Effect of various levels of iron on morphological, biochemical, and physiological properties of *Glycine max* var. Pershing

Somayeh Pooladvand[1], Mahlagha Ghorbanli [2]* and Mozhgan Farzami Sepehr [3]

1. Department of Biology, Faculty of Science, Islamic Azad University, Tehran Shomal Branch, Tehran, Iran.
2. Department of Biology, Faculty of Science, Islamic Azad University, Gorgan Branch, Gorgan, Iran
3. Department of Biology, Islamic Azad University, Saveh Branch, Saveh, Iran

Abstract

Iron is a necessary mineral for plants' biological redox system and an important component of many enzymes. In the present study, effect of iron on morphological, biochemical, and physiological properties of soybean were investigated. The experiments were arranged in a completely randomized design with three replicates. Analysis of the data was carried out using SPSS with multiple range Duncan test ($P < 0.05$) as the statistical tool for comparison of means. The findings suggested that $FeSO_4$ increased seed production, nodule formation and the number of pods and leaves. However, higher concentrations of $FeSO_4$ reduced nodules and leaf numbers. It was also observed that antioxidant enzymes activity in roots and shoots gradually increased with an increase in $FeSO_4$ concentration. The iron content in the treated plants increased in proportion to the increase in $FeSO_4$.

Keywords: iron; soybean, catalase; ascorbate peroxidase; superoxide dismutase

Pooladvand, S., M. Ghorbanli and **M. Farzami Sepehr.** 2012. Effect of various levels of iron on morphological, biochemical, and physiological properties of *Glycine max* var. Pershing'. *Iranian Journal of Plant Physiology* 2 (4), 531-538.

Introduction

All living things including plants need food for growth. Soil is the supplier of the majority of nutrients needed by plants. In many acid soils around the world as well as in half of the cultivated lands with the potential to produce food, heavy metals are the main factor limiting the plant growth. Iron is the first rare element recognized as necessary for plants and animals, playing an important role in biochemical and physiological processes (Hemalatha and Selvaraj, 2011). It works as a key enzymes co-factor that plays a role in plant hormone synthesis and is engaged in many electron transportation reactions (Kerkeb and Connolly, 2006). As iron enters root cells, it must be transported to the leaves. In xylem iron is transported as Fe (III) and probably makes complexes with citrate (Cataldo et al., 1988). Transportation of iron in apoplast is necessary for its absorption processes by root cells (Zhang et al., 1991). Is has been suggested that reduction in iron and its transportation allover mesophyll cells plasma membrane is a very important step that can disturb iron shortage through increasing apoplast pH (Kosegarten et al., 1999). Iron shortage mostly causes the young leaves to turn yellow, a condition which is usually called leaf chlorosis (Salama et al., 2009). Excessive iron on the other hand, results in the oxidative stress. Oxidative stresses in turn result in overactivity of reactive

*Corresponding author
E-mail address: ghorbanli@yahoo.com
Received: June, 2012
Accepted: August, 2012

Fig. I. The number of nodules in soybean Var pershing; from left to right: control, 100, 200, and 300 mg/L $FeSO_4$.

oxigen species (ROS), superoxide radicals (O^{-}_{2}), hydrogen peroxide (H_2O_2), and hydroxyl radicals all of which being a general effect of biologic and abiotic stress (Polle and Rennenberg, 1993). In some cases it is possible to alleviate the symptoms of iron shortage and toxicity by increasing or decreasing the concentration of nutrients as, for instance, adding extra potassium (K) was reported to improve iron toxicity in rice (Li et al., 2001). This study aims at investigating the effect of iron on morphological, biochemical, and physiological properties of soybean.

Material and Methods

Commercially available soybean seeds were obtained from Iran Genetic Center under scientific name 'Glycine max L'. The healthy seeds were sterilized using 1:9 (w/v) diluted sodium hypochlorites for 20 minutes. The obtained seeds were put into plastic pots with the capacity of 2 or 3 times more than the seeds volume. A glass of water with inoculation liquid (*Bradyrhizobium japonicum*) was added to the seeds to cover their surface. Then, the seeds were spread on a clean surface in shadow for 5 minutes to air dry. The seeds were then sown in a strip of plots at the depth of 3-5 centimeters below the surface of the soil in rows 50 – 60 centimeters away from each other. Irrigation was done every 3 days through the main stream between the plots. The first and the second treatments of 100, 200, and 300 mg $FeSO_4$ were applied on 7^{th} and 10^{th} week after sowing the seeds, respectively. On week 12 the plants were harvested.

Protein extraction

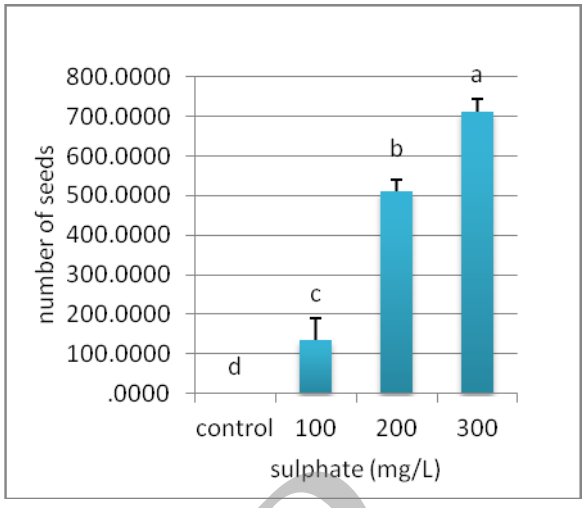

Fig. II. Comparison of the number of seeds at various $FeSO_4$ concentrations

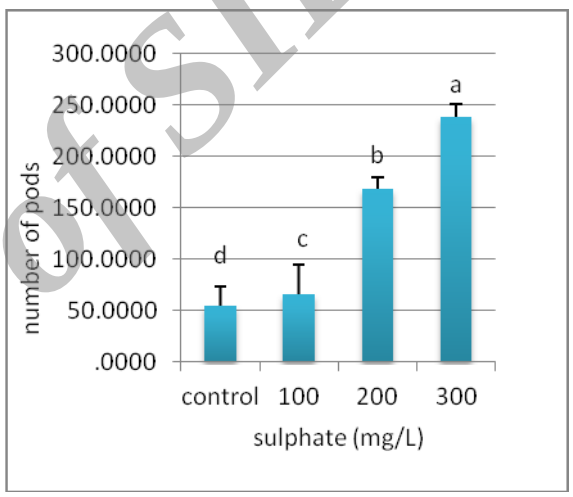

Fig. III. Comparison of the number of pods at various $FeSO_4$ concentrations

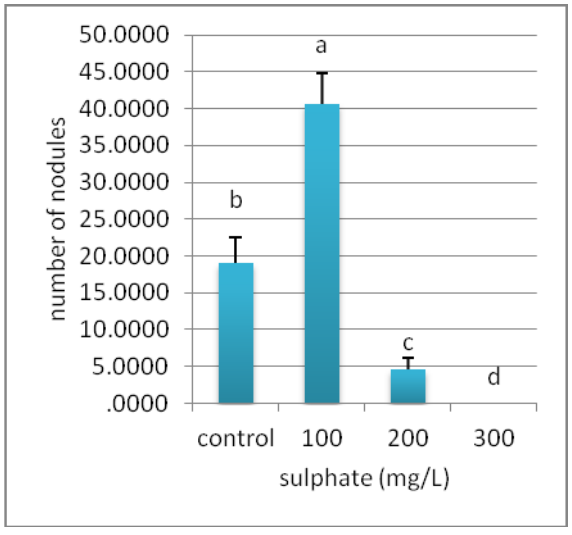

Fig. IV. Comparison of the number of nodules at various FeSO4 concentrations

To extract protein content for measuring enzymes, 0.5 g wet plant matter was milled with 5 ml Tris-glycine buffer to obtain a homogenous solution. The milling was continued for 10 minutes in an ice bath after which the top transparent extract was put into eppendorf and centrifuged12000 rpm using a refrigerated centrifuge for 10 minutes and at 4 °C. The supernatant solution was distributed between several eppendorfs and their characteristics were recorded on each eppendorf. The extracts were kept in the freezer at -20 °C until they were used for the experiment.

Measuring catalase, superoxide dismutase and ascorbate peroxidase activity

Catalase activity was measured using the method described by (Pereira et al., 2002) through analyzing decrease in H_2O_2 content at 240 nm. Also, Sairam and Srivastava (2001) method was used to determine superoxide dismutase content spectrophotometrically at 560 nm and 23 $\pm$ 2 °C. Ascorbate peroxidase activity was measured through recording ascorbate oxidation at 290 nm based on the method of Nakano and Asada (1981).

Measuring iron concentration

To measure iron concentration, digestion method with two-acid mixture was used. The acids used in the study included 4:9 mixture of $HClO_4$: HNO_3 from left to right. After extraction, iron concentration was measured with an atomic absorption equipment (Perkin Elmer 4110) (Lozak et al., 2002). Statistical analysis of the data was carried out using SPSS comparing the means by multiple range Duncan test ($P < 0.05$) with 3 replications for each study. The graphs were prepared using Excel.

Results

Adding 100 mg/L iron significantly increased the number of nodules in the treated plants (Fig. I). The findings also suggested that the number of seeds (Fig. II) and pods (Fig. III) in the treatments with various concentrations of $FeSO_4$ remarkably increased compared with the control plants. As concentration of $FeSO_4$ increased, the number of nodule reduced compared with control plants, and in fact, no nodule was observed at concentration 300 mg/L (Fig. IV). The leaf number also increased with an increase in $FeSO_4$ concentration (Fig. V). However, the higher concentrations of $FeSO_4$ generally reduced the leaf number.

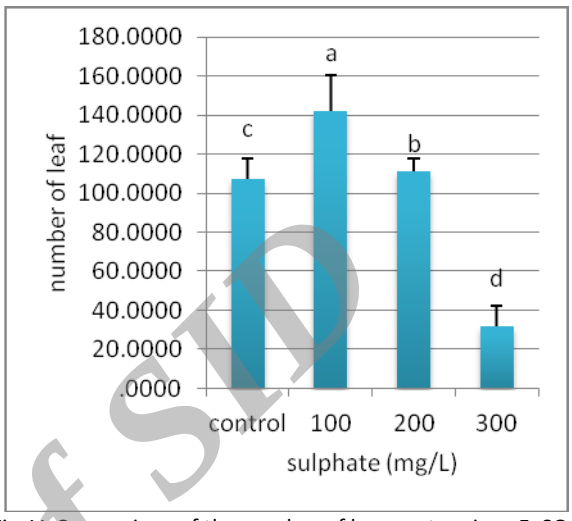

Fig. V. Comparison of the number of leaves at various FeSO4 concentrations

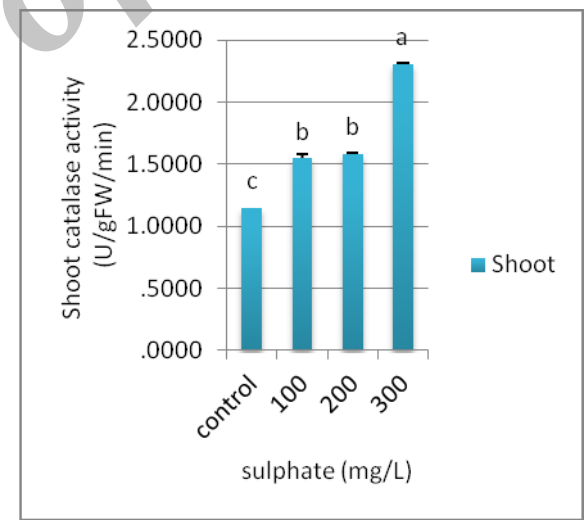

Fig. VI. Catalase activity in soybean shoots

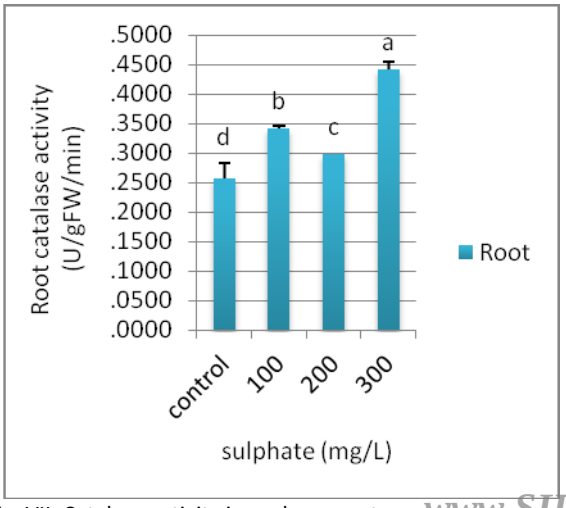

Fig. VII. Catalase activity in soybean roots

Catalase, ascorbate peroxidase and superoxide dismutase activity

All three enzymes in the plants under study had a gradual increase with an increase in $FeSO_4$ concentration and the highest levels of activities for these enzymes were recorded at 300 mg/L concentration while the lowest activity was observed in control plants' roots and shoots (Table 2).

Iron content

Addition of $FeSO_4$ increased root and shoot iron contents (Table 3, Figs. XII and XIII). While the lowest iron level was observed in control roots and shoots, the highest level was recorded for the plants treated with 300 mg/L $FeSO_4$. Root iron contents were higher than those in shoots.

Discussion

In this study, increasing iron concentration in all treatments decreased number of seeds and pods. As Fig. (II) shows, the number of seeds in control plants was 0 while at 100mg/L $FeSO_4$ there were pods with 2, 3, and sometimes 0 seeds which were watery. The seeds at 200 mg/L $FeSO_4$, were bigger and almost hard and at 300 mg/L $FeSO_4$, there were 3 hard big full seeds in each pod. Seed formation at high $FeSO_4$ concentration could be attributed to higher iron absorption at this concentration as maximum heavy metal absorption occurs during seed formation stage. Basavarajappa et al. (1992) reported increased yield for cottonseeds treated with 5 kg/hectare $FeSO_4$. Similarly, Kuzhandaivel and Venkatesan **(**2011) observed that application of $FeSO_4$ (45 ppm) and iron complex significantly increased production and growth of sorghum hybrid CSH-5 seeds. Increased callus and leaf number were also observed up to 100 mg/L $FeSO_4$; however, at higher concentrations callus and leaf number reduced and even at 300 mg/L $FeSO_4$ no callus was observed. This could be explained by the idea that the plant does not need calluses at high $FeSO_4$ concentrations. Soybean's iron requirement for callus formation is limited (Awlad et al., 2003). In control plants

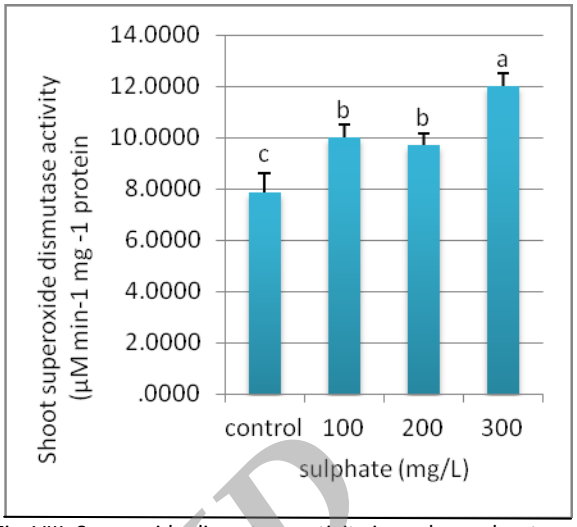

Fig. VIII. Superoxide dismutase activity in soybean shoots

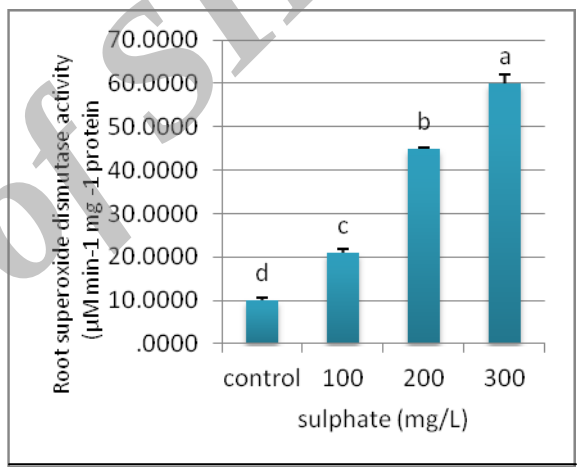

Fig. IX. Superoxide dismutase activity in soybean roots

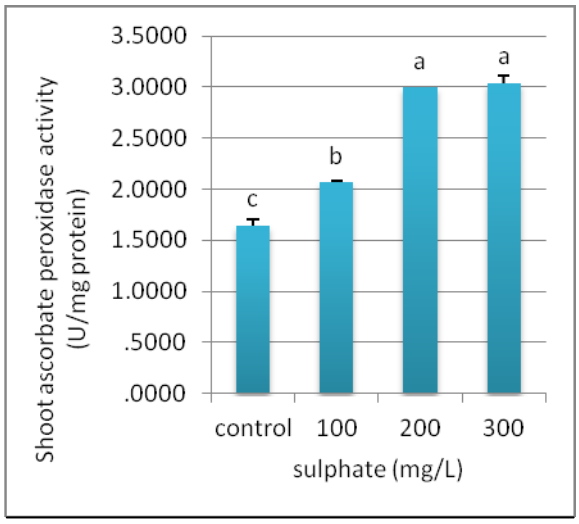

Fig. X. Ascorbate peroxidase activity in soybean shoots

with less iron supply, fewer calluses were observed in previous studies (Abdelmajid and

Chedly, 2003; Awlad et al., 2003; Vadez et al., 2000). Abdelmajid and Chedly (2003) explained that in cereals nodules, iron is necessary for leghemoglobin biosynthesis as it regulates oxygen contents within cells. Singh et al. (1995) reported that using $FeSO_4$ and iron citrate increased pod formation. Lalit Bhatt et al. (2004) in their experiment on the effect of micronutrients on growth and performance of tomatoes found that applying $FeSO_4$ (100 ppm) through leaf led to the maximum number of branches per plant, leaf number, leaf area, wet plant weight, and dry stem weight.

Iron is the main part of a cofactor for many antioxidant enzymes. On the other hand, it can function as a prooxidant since it it catalizes free radicals through fenton reaction (Minotti and Aust, 1987). It has been shown that many enzymes such as catalase and superoxide dismutase in order to perform well, play a role in controlling reactive oxygen species (ROS). Production of ROs, like superoxide onion radical ($O^{\cdot-}_2$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxide radicals (OH) can damage many cellular compounds such as proteins, membrane lipids, and nucleic acids. Plant cells respond to ROS formation by increased production of metaloenzymes such as superoxide dismutase, catalase, peroxidase, and specially ascorbate peroxidase which protect them against oxidative damage causing many oxidative stresses (Halliwell and Gutteridge, 1987). Superoxide dismutase is an important enzyme in various parts of the plants which is engaged in antioxidant processes. It is a key scavenger of ($O^{\cdot-}_2$) and its enzymatic activity results in the production of H_2O_2 and O_2 (Bowler et al., 1992). Increased superoxide dismutase activity may also occur for storing superoxide radicals accumulated in leaves suffering from iron shortage (Sun et al., 2007).

In the present study, superoxide dismutase activity increased at high concentrations of $FeSO_4$. As it was mentioned before, reactive oxygen species are produced at these high concentrations and, as a result, superoxide dismutase enzyme increases to control ROS. Increased superoxide dismutase activity was also reported to increase under the iron stress in flax cultivars (Salama et al., 2009).

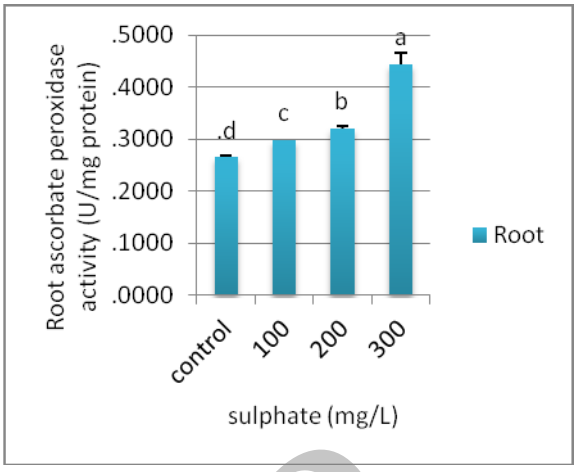

Fig. XI. Ascorbate peroxidase activity in soybean roots

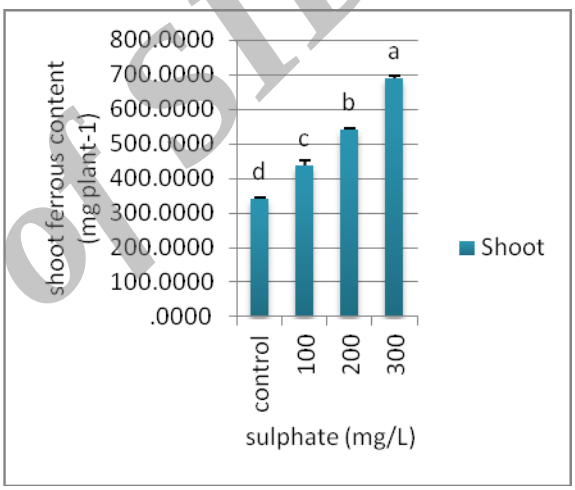

Fig. XII. Soybean shoot iron contents at different concentrations of $FeSO_4$

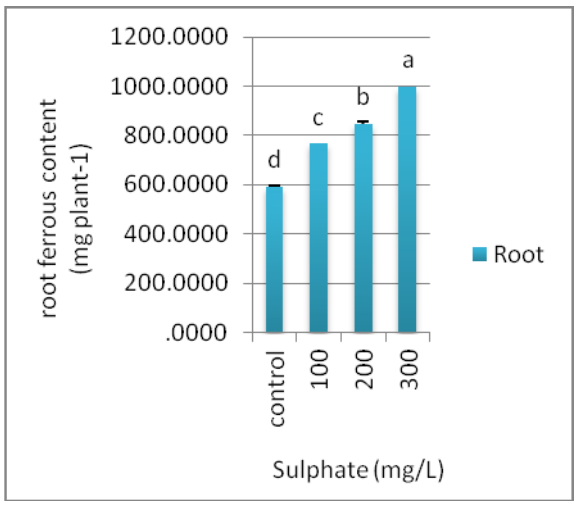

Fig. XIII. Soybean root iron contents at different concentrations of FeSO4

Ascorbate peroxidase and catalase activity in this study also increased with an increase in $FeSO_4$ concentration. Increased catalase activity in the present study agrees with the findings of previous studies on other plants, e.g. Catalase changes H_2O_2 into water and molecular oxygen. The main function of catalase is metabolizing peroxide decomposition in peroxisome after converting glycolate through photorespiration (Qureshi et al., 2007).

Iron absorption in plants depends on its concentration in nutrition solution. Accumulation of iron in roots and shoots of soybeans under study increased with an increase in $FeSO_4$ concentration. Plants behave differently in their absorption of heavy elements and distribution of these elements in their tissues. In this study, iron concentration in roots was more than in shoots and as $FeSO_4$ concentration in the nutrition solution increased, so did iron content in leaves and roots. This is in agreement with the studies reported on strawberry cultivars (Erdal et al., 2004), wheat (Kalian et al., 1992), rice (Mehraban et al., 3008), and green peas (Del Rio, 1978).

Table 1
Comparison of the number of seeds, pods, leaves, and nodules in soybean

	control	100 mg/L	200 mg/L	300 mg/L
Number of seeds	0± 0 d	133/3± 56/08 c	511± 27/87 b	711± 32/45 a
Number of pods	54/3± 19/6 d	66/6± 28/04 c	168/6± 10/78 b	238± 12/12 a
Number of nodules	19± 3/605 b	40/66±4/16 a	4/66± 1/52 c	0± 0 d
Number of leaves	107± 10/53 c	142/3± 18/14 a	111/3± 6/65 b	31/6± 10/5 d

Table 2
Comparison of mean catalase, ascorbate peroxidase and superoxide dismutase enzymes activity

	Control	100 mg/L	200 mg/L	300 mg/L
Root catalase activity	0.25 ± 0.02 d	0.34±0.004 b	0.29±0.001 c	0.44 ± 0.11 a
Shoot catalase activity	1.14 ±0.005 c	1.54 ±0.03b	1.58 ±0.005 b	2.3± 0.01 a
Root superoxide dismutase activity	7/83±0/76 c	10 ±0/5 b	9/7±0/4 b	12±0/49 a
Shoot superoxide dismutase activity	10/04±0/48 d	20/98±0/88 c	44/8 ±0/4 b	60 ±2/003 a
Root ascorbate peroxidase activity	0/26 ±0/003d	0/29±0/001 c	0/32±0/003 b	0/44± 0/02 a
Shoot ascorbate peroxidase activity	1/64 ±0/52 c	2/06±0/01b	2/9± 0/004 a	3/03 ±0/06 a

Table 3
Mean iron content in soybean roots and shoots

	control	100 mg/L	200 mg/L	300 mg/L
Root Iron Content	592/6±5/3 d	765/8 ±2/5 c	846/6±12/5 b	996/9± 2/6 a
Shoot Iron Content	342/5±2/2 d	437/7± 15/8 c	540/1± 3/8 b	691/1± 3/9 a

References

Acosta M., P. M. Lcivello, A. R. Chaves and **G. A. Martinez.** 2005. 'Effect of ethephon and 6-benzylaminopurine on chlorophyll degrading

enzymes and peroxidase-linked chlorophyll bleaching during post-harvest senescence of broccoli (*Brassica oleracea* L.) at 20 °C'. *Postharvest Biology and Technology* 35: 191–199.

Abdelmajid K., M. Gharsalli and **C. Abdelly**. 2003. 'Differences in response to iron deficiency among some lines of common bean'. *Journal of Plant Nutrition*, 26(10 & 11): 2295-2305.

Abd El-Rahman S. Z., H. S. El-Beltagi and **EL-Hariri D. M.** 2009. 'Effect of Fe deficiency on antioxidant system in leaves of three flax cultivars . *Academic Press,* 1(3)122-128.

Awlad H. M., M. A. H. Chowdhury and **N. M. Talukder.** 2003**.** 'Effect of Sulphur and Zinc on nodulation , dry matter yield and nutrient content of soybean'. *Pakistan Journal of Biological Sciences*, 6(5): 461 – 466.

Basavarajappa, R. 1992.' Response of cotton cv. Abaditha (*Gossypium hirsutum* L.) to soil and foliar application of micronutrients under rainfed conditions'. *M.Sc. (Agri.) Thesis*, Univ. Agric. Sci., Dharwad.

Bowler C. M. Van Montagu and **D. Inzé** .1992**.** 'Superoxide dismutase and stress tolerance'. *Annual Rev Plant Physiol Plant Mol Biol*. 116: 83-43

Cataldo D. A., K. M. McFadden, T. R. Garland and **R. Wildung.**1988. 'Organic constituents and complexation of nickel (II), iron (III), cadmium (II), and plutonium (IV) in soybean xylem exudates'. *Plant Physiol.* 86(3):734-739.

Halliwell, B. and **J. M. Gutteridge** .1987. 'Protection against oxidants in biological system: the superoxide theory of oxygen toxicity'. In: Halliwell, B. and Gutteridge, J. M. (eds.). Free Radicals in Biology and Medicine. 86-123.Clarendon Press. Oxford.

Kuzhandaivel H. and **S. Venkatesan.** 2011. 'Impact of iron toxicity on certain enzymes and biochemical parameters of tea'. *Asian Journal of Biochemistry,* 6(5) : 384-394.

Kerkeb, L. and **E. Connolly. 2006**. 'Iron transport and metabolism in plants'. *Genet. Eng.* 27: 119-140.

Kosegarten H. ,B. Hoffmann and **K. Mengel** .**1999.** Apoplastic pH and Fe^{3+} reduction in intact sunflower leaves. *Plant Physiol,* 121: 1-11.

Lalit Bhatt B., K. Srivastava and **M.P. Singh.** 2004**.** 'Studies on the effect of foliar application of micronutrients on growth, yield and economics of tomato (*Lycopersicon esculentum* Mill.)'. *Progressive Horticulture*,36(2) :331-334.

Li H., X. Yang and **A. Luo**.2001. 'Ameliorating effect of potassium on iron toxicity in hybrid rice'. *J Plant Nutr*. 24: 1849-1860.

Lozak A., K. Soltyk, P. Ostapczuk and **Z. Fijalek .** 2002 **.** 'Determination of selected trace elements in herbs and their infusions'. *Sci. Total Environ.* 289: 33-40.

Pooyan M. A. Abdol Zadeh and **R. Sadeghipour.** 2008. 'Iron toxicity in rice (*Oryza sativa* L.), under different Potassium nutrition'. *Asian Journal of plant sciences*. 7(3) :251-259.

Minotti, G. and **S. D. Aust** .1987. 'The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen Peroxide'. *J. Biol. Chem.* 262 :1098-1104.

Nakono, G. and **K. Asada.** 1981. 'Hydrogen peroxide is scavenged by ascorbate - specific peroxidase in spinach chloroplasts'. *Plant Cell Physiol*.22 :867-880.

Periera, G. J. G., S. M. G. Molina, P. J. Lea and **R. A. Azevedo.** 2002. 'Activity of antioxydant enzyme in response to cadmium in *Crotalaria juncea*'. *Plant soil*, 239 :123-132.

Polle, A. and **H. Rennenberg.** 1993. 'Significans of antioxidants in plant adaptation to environmental stress'. In: Plant adaptation to environmental stress, Mansfield, T., L. Fowden and F. Stoddard(Eds.). Chapman and Hall, London, 263-273.

Qureshi M. I., M. Z. Abdin. S. Qadir and **M. Iqbal .**2007**.** 'Lead induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. *Biol Plantarum* ,51: 121-128.

Sairam, R. K. and **G. C. Srivastava.** 2001. 'Water stress tolerance of wheat *Triticum aestivum* L.: Variation in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotype'. *Journal of Agronomy and Crop Science,* 186: 63-70.

Singh, A. L., Y. C. Joshi, V. Chaudhari and **P. V. Zala.** 1995**.** 'Effect of different sources of iron and sulphur on leaf chlorosis, nutrient uptake and yield of groundnut'. *Fertilizer Research*, 24 :85-96.

Sun, B., Y., K. Jing, L. Chen, F. Song, L. Chen and **L. Zhang** .2007**.** 'Protective effect of nitric oxide on iron deficiency-induced oxidative stress in maize (*Zea mays*)'. *J. Plant Physiol*. 164 :536-543.

Vadez, V., T. R. Sinclair, R. Serraj and **L. C. Purcell.** 2000**.** 'Manganese application alleviates the water deficit-induced decline of N2 fixation'. *Plant, Cell and Environment*, 23 :497-505.

Zhang F.S., V. Römheld and H. **Marschner.**1991**.** 'Role of the root apoplasm for iron acquisition by wheat plants'. *Plant Physiol*. 97 :1302-1305.