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Research Article

Role of Biostimulant Priming Applications on Germination, Growth and Chlorophyll Content of Sunflower (*Helianthus annuus* L.) Cultivars under Salinity Stress

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ABSTRACT

Salinity, which is one of the abiotic stresses, has become an important obstacle in agricultural areas. The use of humic acids (HA) as a biostimulant is increasing day by day and it is tried to increase the resistance of plants against stress. In this study, the effects of HA application of 0-15ml L^{-1} (4 concentrations) on the resistance to salt (S) 0-150 mM L^{-1} (4 concentrations) stress in 3 sunflower cultivars (Maximus (C1), Sirena (C2), Reyna (C3) were investigated under laboratory conditions. In the study; germination percentage (GP), mean germination time (MGT), salt tolerance percentage (STP), seeding length (SL), root length (RL), relative water content (RWC), real water content (GSI), total chlorophyll (Chl), chlorophyll stability index (CSI) parameters were examined. As a result of the study, HA applications played a role in reducing the negative effects of salt stress on the examined parameters. It was concluded that HA can be evaluated as an effective material that can be used to increase resistance and tolerance of plants against salt stress.

1. Introduction

Plants are exposed to biotic and abiotic stresses throughout their lives. The most important of these are cold, drought, salinity, flooding and heavy metals (Gontia-Mishra et al. 2014). Today, salinity has become a major problem in agricultural areas all over the world (Gürsoy 2020; 2022). Salt stress is considered one of the most widespread abiotic stresses and very important hampers crop production, especially in arid and semi-arid areas (Hernández 2019). Salinity stress limitations plant growth and development, can induce drastic yield reduction (Alharby et al. 2021; Shahzad et al. 2021). Growth and development in plants begins with germination and plants need to adapt to environmental conditions in order to survive. However, germination is the most sensitive period in the life of plants and is especially important for seedling development.

The priming of seeds allows the acceleration of germination as well as a better growth a greater tolerance to abiotic stress and higher yields (Boucelha et al. 2019). Today, the use of biostimulants has become widespread in order to reduce the effects of stress factors, to ensure sustainability in agriculture and to ensure plant growth (Frioni et al. 2021). With the use of biostimulants, the resistance of plants to abiotic stress was increased and the quality of agricultural production (Bell et al. 2022). Du Jardin (2015), identified seven categories of biostimulants: (i)humic and fulvic acids, (ii)protein hydrolysates and other N-containing compounds, (iii)seaweed extracts and botanicals, (iv)chitosan and other biopolymers, (v))inorganic compounds, (vi)beneficial fungi and (vii)beneficial bacteria. Humic acid is believed to have an important role in plant growth regulator as a biostimulant (Saidimoradi et al. 2019).

Sunflower (*Helianthus annuus* L.) has a very important place in the production of oil crops in the world and in our country (Beyaz et al. 2018).

The aim of this study is to determine the effects of humic acid and salt applications on germination, seedling growth, chlorophyll content, chlorophyll stability index and salt tolerance index of sunflower varieties.

2.Material and Method

The research was carried out at the Aksaray University Guzelyurt Vocational School and some analyzes were done at Aksaray University Scientific and Technological Research Laboratory (ASÜBTAM). In this study sunflower cultivars (Maximus (C1), Sirena (C2), Reyna (C3) were used. Seeds of sunflower for sterilization, they were kept in 5% sodium hypochlorite solution for 10 minutes and then rinsed several times in distilled water then they were dried at room temperature to their initial weight. Seeds were primed for 24 hours and each

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HA [0 (control) (HA1), 5ml L^{-1} (HA2), 10 ml L^{-1} (HA3), 15 ml L^{-1} (HA4)] doses. For each HA dose, 50 seeds were placed in sterile petri dishes on Whatman No:1 blotting papers and 10 ml of different doses of salt [0 (control) (S1), 50 mM L^{-1} (S2), 100mM L^{-1} (S3), 150mM L^{-1} (S4)] concentrations were added. Only water was added to the control petri dish. Filter papers were changed every 2 days and 10 ml of salt containing solutions were added. In order to prevent evaporation the petri dishes are wrapped with parafilm. The research randomized plots experimental design were made with 3 replication according to the trial pattern. Seeds were counted daily and those with a root length of 2mm were considered germinated (ISTA 2003). In the study; germination percentage (GP), mean germination time (MGT), salt tolerance percentage (STP), seedling length (SL), root length (RL), relative water content (RWC), real water content (GSI), total chlorophyll (Chl), chlorophyll stability index (CSI) parameters were examined.

Measurements

Germination percentage (%)

Germination percentage was calculated using the formula below.

Mean germination time (day)

$MGT = \Sigma(Dn)/\Sigma n$,

where, n is the seed number germinated on day D, and D is the number of days from the beginning of the germination test (Orchard 1977).

Percentage of salt tolerance (%)

Salt tolerance (%) = (DWSP / DWCP) x 100

DWSP: Dry weight of plant in salt application

DWCP: Dry weight of plant in control application (Uzun Kayıs and Ceyhan 2015)

Determination of relative and real water contents

In order to determine the relative and real water content in the leaf tissues, leaf samples taken from plants belonging to the sunflower cultivars in the control and stress groups were weighed and their fresh weight was determined, then they were placed in glass tubes containing 5 ml of distilled water and kept in the light for 24 hours. At the end of this period, the hydrated leaf samples were weighed again and their weight in turgor condition was determined. Later, these leaf samples will be dried in the oven at 80°C for 48 hours and their dry weight will be determined again. Finally, the relative and actual water contents will be found according to the formulas below (Ritchie et al. 1990; Arslan 2018).

RWC(%) = (FW − DW)/(TW − DW) x 100 (Relative water content)

GSI(%) = (FW − DW)/FW x 100 (Real water content)

FW: fresh weight, TW: turgor weight, DW: dry weight

Chlorophyll (mg g^{-1})

Samples taken from seedlings (0.25 g) of sunflower cultivars grown in the laboratory with HA and S application were homogenized with 80% acetone, then filtered and made up to 25 ml with acetone. Then the samples were read in the spectrophotometer at 663 and 645 nm and chlorophll was calculated with the following formula (Lichtenthaler and Welburn 1983).

Chlorophyll a (mg g^{-1}) = (12.7*663 nm)-(2.69*645 nm)*V/W*10000

Chlorophyll b (mg g^{-1}) = (22.91*645 nm)-(4.68*663 nm)*V/W*10000

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Determination of chlorophyll stability index

Determination of the chlorophyll stability index is important in terms of showing the tolerance capacity of the plant against stress. (Mohan et al. 2000)

Leaf sample from the treated plant = 1.000 mg leaf sample was kept in a test tube with water at 55 °C for 1 hour.

Leaf sample from the plant in the control plot = 1.000 mg leaf sample was kept in a test tube containing water at room temperature for 1 hour.

The chlorophyll stability index will be calculated with the help of the following equation by reading both samples in a spectrophotometer at 652 nm.

$$CSI = \frac{\text{Absorbance value of the treated sample}}{\text{Absorbance value of the control}} \times 100$$

Statistical analysis

The experimental data obtained at the end of the research, was subjected to analysis of variance using MSTAT-C computer software. Duncan test was applied to determine the significance levels of the differences between means of applications.

3.Results and Discussion

The variance analysis results of this study, which was conducted to determine the effects of biostimulant applications on the germination parameters, seedling growth, salt tolerance percentage, chlorophyll, chlorophyll stability index, relative and actual water content of sunflower varieties under salt stress, are given in Table 1. When Table 1 is examined, it is seen that the interaction of Cultivars × HA Doses × S Doses is significant at the 1% level in the others, except for the MGT feature. In the MGT parameter, the triple interaction was significant at the 5% level. The applied biostimulant doses had a significant effect on the germination parameters and seedling growth of the cultivars under salt stress. However cultivars, HA doses and Salt doses are important at the 1% level, except for the GP feature. On the other hand, it was determined that the bilateral interactions were statistically significant at the level of 1% in the GP feature, except for the interaction (HA Doses × S Doses). In the triple (Cultivars × HA Doses × S Doses) interaction, significant results were determined at the level of 5% for MGT and 1% for all other properties.

Table 1
Analysis of variance on the investigated parameters in sunflower cultivars humic acid and salt treatments

V.S.	D.F.	GP	MGT	SL	RL	RWC	GSI	Chl	CSI	STP
						F Value				
Cultivars	2	20.69**	27.93**	13.51**	331.04**	64.68**	40.75**	35.31**	225.03**	68.54**
HA Doses	3	2.01	20.65**	394.78**	79.50**	84.92**	26.08**	17.13**	295.92**	19.86**
Cultivars × HA Doses	6	9.57**	4.61**	51.82**	176.95**	61.88**	27.27**	7.73**	121.81**	17.11**
S Doses	3	272.79**	234.93**	550.57**	69.84**	470.47**	122.17**	156.18**	47.23**	137.62**
Cultivars × S Doses	6	12.42**	13.27**	8.06**	10.88**	5.42**	3.42**	6.84**	13.51**	5.27**
HA Doses × S Doses	9	1.71	2.78**	13.01**	12.04**	9.89**	4.89**	2.86**	49.78**	3.06**
Cultivars × HA Doses × S doses	18	3.60**	1.99*	6.62**	13.70**	9.04**	2.40**	3.49**	8.43**	2.97**
Error	96	1.56	0.028	0.111	0.025	0.561	1.76	0.051	0.471	2.03
CV%		1.30	9.22	5.80	8.45	1.07	1.88	8.69	1.02	1.86

**:significance level at $p<0.01$, *:significance level at $p<0.05$. VS: Variation source, DF: Degrees of freedom, GP: Germination Percentage, MGT: Mean Germination Time, SL: Shoot Length, RL: Root Length, RWC: Relative Water Content, GSI: Real Water Content, Chl: Total Chlorophyll, CSI: Chlorophyll Stability Index, STP: Salt Tolerance Percentage

Table 2
Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on GP (%)

C× HA S Doses	GP (%)												Mean
	Maximus				Sirena				Reyna				
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
S1	99.67 a	100.0 a	100.0 a	99.67 a	99.67 a	100.0 a	97.33 a-e	100.0 a	99.33 ab	99.67 a	100.0 a	100.0 a	99.61 A
S2	96.33 b-f	98.00 a-d	98.67 abc	97.00 a-f	97.67 a-d	97.67 a-d	98.00 a-d	99.00 ab	98.00 a-d	98.00 a-d	97.33 a-e	98.00 a-d	97.81 B
S3	95.00 d-g	96.33 b-f	97.00 a-f	96.33 b-f	95.00 d-g	97.67 a-d	97.67 a-d	97.33 a-e	97.33 a-e	94.33 e-h	95.00 d-g	94.00 fgh	96.08 C
S4	89.00 ıj	92.67 gh	91.67 hı	89.00 ıj	91.67 hı	95.67 c-g	95.00 d-g	95.67 c-g	93.00 gh	87.00 j	91.67 hı	87.00 j	91.58 D
Mean	95.00 C	96.75 AB	96.83 AB	95.50 BC	96.00 BC	97.75 A	97.00 AB	98.00 A	96.92 AB	94.75 C	96.00 BC	94.75* C	
LSD%1	2.680												

* Dissimilar letters in the column show different groups

When the Duncan test results of the examined traits are examined, it is seen that the lowest germination in terms of GP trait (Table 2) was obtained with 87.0% from S4 salt dose C3 variety and HA2 and HA4 humic acid doses. However, the highest germination was obtained in all 3 cultivars in S1 application and in all other applications (HA2, HA3, HA4) except the control dose (H1) of HA. It was observed that HA application increased the germination rate at all S doses. Gürsoy et al. (2016) applied 4 doses of HA in 3 different growth periods in their study with the winter rapeseed variety Bristol. As a result of the study, they reported that HA applications had a positive effect on plant growth. Kahraman (2017) reported that HA caused an increase in many parameters examined as a result of his study in the form of a 2 year field trial with HA application in cowpea cultivars. Sofi et al. (2018) applied HA and S to alfalfa seeds. As a result of the study, they reported that HA application under salt stress increased seed germination.

Table 3
Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on MGT (day)

C × HA S Doses	MGT (day)												Mean
	Maximus				Sirena				Reyna				
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
S1	1.33 ı-m	1.28 klm	1.22 lm	1.20 m	1.60 e-k	1.50 f-m	1.52 e-m	1.29 klm	1.44 g-m	1.75 d-g	1.75 d-g	1.73 d-g	1.46 C
S2	1.55 e-k	1.52 e-m	1.43 g-m	1.43 g-m	1.63 e-j	1.55 e-k	1.60 e-k	1.38 h-m	1.84 de	1.64 e-ı	1.43 g-m	1.30 j-m	1.53 C
S3	1.83 def	1.66 e-h	1.54 e-l	1.50 g-m	2.19 bc	2.26 bc	2.30 bc	1.75 d-g	2.04 cd	1.85 de	1.63 e-j	1.57 e-k	1.84 B
S4	2.43 b	2.49 b	2.42 b	2.19 bc	2.43 b	2.93 a	2.97 a	2.17 bc	2.36 b	2.37 b	1.99 cd	2.02 cd	2.40 A
Mean	1.78 CDE	1.74 DEF	1.65 EF	1.58 F	1.96 ABC	2.06 AB	2.09 A	1.65 EF	1.92 A-D	1.90 BCD	1.70 EF	1.66* EF	
LSD%1	0.2712												

* Dissimilar letters in the column show different groups

When Table 3, which includes the averages of the MGT parameter, is examined, the highest average germination time was determined as 2.97 days at the S4 dose. The lowest MGT was obtained from S1 HA4 application as 1.20 days. It was determined that MGT decreased as the doses of HA applications increased, however, the HA4 dose was effective in decreasing MGT. Ebrahimi and Miri (2016) applied 3 HA doses (0, 15 and 30 g L^{-1}) in their study in which they investigated the effects of HA on the germination properties of *Borago officinalis* and *Cichorium intybus* plants. As a result of the study, the results showed that application of 30 g l^{-1} humic acid was effective in germination of the plant species and stimulated the plants germination. Gürsoy and Kolsarıcı (2017) determined that the application had a positive effect on yield and yield elements

as a result of their study in which they applied HA to the summer rapeseed plant in a leonardite environment. Bulut (2020) reported that humic acid can be applied as an organic supplement against salt stress in his study in which he applied HA to reduce the effect of salt stress on corn seeds.

Table 4
Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on SL (cm)

C × HA S Doses	SL (cm)												Mean
	Maximus				Sirena				Reyna				
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
S1	6.23 g-j	6.80 e-h	7.57 b-e	8.69 a	5.25 l-o	6.20 g-k	6.95 d-g	9.00 a	5.16 l-o	6.85 e-h	7.44 cde	8.85 a	7.08 A
S2	5.15 l-o	5.95 ı-l	6.33 g-j	7.67 bcd	4.88 m-p	5.61 j-m	5.88 ı-l	8.55 a	4.60 n-q	7.14 c-f	6.55 f-ı	7.77 bc	6.34 B
S3	4.55 o-r	5.00 mno	5.17 l-o	6.59 f-ı	3.92 qrs	4.60 n-q	4.84 m-p	7.43 cde	4.00 qrs	8.27 ab	5.25 l-o	6.10 h-k	5.48 C
S4	3.37 st	3.63 st	4.10 p-s	3.46 st	2.93 t	3.89 qrs	3.90 qrs	6.25 g-j	2.94 t	5.42 k-n	3.77 rs	4.97 mno	4.05 D
Mean	4.83 E	5.35 D	5.79 C	6.60 B	4.25 F	5.07 DE	5.39 D	7.81 A	4.18 F	6.92 B	5.75 C	6.92* B	
LSD%1	0.7149												

* Dissimilar letters in the column show different groups

It was observed that salt stress shortened the seedling length, whereas humic acid doses increased the seedling length in all cultivars. Depending on the salt and humic acid doses, the longest seedling length was obtained in Sirena variety (Table 4). Gürsoy et al. (2016) reported that HA caused the lengthening of the plant in their study where they applied HA to the winter rapeseed plant. Berekati et al. (2019) reported that HA significantly increased plant height in their study in the form of foliar application of HA in rapeseed plants.

Table 5
Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on RL (cm)

C × HA S Doses	RL (cm)												Mean
	Maximus				Sirena				Reyna				
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
S1	1.20 p-s	1.56 l-p	1.38 n-r	2.00 g-j	3.07 bc	3.00 bcd	1.58 l-p	3.22 b	2.29 fg	2.27 fgh	2.34 efg	2.43 ef	2.19 A
S2	1.01 rst	1.66 j-o	1.37 n-r	2.00 g-j	2.67 de	2.88 bcd	1.40 n-r	1.45 m-q	1.90 h-l	1.88 h-l	1.99 g-k	1.64 j-o	1.82 B
S3	0.91 st	1.20 p-s	1.38 n-r	2.19 f-ı	2.81 cd	3.08 bc	1.39 n-r	1.26 o-s	1.85 ı-l	1.66 j-o	1.80 ı-m	1.59 l-p	1.76 BC
S4	0.71 t	1.14 qrs	1.39 n-r	2.09 f-ı	2.44 ef	3.80 a	1.30 n-s	1.12 qrs	1.64 j-o	1.70 j-n	1.60 k-o	1.59 l-p	1.71 C
Mean	0.96 F	1.39 E	1.38 E	2.07 C	2.75 B	3.19 A	1.41 E	1.76 D	1.92 CD	1.88 D	1.94 CD	1.81* D	
LSD%1	0.3393												

* Dissimilar letters in the column show different groups

Table 5 shows the effects of humic acid on sunflower cultivars under different salt stress on root lenght. Root length varied statistically, depending on humic acid doses, salt stress and cultivars (Table 1). Even though salt stress increased root length increased with HA applications. Therefore, even if the S dose is the highest, it is clearly seen that the root length increases with the effect of HA. In general, it was determined that the root length increased with the effect of HA in other applications. Tunçtürk et al. (2020) applied humic acid to the broad bean plant under salt stress conditions. As a result of the study, they reported that they determined that humic acid had positive effects on root development and length.

Table 6
Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on RWC (%)

C × HA S Doses	RWC (%)												Mean
	Maximus				Sirena				Reyna				
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
S1	71.31 c-ı	72.20 cde	72.67 bcd	74.63 a	71.90 cde	73.03 abc	72.90 bcd	74.30 ab	71.93 cde	71.63 c-g	72.49 cd	70.50 e-j	72.46 A
S2	69.60 h-k	71.00 d-ı	71.40 c-h	72.67 bcd	69.83 g-k	72.13 cde	72.00 cde	72.00 cde	69.43 ıjk	70.44 e-j	71.87 c-f	70.37 e-k	71.06 B
S3	67.43 lm	70.42 e-j	69.10 jkl	71.90 cde	66.33 mn	68.90 jkl	70.00 f-k	71.07 d-ı	67.60 lm	69.53 h-k	71.22 c-ı	63.27 o	68.90 C
S4	65.10 n	66.23 mn	69.93 g-k	68.53 kl	63.30 o	65.97 mn	67.53 lm	70.00 f-k	63.27 o	66.20 mn	69.00 jkl	59.97 p	66.25 D
Mean	68.36 E	69.96 CD	70.78 BC	71.93 A	67.84 E	70.01 CD	70.61 BC	71.84 A	68.06 E	69.45 D	71.14 AB	66.03* F	
LSD%1	1.607												

* Dissimilar letters in the column show different groups

In the RWC feature (Table 6) the lowest RWC was determined as 59.97% in S4HA4. The highest RWC was determined as 74.63% in S1HA4. On the other hand, when looking at the general average values, the highest

RWC was obtained from HA4. It is seen that RWC also increases with the increase of HA doses. Therefore, it was determined that HA application against salt stress increased RWC. Akladious and Mohamed (2018) applied calcium nitrate and humic acid to pepper plants grown under salt stress. As a result of the study, they reported that the applications caused an increase in RWC. Compared to the control, they reported that the highest RWC was obtained from (Ca1+HA2) (Ca1 (control) + HA2 (humic acid 750mg kg^{-1}) application. Karimian et al. (2019) S stress in their study on Salvia splendens plant as a greenhouse experiment. They applied HA application in the form of foliar spraying, and reported that RWC increased as a result of the study.

Table 7
Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on GSI (%)

C × HA S Doses	GSI (%)												Mean
	Maximus				Sirena				Reyna				
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
S1	72.12 a-f	72.70 a-e	73.30 a-e	74.60 a	72.33 a-e	73.20 a-e	73.67 abc	74.83 a	73.37 a-d	72.27 a-e	73.43 a-d	71.10 b-h	73.08 A
S2	70.73 b-h	72.13 a-f	72.07 a-f	73.83 ab	70.33 b-ı	73.63 abc	72.67 a-e	73.00 a-e	70.30 c-ı	71.07 b-h	72.57 a-e	68.63 g-k	71.75 B
S3	68.77 f-k	72.10 a-f	70.93 b-h	72.43 a-e	68.27 h-k	72.63 a-e	70.73 b-h	72.17 a-f	68.30 h-k	69.97 d-j	71.43 a-h	62.37 mn	70.01 C
S4	66.03 kl	68.23 h-k	71.70 a-g	70.00 d-j	64.07 lm	66.90 jkl	68.60 g-k	71.77 a-g	64.17 lm	67.17 ı-l	69.80 e-j	60.47 n	67.41 D
Mean	69.41 D	71.29 BC	72.00 AB	72.72 AB	68.75 D	71.59 ABC	71.42 ABC	72.94 A	69.03 D	70.12 CD	71.81 AB	65.64* E	
LSD%1	2.852												

* Dissimilar letters in the column show different groups

When the GSI parameter is examined (Table 7), the lowest GSI was determined as 60.47% in the S4HA4 application, while the highest 74.83% was determined in the S1HA4 application. Although GSI decreased as salt stress increased, it increased slightly with HA applications. Arslan (2018) investigated the photosynthetic activities of C3 and C4 plants under water constraint conditions and reported that stress conditions cause a decrease in the actual water content of the plants. Similarly, in this study with salt stress, GSI decreased and increased slightly with HA applications.

Table 8
Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on Chl (mg g^{-1})

C × HA S Doses	Chl (mg g^{-1})												Mean
	Maximus				Sirena				Reyna				
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
S1	3.00 b-e	3.19 a-d	3.24 abc	3.31 ab	3.09 b-e	3.19 a-d	3.29 ab	3.70 a	3.06 b-e	2.96 b-f	2.57 e-k	3.70 a	3.19 A
S2	2.04 jkl	2.35 g-l	2.34 g-l	2.40 f-l	2.93 b-f	2.59 e-j	2.95 b-f	3.13 b-e	3.00 b-e	3.18 a-d	2.26 g-l	3.19 a-d	2.70 B
S3	1.86 lm	2.19 h-l	2.29 g-l	2.40 f-l	2.66 d-ı	2.17 h-l	2.25 g-l	2.58 e-k	2.64 d-ı	2.69 c-h	2.15 h-l	2.60 e-j	2.38 C
S4	1.45 m	1.90 lm	1.93 lm	1.98 lm	1.91 lm	2.00 kl	2.10 ı-l	2.79 b-g	2.11 h-l	2.36 g-l	2.64 d-ı	2.04 jkl	2.10 D
Mean	2.09 E	2.41 D	2.45 CD	2.52 CD	2.65 BCD	2.49 CD	2.65 BCD	3.05 A	2.70 BC	2.80 AB	2.40 D	2.88* AB	
LSD%1	0.4846												

* Dissimilar letters in the column show different groups

In terms of Total Chlorophyll (Table 8), the lowest Chl was determined as 1.45 mg g^{-1} in S4HA1 and the highest in S1HA4 with 3.70 mg g^{-1}. Although it was S1 in each application, it was determined that chlorophyll increased with HA application. It was determined that Chl increased with the increase of HA at other S doses. El-Ghamry et al. (2009) showed that application of humic acid and amino acids increased chlorophyll a and b. Akladious and Mohamed (2018) applied calcium nitrate and humic acid to pepper plants grown under salt stress. As a result of the study, they reported that salt stress applications decreased Chl a and Chl b, but the applications caused an increase in photosynthetic pigments. They reported that the most effective application was obtained from (Ca1+HA2) (Ca1 (control) + HA2 (humic acid 750mg kg^{-1}) application.

Table 9
Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on CSI (%)

C × HA S Doses	CSI (%)												Mean
	Maximus				Sirena				Reyna				
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
S1	68.70 e-ı	69.03 e-h	70.97 bcd	70.10 c-f	66.42 j-m	64.40 n-s	69.47 d-g	72.97 a	68.83 e-ı	64.37 n-s	66.33 klm	63.43 qrs	67.92 A
S2	68.14 ghı	68.59 e-ı	70.13 cde	69.65 d-g	65.17 m-p	64.34 n-s	68.40 f-ı	72.43 ab	67.67 h-k	64.00 o-s	65.85 lmn	63.83 p-s	67.35 B
S3	66.00 lmn	67.99 g-j	69.42 d-g	68.05 ghı	63.05 s	65.77 lmn	69.67 d-g	71.60 abc	63.00 s	63.50 p-s	68.92 e-ı	64.43 n-s	66.78 C
S4	63.57 p-s	67.32 ı-l	69.32 d-h	65.55 mno	60.34 t	63.26 rs	70.80 cd	70.73 cd	60.70 t	65.00 m-q	71.40 abc	64.97 m-r	66.08 D
Mean	66.60 D	68.23 C	69.96 B	68.34 C	63.74 F	64.44 EF	69.58 B	71.93 A	65.05 E	64.22 F	68.13 C	64.17* F	
LSD%1	1.473												

* Dissimilar letters in the column show different groups

When the averages are examined in terms of chlorophyll stability index (CSI) feature (Table 9), the lowest CSI was determined as 60.34% in S4HA1 application, and the highest in S1HA4 application as 72.97%. Although there were decreases in CSI with salt applications, an increase in CSI was determined as the doses of HA applications increased. Mohan et al. (2000) reported that chlorophyll stability index (CSI) is an indicator of the stress capacity of plants and that high CSI value indicates that stress has no effect on chlorophyll. At the same time, they reported that high CSI is an indicator that the plants resistance to stress, dry matter production and productivity will be high. In this study, the resistance of plants to salt stress increased with the increase of CSI with HA applications.

Table 10
Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on STP (%)

C × HA S Doses	STP (%)												Mean
	Maximus				Sirena				Reyna				
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA 4	HA1	HA2	HA3	HA4	
S1	79.02 a-g	80.83 a-d	80.97 bc	81.47 ab	78.57 a-ı	77.00 e-j	78.53 a-ı	78.97 a-g	80.77 a-d	79.30 a-e	81.50 ab	78.17 a-ı	79.59 A
S2	78.66 a-h	79.03 a-g	78.87 a-g	79.30 a-e	76.90 e-j	75.07 h-n	76.83 e-j	77.87 b-ı	81.60 a	77.67 c-ı	79.93 a-e	76.30 e-l	78.17 B
S3	77.03 e-j	76.33 e-l	77.67 c-ı	75.33 g-m	72.87 l-o	72.30 mno	75.57 f-m	73.30 k-o	79.57 a-e	73.13 k-o	76.97 e-j	72.03 mno	75.18 C
S4	73.57 j-o	76.60 e-k	77.27 d-ı	79.07 a-f	72.23 mno	71.10 o	71.77 no	68.00 p	74.97 ı-n	71.33 o	77.97 a-ı	67.73 p	73.47 D
Mean	77.07 BC	78.20 AB	78.69 A	78.79 A	75.14 DEF	73.87 EF	75.68 CD	74.53 DEF	79.22 A	75.36 DE	79.09 A	73.56* F	
LSD%1	3.055												

* Dissimilar letters in the column show different groups

When the salt tolerance percentage (STP) parameter (Table 10) is analyzed, the lowest STP was determined in S4HA4 with 67.73%, and the highest was determined in S1HA3 application as 81.50%. Although STP decreased with salt applications, it was determined that S tolerance increased with HA applications, especially in HA3 and HA4. Uzun Kayis and Ceyhan (2015) reported that percentage of salt tolerance of lentil varieties ranged from 29.79% to 58.87% and this depending on salt levels, salt tolerance of varieties different from each other.

4.Conclusion

In this study, the effects of different doses of HA applications on sunflower seeds under salt stress were determined. As a result of the study, it was determined that HA applications had positive effects on the germination parameters, early seedling growth, chlorophyll content, chlorophyll stability index, and salt tolerance percentage of sunflower seeds under stress conditions. It was determined that HA4 (15ml L^{-1}) application gave better results against salt stress, and C2 (Sirena) from the cultivars used in the experiment gave the best results. Besides, applications should be made in other plants and under various stress conditions and their results should be evaluated.

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