogenously increased the anti-oxidative capacity of wheat (Hu *et al*. 2007) and scavenged the excess ROS in rice (Yu *et al*. 2005). Also, the protective effect of SNP on *Arabidopsis thaliana* seedlings exposed to the toxic levels of Pb (Phang *et al*. 2011) and alleviating the Pb-inhibitory effects on seed germination root growth of *Lupinus luteu*s (Kopyra and Gwozdz 2003) and seed germination and shoot growth of wheat (Yang *et al*. 2010) has been reported. Although in recent researches SNP has been used as the NO donor to counteract the

negative effect of HM, no information is available on the role of exogenous arginine as the precursor of NO in the antioxidative responses of the plants under heavy metal stresses. The protective effects of arginine in plants have been recorded in previous studies. For example, Zhang *et al.* (2013) indicated that the treatment with exogenous arginine could reduce the chilling damage of the tomato fruit during cold storage. Our previous researches also showed that the pretreatment of plants with arginine could reduce the negative effects of drought (Nasibi *et al.* 2011), nickel (Nasibi *et al.* 2013), cold (Nasibi *et al.* 2013) and salinity (Nejadalimoradi *et al.* 2014) stress.

Safflower (*Carthamus tinctorius* L.) is an annual crop with a wide geographical distribution. It has long been used for coloring and flavoring food. The edible oil of safflower can be used for industrial purposes, the preparation of drugs and also cosmetics production. Safflower possesses interesting characteristics in terms of heavy metal accumulation. Thus, in this study, we investigated the effect of arginine pretreatment on oxidative parameters of *Carthamus tinctorius* under lead toxicity. Also, LNAME was applied with arginine as a nitric oxide synthase inhibitor in some treatments. Because NO can be produced in plants by the non-enzymatic system such as nitrate reductase enzyme, methylene blue (MB) was also used as a nitric oxide scavenger. Comparing these responses can be useful in understanding the physiological and biochemical mechanisms of arginine in plants to cope with the Pb stress.

Materials and Methods

Safflower *(Carthamus tinctorius* L cv. Sina) seeds were primed with the deionized water (as the control), 10 µM arginine with 10 µM MB or without MB and 10 µM arginine + 20 µM LNAME for 24h (four treatments). After priming of the seeds with these solutions, seeds were germinated in pots (10-cm diameter) containing perlite and maintained under the conditions of 16 h photoperiod, the humidity of 40%, day/night temperature of 25/16 °C in the greenhouse of the Department of Biology, Shahid Bahonar University of Kerman, Iran. The experiment was conducted as the completely randomized design with 10 replications per treatment (each pot with 10 seedlings was considered as one experimental unit). Pots were watered daily with 15 ml half-strength Hoagland solution for seven days. Then, the pots for each treatment were divided into two groups. One group received the Hoagland solution (as the control) and the other group was irrigated with the Hoagland solution added with 1mM Pb $(NO_3)_2$ for seven days. To prevent the precipitation of lead phosphate, KH_2PO_4 was removed from the solution in the control and all Pb treatments. The shoot and roots of 14-day-old seedlings were harvested and stored at -20 °C until further analyses.

Plant growth: Shoot and root length of 14-day-old seedlings were measured as the growth characters.

Lipid peroxidation: The lipid peroxidation was measured as described by Heath and Packer (1968), using thiobarbituric acid reactive

substances (TBARS). Fresh root and shoot samples were ground in 0.25% thiobarbituric acid (TBA) in 10% TCA, using mortar and pestle. The mixture was heated at 95 °C for 30 min, then cooled in an ice bath and centrifuged at 10000×g for 10 min. The absorbance of the supernatant was read at 532 nm while a total of 0.25% TBA in 10% TCA served as the blank. The concentration of lipid peroxides together with the oxidative-modified proteins of plants was quantified and expressed as total TBARS as nmol g^{-1} fresh weight, using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

The fresh seedlings (0.5 g) were ground in 5 ml of 0.1 % (m/v) TCA. The mixture was centrifuged at 10000 *g* for 20 min. One ml of the supernatant was mixed with 4 ml of 0.5% TBA dissolved in 10% TCA. This mixture was placed at 95 °C for 30 minutes and then cooled in an ice bath. After centrifugation at 10000 *g* for 15 min, the absorbance of the supernatant was recorded at 532 and 600 nm. The concentration of lipid peroxides together with the oxidative-modified proteins of plants was quantified and expressed as total TBARS as nmol g^{-1} fresh weight, using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Antioxidative enzyme activity: For the determination of enzyme activity, 1 g of fresh seedlings was homogenized in 3 ml of 25 mM sodium phosphate buffer (pH= 6.8) with 3% polyvinylpyrrolidone. The homogenate was centrifuged at 13000 *g* for 1 h and the supernatant was used for the protein determination and enzyme assays. All steps were carried out at 4 °C.

The ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured immediately in the fresh extracts according to the method of Nakano and Asada (1981). The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH=7.0), 0.1 mM H2O2, 0.5 mM ascorbate, 0.1 mM EDTA and 150 µl fresh extract. APX activity was estimated by a decrease in the absorbance at 290 nm (extinction coefficient of 2.8 mM^{-1} cm^{-1}). One unit of the enzyme was the amount necessary to decompose 1 µmol of substrate/min at 25 ºC.

The glutathione peroxidase (GPX; EC 1.11.1.7) activity was measured using the method of Plewa *et al.* (1999). In the homogenates, the increase in the absorption at 470 nm due to the formation of tetraguaiacol was recorded (using the extinction coefficient of 26.6 mM^{-1} cm^{-1}). The reaction mixture contained 20 µl of the plant extract, 50 mM buffer K-phosphate with pH= 7, 0.1 mM EDTA, 10 mM guaiacol and 10 mM H_2O_2 (Plewa *et al.* 1999). The results were expressed as µmol H2O2 min^{-1} g^{-1} dry matter

The catalase (CAT; EC 1.11.1.6) activity was determined by monitoring the initial rate of H_2O_2 disappearance. The concentration of H_2O_2 was measured at 240 nm using the extinction coefficient of 40 mM^{-1} cm^{-1} for H_2O_2 (Velikova *et al.* 2000).

The total protein content of the extracts was determined according to the spectrophotometric method of Bradford (1976), using BSA as the standard.

Pb concentration of the leaves and roots: The plants were collected at the end of the experiment. Dried samples were weighed to obtain the biomass. Digestion of the powdered plant material was carried out in a microwave oven by HNO3 solution at 140 °C (SpektrAA 300, Varian and Mulgrave, Australia)

Translocation factor: The Pb translocation from the roots to the shoots was measured by TF, which is given as below:

TF= C_{shoot}/C_{root} , where C_{shoot} and C_{root} are the metal concentration in the shoots (µg g^{-1}) and roots (µg g^{-1}), respectively. TF>1 indicates the translocation of metals from the roots to the shoots (Zhang *et al.* 2002; Fayiga and Ma 2006).

Statistical analyses: Data were subjected to analysis of variance and significant differences among means were determined by Duncan's multiple range test ($p \leq 0.05$). Data were analyzed by the SPSS (version 12) software.

Results

Effects of Arg pretreatment on Plant growth

Treatments with 1mM of the lead cause a significant decrease in the length of root and shoot in seedlings of *Carthamus tinctorius*. However, Arg-pretreated seedlings had longer roots than the control seedlings. In the Pb-exposed plants which were pretreated with Arg and methylene blue, the protective effect of Arg was reduced. In addition, in plants that were pretreated with Arg+LNAME the positive effect of Arg decreased significantly on the Pb-exposed plants; however, the root length of these plants increased in comparison with the non-pretreated plants. Pb treatment caused a decrease in shoot growth compared with the control. Arg pretreatment increased the shoot growth of *Carthamus tinctorius* seedlings both in the control and Pb-treated plants in comparison with un-pretreated plants. Use of Arg with either MB or LNAME reduced the protective effect of Arg in the un-pretreated plants but had no significant effects on the plants which were under the toxic level of Pb (Figure 1).

Effects of Arg pretreatment on lipid peroxidation and MDA content

Malondialdehyde (MDA) content was measured as a lipid peroxidation index. Treatment of plants with Pb increased the MDA content in roots and shoots of the plants (Figure 2). However, in all cases levels of lipid peroxidation in shoots were lower than in the roots. Pretreatment of the plants with Arg decreased the lipid peroxidation significantly in the root and shoot of the plants which were under Pb stress. Application of MB with Arg decreased the positive effect of Arg in the reduction of lipid peroxidation in plants under Pb stress. In those plants which were pretreated with LNAME, also the peroxidation of lipid increased under the stress condition in comparison with Arg- pretreated plants.

Effects of Arg pretreatment on antioxidant enzyme activity

Pb treatment did not affect the GPX activity in the shoots and roots. Pretreatment of the plants with Arg increased GPX activity in the shoots but decreased it in the roots of the plants under Pb stress compared to control plants (Figure 3).

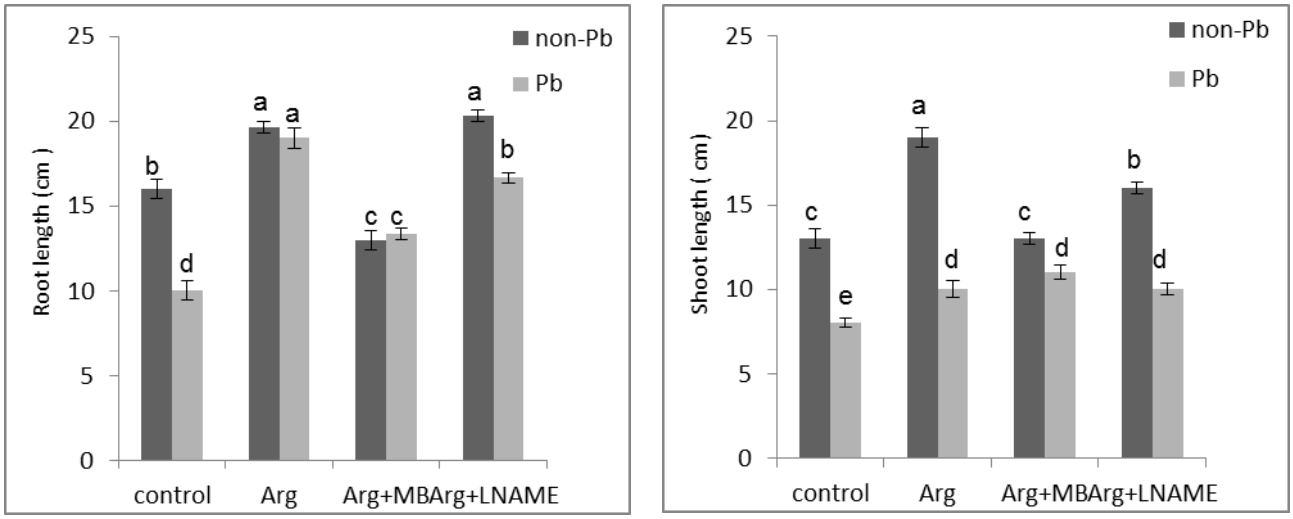

Figure 1. Shoot and root length of 14-day seedlings of *Carthamus tinctorius* after seven days of the Pb stress. Each value represents the mean of 10 replicates. Bars indicate the standard errors. Different letters over the bars indicate significant differences ($p \leq 0.05$).

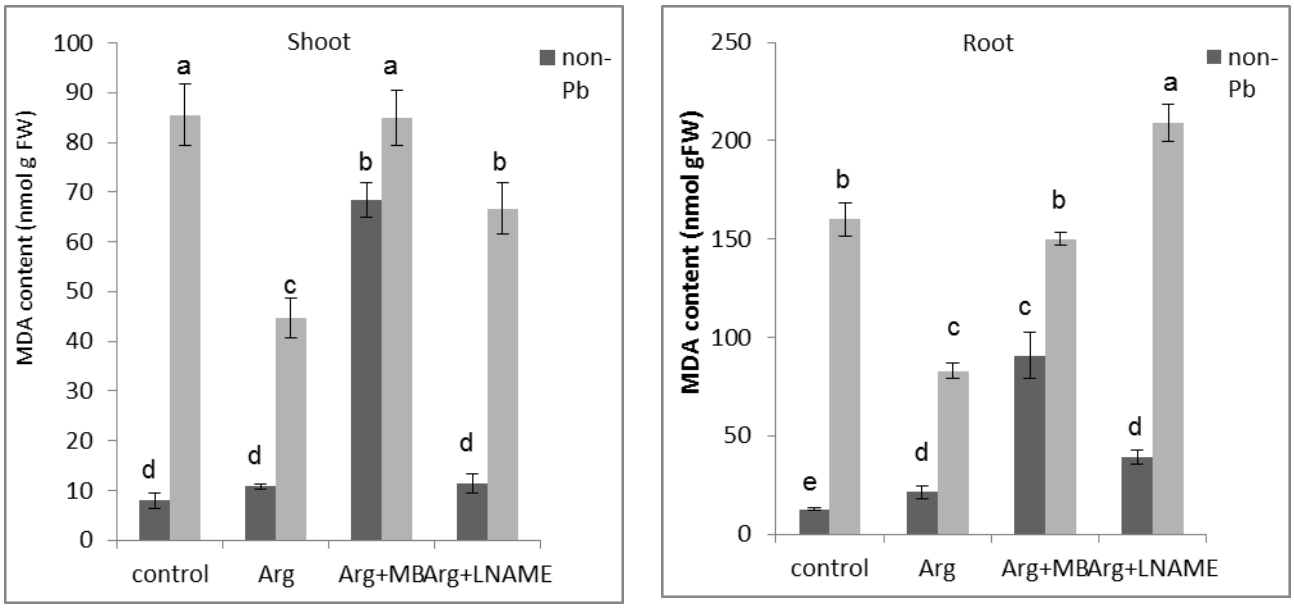

Figure 2. Malondealdehyde content of the root and shoots in 14-day seedlings of *Carthamus tinctorius* after seven days of the Pb stress. Each value represents the mean of 10 replicates. Bars indicate the standard errors. Different letters over the bars indicate significant differences ($p \leq 0.05$).

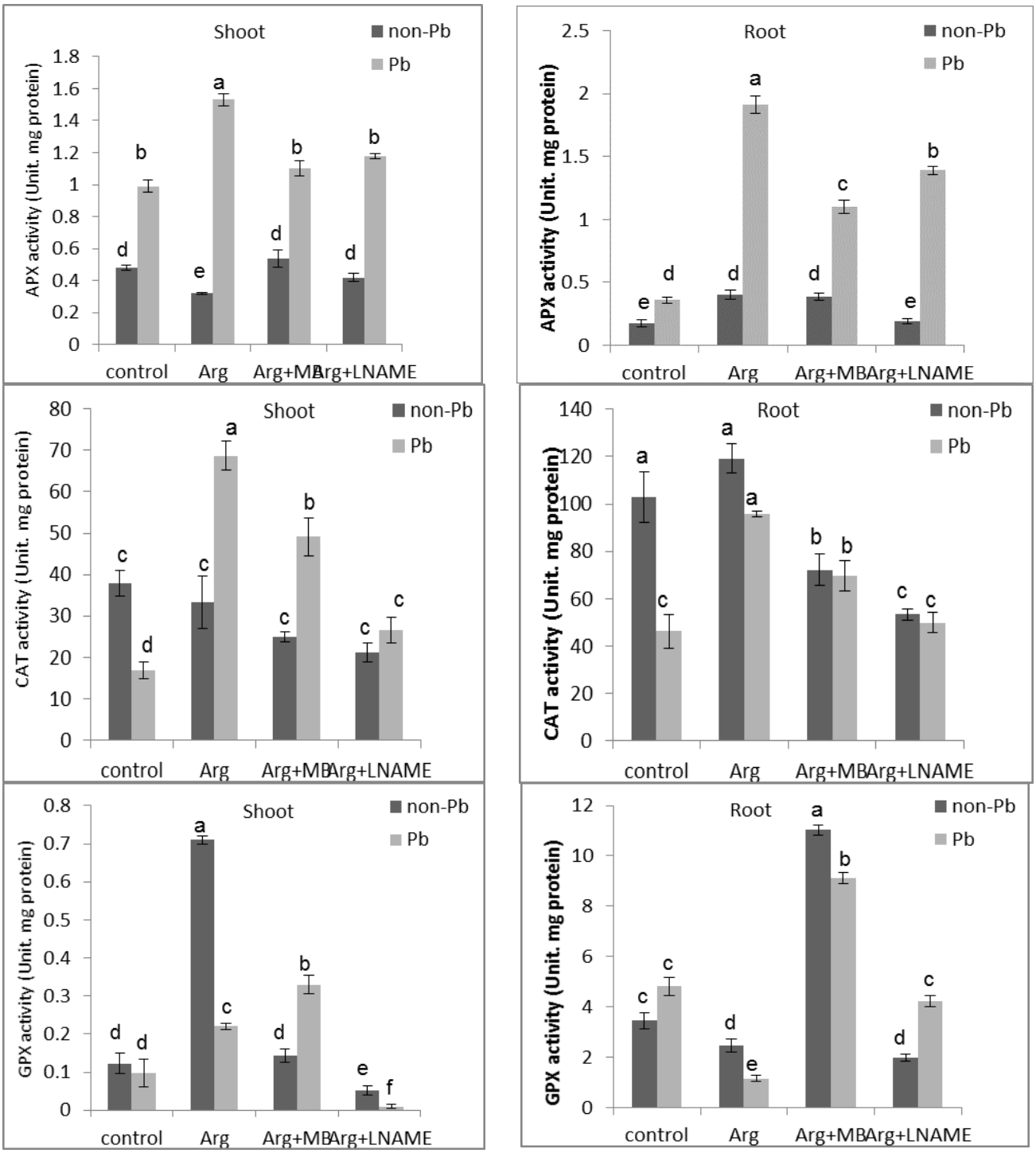

Figure 3. Changes in the protein (a) and activity of CAT (b), APX(c) and GPX (d) enzymes in the 14-day seedlings of *Carthamus tinctorius* after seven days of the Pb stress. Each value represents the mean of 10 replicates. Bars indicate the standard errors. Different letters over the bars indicate significant differences ($p \leq 0.05$).

Pretreatments that contained Arg+MB, increased the activity of GPX in the roots and shoots, compared to the control. Application of LNAME with Arg as pretreatment, declined the GPX activity in the shoots while it did not change this enzyme in the roots under Pb stress.

APX activity increased in the shoot and roots of the plants which were exposed to Pb stress. Arg pretreatment of the plants increased the APX activity in the Pb-stressed plants. When Arg was used with either MB or LNAME the effect of Arg in the activity of APX reduced in the roots and shoots of the plants in the Pb-treated plants.

CAT activity decreased in the roots and shoots of the Pb-stressed plants and Arg application increased the enzyme activity in these organs (Figure 3). Application of Arg with either MB or LNAME decreased the CAT activity in the Pb-stressed plants.

Effects of Arg pretreatment on the Pb content and translocation factor

Pb content in the roots was at least three times higher than those of the shoots (Figure 4). Arg pretreatment decreased the Pb content in the roots and shoots of the plants compared to the control plants. When Arg was applied with either MB or LNAME, the concentration of Pb increased in both roots and shoots when compared with the plants which were treated with Arg. The higher concentration of Pb was measured in the root tissue of the plants which were pretreated with Arg+MB (Figure 4).

The calculation of the TF factor showed that in all treatments the TF factor was lower than one (<1), however, pretreatment of the plants with Arg decreased the TF factor, and application of Arg+MB and Arg +LNAME increased this factor as compared to the Arg-treated plants. The lowest TF factor was observed in plants that were pretreated with Arg (Figure 4).

Discussion

In this research, the effects of Arg were reversed by methylene blue, as a NO scavenger, which assumed that NO had the role in the protection of root growth under Pb stress conditions. Root growth is more susceptible to HM than shoot growth (Titov *et al.* 1996; Seregin and Ivanov 1997; Obroucheva *et al.* 1998). In wheat, the NO donor of sodium nitroprusside could reduce the Pb-preventing effects on seed germination and shoot growth, which was blocked by guanylyl cyclase inhibitor methylene blue (Yang *et al.* 2010).

Pagnussat *et al.* 2002 described NO as an inductor of root morphogenesis, suggesting the role of nitric oxide in the auxin-signaling pathway. Gouvea *et al.* 1997 had a similar assumption because they observed a root tip elongation induced by the NO-releasing compounds. Kolbert *et al*. 2016 also reported that in the Arabidopsis plant, lead stress resulted in overproduction of NO and enhanced the primary and lateral roots. A similar explanation about NO function may also be possible in this study. An increase in root growth of the plants which were pretreated with Arg+LNEME as compared to either un-pretreated plants or Arg+MB treated plants, suggests that NO may be provided from

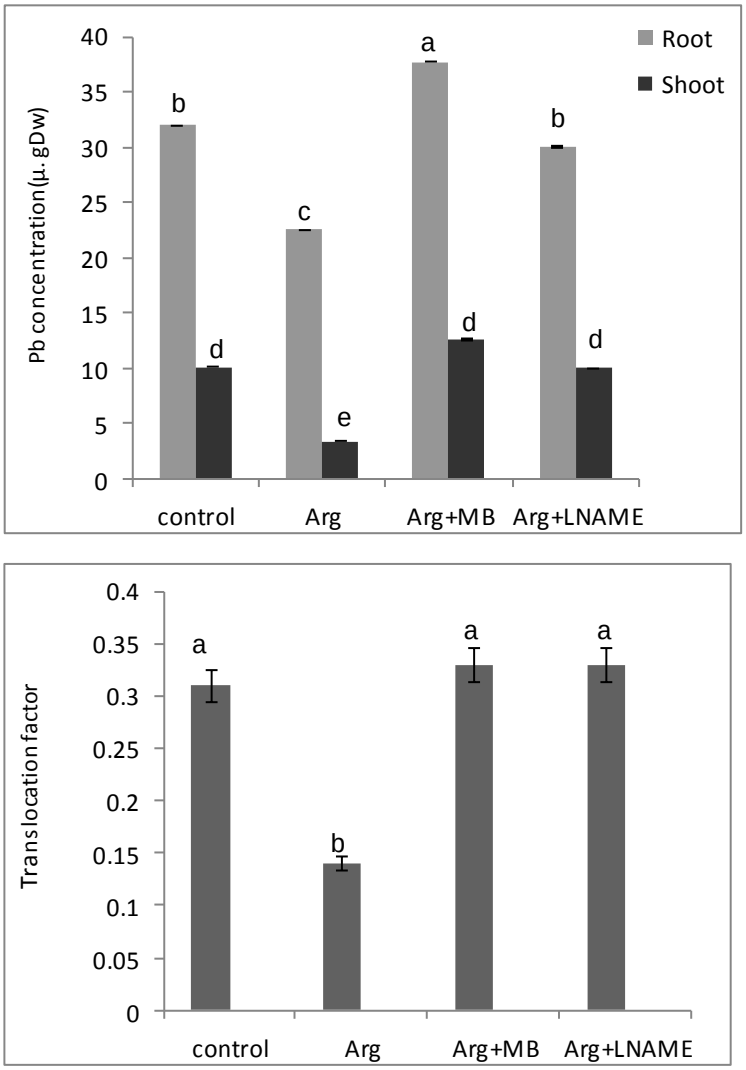

Figure 4. Pb concentration and translocation factor (TF) in 14-day seedlings of *Carthamus tinctorius* after seven days of the Pb stress. Each value represents the mean of 10 replicates. Bars indicate the standard errors. Different letters over the bars indicate significant differences ($p \leq 0.05$).

other pathways such as polyamine dependent NO biosynthesis or NR pathway in these plants.

MDA was measured as an index of Pb-induced ROS formation. MDA level was high in the Pb-stressed plants, and Arg pretreatment somehow inhibited this effect in the roots and shoots of the Pb-treated plants. This effect reversed or decreased when Arg was applied with methylene blue or LNAME, which suggests that NO may act as a good scavenger of ROS and/or a stabilizer of the membrane. It has been proven that Pb induces oxidative stress in plants. Under normal conditions, the total amount of ROS formed in the plant is determined by the balance between the multiple ROS-producing pathways and the ability of the enzymatic and non-enzymatic mechanisms to deal with them. Under stress, ROS formation is high, and this could result in oxidative damage, depending on certain enzyme activities and soluble antioxidants contents. As is shown in

Figure 3, plants have evolved a complex antioxidant enzymes system to avoid the harmful effects of ROS in the shoots and roots. It was observed that arginine pretreatment prevented Pb-induced increment in the GPX activity in the roots, but this effect was reversed in the pretreatments with methylene blue and LNAME that indicate NO could be acting directly as an antioxidant. In this study, Pb treatment caused a decline in the CAT activity in the safflower roots and shoots. However, Arg pretreatment to some extent reversed this reduction. This effect of Arg may be related to the NO production because when Arg was used with MB or LNAME, the effects of Arg on CAT activity decreased.

APX activity increased in the shoots and roots of the the Pb-stressed plants. In roots of the plants which were pretreated with Arg+MB and Arg+LNAME and then exposed to lead, it was seen a significant increase in the activity of APX, and in this case, it may be NO itself acts as a ROS scavenger or as an antioxidant agent against ROS. Decrease in NO levels in these treatments due to the application of NO scavenger (methylene blue) and NO biosynthesis enzyme inhibitor (LNEME) may increase the level of ROS. Increase in MDA content, with the rise in APX and GPX activities, has shown the oxidative damage at the cellular level in methylene blue and LNAME pretreatments and confirms the role of NO produced from Arg. Several reports have demonstrated that heavy metal stress has positive and negative changes in the NO content. For example, some researches showed that NO levels increased in the plants which were subjected to Pb and other heavy metal stress, although it varies with the condition, specificity and type of the metal (Corpas and Barroso 2015; Kolbert 2016; Okant and Cengiz 2019). While in other researches, reduction in the endogenous NO was reported (Xu *et al.* 2010). Therefore, from this point of view, NO was regarded as an alternative method for reducing or balancing the adverse effects of various environmental stresses (Corpas and Barroso 2015). Interestingly, some researches have showed that the application of NO may alleviate the adverse effects of Pb or other heavy metal stress (Phang *et al.* 2011; Zafari *et al.* 2016; Okant and Kaya 2019; Syed Nabi *et al.* 2019). Similarly, Wang *et al.* (2013) reported that SNP usage significantly decreased Cd-induced oxidative stress and lipid peroxidation and assumed that this happened either because of the direct scavenging of ROS or by elicitation of antioxidative enzyme activities.

The present result confirm the previous research which were shown that NO may be generated from other pathways such as polyamines (Tun *et al.* 2006) and NR (Rockel *et al*. 2002), because, in almost all of parameters which were measured in this experiment, methylene blue accelerated the adverse effects of Pb stress in comparison with LNAME. Also, the protective role of NO may be through its direct function as an antioxidant as well as a second messenger in signaling pathways and induced the antioxidant enzyme activity.

Two mechanisms by which NO might quench stress has been assumed. First, NO might action as an antioxidant by directly scavenging ROS, such as superoxide radicals, to make peroxy ($ONOO^-$), which is extremely less toxic than

peroxides and thus limit cellular destruction. Second, NO could react as a signaling molecule in the cascade of events leading to changes of gene expression (Mata and Lamattina 2003).

Conclusions

The data of this research recommended the positive effect of NO in the alleviation of heavy metal, especially the Pb stress. Although the heavy metal tolerance of this plant have been previously studied, to our knowledge, there is no study dealing with the protective role of exogenous arginine against the Pb-induced oxidative stress. The antioxidant role of arginine in the protection of the plant under lead stress which was reported here for the first time and since its practical effect is similar to SNP (NO-releasing compound), it could be suggested as an alternative to SNP. Arginine pretreatment, similar to some amino acids, may permit a relative growth status to be maintained in the soil subjected to the environmental HM contamination such as Pb. Considering the environmental anxieties over SNP due to releasing cyanide compounds in the environment, arginine could be used as an environmentally-friendly compound. So the application of Arg instead of SNP, as an inexpensive chemical and less toxic to the ecosystem, on agricultural farmlands contaminated with HMs, may be beneficial in terms of increasing agricultural yield and safe food production.

Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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استفاده از ال-آرژنین به منظور کاهش آسیب‌های اکسیداتیو در گیاهچه‌های گلرنگ تحت سطوح سمی سرب

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چکیده

تنش سرب هنگامی که با غلظت بالا در محیط اطراف وجود دارد، بر هموستاز و متابولیسم مواد مغذی گیاه تأثیر منفی می‌گذارد. در این تحقیق اثر تیمار نیترات سرب با غلظت ۱ میلی مولار بر گیاهچه‌های ۱۴ روزه گلرنگ، پیش تیمار شده با آرژنین به عنوان پیش ساز نیتریک اکساید، متیلن بلو (MB)، جاروبگر نیتریک اکساید، و ان- نیترو- ال آرژنین متیل استر (LNAME)، ممانعت کننده بیوسنتز نیتریک اکساید، در گلخانه گروه زیست شناسی دانشگاه شهید باهنر کرمان مورد مطالعه قرار گرفت. تیمار سرب موجب ایجاد تنش اکسیداتیو، کاهش رشد ریشه و ساقه و افزایش محتوای مالون دی‌آلدئید (MDA) در گیاهچه‌ها شد. تنش سرب همچنین فعالیت آنزیم آسکوربات پراکسیداز را افزایش و فعالیت آنزیم کاتالاز (CAT) را کاهش داد. پیش تیمار آرژنین با افزایش طول ریشه و ساقه و کاهش محتوای MDA موجب کاهش اثرات زیانبار تنش سرب شد. علاوه بر این، پیش تیمار آرژنین اثر سرب بر فعالیت آنزیم CAT در ریشه گیاهان را بهبود بخشید. در بسیاری از متغیرهای مورد اندازه‌گیری، MB و LNAME اثر تعدیلی پیش تیمار آرژنین را خنثی کردند. بنابراین، به نظر می‌رسد آرژنین از طریق تولید نیتزیک اکساید موجب بروز اثرات مثبت در گیاهچه‌ها شده است. تجزیه داده‌ها نشان داد که در حضور آرژنین، جذب و انتقال سرب کاهش یافته و کاربرد آرژنین با LNAME یا MB این اثرات مثبت آرژنین را خنثی می کند. به نظر می‌رسد که استفاده از آرژنین می‌تواند از طریق جلوگیری از جذب سرب و مهار مستقیم گونه‌های فعال اکسیژن یا فعال سازی آنزیم‌های آنتی اکسیدان، سمیت سرب را کاهش دهد. از طرف دیگر نتایج مربوط به استفاده از LNAME و MB نشان داد که اثر مثبت آرژنین احتمالا به دلیل توانایی آرژنین در تولید نیتریک اکساید است.

واژه‌های کلیدی: آنزیم‌های آنتی اکسیدان؛ عامل ترانسلوکاسیون؛ فلزات سنگین؛ نیتریک اکساید