Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2538-2470 (Online)
Vol. 6, No. 2, 2020
http://JCNS.iauahvaz.ac.ir **OPEN ACCESS**

Assessment Effect of Water Deficit Stress and Foliar Application of Zinc Sulfate on Seed yield, Grain Protein and Antioxidant Enzymes

Mojtaba Afshari[1,2]*

1- *Department of Agronomy, Khuzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran.*

2- *Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.*

RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *24 Mar. 2020*
Received in revised form: *29 Apr. 2020*
Accepted Date: *28 May. 2020*
Available online: *30 Jun. 2020*

To Cite This Article:
Mojtaba Afshari. Assessment Effect of Water Deficit Stress and Foliar Application of Zinc Sulfate on Seed yield, Grain Protein and Antioxidant Enzymes. ***J. Crop. Nutr. Sci.,*** 6(2): 13-25, 2020.

ABSTRACT

BACKGROUND: Microelements are inorganic compounds involved in the synthesis of enzymes and biologically active substances.

OBJECTIVES: To evaluate the physiological traits of maize to $ZnSO_4$ and $FeSO_4$ under drought stress, a field experiment was conducted on maize plants grown under different soil moistures and treated with foliar $ZnSO_4$ and $FeSO_4$ applications.

METHODS: The experiment was laid out as split-split plot in a randomized complete block design with three replicates. The main plot consisted of three levels of water deficit stress comprised of complete irrigation (control), no irrigation at vegetative growth stage (12-14 leaf), and no irrigation at early seed growth stage. Water deficit stress treatment was specific to the above-mentioned stages, after which and until the end of the growth period, the water requirement of the plant was fulfilled. The sub-plot contained foliar solution of zinc sulfate at three concentrations (0, 5 and 10 $gr.l^{-1}$) and sub-sub-plot included iron sulfate foliar solution at three concentrations (0, 3 and 6 $gr.l^{-1}$).

RESULT: Drought stress especially at early seed growth stage significantly decreased grain yield, and Fv/Fm ratio but, activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR) under drought stress were increased. Foliar applied $ZnSO_4$ and $FeSO_4$ increased grain yield by 15.22, 10.73 and 10.74% under normal irrigation, no irrigation at vegetative growth stage and no irrigation at early seed growth stage, respectively. Also further increased the antioxidant enzyme activities and enhanced total phenol content of maize under drought stress. Combined application of $ZnSO_4$ and $FeSO_4$ resulted in alleviating maize plant drought stress by Zn and Fe-mediated improvement in photosynthetic gas exchange attributes. Besides the foliar application of $ZnSO_4$ and $FeSO_4$ regulated physiological processes in maize plants and alleviated the adverse effects of water stress. Results showed that $ZnSO_4$ and $FeSO_4$ could be used for improving maize growth under drought stress.

CONCLUSION: The highest grain yield of maize was recorded for non-drought stress treatment and was followed by foliar application of both $ZnSO_4$ and $FeSO_4$, while severe water stress and non-application of Zn and Fe resulted in the minimum grain yield.

KEYWORDS: *Catalase, Glutathione reductase, Irrigation, Microelement, Peroxidase.*

***Corresponding Author:** Mojtaba Afshari ✉ mojtabaafshari2014@gmail.com

1. BACKGROUND

Maize is the third most important crop in the world after wheat and rice producing almost 100 million hectares in developing countries which its production in developing countries is approximately the 100 million hectares (FAOSTAT, 2012). Water deficit is one of the major environmental factors limiting crop growth and productivity in many areas across the world (Falqueto *et al.,* 2017). In maize, water requirement is low at early growth stages, whereas the most sensitive to drought stress during reproductive growth stages. Thus, pollination and fertilization period at the time of drought stress will result in significant yield loss. (Ahmed-Amal and Mekki, 2005). Exposure of plants to environmental stresses leads to the generation of reactive oxygen species (ROS), such as H_2O_2 (Munne-Bosch and Penuelas, 2003). Production of ROS in plant cells during abiotic stress is mainly the outcome of enhanced photorespiration resulting in the production of H_2O_2 (Baishnab and Ralf, 2012; Kerchev *et al.,* 2016). These reactive species cause oxidative damage and impairing the normal functions of cells (Foyer and Fletcher, 2001). Several reports have shown the deleterious effects of ROS, whose production is stimulated under water stress (Yang *et al.,* 2014). Drought resistance in plants increases with changes in morphological, physiological and biochemical responses such as changes in plant structure, growth rate, tissue osmotic potential, and antioxidant defense against water deficit (Anjum *et al.,* 2011; Ashraf *et al.,* 2015). Intervention of enzymatic and non-enzymatic antioxidant system ensure resistance to environmental stress conditions such as drought (Sharma *et al.* 2012). Zinc (Zn) and iron (Fe) are the most important micronutrients needed for plant growth and have a significant effect on plant yield, and also play an important role in the synthesis of many enzymes. Chlorophyll biosynthesis and energy transfer are also carried out by iron (Gill and Tuteja, 2011). Results showed that foliar application of Zn increased the seed yield of corn (Hussain *et al.,* 2012; Tabatabai *et al.,* 2015). Zinc plays an important role in reducing ROS generation and protects cells from the damaging effects of ROS (Cakmak, 2000). Zinc sulfate has been reported to play an important role in regulating the stomatal closure and maintaining ionic balance in the plant to reduce drought stress and also significantly increase SOD, POD and CAT activities in response to drought stress (Tabatabai *et al.* 2015; Yavas and Unay, 2016). Iron also plays an important role in reducing stress due to salinity, drought and heavy metal stress by activating plant enzymatic antioxidants like catalase (CAT), peroxidase, and superoxide dismutase (SOD) that act as a scavenger of reactive oxygen species (ROS) (Tripathi *et al.,* 2018). Drought has become a source of critical abiotic stress that impacts maize growth and productivity. The benefits of Zn and Fe have been documented in studies with some stressful conditions, but no research has focused on the combined effects of Zn and Fe on growth and

physiological responses of maize to interrupted irrigation at different growth stages of maize, given that maize susceptibility to drought varies at different stages of growth.

2. OBJECTIVES

To evaluate the physiological traits of maize to $ZnSO_4$ and $FeSO_4$ under drought stress, a field experiment was conducted on maize plants grown under different soil moistures and treated with foliar $ZnSO_4$ and $FeSO_4$ applications.

3. MATERIALS AND METHODS

3.1. *Growth Conditions and treatments*

In order to evaluate effect of iron and zinc on yield and some antioxidant enzymes of maize under water deficiency conditions, an experiment was conducted during the summer of 2016 (growing seasons) in an experimental field located in northwest of Ahvaz. The latitude of the test site was: 31° 20′ N, longitude was 48° 40′ E and altitude was 22.5 m. The experiment was laid out as split-split plot in randomized complete block design with four replicates. The main plot consisted of three levels of water deficit stress comprised of complete irrigation (control), no irrigation at vegetative growth stage (12-14 leaf), and no irrigation at early seed growth stage. Water deficit stress treatment was specific to the above-mentioned stages and after that till the end of the growth period, the water requirement of the plant was provided. The sub-plot contained foliar solution of zinc sulfate in three concentrations (0, 5 and 10 $g.l^{-1}$) and iron sulfate foliar solution in three concentrations (0, 3 and 6 $g.l^{-1}$). Prior to cultivation, soil samples were taken from 0-30 cm and 30-60 cm soil depth to determine the physical and chemical properties of the soil which is presented in table 1. In the experiment, each plot consisted of 4 rows, 1.5 m long and spaced 20 cm apart. In this study, seed of maize hybrid 704, which is one of the late hybrids, was used. Seed cultivation was done in a row by a row-crop machine in two crop years in August. Seeds were sown in a 3–5 cm depth. Extra seedlings were thinned at 2–4 leaf stage. The first irrigation was done immediately after planting and then until the full establishment of seedlings (4 to 5 leaves) irrigation was done routinely and providing 100% water requirement of the plant. Manual weeding and chemical control were performed during the weed control period. Foliar application of zinc and iron was carried out at the specified concentrations using a damp sprayer after calibration at the pressure of 1 atmosphere at two times in a six to eight-leaf stage and in a 12-leaf stage. The foliar solution of iron and zinc compounds consisted of $FeSO_4$, $7H_2O$ and $ZnSO_4$. $7H_2O$). Harvesting was carried out after physiological maturity and when grain moisture reached about 20%.

Table 1. Physical and chemical properties of the tested soil

Soil depth (cm)	Soil texture	Organic Carbon (%)	pH	EC ($ds\ m^{-1}$)	Total N (%)	Available P ($mg.kg^{-1}$)	Available K ($mg.kg^{-1}$)
0-30	Silty loam	0.76	7.4	2.5	0.05	7.2	264
30-60	Silty loam	0.52	7.7	2.1	0.04	6.4	217

3.2. Measured traits

In this experiment, the following traits were measured: *Fv/Fm* ratio, malondialdehyde (MDA) accumulation (nmol g^{-1}), antioxidant enzymes assays, total phenol content (mg gallic acid g^{-1} of leaf fresh weight) and yield.

3.2.1. Fv/Fm ratio

The maximum photochemical efficiency of photosystem II (*Fv/Fm*) was measured applying a fluorometer (Walz, Effeltrich, Germany) after 30 min of dark adaptation. The *Fv/Fm* ratio was calculated as: *Fv/Fm* = (*Fm- F0*) /*Fm*. Where, *Fm* and *F0* represented the maximum and minimum yields of dark-adapted leaves, respectively. Proline content was determined in fully expanded uppermost leaves using the method of Bates *et al*. (1973).

3.2.2. MDA concentration

The concentration of malondialdehyde (MDA) which is a product of lipid peroxidation was assessed by the thiobarbituric acid (TBA) according to Wang *et al*. (2009) and was calculated on a fresh weight basis, using the following formula: MDA (nmol.g^{-1} FW) = 6.45 (OD532 – OD600)–0.56 (OD450) × 1000.

3.2.3. Enzyme (CAT, POD, SOD and GR) extraction and assay

Fresh foliar tissue (0.2 g) from fresh seedlings (uppermost leaves) was harvested, weighed, washed with distilled water and then homogenized with a mortar and pestle with 5 ml chilled sodium phosphate buffer (50 mM, pH 7.8). The homogenates were centrifuged at 15,000 g for 15 min at 4°C. The supernatant was stored at 4°C and used for CAT, POD, SOD and GR assays. CAT activity was measured by the method of Blume and McClure (1980). The unit for CAT activity was micromoles of hydrogen peroxide oxidized per minute per milligram of protein. POD activity was determined spectro photometrically, by measuring the oxidation of o-dianisidine (3, 3- dimethoxybenzidine) at 460 nm as described by Ranieri *et al*. (2001) and expressed as units (µmol of dianisidine oxidized per minute) per mg of protein. SOD activity was estimated by recording the decrease in absorbance of superoxide–nitroblue tetrazolium complex by the enzyme (Cavalcanti *et al*., 2004). About 3 ml of reaction mixture, containing 0.1 ml of 13 mM L-methionine, 0.1 ml of 75 µM *p*- nitroblue terazolium chloride (NBT), 0.1 ml of 100 µM EDTA, 0.1 ml riboflavin (2µM) in a 1.5 ml of 50 mM potassium phosphate buffer pH 7.8, 50 µL of the enzymatic extract and distilled water to make up the volume to 3 ml. The reaction was started under illumination of fluorescent lamp (30 W) at 25 ^{o}C and stopped 5 min later by turning it off. The blue formazane produced by NBT photo–reduction was measured as increase in absorbance at 560 nm. The control reaction mixture had no enzyme extract (with maximal color formation). The blank solution had the same complete reaction mixture, but was kept in the dark. One SOD unit was defined as the amount of enzyme required to inhibit 50% of the NBT photo – reduction in comparison with tubes lacking the plant extract and expressed as a unit of

enzyme activity per mg of protein. Glutathione reductase (GR) activity was identified by following the rate of NADPH oxidation at 340 nm according to Balabusta *et al*. (2016). The assay mixture including 0.5 mM NADPH, 10 mM GSSG, 6.25 mM $MgCl_2$ in 0.1 M phosphate buffer (pH 7.5), and 100 µl of the enzyme extract in the total volume of 400 µl. GR activity was expressed as micromoles of NADPH oxidized during 1 min per 1 mg of proteins (µ mol min^{-1} mg $protein^{-1}$). Protein content of the extracts was determined according to the method of Bradford (1976).

3.2.4. Total phenol content (*TPC*)

Phenols were extracted according to Sarrou *et al*. (2015). Fresh leaves (0.2 g) separated from seedlings, was homogenized with a cooled mortar and pestle at 4°C. They were then centrifuged at 4°C (12000 ×g for 20 min) and the supernatants were stored for the assays. Total phenol concentration assayed using the Folin-Ciocalteu reagent, according to Scalbert *et al*. (1989). Each measurement was repeated three times and total phenolic content was expressed as mg gallic acid g^{-1} of leaf fresh weight (F.W).

3.2.5. Seed yield

To determine crop yield, 30 plants (10 plants from each replicate) were sampled randomly and harvested at maturity stage. The harvested plants were sun-dried (in an open place area) and were shelled manually to record grain yield (GY) per plant.

3.3. Statistical analysis

All the data were subjected to analysis of variance (ANOVA) and means were then separated using the least significant difference (LSD) using SAS (version 8.2; SAS Institute, Cary, NC, USA) and MSTAT-C software (Freed and Scott, 1989).

4. RESULT AND DISCUSSION

4.1. Fv/Fm ratio

Chlorophyll fluorescence is very useful to study the effects of environmental stresses on photosynthesis in plants (Afrousheh, 2010). Results from an analysis of variance also revealed that drought stress, $ZnSO_4$, $FeSO_4$ and interactive drought stress × $ZnSO_4$ at 1% and drought stress × $FeSO_4$ at 5% probability levels had a significant impact on *Fv /Fm*. *Fv/Fm* ratio showed significant change in response to interactive effect of drought stress × $ZnSO_4$ × $FeSO_4$ (Table 2). Drought stress by stopping irrigation during different stages of maize growth (non-irrigation at vegetative and the early stage of seed growth) caused a significant decrease *Fv/Fm* ratio. However, the smallest reduction of these parameters was observed with 10 $g.l^{-1}$ $ZnSO_4$. In the current research, foliar application of $ZnSO_4$ improved *Fv/Fm* ratio under water stress. Similar findings have been also reported in the other crops where the use of $ZnSO_4$ improved drought tolerance (Karim *et al*., 2012). It was reported that Zn application under adverse conditions increase chlorophyll content and photosynthesis rate, and improve plant growth (Tavallali *et al*., 2010).

No iron sulfate intake in conditions of constant concentration of zinc sulfate (10 $g.l^{-1}$) decreased *Fv/Fm* ratio under non-irrigation at the early stage of seed growth. By increasing amounts of $ZnSO_4$ and $FeSO_4$ increased the *Fv/Fm* ratio in the non-irrigation at the vegetative growth stage and at the non-irrigation at the early stage of seed growth treatment. This increase was more noticeable in non-irrigation at the early stage of seed growth treatment. The lowest amount of *Fv/Fm* ratio was obtained in non-using of zinc and iron treatments (with mean of 0.38) at the non-irrigation at the early stage of seed growth treatment (Table 3). The lack of micro elements changed the physiological state of investigated maize plants, especially in drought stress conditions.

Table 2. Values of mean squares in the analysis of variance of *Fv/Fm* ratio, *MDA*, *CAT* and *POD* enzyme

S.O.V	df	(*Fv/Fm*)	*MDA*	*CAT*	*POD*
Replication	2	0.00005^{ns}	1.07^{**}	0.13^{ns}	0.17^{ns}
Drought stress (D)	2	0.61^{**}	3098.33^{**}	1117.18^{**}	6115.92^{**}
Error I	4	0.0001	0.17	0.21	0.42
$ZnSO_4$ (Z)	2	0.12^{**}	153.40^{**}	57.84^{**}	160.24^{**}
D × Z	4	0.003^{**}	15.12^{**}	4.30^{**}	17.86^{**}
Error II	12	0.0002	0.59	0.06	0.19
$FeSO_4$ (F)	2	0.004^{**}	0.89^{**}	5.40^{**}	16.20^{**}
D × F	4	0.0004^{*}	0.17^{ns}	0.49^{ns}	2.90^{**}
F × Z	4	0.0002^{ns}	0.23^{ns}	0.59^{*}	2.34^{**}
D × Z × F	8	0.0004^{**}	0.22^{ns}	0.69^{**}	0.80^{*}
Error III	36	0.0001	0.13	0.20	0.27
CV (%)	-	1.48	1.09	4.73	1.26

* and **: Significant at the 5% and 1% probability levels, respectively., ns: Non- Significant.
Fv/Fm: photochemical efficiency of photosystem II, MDA: Malondialdehyde, CAT: Catalase,

4.2. MDA concentration

In terms of MDA the main effects of drought stress, $ZnSO_4$ and $FeSO_4$ and interactive effect of drought stress × $ZnSO_4$ were regarded as significant at 1% probability level (Table 2). Nonetheless, foliage-applied $ZnSO_4$ and $FeSO_4$ enhanced the MDA content leading to improved drought tolerance of maize. The highest concentration of MDA content was obtained in the absence of application of zinc sulphate and iron sulphate in water deficit condition at the early stage of seed growth, which increased by 52.40 % compared to the same treatment under full irrigation conditions (Table 3). Damage caused by drought in plants necessitates studies on the mechanisms of drought tolerance in plants to avoid significant loss of the yield under water stress conditions. Malondialdehyde is a chemical marker that appears in the water deficit stress of the plant, damages the cell wall and destroys the plant, and results show that the no irrigation at the early stage of seed growth, occurrence. In the absence of Zn and Fe concentrations, the highest concentration of malondialdehyde is present.

Table 3. *Fv/Fm* ratio and *MDA*, *CAT* and *POD* enzyme means comparison against control, as affected by foliar application of $ZnSO_4$ and $FeSO_4$

Drought stress	$ZnSO_4$ ($g.l^{-1}$)	$FeSO_4$ ($g.l^{-1}$)	Fv/Fm	MDA	CAT	POD
Full irrigation	0	0	0.75 e	25.87 j	2.20 r	25.13 q
		3	0.76 e	25.48 j	2.97 q	25.04 q
		6	0.76 e	25.52 j	3.13 pq	25.60 pq
	5	0	0.79 d	24.74 k	3.61 opq	26.70 no
		3	0.81 c	24.73 k	3.82 op	26.43 op
		6	0.79 d	24.48 k	3.90 o	26.51 o
	10	0	0.84 b	23.75 l	4.19 o	26.93 no
		3	0.86 a	23.83 l	4.16 o	27.38 n
		6	0.85 ab	23.51 l	4.29 o	27.51 n
Non-irrigation at vegetative growth stage	0	0	0.55 m	34.36 g	5.95 n	35.56 m
		3	0.57 l	34.19 g	6.31 n	37.14 l
		6	0.57 l	34.04 g	7.09 m	38.33 k
	5	0	0.62 i	30.13 h	7.89 l	38.74 jk
		3	0.64 h	30.00 h	8.80 k	39.35 j
		6	0.64 h	29.92 h	9.29 jk	40.81 i
	10	0	0.67 g	28.58 i	9.88 ij	43.11 h
		3	0.70 f	28.51 i	10.43 i	44.84 g
		6	0.70 f	28.37 i	11.41 h	44.43 g
Non-irrigation at the early stage of seed growth	0	0	0.38 q	49.39 a	13.82 g	51.23 f
		3	0.44 p	48.73 b	14.60 f	53.53 e
		6	0.45 p	48.54 b	15.31 ef	55.42 d
	5	0	0.48 o	46.01 c	15.97 de	55.95 d
		3	0.50 n	45.21 d	16.48 cd	57.12 c
		6	0.50 n	44.84 d	17.84 ab	57.98 bc
	10	0	0.58 kl	42.28 ef	17.85 ab	58.26 b
		3	0.60 j	41.98 f	18.12 a	59.58 a
		6	0.59 jk	42.76 e	17.15 bc	58.79 ab
LSD $_{(0.05)}$	-		0.02	0.62	0.74	0.86

Means followed by the same letter are not significantly different by the LSD Multiple Range test at P ≤ 0.05.

Fv/Fm: photochemical efficiency of photosystem II, MDA: Malondialdehyde, CAT: Catalase, POD: Peroxidase.

Also in other stages with the use of these elements it is controlled to increase the concentration of malondialdehyde so that under complete irrigation conditions (control) which is not interrupted. By increased Zinc sulfate and iron sulfate consumption led to the minimum concentration of malondialdehyde.

4.3. Enzyme (CAT, POD, SOD and GR) extraction and assay

The results obtained from Table 2 and 4, demonstrated the significant main and interactive effects (at 1% probability level) of drought stress, $ZnSO_4$ and drought stress × $ZnSO_4$ foliar applications on CAT, POD, SOD, GR, TPC.

The interactive effects of drought stress in the $FeSO_4$ was regarded as non-significant only in terms of CAT. The interactive effect of $FeSO_4$ × $ZnSO_4$ and drought stress × $ZnSO_4$ × $FeSO_4$ at 1% and 5% probability levels had a significant impact on all above-mentioned enzymes. According to our results, it was founded that drought stress increased the activities of CAT, POD, SOD and GR. However, addition of $ZnSO_4$ and $FeSO_4$ in water stressed plants caused a further increase the activity of the above-mentioned enzymes. The highest CAT, POD, SOD and GR enzyme was obtained in consumption of 10 $g.l^{-1}$ zinc sulfate and 3 $g.l^{-1}$ iron sulfate in water deficit condition at the early stage of seed growth. Also, the lowest amount of antioxidant enzymes was obtained under complete irrigation conditions (Table 3 and 5).

Table 4. Values of mean squares in the analysis of variance of antioxidant enzymes assays, TPC and grain yield

S.O. V	df	SOD	GR	TPC	Grain Yield
R	2	0.26^{ns}	0.27^{*}	1.07^{**}	241.16^{**}
Drought stress (D)	2	17721.96^{**}	458.65^{**}	1731.07^{**}	60153.23^{**}
Error	4	1.95	0.44^{ns}	0.01	11.34
$ZnSO_4$ (Z)	2	47.17^{**}	56.74^{**}	20.22^{**}	1034.94^{**}
D × Z	4	44.49^{**}	14.12^{**}	1.42^{**}	205.97^{**}
Error	12	0.36	0.04^{ns}	0.11	53.51
$FeSO_4$ (F)	2	6.45^{**}	2.88^{**}	0.03^{ns}	5.18^{**}
D × F	4	2.78^{**}	1.13^{**}	0.06^{ns}	2.54^{ns}
F × Z	4	2.41^{**}	0.78^{**}	0.06^{ns}	2.01^{ns}
D × Z × F	8	1.54^{**}	0.67^{**}	0.03^{ns}	2.06^{ns}
Error	36	0.21	0.07	0.06	5.14
CV (%)	-	0.94	3.91	1.45	2.39

* and **: Significant at the 5% and 1% probability levels, respectively., ns: Non- Significant.
SOD: Superoxide dismutase, GR: Glutathione reductase, TPC: total phenol content.

In response to drought stress, the activities of CAT, POD and SOD have been reported to be increased (Morteza-Salekjalali *et al*., 2012; Yavas and Unay, 2016) which further support our results. It has been reported that in drought tolerance of maize there is direct or indirect contribution of antioxidant (Farooq *et al*., 2009). In the current investigation $ZnSO_4$ and $FeSO_4$ improved drought tolerance of maize plants which was reflected in an enhanced activity of the above-mentioned enzymes. The Superoxide dismutase (SOD), catalase and other antioxidant enzymes by absorbing the oxygen or oxidative species from the lipid environment and biologically to remove free cell radicals, keep the reactive oxygen groups at the low concentrations, and catalyze the degradation of the hydrogen peroxide in the cell membrane. Therefore, they constitute an important biological defense mechanism against the free radical damage (Mansouri *et al*., 2018).

4.4. Total phenol content (*TPC*)

Applying $ZnSO_4$ ($P \leq 0.01$) affected total phenol content in maize in complete irrigation conditions and cut off irrigation. Also, interactive effect of drought stress × $ZnSO_4$ were regarded as significant at 1% probability level (Table 4). The lowest phenol content was related to the highest level of $ZnSO_4$ (10 $g.l^{-1}$) in water deficit condition at the early stage of seed growth, which increased compared to full irrigation treatment. Application of iron sulfate at constant concentration of zinc sulfate did not show any significant difference in stopping irrigation at vegetative growth stage and at the early stage of seed growth. Under complete irrigation conditions, no significant change was observed in phenol content between two concentrations of 5 $g.l^{-1}$ and 10 $g.l^{-1}$ $ZnSO_4$ and the lowest values of this trait were obtained under complete irrigation conditions and no micronutrients intake (Table 5). In support of our findings, $ZnSO_4$ has been reported that increase TPC (Ma *et al*., 2017) thereby improving drought tolerance of maize plants. Phenolic compounds are components of the cell's antioxidant defence system. These compounds can act as extinguishing or sweeping oxygen free radicals or other active oxygen species. Due to the role of phenolic compounds in reducing or inhibiting lipid auto oxidation, scavenging free radicals, deactivating single oxygen or decomposing peroxides, these compounds act as an antioxidant essential for antioxidant and anti-inflammatory protection (Ksouri *et al*., 2007). Phenolic compounds are also synthesized in the plant cells under optimum environmental conditions, but different environmental stresses alter their content in the cell (Kliebenstein, 2004).

4.5. Grain Yield

The results obtained from Table 4, showed the significant effects (at 1% probability level) of drought stress, $ZnSO_4$ and $FeSO_4$ foliar applications on grain yield. The interactive effects of drought stress × $ZnSO_4$ had a significant impact on grain yield ($P \leq 0.01$). The highest grain yield (153.79 g/plant) was obtained in full irrigation treatment and the lowest in the non-irrigation at the early stage of seed growth. The lack of irrigation at early stage of seed growth resulted in a significant decrease in grain yield compared to complete irrigation. Foliar application of zinc sulfate at non-irrigation at vegetative growth stage and early stage of seed growth resulted in a significant increase in grain yield. 10 $g.l^{-1}$ $ZnSO_4$ concentration increased grain yield compared to 5 $g.l^{-1}$ concentration, significantly (Table 5). Similar result was reported by Ma *et al*. (2017), who found foliar application of Zn increased grain yield. There was no significant difference between 3 and 6 $g.l^{-1}$ $FeSO_4$ in terms of seed yield (Table 5). Grain yield is related to the rate and duration of grain filling. Water deficiency at grain filling period (GFP) can impair photosynthesis, resulting in reduced remobilization of assimilates to grain and reduced GFP, resulting in lower grain weight (Farooq *et al*., 2014).

Table 5. Antioxidant enzymes, total phenol content and yield means comparison against control, as affected by foliar application of $ZnSO_4$ and $FeSO_4$

Drought stress	$ZnSO_4$ ($g.l^{-1}$)	$FeSO_4$ ($g.l^{-1}$)	*SOD*	*GR*	*TPC*	Grain Yield
Full irrigation	0	0	25.06 o	2.75 n	8.25 hi	130.13 e
		3	25.42 o	2.82 n	8.31 h	132.64 de
		6	25.10 o	2.73 n	7.88 i	134.14 d
	5	0	24.08 p	2.97 n	8.90 g	148.60 c
		3	23.84 p	2.96 n	8.90 g	147.10 c
		6	23.33 pq	2.87 n	8.84 g	149.80 bc
	10	0	22.77 q	2.92 n	8.98 g	154.80 a
		3	22.70 q	2.86 n	9.00 g	153.50 ab
		6	22.69 q	2.78 n	8.93 g	154.50 a
Non-irrigation at vegetative growth stage	0	0	42.03 n	3.96 m	16.31 f	83.64 i
		3	44.02 m	4.71 l	16.57 f	84.38 hi
		6	44.23 m	5.02 kl	16.54 f	84.43 hi
	5	0	45.62 l	5.25 k	17.89 e	87.48 gh
		3	46.61 k	5.91 j	17.77 e	88.22 g
		6	47.50 j	7.24 i	17.83 e	87.26 ghi
	10	0	48.50 i	8.65 g	18.89 d	93.27 f
		3	49.29 h	8.68 g	18.70 d	93.42 f
		6	48.59 hi	7.63 i	18.84 d	93.70 f
Non-irrigation at the early stage of seed growth	0	0	69.70 g	8.08 h	23.33 c	48.45 k
		3	72.54 f	9.03 fg	23.73 c	49.11 k
		6	74.29 e	9.13 f	23.66 c	48.45 k
	5	0	74.70 de	10.17 e	24.81 b	50.25 k
		3	75.26 cd	10.75 d	24.78 b	51.01 jk
		6	75.98 c	11.64 c	24.78 b	50.92 jk
	10	0	77.31 b	12.75 b	25.50 a	54.17 j
		3	78.07 a	13.84 a	25.59 a	54.22 j
		6	77.20 b	14.17 a	25.52 a	54.28 j
LSD $_{(0.05)}$			0.76	0.44	0.41	1.67

Means followed by the same letter are not significantly different by the LSD Multiple Range test at $P \leq 0.05$.
SOD: Superoxide dismutase, GR: Glutathione reductase, TPC: total phenol content.

In other hand micronutrient elements such as the zinc and iron are essential for growth plant and involved in physiological processes such as photosynthesis, hormone production and chlorophyll formation and their deficiency can cause nutrient imbalances and ultimately reduce quantity and quality of product (Malakoti *et al.*, 2005). Generally, plant dry matter content is a critical criterion for determining seed yield, and the basis way for increasing grain yield is having sufficient dry matter in the plant (Song *et al.,* 2013). In this study, it seems that the interaction of foliar application of micronutrients has an important role in increasing the amount of dry matter and consequently grain yield.

5. CONCLUSION

Results of this experiment showed that application of Zn + Fe nutrient had better effect on grain yield compared to single application of them and control. The highest grain yield of maize was recorded for non-drought stress treatment and was followed by foliar applied both ZnSO4 and FeSO4, while severe water stress and non-application of Zn and Fe resulted in minimum grain yield. This study provides useful information for underlying physiological and biochemical mechanisms involving $ZnSO_4$ and $FeSO_4$ foliar application and plant tolerance to drought stress. Furthermore, this study provides evidence for the use of $ZnSO_4$ and $FeSO_4$ application in arid and semiarid environments to increase grain yield. Drought stress reduced maize agronomic traits and increased activity of antioxidant enzymes such as catalase, superoxide dismutase and glutathione reductase, which increased above enzymes under drought stresses indicates the effect of these enzymes in reducing oxidative stress damages and their important role in counteracting reactive oxygen species.

ACKNOWLEDGMENT

The authors appreciate the cooperation and help of the laboratories of College of Agriculture, Islamic Azad University, Ahvaz branch, during these experiments.

FOOTNOTES

FUNDING/SUPPORT: This study was done by support of Department of Agronomy, Islamic Azad University, Ahvaz Branch.

REFRENCES

Afrousheh M., H. Hokmabadi. and H. Mirseyed Hosseini. 2010. Effect of nitrogen, iron, magnesium, manganese and molybdenum deficiencies on biochemical and eco physiological characteristics of pistachio seedling (*Pistacia vera*). *In* : Zakynthinos G. (Ed.). XIV GREMPAMeeting on Pistachios and Almonds. Zaragoza: CIHEAM/FAO/AUA/TEI Kalamatas/ NAGREF. pp. 53-63 (Options Méditerranéennes: Série A. Séminaires Méditerranéens; No. 94).

Ahmed-Amal, O. and B. B. Mekki. 2005. Yield and yield components of two maize hybrids as influenced by water deficit during different growth stages. Egypt J. Appl. Sci. 20: 64–79.

Anjum, S. A., L. C. Wang, M. Farooq, M. Hussain, L. L. Xue. and C. M. Zou. 2011. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J. Agron. Crop Sci. 197: 177-185.

Ashraf, U., A. S. Kanu, Z. Mo, S. Hussain, S. A. Anjum. and I. Khan. 2015. Lead toxicity in rice: effects, mechanisms, and mitigation strategies—a mini reviews. Environ. Sci. Pollut. Res. 22: 18318–18332.

Baishnab, C. T. and O. Ralf. 2012. Reactive oxygen species generation and signalling in plants. Plant Signal Behav. 7: 1621–1633.

Balabusta, M., K. Szafranska. and M. M. Posmyk. 2016. Exogenous melatonin improves antioxidant defense in cucumber seeds (*Cucumis sativus* L.) germinated under chilling stress. Front Plant Sci.

Bates, L. S., E. P. Waldren. and I. D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant and Soil. 39: 205-207.

Blume, D. E. and J. McClure. 1980. Developmental effects of Sandoz 6706 on activities of enzymes of phenolic and general metabolism in barely shoots grown in dark or under low of high intensity light. Plant Physiol. 65: 234-238.

Bradford, M. N. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Chem. 72: 248–254.

Cakmak, I. 2000. Tansley Review No. 111– possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New Phytol. 146: 185–205.

Cavalcanti, F. R., J. T. Oliveira, A. A. S. Martins-miranda, R. A. Viegas. and J. A. G. Silveira. 2004. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. New Phytologist. 163: 563–571.

Falqueto, A. R, R. A. S. Junior, M. T. G. Gomes, J. P. R. Martins, D. M. Silva. and F. L. E. Partelli. 2017. Effect of drought stress on chlorophyll a fluorescence in two rubber tree clones. Sci. Hort. 224: 238–243.

FAOSTAT. 2012. http://faostat.fao.org/site/567/desktopdefault.aspx#ancor

Farooq, M., M. Hussain, K. H. Siddique. 2014. Drought stress in wheat during flowering and grain-filling periods. Critical Rev. Plant Sci. 33(4): 331-349.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita. and S. M. A. Basra. 2009. Plant drought stress: effects mechanisms and management. Agron. Sustain. Dev. 29: 185-212.

Foyer, C.H. and J. M. Fletcher. 2001. Plant antioxidants: colour me healthy. Biol. 48: 115–120.

Gill, S. S. and N. Tuteja. 2011. Cadmium stress tolerance in crop plants probing the role of sulfur. Plant Signal. Behav. 6: 215–222.

Hussain, S., M. A. Maqsood, Z. Rengel. and T. Aziz. 2012. Biofortification and estimated human bioavailability of zinc in wheat grains as influenced by methods of zinc application. Plant Soil. 361: 279-290.

Karim, M. R., Y. Q. Zhang, R. R. Zhao, X. P. Chen, F. S. Zhang. and C. Q. Zou. 2012. Alleviation of drought stress in wheat by late foliar application of zinc, boron, and manganese. J. Plant Nutr. Soil Sci. 175: 142–151.

Kliebenstein, D. J. 2004. Secondary metabolites and plant, environment interactions: a view through *Arabidopsis thaliana* tinged glasses. Plant Cell and Environ. 27: 675-684.

Kerchev, P. I., C. Waszczak. and A. Lewandowska. 2016. Lack of glycolate oxidase-1, but not glycolate oxidase-2, attenuates the photo respiratory phenotype of catalase-2 deficient Arabidopsis. Plant Physiol. 171: 1704–1719.

Ksouri, R., W. Megdiche, A. Debez, M. Falleh, C. Grignon. and C. Abdelly. 2007. Salinity effect on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*, Plant Physiol. Biochem. 45: 244-248.

Ma, D., D. Sun, C. Wang, H. Ding, H. Qin, J. Hou, X. Huang, Y. Xie. and T. Guo. 2017. Physiological responses and yield of wheat plants in zinc-mediated alleviation of drought stress. Frontier in Plant Sci. 8: 860.

Mansori, M., A. Jorfi. and M. Mojadam. 2018. Assess enzymatic and non-enzymatic defense role of antioxidants in reduce effects of Salinity. Natl. Conf. Agri. Nat. Res. Livestock. Ardakan Univ. Iran. (Abstract in English)

Malakouti, M. J. and M. M. Tehrani. 2005. The role of Zinc in increasing quantitative and qualitative products and improving community health. Agri. Edu. Pub. 194 pp. (Abstract in English)

Munne-Bosch, S. and J. Penuelas. 2003. Photo and anti oxidative protection, and a role for salicylic acid during drought and recovery in field grown Phillyrea angustifolia plants. Planta. 217: 758–766.

Morteza Salekjalali, R., R. Haddad. and B. Jafari. 2012. Effects of soil water shortages on the activity of antioxidant enzymes and contents of chlorophylls and proteins in barley. Am-Eur. J. Agri. Environ. Sci. 12(1): 57-63.

Ranieri, A., A. Castagna, B. Baldan. and G. F. Soldatini. 2001. Iron deficiency differently affects peroxidase isoforms in sunflower. J. Exp. Bot. 52(354): 25–35.

Sarrou, E., P. Chatzopoulou, K. Dimassi-Theriou, I. Therios. and A. Koularmani. 2015. Effect of melatonin, salicylic acid and gibberellic acid on leaf essential oil and other secondary metabolites of bitter orange young seedlings. J. Essent. Oil Res. 27(6): 487-496.

Sharma, P., A. B. Jha, R. S. Dubey. and M. Pessarakli. 2012. Reactive oxygen species, oxidative damage and ant oxidative defence mechanism in plants under stressful conditions. J. Bot: 26p. Doi: 10.1155/2012/217037.

Song, G. Y., Z. J. Xu. and H. S. Yang. 2013. Effects of N rates on N uptake and yield in erect panicle rice. Agri. Sci. 4: 499–508.

Tabatabai, S. M. R., M. Oveysi. and R. Honarnejad. 2015. Evaluation of some characteristics of corn under water stress and zinc foliar application. Gmp Rev. 16: 34–38.

Tavallali, V., M. Rahemi, S. Eshghi, B. Kholdebarin. and A. Ramezanian. 2010. Zinc alleviates salt stress and increase antioxidant enzyme activity in the leaves of pistachio seedings. Turk. J. Agric. For. 34: 349–359.

Tripathi, D. K., S. Singh, S. Gaur, S. Singh, V. Yadav, N. K. Dubey, D. K. Chauhan. and S. Sahi. 2018. Acquisition and homeostasis of Iron in higher plants and their role in abiotic stress tolerance. 5: 86 Frontiers Environ. Sci.

Wang, F., B. Zeng, Z. Sun. and C. Zhu. 2009. Relationship proline and Hg^{+2} induced oxidative stress in tolerant rice mutant. Archives of Environ. Contamination and Toxicol. 56: 723-731.

Yang, P. M., Q. C. Huang, G. Y. Qin, S. P. Zhao. and J. G. Zhou. 2014. Different drought-stress responses in photosynthesis and reactive oxygen metabolism between tetraploid and diploid rice. Photosynthetica. 52: 193–202.

Yavas, I. and A. Unay. 2016. Effects of zinc and salicylic acid on wheat under drought stress. J. Anim. Plant Sci. 26: 1012–1018.