SAFFLOWER IN RESPONSE TO VARYING LEVELS OF ZINC SUPPLY

NALINI PANDEY AND C.P. SHARMA

Botany Department, Lucknow University, Lucknow-226 007, India
(Accepted April, 1998)

To establish a guideline for the diagnosis and correction of zinc deficiency, safflower (*Carthamus tinctorius* L. cv. T65) was grown in purified sand culture at zinc supply ranging from 0.001 to 10 µM Zn L^{-1}. Optimum dry matter and economic yield of safflower was obtained at 1.0 µM Zn L^{-1}. Plants grown below 1.0 µM Zn L^{-1} developed visible foliar symptoms of zinc deficiency. At 0.001 µM Zn L^{-1} foliar symptoms were severe and economic yield reduced to almost nil. Deficiency of Zn reduced leaf tissue concentration of Zn and induced changes in enzyme activity. At suboptimal levels of Zn supply activity of carbonic anhydrase and aldolase were decreased and that of ribonuclease increased. At 64 DAS when plants developed visible foliar effect of zinc deficiency, leaf tissue concentration of 21 µg Zn g^{-1} dry matter and 30 µg Zn g^{-1} dry matter represent the threshold concentration for Zn deficiency and toxicity respectively. Less than 15 µg Zn g^{-1} dry matter is indicative of severe deficiency associated with 50% decrease in relative yield.

Key Words: Enzymes, Safflower, Zn.

Zinc deficiency reduces plant growth and the activity of Zn enzymes like carbonic anhydrase, aldolase and alcohol dehydrogenase (Snir, 1983; Sandmann and Bogor, 1983; Dell and Wilson, 1989. Zinc also plays an important role in regulating the photosynthetic electron transport and CO_2 fixation (Pandey and Sharma, 1989; Hu and Sparks, 1991). Critical Zn levels for maximum plant growth have been reported for several crops such as subterranean clover (Reuter *et al.*, 1982), sorghum (Ohki, 1984) and eucalyptus (Wallace *et al.*, 1986). No study has, however, been made of Zn nutrition of safflower. As availability of Zn is low in most soils of India (Katyal and Sharma, 1991), and safflower is an important oilseed crop, the present study was undertaken to establish a nutritional guideline for the diagnosis and correction of Zn deficiency in safflower. The influence of variable Zn supply on morphological attributes, biomass and economic yield, tissue Zn and activity of certain enzymes was investigated in leaves of safflower grown in purified sand.

MATERIALS AND METHODS

Safflower (*Carthamus tinctorius* L. cv. T-65) was grown in glass house in purified sand in 5L corning glass pots having a central drainage hole lined with glass wool and covered with an inverted watch glass. The silica sand was purified by two successive treatments of hot 17% (w/v) HCl and 1% (w/v) oxalic acid in a 'Keebush' sand digestor. There were four pots in each treatment. Initially six plants were main-tained in each pot which was reduced to two plants 30 days after sowing (DAS). Zinc was supplied at six levels-0.001, 0.01, 1.0, 2.0 and 10 µM Zn L^{-1}. The composition of nutrient solution (L^{-1}) excluding Zn was: 4mM KNO_3, 8 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 0.33 mM H_3BO_3, 0.1 mM Fe EDTA, 10 µM $MnSO_4$, 1µM $CuSO_4$, 0.01 µM Na_2MoO_4, 0.1 µM $NiSO_4$ and 0.1 µM $CoSO_4$.

Solutions of micronutrients except iron were prepared from twice recrystalized salts. Iron was supplied as Fe-EDTA prepared from disodium salt of ethylenediamine tetra-acetic acid (Na_2 EDTA) and AR grade $FeSO_4$ (Sharma, 1996).

Dry matter yield was determined 64 and 124 DAS. Plants were harvested 124 DAS for determination of economic yield. Tissue concentration of Zn in leaves was determined in upper leaves (1 to 4 from top) 64 DAS when plants showed characteristic symptoms of zinc deficiency. These were thoroughly washed and dried in an electric oven (70°C) and digested in 10:1 nitric acid-perchloric acid mixture for estimating Zn by atomic absorption spectrophotometry. Critical values for sufficiency (adequacy), threshold of deficiency and toxicity (90% optimal yield) and severe deficiency (50% optimal yield) have been worked out through plot of relative yield at harvest and leaf tissue concentration of Zn 64 DAS (Sharma, 1996). At 65 DAS activity of carbonic anhydrase (E.C.4.2.1.1), aldolase (E.C.4.1.2.13) and ribonuclease (E.C.3.1.1.22), were determined in the crude extracts

of leaf (1 to 4 from top), by the methods described earlier (Sharma *et al.,* 1981). Specific activity of the enzymes was expressed in terms of soluble protein.

Results have been presented as mean of two determinations and significance of the results has been tested at P=0.05.

RESULTS

Visible effects: Variation in Zn supply caused visible difference in growth of plants 30 DAS. Compared to plants supplied 1.0 µM Zn L^{-1}, growth was depressed at lower or higher levels of Zn. Growth became more pronounced with age. Severe depression in growth was observed in plants receiving 0.001 µM Zn L^{-1}. The effect was particularly marked in height and development of the branches. In plants grown with 0.001 and 0.01 µM Zn L^{-1}, the expansion of lamina of young leaves was restricted. The young leaves of these plants were small and narrow. Compared to leaves of plants grown with 1.0 µM Zn L^{-1}, leaves of Zn deficient plants were thick, leathery and dull green. Similar, but milder, symptoms were observed at 0.1 µM Zn L^{-1} at 50 DAS. The young leaves of plants receiving 0.001 and 0.01 µM Zn L^{-1} developed bronze coloration near the margins. The old leaves of these plants developed chlorosis along the apical margins. The chlorotic areas later turned necrotic and scorched. The veins of the old leaves, as also the basal part of the stem, developed orange brown pigmentation. Safflower plants raised at 2 and 10 µM Zn L^{-1} developed visible effects of toxicity. These plants appeared stunted and bore smaller leaves than plants supplied 1.0 µM Zn L^{-1}.

Low as well as toxic supply of Zn delayed flowering by eight days, the effect was most severe in plants grown with 0.001 and 0.01 µM Zn L^{-1} when flowering was delayed by twelve days. In these plants the size and number of the heads were highly reduced and the development of both the ray and disc florets was retarded. Because of delayed emergence of the stigma from the corolla tube large proportion of the disc florets of Zn deficient plants were unfertilized and failed to produce seeds.

Plant yield: The dry matter and economic (seed) yield increased with increase in Zn supply from 0.001 to 1.0 µM Zn L^{-1}. Further increase in Zn supply caused yield decrement. The effect of low and toxic levels of Zn supply on dry matter yield became

Table 1: Dry matter and seed yield attributes of safflower grown at variable Zn supply.

Yield Attributes	µM Zn L^{-1}						LSD at P= 0.05
	0.001	0.01	0.1	1.0	2.0	10	
Dry matter g plant^{-1}							
64 DAS	2.49	2.96	3.52	5.04	3.92	2.9	0.53
124 DAS	4.54	7.11	11.72	32.39	22.57	16.32	5.19
No. of Head Plant^{-1}	7	9	11	16	14	12	3
No. of Seeds Plant^{-1}	5	20	100	301	254	209	10
g Head wt. plant^{-1}	2.29	3.31	7.47	26.44	17.64	13.26	2.19
g Seeds plant^{-1}	0.05	0.17	1.90	7.64	4.49	3.40	1.09
100 Seed wt. (g)	-	-	0.19	2.54	1.76	1.62	0.91

pronouced at 124 DAS. Maximum dry matter and economic yield was obtained at 1.0 µM Zn L^{-1} (Table 1). The number and weight of heads and of seeds was also reduced at low and toxic levels of Zn (Table 1). In the Zn deficient plants, the decrease in seed yield was more pronounced than in vegetative yield. At 124 DAS, compared to that at 1.0 µM Zn L^{-1}, the vegetative yield was reduced by 85% and 78% at 0.001 and 0.01 µM Zn L^{-1} respectively, whereas seed yield was decreased by 99% and 97% respectively.

Tissue Zn: Increase in Zn supply from 0.001 to 10 µM Zn L^{-1} increased Zn concentration in the young leaves. Maximum dry matter yield was associated with 24 µg Zn g^{-1} dry matter in leaf. The threshold values for deficiency and toxicity, defined
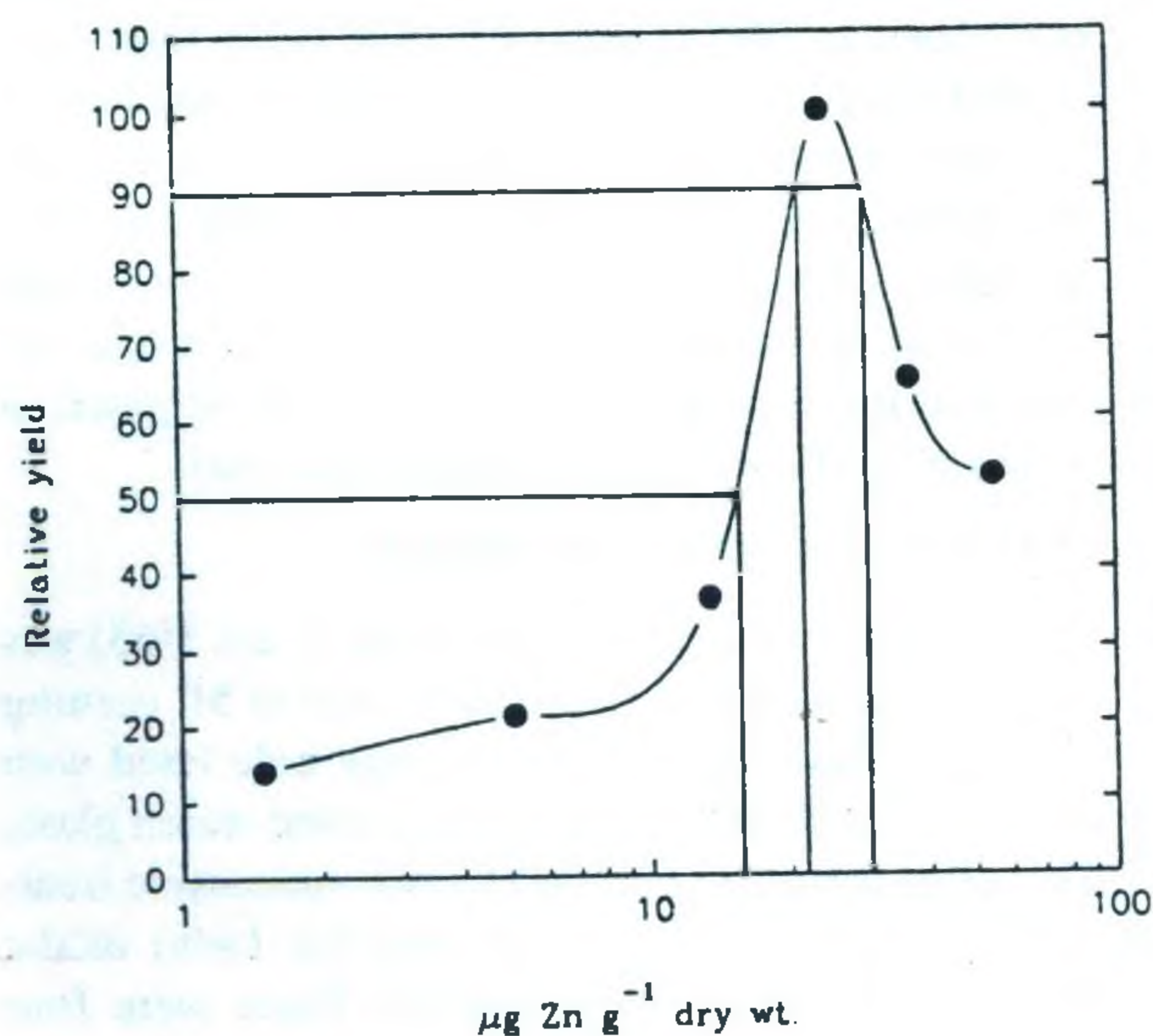

Figure 1. Values of deficiency, threshold of deficiency and threshold of toxicity in young leaves of safflower grown at variable Zn supply.

as the nutrient concentration in the tissue associated with 90% optimal yield were 21 and 30 µg Zn g^{-1} dry matter respectively. The deficiency values of Zn in leaves associated with 50% depression in dry matter yield (compared to the optimal) was 15 µg Zn g^{-1} dry matter (Fig. 1).

Enzyme activity : The activity of carbonic anhydrase was determined by the level of Zn supply. Plants grown with 0.001 to 0.1 µg Zn L^{-1} showed very low activity of carbonic anhydrase. Increasing Zn supply increased the activity of the enzyme which reached the maximum at 10 µg L^{-1} Zn supply. Aldolase activity was influenced by the level of Zn supply. Compared to that in plants grown with 2 and 10 µg Zn L^{-1} the aldolase activity was much lower at less than 1.0 µg Zn L^{-1}. The extent of the decrease in the activity of aldolase due to Zn deficiency was related to the Zn supply. Zinc effect on the activity of ribonuclease was just the opposite. The activity of ribonuclease increased significantly below 1.0 µg Zn L^{-1} and the magnitude of the effect was largely related to the limitation in Zn supply (Fig. 2).

DISCUSSION

Zinc deficiency symptoms in safflower appeared in two phases. Initially, the young leaves became reduced in size, thick, dull green in color and brittle to touch, but did not develop any chlorosis and leaf wrinkling as reported for some other crops (Sorghum-Ohki, 1984; *Eucalyptus*-Wallace *et al.*, 1986; Subterranean clover-Reuter *et al.*, 1982). At later stages, the old leaves of Zn deficient safflower plants developed chlorosis and necrosis along the leaf margins. This was followed by bronze coloration of the veins and stem. In plants grown at 2 and 10 µg Zn L^{-1} leaf size was reduced due to Zn toxicity. The reduction in size and number of leaves, branching and biomass in plants subjected to Zn deficiency is attributed to inadequacy of Zn needed for maintaining the functional role in plant metabolism (Marschner, 1986). Poor development of head and seeds of Zn deficient plants, is attributed to a role of Zn in anther development and pollen viability (Sharma *et al.*, 1987, 1990). Zinc effect on seed yield of safflower was several magnitude larger than on the vegetative yield, suggesting a role of Zn in determining the sink capacity. Zinc toxicity also appreciably depressed seed yield and growth.

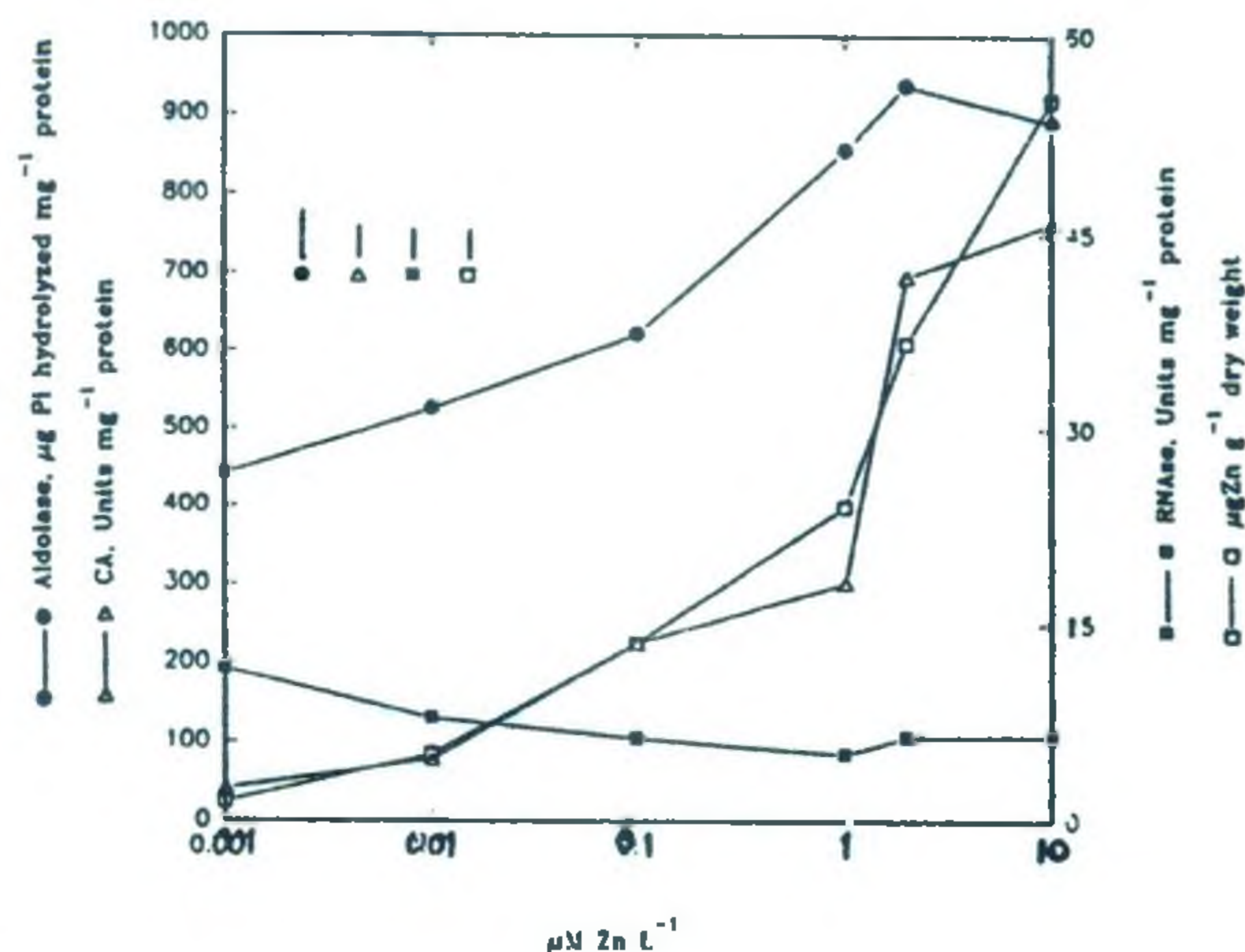Zinc concentration in the young leaves reflected

Figure 2. The tissue concentration of zinc and specific activity of carbonic anhydrase, aldolase and ribonuclease in young leaves of safflower grown at variable Zn supply. Vertical lines represent LSD at P=0.05.

the status of Zn supply. Plot of relative yield at harvest against leaf tissue concentration of Zn at 64 DAS showed that from 21 to 30 µg Zn g^{-1} dry matter represented sufficiency for Zn in safflower, the critical levels for deficiency and toxicity of Zn being 21 and 30 µg Zn g^{-1} dry matter respectively. Less than 15 µg Zn g-1 dry matter indicates severe deficiency (50% optimal yield).

As in some other plants (Dwivedi and Takkar, 1974; Sharma *et al.*, 1987) Zn deficiency caused an increase in the ribonuclease activity in safflower. A decrease in the activity of aldolase under Zn deficiency suggests a requirement of Zn as a cofactor (O'Sullivan, 1979; Sharma *et al.*, 1981; Sandmann and Boger, 1983). The decrease in carbonic anhydrase activity observed in Zn deficient safflower is in consonance with observations made for other plants-*Citrullus* (Sharma *et al.*, 1981), *Pecan* (Snir,1984), *Eucalyptus* and *Trifolium* (Dell and Wilson, 1989). The decrease in the activity of aldolase and carbonic anhydrase was associated with induction of Zn deficiency symptoms. However, the decrease in activity of carbonic anhydrase was more significant than in aldolase, and gave a better indication of the extent of Zn deficiency. Therefore, carbonic anhydrase and the leaf tissue Zn serve as suitable indicators of Zn status of safflower. Increase in ribonuclease under Zn deficiency, as observed here, has also been suggested as indication of Zn

deficiency, but this may not be specific as the deficiency of several other elements is also reported to cause enhancement in this enzyme.

REFERENCES

Dell B & S A Wilson 1989 Zinc nutrition and leaf carbonic anhydrase activity of *Eucalyptus maculata* seedlings and *Trifolium subterraneum*. *Plant Science* **113** 287-290.

Dwivedi R S & P N Takkar 1974 Ribonuclease activity as an index of hidden hunger of zinc in crops. *Plant Science* **40** 173-181.

Hu H & D Spark 1991 Zinc deficiency inhibits chlorophyll synthesis and gas exchange in 'Stuart Pecan'. *Hortscience* **26** 267-268.

Katyal J C & B D Sharma 1991 Extractable and total zinc, copper, manganese and iron in Indian soils and their association with some soil properties. *Geoderma* **49** 165-179.

Marschner H 1986 *Mineral Nutrition of Higher Plants* Academic Press, New York.

Ohki K 1984 Zinc nutrition related to critical deficiency and toxicity levels of sorghum. *Agronomy J* **76** 253-256.

O'Sullivan M 1979 Aldolase activity in plant, as an indicator of zinc deficiency. *J Science Food Agric* **21** 607-609.

Pandey N & C P Sharma 1989 Zinc deficiency effect on photosynthesis and transpiration in safflower and its reversal on making up the deficiency. *Indian J Exp Biol* **27** 376-377.

Reuter D J J F Loneragan A D Robson & D Plaskett 1982 Zinc in subterranean clover (*Trifolium subterraneum L: Seaton Park*). Effect of zinc supply on distribution of zinc and dry weight among plant parts. *Aust J Agric Res* **33** 989-999.

Sandman G & P Bogor 1983 The enzymological function of heavy metals and their role in electron transfer processes of plants In: *Encyclopedia of Plant Physiology, New Series*, Vol. 15A (Lauchli A & R L Bieleski eds) Springer Verlag Berlin and New York. p 563-596.

Sharma C P 1996 (ed) *Deficiency Symptoms and Critical Concentration of Micronutrients in Crop Plants & Micronutrients Project* (ICAR) Lucknow University Centre Lucknow p 1-12.

Sharma C P, J P Gupta & S C Agarwala 1981 Metabolic changes in *Citrullus* subjected to zinc stress. *J Plant Nutr* **3** 337-344.

Sharma P N, C Chatterjee, C P Sharma & S C Agarwala 1987 Zinc deficiency and anther development in maize. *Plant Cell Physiol* **28** 11-18.

Sharma P N, C Chatterjee, S C Agarwala & C P Sharma 1990 Zinc deficiency and pollen fertility in maize (*Zea mays*) In *Plant Nutrition-Physiology and Applications* (M L Van Beusichem ed) Kluwer Academic Publishers. p 261 265.

Snir I 1983 Carbonic anhydrase activity as an indicator of zinc deficiency in pecan leaves. *Plant Soil* **74** 287-289.

Wallace I M B Dell & J F Loneragan 1986 Zinc nutrition of Jarrah (*Eucalyptus marginata Donnex Smith*) seedlings. *Aust J Bot* **34** 41-51.