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Effect of low irrigation and Zn and SiO_2 Nano-fertilizers and Conventional Fertilizers on Morphophysiological traits and seed yield of Sunflower

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ABSTRACT: In order to investigate the effect of foliar application of conventional and nano-fertilizers (ZnO and SiO_2) on yield, morphological and physiological traits and harvest index of sunflowers under water deficit stress an experiment was conducted as a split-plot based on a Randomized Complete Block Design with three replications at experimental field of Islamic Azad University, Birjand Branch, Birjand, Iran in 2014. The main plot was devoted to irrigation at two levels (irrigation after 100 and 200 mm cumulative evaporation from evaporation pan) and the sub-plot was devoted to foliar spray of ZnO and SiO_2 at seven levels (nano ZnO, nano SiO_2, ZnO, SiO_2, nano ZnO + nano SiO_2, ZnO + SiO_2, and control with no foliar spray). Means comparison showed that water deficit stress decreased plant height, head diameter, stomatal conductance, chlorophyll index, seed yield and harvest index of seed by 20.8, 16.9, 27, 2.4, 50.3 and 24.9%, respectively as compared to no-stress conditions.Also foliar application of conventional ZnO fertilizer increased head diameter, seed yield, harvest index for seed in plant and seed in head by 10.2, 59.7, 36.5 and 23.4%, respectively. In total, it is recommended treatment of irrigation after 100 mm cumulative evaporation and to apply conventional ZnO in the cultivation of sunflower under conditions similar to Birjand, Iran.

Keywords: Sunflower, low irrigation, nano-fertilizers, morphological and physiological traits.

INTRODUCTION

Iran with mean annual precipitation of 240 mm is categorized in arid zones of the world. High evapotranspiration, limited water resources and other parameters invokes the interests for studying the effect of water stress and selecting drought-resistant cultivars (Seghatoleslami *et al.,* 2004). Plants face various environmental stresses during their growth, each one having different effects on their growth, metabolism and yield depending on their sensitivity and growth stage (Heidari, 2006). The loss of yield due to stomatal closure, stunted growth, deficiency of assimilates for filling the grains, and the shortening of the grain filling period are some important consequences of drought for plants (Reddy *et al.,* 2004).

On the other hand, the application of conventional fertilizers has been criticized in recent years due to their adverse effects on environment and food quality. Therefore, the application of new methods for fertilizing soil and feeding plants has been taken into consideration by researchers one of which is the application of nano-fertilizers. In fact, nanotechnology has offered opportunities for improving nutrients use efficiency and minimizing the costs of environment protection (Naderi and Abedi, 2012). One of the most important applications of nanotechnology in the field of water and soil is the application of nano-fertilizers for feeding plants (Rezaei *et al.*, 2009).

Sunflowers are important oilseeds in the world whose oil has a high quality because of its unsaturated fatty acids and the lack of cholesterol (Nezami *et al.*, 2008). High yield as well as wide adaptability, photosynthesis capacity and harvest index allows sunflowers to grow under diverse environmental conditions (Agele *et al.*, 2007). The study of the effect of water deficit stress on sunflower yield showed the significant effect of irrigation interval on its seed yield and the loss of seed yield under water deficit stress (Rahimizade *et al.,* 2010). The treatment of water deficit stress at three growth stages of sunflowers (head emergence, flowering and seed filling) significantly influenced their seed yield (Babaeian *et al.,* 2010). The shortening of seed filling period and early senescence of leaves can be mentioned as possible reasons for higher yield loss under stress at seed filling period than under stress at head emergence (Felent *et al.,* 1996).

Mozafari *et al.* (1996) related the loss of harvest index to the decrease in head diameter. Flent *et al.* (1996) revealed that harvest index was increased under mild stress but it started to decrease as water deficit stress was intensified.

Karimzadeh *et al.* (2002) reported that the loss of harvest index under moisture stress was lower than the loss of seed yield. Sidhara and Prasad (2002) found a very good linear relationship between harvest index and seed yield.

Water deficit stress disturbs plant's nutritional balance. The foliar spray of trace nutrients improves plant's growth under water deficit conditions (Paygzar *et al.*, 2009). Zn is an important trace element whose presence is necessary for the metabolic activities of the plants (Hassegawa, 2008). Although the plants' Zn requirement is very slight, if it is not available, the plants will suffer the physiological stresses of various enzymatic systems inefficiency and other Zn-related metabolic activities (Baybordi, 2006). In addition, the addition of SiO_2 to plant medium reduces the penetrability of the plasma wall of the leaf cells resulting in the loss of lipid peroxidation and also, SiO_2 protects cellular wall against heat and drought stress (Liang, 1999; Zhu, 2004).

SiO_2 increases vegetative growth and dry matter production (Agarie *et al.,* 1993). Kaya *et al.* (2006) showed that chlorophyll content and photosynthesis rate of maize were decreased under water deficit stress but the application of SiO_2 increased these traits and improved the plant growth and its production. Moaveni and Kheiri (2011) revealed that TiO_2 nano-particles significantly affected the yield of maize. It is shown that SiO_2 and TiO_2 particles increased reductase nitrate activity and the capability of water and fertilizer uptake and use in soybean (Lu *et al.,* 2002). Sepehr *et al.* (2004) indicated that the application of micronutrients significantly affected the plant height, head diameter, leaf number and seed yield of sunflowers. In addition, it has been shown that the application of micronutrients can improve the resistance of the plants to such environmental stresses as drought and salinity (Baybordi, 2004).

In a study on the effect of irrigation interval and micronutrient fertilizers on sunflowers, it was revealed that the simple effects of irrigation and micronutrients were significant on seed yield. It was also found that although the application of micronutrients had greater effect on seed yield under no-stress conditions, the positive influence of fertilization on crop yield was very promising under drought stress (Rahimizade *et al.,* 2010).

Given the fact that water deficiency, especially at mid-growing season in summer, is one of the main limiting factors of production in arid regions like Southern Khorasan, Iran, the study of the effect of water deficit stress on plant growth and sound management of fertilizers in sunflower fields is of a vital importance. Therefore, the objective of the present study was to investigate the effect of foliar application of conventional oxides and Zn and SiO_2 nano-fertilizers on yield, morphological and physiological traits and harvest index of sunflowers under water deficit stress.

MATERIALS AND METHODS

The present study was conducted in research farm of Department of Agriculture of Islamic Azad University of Birjand, Iran (Long. 59°13' E., Lat. 32°53' N., Alt. 1491 m.) in 2014. The soil properties of the study field are listed in Table 1.

The study was a split-plot experiment based on a Randomized Complete Block Design with three replications. The main plot was devoted to irrigation at two levels (irrigation after 100 and 200 mm cumulative evaporation from evaporation pan) and the sub-plot was devoted to foliar spray of Zn and SiO_2 at seven levels (nano ZnO, nano SiO_2, ZnO, SiO_2, nano ZnO + nano SiO_2, ZnO + SiO_2, and control with no foliar spray). The experimental plots included four planting rows with the length of 6 m and inter-row spacing of 50 cm. The foliar spray was conducted at two stages (two weeks before the initiation of flowering and two weeks after flowering). The concentration of nano ZnO and nano SiO_2 was 0.5:1000 and the concentration of the conventional oxides of Zn and SiO_2 was 5:1000.

Field preparation was started with plowing in autumn followed by leveling during mid-March, 2014. Then, the furrows and ridges were constructed by tractor and furrower in mid-May. Before sowing, the soil was analyzed and according to the results of this analysis (Table 1), the field was fertilized with 50 kg ha^{-1} urea, 50 kg ha^{-1} triple superphosphate and 100 kg ha^{-1} potassium sulfate.

The seeds of sunflower were sown by hand on both sides of ridges on May 28. The inter-plant spacing was adjusted to 15 cm in final thinning. To ensure uniform emergence, the plots were irrigated every 4 days until full emergence and the weeds were controlled by hand weeding. Urea fertilizer at the rate of 160 kg ha^{-1} was applied as heading in all treatments 60 days after sowing.

When the backs of heads became brownish yellow in 90% of the plants, the final harvest was carried out. The readings were not recorded on side rows and 0.5 m of both sides of the rows because of marginal effect. The studied morphological traits included plant height, stem diameter, head diameter and leaf number per plant measured on 10 plants. Two middle rows with the area of $2m^2$ were harvested and following counting the number of heads and winnowing the seeds, seed yield was determined and harvest indices were calculated by the following equations:

$$\text{Harvest index of seeds per plant} = \left(\frac{\text{seed yield}}{\text{biological yield}}\right) \times 100$$

$$\text{Harvest index of seeds per head} = \left(\frac{\text{seed yield}}{\text{head with seed yield}}\right) \times 100$$

$$\text{Harvest index of heads per plant} = \left(\frac{\text{head with seed yield}}{\text{biological yield}}\right) \times 100$$

Table 1: Results of soil analysis.

pH	EC(ms/cm)	$CaCo_3$ (%)	OC (%)	Sand (%)	Silt (%)	Clay (%)	Soil texture	N(total) (%)	P(ava) Ppm	K(ava) ppm	Fe $mg.kg^{-1}$	Cu $mg.kg^{-1}$	Zn $mg.kg^{-1}$	Mn $mg.kg^{-1}$
7.42	9.21	24.5	0.067	56	30	14	Lom sandy	0.08	16.4	5.1	2.73	0.83	0.68	5.71

Stomatal conductance was measured by porometer SC-1 and leaf chlorophyll index was measured by SPAD 94 days after planting on six plants from the underneath surface of the third leaf from the ground.

In the end, data were statistically analyzed by MSTAT-C software package and the means were compared by Duncan Multiple Range Test at 5% level.

RESULTS AND DISCUSSION

A. Morphological traits

Analysis of variance showed that the simple effects of irrigation and fertilization were significant on plant height and head diameter at 5% level, but the number of leaves and stem diameter were not impacted by irrigation and fertilization. In addition, the interaction between irrigation and fertilization was significant for morphological traits (Table 2). Means comparison showed 20.8 and 16.9% loss of plant height and head diameter under irrigation after 200 mm cumulative evaporation as compared to irrigation after 100 mm cumulative evaporation, respectively (Table 3).

The loss of plant height with the loss of soil moisture under water deficit stress (irrigation after 200 mm cumulative evaporation) can be attributed to the disruption of photosynthesis and the decrease in the assimilation for feeding the growing parts of the plant. As the final result, the plant cannot realize its height potential. The results of Neilson and Nelson (1998) and Nabati (2004) suggest that the loss of water potential of meristem tissues due to water deficit stress reduces pressure potential below the level required for cell elongation. Thus, plant height decreases. This finding is in agreement with some studies on sunflower including Daneshian *et al.* (2008), Ghafari and Pashapour (2006) and Goksoy *et al.* (2004) who reported lower plant height under water deficit stress.

It seems that the nutrient requirement of the seeds is mostly supplied from the reserves of head under water deficit stress resulting in the loss of head diameter. On the other hand, the loss of head diameter can be associated with the loss of assimilates under water deficit stress that reduces the number of seeds. The findings related to the loss of head diameter under stress are in agreement with Jaafarzadeh-Kenarsari and Pustini (1997) and Goksoy *et al.* (2004).

Means comparison revealed that the highest plant height (80.83 cm, on average) was related to the application of SiO_2 nano-fertilizer which was 17.3% higher than that under no foliar spray treatment (Table 4). The highest head diameter (7.89 cm, on average) was obtained under ZnO + SiO_2 nano-fertilization which was 18.8% higher than that obtained under no foliar spray treatment (Table 4). Nonetheless, all treatments of Zn and SiO_2 were categorized in the same statistical group in terms of plant height and head diameter.

The results of the present study regarding plant height as affected by the application of ZnO are consistent with those reported by Kherandish (2000), Khalili Mahaleh *et al.* (2006) and Rose *et al.* (2002). Sepehr and Malakooti (1997) also reported the positive role of Zn and Fe foliar spray along their soil application in increasing head diameter. Mozafarian *et al.* (2011) stated that SiO_2 nano-fertilizer increased shoot length.

Table 2: Mean of squares for the effect of irrigation and fertilizer on sunflower traits.

SOV	df	Plant height	Leaf number	Stem diameter	Head diameter	Seed yield	Harvest index seed per plant	Harvest index seed per head	Harvest index head per plant	Stomata conductivity	Cholorophyle index
Irrigation (A)	1	*	n.s	n.s	*	*	*	*	*	*	*
Fertilizer (B)	6	*	n.s	n.s	*	*	*	*	*	*	*
A × B	6	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

^{ns} Non Significant and *, ** Significant at 0.05 and 0.01 probability level, respectively

Table 3: The means comparison of sunflower traits in irrigation levels.

Irrigation (mm cumulative evaporation)	Plant height (cm)	Leaf number	Stem diameter (mm)	Head diameter (cm)	Seed yield (kg. ha^{-1})	Harvest index seed per plant (%)	Harvest index seed per head (%)	Harvest index head per plant (%)	Stomata conductivity (mmol. m^{-2} s^{-1})
200	68.58b	18.29a	8.98a	6.75b	541.65b	37.80b	54.04b	110.07b	43.04b
100	85.31a	20.41a	9.88a	8.12a	1089.20a	45.63a	59.82a	150.73a	44.12a

Means followed by the same letters in each column are not significant according to Duncan's multiple range test (P<0.05)

Table 4: The means comparison of sunflower traits in fertilizer levels.

Fertilizer	Plant height (cm)	Leaf number	Stem diameter (mm)	Head diameter (cm)	Seed yield (kg. ha^{-1})	Harvest index seed per plant (%)	Harvest index seed per head (%)	Harvest index head per plant (%)	Stomata conductivity (mmol. m^{-2} s^{-1})	Cholorophyle index
Control	68.88b	18.74a	8.82a	6.64b	649.49b	20.57b	38.60b	52.94b	130.42a	45.45a
Conventional ZnO	79.72a	18.47a	9.19a	7.32a	1037.16	28.08a	47.62a	57.19ab	136.51a	43.82ab
Nano ZnO	79.05a	19.85a	9.51a	7.56a	809.29	24.60ab	40.44ab	60.51a	124.53ab	42.38b
Conventional SiO_2	71.11ab	19.88a	9.16a	7.63a	843.06	24.99ab	40.92ab	60.83a	126.12ab	42.90b
Nano SiO_2	80.83a	20.16a	9.50a	7.68a	917.80	24.99ab	43.65ab	56.95ab	141.91a	42.38b
Conventional ZnO + SiO_2	80.27a	19.02a	10.10a	7.32a	715.07	22.29ab	41.87ab	53.00b	119.97b	44.36ab
Nano ZnO + SiO_2	75.25ab	19.30a	9.70a	7.89a	736.09ab	22.40ab	38.90 b	57.08ab	133.34a	43.76ab

Means followed by the same letters in each column are not significant according to Duncan's multiple range test (P<0.05)

B. Physiological traits

Irrigation and fertilization significantly influenced stomatal conductance of sunflowers but their interaction was not significant for it (Table 2). Means comparison revealed 26.9% loss of stomatal conductance under irrigation treatment after 200 mm cumulative evaporation than that after 100 mm cumulative evaporation (Table 3). The first response of most species to water deficiency is the closure of stomata to prevent water loss through transpiration whose consequence is the loss of stomatal conductance. The induction fo stomatal closure under water deficit stress is an important function of abscisic acid and the control of stomatal conductance is a mechanism for counteracting water deficiency. Under water deficit conditions, the concentration of abscisic acid increases in roots and starts to go to leaves where it induces stomatal closure to reduce transpiration. In other words, the decrease in water availability to plants results in lower stomatal conductance (Tardieu and Davies, 1993) which is in agreement with the results of Pankovic *et al.* (1999) about sunflowers, Dreesmann *et al.* (1994) about beets and Daneshmand *et al.* (2008) about canola.

According to means comparison, the lowest stomatal conductance was observed under the application of conventional ZnO + SiO_2 and the highest one under the foliar spray of SiO_2 nano-fertilizer (Table 4).

Analysis of variance indicated that the simple effects of irrigation and fertilization were significant on chlorophyll index at 5% level but their interaction was not significant for it (Table 2). Means comparison showed that chlorophyll index was 2.4% higher under no-stress conditions than under stress (Table 3). Leaf chlorophyll is a parameter that may be influenced by water stress. Zarco-tejada *et al.* (2009) mention leaf chlorophyll as one of the most important indices of the environmental pressures on plants and believe that chlorophyll is decreased in plants under stress resulting in the variation of light absorption ratio and the loss of light absorption by plants. Voleti *et al.* (1998) related the loss of chlorophyll index under water deficit stress to the destruction of pigments and/or the decrease in their buildup due to the disruption of the activities of the enzymes responsible for the synthesis of photosynthesizing pigments.

Furthermore, the loss of chlorophyll amount under drought stress can be caused by the increased production of oxygen radicals in cells that lead to peroxidation and consequently, the dissolution of these pigments (Schutz and Fangmeir, 2001). Some researchers, too, blamed the dissolution of chlorophyll due to activities of chlorophyllase, peroxidase and phenol compounds for the loss of chlorophyll concentration under water deficit stress (Ahmadi and Ciocemardeh, 2004).

Means comparison revealed that the highesst leaf chlorophyll index (45.45, on average) was seen in control (no spray) which was significantly higher than that obtained under the application Zn and SiO_2 nano-fertilizers by 7.24% (Table 4).

C. Seed yield

Analysis of variance showed that seed yield was significantly influenced by irrigation and fertilization but it was not influenced by their interaction (Table 2). Water deficit stress decreased seed yield by 50.3% as compared to no-stress conditions (Table 3) which can be related to the loss of leaf area and photosynthesis rate and the increase in the allocation of more assimilates to roots than to shoots. The loss of seed yield under water deficit stress is in agreement with the findings reported by Jafarzadeh Kenarsari and Poustini (1997), Erdem *et al.* (2006) and Goksoy *et al.* (2006) about sunflowers. In addition, the loss of leaf durability, early senescence and the adverse impact of water deficiency on current photosynthesis can be listed as the other reasons for the loss of sunflower seed yield under water deficit conditions.

Means comparison showed that the highest seed yield (1037.16 kg ha^{-1}, on average) was obtained under the foliar spray of ZnO which was 59.7% higher than that under control (Table 4). Micronutrients enhance seed yield through improving photosynthesis rate and leaf area duration (Ebrahimian *et al.*, 2008). There are numerous reports regarding the positive influence of Zn on the yield of plants (Grewal and Wiliams, 2000; Sheykhbaglo *et al.*, 2009; Thalooth *et al.*, 2006; Bukvic *et al.*, 2003). The loss of seed yield in control can be associated with the loss of head diameter and the number of seeds per head. K fertilization and Zn and P foliar spray increased seed yield in cotton, too (Savan *et al.*, 2008). However, some studies report the significant increase in the yield of different species under the application of nano-particles (Feizi *et al.*, 2010; Jaberzadeh *et al.*, 2010; Moaveni and Kheiri, 2011) which is inconsistent with the results of the present study.

D. Harvest index

According to the results of analysis of variance, the simple effects of irrigation and fertilization were significant on the harvest index of seeds per plant, seeds per head and heads per plant at 5% level, but their interaction was not significant for these traits (Table 2). Means comparison revealed 33.1, 20.7 and 10.7% increase in harvest index of seeds per plant, seeds per head and heads per plant under the treatment of irrigation after 100 mm cumulative evaporation as compared to the treatment of irrigation after 200 mm cumulative evaporation, respectively (Table 3).

Lower harvest index under water deficit stress implies that water deficit affects reproductive parts and the accumulation of dry matter in head stronger than the vegetative parts of sunflowers. In fact, harvest index expresses how assimilates are allocated to economical parts of the plant (seed and head) versus total produced matter reserved in plant. Since seed and head yields are the components of harvest indices, the variation of harvest indices greatly depends on the variation of seed and head yields (Alizadeh *et al.*, 2007). Rezaye Soukhtabbandani and Ramezani (2010) stated that water deficit is one of the factors that limit plant growth and development and disrupts carbohydrates partitioning to seed and head in addition to reducing produced dry matter. This disruption reduces harvest index. Pandey *et al.* (2000), too, identified the sensitivity of reproductive growth to adverse conditions as compared to vegetative growth as the reason for lower harvest index under water deficit stress. Given the important role of water in assimilate mobilization to seeds, it is likely that water deficiency during seeds filling period reduces or even stops the mobilization of assimilates which results in lower harvest index. In addition, water deficit stress at the start of flowering stage decreases seed yield and seed harvest index through reducing the number of seeds per plant. In a study on the effect of normal and moisture stress conditions on sunflowers, Fereres and Fernandez (1986) found a correlation between harvest index and seed yield under stress conditions. They related the loss of harvest index under water deficit stress to the loss of head diameter and seed number per head which is in agreement with our findings.

Means comparison revealed that the lowest harvest index of seeds per plant, seeds per head and heads per plant (20.57, 38.60 and 52.94%, respectively) were obtained in control treatment (no foliar spray). Harvest indices of seeds per plant and seeds per head showed 26.7 and 18.9% decrease as compared to the application of conventional ZnO and harvest index of heads per plant exhibited 12.9% loss as compared to the application of conventional SiO_2 (Table 4) implying that fertilizers play an important role in mobilizing assimilates to the seeds of sunflowers through extending seeds filling period and improving leaf area duration. So they play a positive role in increasing the amount of assimilates mobilized to seeds which finally increases harvest indices as compared to no-foliar spray treatment.

CONCULSION

In total, it was found that the application of water deficit stress on sunflowers reduced seed yield by 50.3%. Moreover, the application of ZnO significantly influenced vegetative traits and economical yield of sunflowers. Thus, it is recommended to treat irrigation after 100 mm cumulative evaporation and to apply conventional ZnO (5:1000) in the cultivation of sunflower under conditions similar to Birjand, Iran.

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