Effect of different concentrations of Zinc and their interaction with Sodium nitroprusside (SNP) on physiological and biochemical parameters of *Plantago major L.*

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Abstract

Zinc is a necessary micronutrient in plants whose deficiency can alter essential functions in plant metabolism. High concentrations of Zn can be potentially toxic to plants causing phytotoxicity by the formation of reactive oxygen species. On the other hand, sodium nitroprusside (SNP), a donor of nitric oxide (NO) can protect cells from oxidative damage produce by reactive oxygen species. In this study, we examined the effect of different concentrations of Zn (0, 50, 100, 300, and 500 µM) on growth and physiological parameters of *Plantago major L*. under various concentrations of Sodium nitroprusside (0, 50, 100, and 200 µM). The results showed that Zn treatment decreased fresh and dry weight and increased the contents of malondealdehyde, antioxidant, and osmolyte. The starch content on the other hand decreased. Moreover, application of different concentrations of sodium nitroprusside (especially 100 µM) as a donor of nitric oxide, had a favorable effect as it improved the heavy metal stress through increasing the plant's tolerance against zinc toxicity. However, high concentration of Sodium nitroprusside had a deterrence effect on morphology and physiology of *Plantago. major L*.

Keywords: antioxidant, osmolyte, *Plantago major L*, heavy metal stress, sodium nitroprusside, zinc

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Introduction

Heavy metals are environmental pollutants found all over the industrial communities (Lasat et al., 2002). The main problem with these elements is that unlike biological pollutants, they are not decomposable and this makes them one of the most hazardous groups of environmental pollutants (Kabata-Pendias, 2001). While at low concentrations, Zn is considered as a necessary micronutrient for plants, high concentrations of this heavy metal can damage plants. High concentration of Zn affects mitotic activities, photosynthesis, fluidity and permeability of the cell wall and even can destroy the cells (Rout and Das, 2003). These effects may be due to the reduction in necessary elements such as Fe, Cu, and Mn (Ebbs and Kochian, 1997),

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oxidative damage in cell walls (Cuypers et al., 2001), and interference with photosynthesis performance (Vangronveld and Clijsters, 1997).

Also, Zn toxicity can induce production of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. Through peroxidation of lipids and therefore destroying the cell wall (Beligni and Lamatina, 1999), destroying proteins (Youssefian et al., 2001), inactivating the enzymes, and interference with the performance of DNA (Tian and Li, 2006), these radicals induce secondary stress in plants which results in serious damages in the cellular structures of the plants. Lin et al. (2012) reported that Zn ions at high concentrations because of producing too much ROS induce oxidative damage in plants.

Sodium nitroprusside (SNP) is a compound that releases nitric oxide and as a solution is highly sensitive to the light and its decomposition is speeded up with oxygen and high temperature (Wieczorek et al., 2006). This free radical controls various physiological processes such as cell division, wood differentiation, chlorophyll biosynthesis, and regulation of photosynthesis, induction of seed germination, reducing seed dormancy and generation of organs and root growth, lignification of cell walls, and planned demise of the cells (Siddiqui et al., 2011). It is also suggested that nitric oxide increases plant tolerance to heavy metals (Kumari et al., 2010).

Hsu and Kao (2004) reported the direct effect of SNP on rice leaves under Cadmium stress noting that reduction in malondealdehyde contents after application of NSP is because of the reduced ROS in plant organs. Kumari et al. (2010) found that application of SNP as a donor of nitric oxide improves chickpea plant growth under Cadmium stress and reduces Cadmium levels in the plant organs.

Plntago major L. belonging to Plantaginaceae is a medicinal plant with important compounds such as phenolic compounds (caffeic acid derivatives), flavonoids, alkaloids, terpenoids, and vitamin C. The plant contains high concentrations of phenol which may explain their high antioxidant capabilities (Samuelsen, 2000).

The harmful effects of high concentrations of Zn on plants' physiological processes and growth, and extensive application of nitric oxide as an effective antioxidant was the focus of the present study which aimed at evaluating the effects of nitric oxide on inducing tolerance against toxicity in *Plntago major L.*

Materials and Methods

Plntago major L. seeds obtained from Pakan Bazr Co. in Isfehan, Iran were sterilized with hypochlorite sodium 10% for 10 minutes before they were washed several times with distilled water. The sterilized seeds were sown in pots containing perlite and irrigated with distilled water and after germination they were nourished at appropriate intervals with Hoagland solution until the three-leaf stage. The plants were then treated with ZnSO4 at 0 (control), 100, 300, and 500 mM concentrations, and sodium nitroprusside as a donor of nitic oxide at 0 (control), 50, 100, 200 mM along with Hoagland solution.

After the period of applying stress, the plantlets were removed from the pots, roots and shoots were separated, and fresh and dry weights of the samples after drying them in an oven set at 70° C were measured.

Lipid peroxidation assays

Peroxidation of cell wall lipids' assay was carried out using thiobarbituric acid test (TBAT) and measuring malondealdehyde content. Fresh leaf and root tissues (0.2 g) were homogenized in 5 mL trichloroacetic acid (TCA) 0.1% and then the resulting solution was centrifuged at 6000 g for 5 mins. Four mL trichloroacetic acid 20% containing 0.5% thiobarbituric acid were added to 1 mL supernatant solution. The solution was incubated in hot water bath (95° C) for 30 mins and then immediately cooled in ice bath before it was centrifuged at 6000 g for 10 mins. The absorption of supernatant was recorded at 532 nm and nonspecific absorption at 600 nm was subtracted from it. Concentration of malondealdehyde was calculated using the correction factor 0.155 ($\mu mol^{-1} cm^{-1}$) and recorded based on $\mu mol^{-1} cm^{-1}$ (Health and Packer, 1968).

Table 1
Analysis of variance of the effects of Zn, SNP, and interaction of effects of these treatments on total fresh and dry weights, malondealdehyde, proline, and soluble sugars of the roots and leaves of *Plantago major L*.

	df	Leaf Soluble Sugars	Root Soluble Sugars	Leaf Proline	Root Proline	Root MDA	Leaf MDA	Total Dry Weight	Total fresh Weight
Zn	4	۱۰۳۲**	۱۶۴۶**	۱۸.۸**	۲۲.۲**	۶۹۹.۵**	۲۴۳.۸**	۰.۱۱**	۰.۸۴۴**
SNP	3	۱۶۰**	۲۵/۸۵**	۰.۸۷**	۱.۴۲**	۷۷.۸**	۷۷.۶**	۰.۰۳۱**	۰.۰۵۶**
Zn*SNP	12	۹۱**	۴۸/۳**	۰.۴۵**	۰.۳**	۳.۱۴**	۴.۱۳**	۰.۰۰۱**	۰.۰۰۵**
Error		۰.۸۸۷	۰.۵۷۸	۰.۰۰۷	۰.۰۰۵	۰.۰۶۱۸	۰.۱۵۲۸	۰.۰۰۰۲	۰.۰۰۰۵
CV		۲.۱	۱.۶	۱.۳	۱.۴	۰.۹	۱.۲	۲.۳	۱.۰۷

**: significant at P≤0.01

Carbohydrates assay

Soluble and insoluble sugar assays were carried out using the method of (Kochert, 1978) and phenol sulfuric acid. After assaying the soluble and insoluble sugars, a standard curve was prepared for glucose and inserting the obtained OD in the equation, changes in the soluble and insoluble sugars level were determined and the carbohydrate level was recorded in mg per dry weight using the standard curve.

Proline assay

The proline content of the fresh roots and shoots were assayed using ninhydrin and the method of Bates et al. (1973) spectrophotometrically. Results were recorded in µ g per gram fresh weight.

Glycine betaine assay

Glycine betaine content was assayed based on the method of Grattan and Grieve (1992) and using 0.3 g dry plant matter and iodide potassium, sulfuric acid 2N solution, and 1,2,dichloroethane. The standard curve was used and the absorption of samples was calculated at 365 nm based on µ g per gram dry weight.

Guaiacol peroxidase enzyme activity assay

Guaiacol peroxidase enzyme (GPX) activity was assayed using the method reported by Dazy et al. (2008). Reaction environment contained potassium phosphate 25 mM buffer (PH 6.8), hydrogen peroxide 40 mM, and Guaiacol 20 mM. The reaction started after adding 100 µL enzyme extract in the final volume of 3 mL. Increased absorption due to formation of tetra guaiacol was recorded at 470 nm for 3 mins. Enzyme activity was then expressed as the changes in absorption per minute for each gram fresh weight.

Polyphenol oxidase enzyme activity assay

Polyphenol oxidase enzyme activity was assayed using the method of Raymond et al. (1993). A number of test tubes were first kept at 40° C in a bath before phosphate buffer 0.2 molar pH=6 and pyrogallol 0.02 molar were added to them. When the temperature of the tubes was 40° C, 0.2 mL extract was added to each and the changes in absorption were read at 430 nm.

Statistical analysis

The data obtained from various assays were submitted to statistical analysis using Microsoft Excel 2010.

Results

Fresh and dry weights

Analysis of variance showed that most concentrations of Zn and SNP, alone and in combination, had significant effects (P≤0.01) on fresh and dry weights of roots and shoots (Table 1). Comparison of means suggested that application of low concentrations of SNP (particularly 100 mM) increased fresh and dry weights of *Plantago major L*. under various concentrations of Zn as the most pronounced effect on the growth parameters was recorded under 100 mM concentration of SNP while high

Table 2
Comparison of mean fresh and dry weights, leaf and root malondealdehyde contents, leaf and root proline contents, and leaf and root glycine betaine contents of *Plantago. major L.* under various levels of Zn and FNP

Zn	SNP	Root Glycine Betaine (mg.g^{-1} FW)	Leaf Glycine Betaine (mg.g^{-1} FW)	Root Proline (nmol.g^{-1} FW)	Leaf Proline (nmol.g^{-1} FW)	Root Malondealdehyde (nmol.g^{-1} FW)	Leaf Malondealdehyde (nmol.g^{-1} FW)	Dry Weight g per plant	Fresh Weight g per plant
0	0	۲.۱۱۶o	۳.۲۸۱mn	۳.۲۱o	۴.۴۵l	۱۷.۵۶q	۲۶.۵o	۰.۷۴۳ a	۲.۷ a
50	0	۲.۱۰۱o	۳.۲۹۹m	۳.۴۳n	.۲m	۱۶.۳۲r	۲۷.۰۳no	۰.۷۳ ab	۲.۶۸ ab
100	0	۲.۱۴۴n	۳.۲۶۵n	۳.۵۸m	۴.۸۵k	۱۷.۹۹q	۲۷.۴n	۰.۷۱ bc	۲.۶۹۳ a
200	0	۲.۲۵۸l	۳.۳۲۷l	۳.۸۹l	۵.۶۳j	۲۱.۴۴n	۳۳.۶۶i	۰.۶۳ d	۲/۵۱۶e
0	50	۲.۱۸۵m	۳.۳۰۱m	۳.۶۳m	۵.۸۸l	۲۲.۲m	۳۲.۵۹j	۰.۷۵ a	۲.۶۸۳ab
50	50	۲.۲۹۹k	۳.۳۴۲l	۴.۱۱k	۵.۸۶l	۱۹.۴۶p	۳۱.۵۶k	۰.۷۴ a	۲.۷۱ a
100	50	۲.۳۷۶j	۳.۳۸۵k	۳.۹۳l	۶.۱۷h	۲۰.۰۴o	۲۹.۵۲m	۰.۷۳۳ab	۲.۷۰۶ a
200	50	۲.۳۹۵j	۴.۱۲۶j	۴.۶۴j	۶.۵۵g	۲۴.۳۲k	۳۵.۶۹fg	۰.۶۵۳ d	۲.۶۴ c
0	100	۲.۶۴۳i	۴.۳۸۱i	۵.۲۵h	۶.۲۲h	۲۵.۱۶j	۳۴.۷۳h	۰.۶۹ c	۲.۶۱۳cd
50	100	۲.۷۱h	۴.۴۹۹fg	۵.۸۳f	۶.۵۴g	۲۳.۷۵l	۳۳.۳۸i	۰.۷۳ ab	۲.۶۵ bc
100	100	۲.۷۴۵g	۴.۴۸۶g	۶.۰۶e	۶.۱۷h	۲۱.۹۶m	۳۰.۶۵l	۰.۷۵ a	۲.۶۹۳ a
200	100	۲.۸۱۲e	۴.۵۴۳e	۶.۴۵c	۶.۸f	۲۶.۶۹i	۳۶.۳۳ef	۰.۶۳۳ d	۲.۵۸۳ d
0	300	۲.۶۹۸h	۴.۳۸۲i	۵.۰۷l	۶.۷۷f	۳۲.۳۳f	۳۶.۱۲f	۰.۶ e	۲.۴۰۳ g
50	300	۲.۷۸۳f	۴.۴۰۸h	۵.۶۷g	۶.۵۳g	۳۱.۳۹g	۳۵.۰۳gh	۰.۶۴۶ d	۲.۴۶ f
100	300	۲.۸۲۷e	۴.۵۴e	۵.۹۱f	۷.۱۳e	۲۸.۵۹h	۳۳.۳۵i	۰.۶۸۶ c	۲/۵۰۳e
200	300	۲.۸۲۴e	۴.۵۱۲f	۶.۳۲d	۷.۳۸d	۳۴.۳۷d	۳۷.۰۲e	۰.۵۶۶ f	۲/۳۹۳g
0	500	۳.۵۰۱d	۵.۱۲۱d	۶.۸۵b	۸.۴۹a	۳۷.۷۵b	۴۰.۲c	۰.۴۷ h	۲/۰۲۳ j
50	500	۳.۸۵۸b	۵.۲۴۷b	۷.۱۶a	۸.۱۹b	۳۵.۴۷c	۴۱.۰۸b	۰.۵۰۳ g	۲/۰۶۶ i
100	500	۳.۹۰۴a	۵.۳۳۸a	۶.۷۷b	۸.۵۷a	۳۳.۶۲e	۳۸.۵۱d	۰.۵۵ f	۲.۱۸۳h
200	500	۳.۵۷۳c	۵.۲۱۵c	۶.۴۴c	۷.۶۶c	۴۱.۱۸a	۴۳.۹۷a	۰.۴۳ i	۱.۹۲ k

concentrations of SNP had deterring effects and could not mitigate the effects of Zn (Table 2).

Malondealdehyde Content

Table of analysis of variance (Table 1) reveals significant effects of Zn, SNP, and their interaction on malondealdehyde content of leaves and roots (P≤0.01). Results obtained from comparisons of means suggest that increase in Zn concentrations increased malondealdehyde content and e.g., 500 mM Zn increased malondealdehyde contents of leaves and roots by 51% and 100%, respectively. In comparison with other concentrations, interaction of SNP 200 µM with various concentrations of Zn significantly increased lipid peroxidation and malondealdehyde content. Therefore, it can be concluded that low concentrations of SNP (particularly, 100 µM) reduces malondealdehyde content of the plant under Zn stress and protects cell walls from peroxidation while high concentrations (e.g., 200 µM) increases malondealdehyde content resulted from lipid peroxidation (Table 2).

Proline content

Analysis of variance showed that various concentrations of Zn, SNP, and their combined treatment had significant effects (P≤0.01) on proline contents of leaves (Table 1). Results of interaction of effects of Zn and SNP suggest that in all treatments of Zn except for 500 µM application of 200 µM SNP increased proline content of leaves significantly and the maximum proline content was recorded in the combined treatments of Zn 100 µM + SNP 200 µM showing 100% increase compared with the control plants. Also, under Zn 500 µM treatment, application of 50 µM SNP increased proline content by 100% in comparison with the control.

Table 3
Analysis of variance of effects of Zn, SNP, and their interaction on glycine betaine, guaiacol peroxidase, and polyphenol oxidase contents of leaves and roots of *Plantago. major L*.

	df	Root Polyphenol Oxidase	Leaf Polyphenol Oxidase	Root Guaiacol peroxidase	Leaf Guaiacol peroxidase	Leaf Insoluble Sugar	Root Insoluble Sugar	Leaf Glycine Betaine	Root Glycine Betaine
Zn	4	۰.۱۲۵**	۰.۱۳۶**	۰.۰۰۰۳۸**	۰/۰۰۴۹**	۴۴۷۸**	۲۳۴۱**	۷.۴**	۴/۴**
SNP	3	۰.۰۵۴**	۰.۰۴۶**	۰.۰۰۰۱۷**	۰.۰۰۸۷**	۷۴.۹۸**	۸۴.۲۸**	۰.۱۷**	۰.۰۸**
Zn*SNP	12	۰.۰۰۲۷**	۰.۰۰۲۳**	۰.۰۰۰۰۱**	۰.۰۰۰۲**	۲۶**	۱۸.۷**	۰.۰۹**	۰.۰۲۶**
Error		۰.۰۰۰۰۴۲	۰.۰۰۰۰۴۲	۰.۰۰۰۰۰۰۳۲	۰.۰۰۰۰۲۷	۴.۱۷۳	۳.۳۳۹	۰.۰۰۰۱۷	۰.۰۰۰۱۴۸
CV		۰.۷	۰.۶	۱.۲	۲.۸	۲.۷	۴.۵	۰.۴	۰.۵

plants with Zn 500 µM and SNP 100 µM increased proline content by 92% while under other concentrations of Zn, increase in the proline content resulted from NSP 200 µM treatment (Table 2).

Glycine betaine content

The effects of various concentrations of Zn and NSP and also the interaction of these factors on glycine betaine content of leaves and roots (Table 3) were significant (P≤0.01). Comparison of mean effects of Zn and SNP interaction showed that under Zn treatments, all concentrations of SNP increased glycine betaine contents compared with plants treated with Zn alone and SNP 100 and 200 µM showed more increasing effects on this osmolyte. Maximum and minimum glycine betaine contents of leaves and roots were observed in combined treatments of Zn 50 + SNP 0 and Zn 500 + SNP 100, respectively (Table 2).

Soluble sugars

As Table of analysis of variance (Table 1) shows, various concentrations of Zn, SNP, and their interaction had significant effects (P≤0.01) on soluble sugar contents of leaves and roots. Applying various concentrations of SNP to Zn treatment and interaction of effects of these two treatments showed that in leaves under 50 and 100 µM Zn treatments, NSP at 100 and 200 concentrations had more pronounce effects on increasing soluble sugar contents of the plants under study while in 300 and 500 µM Zn treatments, applying 50 and particularly 100 µM SNP resulted in the maximum soluble sugars. Also combined treatment of Zn 500 + SNP 50 resulted the maximum soluble sugar contents in roots showing 79% increase compared with the control (Fig. I).

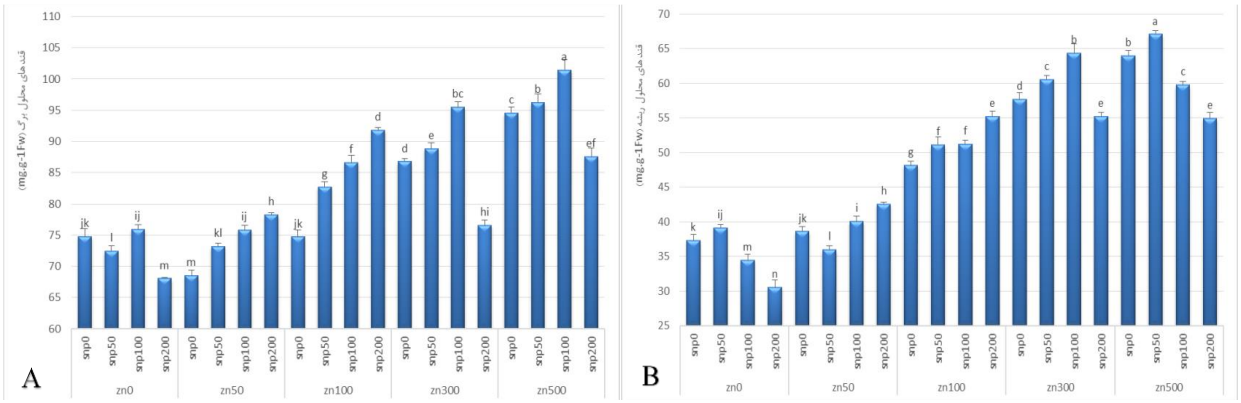

Fig. I. Effect of various concentrations of SNP (0, 50, 100, and 200 µM) on soluble sugar contents of leaves (A) and roots (B) of *Plantago major L*. under 0, 50, 100, 300, and 500 µM Zn treatments

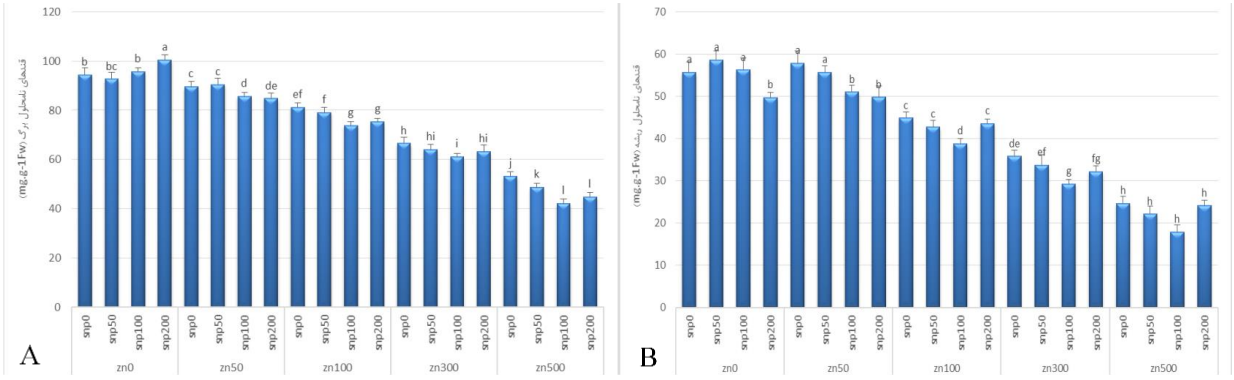

Fig. II. Effect of various concentrations of SNP (0, 50, 100, and 200 µM) on insoluble sugar contents of leaves (A) and roots (B) of *Plantago major L*. under 0, 50, 100, 300, and 500 µM Zn treatments

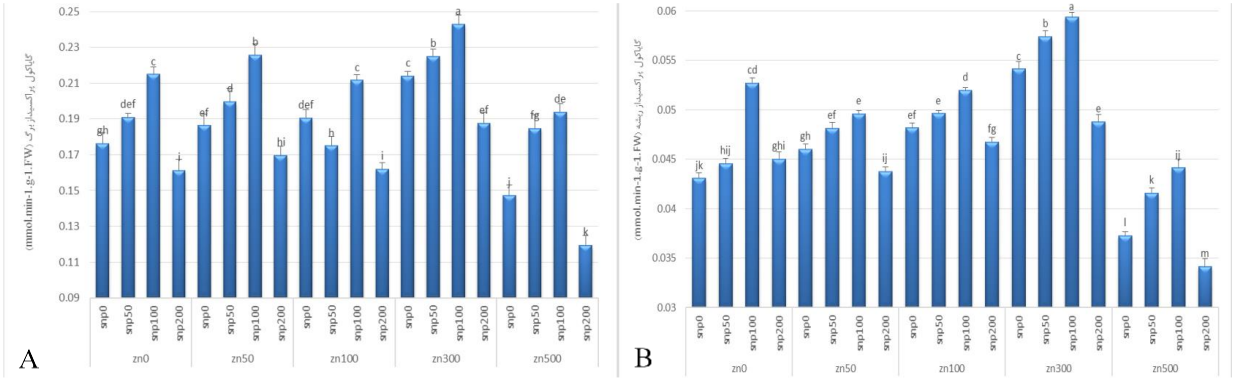

Fig. III. Effect of various concentrations of SNP (0, 50, 100, and 200 µM) on guaiacol peroxidase enzyme activity of leaves (A) and roots (B) of *Plantago major L*. under 0, 50, 100, 300, and 500 µM Zn treatments

Insoluble sugars

Results of ANOVA suggests that the effects of various concentrations of Zn, SNP, and their interaction on insoluble sugar contents of leaves and roots were significant at P≤0.01 (Table 3). Comparison of means showed that insoluble sugars follow a regular reduction trend with increasing Zn concentration. Application of SNP 50 µM to the plant treated with various concentrations of Zn increased insoluble sugar contents of leaves and roots of *Plantago. major L*. The maximum insoluble sugar content was recorded in the combined treatment of Zn (50, 100, 300, 500 µM) + SNP 50 µM (Fig. II).

Guaiacol peroxidase enzyme activity

Analysis of variance (Table 3) showed a significant effect of various concentrations of Zn, SNP, and their interaction on guaiacol peroxidase enzyme activity (P≤0.01). Comparison of means revealed that guaiacol peroxidase enzyme activity in roots and shoots reduced with the increase in Zn concentration. Application of 100 µM SNP increased the activity of this enzyme by 22% and 20% in shoots and roots, respectively compared with control while higher concentrations (200 µM) had a deterring effect on the guaiacol peroxidase enzyme activity (Fig. III).

Polyphenol oxidase enzyme activity

Results of ANOVA (Table 2) showed that the effect of various concentrations of Zn, SNP, and their interaction on polyphenol oxidase enzyme activity of leaves and roots of *Plantago. major L*. was significant (P≤0.01). Comparison of means showed that higher concentrations of Zn particularly 500 µM reduced enzyme activity by 23% and 17% in leaves and roots compared with control plants. Also, in all concentrations of Zn,

only low concentrations of NSP led to increasing trend in polyphenol oxidase enzyme activity while high concentrations of NSP had a decreasing effect on the enzyme activity (Fig. IV).

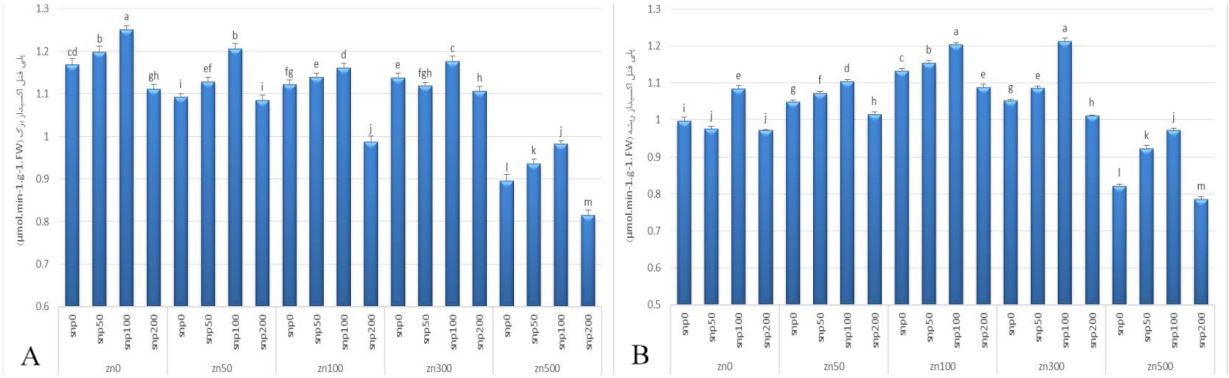

Fig. IV. Effect of various concentrations of NSP (0, 50, 100, 200 µM) on phenol peroxidase activity of leaves (A) and roots (B) of *Plantago major L.* treated with various concentrations of Zn (0, 50, 100, 300, 500 µM)

Discussion

The findings of the present study showed that increasing Zn concentration alone in the nutrition solution caused a reduction in growth parameters of the plants under study while application of low concentrations of SNP (as a nitric oxide releasing agent) alone or in combination with Zn had an increasing effect on growth parameters. Also, high concentrations of SNP had a deterring effect on the growth parameters. Since they compose the structure of many enzymes and proteins, heavy metals such as Cu and Zn are necessary for the normal growth of plants. However, increased concentrations of these and other heavy metals in soil can lead to the symptoms of toxicity and restrain in growth of most plants (Hall, 2002). In line with the findings of this study, Sharma et al. (2010), studying the effects of various concentrations of Zn (0, 10, 20, 50, 75, and 100 µM) on *Cicer arietinum* found that Zn concentrations up to 50 µM results in a positive effect on biomass production. However, increasing the heavy metal concentration reduced the plants' biomass as compared with control plants. These researchers argued that reduction in the growth pattern of the plants treated with heavy metals can be the result of blocking metabolic processes by heavy metals. Similarly, research studies on Zn and Cd suggested that at low concentrations these heavy metals increased growth parameters in soybeans while increasing Zn and Cd concentrations reduced growth parameters (Gupta et al., 2016). It is likely that as a micronutrient, Zn increases photosynthesis and protein metabolism and therefore the plant growth is increased under low concentrations of Zn (Srivastava, 2009).

In this study the contents of guaiacol peroxidase, polyphenol oxidase, and malondealdehyde as the indexes of lipid peroxidation increased following the increase in Zn concentration of the nutrition solution for *Plantago major L*. and while applying low concentrations of NSP in the treatments improved enzyme activities, higher concentrations had an adverse effect on antioxidant enzymes and lipid peroxidation. Evidently, antioxidant system plays an important role in the tolerance of the plants against stress conditions and this is why the activities of one or several of these enzymes or antioxidants increase under stress conditions and this increase is attributed to the increased tolerance of the plants against stress (Fecht-Christoffers et al., 2003).

One of the most important harmful effects of heavy metals on plants is excessive production of reactive oxygen species (ROS) which cause oxidative stress in plants (Hassan and Mansoor, 2014). In order to evaluate the oxidative stress, malondealdehyde content of the plants is assayed as a lipid peroxidation and oxidative damage index in plants exposed to heavy metals (Chamseddine et al., 2009). Magdy and Azooz (2013) studied the

effects of Zn and Pb on *Hibiscus esculentus* and argued that increased level of lipid of peroxidation suggest that Zn and Pb induced oxidative stress and increased antioxidant activity in the plants under study. Also, it is suggested that increase in malondealdehyde contents of tomato leaves under Cu stress can be attributed to the damage in photosynthesis organ, reduced retrieving effects of antioxidants, and reduced metabolic energy of the cells for fulfilling the plant needs (Chamseddine et al., 2009). Another reason for increased lipid peroxidation is inducing lipo oxinage activity in the presence of heavy metals. This is an initiating enzyme for lipid peroxidation (Chaffai et al., 2005). On the other hand, it was found that the nitric oxide resulted from NSP reduced malondealdehyde content caused by lipid peroxidation in sunflower under cadmium stress (Laspina et al., 2005). Nitic oxide as a gas signaling molecule plays a major role in regulation of many key physiologic processes (Besson-Bard et al., 2008). Depending on its concentration and position, nitric oxide can exert both positive and negative effects on plant cells (Qiao and Fan, 2008). Wang et al. (2013) also reported that low concentrations of nitric oxide have protective effects against Cd toxicity which confirms the findings of the present study.

There are many studies reporting that nitric oxide, through increasing the activities of hydrogen peroxide scavenging enzymes, protects plants against oxidative damage (Lamattina et al., 2003). Two mechanisms are referred to in the literature on the protective effects of nitric oxide against oxidative stress: firstly, nitric oxide may directly remove ROS toxicity e.g., through superoxide anions to form peroxi nitrite with lower toxic effects and therefore, limit the cell damage (Martinez, 2000). Secondly, nitric oxide can perform as a signaling molecule activating cellular antioxidant system (Lamattina et al,m 2003).

Another strategy to fight against abiotic stresses is synthesis of adaptable and osmotic protective compounds. Proline, glycine betaine, and soluble sugars are among the most important adapting solutions whose positive effects on modification of osmotic stresses and protection of plasma membrane have been reported Pérez-Clemente et al., 2013). Findings of the present study suggest that osmolyte contents (proline, glycine, betaine, and soluble sugars) increased in leaves and roots of *Plntago major L.* with an increase in the concentration of Zn while insoluble sugar content showed a regular reducing trend with increased Zn concentration. Proline and glycine betaine are important osmolytes that help with osmotic regulation of the cells under stress (Ahmad et al., 2015). Proline plays roles in keeping water equilibrium, maintaining the stability of proteins, maintaining the 3-dimensional structure of proteins and enzymes, and establishing membranes and protein synthesis device, scavenging hydroxyl radicals, reducing the risks due to ROS generation, and regulating the cell pH (Verbuggen and Hermans, 2008). Glycine betaine is the most common adaptable organic solution that exists in most microorganisms, plants, and animals and is considered as the most prevalent known ammonium compound in plants that respond to stress (Yang et al., 2003). Glycine betaine plays the most important role in protecting thylakoid membrane and therefore, in performance of photosynthesis photosystems (Tian et al., 2013). Protective nature of osmolytes under nitric oxide treatment is confirmed in the study reported by Dong et al. (2012). Also, Aly and Mohamed (2012) found similar results reporting that the plants grown under Cu stress had increased proline content. In fact, proline stops membrane damage playing a protective role against lipid peroxidation under heavy metal stress (Thounaojam et al., 2012). Sugars are required as players of a complex communication system for coordination of plants' metabolism, growth, and response to environmental changes (Rolland et al., 2006). Some studies have shown that through increasing heavy metal concentration, the intercellular water balance is disturbed and this results in meta-structural changes in the cell organelles and the enzymes in the sugar metabolism path and by increasing the concentration of the heavy metals, invertase enzyme activity is reduced. Following the decrease in transfer of water to leaves and concentration of heavy metals in cells, carbohydrates are increased in leaves. In fact, increased carbohydrate is an adaptation mechanism for maintaining osmotic potential of the plants under heavy metal elements. Accumulation of carbohydrates is

effective in maintaining cell membrane and osmotic regulation (Sato et al., 2004). Some studies report that increased soluble sugar concentrations in leaves is probably due to the interference with starch hydrolysis process (Drzewiecka et al., 2012). Reduction in the starch content observed in this study is a reason for the claim that starch breaks into soluble sugars (Alaoui et al., 2003).

Conclusion

Studies show the clash between oxidative stress under Zn toxicity and the plants' defense or adaptation strategies. On the other hand, SNP (particularly 100 µM) reduced stress level in the plants under Zn toxicity. SNP through activating antioxidant enzymes plays a key role in reducing oxidative stress and through keeping integrity of the bio membranes, reducing active oxygen species, and modification of the metabolism in some cellular compounds, improves the plants' tolerance threshold against Zn toxicity.

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