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RESEARCH ARTICLE

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Effects of manganese toxicity on activation of antioxidant enzymes, Anthocyanin and total phenolic content in Sunflower *(Helianthus annuus* L.*)*

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ABSTRACT

Various biochemical parameters in response to different concentrations of MnSO4 on sunflower(Helianthus annuus L.) were measured. Seeds were germinated in petri dishes, the five days old seedlings were transferred to pots containing sand in a growth chamber with 16h light period per 24h and day/night temperatures of 25 and 18 °C respectively and irrigated with Hoagland solution. Twenty days old plants were treated with MnSO4 solution at 0, 250, 350 and 1500 µM in nutrient solutions and harvested after 30 days for measurements of biochemical and physiological parameters. One of the main factors limiting toxicity plant growth in acid soils is special. On the other hand, the critical concentration for the toxicity of manganese abroad range of plant species and genotype will vary and depend on environmental conditions such as temperature and nutrient conditions. The results showed that with increasing the concentration of MnSO4 Antioxidant enzymes, anthocyanin content and phenol content increased in the environment containing MnSO4.

Key words*: Anthocyanin, phenolic , MnSO4, Antioxidant enzymes.*

INTRODUCTION

Heavy metals are defined as that group of elements that have specific weights higher than about 5 g/cm^3. A number of them (Co, Fe, Mn, Mo, Ni, Zn, Cu) are essential micronutrients and are required for normal growth and take part in redox reactions, electron transfers and other important metabolic processes in plants. Metals which are considered nonessential (Pb, Cd, Cr, Hg etc.) are potentially highly toxic for plants [30,25,24]. Large areas of land are contaminated with heavy metals (the main group of inorganic contaminants) resulting from urban activities, agricultural practices and industry [18,9]. Excessive concentrations of trace elements (Cd, Co, Cr, Hg, Mn, Ni, Pb and Zn) are toxic and lead to growth inhibition, decrease in biomass and death of the plant [36]. Heavy metals inhibit physiological processes such as respiration, photosynthesis, cell elongation, plant-water relationship, N-metabolism and mineral nutrition [38].

Manganese (Mn) is an essential micronutrient required for the normal growth of higher plants. Like other heavy metals, however, Mn may become toxic when present in excess [22]. Acid soils comprise up to 50% of the world's potentially arable lands. After aluminum (Al), Mn toxicity is probably the most important factor limiting plant productivity in acid soils [12]. Manganese is involved in photosynthesis, respiration, and activation of antioxidative enzymes [22,29]. Acceleration in the enzyme activities such as peroxidase and catalase are believed to play a metabolic role under conditions of metals stress and therefore mayhave a subtle role in metal tolerance [27]. Peroxidases are considered to be heavy metal stress-related enzymes and are used as stress markers in metal poisoning situations [17] .

It was shown that, increase in their activity protects plants to various stress factors [14]. In many plant species, excessive uptake of heavy metals such as Ni, Pb and Cd induces a strong increase of peroxidase activities and qualitative changes to their isozyme patterns [6,20]. Although the effects of heavy metals on the activity of oxygen

radical detoxifying peroxidases have been widely reported, their involvement in the defense mechanisms of plant tissues against metal-induced damages remains controversial [6]. anthocyanin and phenol content increased in the environment containing MnSO4. In this study , Effects of MnSO4 stress on some physiological parameters in sunflower *(Helianthus annuus* L.*)* studied.

MATERIALS AND METHODS

Plants Culture

Seeds of sunflower (*Helianthus annuus* L. cv. Record) provided by Seed and Plant Improvement Institute (SPII) (Karaj, Iran) . Seeds were sterilized in 5% (W/V) sodium hypochlorite (15 min) and washed five times with sterile distilled water. Seeds were germinated in petri dishes, the five days old seedlings were transferred to pots containing sand in a growth chamber with 16h light period per 24h and day/night temperatures of 25 and 18 °C respectively and irrigated with Hoagland solution (Hoagland and Arnon, 1950). Twenty days old plants were treated with MnSO4 solution at 0, 250, 350 and 1500 µM in nutrient solutions and harvested after 30 days for measurements of biochemical and physiological parameters .

Determination of Peroxidase and Catalase Activity

1.Catalase (CAT)

Catalase was determined according to the method published by Chaoui *et al.* (1997). The disappearance of H2O2 was evaluated by measuring the decrease in absorbance at 240 nm (molar extinction coefficient: e = 36.6 mM_1 cm_1) of a reaction mixture containing 100 lL of H2O2 (100 mM), 100 lL of plant extract and completed to 1 mL final volume with 125 mM potassium phosphate buffer (pH 7.0).

2. Guaiacol peroxidase (GPX)

Guaiacol peroxidase was assayed according to the method developed by Roy et al. (1996). The reaction mixture consisted of 100 lL plant extract, 100 lL guaiacol (22 mM), 100 lL H2O2 (100 mM) and completed to 1 mL final volume with 125 mM potassium phosphate buffer (pH 7.0). The increase in absorbance was measured spectrophotometrically at 470 nm (e = 26.6 mM_1 cm_1).

3. Ascorbate peroxidase (APX)

Ascorbate peroxidase was determined according to Garcia- Limones et al. (2002). The reaction mixture consisted of 100 lL ascorbate (5 mM), 100 lL H2O2 (100 mM), 100 lL EDTA (1 mM), 100 lL of plant extract and was completed to a final volume of 1 mL with 125 mM potassium phosphate buffer (pH 7.0). The oxidation of ascorbate was determined by the increase in absorbance at 290 nm (e = 2.8 mM_1 cm_1).

Estimation of Anthocyanin and Total Phenolic:

Contents of anthocyanin and total phenolic were measured according to Zhang and Quantick (1997). The fronds (1 g FW) were extracted with 1% HCl-methanol (5 mL), the extract was filtered and the filtrate was diluted with 1% HCl-methanol to 10 mL. Absorbance of the diluent was measured at 600 and 530 nm for anthocyanin, and at 280 nm for total phenolics. Anthocyanin content was expressed as the change of 0.1 unit of difference between A530 and A600. Total phenolic was calculated from a standard curve made with gallic acid.

Statistical analysis.

The research was conducted using completely randomized design with four replications. The significance of salinity treatments was analyzed by analysis of variance (AVOVA) using SAS software (SAS Institute Inc., Cary, NC). Differences of $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Data presented in Fig. 1,2,3 indicated that the effect MnSO4 on Catalase(CAT), Guaiacol peroxidase (GPX) and Ascorbate peroxidase (APX) activities in sunflower *(Helianthus annuus* L.*)* Catalase(CAT), Guaiacol peroxidase (GPX) and Ascorbate peroxidase (APX) activities of *Helianthus annuus* L. increased in the environment containing MnSO4 in contrast to control. This stimulating influence in antioxidant enzymes activity was observed throughout experimental period.

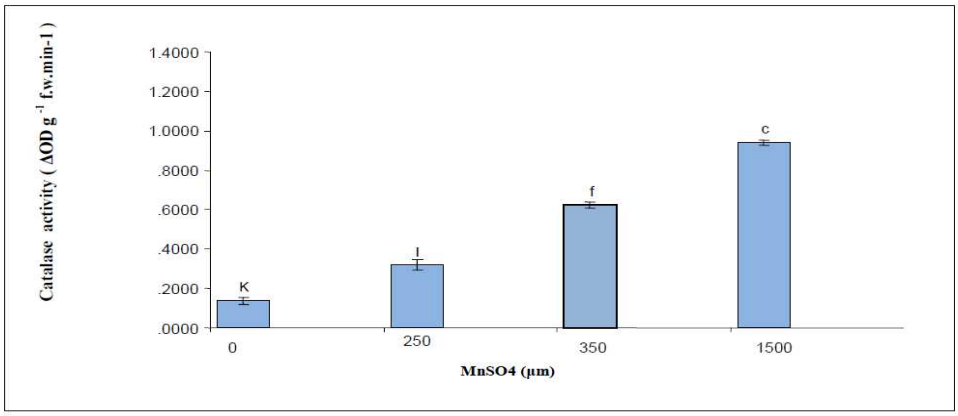

Figure 1. Effects of different concentrations of MnSO4 on catalase (CAT) activity(ΔOD g^{-1} f.w.min-1) in sunflower *(Helianthus annuus* L.*)* Data are means ± SD of four replicates. numbers followed by the same are not significantly different (P>0.05).

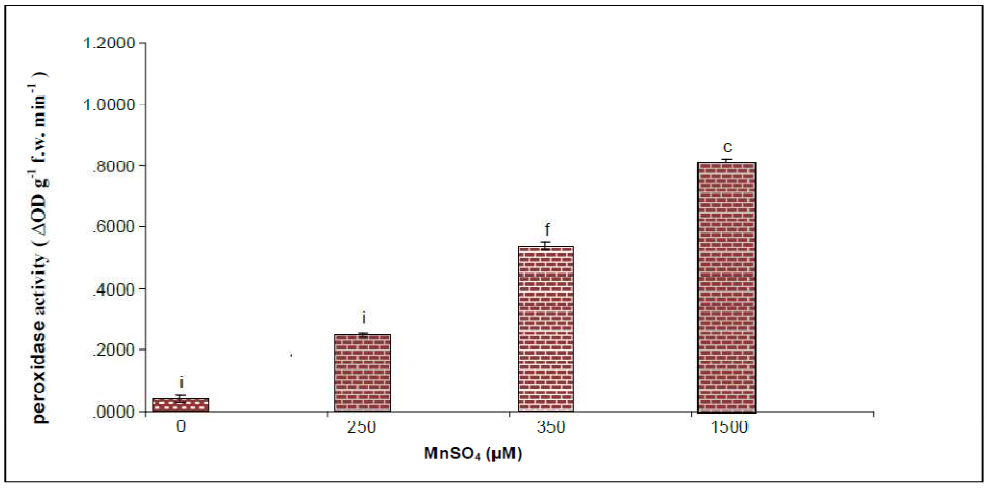

Figure 2. Effects of different concentrations of MnSO4 on Guaiacol peroxidase (GPX) activity(ΔOD g^{-1} f.w.min^{-1}) in sunflower *(Helianthus annuus* L.*)* . Data are means ± SD of four replicates. numbers followed by the same are not significantly different (P>0.05).

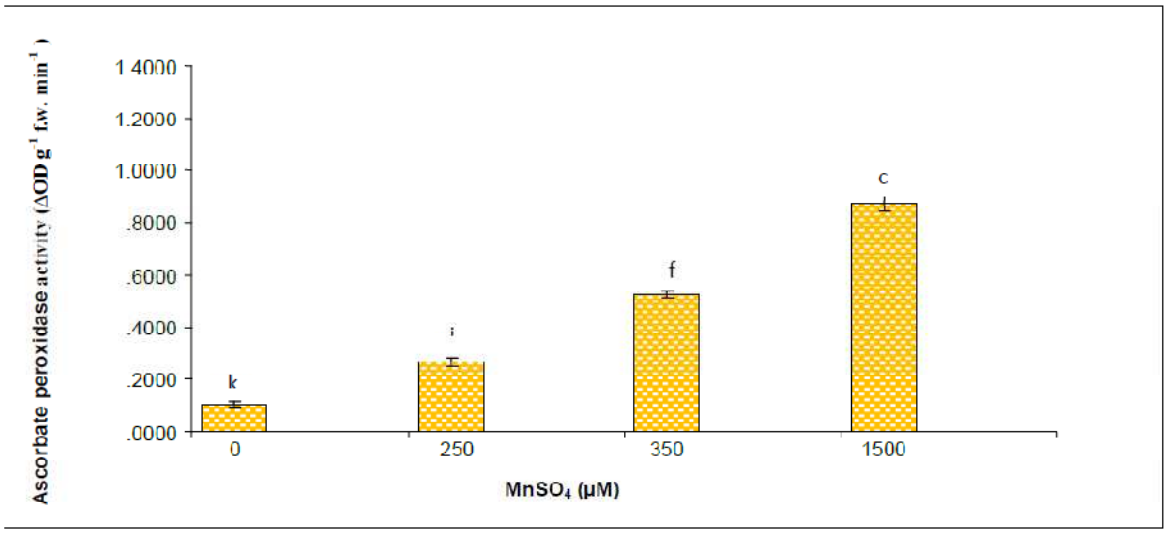

Figure 3. Effects of different concentrations of MnSO4 on Ascorbate peroxidase (APX) activity (ΔOD g^{-1} f.w.min^{-1}) in sunflower *(Helianthus annuus* L.*)* . Data are means ± SD of four replicates. numbers followed by the same are not significantly different (P>0.05) .

Mn toxicity can induce oxidative stress through direct generation of reactive oxygen species (ROS) from Mn ions in the Fenton reaction [19], or direct transfer of electrons in single reaction, leading to a rise ROS level [4,10]. Oxidative stress is characterized by increase in reactive oxygen species as a result of insufficient antioxidant defens.

Responses of organisms to oxidative stress induced the use of antioxidant enzymes .To minimize cellular damage caused by ROS, plants have evolved a scavenging system composed of antioxidants such as ascorbate (ASC) and reduced glutathione (GSH) and antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT) and guaiacol peroxidase (GPX) [7,31]. Antioxidant enzymesmay convert the H2O2 to the H2O in the plant cells and neutralized the toxicity effects of H2O2 [1]. The relationship between total SOD activity and ascorbate peroxidase activity was clear in cells subjected to copper, zinc and manganese treatments. This suggests the existence of a functional link between both enzymes in tobacco cells, which initiates the removal of the toxic effects of H_2O_2 generated during metal treatments. [8].

Data presented in Fig. 4 and Table 1, indicated that the effect of MnSO4 on Anthocyanin and Total Phenolic content in sunflower *(Helianthus annuus* L.*)*. Anthocyanin and Total Phenolic content of *Helianthus annuus* L. increased in the environment containing MnSO4 in contrast to control. This stimulating influence in Anthocyanin and Total Phenolic content was observed throughout experimental period. More recent studies have found that anthocyanins are produced in response to various types of stress, including metal stress [5,15]. In general, anthocyanins are believed to increase the antioxidant response of plants in order to uphold the regular physiological status in tissues directly or indirectly affected by biotic or abiotic stressors [35,26,23]. It was suggested that phytohormones can regulate the synthesis of basic antioxidant enzymes, and some of the isoforms of antioxidant enzymes are also implicated in phytohormone catabolism [33].

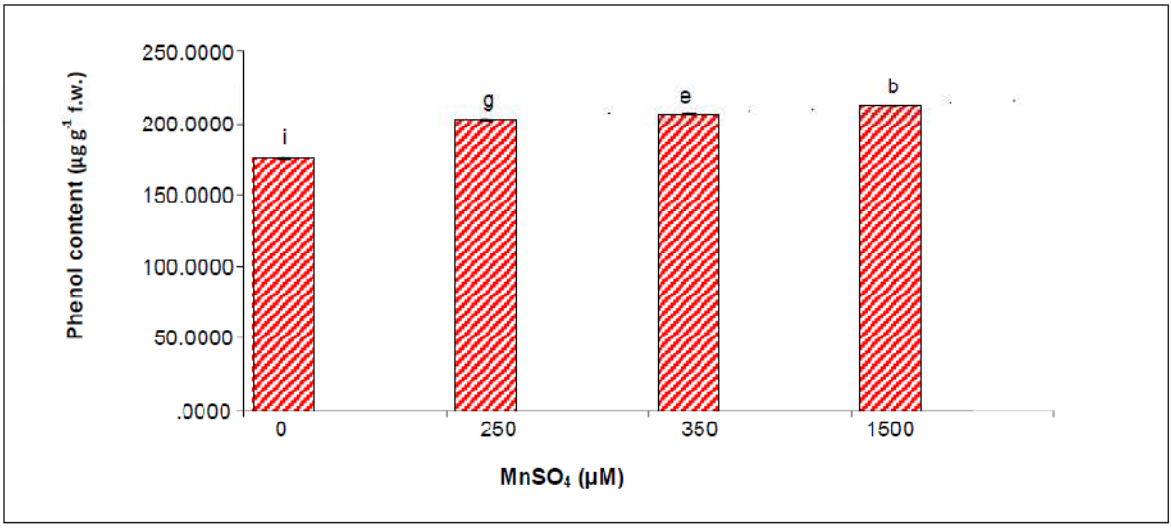

Figure 4. Effects of different concentrations MnSO4 of on total Phenolic content(µg g^{-1}f.w.) in sunflower *(Helianthus annuus* L.*)*. Data are means ± SD of four replicates. numbers followed by the same are not significantly different(P>0.05).

Table 1. Effects of different concentrations of MnSO4 on anthocyanin content(mg g^{-1}f.w.) in sunflower *(Helianthus annuus* L.*)*.

MnSO4 (µm)	0	250	350	1500
anthocyanin content(mg g^{-1}f.w.)	0/628±0/009 ^{l}	0/755±0/004 ^{t}	0/857±0/01 ^{f}	1/08±0/01 ^{c}

Data are means ±SD of four replicates. numbers followed by the same are not significantly different(P>0.05).

All plants produce an amazing diversity of secondary metabolites. One of the most important groups of these metabolites are phenolic compounds. Phenolics are characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups. They are mainly synthetized from cinnamic acid, which is formed from phenylalanine by the action of L-phenyloalanine ammonia-lyase PAL (EC 4.3.1.5), the branch point enzyme between primary (shikimate pathway) and secondary (phenylopropanoid) metabolism [11].

During heavy metal stress phenolic compounds can act as metal chelators and on the other hand phenolics can directly scavenge molecular species of active oxygen. Phenolics, especially flavonoids and phenylopropanoids, are oxidized by peroxidase, and act in H2O2- scavenging, phenolic / ASC / POX system. Their antioxidant action resides mainly in their chemical structure. There is some evidence of induction of phenolic metabolism in plants as a response to multiple stresses including heavy metal stress. Arora *et al.,* (2000) show that phenolics especially flavonoids are able to alter peroxidation kinetics by modifying the lipid packing order. They stabilize membranes by decreasing membrane fluidity in a concentration-dependent manner and hinder the diffusion of free radicals and restrict peroxidative reaction [2,3] .

According to Verstraeten *et al*. (2003), in addition to known protein-binding capacity of flavanols and procyanidins, they can interact with membrane phospholipids through hydrogen bonding to the polar head groups of phospholipids. As a consequence, these compounds can be accumulated at the membranes' surface, both outside and inside the cells. Through this kind of interaction, as they suggest, selected flavonoids help maintain membranes' integrity by preventing the access of deleterious molecules to the hydrophobic region of the bilayer, including those that can affect membrane rheology and those that induce oxidative damage to the membrane components. An increase of phenolics correlated to the increase in activity of enzymes involved in phenolic compounds metabolism was reported [21], suggesting *de novo* synthesis of phenolics under heavy metal stress.

CONCLUSION

The effect of heavy metals on plants resulted in growth inhibition, structure damage,a decline of physiological and biochemical activities as well as function of plants .

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