

The Effect of Zinc on Yield, Yield Components and Micronutrient Concentrations in the Seeds of Safflower Genotypes (*Carthamus tinctorius* L.)

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Abstract

The objective of this study was to determine the effect of zinc (Zn) on yield, yield components and Zn, manganese (Mn), copper (Cu) and iron (Fe) concentrations in seeds of four safflower (*Carthamus tinctorius* L.) genotypes. The research was performed, in a randomized complete block design with three replications. Analysis of variance showed highly significant differences for all the examined traits except harvest index in both years of study. Zinc application significantly affected the plant height, seed weight per plant and 1000-seed weight in the first year and the plant height, seed weight per plant, biological yield per plant, 1000-seed weight and harvest index of the genotypes during the second year. The results of the study showed that Zn uptake differed substantially among the safflower genotypes tested. The Zn, Cu and Mn concentrations in the seeds were increased by Zn application.

Keywords: biological yield, micronutrients, safflower, seed quality, seed yield, zinc deficiency

Introduction

Zinc (Zn) deficiency is one of the most widespread nutritional problems, affecting nearly one-third of the world population (WHO, 2002). In addition, it is known that Zn deficiency not only produces yield losses in plants (Cakmak *et al.*, 1999) but also causes a low accumulation of Zn in the edible parts and seeds of plants (Welch and Graham, 2004). Low Zn intake in the diet results in Zn deficiency in human populations. Serious nutritional problems have been reported, particularly in developed and developing countries, resulting from the consumption of foods low in Zn (Hotz and Brown, 2004; Gibson *et al.*, 2007; Harris *et al.*, 2008; Khoshgofarmanesh *et al.*, 2010; Nriagu, 2010).

Breeding Zn-resistant plant species and varieties and applying Zn-containing fertilizers to correct Zn deficiencies are two approaches to the improvement of Zn concentration in seeds. To obtain genotypes resistant to Zn deficiency through plant breeding requires a long period of time (Graham and Rengel, 1993; Cakmak *et al.*, 1996; Bouis, 2003; Pfeiffer and McClafferty, 2007; Wissuwa *et al.*, 2008). However, increases in yield and in the level of Zn in seeds are possible and relatively easy with Zn fertilization (Phattarakul *et al.*, 2012; Zou, 2012).

The effects of Zn fertilization on the growth and yield of many plants (Yilmaz *et al.*, 1997; Grewal and Williams, 2000; Khoshgofarmanesh *et al.*, 2004; Phattarakul *et al.*, 2012; Zou, 2012) have been studied, but few studies have

investigated the response of safflower (*Carthamus tinctorius* L.) to Zn fertilization.

Safflower, a multipurposed crop, has been grown for centuries in various regions of the world in view of the orange-red dye obtained from its petals, its many medicinal properties, its feed value and especially its high-quality oil, which is rich in polyunsaturated fatty acids. Interest in this crop has increased in recent years, particularly due to its production in areas with limited rainfall, the preference of consumers for healthy oil, the medicinal uses of the flowers and the extraction of edible dyes from the flowers (Singh, 2007). Safflower is an important oil crop that is cultivated mainly for its seed, which is used to obtain edible oil and as birdseed, and may potentially be produced under low-input conditions (Gyulai, 1996; Peterson, 1996; Elfadl, 2009).

In this paper, the effects of Zn application on the soil, on yield and yield components and the accumulation of Zn, Mn, Cu and Fe in seed under low-Zn field conditions with different safflower genotypes were studied over two years.

Materials and methods

This research was conducted during two years, 2009 and 2010, at the Faculty of Agriculture of Eskisehir Osmangazi University, Eskisehir (39° 48' N; 30° 31' E; 789 m elevation). The field experiments included four released safflower varieties ('Yenice', 'Dincer' and 'Remzibey' in Turkey and 'UC-1' in the U.S.) and one high-yielding line

(V-50/63 from Iran). The botanical characteristics of the genotypes were as follows: 'Dincer', non-spiny capitulum and orange petals; 'Yenic', non-spiny capitulum and red petals; 'Remzibey', spiny capitulum; 'UC-1', spiny capitulum and yellow petals; and 'V-50/63', non-spiny capitulum and red petals.

Soil samples were air-dried, passed through a 2 mm sieve and analyzed for pH, CaCO₃, organic matter and texture using standard procedures (Rowell, 1996). Plant-available concentrations of Zn, Fe, Cu, and Mn in the soils were determined according to the methods of Lindsay and Norwell (1978) by extraction with DTPA (Diethylenetriamine Pentaacetic Acid). The soil was alkaline, silty loamy and calcareous with a low content of organic matter and had similar amounts of DTPA-extractable Zn, Fe, Mn and Cu (Tab. 1). The amount of DTPA-extractable Zn was less (0.4 mg kg⁻¹) than the amount (0.7 mg kg⁻¹) needed for optimum plant growth (Sillanpaa, 1990).

The long-term (1991-2010) total rainfall, average temperature and relative humidity at the study location

Tab. 1. Physical and chemical properties of the soils in experimental site

Character	Units	Eskisehir
pH (H ₂ O)		7.6
Organic matter	%	1.04
CaCO ₃	%	6.0
Texture		Silty loam
DTPA extractable Fe	mg kg ⁻¹	3.4
DTPA extractable Zn	mg kg ⁻¹	0.4
DTPA extractable Cu	mg kg ⁻¹	0.3
DTPA extractable Mn	mg kg ⁻¹	3.5

Tab. 2. Monthly temperature, precipitation and relative humidity, Eskisehir (2009 and 2010)

Months	Temperature (°C)			Precipitation (mm)			Relative humidity (%)		
	2009	2010	Long term*	2009	2010	Long term*	2009	2010	Long term*
March	4.6	6.7	4.9	39.8	27.7	29.6	60.5	59.3	64.2
April	10.0	10.2	9.7	26.0	41.2	44.3	55.7	61.2	62.3
May	14.8	16.4	14.9	28.9	5.7	39.4	50.7	55.3	59.3
June	20.4	19.4	19.2	7.9	46.6	24.4	41.0	59.8	55.0
July	22.2	23.3	22.0	11.4	14.3	13.4	42.9	59.7	51.9
August	21.0	25.3	22.0	2.0	1.5	9.0	42.2	52.0	53.0
Mean	15.5	16.8	15.4				48.8	57.8	58.0
Total				116	137	160.1			

*Long-term average, 1991-2010

of seed from each plot were dried for 24 h at 105 °C. After the samples were grounded, they were passed through a nylon sieve (0.5 mm), and 0.2 g of each sample was placed in Pyrex reactors in a microwave digestion unit. HNO₃ and H₂O₂, in a proportion of 3:2, were placed in the reactors. The samples were mineralized at 200 °C for half an hour. The samples were then filtered and diluted to a volume of 100 ml with ultra-pure distilled water. The elements were measured in Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES, Jobin, Yvon-Paris). The data presented are the averages of at least three independent experiments, each consisting of three replications. The quality of the analytical process was also controlled through the use of NCS DC73350

were 160.1 mm, 15.4 °C and 58.0%, respectively (Tab. 2). The rainfall, average temperature and relative humidity were higher in 2010 than in 2009. The long-term average temperature was approximately equal to the average temperature during the 2009 growing season.

The experiment followed a randomized complete block design with three replications. Each plot (5.4 m²) consisted of six rows. The plots were sown at the end of March in 2009 and in the first week of April in 2010, using a seeding rate of 40 kg ha⁻¹ in rows with 30-cm spacing, on a well-prepared seed bed. At planting time, phosphorus (P) and nitrogen (N) were applied at a standard rate of 50 kg ha⁻¹ P₂O₅ as diammonium phosphate (18-46-0) and 80 kg ha⁻¹ N as ammonium nitrate (33-0-0). Plants were irrigated once during emergence and thinned at the rosette stage. Weeds were controlled by hand weeding. Trials were established under rainfed conditions without (-Zn control) and with soil Zn (+Zn: 23 kg Zn ha⁻¹ as ZnSO₄·7H₂O) applications. Zinc sulfate (ZnSO₄·7H₂O) is a common source of Zn fertilizer because it is highly soluble in water and exists in various forms (Hawkins, 1973; Mordvedt and Gilkes, 1993; Yilmaz *et al.*, 1997; Brennan, 2002; Harris *et al.*, 2008).

Yield components were recorded from 10 randomly selected plants in both years. The biological yield per plant, plant height, number of capitula per plant, seed yield per plant, 1000-seed weight, harvest index and seed yield were evaluated for both years. Seed yield in t ha⁻¹ was obtained by harvesting the four central rows of the plot by hand. Approximately three months after harvest, as suggested by the International Seed Testing Association (ISTA, 2008), the 1000-seed weight was calculated by counting eight replicates of 100 seeds from each plot and expressing the weight in grams.

At maturity, approximately 10 g of each seed sample were collected from the plots of each genotype. Sub-samples

certified reference material (poplar leaves). The analysis of these standard reference materials showed good accuracy, with the recovery rates of the metals ranging between 90.3% and 108.9%. The recoveries were compared with the American Organization of Analytical Chemists criteria (AOAC, 1993).

All data were subjected to an analysis of variance (ANOVA) for each character. Mean differences were tested with a least significant difference (LSD) test at a P<0.05 and P<0.01 significance levels. The MSTAT-C computer program (MSTAT, 1984) was used for the analysis.

Results and discussion

Effect of zinc fertilizer on safflower yield components

The results of the ANOVA showed significant effects ($P < 0.05$ or $P < 0.01$) of Zn application on plant height, seed weight per plant and 1000-seed weight in both years. Additionally, the biological yield and harvest index were also significant in the second year (Tab. 3). Genotypic differences were significant ($P < 0.05$ or $P < 0.01$) for all the yield components except the harvest index in both years.

Zinc application produced an increase in the mean of all yield components (Tab. 5 and Tab. 6). Zinc application significantly affected plant height, seed weight per plant and 1000-seed weight in the first year and plant height, seed weight per plant, biological yield per plant, 1000-seed weight and the harvest index of safflower genotypes in the second year. Zinc application significantly affected the biological yield per plant and harvest index of safflower genotypes in the second year but not in the first year. The reason for this difference may be that the years differed climatically. The precipitation rates and temperatures were notably higher in the second year than in the first year. The

precipitation rates and the temperature between March and June during vegetative growth were markedly higher in the second year than in the first year. Sufficient vegetative growth increased the Zn uptake of the plant (Tab. 2). Among the genotypes, the highest average biological yield per plant and the highest harvest index were obtained from 'Yenice' (29.17 g and 36.38%, respectively) in the first year. In the second year, the highest average biological yield per plant was obtained in 'Dincer' (20.40 g) and the highest harvest index in 'Remzibey' (31%).

The mean plant height with applied Zn was 11% higher in the first year than the corresponding value without Zn, and 4% higher in the second year. Among the genotypes, the highest average plant height was obtained from 'V-50/63' (85.94 cm) in the first year and from 'Yenice' (93.26 cm) in the second year. Zn deficiency is known to be characterized by short internodes or stunted plants and reduced plant growth (Mengel and Kirkby, 2001). Movahhedy-Dehnavy *et al.* (2009) observed significant differences in plant height in safflower treated with foliar-applied Zn. The plants treated with Zn were 2-4 cm taller than those without Zn.

Tab. 3. Analysis of variance for effects of genotype and Zn application on yield components of safflower in two years (mean square)

Source	df	Plant Height	Number of Capitula	Seed Weight per Plant	Biological Yield per Plant	Thousand Seed Weight	Harvest Index
2009							
Genotypes (G)	4	944.26**	14.82**	33.46**	174.36*	0.54**	149.80ns
Zn Appl. (A)	1	417.76**	0.73ns	4.09*	3.32ns	0.42**	63.45ns
G x A	4	117.72ns	1.39ns	2.80*	13.22ns	0.72**	35.60ns
2010							
Genotypes (G)	4	890.41**	1.70*	3.44*	30.18**	1.17**	35.68ns
Zn Appl. (A)	1	78.70*	0.59ns	21.96**	54.02**	0.29**	196.96*
G x A	4	49.10*	1.36ns	3.26*	12.85ns	3.00**	77.18ns

* Significant at 5% level; ** significant at 1% level; ns: not significant

Tab. 4. Analysis of variance for effects of genotype and Zn Application on seed yield, Zn, Cu, Mn and Fe concentrations of seeds of safflower in two years (mean square)

Source	df	Seed yield	Zn	Cu	Mn	Fe
2009						
Genotypes (G)	4	447699.15**	114.73**	61.28**	19.67**	613.15*
Zn Appl. (A)	1	4543.62ns	178.60**	214.40**	634.80**	0.43ns
G x A	4	136442.48**	12.15**	60.44**	11.19**	120.03ns
2010						
Genotypes (G)	4	370513.76**	23.36**	1.12**	9.52**	265.30*
Zn Appl. (A)	1	169831.72ns	125.99**	187.50**	144.32**	62.43ns
G x A	4	20658.40ns	5.04**	0.27**	4.90**	100.55ns

* Significant at 5% level; ** significant at 1% level; ns: not significant.

The 1000-seed weight and seed weight per plant have the greatest influence on the seed yield of safflower (Mahasi *et al.*, 2006; Arslan, 2007). The mean 1000-seed weight of varieties grown with applied Zn was 8% higher in the first year and 5% in the second year than that for genotypes grown without Zn. Among the genotypes, the highest 1000-seed weight was obtained in 'Dincer' (4.56 g) in the first year and in 'Remzibey' (4.41 g) in the second year. The highest seed weight per plant was obtained in 'Yenice' (10.01 g) and in 'Dincer' (6.31 g) in the first and second year, respectively. Previous studies have shown that the

1000-seed weight is the yield component most affected by Zn fertilization (Kinaci and Kinaci, 2005; Mirzapour and Khoshgofar, 2006), whereas other yield components were not significantly affected by Zn (Kamali *et al.*, 2009).

The genotype-Zn interaction was significant for seed weight per plant and 1000-seed weight ($P < 0.01$) in both years and for plant height in the second year. The highest value of the genotype-Zn interaction for seed weight per plant was found in 'Yenice' in 2009 and in 'Dincer' in 2010. 'Dincer' and 'Remzibey' showed the highest value of the genotype-Zn interaction for 1000-seed weight in 2009 and

in 2010, respectively. The highest value of the genotype-Zn interaction for plant height was found in 'Yenice' and in 'V50/63' in 2010. These results indicated that the effects of Zn applications depended on the safflower genotypes. Genotypic differences related to Zn deficiency have been discussed by several researchers (Rengel, 2001; Baligar *et al.*, 2001), and the reason that certain species are able to use soil Zn more efficiently than others is not completely clear (Mengel and Kirkby, 2001).

Seed yield and Zn, Fe, Mn and Cu concentrations in safflower

The seed yield of safflower was not affected significantly by Zn application in none of the years (Tab. 4), although Zn application increased the seed yield (Tab. 7). Seed yield was higher in 2010 than in 2009. The effect of the Zn-genotype interaction on seed yield was significant.

Both with and without Zn application, mean seed yield

was higher in the second year than in the first year. Without Zn application in the first year, the mean seed yield was highest in 'Yenice' and lowest in 'UC-1'. Without Zn application in the second year, the mean seed yield was highest in 'Dincer' and lowest in 'V50/63'. Zn application produced a much greater increase in seed yield in the first year (mean 22%) than in the second year (mean 13%). The effect of Zn application on seed yield varied among the genotypes tested in both years. In the first year, Zn application produced the highest increase in seed yield in 'V50/63' (38%), whereas the smallest increase was obtained in 'Remzibey' (9%). Positive effects of Zn application on the seed yield of safflower genotypes have been demonstrated by Movahhedy-Dehnavy *et al.* (2009).

An ANOVA showed significant effects ($P < 0.01$) of Zn application in both years on the Zn, Mn and Cu concentrations of safflower seeds. The genotype-Zn interaction was significant for the Zn, Mn and Cu concentrations ($P < 0.01$) in both years. Only the genotype

Tab. 5. Mean yield components of safflower genotypes under -Zn and +Zn conditions in two years

Genotypes	Plant height (cm)			Number of capitula			Seed weight per plant (g)		
	-Zn	+Zn	Mean	-Zn	+Zn	Mean	-Zn	+Zn	Mean
2009									
V50/63	77.82	94.06	85.94	5.07	4.73	4.90	5.55 d	5.78 cd	5.66
Yenice	72.69	88.94	63.83	8.76	9.43	9.09	8.73 b	11.29 a	10.01
Dincer	66.94	64.97	80.81	6.27	5.23	5.75	6.76 c	5.58 d	6.17
Remzibey	64.30	63.36	65.95	5.80	7.03	6.41	3.76 e	4.63 de	4.19
UC-1	51.82	59.55	55.68	5.93	6.97	6.45	3.71 e	4.92 ed	4.31
Mean (Zn)	66.71B	74.18A		6.37	6.68		7.01B	7.55A	
2010									
V50/63	82.20 b	92.86 a	87.53	3.98	5.53	4.75	3.53 c	5.85 b	4.69
Yenice	91.20 a	95.32 a	93.26	6.03	5.27	5.65	4.78 b	4.72 b	4.75
Dincer	75.55 bc	72.68 cd	74.11	5.70	6.53	6.11	4.37 b	8.25 a	6.31
Remzibey	68.21 de	74.57 cd	71.39	5.41	4.90	5.15	4.36 b	5.60 b	4.98
UC-1	64.50 e	62.43 e	63.46	5.60	5.90	5.75	3.76 c	4.93 bc	4.34
Mean (Zn)	76.33B	79.57A		5.34	5.63		4.16B	5.87A	

Note: Values are means of three independent replicates. For each trait, means followed by different letters are significantly different from each other for "genotype x Zn interaction" (LSD test, $P < 0.05$ and $P < 0.01$).

Tab. 6. Mean yield components of safflower genotypes under -Zn and +Zn conditions in two years

Genotypes	Biological yield per plant (g)			Thousand seed weight (g)			Harvest Index (%)		
	-Zn	+Zn	Mean	-Zn	+Zn	Mean	-Zn	+Zn	Mean
2009									
V50/63	17.84	17.63	17.73	3.59 c	3.90 bc	3.74	31.31	33.36	32.33
Yenice	28.05	30.29	29.17	4.20 b	4.34 b	4.27	32.15	40.62	36.38
Dincer	19.56	15.94	17.75	4.19 b	4.94 a	4.56	39.24	34.97	37.10
Remzibey	17.02	17.59	17.30	4.16 b	3.94bc	4.05	22.28	28.51	25.39
UC-1	13.88	18.23	16.05	4.06 b	4.28 b	4.17	27.67	29.74	28.70
Mean (Zn)	19.27B	19.94A		4.04B	4.28A		30.53B	33.44A	30.13
2010									
V50/63	12.83	20.24	16.53	3.20 f	3.27 f	3.23	27.85	29.18	28.51
Yenice	18.56	19.72	19.14	3.99 cd	4.17 bc	4.08	25.80	23.84	24.82
Dincer	19.55	21.26	20.40	3.70 e	3.73 e	3.71	22.41	38.63	30.52
Remzibey	16.08	15.89	15.98	4.19 b	4.64 a	4.41	26.99	35.01	31.00
UC-1	13.42	16.77	15.09	3.81 ed	4.06 bc	3.93	28.02	30.04	29.03
Mean (Zn)	16.09	18.78		3.78B	3.97A		26.21	31.34	28.77

Note: Values are means of three independent replicates. For each trait, means followed by different letters are significantly different (LSD test, $P < 0.05$ and $P < 0.01$).

had a significant effect on Fe concentration in both years (Tab. 4).

In both years, the Zn concentration in the seed was increased by Zn application. In the first and second year, the mean Zn concentration in the seeds was 36 mg kg⁻¹ and 38 mg kg⁻¹, respectively, without Zn application, whereas the mean Zn concentration was 41 mg kg⁻¹ and 43 mg kg⁻¹, respectively, with Zn application (Tab. 7). Movahhedy-Dehnavy *et al.* (2009) found that Zn application changed the Zn concentration of safflower seeds. Our results were similar to theirs. 'Remzibey' and 'Dincer' varieties showed the highest increase in seeds Zn concentration as a result of Zn application. These results showed that these genotypes can be used for human and animal nutrition. Zinc deficiency in humans and animals has been a serious nutritional problem in Turkey (Cakmak *et al.*, 1999) and in the world (Alloway, 2008). Safflower varieties that show a greater increase in seed Zn in response to Zn application

may aid in the solution of this problem. Wissuwa *et al.* (2008) found significant genotypic differences in the concentrations of Zn in grain.

The Cu and Mn concentrations in the seed in all the genotypes increased with Zn application in both years. The Cu, Mn and Fe concentrations in the seeds were 3.96, 5.15 and 50.32 mg kg⁻¹, respectively, without Zn application and 9.31, 14.35 and 50.57 mg kg⁻¹, respectively, with Zn in the first year. In the second year, the Cu, Mn and Fe concentrations in the seeds were 4.75, 4.47 and 61.71 mg kg⁻¹, respectively, without Zn application and 9.75, 8.86 and 58.83 mg kg⁻¹, respectively, with Zn. Copper and Mn were affected by Zn application, whereas the Fe concentration in the seed decreased or did not change markedly. Kaya and Higgs (2002) reported that Zn application might prevent the uptake and transfer of Fe in shoots and fruits.

Tab. 7. Seed yield, Zn, Cu, Mn and Fe concentrations of seeds

Genotypes	Seed yield (kg/ha)			Zn (mg/kg)		Cu (mg/kg)		Mn (mg/kg)		Fe (mg/kg)	
	-Zn	+Zn	%	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn
2009											
V50/63	1238.2 bc	1706.0 a	38	33.40 h	34.37 g	2.75 d	3.30 d	5.53 ef	16.80 a	36.93	50.00
Yenice	1254.6 bc	1412.6 bc	13	36.90 f	42.93 c	5.80 bc	6.10 b	3.35 f	10.53 cd	50.10	47.77
Dincer	921.1 ef	1249.5 bc	36	40.83 d	43.97 b	4.70 bcd	15.75 a	7.95 ed	15.10 ba	55.17	50.40
Remzibey	953.6 def	1038.3 dce	9	38.47 e	46.73 a	3.60 cd	5.03 bc	5.90 ef	13.33 bc	38.09	43.13
UC-1	738.7 f	828.2 ef	12	30.90 i	36.90 f	2.95 d	16.35 a	3.03 f	16.00 ab	71.33	61.53
Mean (Zn)	1021.0	1247.0	22	36.00 B	41.00 A	3.96 B	9.31A	5.15B	14.35A	50.32	50.57
2010											
V50/63	999.0	1248.5	25	38.05 g	39.23 e	4.60 de	9.50 b	4.75 cd	9.85 ab	53.33	62.64
Yenice	1138.3	1425.4	25	39.13 e	42.87 c	4.23 e	9.70 ab	4.70 cd	9.40 ab	77.90	65.43
Dincer	1666.8	1756.3	5	40.37 d	45.87 a	4.90 cd	9.83 ab	5.60 c	8.63 b	59.60	59.83
Remzibey	1554.8	1679.7	0	38.33 f	44.10 b	4.23 e	9.57 b	4.00 cd	10.85 a	56.87	52.00
UC-1	1578.6	1580.0	8	36.23 h	40.53 d	5.77 c	10.13 a	3.30 d	5.55 c	60.87	54.23
Mean (Zn)	1388.0	1538.0	13	38.00B	43.00 A	4.75B	9.75A	4.47B	8.86A	61.71	58.83

Note: Values are means of three independent replicates. For each trait, means followed by different letters are significantly different (LSD test, P < 0.05 and P < 0.01).

Conclusions

The results of the study showed that the yield and yield components of safflower genotypes differed substantially in response to Zn application. It can be concluded that safflower genotype 'V50/63' showed a higher yield with Zn application and that the yield of 'Remzibey' was not strongly affected by Zn deficiency. However, further detailed studies should be conducted to understand the reasons underlying the differential Zn efficiencies of safflower genotypes. 'Remzibey' might be used as a genetic source for obtaining Zn-efficient safflower genotype in breeding programs. The line 'V50/63' was found to be very sensitive to Zn deficiency.

The seed yield and the Zn concentration in the seeds, in the studied safflower genotypes, responded well to Zn application. Therefore, Zn application should be considered by safflower growers in Zn-deficient areas.

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