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ZnO affects soybean grain yield, oil quantity, quality, and leaf antioxidant activity in drought stress conditions

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Abstract

Lack of available water in arid and semi-arid regions has a negative effect on the soybean production (*Glycine max* L.). Nano zinc chelate (ZnO) has the potential to alleviate this issue through overcoming zinc deficiency and thereby improve plant function. Therefore, to evaluate the effectiveness of ZnO application on soybean in drought stress conditions, a split plot experiment based on randomized complete block design was conducted. Irrigation treatments (100%, 80%, 60% and 40% water requirement) was the main plot and foliar application of nano-zinc (2 ml.L^{-1} foliar treatment vs. no-application control) was the subplot. The results showed that drought stress had negative effects on plant yield and productivity. Under drought, grain yield, seed oil percentage, oil yield, palmitic acid, stearic acid, cis-oleic acid and linoleic acid were decreased. ZnO application significantly increased proline content, catalase and peroxidase activities. However, the percentage of palmitic acid, stearic acid, linoleic acid and α-linoleic acid decreased with the ZnO foliar spray. In general, results showed that, ZnO treatment, with enhancing antioxidant enzymes activity and changing physiological parameters decreased adverse effects of drought stress on soybean plants.

Keywords: Catalase, Fatty acids, Foliar spray, Peroxidase

Introduction

Soybean is a major agricultural crop worldwide and according to FAO, 351.3 million tons of soybean was produced in 2017 (FAO, 2018). Soybean is mainly grown for protein, forage, and oil (Jahanzad *et al*., 2015). As the leading oil crop worldwide, soybean seeds contain 22% oil content. (Liu and Li, 2010; Kobraee and Shamsi, 2013). Soybean oil contains unsaturated fatty acids including oleic acid, linoleic acid, and linolenic acid. These unsaturated fatty acids often lower serum cholesterol levels, reduce the chance of cardiovascular ailments, and therefore, improve human health (Liu and Li, 2010).

Deficiency of water resources is a major challenge for achieving global food security goals. An inevitable consequence of drought stress is increase in reactive oxygen species (ROS) production in the chloroplasts, the peroxisomes and the mitochondria. The production of ROS is the initial response of the plant to drought stress, which in turn activates second messengers for plant protective responses (Farooq *et al.*, 2009). In mild drought stress conditions, ROS act as molecular signals and cause plant cell responses to water scarcity (Farooq *et al.*, 2016). However, a severe drought condition induces the generation of ROS such as superoxide, hydrogen peroxide, hydroxyl and alkoxy radicals (McCord, 2000). It affects cellular structure and proteins, results in the death of plant cell (Smirnoff, 1993).

One of the typical effects of drought stress is the reduced solubility and uptake of nutrients by the plant roots (El-Fouly *et al*., 2011). Therefore, soils in arid and semi-arid regions have often lowered soil fertility companed with the temperate regions (Karymi *et al.*, 2016; Ashraf *et al.*, 2012). One strategy to alleviate the drought stress is application of macro and microelements to plants (Waraich *et al.*, 2011; Kobraee and Shamsi, 2013; Dimkpa *et al.*, 2017). Zinc is one of the essential micronutrients that affects the structure and function of the plant and is considered a cofactor for key cellular enzymes (Marschner, 1986; Dimkpa *et al.*, 2017). Zinc deficiency is very common in arid and semi-arid regions. Zinc is less soluble in alkaline soils and thus is unavailable for plant uptake by roots; a typical issue of arid and semi-arid soils regions (Marschner, 1993; Karymi *et al.*, 2016). One strategy to overcome zinc deficiency in semi-arid soils is foliar application of ZnO (Kobraee and Shamsi, 2013; Yadavi *et al.*, 2014). Direct contact of the solution with the leaf

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surface and its short transfer pathway to leaf photosynthetic active centers and higher absorption efficiency of the leaf than the root (due to the elimination of the effects of soil colloids) have caused increasing application of the ZnO foliar spray (Karymi *et al.,* 2016; Movahhedy-Dehnavy *et al.,* 2009). Previous research has shown positive effects of foliar application of nano-zinc on yield, yield components and grain protein of chickpea (*Cicer arietinum* L.) in drought stress conditions (Shaban *et al.,* 2012). Improved crop growth and yield in mungbean and chickpea has been also reported by foliar addition of zinc in water-limited conditions (Thalooth *et al.*, 2006; Shaban *et al.*, 2012).

Today, the use of compounds containing engineered nanoparticles is increasing in various fields of science, including agriculture (Ruffini and Cremonini, 2009; Dimkpa *et al.,* 2017). Nano particles are the atoms or molecules lower than 100 nm. A reduction in size of such particles increased the special surface of any particles inside fertilizers and accordingly led to release more energy from their surface. Because of their special properties derived from their size, nanoparticles cause many morphological and physiological changes in plants. The efficiency of nanoparticles is determined by the chemical composition, size, surface, reactivity and concentration and their effects are dependent on the aforementioned characteristics (Khodakovskaya *et al.*, 2012; Garcia-Gomez *et al.,* 2017). In recent years, novel findings have been obtained on the effects of nanoparticles and their absorption by the plant. In this regard, reports show the positive and negative effects of nanoparticles on the growth and development of plants, and this type of effect depends on the composition, concentration, size, and physical and chemical characteristics, as well as plant species (Ma *et al.*, 2010; Dimkpa *et al.*, 2017). These findings showed that the application of nanoparticles containing micro nutrient such as zinc and copper in plant subjected to drought stress could play an important role in improving soybean yield (Dimkpa *et al.*, 2017). In addition, with the development of nanotechnology, nano chelates of micro nutrients such as zinc is progressively expanding to increase the absorption and transfer efficiency (Mohammadi, 2015). Therefore, in this study, the effect of ZnO foliar spray was investigated on antioxidant enzyme activity, grain yield, oil quality, and soybean oil quality under the drought stress conditions.

Materials and methods

Location of the experiment: The present experiment was carried out at Research Field, Faculty of Agriculture, University of Lorestan, Iran in 2016. The experimental site was located at 48’ 17” longitude, 33’ 26” latitude, with an altitude of 1148 meters above sea level. Before cultivation, the soil analysis was carried out at a depth of 0-30 cm by Jons (2001) methods (Table 1). According to the recommendations of the soil analysis, 5 tons per hectare of sheep manure were used. Considering the nitrogen fixation by plant itself, urea fertilizer was used up to 50 $Kg.ha^{-1}$ before cultivation. In addition, 100 kg of triple superphosphate and 250 kg of potassium sulfate was also added per hectare and the soil preparation operation was performed. Each experimental unit was 15 square meters (5 × 3) and consisted of five rows of 5 meters length. The distance between the main plots and subplots was 3 and 1.5 meters, respectively, and the distance between repetitions was 5 meters. The distance between soybean plants was 5.5 cm in each row and the spacing between the rows was 60 cm. Before cultivation, soybean seeds (Kowsar cultivar) inoculated with bacteria and weeds were controlled manually during the growth period.

Experimental treatments: The treatments included irrigation at four levels: 100% water requirement, 80% water requirement, 60% water requirement and 40% water requirement in the main plots, and ZnO foliar spray at the control level (distilled water) and 2 $ml.L^{-1}$ ZnO application (included 20% zinc absorbable ion, 2 liters per hectare) in the subplots. ZnO was purchased from the Knowledge-based Sepehr Parmis Company in Zanjan.

Drought stress treatments were applied from the start of flowering to full maturity. Irrigation treatments were made based on soil moisture deficiency, and were scheduled according to the time and depth of irrigation. To determine the appropriate irrigation time, soil moisture monitoring was performed and irrigation was carried out before soil moisture was discharged more than the threshold of the intended treatment. Maximum allowable moisture discharge coefficient for soybean was considered as 0.55 AWC (Available Water Content) (Allen *et al.,* 1998). The volume of water required for each irrigation treatment was calculated by equation (1), taking into account the area of each plot and applying the corresponding stress coefficients. The net and gross depth of irrigation were determined by Eq. (2) and (3) to replace the soil moisture. During the season, the volume of water input to each plot was controlled by volume meter.

$$V = d_g \times A \times f \quad (1)$$

Where A: area of each plot (m), dg: gross depth of irrigation water (mm), V: irrigation water volume (liters), and f: stress coefficient

$$dn = (\theta fc - \theta i) \times pb \times Zr \quad (2)$$

$$dg = dn/E \quad (3)$$

Where dn: net depth of irrigation water (mm), fc: soil moisture weight at soil capacity point, iθ soil moisture weight before irrigation, pb: specific apparent mass of the soil (g/cm3), Zr: depth of root (mm), dg: gross irrigation depth (mm). The calculation of gross irrigation depth was based on 95% efficiency.

ZnO foliar spray treatment was performed at 50% flowering and at 50% podding of soybean (containing 20% absorbable zinc ion) in 1000 liters of water per hectare in the early early morning hours. In control treatment, distilled water was applied for foliar

Table 1- Soil analysis of texture, elements, acidity and electrical conductivity before sowing of soybean

Soil texture	(%)			pH	EC (dS/m)		Lime(%)
	Clay	Silt	Sand	7.8	0.55		16.5
Clay	41	28	31				
Elements							
K available (mg kg^{-1})	P available (mg kg^{-1})	O.C (%)	Cu	Zn	Mn		Fe
			(mg kg^{-1})				
345	6.8	1.11	1.3	0.62	10.1		4.10

spraying. The EC and pH of the fertilizer solution was 11 µS/cm and 6.8, respectively.

Measurements: To measure proline and antioxidant enzymes, at the generative stage (two months after stress), two fully developed young leaves were selected from each replicate and transferred to the laboratory in the refrigerator. After homogenizing the samples, leaf proline was measured by the method of Bates *et al.* (1973). Beers and Sizer (1952) method was used to measure catalase activity (CAT), and Zhang *et al.* (1995) method to measure the activity of peroxidase enzyme (POD).

At maturity (133 days after sowing), after removing the margins of the plots, a surface area of three m^2 was harvested manually and the grain yield per unit area was determined. In order to prepare the necessary samples to determine the quality of the seeds, 50 gr of seeds were removed from each plot. After milling the samples, oil extraction was carried out by a soxhlet using a polar ether solvent.

To measure the zinc concentration in soybean, the extract was first prepared by the wet oxidation method (Westerma, 1990). Then, the concentration of zinc element was determined using ICP (ICP-OES Integra XL, GBC, Australia). For the analysis of fatty acids, the method of methyl ester preparation from oil was used and then, the Varian CP-3800 gas chromatography apparatus and CPsill-88 column were used to determine the percentage of fatty acids (British Pharmacopoeia, 2009).

Experiment design and Data analysis: The experiment was carried out in a split plot design in a Randomized Complete Block design (RCB) with three replications. Data analysis variance was performed by SAS 9.1 software. Comparisons of mean specifications were performed at a probability level of 5% with Duncan multiple range test. Excel 2007 was also used to draw graphs.

Results

Catalase and peroxidase: The interaction effects of drought stress and ZnO was significant on the activity of catalase ($P \leq 0.01$) and peroxidase ($P \leq 0.05$) (Table 2). With increasing drought stress, the activity of catalase enzyme was significantly increased by the ZnO foliar spray. Although there were no significant differences between the use of zinc and control at the highest level of stress (40% water requirement), at each of 100, 80 and 60% water requirement levels, the catalase activity in ZnO treatment was greater compared to the control (Figure 1 a). By decreasing water availability, leaf enzymes significantly increased leaf peroxidase activity. The highest activity was obtained by the combined treatment of 40% water requirement ZnO foliar spray (Figure 1 b). The use of ZnO increased the activity of peroxidase under normal moisture conditions as well as different levels of drought stress (Figure 1 b).

Proline: Proline content of the leaf was affected by the ZnO foliar spray and water deficit treatments ($P \leq 0.01$) (Table 2). With the increase in the intensity of water deficit stress, leaf proline content increased in the application and non-application treatments (Figure 2). Under non-stress conditions (100% irrigation), the application of ZnO on leaves caused a significant increase in proline content of soybean leaves. In each of 80 and 60% water stress treatments, proline content of leaves was not affected by foliar spray treatment. At the highest stress level (40% water requirement), ZnO foliar spray significantly increased leaf proline content (Figure 2).

Grain yield: Interactions of ZnO foliar spray and the drought treatments were significant on soybean grain yield ($P \leq 0.05$, Table 2). Reduction of the water availability decreased the grain yield, so that the highest level of stress (40% water requirement) had a 65% reduction in grain yield compared to normal irrigation. Under non-stress conditions, the use of ZnO foliar spray did not have any effect on grain yield, but under low water stress conditions, application of zinc significantly improved the yield (Figure 3 a).

Grain zinc concentration: When meeting the 100% water requirement, ZnO foliar spray had no effect on zinc of grain, but in 80% water requirement, the ZnO had a significant effect on the zinc content of grain compared to the control (Figure 3 b). At the other levels of drought stress, zinc content of the grain was not affected by ZnO foliar spray (Figure 3 b). The grain zinc content had a positive and significant correlation with grain yield (Table 3). Although in the experimental treatments there was no significant difference in zinc concentrations, considering the higher grain yield of the ZnO foliar spray treatment compared to the control at each stress levels of 80 to 40% water requirement, it can be deduced that the total zinc content in harvested area under ZnO treatment was more than the control (Figure 3).

Grain oil content: Interaction of the ZnO foliar

Table 2- Analysis of variance of biochemical traits, seed and oil yield and fatty acids.

S.O.V	d.f	Proline	Catalase	Peroxidase	Seed yield	Seed zinc content	Seed oil content	Oil yield
Replication	2	0.64**	0.08^{ns}	0.005*	61599*	60.41^{ns}	17.34**	1712**
Irrigation (I)	3	0.70**	2.05**	0.001^{ns}	167190**	6.33	10.85**	5950**
Error a	6	0.02	0.05	0.0007	5826	19.79	0.016	34
Zn	1	0.92**	1.46**	0.0002	27263**	10.30	36.95**	257566*
I × Zn	3	0.60**	1.85**	0.002*	10443*	55.76*	0.06*	37240*
Error b	8	0.01	0.03	0.0004	2202	11.21	0.014	2
C.V (%)	-	14.3	7.9	17.0	14.3	5.2	0.6	1.4

ns,* and **: are non-significant, significant at 5 and 1% probability levels, respectively.

Continue of table 2-

S.O.V	d.f	Palmitic acid	Stearic acid	Oleic acid	cis-Oleic acid	Linoleic acid	α-Linoleic acid
Replication	2	2.57**	0.82**	5.37**	0.036**	1.79**	0.92**
Irrigation (I)	3	1.20**	0.19**	9.29**	0.072**	7.81**	0.17**
Error a	6	0.01	0.01	0.01	0.001	0.01	0.01
Zn	1	1.43**	1.14**	8.12**	0.066**	0.12**	1.42**
I × Zn	3	2.18**	0.17**	18.00**	0.17497**	27.76**	0.39**
Error b	8	0.01	0.01	0.01	0.001	0.01	0.01
C.V (%)	-	1.0	1.5	1.1	1.5	1.0	1.2

ns,* and **: are non-significant, significant at 5 and 1% probability levels, respectively.

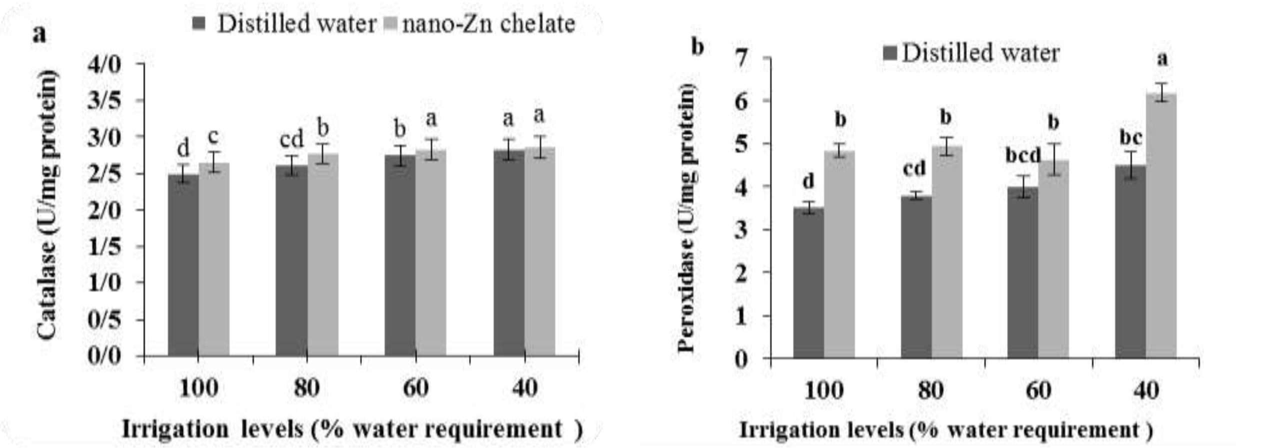

Figure 1- Mean comparison of irrigation levels and foliar application of nano-zn chelate on leaf catalase (a) and peroxidase (b). Columns with at least one same letter have not significant differences at 0.05 probability level using multiple range test. The bars indicate standard error (n=3).

spray and the drought treatments on the oil content of the grain were significant (P≤0.05) (Table 2). The use of ZnO prevented the significant reduction of grain oil content under drought conditions. As the drought stress increased to 60% and 40% water requirement, the oil content of the grain decreased if no ZnO was used. At each level of irrigation, ZnO foliar spray significantly increased the oil content of the grain (Figure 4 a).

Oil yield: Drought stress reduced oil yield per unit area. The ZnO improved the oil yield in both control and each drought stress levels (Figure 4 b). Considering the significant correlation between the grain content and oil yield (0.73), grain oil percentage (0.74) and grain yield per unit area (0.73) (Table 3), and also the increase of the mentioned specification due to the use of ZnO, it could be said that the application of ZnO had been effective in increasing the quantity and quality of soybean grain under the drought stress.

Fatty acids of grain oil: Interaction of the ZnO foliar spray and water deficiency treatments on saturated and unsaturated fatty acids were significant (P≤0.01) (Table 2). Drought stress and ZnO foliar spray had a significant effect on the fatty acids of the oil. With the progress of drought stress, the percentage of palmitic acid in treatments without ZnO decreased significantly. Although at levels of 80 and 100% of water requirement, the percentage of palmitic acid (C16: 0) decreased due to the ZnO, but at 60% water

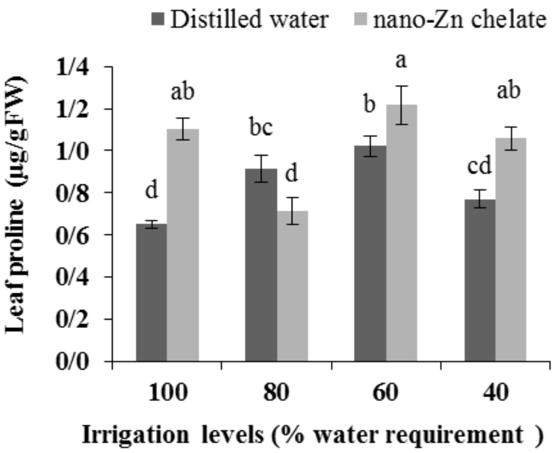

Figure 2- Mean comparison of irrigation levels and foliar application of nano-zn chelate on leaf proline. Columns with at least one same letter have not significant differences at 0.05 probability level using multiple range test. The bars indicate standard error (n=3).

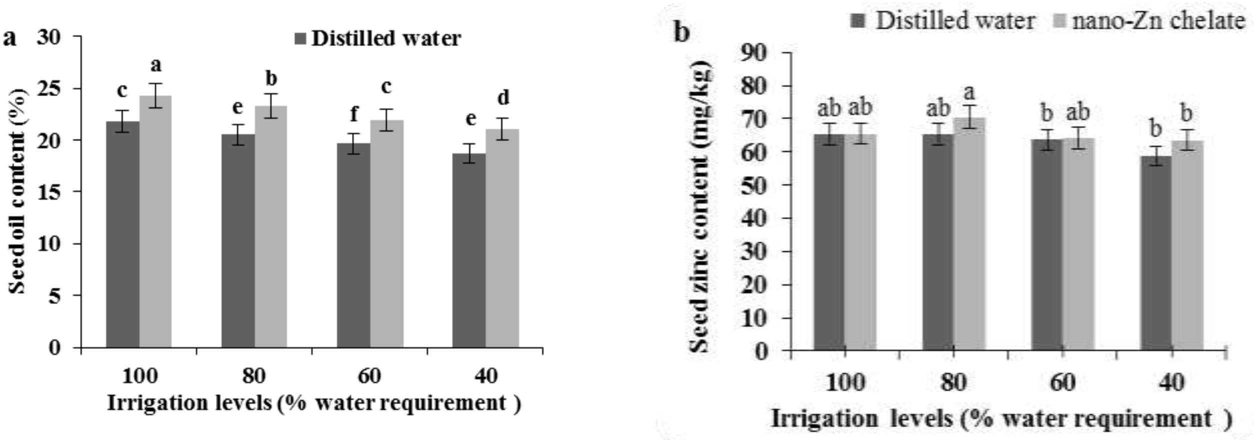

Figure 3- Mean comparison of irrigation levels and foliar application of nano-zn chelate on seed yield (a) and zinc content (b). Columns with at least one same letter have not significant differences at 0.05 probability level using multiple range test. The bars indicate standard error (n=3).

Table 3- Correlation between biochemical traits, seed and oil yield and fatty acids.

Traits	1 Catalase	2 Peroxidase	3 Proline	4 Seed zinc content	5 Seed yield	6 Seed oil content	7 Oil yield	8 Palmitic acid	9 Stearic acid	10 Oleic acid	11 cis-Oleic acid	12 Linoleic acid	13 α-Linoleic acid
1	1												
2	0.72^{*}	1											
3	0.46^{ns}	0.40^{ns}	1										
4	-0.29^{ns}	0.02^{ns}	-0.20^{ns}	1									
5	-0.73^{*}	-0.23^{ns}	-0.30^{ns}	0.73^{*}	1								
6	-0.24^{ns}	-0.23^{ns}	0.13^{ns}	0.74^{*}	0.82^{*}	1							
7	-0.66^{*}	-0.15^{ns}	-0.24^{ns}	0.73^{*}	0.99^{**}	0.87^{**}	1						
8	-0.63^{*}	-0.44^{ns}	-0.16^{ns}	0.10^{ns}	0.31^{ns}	-0.04^{ns}	0.21^{ns}	1					
9	-0.34^{ns}	-0.69^{*}	-0.33^{ns}	-0.23^{ns}	-0.15^{ns}	-0.53^{ns}	-0.25^{ns}	0.67^{*}	1				
10	0.60^{ns}	0.42^{ns}	-0.10^{ns}	0.06^{ns}	-0.34^{ns}	-0.12^{ns}	-0.33^{ns}	-0.04^{ns}	-0.21^{ns}	1			
11	-0.41^{ns}	0.05^{ns}	-0.06^{ns}	0.41^{ns}	0.47^{ns}	0.35^{ns}	0.41^{ns}	0.78^{*}	0.17^{ns}	0.14^{ns}	1		
12	-0.19^{ns}	-0.06^{ns}	-0.22^{ns}	-0.03^{ns}	0.20^{ns}	-0.23^{ns}	0.25^{ns}	-0.48^{ns}	-0.61^{ns}	-0.85^{**}	-0.44^{ns}	1	
13	-0.31^{ns}	-0.49^{ns}	-0.19^{ns}	-0.65^{ns}	-0.16^{ns}	-0.45^{ns}	-0.17^{ns}	-0.08^{ns}	0.16^{ns}	-0.63^{*}	-0.57^{ns}	$.48^{ns}$	1

ns,* and **: are non-significant, significant at 5 and 1% probability levels, respectively

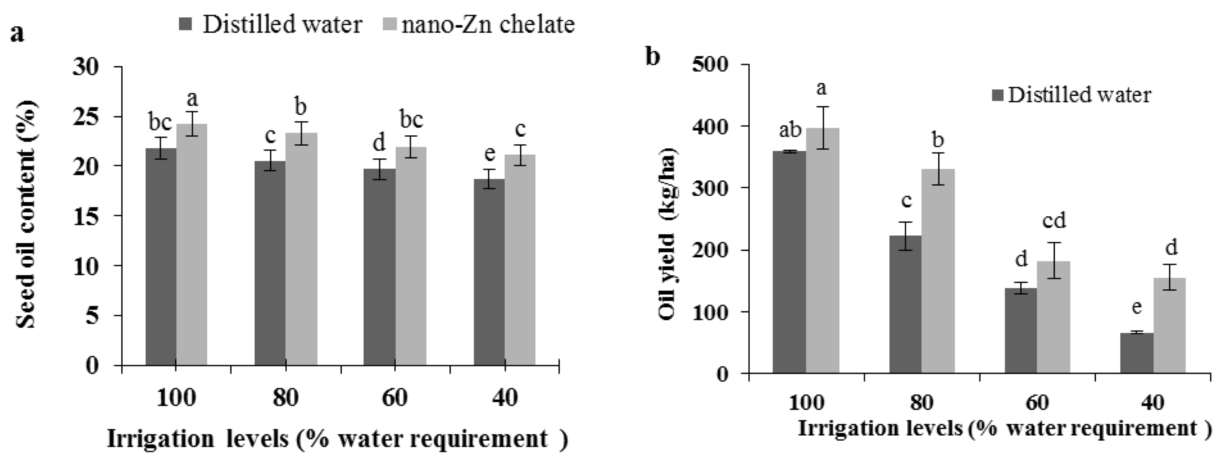

Figure 4- Mean comparison of irrigation levels and foliar application of nano-zn chelate on seed oil content (a) and oil yield (b). Columns with at least one same letter have not significant differences at 0.05 probability level using multiple range test. The bars indicate standard error (n=3).

requirement it did not have any significant effect, and at 40% of water requirement, it increased the palmitic acid (Figure 5 a).

Generally, with increasing drought stress, the percentage of stearic acid (C18: 0) decreased. In each level of water deficiency stress, spraying of the ZnO decreased the stearic acid in comparison to distilled water spraying (Figure 5 b).

With increasing drought stress, the percentage of oleic acid (C18: I) increased. However, under non-stress conditions, the percentage of oleic acid in distilled water spraying was significantly higher than that of ZnO spraying. However, by applying the water deficiency stress, the percentage of oleic acid in ZnO treatment significantly increased (Figure 5 c).

With the increase of water deficiency, the percentage of *cis*-oleic fatty acids (*cis*-C18: I) decreased. The ZnO at the no-stress level and 80% water requirement, decreased *cis*-oleic acid. However, at higher levels (60 and 80% water requirement), the percentage of *cis*-oleic acid increased by the ZnO significantly (Figure 5 d).

The percentage of linoleic acid (C18: II) decreased by increasing water deficiency. The highest and lowest percentage of linoleic acid were obtained in no water stress conditions and ZnO and distilled water spraying, respectively (Figure 5 e). The α-linoleic acid (C18: II) increased by water shortage. At every level of water deficiency, the percentage of α-linoleic acid was lower in the ZnO treatment in comparison to distilled water spraying. The highest and lowest amounts of α-linoleic acid were obtained in the treatment without ZnO at 40% water requirement and the application of ZnO at 80% water requirement (Figure 5 f).

In general, the percentage of palmitic acid and stearic acid in distilled water treatment was reduced with increasing drought stress and the total concentration of these fatty acids in ZnO treatment was lower than that of distilled water spraying. Total amount of unsaturated fatty acids (oleic acid, *cis*-oleic acid, linoleic acid and α -linoleic acid) was higher in the ZnO treatment in comparison to distilled water spraying under both stress and full irrigation conditions (Figure 5). Application of the ZnO under no-water deficiency stress (100% water requirement) reduced the percentage of oleic and *cis*-oleic acids, but increased the linoleic and α-linoleic acids. In other words, by an increase in the unsaturated fatty acids, the quality of the oil was improved. In drought stress conditions, zinc spraying increased the percentage of unsaturated fatty oleic acid and *cis*-oleic acid; but decreased linoleic acid and α-linoleic acid.

Discussion

The results of current study showed that with increasing drought stress, leaf proline and activity of catalase and peroxidase enzymes increased. Also, the previous studies have indicated increased proline content and antioxidant activity in drought stress conditions (Labanauskas *et al*., 1981; Karymi *et al*., 2016; Garcia-Gomez *et al*., 2017). Environmental stresses cause an over-production of ROS and induction of oxidative damage (McCord, 2000; Farooq *et al*., 2016). In such conditions, various types of adaptations may occur to protect the plant, including the production of osmolytes and stimulating the antioxidant enzymes (Farooq *et al.*, 2009; Garcia-Gomez *et al.*, 2017). According to the results, it seems that application of 60% and 40% water requirement has the most effect on stimulation of antioxidant systems of soybean. The ZnO, in low water levels, increased proline, and activity of catalase and peroxidase. The recent studies have shown that the ZnO foliar spray under drought stress conditions increases the amount of osmolytes such as proline and soluble carbohydrates in the soybean leaf and improves the antioxidant activity via catalase, peroxidase and superoxide dismutase enzymes (Karymi *et al.*, 2016) which was consistent with the results obtained in this research. The zinc, as an essential micronutrient in plants, has structural role in some enzymes (such as

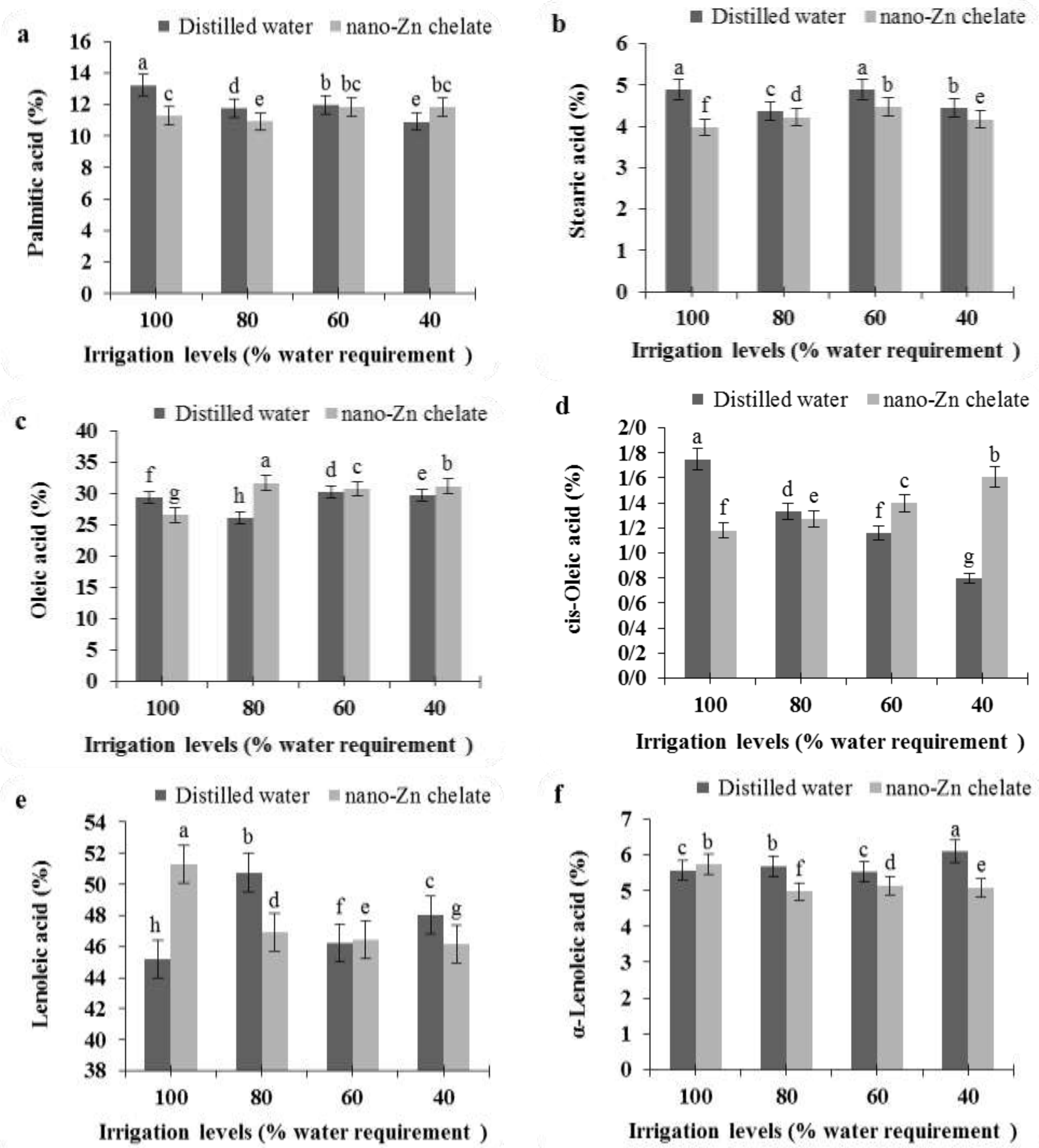

Figure 5- Mean comparison of irrigation levels and foliar application of nano-zn chelate on seed fatty acids: Palmitic acid (a), Stearic acid (b), Oleic acid (c), *cis*-Oleic acid (d), Lenoleic acid (e), α-Lenoleic acid (f). Columns with at least one same letter have not significant differences at 0.05 probability level using multiple range test. The bars indicate standard error (n=3).

SOD-Zn) and co-factor of enzymes in the synthesis of proteins, DNA and RNA, photosynthesis, auxin synthesis, cell division, and fertilization (Kobraee *et al.*, 2011). Zinc maintains membrane integrity under stress conditions in an indirect manner (Bharti *et al.*, 2013), which is associated with an increase in the production of cellular protecting compound due to the presence of zinc (Garcia-Gomez *et al.*, 2017). Considering the reduction of nutrient uptake capacity by root in drought stress conditions (Egli *et al.*, 2004; Siddique, 2016), it seems that ZnO foliar spray causes a modification of the deficiency of this nutrient in the plant. In this way, all processes associated with it, which have a cell protective function, have improved. The structural and co-factor roles of zinc in enzymes (Kobraee and Shamsi, 2013) increased the scavenging of free radicals in soybean leaves.

Despite the slight changes in zinc grain content by the zinc treatments, higher grain yield was obtained in plants treated with ZnO resulted in more zinc extraction by the zinc treatment. Also, the result of a study indicated that zinc extraction increased with increasing grain yield due to its foliar application in safflower (Movahhedy-Dehnavy *et al.*, 2009). The results of Dimkpa *et al.* (2017) showed that by using micronutrients such as zinc in water deficiency stress, the amount of nutrient accumulation in soybean shoot increased significantly, which was consistent with the results of the present study. In spite of little changes occurred in oil content of grain, higher grain yield in the plants treated with Nano-Zn chelate fertilizer is contributed to absorbing more zinc by ZnO-treated plants as compared to the controls. The results of other researchers also indicated that an increase at level of absorbing zinc accompanied with enhancing in grain yield was due to applying spraying the fertilizers on leaves of sunflower (Movahhedy-Dehnavy *et al*., 2009). In our research, it seems that the rate of zinc used in order for enhancing its content in soybean grains was not sufficient, or an adequate rate of zinc in soil may nullify effect of treatments on zinc content in grains.

With the progress of drought stress at the reproductive stage, grain yield, oil yield and oil percent decreased, but the ZnO mitigated drought effects on the mentioned traits. Studies on various species such as sunflower (Movahhedy-Dehnavy *et al.*, 2009), chickpea (Shaban *et al.*, 2012), bean (Yadavi *et al.*, 2014) and soybean (Karymi *et al*., 2016; Ghasemi-Golezani *et al.*, 2015; Kobraee and Shamsi, 2013; Bertolli *et al.*, 2012) indicated a negative effect of drought stress on quantitative and qualitative traits related to grain yield and even on the quantity and quality of the grain oil, which was consistent with the present study. Decreasing in the percentage of grain oil by drought stress may be due to the drought inhibitory effects on all parts of the plant. At this situation, the non-structural carbohydrates such as glucose, fructose and sucrose are decreased; and as a result, the sugars limitation during grain filling will occur, resulting in a shortage of the grain oil (Farooq *et al.*, 2016). In addition, increasing the ROS by drought causes high damage to the cellular structure and biological macromolecules (Smirnoff, 1993; Farooq *et al.*, 2016). In fact, ROS cause oxidation of fats (Foyer, 2005; Farooq *et al.*, 2016). It can be stated that accumulation of ROS by drought during grain filling made the oxidation of saturated fatty acids of soybean oil, thereby, reducing the percentage of the grain oil and, consequently, the oil yield per unit area. In line with these results, researchers have also suggested that in addition to reducing the soybean oil, drought stress changes the composition of grain's fatty acids as well (Dornbos and Mullen, 1992). The foliar application of zinc and manganese have increased grain yield and oil yield of sunflower under stress conditions (Movahhedy-Dehnavy *et al.*, 2009). It has also been affirmed that the positive effects of using nano-iron chelate in the improvement of the yield of soybean oil (Mohammadi, 2015). In another study, the spraying of micronutrients (zinc, manganese and iron) also reduced the effects of stress and increased soybeans yield (Kobraee and Shamsi, 2013), which was consistent with the positive results obtained from the application of ZnO in this experiment.

The oil quality depends on the ratio of unsaturated fatty acids to saturated fatty acids (Movahhedy-Dehnavy *et al.*, 2009; Mohammadi, 2015). In this experiment, zinc application reduced the total amount of saturated fatty palmitic acid and stearic acid by 2.7% and increased the quality. Previous findings have shown that the use of nano-iron chelate increased the percentage of unsaturated fatty acids of linoleic acid and oleic acid in soybeans (Mohammadi *et al.*, 2015). In current study, drought stress during grain filling had a slight effect on the composition of fatty acids. Although the previous findings on soybean showed that the drought stress increased the stearic acid and decreased oleic acid (Dornbos and Mullen, 1992), in the present experiment, with increasing drought, the average data of treatments with the zinc foliar spray indicated a decrease in the relative percentage of stearic acid but an increase oleic acid. In drought conditions, the oleic acid and linoleic acid increased and *cis*-oleic acid was decreased. Dornbos and Mullen (1992) showed that linoleic and linolenic acids decreased by 11.1% under high-temperature stress during grain filling, and, by contrast, oleic acid increased by 10%. They suggested that it might be due to the limitation of the conversion of oleic acid as a precursor of linoleic and linolenic acids, thereby the percentage of oleic acid increased, and linoleic and linolenic acids decreased. The type of stress, soybean cultivar, and climatic conditions seem to have contributed to the contrariness of the results of this experiment with the previous study.

In general, the results of this experiment indicated positive effects of the ZnO in the drought stress conditions for the improvement of the grain yield, oil percentage and yield, and increase the quality of oil by increasing the unsaturated fatty acids. Improvements of the activity of catalase and peroxidase enzymes, along with proline increase, were other positive effects of ZnO. Therefore, it is advisable to use ZnO foliar spray in order to improve the quantity and quality of yield and to increase the resistance of soybeans to drought stress conditions.

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