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Effect of the Selenium and Zinc Concomitant Use on Some Morphological and Physiological Properties of Spring Safflower (C.V. Arak 2811)

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ABSTRACT: Present study carried out to observe the effect of selenium and zinc application on spring safflower. This experiment was done using two-factor factorial experiment based on randomized complete blocks design in four replications in 2013. This work had two factors which the first factor was selenium foliar application in four levels (0 g/ha (control), 10 g/ha, 15 g/ha and 20 g/ha) and the second factor was zinc foliar application in three levels (control, 2 g/lit zinc sulfate and 0.5 g/lit zinc nanoparticles). Achieved results some safflower properties in this study (seed yield, biological yield, harvest index, seed zinc concentration) indicated that selenium application created significant effect on most of traits. It's needed to be noticed that 15 g/ha selenium and 2 g/lit zinc concomitant use increased seed yield by 22.19 percent. In general it can be stated that the application of selenium by 15 g/ha increased seed yield and in the rate of 20 g/ha leads to decreasing on its amount.

Keywords: Selenium, zinc, safflower, seed zinc concentration.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) as an important oilseed crop had been cultivated in arid and semi-arid regions of the world, such as India and elsewhere in the Middle East and Africa (Ravi *et al.,* 2008). This native, valuable and oily plant was cultured years away in this country.

Interest in the role of selenium in plants greatly intensified in the last two decades. Selenium in trace amounts is one of the beneficial micronutrients for human and animals' nutrition. However, high doses of selenium may have toxic effects for animals (Lemly, 1997; Nigam et al., 1969; Wilber, 1980) and humans (Von Vleet and Ferrans, 1992). Selenium concentrations range from very small and necessary amounts to fatal amounts are quite narrow. The minimum level of nutrition for the animals is about 0.05 to 0.10 mg selenium per kg of dry matter, while exposure at levels 2 to 5 mg selenium per kg of dry matter it will cause toxicity (Wilber, 1980; Wu *et al.,* 1996). The first report about the nutritional benefits of selenium was published in 1957 (Schwarz and Foltz, 1957). In 1973, selenium is shown as the part of the important antioxidant enzyme formation process called glutathione peroxidase (GSH) (Rotruck *et al.,* 1973). Among the other selenium therapeutic properties are include suppression of cancer (Jansson, 1980) and the elimination of specific symptoms associated with AIDS.

Selenium is an essential element for many organisms; however, selenium isn't mentioned as an essential element (Terry *et al.,* 2000; Ferri *et al.,* 2007; Ellis and Salt, 2007). The plants shows a variety of physiological responses and some species do store large amounts of selenium in themselves. But selenium is toxic to some plants, so that these plants are so susceptible to large amounts of selenium in soil and water (Terry *et al.,* 2000). In general, selenium is an essential element for thirty selenoenzymes and selenoprotein and it is an important component of enzymes that protect cells against free radicals. Also incorporation of selenium into proteins, protects tissues and membranes against oxidative damage (Terry *et al.,* 2000; Turakainen *et al.,* 2004). Although selenium is an essential element for plants, but it is not necessary and even toxic. But nonetheless low concentrations of selenium have beneficial effects on the metabolism of plant cells and regulates the absorption of some ions. Plants treated with selenium increases the amount of enzymes eliminator the H_2O_2 (particularly ascorbate peroxidase and glutathione peroxidase) and antioxidant compounds (such as ascorbate, glutathione and proline) (Hasanuzzaman *et al.,* 2010; Khattab, 2004; Krzysztof *et al.,* 2008) and that is why selenium can reduce the amount of H_2O_2 in plants (Rios *et al.,* 2008). Studies have shown that the soil in some areas (such as Northern Europe) are faced with selenium deficiency (Gao and Tanji, 2000; Wu and Huang, 1991). Selenium foliar application increased the amount of antioxidant enzymes and drought resistance raises (Dhillon, 2002).

Some reports (Pennanen *et al.,* 2002) showed that treatment of selenium increases the length of root and shoot. Low concentrations of selenium enhances plant growth by increasing the synthesis of photosynthetic pigments, carbon fixation and synthesis and hydrolysis of starch and sucrose but in high levels leads to chlorophyll reduction and reducing carbohydrate synthesis and subsequent reduction in plant growth (Han-Wens *et al.,* 2010; Tailin *et al.,* 2001). Wen Han and colleagues (2010) stated that selenite at low concentrations, improves cell division in the root tip meristematic cells and subsequent root growth in garlic but at high levels causes to reduction in cell division in these cells (Madaan and Mudgal, 2011).

Nowadays in addition to macronutrients utilization micronutrients is concerned as an important tool to achieve maximum crop yield (Mosavi *et al.,* 2007). Micronutrients in addition to increasing the quality and quantity of crops, influence on the health of humans and livestock (Sharma *et al.,* 1992). Consumption of micronutrient in deficiency cases, especially by the spray can improves safflower yield and yield components (Lewis & McFarlane, *et al.,* 1986; Movahedy-dehnavy *et al.,* 2009). Nowadays proved that micronutrients are in all the crops increase yield. Lack of micronutrients are discussed as a limiting factor for safflower yield (Rehm *et al.,* 1981).

Research has shown that the use of zinc before flowering, increase yield and protein content in soybeans (Rose *et al.,* 2002). Some researchers, also in 1970, were introduced vital and effective role of zinc in plants and demonstrated that this element plays a key role in producing protein- enzyme activity such as dehydrogenase proteinase (Kheirandish, 2000; Rose *et al.,* 2002). Also zinc have catalyst, actuator and constructional role in the many plant enzyme systems and zinc increased protein synthesis and reduces the accumulation of amino acids in plants by the transfer of amino acids and reducing the degradation of RNA (Brown *et al.,* 1993). Research results about application of zinc indicate that the application of these elements in different growth stages of safflower can affect the performance of the plant in different ways (Alloway, 2003).

MATERIALS AND METHODS

This study was carried out using a factorial experiment based on randomized complete block design with 4 replications in Arak. The region is located by 34° 6' 35" N 49° 36' 35" E and height Above Sea Level is 2160 meters. Sowing date was done in March 10, 2013. This region having 180-150 days without rain, cool/wet winters and hot/dry summers is as a hot/dry Mediterranean areas moisture regime. Experimental factors included selenium foliar application in four levels (0 g/ha (control), 10 g/ha, 15 g/ha and 20 g/ha) as firs factor and zinc foliar application in three levels (control, 2 g/lit zinc sulfate and 0.5 g/lit zinc nanoparticles) as second factor. It is need to be noticed that just water was sprayed in control treatments. Treatments were done two weeks before flowering. Foliar application was done in this way that solution drops was so flowing on all parts of the safflower that aerial extremities were completely soaked. Sprayer nozzles were put at a height of 40 cm above the plant. Ultimate harvest was performed simultaneously at all the blocks in mid-August.

A. Statistical Analysis

To analyze this study's data were applied ANOVA procedure of SAS software. For mean comparisons Duncan test was used by a probability level of 5%.

RESULTS AND DISCUSSION

A. Seed yield

Results of analysis of variance showed that in addition to the main effects of the experimental factors, interaction factors also had a significant effect on seed yield. So that basis of the main effects of comparisons average (Table 2), application of 15 g/ha Se (the highest level: average of 1126.27 kg/ha) leads to increase 14.56 % in compare to 20 g/ha Se (the lowest level: average of 983.08 kg/ha). Due to an increase in the use of selenium yield can be attributed to the fact that Se increases the absorption of micronutrients, which are beneficial to plant growth. Also the role of Se in small amounts can increase the amount of H_2O_2 eliminator enzymes (Hasanuzzaman *et al.,* 2010; Khattab, 2004; Krzysztof *et al.,* 2008) that is toxic (Rios *et al.,* 2008). However, when the amount exceeds a certain limit the application of selenium can be toxic. However, when the amount exceeds a certain limit the application of selenium can be toxic (Terry *et al.,* 2000). Some reports have also reported similar results with the results of this study (Timothy, 2001; Smith and Peery, 1996). Also it has been observed that among the zinc doses application, the highest yield was related to 2 g/lit on the average of 1111.85 kg/ha in comparison to the control (non-application) which showed 10.2% increase. This increase can be due to deficiency of micronutrients such as zinc. Sharafiand colleagues (2000) reported that increased levels of micronutrients increases these elements acceptors in plants. The results of this test with results Silspoor (2007) are also compatible.

B. Biological yield

In this test the biological yield same as the seed yield, effects by the main and interaction effects. As indicated in Table comparisons of mean effects (Table 2), application of 15 and 20 g/ha Se produce maximum and minimum amounts of biological yield by 14.81% increase in safflower respectively. Same as seed yield can be stated that application of Se up to a certain amount leads to increase in the vegetative and reproductive growth of the plant due to the beneficial effects and vice versa excessive application causes negative effects of selenium and then of course it will reduce the amount of vegetative and reproductive growth.

Table 1: Analysis of variance.

SOV	df	Seed yield	Biological yield	Harvest index	Seed zinc concentration
Replication	3	945.44 ^{ns}	20387.03 ^{ns}	0.25 ^{ns}	6.76 ^{ns}
Selenium	3	44913.87 **	556719.04 **	0.82 ^{ns}	2.77 ^{ns}
Zinc	2	41088.13 **	65707.72 **	11.65 **	10.21 *
Se * Zn	6	5557.67 *	64177.87 **	0.18 ^{ns}	51.91 **
Error	33	1859.30	11514.03	1.45	2.67
CV%	------	4.06	2.82	4.31	5.27

*, ** and ns: Significant at the 5% and 1% level of probability and non-significant, respectively.

Table 2: Analysis of variance.

SOV	df	plant height	heads per plant	Seed per head	1000 seed weight
Replication	**3**	49.89*	3.72*	1.64^{ns}	6.44^{ns}
Selenium	**3**	91.32**	23.56**	52.50**	20.67*
Zinc	**2**	55.97*	4.42*	18.57*	0.32^{ns}
Se * Zn	**6**	4.30^{ns}	1.11^{ns}	4.14^{ns}	3.65^{ns}
Error	**33**	15.38	1.22	5.51	6.75
CV%	**-----**	6.81	5.70	6.87	10.55

*, ** and ns: Significant at the 5% and 1% level of probability and non-significant, respectively

Table 3: Mean comparison of main effects.

Experimental treatments		Seed yield (kg/ha)		Biological yield (kg/ha)		Harvest index (%)		Seed zinc concentration (mg/kg)	
Se	0 g/ha	1047.06	c	3768.80	b	27.77	a	30.61	a
	10 g/ha	1087.49	b	3838.22	b	28.33	a	31.59	a
	15 g/ha	1126.27	a	4049.48	a	27.79	a	31.16	a
	20 g/ha	983.03	d	3526.91	c	27.90	a	30.59	a
Zn	0 g/lit	1010.50	c	3731.72	b	27.10	b	30.07	b
	0.5 g/lit	1060.58	b	3795.94	ab	27.95	ab	31.37	a
	2 g/lit	1111.85	a	3859.89	a	28.80	a	31.53	a

Mean in each column, followed by similar letter(s) not significantly different at 5% probability level, using DUNCAN test.

Table 3: Mean comparison of interaction effects.

Experimental treatments		Seed yield (kg/ha)		Biological yield (kg/ha)		Harvest index (%)		Seed zinc concentration (mg/kg)	
0 g/ha Se	**0 g/lit Zn**	993.45	de	3671.17	d	27.06	b	33.02	ab
	0.5 g/lit Zn	1034.86	d	3764.37	cd	27.50	ab	28.51	b
	2 g/lit Zn	1112.85	b	3870.83	bc	28.75	a	30.31	b
10 g/ha Se	**0 g/lit Zn**	1019.21	d	3688.86	d	27.66	a	27.25	bc
	0.5 g/lit Zn	1100.73	bc	3881.48	b	28.36	a	34.32	a
	2 g/lit Zn	1142.53	b	3944.30	b	28.98	a	33.21	a
15 g/ha Se	**0 g/lit Zn**	1050.92	cd	3934.32	b	26.73	b	26.58	b
	0.5 g/lit Zn	1113.92	b	3991.57	b	27.91	a	33.21	a
	2 g/lit Zn	1213.97	a	4222.53	a	28.75	a	33.68	a
20 g/ha Se	**0 g/lit Zn**	978.40	e	3632.53	de	26.94	b	33.43	a
	0.5 g/lit Zn	992.80	e	3546.32	ef	28.03	a	29.42	b
	2 g/lit Zn	978.03	e	3401.88	f	28.74	a	28.91	c

Mean in each column, followed by similar letter (s) not significantly different at 5% probability level, using DUNCAN test.

In previous reports also stated that the use of selenium in certain amounts were increased root growth and aboveground plant parts and undesirable increase the amount of selenium reduces the yield (Hawrylak-Nowak, 2008; Jahid *et al.*, 2010; Tailin *et al.*, 2001). The report also states that the use of small amounts of selenium increases the biological function compared to control (Alda *et al.*, 2011). As is clear from the results of this study more Zn utilization increase the biological function too. According to the average comparison results can be seen that the use of 2 g/lit Zn, increasing the biological yield by the rate of 3.43% compared to control. The reason for this is probably due to the high absorption of this element parallel with excessive application of Zn and in fact compensation the lack of micronutrients increase vegetative growth and increase biomass production of plant.

Table 4: Mean comparison of main effects.

Experimental treatments		plant height (cm)		heads per plant		Seed per head		1000 seed weight (g)	
Se	0 g/ha	54.10	c	19.23	b	33.07	b	23.88	ab
	10 g/ha	56.74	bc	20.28	a	35.03	a	25.35	ab
	15 g/ha	58.73	ab	20.68	a	36.55	a	26.02	a
	20 g/ha	60.53	a	17.54	c	31.83	b	23.16	b
Zn	**0** g/lit	55.57	b	18.85	b	32.95	b	24.68	a
	0.5 g/lit	57.70	ab	19.57	ab	34.35	ab	24.69	a
	2 g/lit	59.30	a	19.88	a	35.07	a	24.44	a

Mean in each column, followed by similar letter (s) not significantly different at 5% probability level, using DUNCAN test.

Table 4: Mean comparison of interaction effects.

Experimental treatments Se	Zn	plant height (cm)		heads per plant		Seed per head		1000 seed weight (g)	
0 g/ha	**0** g/lit	51.85	c	18.25	cd	31.65	bc	23.52	a
	0.5 g/lit	53.68	bc	19.40	bc	33.40	b	24.06	a
	2 g/lit	56.78	b	20.06	a	34.16	b	24.07	a
10 g/ha	**0** g/lit	55.88	b	19.47	b	32.97	b	24.90	a
	0.5 g/lit	57.04	b	20.42	a	35.58	a	25.32	a
	2 g/lit	57.30	b	20.39	a	36.46	a	25.82	a
15 g/ha	**0** g/lit	57.17	b	19.92	ab	34.96	ab	25.60	a
	0.5 g/lit	58.94	ab	20.85	a	36.59	a	26.23	a
	2 g/lit	60.07	a	20.28	a	38.11	a	26.23	a
20 g/ha	**0** g/lit	57.39	b	17.78	d	32.22	b	24.71	a
	0.5 g/lit	61.16	a	17.61	d	31.83	b	23.14	ab
	2 g/lit	63.05	a	17.25	d	31.43	c	21.62	b

Mean in each column, followed by similar letter (s) not significantly different at 5% probability level, using DUNCAN test.

The report found that foliar application of zinc increased biological yield by increase in rates of photosynthesis, the initial growth and nitrogen fixation (Ved *et al.,* 2002). Also Homayouni and colleagues (2013) reported that biological yield increased In effect the rate of 3 per thousand by 6% compare to the control treatment.

C. Harvest index

Unlike the Se main effect and interaction between Se and Zn, using the spraying of Zn could cause significant differences between the various treatments compare to control. Whatever applying the amount of Se was increased, the amount of the difference were added more and more between the 0, 0.5 and 2 g/lit Zn. In fact, increasing the Zn applied leads to of 6.28% difference in harvest index between the 2 g/lit Zn and control treatment. Actually application of Zn is causing the rising trend in harvest index which suggest that the removal of the zinc deficiency, adds the dry matter amount allocated to the economic sector's plant. Other researchers have also reported that concomitant use of iron and zinc cause to achieve the highest harvest index (Shirani-Rad *et al.,* 2011). Given that the treatments sort on yield is are likewise the harvest index, It can be stated that one of the causes of these results in seed yield are affected by similar results in thousand seed weight. In accordance with these results, the report also states that the application of small amounts of selenium in the barley is increased thousand seed weight per plant (Habibi, 2013). Also some researchers reported lake of effectiveness of Se application on thousand seed weight (Zahedi *et al.,* 2009).

D. Zinc concentration

As it can be seen in the analysis of variance, zinc main effect and the interaction of factors could cause significant changes in the concentration of zinc in the seed. Considering meaningfulness of zinc application on the concentration of seeds zinc, by increasing the amount of zinc applied, it is added to the absorption of zinc in the seeds. Somehow that the application of zinc 0.5 and 2 g/lit allocated the highest amount of zinc concentration in the seed, 31.37 and 31.53 mg/kg respectively which showed 4.30% and 4.84% increase in compare to control. In terms of the absorption in the seeds the results indicate the medium needs of soil to adding the zinc, because differences between the values of 5.0 and 2 g/lit zinc are not statistically significant. Ravi and colleagues (2008) reported that application of zinc in safflower enhances zinc absorption compared to the control and of course concurrent use of other micro nutrients such as iron along with zinc, leads to intensification of zinc absorption. Some other reports also stated that application of zinc enhanced zinc concentration in stem of seed (Tolay *et al.,* 2011).

E. Plant height

As a result, foliar application selenium had the significant effect on Safflower plant height. However, the interaction between the two factors were not cause to significant effect on plant height.

So that an increase in selenium dose increases more and more safflower plant height. In fact, the effect of selenium toxicity in this trait did not cause negative effects. The results is consistent with the results of Elda and colleagues (2011) who reported an increase in plant height in parallel to increase the use of selenium.

Increase in zinc use rate increased plant height of safflower. This result can be related to poor soil in terms of micronutrient deficiency such as zinc. Others have reported similar results with this research (Homayouni *et al.,* 2013; Thalooth *et al.,* 2006; Gupta *et al.,* 2003). Other researchers have also reported similar results in the flax plant (Bakry *et al.,* 2012).

F. Number of heads per plant

The main effects of the factors unlike those interactions were affected significantly the number of heads per plant. Concurrent with the increasing application of selenium to 15 g/ha, was added to the number of heads per plant. In operation 20 g/ha of selenium reduces the number of heads per plant, even to the extent of less than control. In this experiment, it was observed that the highest number of heads per plant in application of 15 and 10 g/ha Se, 20.68 and 20.28 respectively which express 7.54 and 5.46 % increase respectively. Well as minimum number of heads per plant achieved in application of 20 g/ha Se with an average of 17.54 (9.63 % reduction compared to control) in the plant. According to these results, it seems that selenium has been shown to improve survival and pollen fertilization on plant. It was also reported that selenium prevents the degeneration of chlorophyll (Seppanen *et al.,* 2003). Similar results reported in the other findings (Zahedi *et al.,* 2009). In the other study also found that the application of 10 and 20 mcg of selenium reduces the number of bolls per plant is canola which can be considered that probably special amount of selenium was leading to toxicity and reduced the quality and quantity of pollination and fertilization (Hajiboland and Keivanfar, 2012). It was also reported that the addition of Se delayed monocarpic senescence in the soybean that consequently application of Se 75 days after sowing leads to increase in number of pods per plant compare with control (Djanaguiraman *et al.,* 2004).

G. Number of seeds per head

Individually application of selenium and zinc could cause a significant impact on the number of seeds per head, but they did not cause significant differences in their simultaneous application. Due to the significantly of selenium it was found that treatment of 15 and 10 g/ha Se (average of 36.55 and 35.03 respectively) create 10.52 and 5.92 % increase compared to the control, respectively, which were the highest numbers. However, the lowest number obtained in application of 20 g/ha Se with average of 31.83which indicate 3.89 % reduction rather than control treatment. The reason of this increase in the number of seed per head is obviation of selenium deficiency as a necessary element.

But since the toxicity of selenium is a multiple of its peers can be toxic in large amounts to the creation of reducing the influence of other nutrients. The report also states that utilize the Se increases the number of seed per three varieties of canola (Zahedi *et al.,* 2009). Also Zahedi and colleagues (2011) reported that application of 15 g/lit selenium increases the number of seeds per rapeseed boll compared to control. It is worth noting that in their research application of 30 g/lit Se increases seeds per head but the rate of increase was less compared to 15 g/lit. This could indicate that the reduction in the rate of increase in the number of seeds per boll treated with 30 g / lit, compared to 15 g / lit probably is due to the low levels of toxicity. But the reason behind this increase compared to control treatments can be more resistant to high levels of selenium in the rapeseed plant.

H. One thousands seeds weight

Achieved results declared that in this study, unlike the application of zinc, foliar application of selenium cause significant differences in the amount of safflower seed weight. So that the use of selenium in the amount of 15 g/ha produced plants with highest seed weight with an average of 26.02 g which in compare to 20 g/ha by average of 23.16 g, indicated 12.34 % increase in plant seed weight.

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