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# STRATEGIES OF COPPER TOLERANCE IN ROOT AND SHOOT OF BROAD BEAN (*Vicia faba* L.)

**Arafat Abdel Hamed Abdel Latef[1,2,*] and Mona Fawzy Abu Alhmad[1]**

**[1]Department of Botany, Faculty of Science, South Valley University, Qena, Egypt;**
**[2]Department of Biology, Faculty of Education, Sebha University, Libya**
**[*]Corresponding author's e-mail: moawad76@gmail.com**

Changes in dry mass and some physiological parameters were investigated in root and shoot of broad bean (*Vicia faba* L.) exposed to different concentrations of $CuSO_4$ (0, 100, 200, 300 and 400 mg $kg^{-1}$) for 15 days. Low Cu concentration did not affect dry mass of root and shoot. However, the negative effect of copper on dry mass was noticed in root than shoot especially at the higher concentrations of copper due to the accumulation of Cu was higher in root than that in shoot. Cu induced changes in the concentration of photosynthetic pigments, Zn, K and Ca. Exposure to Cu induced the accumulation of organic solutes (soluble sugar, soluble protein and total free amino acids) in root than shoot. Level of malondialdehyde (MDA) was correlated with the concentration of Cu in broad bean tissues. Copper stimulated the activity of superoxide dismutase (SOD) and peroxidase (POD) and this stimulation was more obvious in shoot than root.
**Keywords:** Antioxidant enzymes, copper, malondialdehyde, peroxidase, superoxide dismutase, *Vicia faba* L.

## INTRODUCTION

Copper (Cu) is an essential micronutrient for normal plant growth and affects in several physiological processes such as photosynthesis, respiration, distribution of carbohydrates, protein metabolism and antioxidant activity. The plant cells need to maintain the copper concentrations at low levels due to copper at high concentrations becomes toxic as it interferes with photosynthesis and respiration processes, the synthesis of chlorophyll and protein, enzyme activity and membrane integrity, all of which could lead to growth inhibition (Yruela, 2005; Gao *et al*., 2008; Azooz *et al*., 2012).

The excess of copper induced the formation of reactive oxygen species (ROS). ROS include the superoxide anion radical ($O_2^-$), hydroxyl radical (-OH), hydrogen peroxide ($H_2O_2$), and singlet oxygen ($^1O_2$), all of which can pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation and ultimately leading to death of the cells. Plants possess enzymatic mechanism for scavenging of ROS. The antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD) have a vital role to scavenge ROS and thereby prevent oxidative injury (Asada, 1999; Abdel Latef and Chaoxing, 2011).

Copper toxicity is related to disturbances in the uptake of other essential elements (Azooz *et al*., 2012). Ouzounidou *et al*. (1995) and Mocquot *et al*. (1996) reported synergistic, antagonistic or no effect of copper on the uptake of macro and micronutrients, depending on crop species and concentration of copper.

The objectives of this study were (1) to study the tolerance and accumulation of Cu in root and shoot of broad bean (*Vicia faba* L.) plants and (2) to investigate some physiological parameters responsible for Cu tolerance in both root and shoot of broad bean plants.

## MATERIALS AND METHODS

The seeds of broad bean (*Vicia faba* L.) were surface sterilized by immersion in a mixture of ethanol 96% and $H_2O_2$ for 3 min, followed by several washings in sterile distilled water. A greenhouse experiment was conducted in weighed plastic pots containing one kg of well mixed air-dried soil. Copper was applied at the concentrations of 0, 100, 200, 300 and 400 mg $kg^{-1}$ of soil in the form of copper sulphate ($CuSO_4.5H_2O$). The treatments were replicated 3 times in a completely randomized block design. Carefully selected uniform sized broad bean seeds were sowed in each pot (5 seeds/pot). After 15 days, the plants were harvested.

At harvest, root and shoot were separately washed with tap water to remove any adhering debris. The root and shoot dry mass were determined after oven-drying at 70°C for 48 h.

The concentrations of chlorophyll a (Chl. a), chlorophyll b (Chl. b) and carotenoids of youngest fully-expanded leaf 1 were assayed according to Zhang and Zhang (2006). The extraction was made from a 200 mg fresh sample in 20 mL ethanol, acetone and water (4.5: 4.5: 1, v/v/v) mixture and measured at 645 nm, 663 nm and 470 nm with spectrophotometer (*Spectronic Genesys* ZPC, Rochester, NY, USA). Soluble sugar of root and shoot was determined by the anthrone sulfuric acid method described by Badour (1959). The dried tissue of root and shoot was extracted by distilled water. 1 mL of the sugar extract was mixed with 9

mL of anthrone sulfuric acid reagent in a test tube and heated for 7 min at 100°C. The absorbance was measured spectrophotometrically at 620 nm against blank containing only distilled water and anthrone reagent. Soluble protein of root and shoot was determined according to the method described by Bradford (1976), in which 5 mL of the protein reagent were added to 0.1 mL of the extract and the contents mixed on a vortex mixer. The absorbance was measured at 595 nm after 1 h. The concentration of soluble protein was calculated from a previously constructed standard curve for bovine serum albumin. Total free amino acids of root and shoot were extracted and estimated according to the method of Lee and Takahashi (1966). About 0.1 mL of the water extract containing free amino acids was mixed with 1.9 mL of ninhydrin-citrate-glycerol mixture in a test tube for 20 min at 100°C. The absorbance was measured at 570 nm against a blank (only distilled water and the same reagent). For mineral composition determination (Cu, Zn, K and Ca), dried samples of root and shoot were ground and digested in concentrated acid ($HNO_3$: $HClO_4$, 2:3 v/v) at 140–160°C. After cooling, the extracts were diluted with 1 M HCl and made up to 25 mL (Allen, 1989) and analyzed by atomic absorption spectrometry. Malondialdehyde (MDA) was measured according to the thiobarbituric acid (TBA) reaction as described by Zhang and Qu (2004). Root and shoot samples were homogenized with 5% trichloroacetic acid and centrifuged at 4,000 g for 10 min. 2 mL of extract was added to 2 mL 0.6% TBA placed in a boiling water bath for 10 min, and absorbance was read at 532, 600, and 452 nm. The MDA content was calculated according to the formula: $6.45\times (A_{532}-A_{600}) - 0.56\times A_{450}$. For enzyme extracts and assays, 500 mg fresh root and shoot were frozen in liquid nitrogen and then ground in 4 mL solution containing 50 mM phosphate buffer (pH 7.0), 1% (w/v) polyvinylpolypyrrolidone, and 0.2 mM ascorbic acid. The homogenate was centrifuged at 15, 000 g for 30 min, and the supernatant was collected for enzyme assays. The activity of superoxide dismutase (SOD, EC 1.15.1.1) of root and shoot was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), according to Stewart and Bewley (1980). The reaction mixture (3 mL) contained 13 mM methionine, 75 mM NBT, 100 mM EDTA, 50 µL of enzyme extract within 50 mM phosphate buffer (pH 7.8). The reaction was started with 2 mM riboflavin by exposing the cuvette to a 15-W fluorescent tube for 10 min. The absorbance of each reaction mixture was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT. Peroxidase (POD, EC 1.11.1.7) activity of root and shoot was measured by following the change of absorption at 470 nm due to guaiacol oxidation. The activity was assayed for 1 min in a reaction solution (3 mL final volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM $H_2O_2$ and 0.15 mL enzyme extract (Polle *et al*., 1994).

***Statistical Analysis*:** Experimental data were subjected to one way analysis of variance and the means were separated by the least significant difference (LSD) (Steel and Torrie, 1960).

## RESULTS

The data in Table 1 showed that, the dry mass of root and shoot did not change significantly up to the level of 100 mg $k^{-1}$ $CuSO_4$, beyond this concentration a significant reduction was recorded compared to control plants especially at the highest dose of copper (400 mg $k^{-1}$) and in root than shoot. The reduction in dry mass of root and shoot at the highest dose of Cu was 56 % and 42 % respectively versus the control.

The concentrations of Chl. a and Chl. b gradually decreased by increasing the copper concentration in the soil. This inhibitory effect was more pronounced at the highest dose of copper and in Chl. b (40 %) than Chl. a (25%) versus the control (Table 1). On the other hand, the concentration of carotenoids gradually increased by increasing the copper concentration in the soil. The maximum increase in carotenoids was recorded at the highest level of Cu. This increase was 49 % over the control (Table 1).

The results in Table 2 showed that, there was a significant accumulation in the concentration of soluble sugar in root of broad bean as a result of addition Cu to the soil. The maximum accumulation of soluble sugar was 2.6 fold at the highest level of Cu as compared to control plants (Table 2). In shoot, the concentration of soluble sugar gradually

**Table 1. Effect of different concentrations of copper on dry mass (g plant$^{-1}$), chlorophyll (Chl. a and Chl. b) and carotenoids concentration (mg g$^{-1}$ FW.) of broad bean (*Vicia faba* L.)**

| Treatment $CuSO_4$ (mg $kg^{-1}$) | Dry mass root | Dry mass shoot | Chl. a | Chl. b | Carotenoids |
|---|---|---|---|---|---|
| 0 | 0.025 | 0.217 | 0.77 | 0.32 | 1.18 |
| 100 | 0.023 | 0.214 | 0.73 | 0.30 | 1.35 |
| 200 | 0.020 | 0.180 | 0.66 | 0.26 | 1.44 |
| 300 | 0.014 | 0.140 | 0.62 | 0.24 | 1.62 |
| 400 | 0.011 | 0.125 | 0.58 | 0.19 | 1.76 |
| LSD 5% | 0.003 | 0.036 | 0.08 | 0.05 | 0.18 |

**Table 2. Effect of different concentrations of copper on soluble sugars, soluble protein and total free amino acids concentration (mg $g^{-1}$ DW) of broad bean (*Vicia faba* L.)**

| Treatment $CuSO_4$ (mg $kg^{-1}$) | Soluble sugar root | Soluble sugar shoot | Soluble protein root | Soluble protein shoot | Total free amino acid root | Total free amino acids shoot |
|---|---|---|---|---|---|---|
| 0 | 24.95 | 38.88 | 46.35 | 107.10 | 2.08 | 4.06 |
| 100 | 35.64 | 45.87 | 77.40 | 142.64 | 2.12 | 4.38 |
| 200 | 46.98 | 51.32 | 99.45 | 144.45 | 2.94 | 4.71 |
| 300 | 54.35 | 62.89 | 88.20 | 145.35 | 3.13 | 4.85 |
| 400 | 66.74 | 68.04 | 105.75 | 166.95 | 3.54 | 5.08 |
| LSD 5% | 5.76 | 6.98 | 14.09 | 20.23 | 0.31 | 0.79 |

increased under the treatment with copper. As in root, the maximum accumulation of soluble sugar in shoot of broad bean was recorded at the highest level of Cu (75 %) over control plants (Table 2).

Application of copper in the soil significantly accumulated the concentration of soluble protein in root of broad bean. The maximum accumulation of soluble protein in root was 2.3 fold at the highest level of Cu versus untreated plants (Table 2). In shoot, the treatment with copper increased the soluble protein concentration as compared to the control. This increase was approximately similar at the levels from 100 to 300 mg $kg^{-1}$ $CuSO_4$. The highest level of Cu was recorded the highest value of soluble protein (56 %) over the control (Table 2).

The lowest level of Cu remained the concentration of total free amino acids as control in root of broad bean, above this level a significant accumulation in the concentration of total free amino acids was recorded. The highest value of total free amino acids was recorded at the highest level of Cu (70 %) over the control (Table 2). In shoot, the concentration of total free amino acids was slightly increased under the treatment with Cu and the highest value was recorded at the highest level of Cu (25 %) over the control. This increase in total free amino acids in shoot of broad bean was non-significant at most copper levels (Table 2).

The concentration of Cu significantly accumulated in root and shoot of broad bean as a result of addition Cu to the soil. This increase was much noticed in root than shoot at all used concentrations of Cu. At the highest level of Cu, the concentration of Cu was 2 times in root versus shoot (Table 3).

The concentration of Zn in root of broad bean remained unchanged up to the level of 100 mg $k^{-1}$ $CuSO_4$, then a sudden drop in Zn concentration was recorded with the rise of Cu in the soil. In shoot, there was non-significant change in the concentration of Zn up to the level of 200 mg $k^{-1}$ $CuSO_4$, after that a significant decrease was recorded in comparison to the control (Table 3).

The treatment with copper up to the level of 200 mg $k^{-1}$ $CuSO_4$ induced non-significant change in the concentration of K in root of broad bean, while a drastic reduction was recorded at the higher levels of Cu (Table 3). In shoot, the levels from 0 to 300 mg $kg^{-1}$ $CuSO_4$ induced non-significant change with trend to increase in K concentration. At the highest level of Cu, a significant increase in K concentration and this increase was 28 % over the control (Table 3).

The copper treatments retarded the transport and accumulation of calcium in root and shoot of broad bean. This inhibitory effect was equal in root and shoot at the levels of 100 (about 25%) and 200 mg $k^{-1}$ $CuSO_4$ (about 35%) as compared to control (Table 3).

The data in Table 4 showed that, MDA concentration was significantly accumulated in root at all copper levels as compared to control. In shoot, the accumulation of MDA concentration was significantly at moderate and higher copper concentrations of soil. However, the lowest copper concentration had non-significant increase in MDA (Table 4). At the highest level of Cu, the accumulation of

**Table 3. Effect of different concentrations of copper on the concentration of Cu (µg $g^{-1}$ DW), Zn (µg $g^{-1}$ DW), K (mg $g^{-1}$ DW) and Ca (mg $g^{-1}$ DW) of broad bean (*Vicia faba* L.)**

| Treatment $CuSO_4$ (mg $kg^{-1}$) | Cu root | Cu shoot | Zn root | Zn shoot | K root | K shoot | Ca root | Ca shoot |
|---|---|---|---|---|---|---|---|---|
| 0 | 24.23 | 20.45 | 71.43 | 36.00 | 9.51 | 11.37 | 4.72 | 9.62 |
| 100 | 45.35 | 29.56 | 69.86 | 35.50 | 8.99 | 11.56 | 3.51 | 7.35 |
| 200 | 82.00 | 33.21 | 50.63 | 32.12 | 8.35 | 11.93 | 3.05 | 6.33 |
| 300 | 99.00 | 42.76 | 47.50 | 28.95 | 6.47 | 12.77 | 2.78 | 4.64 |
| 400 | 112.00 | 54.67 | 40.00 | 27.82 | 5.31 | 14.54 | 2.16 | 3.38 |
| LSD 5% | 11.87 | 4.91 | 4.77 | 6.74 | 1.14 | 1.51 | 0.99 | 1.05 |

**Table 4. Effect of different concentrations of copper on Cu concentration on malondialdehyde (MDA) concentration (nmol $mg^{-1}$FW), superoxide dismutase (SOD) and peroxidase (POD) activity ($Umg^{-1}$ FW) of broad bean (*Vicia faba* L.)**

| Treatment $CuSO_4$ (mg $kg^{-1}$) | MDA root | MDA shoot | SOD root | SOD shoot | POD root | POD shoot |
|---|---|---|---|---|---|---|
| 0 | 6.20 | 9.83 | 5.12 | 6.98 | 7.30 | 15.10 |
| 100 | 10.46 | 10.04 | 5.58 | 7.91 | 8.12 | 18.99 |
| 200 | 22.24 | 13.45 | 6.66 | 12.87 | 8.79 | 26.85 |
| 300 | 20.31 | 12.96 | 7.46 | 16.59 | 9.87 | 34.00 |
| 400 | 46.97 | 16.12 | 8.09 | 17.36 | 11.99 | 37.00 |
| LSD 5% | 2.01 | 1.16 | 1.03 | 3.55 | 1.44 | 3.32 |

MDA in root was 2.9 times versus shoot (Table 4).

The activity of SOD and POD in root and shoot of broad bean was increased as a result of treatment with Cu as compared to control (Table 4). This increase was significant at the moderate and higher levels of copper. At the highest concentration of Cu, the increase in SOD activity in root and shoot was 58 % and 149 % respectively over the control (Table 4). At the same level of Cu, the increase in POD activity in root and shoot was 64 % and 145 % respectively versus control plants (Table 4).

## DISCUSSION

The results of this study showed that the higher concentrations of Cu caused significant reduction in dry mass of root and shoot of broad bean. This inhibitory action of excess copper in root and shoot growth is due to inhibition of both cell elongation and division (Arduini *et al*., 1994; Khatun *et al*., 2008; Azooz *et al*., 2012). It is worthy to mention that, the reduction in dry mass was obvious in root than shoot. This could explain, in part due to the accumulation of Cu was more noticed in root than shoot. Previous studies in other plant species have been reported similar results (Hong-yun, 2005; Rahman *et al*., 2011).

Excess of copper caused a decrease in Chl. a and Chl. b of leaf, but this decrease was more in Chl. b than Chl. a, this indicates that Chl. b is more affected than Chl. a. Many studies found chlorophyll reduction by copper treatment (Zengin and Kirbag, 2007; Khatun *et al*., 2008; Ahmed *et al*., 2010). The reduction of chlorophyll in broad bean under copper treatment may be due to inhibition of enzymes acting in chlorophyll synthesis or to degradation of chlorophyll (Mourato *et al*., 2009). On the other hand, increasing concentration of copper enhanced the concentration of carotenoids might be an indication of non-enzymatic antioxidant defense (Ahmed *et al*., 2010).

In this study, copper treatment accumulated soluble sugar concentration in root more than shoot. Similarly Alaoui-Sossé *et al.* (2004) reported Cu induced notable accumulation in soluble sugar in cucumber plants. This accumulation of soluble sugar especially in root of broad bean could possibly provide an adaptive mechanism maintaining favorable osmotic potential under Cu toxicity.

The soluble protein concentration was noticed to be enhanced in root more than shoot of broad bean with increase in the concentration of copper sulphate as compared to control. This increase in soluble protein concentration under excess of copper may be due to increase in metal binding protein and this increase of soluble protein in root may be due the defense mechanism towards metal stress. Similar observation has been reported in banana by Deo and Nayak (2011).

In this study, the accumulation of soluble sugar and soluble protein in root especially at the higher doses of copper was linked to the marked depression of growth. This might confirm the ability of root to synthesis of diverse protein and carbohydrates from state of active growth to state of survival (Abdel Latef *et al*., 2009).

Total free amino acids concentration increased under copper treatment, and this increase was higher in root than shoot, indicating the role of amino acids in detoxification of copper stress due to amino acids play significant role in metal chelation (Hall, 2002; Xiong *et al*., 2006; Azooz *et al*., 2012). Thus, it could be concluded that to tolerate the presence of excess Cu in broad bean plants, the root tissues may react by synthesis and accumulation of soluble sugars, soluble proteins and amino acids more than shoot.

The concentration of Cu was significantly higher in root than shoot, and increased significantly with increasing supplied concentrations of the metal. Similarly, Lin *et al*. (2003) and Rahman *et al*. (2011) reported that Cu concentration on plant tissues increased with the increase in its concentration in the nutrient solution and with the length of exposure period, and that Cu concentration in the roots is generally higher than that in the aerial parts. According to Yurekli and Porgali (2006), the higher accumulation of copper results from a tolerance mechanism developed by the plant in order to reduce the effect of heavy metal stress.

Excess copper mostly decreased the uptake of Zn, K and Ca in broad bean plants. Therefore it could be concluded that copper had antagonistic effect rather than synergistic effect on the uptake of Zn, K and Ca. Many reports (Bouazizi *et al*., 2010; Lequeux *et al*., 2010; Manivasagaperumal *et al*., 2011) stated the deficiency of various mineral contents

under copper toxicity. On the other hand, the increase in K concentration at the highest level of Cu in the shoot may be due to K efflux as part of a mechanism of Cu tolerance.

Copper stress causes physiological disorders of plants and induces the production of ROS (Abdel Latef, 2011). The accumulation of ROS breaks the balance between ROS production and the capacity of plants to scavenge for them, which induces destructive oxidative processes such as membrane lipid peroxidation and protein oxidation (Abdel Latef, 2011). MDA is a product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Rahman *et al*., 2011). Therefore, MDA concentration in the root and shoot of broad bean plants was measured under copper stress. Under copper stress, MDA concentration increased in root and shoot of broad bean. Similar results were observed in root and leave of *Kandelia candel* (Rahman *et al*., 2011) who reported that Cu treatments enhanced the generation of $H_2O_2$ and then caused lipid peroxidation as indicated by increases in the MDA concentrations. Increased MDA levels indicate the occurrence of oxidative stress, and this may be one of the possible mechanisms generating toxicity due to Cu stress in plant tissues. It is worthy to mention that, the MDA concentration in shoot remained lower than that root. The lower MDA in shoot versus root indicated that shoot had a better protection against oxidative damage caused by Cu treatment. SOD plays central role in defense against oxidative stress in all aerobic organisms and is a key antioxidative enzyme that catalyzes the disproportionation of $O_2^-$ to form $H_2O_2$ and $O_2$ (Scandalios, 1993). POD is widely distributed in plants and is one of the key enzymes that modulate the breakdown of $H_2O_2$. POD activity has been suggested as a potential biomarker for sub lethal metal toxicity in plant tissues (Rahman *et al*., 2011). Many studies confirmed that excess copper could promote the production of ROS leading to increase in the activity of antioxidant enzymes as a defense system (Abdel Latef, 2011; Verma *et al*., 2011; Azooz *et al*., 2012). This is in harmony with our results which showed that growing of broad bean in excess copper caused increase in the activity of SOD and POD which can be considered as evidence of stimulation ROS production. The higher activity of SOD and POD in shoot is an indication of shoot ability to cope with ROS. Therefore, the increase recorded in the activity of SOD and POD may be attributed to the adaptive defense system of broad bean organs (especially shoot) against the toxic effect imposed by copper.

In conclusion, the results of this study showed that copper concentrations greatly affected growth, leaves photosynthetic pigments, organic solutes, Cu, Zn, K and Ca accumulation, MDA concentration and SOD and POD activity in root and shoot of broad bean (*Vicia faba* L.). The strategies of root and shoot to cope with copper stress depended on accumulation of organic solutes and oxidative stress defense mechanisms. Root response to high copper concentrations involved mainly soluble sugar, soluble protein and total free amino acids, while shoot response involved mainly SOD and POD enzymes.

## REFERENCES


Abdel Latef, A.A. 2011. Influence of arbuscular mycorrhizal fungi and copper on growth, accumulation of osmolyte, mineral nutrition and antioxidant enzyme activity of pepper (*Capsicum annuum* L.). Mycorrhiza. 21:495-503.

Abdel Latef, A.A. and H. Chaoxing. 2011. Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. Acta Physiol. Plant. 33:1217-1225.

Abdel Latef, A.A., M.A.K. Shaddad, A.M. Ismail and M.F. Abu Alhmad. 2009. Benzyladenine can alleviate saline injury of two roselle (*Hibiscus sabdariffa*) cultivars via equilibration of cytosolutes including anthocyanins. Int. J. Agric. Biol. 11:151-157.

Ahmed, H., A. Hasanain, S. Akhtar, A. Hussain, Abaid-ullah, G. Yasin, A. Wahid and S. Mahmood. 2010. Antioxidant enzymes as bio-markers for copper tolerance in safflower (*Carthamus tinctorius* L.). Afr. J. Biotech. 9:5441-5444.

Alaoui-Sossé, B., P.F. Gent, M.L. Vinit-Dunand, D. Tooussaint, P. Epron and M. Badot. 2004. Effect of copper on growth in cucumber plants (*Cucumis sativus*) and its relationships with carbohydrate accumulation and changes in ion content. Plant Sci. 166:1213-1218.

Allen, SE. 1989. Chemical analysis of ecological materials, 2nd Ed. Blackwell, Oxford, UK.

Arduini, I., D.L. Godbold and A. Onnis. 1994. Cadmium and copper change root growth and morphology of *pinus pinea* and *Pinus pineaster* seedlings. Physiol. Plant. 92: 675-680.

Asada, K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygen dissipation of excess photons. Annu. Rev. Plant Phys. 50:601-639.

Azooz, M.M., M.F. Abou-Elhamd and M.A. Al-Fredan. 2012. Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat (*Triticum aestivum* cv. Hasaawi) at early growing stage. Austr. J. Crop Sci. 4:688-694.

Badour, S.S.A. 1959. Analytisch–chemische Untersuchung des Kaliummangels bei Chlorella im Vergleich mit anderen Mangelzuständen. [Analytical-chemical investigation of potassium deficiency in *Chlore lla* in comparison with other deficiencies]. Ph.D. Dissertation, Göttingen University, Göttingen, Germany.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing

the principle of protein binding. Anal. Biochem. 72: 248-254.

Bouazizi, H., H. Jouili, A. Geitmann and E.E.I. Ferjani. 2010. Copper toxicity in expanding leaves of *Phaseolus vulgaris* L.: antioxidant enzyme response and nutrient element uptake. Ecotox. Environ. Safe. 73:1304-1308.

Deo, B. and P.K. Nayak. 2011. Study of copper phytotoxicity in vitro culture of *Musa acuminata* cv. 'Bantala'. J. Agric. Biotech. Sustainable Dev. 3:136-140.

Gao, S., R. Yan, M. Cao, W. Yang, S. Wang and F. Chen. 2008. Effects of copper on growth, antioxidant enzymes and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. Plant Soil Environ. 54:117-122.

Hall, J.L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. 53:1-11.

Hong-yun, P., Y. Xiao-e and T. Sheng-ke. 2005. Accumulation and ultrastructural distribution of copper *in Elsholtzia splendens*. J. Zhej Univ. Sci. 6:311-318.

Khatun, S., M.B. Ali, E.J. Hahn and K.Y. Paek, 2008. Copper toxicity in *Withania somnifera*: Growth and antioxidant enzymes responses of in vitro grown plants. Environ. Exp. Bot. 64:279-285.

Lee, Y.P. and T. Takanashi. 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. Anal. Biochem. 14:71-77.

Lequeux, H., C. Hermans., S. Lutts and N. Verbruggen 2010. Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol. Biochem. 48:673-682.

Lin, J., W. Jiang and D. Liu. 2003. Accumulation of copper by roots, hypocotyls, cotyledons and leaves of sunflower (*Helianthus annuus* L.). Bioresource Technol. 86:151-155.

Manivasagaperumal, R., P. Vijayarengan, S. Balamurugan and G. Thiyagarajan. 2011. Effect of copper on growth, dry matter yield and nutrition content of *Vigna Radiata* (L.) wilczek. J. Phytol. 3:53-62.

Mocquot, B., J. Vangronsveld, H. Clijsters and M. Mench. 1996. Copper toxicity in young maize (*Zea mays* L.) plants: effects of growth, mineral and chlorophyll contents and enzyme activities. Plant Soil 182:287-300.

Mourato, M.P., L.L. Martins and M.P. Campos-Andrad, 2009. Physiological responses of *Lupinus lutetus* to different copper concentrations. Biol. Plant. 53:105-111.

Ouzounidou, G., M. Ciamporova, M. Moustakas and S. Karrtaglis. 1995. Response of maize plants to copper stress I: Growth, mineral content and ultrastructure of roots. Environ. Exp. Bot. 35:167-176.

Polle, A., T. Otter and F. Seifert. 1994. Apoplastic peroxidases and lignification in needles of Norway (*Picea abies* L.). Plant Physiol. 106:53-60.

Rahman, M.M., Y. Chongling, M.D. M. Rahman and K.S. Islam. 2011. Effects of copper on growth, accumulation, antioxidant activity and malondialdehyde content in young seedlings of the mangrove species *Kandelia candel* (L.). Plant Biosyst. 1:1-11.

Scandalios, J.G. 1993. Oxygen stress and superoxide dismutases, Plant Physiol. 101:7-12.

Steel, R.G. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co, New York, USA.

Stewart, R.C. and J.D. Bewley. 1980. Lipid peroxidation associated with accelerated ageing of soybean axes. Plant Physiol. 65:245-248.

Verma, J.P., V. Singh and J. Yadav. 2011. Effect of copper sulphate on seed germination, plant growth and peroxidase activity of mung bean (*Vigna radiate*). Int. J. Bot. 7:200-204.

Xiong, Z.T., C. Liu and B. Geng. 2006. Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. Ecol. Environ. Safety. 64:273-280.

Yruela, I. 2005. Copper in plants. Braz. J. Plant Physiol. 17:145-156.

Yurekli, F. and Z.b. Porgali. 2006. The effect of excessive exposure to copper in bean plants. Acta Biol. Cracoviensia Ser. Bot. 48:7-13.

Zengin, F.K. and S. Kirbag. 2007. Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings. J. Environ. Biol. 28:561-566.

Zhang, Z.A. and M.S. Zhang. 2006. Experimental guide for plant physiology. High Education, Beijing, China.

Zhang, Z.L. and W. Qu. 2004. Experimental guidance of plant physiology. High Education, Beijing, China.