



SHORT COMMUNICATION

INFLUENCE OF EXCESS COPPER ON SUGARCANE METABOLISM AND NUTRIENT COMPOSITION

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Single bud setts of sugarcane (*Saccharum Officinarum* hybrid, CoLk 8102) planted in polyethylene pots filled with refined sand in complete nutrient solution at three levels of copper, viz. 0.065 (control), 65.0 and 130 (excess) ppm as copper sulphate. Results obtained showed a decrease in area and length of leaf, plant height, root number and length, fresh and dry weight of different plant parts due to excess Cu supply. Excess Cu decreased chlorophyll a, b and carotenoids contents and catalase activity, while peroxidase activity was increased. A significant reduction in calcium uptake was observed under excess Cu. Cu concentration increased significantly in different plant parts with an increase in Cu supply. High levels of Cu depressed the uptake of Fe and Zn in leaf and shoot. Above findings suggested that excess copper adversely affected growth characteristics, availability of essential nutrients and concentration of chlorophyll and carotenoids pigments which may reduce the net photosynthetic rate and ultimately resulting in low biomass of sugarcane plant.

Key words: Copper, enzyme activity, nutrients, photosynthetic pigments, sugarcane

Copper is one of the essential micro-nutrients. Plants require very small amount of microelements (less than 1 ppm); slight deficiency or toxicity can be the cause of severe yield loss or damage of standing crop. Poultry manures, sewage sludges, swine, composted refuse, fly ash, burned tires and copper wires often contain potentially toxic levels of metals and pose a considerable environmental metal risk if their use is unregulated in agricultural fields (Baker 1974). Repeated use of Bordeaux sprays may cause Cu toxicity in plants. Hewitt (1983) observed that Cu consistently induced Fe chlorosis in crops. Generally Cu toxicities have been associated with soil Cu levels of 150 and 400 ppm (Baker 1974). While in plants toxic range is varied from plant to plant. Copper generally accumulates in roots (Jarvis 1978, Jiang *et al.* 2000). In roots, toxic range was from 100 to 300 ppm and 20 to 30 ppm in tops of rice (Chino

1981). In sugarcane, copper toxicity was observed if root copper was in the range of 54- 375 ppm. Present study is aimed to investigate the effect of excess level of copper on growth, metabolism and nutrient availability in sugarcane.

Single bud setts of sugarcane (*Saccharum* sp. hybrid, CoLk 8102) were planted in polyethylene pots filled with refined sand at three levels of copper, viz. 0.065 (control), 65.0 and 130.0 (excess) ppm Cu. Copper was supplied as CuSO_4 in nutrient solution. The composition and methods of preparing complete nutrient solution have been described earlier (Agarwala *et al.* 1985). Nutrient solution was supplied daily except on Sundays when each pot was flushed with pure water to remove deleterious substances from the rooting medium. There were three replications in each treatment. Visible

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symptoms of copper toxicity were observed at 30 days after planting (DAP). To determine growth parameters, viz. leaf area, length, plant height, root number and length, fresh weight, dry weight and Ca, Fe, Mn, Cu and Zn in different plant parts, sugarcane plants were harvested and sampled at 50 days after planting. All plant samples were thoroughly washed with tap water, rinsed with distilled water and oven dried to constant weight at 85°C. Dried plant material was digested in di-acid mixture. Fe, Mn, Cu and Zn were estimated in clear digest by atomic absorption spectrophotometer and expressed as µg/g dry weight. Calcium was determined by a Systronics Flame photometer. Chlorophyll a, b and carotenoids contents were determined in fresh leaves at 45 days after planting by the method of Arnon (1949). Activity of catalase (Euler and Josephson 1927) and peroxidase (Luck1963) was determined in fresh leaves and data were expressed on per mg protein basis. Soluble protein content was determined in enzyme extract by Lowry *et al.* (1951). Treatment means were separated at $P = 0.05$ by the critical difference (CD) as described by Panse and Sukhatme (1985).

A significant decrease in leaf area, leaf length, plant height and root number and length, due to excess doses of Cu in the growing medium was observed (Table1). Similar effect of reduced root growth has been observed by Hill *et al.* (2000) in taro and by Jiang *et al.* (2000) in sunflower where it was observed that excess copper supply restricts root length. Restricted root length leads to depletion of essential nutrients in the restricted rooting zone, which consequently reduced plant growth. Plants exhibited chlorosis due to excess Cu supply and complete necrosis at 130 ppm Cu at later stage of growth. Symptoms of excess copper as chlorosis might be due to displacement of Fe by copper in enzymes which are involved in chlorophyll synthesis (VanAssche and Clijsters 1990, Lanaras *et al.* 1993). The fresh and dry weight of different plant parts was decreased under higher level (65 and 130 ppm) of copper in the growing medium. The decrease in plant weight may be due to reduced rate of photosynthesis (Hill *et al.* 2000) or due to disturbed nitrogen and carbohydrate metabolism at excess copper concentration. Excess Cu also resulted in reduced chlorophyll a, b and carotenoids contents (Table 1).

Table 1. Effect of copper on growth attributes and photosynthetic pigments in sugarcane

Parameters	ppm Cu supply			CD at 5%
	0.065	65.0	130.0	
Root Number per plant	36.00	31.00	31.00	NS
Root length (cm)	6.50	5.43	4.58	0.486
Leaf area (cm ² per leaf)	44.50	24.91	22.86	15.5
Leaf length (cm)	33.00	26.19	24.95	7.79
Leaf width (cm)	1.40	1.16	1.25	NS
Height (cm)	11.80	9.00	9.00	2.01
Chlorophyll a (mg/ g fw)	0.830	0.593	0.494	0.037
Chlorophyll b (mg/ g fw)	0.238	0.166	0.143	0.018
Carotenoids (mg/ g fw)	0.255	0.191	0.157	0.005

Table 2. Effect of copper on specific activity of catalase and peroxidase in sugarcane

Parameters	ppm Cu supply			CD at 5%
	0.065	65.0	130.0	
Catalase (µmol H ₂ O ₂ decomposed mg ⁻¹ protein min ⁻¹)	148	111	78	16
Peroxidase (Δ OD mg protein ⁻¹ min ⁻¹)	6.22	6.50	7.00	0.35

Table 3. Effect of copper on nutrient concentrations in sugarcane

Plant parts	ppm Cu supply			CD at 5%
	0.065	65.0	130.0	
Calcium (mg/ 100 mg dw)				
Leaf	0.573	0.493	0.373	0.044
Shoot	0.635	0.640	0.480	0.048
Root	0.330	0.100	0.067	0.037
Fe ($\mu\text{g/g dw}$)				
Leaf lamina	196.8	162.9	132.89	34.36
Stalk	158.69	133.5	92.99	22.49
Root	1043.7	1060	863.95	NS
Mn ($\mu\text{g/g dw}$)				
Leaf lamina	20.1	24	23.4	NS
Stalk	20.1	22.5	20.1	NS
Root	23.99	24.75	27.75	NS
Zn ($\mu\text{g/g dw}$)				
Leaf lamina	58.8	46.2	44.7	12.04
Stalk	71.1	54.3	55.2	3.21
Root	95.24	65.99	60.75	11.07
Cu ($\mu\text{g/g dw}$)				
Leaf lamina	47.7	23.1	38.1	10.01
Stalk	15.9	68.1	46.8	16.06
Root	51.75	125.99	177.5	16.06

Reduced photosynthetic pigments at excess copper supply might be due to induced Fe deficiency (Daniels *et al.* 1972). The specific activity of catalase decreased with an increase in Cu supply in the growing medium (Table 2). The low activity of catalase may be due to complete or partial replacement of iron from active sites as reported in barley (Agarwala *et al.* 1977) and oat (Luna *et al.* 1994). Higher activity of peroxidase was found in sugarcane leaves at excess copper concentration (Table 2). This enzyme plays a protective role by scavenging reactive oxygen species, *viz.* superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) produced under abiotic stress conditions.

Excess Cu causes significant reduction in calcium uptake in all plant parts (Table 3) which may be due to restricted rooting zone as observed by several workers in other crops (Hill *et al.* 2000, Jiang *et al.* 2000). The increase in Cu concentration in excess Cu treated plants increased copper content in stalk and root and depressed

the uptake of Fe and Zn in leaf, shoot and root tissues. While, Mn content increased due to high Cu supply (Table 3). This might be due to poor root growth (Hill *et al.* 2000, Jiang *et al.* 2000) and displacement of Fe by copper (Van Assche and Clijsters 1990).

Findings suggested excess Cu resulted in poor root growth, low availability of nutrients and disturbed plant metabolism which in turn reduced growth and biomass of sugarcane.

REFERENCES

- Agarwala, S.C., Bisht, S.S. and Sharma, C.P. (1977). Relative effectiveness of certain heavy metals in producing toxicity and symptoms of iron deficiency in barley. *Can. J. Bot.* **55**: 129.
- Agarwala, S.C., Chatterjee, C., Sharma, C.P. and Nautiyal, N. (1985). Copper nutrition of sugarbeet. *J. Exp. Bot.* **36**: 881-888.

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- Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **24**: 1-15.
- Baker, D.E. (1974). Copper: Soil, water, plant relationship. *Proc. Fed. Am. Soc. Exp. Biol.* **33**: 1188-1193.
- Chino, M. (1981). Metal stress in rice plants. In: K. Kitagishi and I. Yamane (eds.), Heavy Metals Pollution in Soils of Japan, pp.65-80. Japan Sci Soc. Press, Tokyo, Japan.
- Daniels, R.R., Stuckmeyer, B.E. and Peterson, L.A. (1972). Copper toxicity in *Phaseolus vulgaris* L. as influenced by iron nutrition. I. An anatomical study. *J. Am. Soc. Hort. Sci.* **9**: 249-254.
- Euler, H. von and Josephson, K. (1927). Uber Katalase. I. Justus Liebig's. *Ann. Chem.* **452**: 158-181.
- Hewitt, E.J. (1983). Essential and functional methods in plants. In: D.A. Robb. and W.S. Pierpoint (eds.) Metals and Micronutrients : Uptake and Utilization by Plants, pp. 313-315. Academic Press, New York.
- Hill, S.A., Susan, C.M. and Russel, S.Y. (2000). Taro responses to excess copper in solution culture. *Hort. Sci.* **35**: 863-867.
- Jarvis, S.C. (1978). Copper uptake and accumulation by perennial ryegrass grown in soil and. solution culture. *J. Sci. Fed. Agric.* **29**: 12-18.
- Jiang, W., Donghua, L. and Li, H. (2000). Effects of Cu²⁺ on root growth, cell division and nucleolus of *Helianthus annuus* L. *Sci. Total Environ.* **256**: 59-65.
- Lanaras, T., Moustaka, M., Symenoides, L., Diamantoglou, S. and Karataglis, S. (1993). Plant metal content, growth responses and photosynthetic measurements on field –cultivated growing on ore bodies enriched in Cu. *Physiol. Plant.* **88**: 307- 314.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.* **193**: 265- 275.
- Luck, H. (1963). Peroxidase. In: H.U. Bergmeyer (ed.), Methods in Enzymatic Analysis, pp.895-897. Academic Press, New York.
- Luna, C.M., Gonzalez, C.A. and Trippi, V.S.(1994). Oxidative damage caused by an excess of copper in oat leaves. *Plant Cell Physiol.* **35**: 11-15.
- Panase, V.G. and Sukhatme, P. (1985). Statistical Methods for Agricultural Workers (4th ed.) ICAR, New Delhi.
- Van Assche, F. and Clinjsters, H. (1990). Effect of metals on enzyme activity in plants. *Plant Cell environ.* **13**: 195-206.