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EFFECT OF ZINC SUPPLY ON GROWTH AND SOME METABOLIC CHARACTERISTICS OF SAFFLOWER AND SUNFLOWER PLANTS

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SUMMARY

The effect of five Zn levels, viz. 0, 2.5, 5.0, 7.5 and 10.0 mg kg^{-1} of soil (designated as Zn_0, $Zn_{2.5}$, $Zn_{5.0}$, $Zn_{7.5}$, $Zn_{10.0}$ respectively) on the specific leaf area (SLA), dry matter production, and some related metabolic parameters of safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) were studied. The first visible symptoms of Zn toxicity were observed as reduction in both specific leaf area (SLA) and dry matter yield especially of shoots along with decreased chlorophyll and carotenoids content. These symptoms were more expressed in safflower than in sunflower plants. Zinc toxicity in safflower was apparent above $Zn_{7.5}$, while in sunflower only at the highest level $Zn_{10.0}$. Net photosynthetic rate decreased at higher zinc doses. In contrast, the respiration rate in both plant species increased progressively with increasing Zn doses. Zinc content in the two tested species increased more in roots than in shoots with increased application of Zn. Zinc applied affected accumulation of other nutrients, but marked differences were noted in Fe and Mn contents of shoot and root in both plants. Also, membrane permeability increased with increasing Zn doses especially in safflower plants.

***Key words*: Net photosynthetic rate, respiration rate, safflower, specific leaf area, sunflower, zinc toxicity**

INTRODUCTION

Zinc deficient soils are common all over the world in both tropical and temperate climates. Zn deficiency is the most widespread micronutrient deficiency (Graham *et al*. 1992 and Rashid *et al*. 1994). The problem of Zn deficiency has been further accentuated by intensive cultivation of high yielding cultivars and use of high doses of mineral fertilizers (Graham and Rengel 1993). A wide variation in response of crops to zinc application is reported (Graham 1991). It is postulated that for wheat, Zn-efficient genotypes yield up to three times more than Zn-inefficient types in Zn-deficient soils (Graham 1991). The use of fertilizers to solve the problem of Zn deficiency is a common practice but crop species exhibit differential tolerance to Zn deficiency (Saxena and Chandel 1992). Microelements such as zinc are essential and are involved in numerous physiological processes, but at high concentrations they are strongly toxic and impair plant growth (Monnet *et al*. 2001). The most characteristic visible symptoms of zinc deficiency are stunted growth due to shortening of internodes and decrease in leaf size (Marschner 1995). Sharma *et al*. (1994) observed decreased photosynthetic rates and reduced leaf chlorophyll content in Zn-deficient leaves of cauliflower. Zn nutrition affected the accumulation of other nutrients in different plants, but marked differences were noted in Fe and Mn contents, especially in shoot (Khan *et al*. 1998). However, because of the similarities in ion radius of bivalent cations (Mn, Fe, Cu, Mg and Zn),

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excess zinc can shift certain physiological equilibrium by local competition at various sites (De Fillipis and Ziegler 1993, Monnet *et al*. 2001).

In the present work we determined effect of Zn on growth and some metabolic characteristics of safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) plants grown under different levels of zinc.

MATERIALS AND METHODS

Seeds of the safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) were obtained from the Faculty of Agriculture, Qena University. Plants were grown in weighed plastic pots (30 cm length and 7 cm diameter) containing 1.5 kg air-dried soil under constant conditions [30/20 ^{o}C day/night (12h) temperature cycles, and light intensity of 110 mol m^{-2} s^{-1}] in a growth chamber. Five different levels of Zn (Zn SO_4 $7H_2O$), viz. 0.0, 2.5, 5.0, 7.0 and 10.0 mg Zn kg^{-1} soil (designated as Zn_0, $Zn_{2.5}$, $Zn_{7.5}$ and $Zn_{10.0}$ respectively) were supplied to the soil. The soil was then thoroughly mixed with the applied Zn levels. Seeds were surface sterilized by soaking in 70 % (v/v) ethanol for 1 min and in sodium hypochloride (1 % active chlorine, v/v) for 5 min, and washed three times with sterile deionized water (DD). Sterile seeds were germinated on filter paper, pre-soaked with DD water at 25 ^{o}C. After 48h, five uniformly germinated seeds were sown at a depth of 1 cm in each pot. The experiment was set up in a completely randomized design with 3 replicates of each treatment. The plants were watered with DD water to field capacity by weighing the pots daily and left to grow under the different Zn doses until the end of experiment.

At the end of experiment (six weeks), the chlorophyll (chl *a* and chl *b*) and total carotenoids contents were estimated in leaves using the spectrophotometric method (Spectronic Genesis ZPC, Rochester, NY, USA) according to Lichtenthaler and Wellburn (1983). Net photosynthetic (oxygen evolution) (P_N) and dark respiration (oxygen consumption) (R_D) rates were determined manometrically using the Warburg method described by Umbreit *et al*. (1959). Membrane permeability was measured by determing the electrical conductivity (EC) of the excised leaves according to Yan *et al*. (1996). Specific leaf area (SLA) was measured by calculating the area of five leaf discs whose weights were then averaged (Martin and Coughtrey 1982). The results were expressed in m^2 g^{-1} dry weight. At maturity, plants were harvested for shoot and root dry matter production. Soil was washed off from the roots under running tap water and then rinsed in DD water. For dry mass determination, both roots and shoots were dried separately at 80 ^{o}C for 48h to constant mass. For determing the Fe, Mg, Mn and Zn in the shoots and roots of both plant species, the dried samples were pre-digested in 10 cm^3 of 70 % (v/v) HNO_3 overnight and then heated at 130 ^{o}C until complete digestion occurred. The volume of acid reduced to about 1 cm^3 digests were made up to 25 cm^3 with 1 % (v/v) HNO_3. The diluted samples were analyzed for Fe, Mg, Mn and Zn contents by an Inductively Coupled Plasma Emission Spectrometry (Zarcinas *et al*. 1987). The experimental data was subjected to analysis by the least significant difference test (L.S.D.) using SPSS program.

RESULTS

Visual Zn-deficiency symptoms, such as reduction in the shoot and root growth and specific leaf area (SLA) were observed in Zn_0 plants (Table 1). Root dry matter in both plant species was reduced, but this reduction was less as compared to shoot growth. All these symptoms were more apparent in safflower than sunflower plants. Total plant dry matter increased from Zn_0 to $Zn_{5.0}$ (safflower) or to $Zn_{7.5}$ (sunflower) and declined with further increase of Zn doses. In most cases, the root: shoot ratio (R/S) in safflower plants was highest at the middle doses of Zn (Table 1). A higher R/S ratio in safflower as compared with control plants indicated that shoot growth was more affected than root growth. The reduction in dry matter production of safflower plants was found at $Zn_{7.5}$ and $Zn_{10.0}$, while in sunflower plants only at highest doses of Zn applied.

Chlorophyll and carotenoids content in leaf of both experimental plants increased from Zn_0 to $Zn_{5.0}$ and declined at higher Zn doses (Table 2). Similarly, net photosynthetic rate (P_N) of both tested plant species increasing gradually up to $Zn_{5.0}$ and thereafter it decreased sharply as compared with plants growing without Zn_0 treatments (Table 2). However a different trend was observed for the respiration rate (R_D), in the

Table 1. Effect of different levels of Zn on specific leaf area (SLA) (m^2 g^{-1} dry weight), dry matter (g $plant^{-1}$) of shoot (S) and root (R) and R/S ratio in safflower and sunflower plants. Significant differences from the control at * P = 0.05 and ** P = 0.01.

Treatment	Safflower					Sunflower				
	Specific leaf area (SLA)	Dry matter				Specific leaf area (SLA)	Dry matter			
		S	R	Total	R/S ratio		S	R	Total	R/S ratio
Zn_0	34.02	3.75	1.88	5.63	0.50	41.72	5.27	2.45	7.72	0.46
$Zn_{2.5}$	36.16*	3.96	2.12*	6.08	0.54*	43.98*	5.68	2.97**	8.65*	0.50**
$Zn_{5.0}$	37.27**	4.01*	2.27**	6.28*	0.57**	44.83**	6.29**	3.23**	9.52**	0.50**
$Zn_{7.5}$	31.14	3.15**	1.65*	4.80*	0.52	44.51*	6.64**	3.28**	9.92**	0.49**
$Zn_{10.0}$	27.80**	2.50**	1.15**	3.65**	0.46**	36.77**	3.80**	1.70**	5.50**	0.45
L.S.D. $_{5\%}$	1.91	0.22	0.19	0.61	0.03	2.06	0.53	0.17	0.77	0.02
L.S.D. $_{1\%}$	2.62	0.30	0.26	0.84	0.04	2.82	0.73	0.23	1.05	0.03

two tested plants. The respiration rate showed an increase with the increase in Zn level (Zn 10.0) in both sunflower and safflower (Table 2).

The concentrations of Fe, Mg and Mn (Table 3) revealed that there was a marked decrease in the concentration of Mg especially in roots of safflower than in sunflower plants. A higher Fe accumulation was observed in shoots of safflower and sunflower plants up to $Zn_{5.0}$ and $Zn_{7.5}$ levels respectively, but above these levels the concentration of Fe sharply decreased. In case of roots of both tested plants, Fe concentrations were significantly reduced under Zn applied, as compared with the control (Zn_0). On the other hand, Mn concentration tended to increase in roots of both experimental plants up to $Zn_{5.0}$ and then these values progressively

Table 2. Effect of different levels of Zn on chlorophyll [g kg^{-1} (dry matter)] content and net photosynthetic rate (P_N) and dark respiration (R_D) rates [µmol (O_2) kg^{-1} (dry matter) $unit^{-1}$ time] in safflower and sunflower plants. Significant differences from the control at * P = 0.05 and ** P = 0.01.

Treatment	Safflower						Sunflower					
	Contents of chlorophyll						Contents of chlorophyll					
	Chl a	Chl b	Car.	Total	P_N	R_D	Chl a	Chl b	Car.	Total	P_N	R_D
Zn_0	5.85	3.19	2.29	11.33	129	30.2	7.67	3.58	1.88	13.13	237	55.4
$Zn_{2.5}$	6.58*	3.38	2.47*	12.43*	137**	33.8**	8.47*	3.81	2.03	14.31*	261**	57.7*
$Zn_{5.0}$	7.65**	3.60	3.11**	14.36**	140**	38.5**	10.03**	4.03*	3.44**	17.50**	273**	66.3**
$Zn_{7.5}$	5.08*	2.85	2.10*	10.03*	122**	41.7**	10.35**	3.86	3.10**	17.31**	277**	70.8**
$Zn_{10.0}$	4.04**	2.17**	1.88**	8.09**	112**	49.8**	6.21**	2.88**	2.07*	11.16*	220**	74.5**
L.S.D.$_{5\%}$	0.62	0.43	0.16	1.06	3.66	2.02	0.77	0.34	0.19	1.55	4.75	2.21
L.S.D. $_{1\%}$	0.85	0.59	0.22	1.45	5.01	2.77	1.05	0.47	0.26	2.12	6.51	3.03

Table 3. Effect of different levels of Zn on Fe, Mg and Mn content [mg g^{-1} (dry matter)] in shoot and root of safflower and sunflower plants. Significant differences from the control at * $P = 0.05$ and ** $P = 0.01$.

Treatment	Safflower					
	Fe content		Mg content		Mn content	
	Shoot	Root	Shoot	Root	Shoot	Root
Zn_0	0.123	0.105	5.70	3.60	0.388	0.196
$Zn_{2.5}$	0.127	0.097*	4.91*	3.11**	0.395**	0.211*
$Zn_{5.0}$	0.134*	0.092**	4.33**	2.82**	0.390	0.235**
$Zn_{7.5}$	0.109**	0.081**	3.77**	2.26**	0.387	0.181*
$Zn_{10.0}$	0.102**	0.076**	3.56**	1.85**	0.386	0.165**
L.S.D. $_{5\%}$	0.009	0.007	0.681	0.285	0.004	0.012
L.S.D. $_{1\%}$	0.012	0.010	0.933	0.390	0.005	0.016
Treatment	**Sunflower**					
	Fe content		Mg content		Mn content	
	Shoot	Root	Shoot	Root	Shoot	Root
Zn_0	0.143	0.135	8.66	4.70	0.570	0.233
$Zn_{2.5}$	0.156*	0.129*	8.08	3.97*	0.572	0.241
$Zn_{5.0}$	0.162**	0.124**	6.37**	3.33**	0.569	0.247*
$Zn_{7.5}$	0.167**	0.118**	6.11**	2.84**	0.571	0.218*
$Zn_{10.0}$	0.116**	0.103**	5.31**	2.53**	0.568	0.197**
L.S.D. $_{5\%}$	0.011	0.005	0.83	0.06	0.004	0.013
L.S.D. $_{1\%}$	0.015	0.007	1.14	0.08	0.005	0.018

decreased. In case of shoots, Mn concentration remained more or less unchanged up to the highest Zn doses in both plants compared with the control (Zn_0) plants (Table 3).

The concentration of Zn in the two tested plants increased with the increasing Zn doses, compared with those of the control plants Zn_0, irrespective of the plant tissue analyzed (Table 4). This increase was more obvious in roots than in shoots, especially in safflower plants. Consequently, electric conductivity (EC %) increased in the both plants by increasing Zn doses (Table 4). It was interesting to note that membrane permeability of safflower plants was higher than that of sunflower plants.

DISCUSSION

The results obtained in the present study exhibit different variations in the seedling growth under different doses of Zn application, in the two tested plants. Specific leaf area (SLA) and dry matter production of shoot and root increased by increasing Zn supply in safflower plants upto $Zn_{5.0}$ while in case of sunflower the increase was upto $Zn_{7.5}$ without any observable toxicity symptoms. In contrast to our results, Rengel and Graham (1995) observed Zn-induced toxicity in wheat at lower levels, which indicates that Zn requirement of safflower and sunflower may be higher than that of wheat. In this respect, Singh *et al*. (1983) reported that Zn requirement and tolerance of different crop species varies greatly. The

Table 4. Effect of different levels of Zn on Zn content [mg. g^{-1} (dry matter)] in shoot and root and membrane permeability [expressed as decrease in electric conductivity (EC %)] of safflower and sunflower plants. Significant differences from the control at * $P = 0.05$ and ** $P = 0.01$.

Treatment	Safflower			Sunflower		
	Zn concentration		EC	Zn concentration		EC
	Shoot	Root	[%]	Shoot	Root	[%]
Zn_0	1.77	2.88	13.6	1.05	1.94	11.6
$Zn_{2.5}$	4.82**	6.70**	19.5**	2.67*	3.85*	13.5*
$Zn_{5.0}$	7.37**	10.8**	25.6**	5.54**	7.60**	16.4**
$Zn_{7.5}$	9.92**	12.4**	26.8**	6.70**	8.75**	18.2**
$Zn_{10.0}$	10.70**	16.5**	28.2	10.3**	9.70**	19.7**
L.S.D. $_{5\%}$	1.77	1.98	2.14	1.53	1.43	1.71
L.S.D. $_{1\%}$	2.42	2.71	2.93	2.10	1.96	2.34

reduction in root and shoot growth of safflower and sunflower under higher doses of Zn indicated that an adequate amount of Zn in the rooting zone is essential for root and shoot growth (Grewal *et al*. 1997). Dry matter production of roots in both tested species under low Zn doses increased without affecting the dry matter of shoot. However, higher Zn doses led to visually observable toxic symptoms and growth of these plants was clearly arrested compared with control Zn_0. This is in accordance with the results of Cakmak *et al*. (1996), Khan *et al*. (1998), and Monnet *et al*. (2001) in wheat, barley, sunflower, and ryegrass plant (*Lolium perenne* L.). Under the highest Zn doses, we also observed the reduction in R/S ratios of both tested species. At low and moderate doses of Zn, the R/S ratios correlated with Zn concentration, indicating thereby that the development of a better root system in plants be genetically controlled. The plants accumulating more shoot biomass may translocate more carbon from shoot to roots for a better root development. Further plants having finer roots have larger surface area (Graham and Rengel 1993 and Dong *et al*. 1995).

Sunflower plants, despite having a larger root dry matter production than safflower plants, had the lowest Zn concentration in root under Zn_0, which might be related with a dilution of Zn in root due to higher root dry matter yield. The total Zn in safflower is much larger than Zn in sunflower. Concomitantly, membrane permeability and membrane leakage was positively correlated with high Zn accumulation (Cakmak and Marschner, 1988). Increased electrolyte leakage is generally considered as an index of membrane damage (Yan *et al*. 1996). Consequently, tolerance index was lower in safflower than in sunflower. This indicated that sunflower plants displayed a very strong capacity to protect itself against Zn toxicity (Graham *et al*. 1992 and Cakmak 2000).

Contents of Chlorophyll and carotenoids increased upto $Zn_{5.0}$ in both plant species. At higher Zn doses toxic symptoms appear, and visual symptoms were chlorosis and necrosis of the leaves. This is in accordance with the results obtained by De Filippis and Pallaghy (1994) and Monnet *et al*. (2001) in *Euglena* and *Lolium perenne* under zinc excess. Hu and Sparks, (1991) reported that Zn plays a key role in photosynthesis, affecting chlorophyll synthesis. The changes in contents of photosynthetic pigments were related to changes in net photosynthetic rate (P_N). One of the major factors which delimit plant growth under zinc stress, is the disruption of the photosynthetic (oxygen evolution) and respiratory (dark O_2-uptake) activities. In the present work, net photosynthesis in the two tested plants decreased at higher Zn applied. The pattern of changes in dark respiration R_D increased from Zn_0 to $Zn_{10.0}$, which can be explained by need for a higher energy allocation for maintenance of ion concentration gradient and active transport processes for the repair of tissues. The increase in maintenance respiration under higher doses of Zn is a

characteristic feature of Zn tolerance. Similar results were found by other authors using other experimental plants. For example, *Silene vulgaris* and *Lycopersicon peruvianum* have developed partial exclusion or specific compartmentation to alleviate zinc stress (Wollgiehn and Neumann 1999) or a reduction in the energy transfer from antennae to the reaction centers, as described by Ralph and Burchett (1998).

In both tested plants, the Mn content increase with increase in Zn content of the shoot system to limit substitution by Zn on the rate of photosynthesis. In our study, under Zn applied the biosynthesis of chlorophyll b was less affected than the biosynthesis of chlorophyll a. Similar results were obtained by De Filippis and Pallaghy (1994) and Monnet *et al*. (2001) in *the Euglena* and *Lolium perenne* under excess Zn. The Mg content was unchanged, whereas Zn content increased in the shoot of both plant species.

Finally it can be said that the tolerance of tested plant species to Zn applied could be linked to enhanced seedling growth and stimulation of net photosynthesis P_N especially under low and moderate Zn doses. The higher Zn accumulation in Zn-sensitive safflower than in Zn-tolerant sunflower was a result of decreased respiratory rate R_D and increased of membrane damage.

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