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SHORT COMMUNICATION

COPPER TOXICITY CAUSES OXIDATIVE STRESS IN *BRASSICA JUNCEA* L. SEEDLINGS

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A relationship between Cu^{2+} ion toxicity and oxidative stress was investigated in *Brassica juncea* L. seedlings by treating with different concentrations of copper (0.2, 0.4 0.6 and 0.8 mM). A uniform decrease in germination, biomass, root and shoot elongation with increasing concentrations marked as the primary signs of copper injury. Seed germination was completely inhibited at 0.8 mM copper. An increasing concentration of copper treatment showed an uniform decrease in chlorophylls and â-carotene composition with a significant accumulation of free proline suggesting an osmoprotection from copper. Copper stress resulted, an increase in lipid peroxidation with increasing copper concentrations. The increase in total peroxide content was accompanied by a decrease in catalase (CAT) and superoxide dismutase (SOD) activity at 0.4 and 0.6mM copper. However, peroxidase (POX) and glutathione reductase (GR) activities increased with increasing copper concentrations. The glutathione, ascorbate and polyphenol contents showed a decrease at a higher metal concentration. These results suggested an induction of oxidative stress in *Brassica juncea* L. seedlings under copper toxicity.

***Key words: Brassica juncea*, copper, oxidative stress, toxicity.**

The inhibition of plant growth and crop production by excess heavy metals such as copper, cadmium, zinc or nickel in contaminated soil is a global agriculture problem. In agriculture, copper is extensively used in the form of fertilizers, growth promoters, fungicides and pesticides (Adriano 1986). Copper is an essential micronutrient of higher plants and plays a vital role in maintaining normal metabolism. It also participates in an electron transfer reactions of photosynthesis in the form of plastocyanin (Raven *et al.* 1999). However, copper at high level becomes strongly phytotoxic to cells and causes an inhibition of plant growth or even death. Studies from some plant species demonstrated that excess of copper in plant growth medium induces formation of reactive oxygen species (ROS) in treated tissues (Groppa *et al.* 2001). Copper induced generation of hydrogen peroxide, hydroxyl radicals or other reactive oxygen species has been directly correlated with the damage to proteins and lipids. Photosynthesis is also sensitive to excessive copper; the pigments and protein components of photosynthetic membranes are the targets (Patsikka *et al.* 2002). In addition, copper toxicity is related to disturbances in the uptake of other essential elements (Van Assche and Clijsters 1990).

The plant antioxidative defense system involves several enzymes and low molecular weight quenchers that are present in plant cells are converted to hydrogen peroxide by the action of superoxide dismutase. Accumulation of hydrogen peroxide a strong oxidant is prevented in the cell by catalase or ascorbate glutathione cycle, where ascorbate peroxidase reduces it to water

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(Iannelli *et al*. 2002). The enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) are involved in the detoxification of $O_2^{\bullet-}$ and H_2O_2 respectively, there by preventing the formation of hydroxyl radicals. Ascorbate peroxidase (APX) an important component of ascorbate glutathione cycle involved in H_2O_2 detoxification (Jimenez *et al.* 1997).The toxicity of copper has been related with oxidative damages and alterations in antioxidant systems in *Avena sativa* (Luna *et al*. 1994) and *Phaseolus vulgaris* (Patiskka *et al*. 2002). However, there are very few reports known about phytotoxicity of copper in *Brassica*. The chief use of *Brassica* seeds in India as oil and condiments for the preparation of pickles and vegetables. Keeping this in view, the present study was carried out to investigate phytotoxic role of copper in causing oxidative stress in *Brassica juncaea* seedlings.

Seeds of *Brassica juncea* L. were surface sterilized by rinsing in 0.1% of $HgCl_2$ followed by rinsing with distilled water for 2 – 3 times. After washing thoroughly the seeds were germinated in petri plates containing Whatman no. 1 filter paper. Further, the filter paper was moistened with solution of copper chloride of different concentrations (0.2, 0.4, 0.6 and 0.8 mM). These petri plates were maintained in laboratory conditions at 28 ± 2ºC for 7 days. The number of germinated seeds was counted after 7 days of incubation and the data were expressed as percentage of the total number of seeds used. Root and shoot length were measured after 7 days and the dry mass was determined after keeping them in an oven at 70ºC for 48hrs.

After 7 days of incubation, seedlings were sampled for various biochemical and enzymic estimations. The total chlorophylls and â-carotene content was estimated following the methods of Arnon (1949) and Jensen (1978). Proline content was estimated as per the method of Bates *et al.* (1973) and total peroxide content as per the method of Sagisaka (1976). Estimation of lipid peroxidation or thiobarbituric acid reactive substances (TBARS) was done as per the method of Heath and Packer (1968). For the assay of enzymes, viz. catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and glutathione reductase (GR), the plant material was homogenized with 5 ml of phosphate buffer (pH 6.8) in a pre-chilled mortar and pestle. The extract was centrifuged at 4ºC for 15 min at 15000 rpm. The supernatant obtained was used as the enzyme extract. The CAT activity in the enzyme extract was then estimated according to the method of Teranishi *et al.* (1974). The SOD activity was estimated according to the method of Dhindsa *et al.* (1981). The POD and GR activity was estimated according to the method of Malick and Singh (1980) and Foyer and Halliwell (1976). Further the analysis of ascorbate, glutathione and polyphenols was made by the methods described by Klein and Perry (1982), Griffith (1930) and Malick and Singh (1980) respectively. All the experimental results were analyzed and compared by ANOVA and Duncan's multiple range test (DMRT).

The effect of different concentrations of copper on germination, biomass, root and shoot lengths of 7 days seedlings of *Brassica juncea* L. are shown in Table 1. The seeds in the control and in 0.2 mM copper showed 100% germination. Further increase in copper concentrations led to reduction in seed germination. At 0.8 mM copper concentration, the germination was completely inhibited. Swamy (1996) has reported that the

Table 1. Effect of different concentrations of copper on growth of 7 days old seedlings of *Brassica juncea* L.

Treatment Cu (mM)	Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)
Control	100	$6.02^{a} \pm 0.05$	$7.20^{a} \pm 0.10$	$0.51^{b} \pm 0.90$	$0.07^{a} \pm 0.13$
0.2	100	$4.87^{b} \pm 0.10$	$6.90^{b} \pm 0.80$	$0.624^{a} \pm 0.12$	$0.06^{b} \pm 0.09$
0.4	70	$2.05^{c} \pm 0.02$	$6.08^{c} \pm 0.14$	$0.28^{c} \pm 0.15$	$0.05^{b} \pm 0.03$
0.6	30	$1.08^{c} \pm 0.08$	$4.52^{d} \pm 0.50$	$0.11^{d} \pm 0.10$	$0.02^{cd} \pm 0.08$
0.8	-	-	-	-	-

Each value is expressed as mean ± S.D. (n=3) and statistically significant at $P < 0.05$

higher concentration of chromium reduced seed germination and growth in *Vigna mungo* L. seedlings. It is evident from our results that the root length was adversely affected as compared to shoot length at higher concentrations of copper. Similarly, Fernandes and Henriques (1991) reported that, the differential effect of copper on root and shoot growth is due to the fact that copper is accumulated mainly in roots and a minor extent in shoots. The fresh and dry weight of seedlings was higher at 0.2 mM but it was lowered with increasing concentrations of copper. Similar results were reported in rice plant treated with copper (Dube *et al.* 2005).

The changes in pigments, proline, lipid peroxidation and total peroxide contents in different concentrations of copper on 7 days old seedlings of *Brassica juncea* L. are shown in Table 2. The total chlorophyll content decreased with increasing concentrations. Decrease in chlorophyll content either due to the inhibitory effect of the metal on chlorophyll biosynthesis or plant specific effect, as reported for other metals in different plants (Panda and Patra 2000). The â-Carotein content increased at lower concentration compared to control but it declined in increasing concentrations. It could probably be due to carotenoids degradation as well as inhibition of carotenoids biosynthesis (Moustakas *et al*. 1997). The proline content increased with increasing concentrations of copper may be due to a decrease in water potential with the imposition of heavy metal treatment (Panda and Khan 2003). Alia *et al.* (1995) reported that proline accumulation in plants has the role of detoxification of active oxygen species produced by metal treatment. Similarly, an increase in lipid peroxidation and total peroxide was recorded with the increasing copper concentrations in *Brassica juncea* seedlings. It has been observed that high concentrations of copper increase lipid peroxidation in rice seedlings which may be due to an excessive generation of hydroxyl radicals (Li-Men Chen *et al.*2000, Ramdevi and Prasad 1998).

Table 2. Effect of different concentrations of copper on pigments, proline, lipid peroxidation and total peroxide content in 7 days old seedlings of *Brassica juncea* L.

Treatment Cu (mM)	Total chlorophyll (mg/g)	β- carotene (mg/g)	Proline (mg/100mg)	Lipid peroxidation (µmol g^{-1} fw)	Total peroxide (µmol g^{-1} fw)
Control	$16.94^{a} \pm 0.10$	$0.58^{a} \pm 0.15$	$0.24^{a} \pm 0.10$	$2.92^{a} \pm 0.12$	$0.92^{a} \pm 0.13$
0.2	$14.66^{b} \pm 0.10$	$0.77^{b} \pm 0.10$	$0.34^{b} \pm 0.80$	$3.42^{b} \pm 0.90$	$0.39^{b} \pm 0.09$
0.4	$11.22^{c} \pm 0.30$	$0.42^{c} \pm 0.02$	$0.62^{c} \pm 0.14$	$4.59^{c} \pm 0.15$	$0.43^{b} \pm 0.03$
0.6	$8.13^{d} \pm 0.30$	$0.39^{c} \pm 0.08$	$1.07^{d} \pm 0.50$	$5.76^{d} \pm 0.10$	$0.56^{c} \pm 0.08$
0.8	-	-	-	-	-

Each value is expressed as mean ± S.D (n=3) and statistically significant at $P < 0.05$

Table 3. Effect of different concentrations of copper on antioxidative enzyme activity in 7 days old seedlings of *Brassica juncea* L.

Treatment Cu (mM)	Peroxidase (unit $min^{-1}mg^{-1}$)	Glutathion reductase (unit mg^{-1})	Superoxide dismutase (unit mg^{-1})	Catalase (unit mg^{-1})
Control	$0.13^{a} \pm 0.05$	$0.42^{a} \pm 0.04$	$7.38^{a} \pm 0.13$	$1.08^{a} \pm 0.01$
0.2	$0.26^{b} \pm 0.06$	$0.55^{b} \pm 0.13$	$39.14^{b} \pm 0.09$	$0.82^{b} \pm 0.23$
0.4	$0.68^{c} \pm 0.03$	$0.62^{c} \pm 0.12$	$5.72^{c} \pm 0.09$	$0.64^{c} \pm 0.08$
0.6	$0.64^{c} \pm 0.02$	$0.88^{d} \pm 0.08$	$2.34^{d} \pm 0.07$	$0.35^{d} \pm 0.14$
0.8	-	-	-	-

Each value is expressed as mean ± S.D (n=3) and statistically significant at $P < 0.05$

A uniform increase in peroxidase and glutathione reductase activities paralleled with a gradual decrease in catalase and superoxide dismutase activity was detected with increase in copper concentrations (Table 3). A significant increase in the peroxidase and glutathione reductase activities suggests their role in detoxification of peroxide produced in *Brassica juncea* under copper treatments. Similar findings for copper and other metals in different plants have been reported by other workers (Subramanium 1998, Panda *et al.* 2003). The superoxide dismutase activities increased at lower concentration (0.2 mM) but it declined at higher concentrations (0.4 and 0.6 mM) of copper. A decrease in superoxide dismutase activity in response to copper suggests an inactivation of superoxide dismutase enzyme by free radicals has also been reported by Panda and Patra (2000) in other metals. The CAT activity decreased with increasing concentrations of copper. The decreased activity of catalase may be due to increase in H_2O_2, which in turn inactivated the enzyme. Similar results were also observed in oat leaf segments after exposure to the toxic level of copper (Luna *et al*. 1994).

A small decrease in ascorbate and polyphenol content was visible at 0.6 mM copper concentration, whereas lower copper concentrations did not affect it. A slight increase in glutathione content was seen at 0.2 mM concentration; at 0.4 mM and 0.6 mM concentrations it decreased (Table 4). The decreasing trend in the cellular non-enzymatic antioxidants like polyphenol, ascorbate and glutathione at higher copper concentrations may suggest their inability to detoxify the reactive oxygen species directly (Gallego *et al.* 1996).

Table 4. Effect of different concentrations of copper on polyphenol, ascorbate and glutathion content in 7 days old seedlings of *Brassica juncea* L.

Treatment Cu (mM)	Glutathione (µmol g^{-1} fw)	Ascorbate (µmol g^{-1} fw)	Polyphenol (µmol g^{-1} fw)
Control	$1.53^a \pm 0.005$	$2.14^a \pm 0.007$	$2.63^a \pm 0.03$
0.2	$1.90^b \pm 0.01$	$1.99^b \pm 0.003$	$2.20^b \pm 0.09$
0.4	$0.83^c \pm 0.09$	$1.95^c \pm 0.008$	$2.03^c \pm 0.01$
0.6	$0.78^d \pm 0.02$	$0..80^c \pm 0.014$	$1.50^c \pm 0.02$
0.8	-	-	-

Each value is expressed as mean ± S.D (n=3) and statistically significant at $P < 0.05$.

From the present investigation, it is evident that copper toxicity causes oxidative stress in 7 days old seedlings of *Brassica junceae* L. and that increase in peroxidase and glutathion reductase activity was perhaps to help in detoxifying the H_2O_2 produced in response to copper treatments.

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