

## Effect of Foliar Spray of Zinc Oxide on Some Antioxidant Enzymes Activity of Sunflower under Salt Stress

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### ABSTRACT

This study investigated the effects of foliar application of normal and nanoparticles of zinc oxide (ZnO) on the growth, proline content, and some antioxidant enzyme activities of sunflower cultivars at different salinity levels. Treatments included five cultivars (*Helianthus annuus* L. cvs. Alstar, Olsion, Yourflor, Hysun36, and Hysun33), two salinity levels (0 and 100 mM NaCl), and three foliar applications (none-sprayed, ZnO normal and nanoparticles at a rate of 2 g/L). Olsion showed the highest proline content and superoxide dismutase activity (SOD) among the studied cultivars under saline condition. Foliar spray of ZnO improved SOD activity and shoot dry weight of sunflower. Nanoparticles of ZnO had positive effect on biomass production of sunflower plants compared to the normal form. According to the result, Olsion and Hysun33 cultivars were suitable for saline conditions, whereas Hysun36 was appropriate for normal condition.

**Keywords:** Nanoparticles, Superoxide dismutase, *Helianthus annuus*, Proline.

### INTRODUCTION

Salinity is excessive accumulation of salt in soil that leads to problems in plant water uptake. Soil salinization occurs in arid regions more than other parts because the amount of rainfall in these areas is not enough to wash the salt from the root zone (Owens, 2001). Salinity reduces plant growth by reducing soil water potential, cells turgor pressure, disrupting the balance of nutrients in the soil and plants, and can also cause toxicity effects (Shilpim and Narendra, 2005). Reactive oxygen species (ROS) are among the most damaging factors to cells under biotic and abiotic stresses. Superoxide (O<sub>2</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl (OH) are reactive oxygen species, which increase under stress conditions

in plants (Neill *et al.*, 2002). Plant cells are equipped with antioxidant defense systems in order to avoid the damaging effects of ROS, which is composed of enzymatic and non-enzymatic components. The most important antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Mittler, 2002). Several reports have been published on the antioxidant enzyme activities of sunflower under salinity (Di Baccio *et al.*, 2004; Rios-Gonzalez *et al.*, 2002; Davenport *et al.*, 2003). Common biochemical response of plant cells to osmotic stress is accumulation of organic metabolites such as proline, betaine, and sucrose (Xiong and Zhu, 2002).

Zinc plays an important role in the germination, plant production, and chlorophyll

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synthesis (Kaya and Higgs, 2002). Zn can reduce the negative effects of ROSs in environmental stresses and its deficiency decreases plant resistance (Cakmak and Marschner, 1988). Antioxidant enzyme activities decrease under lack of micronutrients and stress condition, which lead to decrease of the plant resistance. Zn reduced ROSs production and increased activity of enzymes such as SOD, CAT and POD (Yu *et al.*, 1998).

Although the effects of soil and foliar application of Zn on plant yield have been evaluated, few studies are available about its effects on salt tolerance of plants and difference of efficiency between normal and nano forms of ZnO. The use of nanotechnology is growing in all areas, especially agriculture. The diameter of nano-particles is less than 100 nm, which can alter their physical and chemical properties (Monica and Cremonini, 2009). The use of nano-particle fertilizers have been considered recently. Plant species respond to nano-particles differently. For example, in the study of Zhu *et al.* (2008), *cucurbita maxiama* L. could absorb, transport, and accumulate nano-particles in their tissues, but *phaseolus limensis* L. could not. There are a few reports about the positive effect of nano-particles on crops such as groundnut (Prasad *et al.*, 2012), pea (Pandey *et al.*, 2010) and spinach (Yang *et al.*, 2006).

This experiment was conducted to investigate the effect of normal ZnO and nano-particles of ZnO on the antioxidant enzyme activities and proline content of sunflower cultivars under saline conditions.

## MATERIALS AND METHODS

An experiment with five sunflower (*Helianthus annuus* L.) cultivars, namely, Alestar, Olsion, Hysun36, Yourflor, and

Hysun33, at three levels of Zinc Oxide foliar application (normal ZnO, ZnO nano-particles with 99.9 % purity at the rate of 2 g/L and none-sprayed containing distilled water) and two salinity levels (0 and 100 mM NaCl) in three replications was conducted at the greenhouse of College of Agriculture, Isfahan University of Technology, during April and Jun, 2012. Both forms of ZnO used in this experiment were from US Nano Company. The average size of ZnO nano-particles was 20 nm. The plants were kept under controlled conditions of greenhouse with an 8-h light period at a light intensity of  $390 \text{ mmolm}^{-2}\text{s}^{-1}$ , 25/20 °C day/night temperature, and 65-75% relative humidity. A bulk surface soil (0-30 cm) sample was collected from Golpaygan in the north western of Isfahan. Selected physical and chemical properties of this soil are shown in Table 1. Ninety Polyethylene pots (30 cm height and 20 cm diameter) were first filled with a 5 cm layer of well-washed sand to improve drainage. On top of this sand 10 kg soil was added. Seeds of sunflower cultivars were obtained from the Seed and Plant Improvement Institute, Karaj, Iran. Some characteristics of the cultivars are given in Table 2. Ten sunflower seeds were sown, thinned to four plants per pot after 10 days, and grown for 50 day. At two foliar stages, about 500 mL of a 2g/L  $\text{KNO}_3$  solution was applied to each pot. In a twenty day period after germination (four-leaf stage), NaCl was added to irrigation water in step-wise aliquots of 50 mM up to 100 mM. Final EC of soil was  $12.2 \text{ dS m}^{-1}$  after salt was added. Foliar application of ZnO was applied twice: the first one was used immediately after the end of six-leaf stage and the second foliar application was carried out one week later. Plants were sprayed until the leaves were completely wet and the solution ran off the leaves. Plants

**Table 1.** Some characteristics of the soil used in the experiment.

Texture	Sand %	Silt %	Clay %	pH	EC $\text{dS m}^{-1}$	N %	Available P	Available K $\text{mg kg}^{-1}$	DTPA Zn
Loam	45.5	35	19.5	7.6	2.3	0.08	25	188	0.79

**Table 2.** Some agronomic properties of sunflower cultivars.

Cultivar	Salinity status	Maturity status
Alestar	Intermediate	early
Olsion	Tolerant	semi early
Hysun36	Sensitive	early
Yourflor	Sensitive	early
Hysun33	Tolerant	early

were harvested after 45 days, leaf and roots were washed with deionized water. The roots and shoots were separated for analysis. Plant samples were dried for 72 h at 70 °C and weighed. Leaf Zn concentrations were measured according to the method of Chapman and Pratt (1961) by atomic absorption spectrometry (Perkin-Elmer, Analyst 200, Perkin Elmer, Waltham, MA). Free proline contents were measured according to the method of Bates *et al.* (1973). Young fresh leaf samples were frozen at -80 °C for enzyme analysis.

### Enzyme Assay

All biochemical traits were done at Crop Physiology Laboratory, College of Agriculture, Isfahan University of Technology. To determine antioxidant enzyme activities, fresh leaf samples (0.3 g) from the control and treated plants were ground with liquid nitrogen, and suspended in specific buffer and pH for each enzyme extraction. The homogenates were centrifuged at 14000 rpm for 20 min at 4 °C and the resulting supernatants were used for enzyme assay. The protein concentrations of leaf crude extract were determined according to Bradford (1976).

### Catalase (CAT) Activity

Catalase (CAT, EC 1.11.1.6) activity was assayed at 20 °C in a 3-mL reaction volume containing 2.8 mL of 50 mM potassium phosphate buffer (pH 7 not containing EDTA), 120 µL enzyme extract, and 80 µL 0.5 M

hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Activity was determined by spectrophotometry (HITACHI U-1800) at 240 nm, measuring the decrease in absorbance for 30 s (Aebi, 1984).

### Superoxide Dismutase (SOD)

SOD (EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride, as described by Giannopolitis and Ries (1977). The assay mixture consisted of 50 µL of the enzyme extract, 50 Mm phosphate buffer (pH 7.8), 0.1 µM EDTA, 13 mM methionine, 75 µM nitroblue tetrazolium and 2 µM riboflavin in a total volume of 1.5 mL. Riboflavin was added last and tubes were shaken and placed under fluorescent lighting from two 20 W tubes. The reaction was allowed to proceed for 15 min, after which the lights were switched off and the tubes covered with a black cloth. Absorbance of the reaction mixture was read at 560 nm, and one unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the nitroblue tetrazolium photoreduction rate.

### Ascorbate Peroxidase (APX)

APX (EC 1.11.1.11) activity was measured immediately in fresh extracts and was assayed as described by Nakano and Asada (1981), using a reaction mixture (1 mL) containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM hydrogen peroxide, 0.5 mM ascorbate and 0.1 mM EDTA. The hydrogen peroxide-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm (Extinction coefficient 2.8 mM<sup>-1</sup> cm<sup>-1</sup>).

### Statistical Analysis

The experiment was set up in a completely randomized design with factorial arrangement of treatments and three



replications. Data were analyzed by one-way analysis of variance (ANOVA) using the statistical software SAS (version 9.1). The treatment mean values were compared by least significant difference (LSD) test at 0.05 level of probability.

## RESULTS

### Shoot and Root Dry Weight

Analysis of variance indicated that shoot dry weight was significantly affected by salinity, foliar application, and cultivar, whereas root dry weight was affected only by salinity and cultivar (Table 3). Salt stress of the growth medium caused significant ( $p < 0.01$ ) reductions in shoot and root dry weight (Figure 1). Averaged over the cultivars and foliar application, plants growing under the 100 mM NaCl showed lowered means of shoot and root dry weights by 20% compared with the none-saline condition. Irrespective of the salinity and foliar application, the highest values of shoot and root dry weight belonged to cv. Olsion, however, the lowest values of shoot (16.3 g) and root (2.4 g) dry weight were observed in cvs. Yourflor and Alestar (Figure 1). Foliar spray of ZnO produced a significant growth of sunflower plants measured as dry weight of shoot, whereas the root growth of

sunflower was not affected by foliar spray of ZnO (Table 3). Regardless of cultivars and salinity, the increases under ZnO and nanoparticles treatments in comparison with none sprayed treatment were 7% and 19% for shoot dry weight, respectively. The foliar application of nano-particles ZnO recorded significantly higher shoot dry weight as compared to the normal ZnO form. No significant difference was found among cultivars in case of shoot dry weight under foliar application treatments (Table 3).

The NaCl presence decreased the shoot dry weight of all five cultivars. However, the magnitude of the reduction varied considerably among the sunflower cultivars. The averages of reduction for shoot dry weight were 11%, 15%, 17%, 27%, and 34% in cvs. Olsion, Hysun33, Alstar, Hysun36 and Yourflor, respectively, under saline condition. In fact, the highest and lowest reduction in shoot dry weight of sunflower cultivars as a result of salinity was observed in cvs. Yourfor and Olsion, respectively.

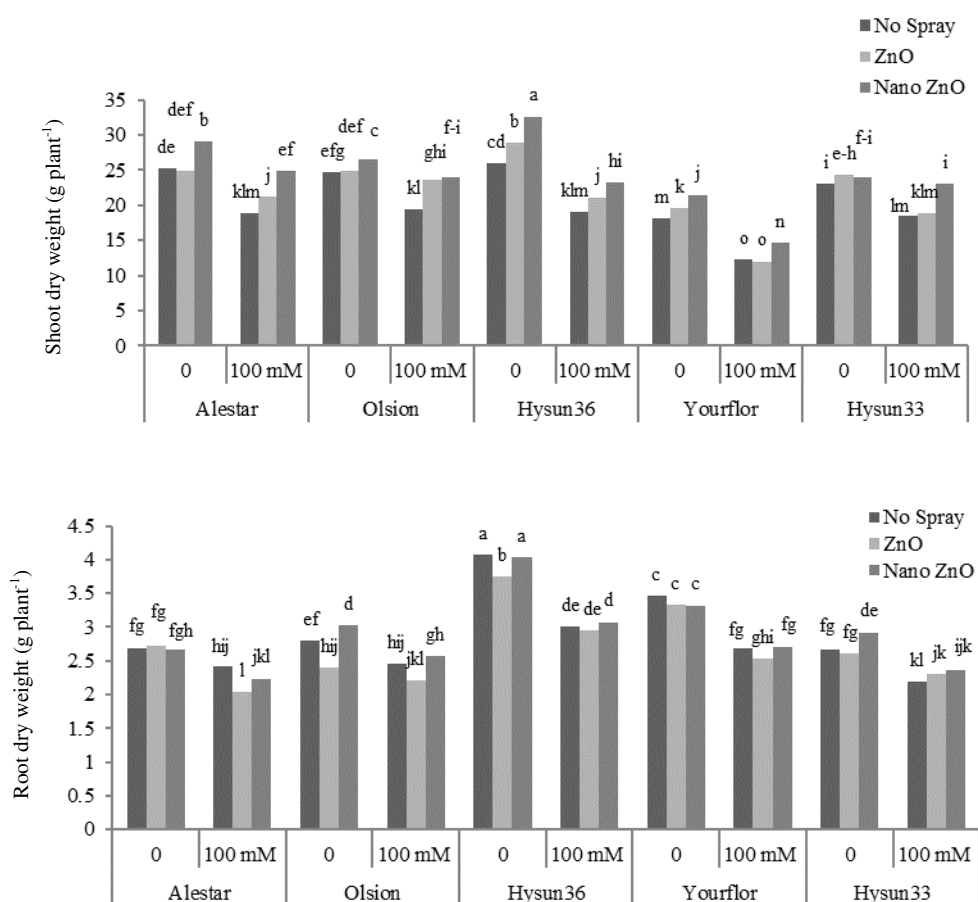
### Proline

Significant effects of salinity, foliar application, and cultivars on proline content are given in Table 3. Regardless of the cultivar and foliar application, leaf proline content (46%) increased under saline treatment (Figure 2). Averaged over the

**Table 3.** Analysis of variance for shoot and root dry weight, Proline content, Zinc concentration (Zn), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activity of sunflower cultivar

Source of Variation	Degrees of freedom	Mean Square						
		Shoot dry weight	Root dry weight	Proline	Zn	SOD	CAT	APX
Replication	2	35.2**	0.07ns	113**	62ns	387**	0.8**	0.3**
Cultivar (C)	4	222**	3.3**	31.5**	192270**	474**	1.1**	0.89**
Salinity (S)	1	617**	7.61**	64.6**	6725**	523**	0.00004ns	0.42**
Foliar spray (F)	2	113**	0.37ns	24.5**	188926**	86.2**	0.006ns	0.0001ns
C*S	4	19.9**	0.26ns	27.2**	425ns	78.9**	0.01ns	0.01*
C*F	8	3.2ns	0.06ns	4.1ns	49285**	16.9ns	0.004ns	0.001ns
S*F	2	1.7ns	0.006ns	12**	1717**	93.6**	0.005ns	0.01ns
C*S*F	8	4.9ns	0.03ns	4.6ns	248ns	15.9ns	0.004ns	0.002ns

ns = non-significant, \* = significant at 0.05 level, \*\*=significant at 0.01 level

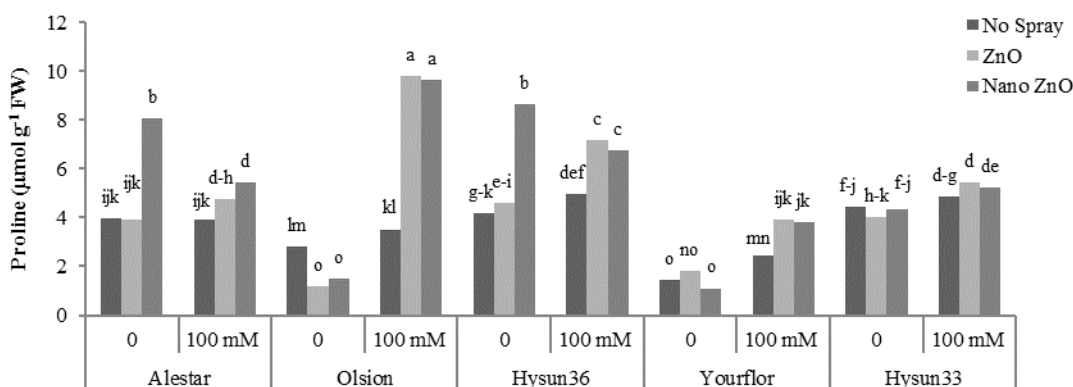


**Figure 1.** Effect of salinity levels and foliar applications of ZnO on shoot and root dry weight of five sunflower cultivars. Bars with the same letter are not significantly different at ( $P < 0.05$ ) according to least significant difference (LSD) test.

salinity and foliar application, the highest and lowest means for proline content were observed in cvs. Hysun33 and Yourflor, respectively. Foliar application of ZnO significantly increased proline content as compared with none sprayed treatment. Irrespective of the cultivar and salinity, enhancement under ZnO and nano-particles ZnO treatments in comparison with none sprayed treatment were 27% and 50% for proline content, respectively. The foliar application of nano-particles ZnO recorded significantly higher proline content ( $5.4 \mu\text{mol g}^{-1} \text{fw}$ ) relative to the normal form ( $4.6 \mu\text{mol g}^{-1} \text{fw}$ ).

The effect of salinity on the proline content varied considerably among the examined sunflower cultivars (Table 3). The amount of proline content was increased by salinity in all cultivars, except cv. Alestar. The magnitude of increases in leaf proline content for cvs. Olsion, Hysun36, Yourflor, and Hysun33 were 322%, 87%, 135%, and 21%, respectively. In contrast, leaf proline content of cv. Alestar decreased (13%) by increasing salinity.

The effect of foliar application on the proline content varied significantly under salt stress (Table 3). The proline content increased by foliar application of ZnO (nano and normal forms) in comparison with none



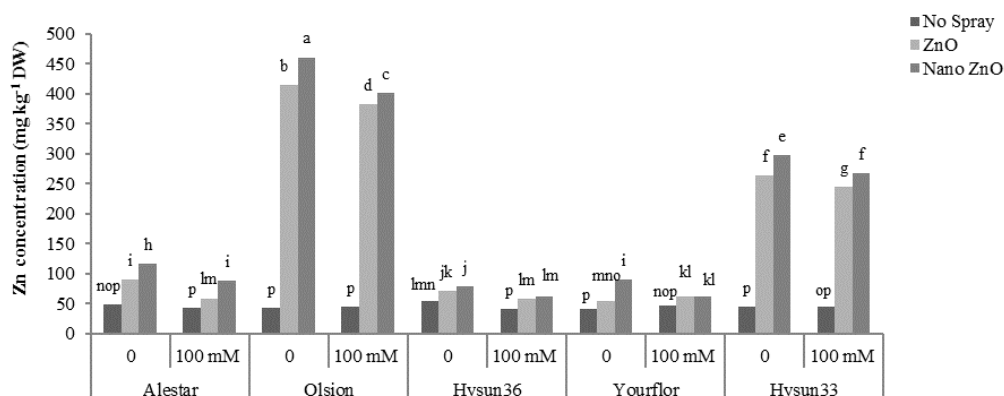
**Figure 2.** Effect of salinity levels and foliar application of ZnO on proline contents of five sunflower cultivars. Bars with the same letter are not significantly different at (P<0.05) according to least significant difference (LSD) test.

sprayed treatment under saline condition. In fact, although the content of proline increased under salt stress, foliar application of ZnO improved proline synthesis. No significant difference was found among cultivars under foliar application treatments in the proline content (Table 3). This shows that proline content of sunflower cultivars was similarly affected by foliar application of ZnO.

### Zn Concentration

The result showed that the effects of

salinity, cultivar, and foliar application of ZnO were highly significant on leaf Zn concentration (Table 3). Averaged over cultivars and foliar spray, reduction of 11% in the mean of leaf Zn concentration was observed under 100 mM of NaCl relative to the none-saline condition. Regardless of the salinity and foliar application, the highest Zn concentration belonged to cv. Olsion. However, the lowest mean for Zn concentration was observed in cvs. Yourflor followed by Hysun33 (Figure 3). Foliar application of ZnO increased leaf Zn concentration as compared with none sprayed treatment significantly (Figure 3).



**Figure 3.** Effect of salinity levels and foliar application of ZnO on Zn concentration of five sunflower cultivars. Bars with the same letter are not significantly different at (P<0.05) according to least significant difference (LSD) test.

Irrespective of cultivars and salinity, the means of Zn concentration under ZnO and nano-particles ZnO treatments were nearly 2.7 and 3.2 fold of those of the none-sprayed treatment, respectively. The foliar application of ZnO nano-particles recorded significantly higher Zn concentration ( $192 \text{ mg kg}^{-1} \text{ DW}$ ) as compared to the normal ZnO form ( $169 \text{ mg kg}^{-1} \text{ DW}$ ).

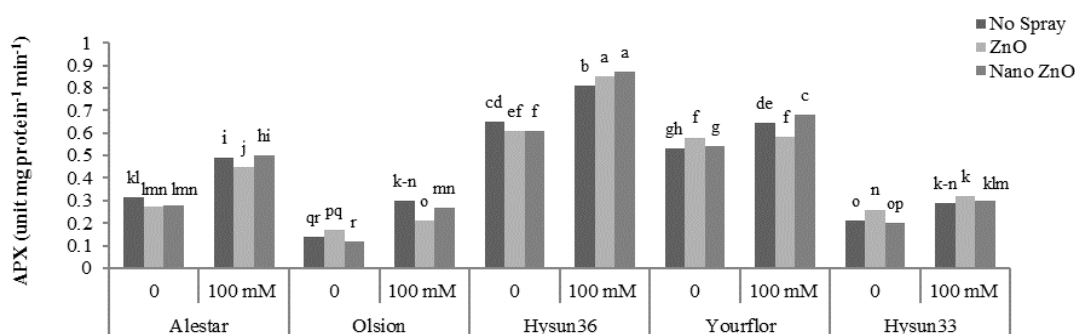
The effect of foliar application on the Zn concentration varied significantly ( $P < 0.01$ ) under salt stress (Table 3). Foliar application of ZnO resulted in an increase in leaf Zn concentration under both saline and non-saline conditions, but the extent of increase in leaf Zn concentration was remarkably greater under non-saline (319%) than saline (282%) treatment. The highest Zn concentration was observed in non-saline treatment by foliar application of nano-particles ( $208 \text{ mg kg}^{-1} \text{ DW}$ ), whereas the lowest Zn concentration was recorded in none-sprayed treatment under saline condition ( $44 \text{ mg kg}^{-1} \text{ DW}$ ).

The effect of foliar application on the leaf Zn concentration varied considerably among the examined sunflower cultivars (Table 3). There were no significant differences among cultivars in view of leaf Zn concentration in none-sprayed treatment. However, leaf Zn concentration was significantly higher in cvs. Olsion and Hysun33 as compared to other tested cultivars when ZnO was sprayed. Irrespective of ZnO particle size,

the extent of the increases in leaf Zn concentration as a result of ZnO spray were 95%, 863%, 38%, 52%, and 507% in cvs. Alstar, Olsion, Hysun36, Yourflor, and Hysun33, respectively. Not only the highest Zn concentration was observed in cv. Olsion ( $431 \text{ mg kg}^{-1} \text{ DW}$ ) when nano-particles were sprayed, but also the lowest Zn concentration belonged to cv. Olsion ( $43 \text{ mg kg}^{-1} \text{ DW}$ ) in none-sprayed treatment. No significant difference was found among cultivars under salt stress in the leaf Zn concentration (Table 3). This shows that Zn concentration of sunflower cultivars was similarly decreased by salt stress.

### Activities of SOD, CAT, and APX in Leaf

SOD activity was significantly affected by cultivar, salinity, and foliar application. The effects of cultivar and salinity were significant on APX activity. CAT activity of sunflower cultivars varied significantly (Table 3). Averaged over cultivars and foliar spray, increase of 41% and 38% in the means of SOD and APX activities were observed in the plants grown under salt stress, respectively, relative to the non-saline condition. Regardless of the salinity and foliar application, the activity of enzyme SOD in cvs. Yourflor followed by Alestar was higher than the other cultivars (Figure 4).



**Figure 4.** Effect of salinity levels and foliar application of ZnO on SOD, CAT and APX activities of five sunflower cultivars. Bars with the same letter are not significantly different at ( $P < 0.05$ ) according to least significant difference (LSD) test.



The maximum and minimum CAT activity was observed in cvs. Hysun33 ( $0.69 \mu\text{mol min}^{-1} \text{g}^{-1} \text{FW}$ ) and Alestar ( $0.11 \mu\text{mol min}^{-1} \text{g}^{-1} \text{FW}$ ), respectively (Figure 4). The highest mean for APX activity ( $0.73 \text{ unit mg protein}^{-1} \text{ min}^{-1}$ ) was recorded in cv. Hysun33, while cv. Olsion showed the lowest APX activity ( $0.2 \text{ unit mg protein}^{-1} \text{ min}^{-1}$ ). Foliar application of nano ZnO increased SOD activity considerably in comparison with other treatments. Averaged over cultivar and salinity, the extent of increase in the SOD activity was 20% by foliar application of nano form of ZnO (Figure 4). However, APX and CAT enzyme activities of five sunflower cultivars remained unchanged under foliar application of ZnO (Figure 4). The positive effect of ZnO spray on the activity of SOD in sunflower was remarkably greater than that of CAT and APX.

Although the increase in salt concentration led to a rise in SOD and APX activities of all cultivars (Figure 4), the magnitude of the increase was different among the examined sunflower cultivars. The extent of increases under saline condition in cvs. Olsion, Hysun33, Alestar, Hysun36, and Yourflor were 86%, 4%, 56%, 21%, and 16% for SOD activity and 78%, 36%, 65%, 35%, and 14% for APX activity, respectively. In the presence of salinity, the highest leaf SOD and APX activity was found in cv. Olsion.

The effect of foliar application on the SOD activity varied significantly ( $P < 0.01$ ) under salt stress (Table 3). Foliar application of ZnO resulted in an increase in SOD activity in both saline and non-saline conditions, but the magnitude of increase in SOD activity was greater under saline condition. The greatest increases in SOD activity were recorded under salt stress by foliar application of nanoparticles of ZnO. In other words, the highest SOD activity ( $21 \text{ unit mg protein}^{-1} \text{ min}^{-1}$ ) was observed under saline condition when nanoparticles of ZnO were sprayed, whereas, the lowest SOD activity ( $11.4 \text{ unit mg protein}^{-1} \text{ min}^{-1}$ ) was recorded under non-saline condition and non-sprayed treatment. Interaction effect of foliar application and cultivar was not significant

in the case of SOD, APX, and CAT activities (Table 3). This showed that SOD, APX and CAT activities of sunflower cultivars were similarly affected by foliar application.

## DISCUSSION

According to the results, the salinity caused significant decrease in shoot, root dry weight, and leaf Zn concentration and increase in the activity of SOD and APX of sunflower cultivars. In addition, salt stress markedly affected proline content and increased all sunflower cultivars, except cv. Alestar. Decrease in shoot dry weight may be a consequence of generation of ROS that is evident from significant increases in CAT, APX, and SOD activity in leaves of the sunflower plants under salinity. Salt stress (150 mM) caused a substantial decrease in the shoot fresh and dry weights of eight sunflower cultivars (Shahbaz *et al.*, 2011). Salinity induced reduction in photosynthetic capacity depends on the amount of photosynthesizing tissue (leaf area), photosynthetic pigments, stomatal and non-stomatal factors that affect the  $\text{CO}_2$  assimilation (gas exchange and metabolism) and finally cause decline in plant growth (Dubey, 2005). Even under optimal conditions, many metabolic processes produce ROS. The production of toxic oxygen formative is increased as a result of all types of environmental stresses. To scavenge ROS, plant cells possess an antioxidant system consisting of low-molecular-weight antioxidants, such as ascorbate,  $\alpha$ -tocopherol, glutathione, and carotenoids (nonenzymatic antioxidants), as well as antioxidant enzymes such as SOD, CAT, and APX (Noctor and Foyer, 1998). The SOD removes superoxide anion ( $\text{O}_2^-$ ) free radicals, accompanied by formation of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), which is then detoxified by CAT and POD (Sudhakar *et al.*, 2001). In the present study, SOD and APX seem to play key roles in the modification of salinity effects.



Accumulation of proline is a main factor that supports plants to sustain growth under saline conditions. The relatively salt-tolerant cultivars adjust to salt stress by enhancing compatible solutes including proline, as these solutes decrease osmotic potential, thereupon protecting cell turgor and water potentials for plant development (Hasegawa *et al.*, 2000). The higher level of proline content in sunflower leaf may be due to expression of gene encoding key enzymes of proline synthesis that is controlled by osmotic and salinity stress. Proline also can play a role as protective agent for cytoplasmic enzymes (Nikolopoulos and Manetas, 1991) and/or scavenging hydroxyl radicals (Hoque *et al.*, 2007).

Significant variation was found among the studied sunflower cultivars in their growth response to salinity. Differences in growth of sunflower cultivars in response to salt stress observed in the present study might have been due to variation in a number of biochemical or physiological traits that are associated with the processes related to the mechanism of salt tolerance such as photosynthesis, nutrient homeostasis, and accumulation of compatible solutes. In this study, cv. Olsion had the highest extent of increase in the proline content and activities of antioxidant enzymes (SOD and APX) and had the lowest reduction in shoot dry weight in comparison with other cultivars under saline condition. This indicated that a positive correlation existed between increase in the content of proline, SOD, and APX activities and tolerance to salt stress of cv. Olsion. These results suggest that cv. Olsion has a greater capacity to acclimatize salt stress by more rapidly developing an antioxidative defense system than other cultivars. The extent of shoot dry weight reduction was maximum in cv. Yourflor under salt stress. Yourflor also had the lowest increase in the APX activity. In fact, cv. Yourflor was identified as a salt sensitive cultivar in this experiment. Differential response of sunflower cultivars inactivity of antioxidative enzymes to environmental stress such as salinity has been previously

reported (Rady *et al.*, 2011; Di Baccio *et al.*, 2004; Rios-Gonzalez *et al.*, 2002).

Zinc is used for protein synthesis, membrane function, and tolerance to environmental stresses (Cakmak and Marschner, 1988). Foliar application is more effective and economical than soil fertilization. According to the results obtained from the present study, shoot dry weight, proline content, Zn concentration, and SOD activity increased by foliar application of ZnO. The effect of foliar application of ZnO on shoot dry weight was more than root dry weight. In accordance with the increase in proline content, Zn concentration, and SOD activity and decrease in detrimental effect of salinity, shoot dry weight was also considerably affected by ZnO application in all cultivars. Between the effects of the two kinds of ZnO, there was significant difference for shoot dry weights, proline content, leaf Zn concentration, and SOD activity. There was a positive response of SOD activity to foliar application of ZnO, particularly nanoparticles, under salt stress. Because Zn is in the molecular structure of SOD, foliar application of ZnO has a positive impact on the formation and activity of this enzyme. Zinc deficiency probably increased ROS levels and, thus, required higher SOD activity. In agreement with our results, SOD activity increased under excess Zn (Madhav Rao and Srestry, 2000; Wang *et al.*, 2009). Although salinity increased SOD activity, the foliar application of ZnO contributed to its production. This may explain the role of Zn in salinity alleviation. In Sanaeiostovar *et al.* (2012) experiment, applied zinc increased the SOD activity of wheat cultivars. Foliar spray of ZnO in two forms of nano and normal can reduce the negative effects of salinity on sunflower growth. Sunflower cultivars were different in their ability to accumulate Zn in both nano and normal ZnO forms. Olsion accumulated much more Zn in leaves followed by Hysun33, especially by foliar application of nano ZnO in comparison with other cultivars. The observed difference of leaf Zn



concentration may be due to diversity in absorbing and accumulation among sunflower cultivars. Maximum Zn concentration was recorded in Olsion followed by Hysun33 and that may be one of the reasons of tolerance to salt stress. This difference in absorption capability of cultivars may be due to the structure of leaf, which leads to the variation in leaf Zn concentration. In the present study, ZnO application effect on the leaf proline content and activity of SOD and APX was dependent on the sunflower cultivars and presence or absence of NaCl. It seems that cv. Olsion could easily tolerate the presence of 100 mM NaCl in the growth media when it was sprayed with ZnO, while at the non-sprayed treatment, salinity caused reduction in growth of this cultivars. The content of proline and SOD activity increased under salt stress by foliar application of ZnO. The results indicate that ZnO spray improved proline and SOD synthesis under salt stress. Although foliar application of ZnO resulted in an increase in leaf Zn concentration under both saline and non-saline conditions, the extent of increase was greater under non-saline than saline medium. NaCl appears to have had a specific inhibitory effect on Zn absorption by the rice cultivar used in the study of Saleh and Maftoun (2008). Zinc application significantly increased the Zn concentration in rice shoots in non-saline as well as saline soil.

Foliar application of ZnO nano-particles increased shoot dry weight, leaf Zn concentration, and SOD activity by, respectively, 11%, 13%, and 19% in comparison with normal form. In addition, proline content was significantly higher in the treatment with ZnO nano-particles as compared to normal ZnO form. These increases may be due to smaller size of particles, which cause faster absorption and transfer. It seems that ZnO, especially nano-particles, can promote plant defense system against stress condition such as antioxidant enzymes and proline. This is in agreement with the earlier reports wherein it was emphasized that Zn only at appropriate

concentrations was required for structural and catalytic components of proteins and enzymes as cofactors, essential for normal growth, and development of plants (Clarke and Berg, 1998), and excessive accumulations of the micronutrient in plants operate as stress factors producing physiological constraints (Ali *et al.*, 2000). Lower Zn concentration was ineffective in alleviating stress and higher zinc concentration inhibited plant growth because of toxicological damage to plants (Jiang *et al.*, 2014). Selection of the appropriate dose of nano-sized particles is very important because high concentration of these particles can damage plant tissues. Considering these aspects, both positive and negative effects of nano-particles have been reported in plants (Prasad *et al.*, 2012; Lin and Xing, 2008). Two different actions may be attributed to toxicity of nano-particles: Release of toxic ions and stress caused by the surface and size of the nano-particles (Brunner *et al.*, 2006). In Lin and Xing (2008) experiment, phytotoxicity of commercially available ZnO nano-particles to ryegrass is reported.

## CONCLUSION

Our study suggests that ZnO in the form of nano-particles is absorbed by sunflower better than its normal form. The mobility of the nanoparticles is very high, which leads to rapid transport of the nutrient to all parts of the plant. Due to its small size, the availability of the nanoparticle of ZnO can be higher compared to the normal form. All these factors may be responsible for higher shoot dry weight of sunflower in ZnO nanoparticles treatment compared to normal form. Further comment on the use of nanoparticles as fertilizers in agriculture requires more experiments, because the major challenge in the application of nano-fertilizers is its impact on the environment and human health.

## REFERENCES

1. Aebi, H. 1984. Catalase in Vitro. *Method Enzymol.*, **105**: 121-126.
2. Ali, G., Srivastava, P. S. and Iqbal, M. 2000. Influence of Cadmium and Zinc on Growth and Photosynthesis of *Bacopa monniera* L. Cultivated in Vitro. *Biol Plant.*, **43**: 599-601.
3. Bates, L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid Determination of Free Proline for Water Stress Studies. *Plant Soil.*, **39**: 205-207.
4. Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal Biochem.*, **72**: 248-254.
5. Brunner, T. J., Wick, P., Manser, P., Spohn, P., Grass, R. N., Limbach, L. K., Bruinink, A. and Stark, W. J. 2006. In Vitro Cytotoxicity of Oxide Nanoparticles: Comparison to Asbestos, Silica, and the Effect of Particle Solubility. *Environ Sci Technol.*, **40**: 4374-4381.
6. Cakmak, I. and Marschner, H. 1988. Zinc-Dependent Changes in ESR Signals, NADPH Oxidase and Plasma Membrane Permeability in Cotton Roots. *Physiol Plant.*, **73**: 132-186.
7. Chapman, H. D. and Pratt, P. F. 1961. *Methods of Analysis for Soil, Plant and Water*. Riverside, CA: University of California, Division of Agricultural Science.
8. Clarke, N. D. and Berg, J. M. 1998. Review: Zinc Fingers in *Caenorhabditis Elegans*: Finding Families and Probing Pathways. *Sci.*, **282**: 2018-2022.
9. Davenport, S. B., Gallego, S. M., Benavides, M. P. and Tomaro, M. L. 2003. Behavior of Antioxidant Defense System in the Adaptive Response to Salt Stress in *Helianthus annuus* L. cells. *Plant Growth Regul.*, **40**: 81-88.
10. Di Baccio, D., Navari-Izzo, F. and Izzo, R. 2004. Seawater Irrigation: Antioxidant Defense Responses in Leaves and Roots of a Sunflower (*Helianthus annuus* L.) Ecotype. *J Plant Physiol.*, **161**: 1359-1366.
11. Dubey, R. S. 2005. Photosynthesis in Plants under Stressful Conditions in Photosynthesis handbooks. 2nd Ed. M. Pessarakli C. R. C. Press, New York. p. 717-718.
12. Giannopolitis, C. N. and Ries, S. K. 1977. Superoxide Dismutases. I. Occurrence in Higher Plants. *Plant Physiol.*, **59**: 309-314.
13. Hasegawa, P. M., Bressan, R. A., Zhu, J. K. and Bohnert, H. J. 2000. Plant Cellular and Molecular Responses to High Salinity. *Annu Rev Plant Physiol Plant Mol Biol.*, **51**: 463-499.
14. Hoque, M. D. A., Okuma, E., Banu, M. N. A., Nakamura, Y., Shimoishi, Y. and Murata, Y. 2007. Exogenous Proline Mitigates the Determintal Effects of Salt Stress More Than Exogenous Betaine by Increasing Antioxidant Enzyme Activities. *J. Plant Physiol.*, **164**: 553-561.
15. Jiang, W., Sun, X. H., Xu, H. L., Mantri, N. and Lu, H. F. 2014. Optimal Concentration of Zinc Sulfate in Foliar Spray to Alleviate Salinity Stress in *Glycine soja*. *J. Agr. Sci. Tech.*, **16**: 445-460.
16. Kaya, C. and Higgs, D. 2002. Response of Tomato (*Lycopersicon esculentum* L.) Cultivars to Foliar Application of Zinc in Sand Culture at Low Zinc. *Sci Hort.*, **93**: 53-64.
17. Lin, D. and Xing, B. 2008. Root Uptake and Phytotoxicity of ZnO Nanoparticles. *Environ Sci Technol.*, **42**: 5580-5585.
18. Madhav Rao, K. V. and Srestry, T. V. 2000. Antioxidative Parameters in the Seedlings of Pigeon Pea (*Cajanas cajan* L. Millspough) in Response to Zn and Ni Stresses. *Plant Sci.*, **157**: 113-128.
19. Mittler, R. 2002. Oxidative Stress, Antioxidants and Stress Tolerance. *Trends Plant Sci.*, **7**: 405-410.
20. Monica, R. C. and Cremonini, R. 2009. Nanoparticles and Higher Plants. *Caryologia.*, **62**: 161-165.
21. Nakano, Y. and Asada, K. 1981. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.*, **22**: 867-880.
22. Neill, S., Desikan, R. and Hancock, J. 2002. Hydrogen Peroxide Signaling. *Curr Opin Plant Biol.*, **5**: 388-395.
23. Nikolopoulos, D. and Manetase, Y. 1991. Compatible Solutes and *in vitro* Stability of *Salsola soda* Enzymes: Proline Incopatiility. *Phytochem.*, **30**: 411-413.
24. Noctor, G. and Foyer. C. H. 1998. Ascorbate and Glutathione: Keeping Active Oxygen under Control. *Annu Rev Plant Physiol Plant Mol Biol.*, **49**: 249-279.



25. Owens, S. 2001. Salt of the Earth. Genetic Engineering May Help to Reclaim Agriculture Land use to Stalinization. *EMBO Reports.*, **2**: 877-879.
26. Pandey, A. C., Sanjay, S. S. and Yadav, R. S. 2010. Application of ZnO Nanoparticles in Influencing the Growth Rate of *Cicer arietinum*. *J Exp Nanosci.*, **5**: 488-497.
27. Prasad, T. N. V. K. V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Raja Reddy, K., Sreepasad, T. S., Sajanalal, P. R. and Pradeep, T. 2012. Effect of Nanoscales Zinc Oxide on the Germination, Growth and Yield of Peanut. *J Plant Nutr.*, **35**: 905-927.
28. Rady, M. M., Sadak, M. S., El-Bassiouny, H. M. S. and Abd El-Monem, A. A. 2011. Alleviation of the Adverse Effects of Salinity Stress in Sunflower Cultivars Using Nicotinamide and  $\alpha$ -Tocopherol. *Aust J Basic Appl Sci.*, **5**: 342-355.
29. Rios-Gonzalez, K., Erdei, L. and Lips, S. H. 2002. The Activity of Antioxidant Enzymes in Maize and Sunflower Seedlings as Affected by Salinity and Different Nitrogen Sources. *Plant Sci.*, **162**: 923-930.
30. Saleh, J. and Maftoun, M. 2008. Interactive Effects of NaCl Levels and Zinc Sources and Levels on the Growth and Mineral Composition of Rice. *J. Agric. Sci. Technol.*, **10**: 325-336.
31. Sanaeiostovar, A., Khoshgoftarmanesh, A. H., Shariatmadari, H., Afyuni, M. and Schulin, R. 2012. Combined Effect of Zinc and Cadmium Levels on Root Antioxidative Responses in Three Different Zinc-efficient Wheat Genotypes. *J. Agron Crop Sci.*, **198**: 276-285.
32. Shahbaz, M., Ashraf, M., Akram, N., Hanif, A., Hameed, S., Joham, S. and Rehman, R. 2011. Salt-induced Modulation in Growth, Photosynthetic Capacity, Proline Content and Ion Accumulation in Sunflower (*Helianthus annuus* L.). *Acta Physiol Plant.*, **33**: 1113-1122.
33. Shilpim, M. and Narendra, T. 2005. Cold, Salinity and Drought Stresses: an Overview. *Arch Biochem Biophys.*, **444**: 139-158.
34. Sudhakar, C., Lakshmi, A. and Giridarakumar, S. 2001. Changes in the Antioxidant enzyme Efficacy in Two High Yielding Genotypes of Mulberry (*Morus alba* L.) under NaCl Salinity. *Plant Sci.*, **161**: 613-619.
35. Wang, C., Zhang, S. H., Wang, P. F., Hou, J., Zhang, W. J., Li, W. and Lin, Z. P. 2009. The Effect of Excess Zn on Mineral Nutrition and Antioxidative Response in Rapeseed Seedlings. *Chemosphere.*, **75**: 1468-1476.
36. Xiong, L. and Zhu, J. K. 2002. Molecular and Genetic Aspects of Plant Response to Osmotic Stress. *Plant Cell Environ.*, **25**: 131-139.
37. Yang, F., Hong, F. S., You, W. J., Liu, C., Gao, F. Q., Wu, C. and Yang, P. 2006. Influences of Nano-anatase TiO<sub>2</sub> on the Nitrogen Metabolism of Growing Spinach. *Biol Trace Elem Res.*, **110**: 179-190.
38. Yu, Q., Osborne, L. and Rengel, Z. 1998. Micronutrient Deficiency Changes Activities of Superoxide Dismutase and Ascorbate Peroxidase in Tobacco Plants. *J Plant Nutr.*, **21**: 1427-1437.
39. Zhu, H., Han, J., Xiao, J. Q. and Jin, Y. 2008. Uptake, Translocation and Accumulation of Manufactured Iron Oxide Nanoparticles by Pumpkin Plants. *J. Environ Monitor.*, **10**: 713-717.

## تأثیر محلول پاشی اکسید روی بر فعالیت برخی آنزیم‌های آنتی‌اکسیدانی آفتابگردان تحت تنش شوری

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### چکیده

مطالعه‌ای برای بررسی تأثیر محلول پاشی اکسید روی به دو فرم معمول و نانو ذرات بر رشد، محتوای پروتئین و فعالیت آنزیم‌های آنتی‌اکسیدانی ارقام آفتابگردان تحت سطوح شوری طراحی شد. تیمارها شامل ۵ رقم آفتابگردان (الستار، السیون، یورفلور، هایسان ۳۶ و هایسان ۳۳) دو سطح شوری (صفر و ۱۰۰ میلی‌مولار کلرید سدیم) و ۳ سطح محلول پاشی (عدم محلول پاشی، محلول پاشی فرم معمول و نانو ذرات اکسید روی در غلظت ۲ در هزار) بود. رقم السیون بیشترین محتوای پروتئین و فعالیت آنزیم سوپراکسیددسموتاز را بین ارقام آفتابگردان تحت تنش شوری دارا بود. محلول پاشی اکسید روی فعالیت آنزیم سوپراکسیددسموتاز و وزن خشک اندام هوایی ارقام آفتابگردان را افزایش داد. نانو ذرات اکسید روی در مقایسه با فرم معمول اثر مثبت بیشتری بر تولید بیوماس گیاه آفتابگردان داشت. مطابق نتایج، ارقام السیون و هایسان ۳۳ مناسب برای کشت تحت تنش شوری و در مقابل رقم هایسان ۳۳ مناسب برای کشت تحت شرایط غیر شور بودند.