Indian J. Plant Physiol., Vol. 8, No. 3 (N.S.) pp. 302-306 (July-Sept., 2003)

MODULATION OF RADISH METABOLISM BY ZINC PHYTOTOXICITY

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Received on 17 July, 2002, Revised on 17 Sept., 2003

SUMMARY

The effect of excess zinc on radish (*Raphanus sativus* L) cv. Jaunpuri was examined by growing plants in refined sand for 24 days in complete nutrient solution; on 25th day zinc as zinc sulphate was added at 0.1 and 0.2 mM (excess) levels. A set of radish plants was also maintained at 0.001 mM Zn and served as control. After 10-12th day of metal supply, the visible symptoms as depression in growth and irregular interveinal chlorosis of mature young leaves of zinc toxicity were observed with 0.2 mM Zn. With ageing, growth almost ceased and chlorosis intensified covering entire lamina, later brown necrotic spots appeared on chlorotic areas, enlarged, coalesced and whole leaf turned necrotic and limped down. These effects were more severe at 0.2 than 0.1 mM Zn. After 40 days of treatments, excess zinc reduced the root development, fresh weight, biomass of radish, concentration of total as well as active iron, chlorophylls a and b and activity of catalase, peroxidase, acid phosphatase and starch phosphorylase in leaves. In radish the accumulation of zinc was more in tops than in roots with application of zinc.

Key words : **Enzyme activity, radish, root growth, zinc excess.**

INTRODUCTION

Zinc when present in larger concentration in soils and due to its excessive uptake by plants growing on these soils might induce zinc toxicity. Usually zinc toxicity leads to chlorosis of young leaves and inhibits root elongation (Godbold *et al.* 1983, Ruano *et al.* 1988). Reports are also available on induced deficiency of Mn or Mg or Fe in zinc toxicity (Woolhouse 1983). Induced Mn deficiency is because high zinc supply strongly decreases the Mn content of plants (Ruano *et al.* 1988). Zinc as a heavy metal is known to occur in highest concentration in the majority of wastes arising from modern industries (Boardman and McGuire 1990). In various agricultural lands where toxicity of zinc prevails often sewage sludge rich in heavy metals or pesticides are applied and plants growing there exhibit sign of zinc toxicity. It has been suggested that the content of zinc vary considerably depending on growth conditions, whereas, appearance of visible effects is relatively independent (Cakmak *et al.* 1996, 1997). In plants excess zinc has been documented to retard several physiological processes, including phloem translocation (Foy *et al.* 1978). The symptoms of zinc toxicity have been reported for several crop plants such as wheat and maize, i.e. light blue green tinge develops at leaf tip and spreads to the base, and finally the leaf dries out (Takkar and Mann 1978). The general symptoms of zinc excess are retardation of growth, plants are stunted and chlorotic. Hampp *et al.* (1976) showed that high Zn inhibits CO_2 fixation in isolated spinach chloroplasts and non-cyclic electron transport was also affected. De Filippis and Pallaghy (1976) observed that high Zn inhibits photosynthesis and respiration in *Chlorella*, inhibition was accompanied by chlorophyll loss.

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Zinc also reported to alter membrane permeability and is one of the primary effects of Zn toxicity. It has been reported that high zinc levels cause membranes to become 'leaky' to K and thus enhances K leakage from maize roots. Observations also suggest that this action of zinc might be mediated indirectly through an interruption of metabolism linked to the transport process (Collins 1981). The responses of excess zinc have frequently been attributed to an interference with iron metabolism. However, chlorosis of leaves induced by excess zinc sometimes has no relationship with the total iron content but instead iron appears to retard the absorption and translocation of zinc (Rosen *et al.* 1977). Ambler *et al.* (1970) have documented that high levels of zinc lower the amount of reductant necessary for iron translocation by soybean roots. Apart from this, the information on phytotoxicity of heavy metals on plant metabolism is sporadic. Hence, efforts have been made to examine some characteristic responses of excess zinc on radish in the present study.

MATERIALS AND METHODS

Radish (*Raphanus sativus* L.) cv. Jaunpuri was grown in refined sand in a glass house at an ambient temperature. The composition of complete nutrient (normal) solution was: 4 mM KNO_3, 4mM Ca $(NO_3)_2$, 2mM $MgSO_4$, 1.5 mM NaH_2PO_4, 100 µM Fe-EDTA, 10 µM $MnSO_4$, 30 µM H_3BO_3, 1 µM $CuSO_4$, 1 µM $ZnSO_4$, 0.2 µM Na_2MoO_4, 0.1 µM $NiSO_4$, 0.1 µM $CoSO_4$ and 0.1 mM NaCl (Agarwala & Chatterjee 1996). Initially the seedlings were maintained in the above mentioned complete nutrient solution for 24 days. On 25th day pots containing radish plants were separated into three lots. Each lot contained four replicates with two plants per replication. The treatments were:

(i) First lot of radish plants was allowed to grow with full nutrient solution to serve as control.

(ii) Second lot of radish plants were supplied with 0.1 mM zinc sulphate.

(iii) Third lot of radish plants were supplied with 0.2 mM zinc sulphate.

These treatments were maintained for 40 days and on 41st day, changes in growth parameters and appearance of visible symptoms of Zn toxicity were observed. Plants were sampled on 41st day and separated into tops and roots for biomass. For analysis of iron and zinc the oven dried plant parts were digested in 10:1 nitric: perchloric acids and analysed by atomic absorption spectrophotometer.

In young leaves at 41st day (15 days after metal supply), the concentration of chlorophylls a and b (Arnon 1949) and activities of catalase (Euler and Josephson 1927), peroxidase (Luck 1963), acid phosphatase (Schmidt 1955) and starch phosphorylase (Srivastava and Krishnan 1961) were assayed. Active iron concentration was also measured in young leaves 16 days after metal supply (Mehrotra and Jain 1992). All determinations were made in triplicate and statistically analysed.

RESULTS AND DISCUSSION

Radish grown in excess zinc in refined sand exhibited growth depression at early stage and at the same time developed visible symptoms of zinc toxicity. The effects were iron deficiency type chlorosis of interveinal areas of middle and young leaves of plants. The symptoms later intensified and covered almost entire lamina. These changes are in consonance with the observations of Rauser (1973) and Woolhouse (1983) for soybean where red brown pigments developed on leaves, as has been observed in radish also. The characteristic visible symptoms of zinc toxicity in radish might be due to presence of higher phenolic substances in persistent zinc excess. High concentration of zinc also affected the formation and productivity of roots in radish. The root formation was poor, roots were less in length, girth and volume as has been observed earlier in several plant species (Takkar and Mann 1978, Godbold *et al.* 1983 and Kopponen *et al.* 2001). The effect of excess zinc on root development is a specific feature.

In radish excess zinc reduced the biomass (Table 1). The reduction in biomass of radish is very pronounced and is partially similar to the results on rice (Chino 1981). The decrease in biomass in excess zinc treated radish might be due to low protein formation resulting in inhibition of photosynthesis, as well as hampered carbohydrate translocation (Samarkoon and Rauser 1979). Stimulation of chlorophyll loss under such conditions has also been observed in *Chlorella* (De Filippis and Pallaghy 1976).

Zinc excess not only decreased the chlorophyll content but also decreased the activities of antioxidant enzymes,

Table 1. Effect of excess zinc on root length, volume, girth, fresh and dry weight and concentration of zinc and iron in top and roots of radish. (±SE).

Parameters	mM Zn supply		
	Control	0.1	0.2
Leaf area (cm^2 $leaf^{-1}$)	34.9±0.4	17.6±0.5	14.4±1.1
Root length (cm)	16.0±0.5	11.2±0.42	9.0±0.0
Root girth (cm)	8.9±0.4	7.7±0.0	7.3±0.3
Root volume (cc)	95±2.8	40.2±1.5	30.0±5.6
Fresh wt. (g $plant^{-1}$)	378±17.0	232±15.0	168±15.0
Dry wt. (g $plant^{-1}$)			
Top	14.7±0.9	9.7±0.0	6.4±0.1
Roots	5.5±0.1	2.4±0.1	1.8±0.1
Tissue Zn (µg g^{-1}dw)			
Tops	51.0±7.2	143.5±0.0	156±1.4
Roots	48.5±0.0	103.5±23.3	130±6.2
Tissue Fe (µg g^{-1}dw)			
Tops	190.0±8.6	165±0.8	145±11.5
Roots	142.5±1.4	110. ±4.3	105±0.0

i.e. catalase and peroxidase. Interference of heavy metals including zinc in excess amounts with normal iron metabolism is known to induce physiological iron deficiency and lowering of chlorophyll synthesis (Agarwala *et al.* 1977, Samarkoon and Rauser 1979). The decrease in concentration of chlorophyll a and b in leaves of excess zinc treated radish might, therefore, be through its effect on iron metabolism (Fig. 1). The decreased activity of peroxidase under excess zinc might be due to unavailability of functional active iron for different biomolecules. The decrease in peroxidase activity in leaves of radish under excess zinc is in contrast to the reports on the effect of heavy metals on iron enzymes in barley and maize (Agarwala *et al.* 1977, Bisht and Mehrotra 1989). The decrease in catalase activity in excess zinc treated radish (Fig. 1) is similar to the observations reported on oxidative stress responsive enzymes under high concentration of heavy metals (Agarwala *et al.* 1977, Bisht and Mehrotra 1989).

In the leaves of radish under excess zinc treatment, the activity of acid phosphatase decreased (Fig. 1) which might be due to high concentration of zinc suppressing phosphorus metabolism by lowering the content of inorganic phosphorus. This suggests a negative correlation between zinc and phosphorus. The activity of starch phosphorylase in the leaves of radish was also drastically decreased under excess zinc level (Fig. 1). Zinc in high amounts is known to bind ubiquinones and cytochrome b in the electron transport chain (Kleiner 1974). The zinc toxicity also has the inhibitory effect on phloem translocation (Samarkoon and Rauser 1979) and at the same time concomitant accumulation of various carbohydrate fractions occur. No definite mechanism is understood but most probably callose deposition on sieve plates have been observed in excess zinc condition (Samarkoon and Rauser 1979, Foy *et al.* 1978).

Under high zinc concentrations, there is not only accumulation of zinc in both tops and roots of radish but it appears that most of the element has been transported from roots to shoots resulting in high zinc concentration in tops of radish (Table 1). Similar results have been reported by Loneragan (1977). The zinc concentration in both tops and roots of radish observed here is much higher (Table 1) than that reported for pea (Melton *et al.* 1970)

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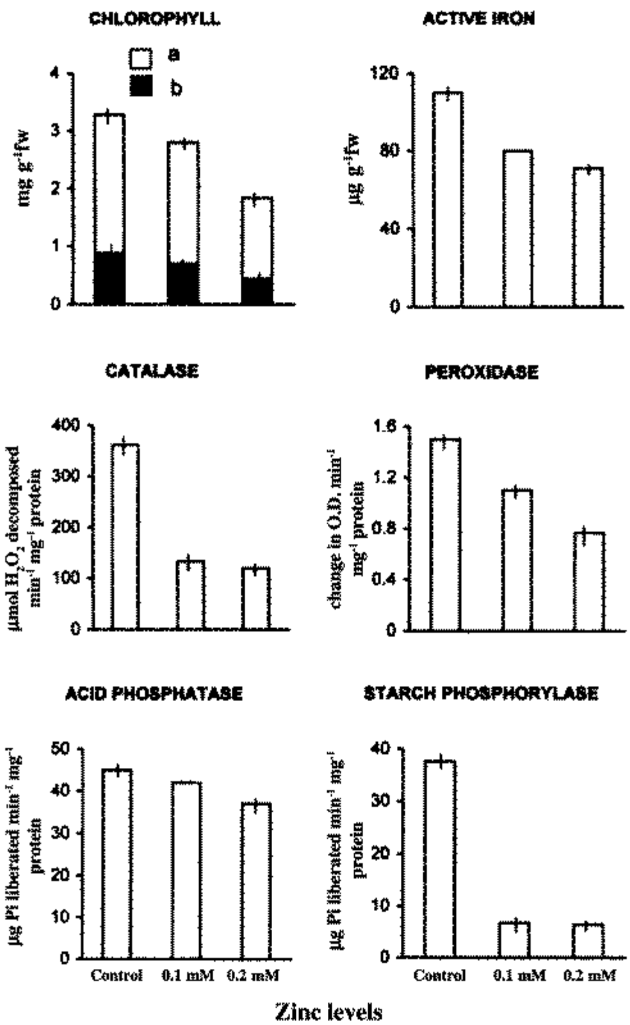

Fig. 1. Effect of excess zinc on chlorophyll a and b, active iron content and activities of catalase, peroxidase, acid phosphatase and starch phosphorylase in radish leaves.

or apple seedlings (Benson 1966). However, in consonance with those findings inhibition of further growth or cessation of root growth or reduced growth was observed in radish. As has been observed earlier for soybean and wheat (Dowdy and Larson 1975, Giordano and Mays 1977), here in radish also it was found that zinc concentration was higher in shoots or leaves than in the storage tissue or roots. These concentrations of zinc in plant tissues could be toxic (Sauerbeck 1982).

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