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RESEARCH ARTICLE

Evaluate the effect of salicylic acid and ascorbic acid on agronomic and antioxidant activities of Sunflower in water deficit conditions

Shilan Davodi[1], Bahram Mirshekari[1], Touraj Mir Mahmoudi[2*], Farhad Farahvash[1], Saman Yazdan Seta[2]

[1] Department of Agronomy, Tabriz Branch, Islamic Azad University, Tabriz, Iran Tabriz, 5153715898, Iran

[2] Department of Agronomy, Mahabad Branch, Islamic Azad University, Mahabad, Iran, Mahabad 433-59135, Iran

*Email: toraj73@yahoo.com

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Abstract

To evaluate the effect of salicylic acid and ascorbic acid foliar application on a number of agronomic and antioxidant activities of sunflower, an experiment was conducted at research farm of Mahabad city, locted in the south of West Azerbaijan Province, Iran during 2016-2018 cropping seasons. The experiment was laid out as a split-plot design based on a completely randomized block design with three replications. Irrigation regimes (irrigation after 60 mm and irrigation after 120 mm evaporation from class A pan respectively) were established as main plots and the foliar applications of ascorbic acid (control, 50 and 100 ppm) and salicylic acid (control, 100 and 200 ppm) were the sub-plots. In the present study, the highest amount of proline content and activity of antioxidant enzymes was recorded under water deficit conditions and foliar application of 100 ppm salicylic acid. Applying salicylic acid increased grain yield under both normal irrigation and water deficit conditions. Also, the highest proline content and activity of antioxidant enzymes were under the treatment of water deficit and application of 50 ppm ascorbic acid. The results showed no significant difference between the combined foliar application of salicylic acid and ascorbic acid in terms of grain yield and oil percentage with their separate application. Therefore, simultaneous use of these two substances under normal irrigation and water deficit conditions was not economical and not recommended.

Keywords

Antioxidants, Catalase, Oil percent, Sunflower

Introduction

Sunflower (*Helianthus annuus* L.) is one of the major and most important non-conventional oilseed crops in the world due to its excellent oil quality. Sunflower grain contains 48-48% of high-quality edible oil, a high percentage of unsaturated fatty acids (60%), which includes oleic acid (16%) and linoleic acid (72%) which can control the cholesterol content of human blood, also Sunflower grain has about 20-27% protein (1).

Although sunflower is known as a drought-tolerant crop or grown under dryland conditions, substantial yield increases are obtained with frequent irrigation.

Water deficit damages the chloroplast thylakoid membrane, disrupts its activity, and sequentially reduces photosynthesis and grain yield in crops (2). Drought stress induces the accumulation of reactive oxygen species

(ROS) inside the cell, which causes oxidative stress to plants (3). ROS can cause extensive disturbance to cellular macromolecules such as lipids, enzymes, and DNA if ROS are not rapidly and effectively removed from plants (4).

Plants can protect themselves from damage caused by oxidative stress via enzymatic and non-enzymatic antioxidant mechanisms (5).

Plants protect themselves by increasing ROS inhibition capacity through antioxidant enzymes (6). Various chemicals such as osmotic protectors, growth regulators, and stress signaling molecules have been used successfully to withstand several biotic and non-biotic stresses (7). Ascorbic acid is one of the most important growth regulatory factors, which regulates many biological processes such as enzymatic responses, cofactors for enzymes involved in photosynthesis regulating processes, biosynthesis of hormones, reproduction of other antioxidants, cell division and cell elongation, Ascorbic acid acts as signal transduction (8) and is known as the most abundant antioxidant that protects plant cells. At present, due to its effect on cell division and differentiation, it is considered a regulator of plant growth and development.

Salicylic acid had a crucial role in regulating plant growth, maturity, flowering and response to abiotic stresses (9). Salicylic acid performs an essential function in responding to unfavorable environmental conditions, including water deficit stress and salinity of water and soil stress. Salicylic acid has excellent agronomic potential to increase tolerance to various stresses in important crops (10). Salicylic acid plays a different role in the plant depending on the concentration used. At low concentrations, it improves antioxidant properties and resistance to environmental stresses, while at high concentrations it causes cell death and cell sensitivity to environmental stresses (9). Has been reported reported ascorbic acid treatment at the concentration of 4 ml under salinity conditions increased bean growth and yield of *Vicia faba* (11). In another study, was observed that treatment with SA (10^{-5} or 10^{-4} ml/l) improved photosynthesis in the plant, increased pollen, leaf area and leaf weight, but had no effect on plant height (12). Has been stated that the bean plants were sprayed with SA at high concentrations of 1 ml.l^{-1} for 30 and 45 days and their shoot dry weight, number of nodules per plant, chlorophyll content improved significantly (13). The researchers found spraying pea with salicylic acid at the concentrations of 10 and 20 mg. l^{-1} increased plant height, leaf number, fresh and dry weight, grain yield and grain yield components (14).

In another study, was revealed that foliar application of SA increased the yield and yield components of beans, including the number of pods per plant, number of seeds per pod, seed weight per plant and weight of 100 seeds in faba bean (15) .Has been reported that 200 ppm acetylsalicylic acid antioxidant effectively increased pea pod length (16).

In a study on chickpea, it was reported that the simultaneous use of salicylic acid and ascorbic acid could increase plant biomass and reduce the adverse effects of dehydration stress on plant growth characteristics (17).

One of the most important centers of sunflower production in Iran is the northwest of the country. In these areas, the sunflower plant is faced with different periods of water deficit stress with different intensities. Finding a solution to moderate the adverse effect of water shortage stress can increase production and cultivation of this crop, Therefore, the present study was conducted with the aim of foliar application of salicylic acid and ascorbic acid on the enzymatic and agronomic properties of sunflower under normal and water deficit conditions.

Materials and Methods

The experiment was conducted in the 2016 - 2018 crop season at Experimental Field Area of Islamic Azad University, Mahabad Branch, West Azerbaijan Province, Iran, which was located between 45°43' N latitude and 36°46′ E longitude. The soil characteristics of the site and the meteorological characteristics of the area are listed in Tables 1 and 2. The experiment was laid out as a split-plot design based on a completely randomized block design with three replications. Irrigation regimes (irrigation after 60 mm evaporation from class A pan and irrigation after 120 mm evaporation from class A pan respectively) were established as main plots and foliar applications of ascorbic acid (control, 50 and 100 ppm) and salicylic acid (control, 100 and 200 ppm) were the sub-plots. The sunflower cultivar studied in this study was (cv. Euroflore).

Table 1. Physical and chemical characteristics of soil testing

Clay %	Silt %	Sand %	K (ppm)	P (ppm)	OC %	pH	EC (ds/m)	Sp %	Depth
22.4	44.4	33.2	280	4.8	0.64	8.2	1.14	38	0.30

Each experimental unit consisted of 4 planting lines with the length of 5 m. The distance between planting rows was considered to be 50 and the distance between the plants was considered to be 20 cm. The seeds were treated with Bavistin with the concentration of 0.25% and then, planted by hand in June at the depth of 4 cm.

Each experimental unit was fertilized with 100 kg of aminophosphate; all the experimental units were irrigated immediately after planting, but the subsequent irrigations were carried out according to the irrigation regimes treatments. Crop management operations such as manual weeding and thinning were performed as required. In the flowering stage, the youngest developed leaves were randomly selected from 10 plants per plot and were immediately frozen in liquid nitrogen and stored at -80 ° C for biochemical analysis. Spraying SA (0, 100 and 200 ppm) and ascorbic acid (0, 50, and 100 ppm) with certain concentrations was done in three times: the first time was done 45 days after planting and another's foliar spraying was done ten days between them. The plants were sprayed early in the morning using a 2 lit hand sprayer.

To measure the activity of catalase, peroxidase and superoxide dismutase enzymes, standard methods were used respectively (18-20). Determination of proline con-

Table 2. Meteorological parameters in the years of 2016-17 in the city of Mahabad

2016				2017				
Monthly evaporation	Monthly rainfall amount	Monthly average temperature		Monthly evaporation	Monthly rainfall amount	Monthly average temperature		Month
		Max	Max			Max	Min	
175.6	7.4	23.7	10.5	119.5	4.9	2.38	6.7	September
51.7	79.2	9.6	2.1	38.9	47.5	10.1	0.7	October
0	4.6	4.5	-3.5	0	1.4	5.1	-5.6	November
0	39.9	7.6	-1.5	0	13.5	7.9	-2.9	January
0	23.4	5.5	-2.7	0	8.4	6.2	-4.2	February
0	59.2	6.8	-2.2	0	20	8	-3.7	March
62.6	31.5	16.6	5.1	66.9	41.2	17.3	4.6	April
185.4	34.9	24.5	9.4	155.9	48.7	24.2	8.9	May
282	7.5	29.8	14.1	282	2.7	29.6	12.7	June
304.5	4.7	32.7	17.7	276.9	4.6	32.1	15.7	July
365.1	2.2	36.1	9.4	316.5	0	35.2	16.9	August
272.8	1.4	31.1	14.8	194.3	11.1	30.7	12.6	January
168	2.9	25.8	10.1	91.4	4.9	25.6	7.1	February

centration in the leaf tissue was used based on Bates method (21).

To measure grain yield when the sunflower heads turned yellow and brown, two middle rows were harvested and two side rows were removed as margins. After separating the seeds from heads, they were measured according to their number and weight and, then, recorded as tons per hectare.

Simple analysis of variance and combined analysis of variance were performed after reviewing and confirming the assumptions based on two-year data. Finally, all the data were analyzed by SAS. 9.2 statistical software and the means were compared by Duncan's Multiple Range Test at the 5% probability level.

Results and Discussion

Proline content

The results showed that, the differences between irrigation ($p < 0.01$), salicylic acid ($p < 0.05$), irrigation × salicylic acid ($P< 0.01$), ascorbic acid ($P< 0.01$), irrigation × ascorbic acid ($p < 0.05$), and salicylic acid × ascorbic acid ($p < 0.05$) were significant on the proline content (Table 3).

Mean comparisons of irrigation × salicylic acid treatments (Table 4) showed that in water deficit conditions, all the three levels of SA had higher proline content than normal conditions. In the present study, the highest amount of proline content was observed in water deficit condition with levels of 100 and 200 ppm SA by 6.09 and 6.30 (µg/g fr. Wt) and the lowest amount was observed in normal conditions with control treatment by 5.06 (µg/g fr. Wt). In the present study, SA treatments treatment induced more accumulation of the free proline in both water-deficit and normal conditions.

The results obtained from the mean comparison (Table 5) showed that the highest proline content was allocated to control the treatment of ascorbic acid under water deficit condition with the average of 6.61 (µg/g fr. Wt). However, there was no significant difference between the mentioned treatment and the application 50 and 100 ppm ascorbic acid under water deficit condition. It can be stated that under water deficit conditioms, the use of ascorbic acid induces proline synthesis, Increasing the proline content in plant tissue improves plant resistance to drought stress. The minimum value was recorded from all 3 levels of ascorbic acid under normal irrigation.

The proline content was highest at the levels of 100 and 200 ppm of SA with the control level of ascorbic acid with the average of 6.52 (µg/g fr. Wt). The minimum value was recorded from The control of both treatments and application of 200 SA with 100 ppm of ascorbic acid.

The results obtained from the mean comparison (Table 6) showed that the highest proline content was allocated to control the treatment of ascorbic acid under water deficit condition with the average of 6.61(µg/g fr. Wt). However, there was no significant difference between the mentioned treatment and applying 50 and 100 ppm ascorbic acid under water deficit condition. The minimum value was recorded from all the 3 levels of ascorbic acid of fertilizer under normal irrigation. In our study, foliar application of SA resulted in the accumulation of proline which in turn preserved the photosynthetic machinery from water deficit by sustaining the construction of Rubisco, increasing water and osmotic potential and decreasing oxidative stress (22). In a study on maize (23) and Mungbean cultivars (24) reported that water deficit increased leaf proline content and foliar application of SA accelerated this increase. In another study revealed that SA during water deficit enhanced proline production in stressed tomato and amaranth plants (25) .

Table 3. Analysis of variance of understudy characters for sunflower in normal and water deficit conditions

SOV	MS								
	DF	Proline	Cata-lase	Superox-ide dis-mutase	Malone dialde-hyde	Number of Grain	TKW	Grain yield	Oil pre-centage
Year (Y)	1	0.12^{ns}	0.03^{ns}	0.01^{ns}	149.34^{ns}	2450^{ns}	11.68^{ns}	0.25^{ns}	39.12^{ns}
Irrigation)I(	1	3.24**	2.97**	4.51**	246.0*	**1114267	**3471.51	15.48**	1319.57**
Y×I	1	0.05^{ns}	5.38**	0.006^{ns}	34.11^{ns}	**56679	0.79^{ns}	0.36^{ns}	48.67^{ns}
E1	8	0.04	0.17	0.121	19.25	12415	5.75	0.28	154.32
Salicylic Acid (SA)	2	0.26*	1.68**	1.87**	110.13^{ns}	**35896	**409.22	1.17**	534.40**
Y×SA	2	0.54^{ns}	0.51^{ns}	0.11ns	55.39^{ns}	9027^{ns}	*42.75	0.18^{ns}	52.34^{ns}
I×SA	2	0.37**	1.03*	0.84**	24.02^{ns}	**56986	10.26^{ns}	0.92**	335.81**
Ascorbic Acid (AsA)	2	0.41**	1.23**	0.96**	804.84**	**34180	**381.96	1.82**	357.86**
Y×AsA	2	0.13^{ns}	0.05^{ns}	0.02^{ns}	45.64^{ns}	2291^{ns}	1.19^{ns}	0.11^{ns}	46.60^{ns}
I× AsA	2	0.35*	1.58**	0.20^{ns}	409.01**	*19671	**51.91	0.17^{ns}	4.12^{ns}
SA ×AsA	4	0.25*	1.15**	0.73**	24.12^{ns}	8818^{ns}	**173.64	0.88*	233.52*
Y×I×SA	2	0.11^{ns}	0.20^{ns}	0.02^{ns}	27.39^{ns}	8235^{ns}	11.58^{ns}	0.08^{ns}	124.53^{ns}
Y×I× AsA	2	0.02^{ns}	0.46^{ns}	0.02^{ns}	35.00^{ns}	2997^{ns}	6.02^{ns}	0.15^{ns}	113.20^{ns}
Y×SA× AsA	4	0.06^{ns}	0.51^{ns}	0.11^{ns}	2.59^{ns}	1611^{ns}	5.45^{ns}	0.40^{ns}	6.71^{ns}
I×SA× AsA	4	0.05^{ns}	0.05^{ns}	0.07^{ns}	62.14^{ns}	1531^{ns}	11.22^{ns}	0.23^{ns}	10.06^{ns}
Y×I×SA× AsA	4	0.04^{ns}	0.16^{ns}	0.01^{ns}	59.2^{ns}	1405^{ns}	3.07^{ns}	0.07^{ns}	124.39^{ns}
E2	48	0.08	0.26	0.12	46.05	6415^{ns}	11.33^{ns}	0.17	80.88
CV%		10.39	19.76	14.61	13.42	11.20	14.65	16.88	19.69

ns, * and **: Non-significant and significant at 5% and 1% levels of probability, respectively

Table 4. Effect of Irrigation levels and salicylic acid treatments on understudy characters for sunflower at two years

Irrigation	Salicylic acid	Proline (mg/ g fr. Wt)	Catalase (Mmol g^{-1} fw da^{-1})	Superoxide dis-mutase (Mmol g^{-1} fw da^{-1})	Malone di alde-hyde (Mmol g^{-1} fw da^{-1})	Number Of Grain	TKW (g)	Grain yield (t/ha)	Oil per-centage
Normal	Control	5.06a	1.40b	0.91c	46.88a	973.0c	74.57a	2.55bc	36.72c
	100ppm	5.85ab	1.83ab	1.39b	49.33a	1126.5b	78.38a	3.16a	43.50bc
	200ppm	5.59b	1.46b	1.29b	50.94a	1197.1a	81.17a	2.85ab	46.31ab
water deficit	Control	6.01a	1.70ab	1.41b	51.72a	882.2d	62.68a	1.97d	45.66ab
	100ppm	6.09a	2.10a	1.83a	5.88a	943.9c	68.27a	2.02d	52.65a
	200ppm	6.01a	1.88ab	1.58ab	52.61a	956.0c	69.16a	2.30c	49.20ab

Table 5. Effect of Irigation ×ascrobic acid intraction treatments on understudy characters for sunflower at two years

Irrigation	Ascorbic Acid	Proline (mg/ g fr. Wt)	Catalase (Mmol g^{-1} fw da^{-1})	Superox-ide dis-mutase (Mmol g^{-1} fw da^{-1})	Malone di aldehyde (Mmol g^{-1} fw da^{-1})	Number Of Grain	TKW (g)	Grain yield (t/ha)	Oil per-centage
Normal	Control	5.81bc	1.29b	1.14a	52.50ab	1074.0b	76.10b	2.53a	39.53a
	50ppm	5.06c	1.65ab	1.34a	47.38bc	1100.8ab	81.56a	3.07a	43.34a
	100ppm	5.03c	1.74ab	1.21a	44.33c	1121.9a	76.47b	2.95a	43.67a
water defi-cit	Control	6.61a	1.76ab	1.40a	55.44a	875.8d	62.47e	1.88a	44.67a
	50ppm	6.16ab	2.14a	1.75a	57.72a	909.5d	70.00c	2.15a	53.13a
	100ppm	5.91abc	1.78ab	1.67a	46.00b	996.3c	67.64d	2.22a	49.69a

Means in each column with the same letter are not significantly different at P<0.05

Table 6. Effect of salicylic acid× ascrobic acid intraction treatments on understudy characters for sunflower at two years

Salicylic Acid	Ascorbic Acid	Proline (mg/ g fr. Wt)	Catalase (Mmol g^{-1} fw da^{-1})	Superoxide dismutase (Mmol g^{-1} fw da^{-1})	Malone di aldehyde (Mmol g^{-1} fw da^{-1})	Number Of Grain	TKW (g)	Grain yield (t/ha)	Oil percentage
	Control	5.32b	1.39d	0.94c	44.58a	904.8f	61.30d	1.99b	37.39b
Control	50ppm	5.77ab	1.41cd	1.21bc	50.75a	950.5ef	72.52c	2.36ab	44.13ab
	100ppm	5.78ab	1.85a-d	1.33bc	52.58a	1038.6abc	72.06c	2.43ab	42.06ab
	Control	6.52a	1.72a-d	1.42ab	46.58a	986.5cde	73.24bc	2.36ab	44.99ab
100ppm	50ppm	6.06ab	2.11ab	1.87a	51.41a	979.5de	75.56b	2.82a	53.83a
	100ppm	5.96ab	2.07ab	1.55ab	53.83	1047.1ab	71.16c	2.59a	45.40ab
	Control	5.88ab	1.47bcd	1.31bc	44.33a	1033.4bcd	73.31bc	2.27ab	43.93ab
200ppm	50ppm	5.73b	2.16a	1.55ab	54.50a	1085.8ab	79.70a	2.66a	47.24ab
	100ppm	5.81ab	2.09ab	1.45ab	55.12a	1091.6a	72.49c	2.72a	52.09a

Means in each column with the same letter are not significantly different at $P<0.05$

In one study, the treatment of wheat with salicylic acid and ascorbic acid was able to moderate the effect of salinity stress on wheat seedlings by increasing the proline content (26).

Superoxide dismutase

Results of combined analysis of variance in two years showed that the effects of irrigation ($P< 0.01$), SA ($P< 0.01$), irrigation × salicylic acid ($P< 0.01$), ascorbic acid ($P< 0.01$) and salicylic acid × ascorbic acid ($p < 0.01$) were significant ($P< 0.01$) on the superoxide dismutase content (Table 3).

In this study, the amount of superoxide dismutase was highest in 100 ppm foliar application of salicylic under water deficit condition by 1.83 µg/g fr. Wt. However, there was no significant difference between this treatment and foliar spraying with 200 ppm SA, while the lowest one was recorded from the control treatment by 0.91 in this condition (Table 4).

Results also revealed the highest superoxide dismutase (1.87 µg/g fr. Wt) was obtained while using 100 ppm salicylic acid with 50 ppm ascorbic acid (Table 6). However, the lowest amount of enzyme activity was recorded in the control of both treatments (0.94 µg/g fr. Wt).

In a study on canola, it was reported that the activity of all antioxidant enzymes (except sod in the root) increased under salinity stress; the foliar application of ascorbic acid could reduce the activity of these enzymes in leaves, but had an effect on enzyme activity in roots (27).

Increase in the activity of SOD during initial periods of drought stress might protect plants from oxidative injury (28).

It is conceivable that foliar application of SA and ascorbic acid-induced activation of SOD was due to its effect on ABA, because it is well known that ABA is included in the induction process of the expression of genes encoding proteins involved in plant stress responses, including the genes of SOD.

The enzyme SOD dismutates O_2- to H2O2 and is present in the cytosol and different cell organelles (29). In a study, SA increased the amount of SOD by 70 % compared to the control treatment in water deficit condition (30). It was found that drought stress and foliar application of triazol increased the amount of superoxide dismutase activity in sunflower leaves (31).

Catalase

The effects of irrigation ($p < 0.01$), year × irrigation ($p < 0.01$), SA ($p < 0.01$), irrigation × salicylic acid ($p < 0.05$), ascorbic acid ($P< 0.01$), irrigation × ascorbic acid ($p < 0.01$) and salicylic acid × ascorbic acid ($p < 0.01$) on the catalase content were significant (Table 3). The result showed the highest catalase content (2.10 µg/g fr. Wt) was gained from water deficit condition with 100 ppm SA treatment and the least amount was observed in normal conditions with the control of SA by 1.40 µg/g fr. Wt (Table 4). Whereas, AA application may act to save plants from oxidative damage caused by water stress (32).

The highest catalase content (2.14 µg/g fr. Wt) was obtained while using 50 ppm ascorbic acid under water deficit condition and the lowest (1.29 µg/g fr. Wt) was observed in the control of ascorbic acid with normal irrigation (Table 5).

Among the treatments of SA with ascorbic acid interactions, the level of 200 ppm SA along with the level of 50 ppm ascorbic acid had the highest amounts of catalase activity (2.16 µg/g fr. Wt); the lowest amount of catalase content was recorded in the control of both treatments (1.39 µg/g fr. Wt) (Table 6).

Malondialdehyde

Based on the results of the analysis of variance (Table 2), the malondialdehyde content was significantly affected by irrigation ($p < 0.05$), ascorbic acid ($p < 0.01$) and interaction of irrigation× ascorbic acid ($p < 0.01$) (Table 3).

In our research, water deficit increased membrane lipid peroxidation through the increase of MDA level, but using ascorbic acid reduced lipid peroxidation because MDA content significantly decreased. The highest amount of malondialdehydes was gained from the control and

100ppm of ascorbic acid under water deficit conditions (by 55.44 and 57.72), while the lowest one was recorded from 50 ppm of ascorbic acid under normal conditions (Table 5).

High endogenous AA and high activities of antioxidant enzymes are limiting factors to alleviate the dangerous effects of oxidative stress (33).

The high accumulation of hydrogen peroxide in the cell causes oxidative stress and the inactivation of key enzymes in photosynthesis (34). It was found that water deficit stress caused a significant increase in H_2O_2 content; an increase in malondialdehyde was a clear indicator of the occurrence of oxidative stress (30). Reduction of membrane lipid peroxidation in SA treatment in Ctenanthe setosa and maize has been reported in the previous studies (35, 36).

Number of Grains per Head

The effects of irrigation ($p < 0.01$), year × irrigation ($p < 0.01$), salicylic acid ($P < 0.01$), irrigation × salicylic acid ($P < 0.01$), ascorbic acid ($P < 0.01$) and irrigation × ascorbic acid ($p < 0.05$) were significant on the biological yield (Table 3).

The highest grain number (1197.1) was obtained in the use of 100 ppm salicylic acid under normal conditions and the lowest number (882.2) was observed in the control treatment under water deficit conditions. Results of the present study showed that applying SA in both environmental conditions increased the number of grains per head (Table 4).

Applying 100 and 200 ppm SA increased the number of grains per head in normal irrigation conditions by 15.15 and 23.03 % respectively and in water deficit conditions of 6.92 and 8.29 % respectively compared to the control treatment in similar conditions (Table 5).

Using SA under normal conditions had a greater effect on increasing the number of grains per head compared to water deficit conditions.

In the present study, applying ascorbic acid also showed a significantly positive effect on increasing the number of grains per head in both conditions, so that under normal conditions, applying 100 ppm ascorbic acid with the average of 1112.9 grains showed the highest number of grains and increased this trait in comparison to the control treatment by 4.45 % (Table 6).

In this study, water deficit stress reduced the number of grains per head, decreased number of grains can be due to reduced photosynthesis, accelerated senescence of leaf and limited photosynthetic resources that reduce the number and weight of grains, in addition, the decrease in the number of grains can be due to the decrease in the number of fertile spikelets due to water deficit stress (37). It seems that the application of ascorbic acid through increasing vegetative growth, accumulation of grain carbohydrates and grain set has a positive effect on increasing the number of grains (32).

It was reported that using salicylic acid and ascorbic acid under salinity stress moderated the harmful effect of this stress on the growth of roots and stems of wheat (26). In a study on wheat, it was observed that the use of ascorbic acid increased the number of grains in wheat under water shortage conditions (38).

The results (Table 4) indicated that the maximum number of grains per head was recorded from applying 200 ppm salicylic acid + 100 ppm ascorbic acid by 1091, whereas the minimum was gained from the control of both treatment and control of salicylic acid + 50ppm ascorbic acid by 904.8 and 950.5 grain respectively. Increasing the number of seeds in foliar application of salicylic acid and ascorbic acid can be attributed to the role of these 2 treatments in reducing competition between vegetative and reproductive organs for photosynthesis products and, thus, increase grain fertility. Therefore, the foliar application of SA and ascorbic acid with the development of root growth and vegetative part of sunflower increased the grain yield components under water deficit stress.

Thousand Kernel Weight

Results revealed that the effects of irrigation ($P < 0.01$), salicylic acid ($P < 0.01$), ascorbic acid ($P < 0.01$), irrigation × ascorbic acid ($P < 0.01$) and salicylic acid × ascorbic acid ($p < 0.05$) were significant ($P < 0.01$) thousand kernel weight (Table 3).

In the present study, application of ascorbic acid showed a significantly positive effect on increasing the thousand kernel weight in both conditions, so that under both conditions, the application of 50 ppm ascorbic acid with the average of 81.56 and 70.00 g increased this trait in comparison with the control treatment (Table 5).

The results (Table 6) showed that the maximum thousand kernel weight was recorded from the application of 200 ppm salicylic acid + 50 ppm ascorbic acid by 79.70 g, whereas the minimum was gained from the control of both treatments by 61.30 g. It was found that the simultaneous use of salicylic acid and ascorbic acid significantly increased thousand kernel weight components compared to the control treatment in bean cultivars (39).

Water deficit induced poor grain growth and resulted in small grains with light weigh. In a study on maize, the use of ascorbic acid + salicylic acid under water stress shortage conditions increased the thousand kernel weight (40).

Grain yield

Results revealed that the effects of irrigation ($P < 0.01$), salicylic acid ($P < 0.01$), irrigation × salicylic acid ($P < 0.01$), ascorbic acid ($P < 0.01$) and salicylic acid × ascorbic acid ($p < 0.05$) were significant ($P < 0.01$) on the grain yield (Table 3).

Results showed that water deficit condition significantly reduced grain yield, so that all levels of salicylic acid in normal conditions had higher grain yield than water deficit condition (Table 4). It was reported that water deficit significantly reduces grain yield in sunflower cultivars (41, 42).

Also, the application of salicylic acid had a positive effect on grain yield. So, in normal conditions, levels of 100 and 200 ppm of salicylic acid increased grain yield by

10.95% and 19.30% compared to the control treatment in the same conditions; however, in water deficit conditions, the only 200 ppm of salicylic acid had a significant effect on grain yield and increased the amount of this trait by 14.34% in comparison to the control treatment (Table 4).

Among salicylic acid and ascorbic acid treatment compounds, the highest grain yield was assigned to 100 and 200 ppm salicylic acid treatment in combination with 50 and 100 ppm ascorbic acid levels. The 4 mentioned compounds increased the grain yield compared to the control of both treatments by 31.91%, 23.16%, 25.18% and 26.83% respectively (Table 6).

The positive effect of salicylic and ascorbic acid foliar application on sunflower grain yield can be attributed to the increase in yield components, ie number of grain and 1000- kernel weight under the effect of these treatments.

In this study, the use of salicylic acid and ascorbic acid improved the activity of antioxidant enzymes and reduced the oxidation of cell membrane lipids, thereby protecting cellular structures. This protective effect was able to improve photosynthesis and modulate the adverse effect of water deficit stress on grain yield and grain yield components.

These outcomes are in agreement withcthe findings by other researchers (43, 44). It was found that with exacerbation of water stress, grain yield decreased but, the use of salicylic acid reduced the adverse effects of water deficit on grain yield, in Black Cumin (*Nigella sativa* L.) (45). In another study, the application of ascorbic acid improved grain yield and grain components under water deficit conditions (38). It has been noted that integrated application of SA and AsA in equivalent dose enhanced yield traits of cotton under water deficit conditions (46).

Oil precentage

In the present study the differences between irrigation ($p < 0.01$), salicylic acid ($p < 0.01$), irrigation × salicylic acid ($P< 0.01$), ascorbic acid ($P< 0.01$) and salicylic acid × ascorbic acid ($p < 0.05$) were significant on the seed oil content (Table 3).

Results showed that water deficit increased seed oil content, but the use of salicylic acid could accelerate this increase (Table 4). Increased essential oil content under water deficit conditions has been assigned to producing high concentrations of terpenes, isoprenoids and phenylpropanoids by plants (47).

In this study, the amount of oil content was highest in 100 ppm salicylic acid in water deficit condition by 52.65%, while the lowest one was recorded from control treatment by 36.72% in normal conditions (Table 4). Significant improvement in oil yield was observed with SA foliar application under water deficit condition in lemongrass varieties (48).

The results (Table 6) indicated that the highest seed oil content was gained from applying 100 ppm salicylic acid + 50 ppm ascorbic acid and 200 ppm salicylic acid + 100 ppm ascorbic acid by 53.83 and 52.09 % respectively; the lowest value was observed in the control of both treatments by 37.39 %. There was no significant difference between the other treatments in terms of grain oil content.

An increase in oil content in salicylic acid treatment can be due to the role of this substance in increasing nutrient uptake, cycle growth, changes in the number of oil glands and biosynthesis of monoterpenes (48). Also, the positive effect of ascorbic acid on increasing oil content can be related to the increased capacity of meristematic cells to synthesize the substrate necessary for oil biosynthesis (49) .It has been stated that using SA + ASC on coriander led to changes in essential oils and volatile composition (50).

Conclusion

In the present study, the highest amount of proline and activity of antioxidant enzymes were recorded under water deficit conditions and foliar application of 100 ppm salicylic acid .Application of salicylic acid also increased grain yield under both normal irrigation and water deficit conditions. Applying ascorbic acid levels in both normal irrigation and water deficit conditions had no significant effect on changes in grain yield and oil content. Therefore, the use of salicylic acid was more effective than ascorbic acid in modulating the effect of water deficit stress. The results also demonstrated no significant difference between the combined foliar application of salicylic acid and ascorbic acid in terms of grain yield and oil % with their separate applications. Therefore, in sunflower, their simultaneous foliar application in sunflower cultivation is not recommended. Therefore, the use of salicylic acid can be an appropriate solution to moderate the effect of drought stress in the study area where the plant experiences different periods of this stress with different intensities.

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Authors contributions

All authors collaborated in the writing and editing of the manuscript.

Compliance with ethical standards

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