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Effects of exogenous application of nano particles and compatible organic solutes on sunflower (*Helianthus annuus* L.)

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ABSTRACT: Mediterranean semi-arid regions in northwestern Iran receive precipitation below potential evapotranspiration and are characterised by excessive heat and terminal drought stress. Exogenous application of some nanoparticles and growth regulators appears to alleviate the adverse effects of abiotic stress and improve plant performance. In the present paper, an attempt is made to evaluate the effects of foliar application of salicylic acid (SA), glycine betaine (GB), ascorbic acid (AA), nano-silica ($nSiO_2$) and nano titanium dioxide ($nTiO_2$) suspensions on growth and yield components of sunflower (*cv.* Azargol) in the semi-arid highland region of Maragheh, Iran. Plants were grown under rain-fed conditions with supplemental irrigation applied three times during the reproductive growth stages of growth. The results revealed that foliar application of SA considerably improved head diameter, chlorophyll content of plant leaves, and days to flowering, while it delayed physiological maturity for a fairly long time. Evaluation of the achene yield component indicated that application of GB significantly increased the weight of achenes when compared with other treatments. The percentage of empty achenes was considerably affected by treatments, the lowest percentage of hollow achenes being recorded for plants sprayed with GB, followed by ones sprayed with AA and $nTiO_2$. The percentage of oil in sunflower achenes was substantially increased by foliar application of nano particles, the highest oil percentage being recorded in plants treated with $nTiO_2$. The results also showed that the highest number of achenes per head, greatest kernel weight, and largest achene size were obtained by spraying with nanoparticle suspensions. Although all treatments improved the achene yield, the effect of $nTiO_2$ was most prominent. The results of the present experiment support the conclusion that foliar application of nanoparticles may alleviate adverse environmental factors and improve the performance of plants in semi-arid regions.

Keywords: compatible solutes, nano silicon dioxide, oil content, terminal drought stress, TiO_2 nanoparticles

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the commercially important oil seed crops in semi-arid environments. Sunflower oil has light colour, a high level of unsaturated fatty acids and no linolenic acid, bland flavour and high smoke points. Sunflower seed has become the world's second most important source of edible vegetable oil and must rank high on any priority list for research attention. It has been estimated that world production of sunflower seed exceeds 41.4 million tonnes from an area of 25.2 million ha of land (Faostat 2014). The annual

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production of sunflower in Iran is close to 78000 tones achieved from 70000 ha (Faostat 2014). However, the average yield of this crop in semi-arid regions is relatively low and estimated at about 1050 kg per hectare. Semi-arid regions in the North West of Iran are characterised by low erratic rainfall with total annual precipitation of close to 400 mm, inter-annual rainfall varying from 25 to 50%. In general, due to the unpredictable precipitation, short rainy season, poor soil fertility, inappropriate agronomic management, terminal drought and heat stress, the yield of rain-fed sunflower in semi-arid Mediterranean regions is less than the expected quantity. Despite all the mentioned restrictions, rain-fed agriculture plays a key role in food security. About 80% of the world's farmland is cultivated as rain-fed, and two thirds of global food production is achieved from these regions (Oweis & Hachum 2012). In recent years, owing to dwindling water resources and increasing food requirements, there is an urgent need to maximise crop yields under conditions of limited water supply, where full or conventional irrigation cannot be considered as acceptable options (Fan *et al.* 2005). In semi-arid Mediterranean regions, water scarcity often occurs during the critical reproductive growth stages of spring crops (Oweis & Hachum 2012). This can adversely affect crop yield quantity and/or quality. The concept of irrigation management has been a contentious subject in these areas. In some cases, it seems that irrigation scheduling to manage supplemental water can maximise the net profit of crops by bridging intra-seasonal dry spells (Fox & Rockström 2003). Supplemental irrigation can be described as the application of small quantities of water to essentially rain-fed crops during times when rainfall fails to provide adequate moisture for normal plant growth, in order to increase and stabilise yields (Tavakkoli & Oweis 2004; Oweis & Hachum 2012). Supplemental irrigation is practised for species generally grown without irrigation and with a good economic profitability.

The strategy of exogenous utilisation of different organic osmolytes, compatible solutes, and growth regulators in semi-arid region seems to be an effective practice for improving crop performance (Anjum *et al.* 2011). This approach is generally applied to mitigate the adverse effect of environmental stress and can lead to enhanced plant performance (Senaratna *et al.* 2003; Wang *et al.* 2010a). Osmo-protectants or compatible solutes are small molecules that protect cells from desiccation by maintaining a high intracellular osmolality (Wani *et al.* 2013). Glycine betaine (GB) is one of the best known compatible solutes, and physiological, biochemical, molecular and genetic evaluations have suggested that GB has a very important role in protection of plants under extreme environmental conditions (Farooq *et al.* 2008). Salicylic acid (SA) is a phenolic compound and naturally occurring plant growth regulator that influences various physiological and biochemical functions in plants (Wang *et al.* 2010b). It can play a role as a regulatory signal mediating the response of plants to biotic and abiotic stresses. Furthermore, SA can regulate different physiological or developmental processes such as seed germination, vegetative growth, photosynthesis, respiration, thermogenesis, flower formation, seed production and senescence (Rivas-San Vicente & Plasencia 2011; Ahmad *et al.* 2014). In recent years, ascorbic acid (AA) has been the focus of intensive research due to its multifunction as a major redox buffer and as a cofactor for enzymes involved in regulating photosynthesis, hormone biosynthesis and regeneration of other antioxidants (Gallie 2013). A water-soluble organic compound with antioxidant properties, ascorbic acid is essential to many aspects of plant growth and protection.

The use of nanotechnology in agriculture has increased considerably in recent years, and numerous benefits of nanoparticle applications have been reported (Siddiqui *et al.* 2015). Nanoparticles have unique physicochemical properties compared with bulky particles: their small size and ability to cross barriers (cell walls and plasma membranes) facilitate effective absorption, while their large specific surface can result in a good level of interaction with intracellular structures (Monica & Cremonini 2009). Consequently, nanoparticles can be used to increase the supply of elements to plant shoots and foliage. Nano-silicon dioxide (nSiO_2) and nano-titanium dioxide (nTiO_2) possess exceptional optical and biological characteristics and have recently caught the attention of plant physiologists. Nanoparticles of TiO_2 are promising as an efficient nutrient source for plants that can be used to improve biomass production by enhancing metabolic activities, photo-catalytic activity and conversion of light energy (Gao *et al.* 2008; Xie *et al.* 2011); and by increasing the activity of enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and peroxidase, as well as by protecting chloroplast membrane structure (Hong *et al.* 2005). Raliya *et al.* (2015) found that foliar spraying of TiO_2 nanoparticles on mung bean seedlings can significantly improve shoot length, root length, root area, root nodule content, leaf chlorophyll content and leaf content of total soluble protein. In addition, foliar application of nano-silicon dioxide has enjoyed greater consideration during the last years (Siddiqui *et al.* 2015). Silicon is the second most abundant element in soils and has been proven beneficial for the healthy growth and development of many plant species. It is recognised that foliar spraying of nano-silicon dioxide on plants improves their growth and performance by enhancing the accumulation of proline, that of free amino acids, content of nutrients, activity of antioxidant enzymes, gas exchange and efficiency of the photosynthetic apparatus (Xie *et al.* 2012; Kalteh *et al.* 2014). It would therefore be rational to assume that exogenous application of nanoparticles can improve the resistance of plants against heat and drought stress in semi-arid Mediterranean regions.

Although nanoparticles can regulate plant growth, they are not classified as plant growth regulators. Despite recent progress in understanding some environmental consequences of nanoparticles and compatible solutes, little research has been devoted to the influence of these materials on the yield and oil content of sunflower in semi-arid regions, and conflicting results are often reported. Therefore, the aim of the present study was to determine the possible effects of these plant growth substances and nano-particles on growth and the yield of sunflower under conditions of supplemental irrigation.

MATERIAL AND METHODS

A field experiment was conducted on an experimental farm in the Maragheh region, Northwest Iran (latitude 37°23' N, longitude 46°16' E and altitude 1485 m) during the spring of 2015. The experimental design was that of a randomised complete block in four replicates. Maragheh's climate is moderately cold and considered to be BSk according to the Köppen-Geiger climate classification (1928). In Maragheh, the average annual temperature is 11.2°C. The average amount of annual precipitation is 353 mm. Between the driest and the most humid months, the difference in precipitation is close to 64 mm. The variation in annual temperature is around 25.2°C. Rainfall from June to October is relatively light, and the highest rate of evapotranspiration then occurs. Application of supplemental irrigation is necessary during the dry spell. Monthly meteorological data (temperature, relative humidity, evapotranspiration and rainfall) for the growing season are shown in Table 1.

The soil of the field was a sandy loam containing 0.4% of organic matter (OM) with pH 7.57 and electrical conductivity (EC) of 0.506 $ds \cdot m^{-1}$. The soil contained 53% sand, 31% silt and 16% clay. The experimental field was ploughed once in early autumn and harrowed twice to bring the soil to fine tilth one week before planting. The recommended dose of fertiliser (150 kg N and 100 kg P_2O_5 ha^{-1}) was applied in the form of urea and triple superphosphate at the time of seed bed preparation.

Achenes of sunflower (*Helianthus annuus* L., cv. Azargol) were obtained from the Seed and Plant Improvement Institute, Karaj, Iran. Sunflower achenes were hand sown on the 28^{th} of March. Each experimental plot consisted of 8 rows, 4.5 m in length, with inter-plant and inter-row distances of 60 and 20 cm, respectively. Plants were grown under rain-fed conditions during vegetative growth, and three supplemental irrigations were applied during different reproductive stages of growth. The first irrigation was applied at the stage of inflorescence emergence (BBCH= 57; inflorescence clearly separated from foliage leaves), the second at the flowering stage (BBCH= 61; beginning of flowering) and the third at the stage of fruit development (BBCH= 71; achenes on the outer edge of the inflorescence are grey and have reached final size). There was no evidence of pests or disease on plants during the experiment. Weeds were controlled by hand-hoeing. Experimental treatments included T_1 [control (spraying with water)], T_2 [foliar application of nano-silicon dioxide suspension (2 mM)], T_3 [foliar spraying with glycine betaine (100 mM)], T_4 [foliar spraying with salicylic acid (1mM)], T_5 [foliar application of ascorbic acid (1 mM)] and T_6 [spraying with nano-titanium dioxide (2 mM)]. The plants were sprayed with the spraying liquids until their leaves reached the point of maximum liquid retention, after which runoff occurred. Foliar treatments were applied at the stage of stem elongation (BBCH= 32) and were repeated at the flowering stage (BBCH= 63). Nanomaterials were purchased from Pishgaman Nano, Iran. The particles were characterised morphologically by scanning electron micrography (SEM) and transmission electron microscopy. Their size was less than 100 nm. Chlorophyll content (SPAD) values were recorded with a SPAD-502 meter (Konica-Minolta, Japan) using fully expanded upper leaves at the flowering stage. Twenty independent SPAD measurements were made per each experimental unit using several different plants. The SPAD-502 meter measures the leaf's absorbance in the red and near-infrared regions. Using these two absorbances, the SPAD-502 meter calculates a numerical SPAD value that is proportional to the amount of chlorophyll present

Table 1. Summary meteorological data for the 2015 season at the Maragheh station.

	Minimum temperature (°C)	Maximum temperature (°C)	Mean temperature (°C)	Precipitation amount (mm)	Mean humidity (%)	Actual crop evapotranspiration (mm)
March	1. 9	13. 5	8	12. 69	49. 7	45. 51
April	6. 1	19. 4	13. 6	25. 9	41. 8	68. 10
May	11. 4	25. 5	19. 5	11. 14	36. 7	89. 56
June	17. 7	32. 7	263	2. 87	23. 9	115. 36
July	22	36. 4	29. 9	0. 35	24	131. 05

Table 2. Influence of different foliar applications on morpho-physiological traits of sunflower (*H. annuus*) plants.

Treatment	PH	LL	LW	SD	HD	CHL	DF	DM	HP
Control	104. 68[b]	14. 23[c]	8. 40[a]	14. 90[d]	11. 65[c]	35. 25[d]	60. 00[b]	103. 50[c]	30. 77[a]
Nano SiO_2	110. 00[ab]	16. 00[ab]	11. 39[a]	17. 46[bc]	13. 26[b]	44. 50[bc]	68. 50[b]	108. 00[b]	28. 25[b]
Glycine betaine	106. 33[b]	17. 20[a]	10. 70[a]	17. 59[bc]	13. 52[b]	40. 50[cd]	80. 75[a]	103. 25[c]	30. 92[a]
Salicylic acid	111. 72[ab]	15. 07[b]	10. 89[a]	16. 09[cd]	14. 10[a]	53. 25[a]	79. 50[a]	109. 50[ab]	28. 97[b]
Ascorbic acid	118. 09[a]	14. 51[c]	9. 30[a]	18. 33[b]	13. 63[ab]	47. 75[b]	69. 25[b]	106. 50[bc]	28. 25[b]
Nano TiO_2	109. 51[ab]	16. 89[a]	8. 56[a]	20. 12[a]	13. 73[ab]	48. 36[b]	67. 25[b]	113. 75[a]	28. 16[b]
Level of significance	NS	**	NS	**	**	**	**	*	*
CV (%)	6. 21	3. 70	21. 82	6. 11	2. 91	8. 06	3. 61	2. 75	3. 89

CV = coefficient of variation, PH: plant height (cm), LL: leaf length (cm), LW: leaf width (cm), SD: stem diameter (mm), HD: head diameter (cm), CHL: chlorophyll content (SPAD units), DF: days to flowering, DM: days to physiological maturity, HP: husk percentage. Values in a column with the same letter (s) or without any letter (s) do not differ significantly, whereas values with dissimilar letters are statistically different. NS = not significant, * = significant at 5% level of probability, ** = significant at 1% level of probability.

in the leaf. Leaf length and width were measured at the end of the flowering stage (BBCH= 69). Observations were recorded on 10 randomly selected plants. The influence of different spray treatments on phenological development of the plants was evaluated through regular monitoring of the field and by recording the days to 50% flowering and days to maturity. Plants were harvested at the stage of physiological maturity, when the back of the head had turned from green to yellow and the bracts were turning brown. At harvest, 10 plants from each replicate for each treatment were sampled randomly and quantified for yield and yield components. The following data were recorded: plant height; stem diameter; head diameter; husk percentage; number of seeds per head; weight of 1000 achenes; kernel weight; achene length; achene thickness; achene width; straw yield; and harvest index. The seed oil percentage was measured using a portable near-infrared seed analyser (Zeltex). Near-infrared (NIR) prediction values are determined by the NIR spectrum obtained from the sample analysed. The NIR seed analyser works on the basis of diffuse transmittance. Light energy that enters the sample is scattered and absorbed within the sample. The analyser measures the spectra exiting the sample and directly displays the oil concentration in achenes. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute 1988), the least significant difference (LSD) being used to compare means of traits ($p < 0.05$). Pair-wise Pearson's correlation coefficients were calculated for 11 quantitative characters with seed yield. Also, correlation coefficients were computed by plotting the first two PCA (principal component analysis) components using Minitab, version 14. The principal component analysis used was based on Everitt & Dunn (1992).

RESULTS

Analysis of variance indicated a highly significant effect of foliar applications on most agro-morphological traits of sunflower (Table 2). Although the effect of treatments on plant height was statistically non-significant, Fisher's least significant difference (LSD) test could be used to separate the means of different treatments. The greatest height of plants was recorded for ones treated with ascorbic acid (AA), whereas the smallest plants were observed under the control conditions (intact plants sprayed with water). Leaf length was significantly influenced by treatment: leaves obtained after application of glycine betaine (GB) were the longest, followed by those that received nanoparticle treatments ($nTiO_2$ and $nSiO_2$). The results revealed that leaf width was not affected by foliar treatments. Stem diameter notably responded to foliar spray in that the thickest stems were obtained by foliar application $nTiO_2$. Comparison of mean values showed that application of $nTiO_2$ and GB increased stem diameter up to 35 and 23%, respectively, over the control. Foliar treatments considerably improved the head diameter: to be specific, the largest heads were recorded in plants treated with salicylic acid (SA), AA and $nTiO_2$ (Table 2). Analysis of variance showed that foliar applications significantly affected chlorophyll content ($p<0.01$). Comparison of mean values of this trait revealed that foliar application of SA increased chlorophyll content (the SPAD value) up to 51% over the control. Similarly, application of $nTiO_2$, AA

Table 3. Yield components of sunflower (*H. annuus*) as affected by different growth regulators in a semi-arid region under conditions of supplemental irrigation.

Treatment	NSH	TAW	KW	AL	AT	AW	STY	HI
Control	297. 49[d]	42. 28[ab]	0. 039[c]	10. 85[d]	2. 50[d]	4. 39[c]	7576[ab]	11. 88[a]
Nano SiO_2	350. 61[a]	42. 84[ab]	0. 049[ab]	11. 86[a]	2. 82[a]	4. 87[a]	7970[ab]	13. 15[a]
Glycine betaine	312. 19[cd]	43. 68[a]	0. 045[b]	11. 27[c]	2. 58[cd]	4. 45[c]	7498[b]	13. 52[a]
Salicylic acid	339. 50[ab]	41. 72[b]	0. 044[bc]	11. 08[cd]	2. 68[bc]	4. 77[ab]	7439[b]	14. 30[a]
Ascorbic acid	324. 97[bc]	42. 00[ab]	0. 045[b]	11. 53[abc]	2. 72[ab]	4. 56[bc]	7857[ab]	12. 91[a]
Nano TiO_2	352. 62[a]	42. 84[ab]	0. 052[a]	11. 75[ab]	2. 70[abc]	4. 67[abc]	8109[a]	14. 59[a]
Level of significance	**	*	*	**	*	*	NS	NS
CV (%)	12. 68	4. 46	8. 02	3. 22	4. 49	5. 40	4. 64	13. 98

CV = coefficient of variation, NSH: number of seeds per head, TAW: 1000-achene weight (g), KW: kernel weight (g), AL: achene length (mm), AT: achene thickness (mm), AW: achene width (mm), STY: straw yield (kg ha^{-1}), HI: harvest index. Values in a column with the same letter (s) or without any letter (s) do not differ significantly, whereas values with dissimilar letters are statistically different. NS = not significant, * = significant at 5% level of probability, ** = significant at 1% level of probability.

and $nSiO_2$ enhanced chlorophyll content up to 37, 35 and 26%, respectively, compared with the control. Exogenous application of compatible solutes and suspensions of nanoparticles influenced both the days to 50% flowering and the days to physiological maturity, at the levels of 0.01 and 0.05, respectively. Foliar treatments generally delayed flowering and maturity. Salicylic acid had an improving effect on chlorophyll content and prolongation of vegetative growth, and plants treated with it showed the longest and most efficient period of development. Foliar applications significantly affected the husk percentage at the level of 0.05. Comparison of mean husk percentage values between different treatments indicated that with the exception of GB, all treatments considerably reduced the amount of husk.

The results of variance analysis of yield components are shown in Table 3. The effect of foliar spray treatments on the number of achenes per head was statistically significant at the level of 0.01. Almost all the treatments improved this trait when compared with the control. Comparison of mean values revealed that application of both nanoparticle suspensions increased the achene number up to 12% over the control. However, no significant difference between SA and nanoparticles was observed. Evaluation of the number of empty achenes revealed that the smallest number of hollow achenes was recorded in plants treated with GB, followed by ones treated with SA and AA, while the largest number of empty achenes was recorded in the control plants (Fig. 1). The weight of 1000 achenes was slightly affected by foliar application of compatible solutes ($p<0.05$), the highest achene weight being recorded in plants treated with GB, whereas the lowest achene weight was observed in plants treated with SA (Table 3). Similarly, assessment of kernel weight indicated that foliar treatment impacted this trait at the level of 0.05. Comparison of mean values of kernel weight showed that foliar application of $nTiO_2$ and $nSiO_2$ increased this component up to 33 and 25%, respectively, over the control. Examination of the morphological characteristics of achenes showed that foliar utilisation of nanoparticles considerably improved achene size, the longest, widest and thickest achenes being obtained by foliar spraying of $nSiO_2$ (Table 3). Investigation of the achene yield showed that foliar treatment marginally affected this trait, the application of $nTiO_2$ increasing the achene yield up to 35% in comparison with untreated plants (Fig. 2). For the other foliar treatments, no significant difference was observed in comparison with the control. Seed oil percentage was noticeably influenced by foliar treatment ($p<0.01$). The highest oil percentage was recorded in plants treated with $nTiO_2$, followed by ones treated with $nSiO_2$. Foliar application of $nSiO_2$ and $nTiO_2$ increased oil percentage up to 4 and 11%, respectively, over the control (Fig. 3). As in the case of achene yield, no significant differences of oil percentages were observed after the other treatments.

The results of correlation analysis were corroborated by principal component analysis (PCA) (Fig. 4). The correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors. In Fig. 4, the most prominent relation is the strong positive association between achene yield, head diameter, achene length, harvest index, stem diameter, leaf with, head number per plant, number of achenes per

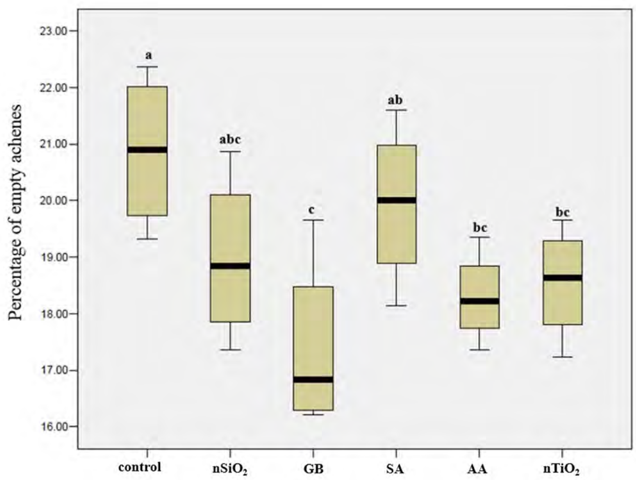

Fig. 1. Percentage of empty achenes out of the total number of achenes in sunflower plants treated with different growth regulators during growth in the semi-arid highland region of Maragheh under conditions of supplemental irrigation. Medians of data marked with the same letter are not significantly different ($P < 0.05$). GB = glycine betaine, SA = salicylic acid, AA = ascorbic acid.

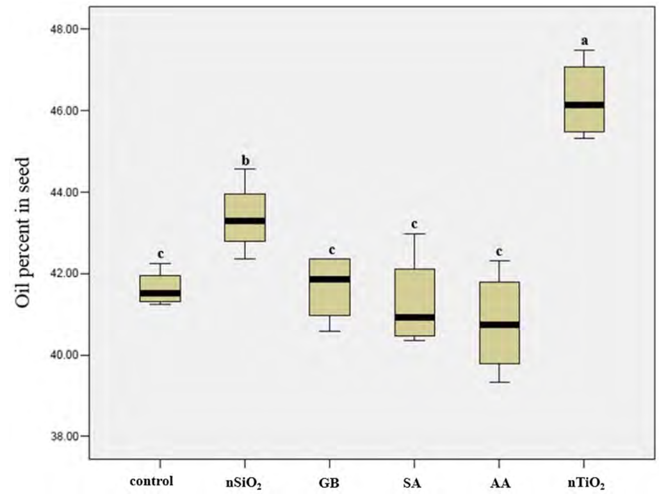

Fig. 3. Percentage of oil in seeds of sunflower plants treated with different growth regulators during growth in the semi-arid highland region of Maragheh under conditions of supplemental irrigation. Medians of data marked with the same letter are not significantly different ($P < 0.05$). GB = glycine betaine, SA = salicylic acid, AA = ascorbic acid.

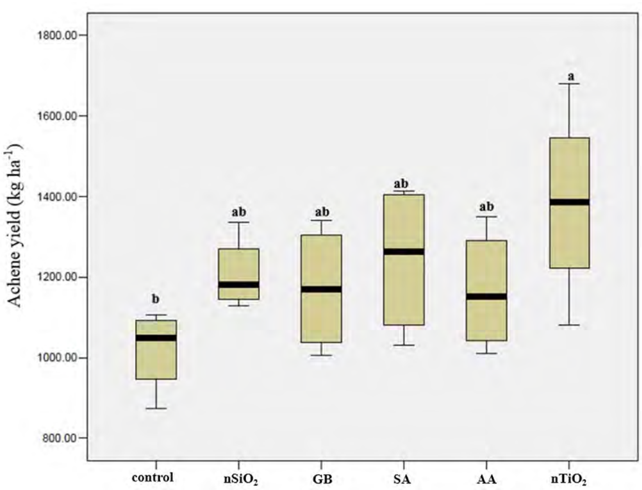

Fig. 2. Achene yield of sunflower plants treated with different growth regulators during growth in the semi-arid highland region of Maragheh under conditions of supplemental irrigation. Medians of data marked with the same letter are not significantly different ($P < 0.05$). GB = glycine betaine, SA = salicylic acid, AA = ascorbic acid.

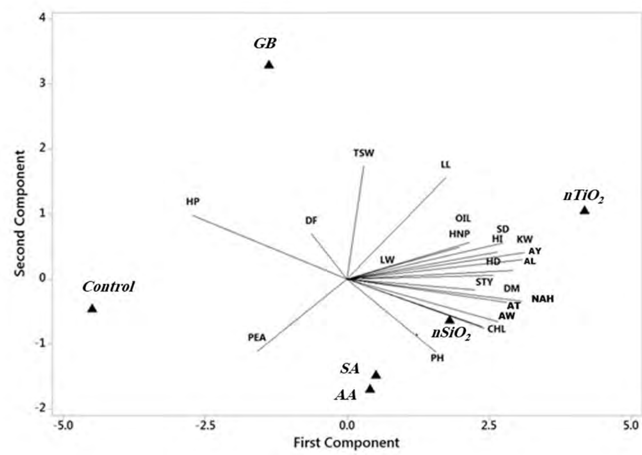

Fig. 4. Biplots for the first and second principal components used for mean agronomic traits in sunflower plants grown in the semi-arid highland region of Maragheh under conditions of supplemental irrigation and treated with different growth regulators. GB = glycine betaine, SA = salicylic acid, AA = ascorbic acid, PH: plant height, LL: leaf length, LW: leaf width, SD: stem diameter, HNP: head number per plant, HD: head diameter, CHL: chlorophyll content, DF: days to flowering, DM: days to physiological maturity, HP: husk percentage, NAH: number of achenes per head, TSW: 1000-seed weight, KW: kernel weight, AL: achene length, AT: achene thickness, AW: achene width, STY: straw yield, HI: harvest index, PEA: percentage of empty achenes, PH: plant height, LW: leaf width.

head, oil percentage and straw yield, as indicated by the small obtuse angles between their vectors (r = cos 0 = +1). Furthermore, PCA analysis showed that the highest values of achene length, oil percentage, head number per plant, stem diameter, kernel weight and achene yield were obtained by foliar application of $nTiO_2$, as is indicated by proximity of the listed attributes to the position of $nTiO_2$ (Fig. 4). On the other hand, the highest values of chlorophyll content, achene weight, achene thickness, days to maturity and the number of achenes in the head were obtained by $nSiO_2$ application. Moreover, evaluation of the correlation coefficient revealed that there is a significant positive correlation between achene yield

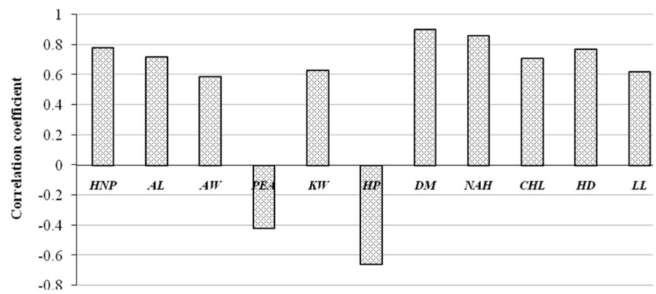

Fig. 5. Coefficient of correlation of 11 quantitative characters with seed yield in sunflower plants. LL: leaf length, HD: head diameter, CHL: chlorophyll content, DM: days to physiological maturity, HP: husk percentage, NAH: number of achenes per head, KW: kernel weight, AL: achene length, AW: achene width, HNP: head number per plant.

and leaf length, head diameter, chlorophyll content, days to physiological maturity, number of achenes per head, kernel weight, achene length, achene width and head number per plant. Finally, a considerable negative correlation was observed between achene yield and the percentage of empty achenes, as well as between achene yield and husk percentage (Fig. 5).

DISCUSSION

The results showed that foliar application of compatible solutes and suspensions of nanoparticles caused a marked increase in growth-related characteristics of sunflower. Terminal heat stress and drought stress caused by high temperatures and low precipitation during achene development are important constraints on sunflower production in semi-arid regions. Our results suggest that compatible solutes may stimulate osmotic adjustment in the sunflower plant. The significant improvement of growth characteristics achieved by exogenous application of compatible solutes (GB and SA) implies that sunflower plants do not normally accumulate significant amounts of osmo-protectants under these stresses. Contrary to previous reports indicating positive effects of ascorbic acid on plant growth (Gallie 2013), we found no differences between AA-treated and control plants for most of the examined morpho-physiological traits. This may be due to the concentration used or the poor response of sunflower as a plant species. The results of the present study are consistent with those of Hussain *et al.* (2009), who found that exogenous application of GB and SA improved the growth and water relations of hybrid sunflower (*Helianthus annuus* L.) under different irrigation regimes. Turgor pressure is central to nearly all plant functions. It is responsible for growth, transport and movement, in addition to being involved in cell metabolism. Foliar treatments notably enhanced leaf length, head diameter and stem diameter over the control. It appears that these treatments improved the water status and turgor pressure by intensifying the plant's osmotic adjustment, which resulted in its enhanced growth. The present findings seem to be consistent with the results of research which showed that exogenous application of SA under drought stress resulted in the maintenance of turgor due to accumulation of significantly greater amounts of different osmotica (glycine betaine, soluble sugars and free amino acids) and improved plant growth in shallot (Ahmad *et al.* 2014). However, nanoparticles had more positive stimulatory effects on some growth characteristics in comparison with other treatments.

Our findings indicated that foliar treatment increased both leaf size and chlorophyll content. From the perspective of source-sink relations, this means that exogenous application of compatible solutes or nanoparticles can improve source strength. However, in order for the achene yield to increase, sink and source strength must be in balance throughout the whole plant. Thus, an increase in whole plant source strength (source activity or source size) must be matched by an equal increase in sink strength (White *et al.* 2015), either through increase in achene numbers, achene size or kernel weight (achene filling). Although some treatments like SA and $nSiO_2$ improved plant growth characteristics and partly increased source size, this was not completely coordinated with sink enlargement, and the highest percentage of empty achenes was recorded for the control plants and those treated with SA and $nSiO_2$. Results of the present experiment revealed that $nTiO_2$ improved both vegetative and reproductive traits. Improved seed filling seems to correlate with an increased source–sink ratio. The smallest number of empty achenes and the highest achene yield were recorded for plants treated with $nTiO_2$. It appears that $nTiO_2$ positively affected transport of photoassimilates and biomass production. These findings agree with the results of Jaberzadeh *et al.* (2013), who reported that application of $nTiO_2$ increased wheat plant growth and significantly improved grain yield under drought stress conditions. However, plant growth and seed setting are controlled by similar physiological and developmental mechanisms, and more detailed investigations are needed for accurate interpretation of the action of $nTiO_2$.

Sunflower is an allogamic plant that needs insects at flowering, and up to 90% of seeds set in flower heads are accessible to pollinators (Nderitu *et al.* 2008). Thus, it is not inconceivable that the action of pollinators and seed setting were affected by flair application treatments. During foliar application, nanoparticles enter the cellular system via stomata and can affect transpiration, influence photosynthesis and interfere with translocation of food material. Moreover, it seems that foliar spray of $nTiO_2$ can affect the performance of stomata. Plants rely upon environmental signals to regulate the water status by osmotic movement of water into their guard cells and opening of the stomata, so that the advantage of increased photosynthesis is balanced against the disadvantage of increased water loss. In this context, Alidoust & Isoda (2013) reported that application of Fe_2O_3 nanoparticles on *Glycine max* increased photosynthetic rates, which was attributed to increased stomatal opening.

Our finding that the percentage of oil in achenes is increased by application of beneficial nanoparticles corroborates these earlier results. Zareii *et al.* (2014) found that foliar application of iron nanoparticles during the reproductive growth stage significantly improved the oil percentage in safflower. Because of their very small size, these particles offer a large contact surface per unit of mass and can pass through different protective barriers. Undoubtedly, nanoparticles compared to bulky particles interact better with intracellular processes, and this can partially explain their greater effectiveness. However, the findings of the present study do not support the results of Ahmed (2013), who suggested that use of salicylic acid and ascorbic acid would be highly helpful as a way to increase oil yield and fatty acid content in sunflower. Under the studied conditions, nanoparticles were more efficient than compatible organic solutes for improving both vegetative growth and yield components. Thus, the application of beneficial nanoparticles such as $nTiO_2$ and $nSiO_2$ seems to be an effective new substitute for foliar spraying with compatible solutes and can be considered a suitable option for agronomic management. Taken altogether, the results of the present study suggest that foliar application of $nTiO_2$ in proper concentrations can be a suitable option for improving sunflower production in semi-arid highland regions. The present findings are consistent with the results of other research indicating that exogenous application of some nanoparticles can significantly improve plant growth (Mandeh *et al.* 2012; Song *et al.* 2013). Titanium dioxide nanoparticles ($nTiO_2$) are promising as an efficient nutrient source that can be used by plants to improve biomass production as a result of enhanced nitrogen assimilation, photoreduction, activities of photosystem II and the electron transport chain and scavenging of reactive oxygen species (Morteza *et al.* 2013; Raliya *et al.* 2015). However, the effectiveness of nano-particles strongly depends on their concentration and varies from plant species to species. It follows that the effects of different concentrations and the interaction nano-particles with cellular processes need to be further elucidated in order to develop a more comprehensive interpretation.

CONCLUSION

Sunflower production in the semi-arid area of north-western Iran depends mainly on the soil's water status throughout the growing season. However, the occurrence of drought in the region is an inevitable matter. We found that the exogenous application of compatible solutes and nanoparticle suspensions as a foliar spray improved the agro-morphological traits of sunflower. The data presented in this study indicate that although foliar application of compatible solutes positively affected vegetative characteristics, their effect on most achene yield components was not considerable. On the other hand, foliar spraying with nanoparticles, and nano-titanium in particular, considerably enhanced the achene yield and yield components. The largest number of achenes per head, greatest kernel weight and highest oil percentage were recorded for plants treated with $nTiO_2$. Based on the obtained results, we are able to conclude that application of $nTiO_2$ can be an appropriate foliar treatment to improve sunflower performance in semi-arid regions.

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REFERENCES

Ahmad MA, Murali PV & Marimuthu G. 2014. Impact of salicylic acid on growth, photosynthesis and compatible solute accumulation in *Allium cepa* L. subjected to drought stress. *International Journal of Agricultural and Food Science* **4**(1): 22-30.

Ahmed N. 2013. Role of plant growth regulators in improving oil quantity and quality of sunflower hybrids in drought stress. *Biological Society of Pakistan* **59**(2): 315-322.

Alidoust D & Isoda A. 2013. Effect of γFe_2O_3 nanoparticles on photosynthetic characteristic of soybean (*Glycine max* L. Merr.): foliar spray versus soil amendment. *Acta Physiologiae Plantarum* **35**(12): 3365-3375.

Anjum SA, Farooq M, Wang LC, Xue LL, Wang SG, Wang L & Chen M. 2011. Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions. *Plant, Soil and Environment* **57**(7): 326-331.

Everitt BS & Dunn G. 1992. *Applied multivariate data analysis*. New York, Oxford University Press.

Fan T, Stewart BA, Payne WA, Wang Y, Song S, Luo J & Robinson CA. 2005. Supplemental irrigation and water: yield relationships for plasticulture crops in the loess plateau of China. *Agronomy Journal* **97**: 177–188.

Faostat 2014. *Food and Agricultural Organization of the United Nations*. Available online: http://www. fao. org/ faostat/en/#data/QC.

Farooq M, Aziz T, Hussain M, Rehman H, Jabran K & Khan MB. 2008. Glycinebetaine improves chilling tolerance in hybrid maize. *Journal of Agronomy and Crop Science* **194**: 152-160.

Fox P & Rockström J. 2003. Supplemental irrigation for dry-spell mitigation of rainfed agriculture in the Sahel. *Agricultural water management* **61**(1): 29-50.

Gallie DR. 2013. L-Ascorbic acid: a multifunctional molecule supporting plant growth and development. *Scientifica* 2013. Article ID 795964, 24 pages, http://dx.doi.org/10.1155/2013/795964.

Gao F, Liu C, Qu C, Zheng L, Yang F, Su M & Hong F. 2008. Was improvement of spinach growth by nano-TiO2 treatment related to the changes of rubisco activase? *Biometals* **21**: 211-217.

Hong F, Yang F, Liu C, Gao Q, Wan Z, Gu F, Wu C, Ma Z, Zhou J & Yang P. 2005. Influences of nano-TiO2 on the chloroplast aging of spinach under light. *Biological Trace Element Research* **104**: 249-260.

Hussain M, Malik MA, Farooq M, Khan MB, Akram M & Saleem MF. 2009. Exogenous glycinebetaine and salicylic acid application improves water relations, allometry and quality of hybrid sunflower under water deficit conditions. *Journal of Agronomy and Crop Science* **195**(2): 98-109.

Jaberzadeh A, Moaveni P, Moghadam HRT & Zahedi H. 2013. Influence of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **41**(1): 201.

Kalteh M, Alipour ZT, Ashraf S, Aliabadi MM & Nosratabadi AF. 2014. Effect of silica nanoparticles on basil (*Ocimum basilicum*) under salinity stress. *Journal of Chemical Health Risks* **4**(3): 49–55.

Köppen W & Geiger R. 1928. *Die klimate der Erde*. Gotha, Verlag Justus Perthes.

Mandeh M, Omidi M & Rahaie M. 2012. In vitro influences of TiO_2 nanoparticles on barley (*Hordeum vulgare* L.) tissue culture. *Biological Trace Element Research* **150**(1-3): 376-380.

Monica RC & Cremonini R. 2009. Nanoparticles and higher plants. *Caryologia* **62**(2): 161-165.

Morteza E, Moaveni P, Farahani HA & Kiyani M. 2013. Study of photosynthetic pigments changes of maize (*Zea mays* L.) under nano TiO_2 spraying at various growth stages. *SpringerPlus* **2**(1): 1-5.

Nderitu J, Nyamasyo G, Kasina M & Oronje ML. 2008. Diversity of sunflower pollinators and their effect on seed yield in Makueni District, Eastern Kenya. *Spanish Journal of Agricultural Research* **6**(2): 271-278.

Oweis T & Hachum A. 2012. *Supplemental irrigation, a highly efficient water-use practice*. ICARDA, Aleppo, Syria.

Raliya R, Biswas P & Tarafdar JC. 2015. TiO_2 nanoparticle biosynthesis and its physiological effect on mung bean (*Vigna radiata* L.). *Biotechnology Reports* **5**: 22-26.

Rivas-San Vicente M & Plasencia J. 2011. Salicylic acid beyond defence: its role in plant growth and development. *Journal of Experimental Botany* **62**(10): 3321-3338.

Senaratna T, Merritt D, Dixon K, Bunn E, Touchell D & Sivasithamparam K. 2003. Benzoic acid may act as the functional group in salicylic acid and derivatives in the induction of multiple stress tolerance in plants. *Plant Growth Regulation* **39**(1): 77-81.

Siddiqui MH, Al-Whaibi MH, Firoz M & Al-Khaishany MY. 2015. Role of nanoparticles in plants. In: Siddiqui MH, Al-Whaibi MH, Mohamad F (eds.), *Nanotechnology and plant sciences: nanoparticles and their impact on plants,* pp. 19-35, Springer International Publishing.

Song U, Shin M, Lee G, Roh J, Kim Y & Lee EJ. 2013. Functional analysis of TiO_2 nanoparticle toxicity in three plant species. *Biological Trace Element Research* **155**(1): 93-103.

Tavakkoli AR & Oweis TY. 2004. The role of supplemental irrigation and nitrogen in producing bread wheat in the highlands of Iran. *Agricultural Water Management* **65**(3): 225-236.

Wang GP, Li F, Zhang J, Zhao MR, Hui Z & Wang W. 2010a. Overaccumulation of glycine betaine enhances tolerance of the photosynthetic apparatus to drought and heat stress in wheat. *Photosynthetica* **48**(1): 30-41.

Wang LJ, Fan L, Loescher W, Duan W, Liu GJ, Cheng JS, Luo HB & Li SH. 2010b. Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves. *BMC Plant Biology* **10**(1): 34.

Wani SH, Singh NB, Haribhushan A & Mir JI. 2013. Compatible solute engineering in plants for abiotic stress tolerance-role of glycine betaine. *Current Genomics* **14**(3): 157.

White AC, Rogers A, Rees M & Osborne CP. 2015. How can we make plants grow faster? A source–sink perspective on growth rate. *Journal of Experimental Botany,* erv447. http://dx.doi: 10. 1093/jxb/erv447.

Xie Y, Li B, Zhang Q & Zhang C. 2012. Effects of nano-silicon dioxide on photosynthetic fluorescence characteristics of *Indocalamus barbatus* McClure. *Journal of Nanjing Forestry University (Natural Science Edition)* **2**: 59–63.

Xie Y, Li B, Zhang Q, Zhang C, Lu K, Tao G. 2011. Effects of nano-TiO_2 on photosynthetic characteristics of *Indocalamus barbatus. Journal of Northeast Agricultural University* **39**: 22-25.

Zareii FD, Roozbahani A & Hosnamidi A. 2014. Evaluation the effect of water stress and foliar application of Fe nanoparticles on yield, yield components and oil percentage of safflower (*Carthamus tinctorious* L.). *International Journal of Advanced Biological and Biomedical Research* **2**(4): 1150-1159.

Botanica SERBICA

REZIME

Efekti egzogene primene nano čestica i kompatibilnih organskih rastvora na suncokret (*Helianthus annuus* L.)

Mohsen Janmohammadi, Saeed Yousefzadeh, Shahryar Dashti, Naser Sabaghnia

Mediteranski polusušni delovi u severozapadnom Iranu dobijaju količinu padavina koja je manja od potencijalne evapotranspiracije i karakterišu se visokim temperaturama i izuzetnim stresom izazvanim sušom. Egzogena primena nekih nano čestica i regulatora rasta izgleda da ublažava negativne efekte abiotičkog stresa i poboljšava performanse biljaka. U ovom radu smo pokušali da ocenimo efekte folijarne primene rastvora salicilne kiseline (SA), glicin betaina (GB), askorbinske kiseline (AA), nano silika ($nSiO_2$) i nano titanijum-dioksida ($nTiO_2$) na rast i prinos komponenti suncokreta u polusušnom brdskom region Marageha u Iranu. Biljke su gajene na otvorenom, nezaštićene od kiše sa dodatnim zalivanjem primenjenim tri puta tokom reproduktivne faze rasta. Rezultati otkrivaju da folijarna primena SA značajno poboljšava prečnik glavice, sadržaj hlorofila u listovima i vreme cvetanja, dok fiziološku zrelost odlaže na dugi vremenski period. Evaluacija komponente prinosa plodova suncokreta pokazuju da primena GB značajno povećala njihovu težinu u poređenju sa drugim tretmanima. Procenat praznih plodova je značajno uslovljen tretmanima, najniži procenat praznih plodova je zabeležen kod biljaka tretiranih GB, slede oni prskani AA i $nTiO_2$. Procenat ulja u plodovima značajno raste pri folijarnoj primeni nano čestica, a najveći procenat je dobijen pri tretiranju sa $nTiO_2$. Rezultati takođe pokazuju da je najveći broj plodova po glavici, njihova najveća masa i veličina, zabeležena nakon prskanja rastvorom nano čestica. Iako svi tretmani povećavaju prinos plodova, efekat $nTiO_2$ je najznačajniji. Rezultati ovog eksperimenta podržavaju zaključak da folijarna primena nano čestica može da ublaži negativne ekološke faktore i da poboljša performanse biljaka u polu-sušnim regionima.

Ključne reči: kompatibilni rastvori, nano silicijum dioksid, sadržaj ulja, terminalni sušni stres, TiO2 nano čestice