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Research Article

Effect of Humic Acid Applications on Physiological and Biochemical Properties of Soybean (*Glycine max* L.) Grown under Salt Stress Conditions

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Abstract: In the study, humic acid was applied to soybean (*Glycine max* L.), which has high economic value and importance, to determine the tolerance level of the plant against salt stress, and physical and chemical changes in the plant were observed. The study was carried out in the climate room of Van Yuzuncu Yil University Faculty of Agriculture, Department of Field Crops in 2019. In the research, İlksoy soybean variety was used. The experiment was carried out in 4 factorial orders according to the factorial experiment was designed based on Completely Randomized Design. In the research, four different Humic acid doses (0, 500, 1000 and 2000 ppm) and 3 different NaCl salt doses (0, 125 and 250 mM) were used. In the study, root length, stem length, root fresh weight, stem fresh weight, root dry weight, stem dry weight, leaf area, chlorophyll content, ion leakage in leaf tissues, lipid peroxidation level (MDA), relative water content and membrane resistance index in leaf tissues were determined. Properties such as index were also examined. As a result of the study, the longest root was 38 cm for the control plots that salt and humic acid didn't apply to the plants. The highest root fresh weight was 2.08 g and the stem fresh weight was 1.87 g of the plots where 500 ppm humic acid dose applied. In addition, the plants with the highest chlorophyll ratio was 51.05 under 250 mM salt applied without humic acid application.

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1. Introduction

Soil salinity creates stress on the plant and causes many changes. Most of the world's water resources (70%) are salty. Therefore, there are yield losses in aquaculture in areas with high salt content (Celik and Karakurt, 2022). Salinity; It is one of the leading sources of abiotic stress that threatens the life of plants (Toprak and Tunçtürk, 2018). Therefore, the effects of stress on different plants grown in the same saline soil differs. While some plants can initiate various morphological, physiological, biochemical and molecular changes depending on the increase in salinity in the environment in which they are grown, and minimize the damages of stress and continue their lives, the mechanisms that can tolerate stress are not sufficient in some plants and their chances of survival decrease (Çulha and Çakırlar, 2011).

Humic acid which facilitates the uptake of water and nutrients by plants is a group of molecules and significantly increase productivity. Humic acid is a commercial product contains many elements which improve the soil fertility and increase the availability of nutrients and consequently increase plant growth and yield. It particularly is used to enhance or reduce the negative effect of salt stress. Many investigators reported that humic acid applications led to a significant increase in soil organic matter which is improves plant growth and crop production (SoilBiotics, 2019).

Glycine max L. is one of the most important oil plants. Soybean (*Glycine max* L.) is the most valuable oilseed crop in human nutrition as a good protein feed and biofuel raw material, and as fodder for detailed livestock and aquaculture. World soybean production is 75.5 million tons and its area is 176.6 million tons of beans. It is a product grown by irrigation. It is also grown in warm conditions in tropical, subtropical and temperate climates (FAO, 2019).

The objectives of this study were: to investigate the integrated effects of humic acid fertilizer on soybean growth and nutrient uptake; to understand the mechanism underlying soybean salt tolerance; to establish the effective-ness of preparations, made on the basis of humic acids on the yields of crops in case of different methods and amounts used; to study plant growth parameters and physiological and biochemical changes in soybean seedlings under salt stress.

2. Materials and Methods

In the present study, Ilksoy soybean variety obtained from Trakya Agricultural Research Institute was used as seed material in the experiments. Experiment was carried out in 48 plastic pots with 500 cc capacity. The factorial experiment was designed based on Completely Randomized Design with four replications, with four different humic acid doses (0, 500, 1000 and 2000 ppm) and three different NaCl salt doses (0, 125 and 250 mM). In the study, 3 seeds were planted in each pot and after germination of the seeds only one the best healthy plant was left and the other two were removed. The seeds were sterilized with 5% sodium hypochlorite for 15 minutes and thoroughly washed with pure water, and then they were ready for planting. The growing media of the seeds was 1/3 perlite and 2/3 soil mixture. After planting, the pots were placed in a 16/8 hour light/dark photoperiod, under 25° C temperature and 65% humidity in a chamber. Plants were applied 100 mg kg^{-1} nitrogen, 45 mg kg^{-1} phosphorus and 75 mg kg^{-1} potassium per plant as basic fertilization from planting (Ertürk, 2011). The experiment conducted in the climate room of the Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University in 2019.

Humic acid doses was mixed into the soil before planting, and 300mg kg^{-1}nitrogen, 150 mg kg^{-1} phosphorus and 200 mg kg^{-1}potassium were applied to each pot as basic fertilization. The salt stress applications were started when the plants reached a certain growth stage (about 1 month later). The application of salt was made by adding the solution prepared with different salt doses as irrigation. At the stage where physiological problems occurred in the plants, the experiment ended and plants were harvested for the necessary analyzes.

Root length (cm), from the most extreme part of the root part of the plants up to the root neck was found by measuring. Stem height (cm), the height of the plants was measured from the soil level to the highest point of the plant. Root fresh weight (g), after separating the root part of the plants representing the applications, root fresh weight was determined using a sensitive balance.

Stem fresh weight (g), after the plants representing the applications were cut at soil level, the fresh stem weight was determined using a sensitive balance. Root dry weight (g), after the harvest, plant samples were kept in the oven at 70 ºC for 48 hours and the root dry weight was calculated. Stem dry weight (g), after the harvest, plant samples were stored in the oven at 70 ºC for 48 hours and the stem dry weight was calculated.

Relative water content in leaf tissues (RWC) (%), to determine the proportional water content of the plants, 4 discs were cut and wet weights was weighed for each leaf immediately after harvest. Leaf discs weighed in ultrapure water at 25ºC for 2 hours, and turgor weights were weighed. The samples were then dried at 110° C for 24 hours to record their weight. Arora et al. (1998) equation was used for calculating.

$$RWC (\%) = [(\text{fresh weight-oven dry weight})/(\text{turgor weight - oven dry weight})] \times 100 \quad (1)$$

Determination of lipid peroxidation levels (MDA): Lipid peroxidation in plants is expressed as malondialdehyde (MDA) content. 0.5 g of the leaf sample was homogenized with 10 ml of 0.1% trichloroacetic acid (TCA) and the homogenate was centrifuged at 15000 g for 5 minutes. 1 ml of the supernatant portion was removed and 0.5% thiobarbituric acid (TBA) dissolved in 4 ml of 20% TCA was added. After the mixture was kept in a 95 °C water bath for 30 minutes, it was rapidly cooled in ice bath and centrifuged at 10000 g for 10 minutes (Sairam and Saxena 2000).

$$MDA \ (nmol \ ml^{-1}) = [(A532-A600) / 155\ 000]\ 10^6 \quad (2)$$

Determination of ion leakage in leaf tissues (%): The wet leaf samples (0.1 g) were taken before harvest and washed with tap water and then with pure water. The plant samples were kept in 10 ml of purified water at 40° C for 30 minutes, this was (C1). The EC were measured again in the sample held in a hot water bath at 100° C for 10 minutes (C2) and ion leakage or membrane permeability in leaf tissues were calculated by the following equation (Sairam, 1994).

$$\text{Ion Leakage in Leaf Tissues} = (C1 / C2) \times 100 \quad (3)$$

Membrane endurance index in leaf Tissues (%): First of all, leaf samples (0.1 g) were washed with tap water and then purified with pure water and the plant samples were kept in 10 ml of pure water for 30 minutes at 40° C and the EC was measured (C1), in the water bath which is kept at 100° C for 10 minutes, the EC was measured again (C2) and the membrane stability index or membrane stability index calculated in the leaf tissues with the following equation (Sairam 1994).

$$\text{Membrane Endurance Index in Leaf Tissues} (\%) = [1- (C1 / C2)] \times 100 \quad (4)$$

Leaf area: The leaves selected as representative of plant saplings were placed on A4 paper and photographed with android device. The leaf area was determined using the Easy Leaf Area program

Chlorophyll content: The chlorophyll content we determined by the portable chlorophyll meter device (Minolta SPAD-502, Osaka, Japan), which indirectly measures the chlorophyll content in the leaf.

Data were subjected to analysis of variance (ANOVA) using COSTAT statistical (version 6.3) package program according to the factorial experiment was designed based on Completely Randomized Design. The means compared with Duncan Multiple Range Test (DMRT) at α=0.05. Also, correlation analysis was done using IBM SPSS statistics (version 22.0) program (IBM Corp., 2013).

3. Findings and Discussion

The effects of humic acid (HA) were determined in controlled growth chamber via applying different concentrations of HA on soybean seedlings grown under different concentrations of salt stress through measuring some plant growth, physiological and biochemical properties.

In the study, while the effect of salt doses on root length, relative water content in leaf tissues, membrane resistance index of leaf tissues, leaf area and total chlorophyll content parameters was not statistically significant, its effect on other parameters was found to be significant.The effect of humic acid applications on root fresh weight and membrane resistance index of leaf tissues was found to be insignificant.The effect of S x HA interaction has statistically influenced on all other parameters, except features such as stem height, relative water content in leaf tissues, MDA, ion leakage in leaf tissues and membrane resistance index of leaf tissues.

Root length values of the soybean plants as a result of different salt dosage applications were determined as 29.7 - 30.0 cm. Although, salt doses negatively affected root length, this effect was not statistically significant. Growth and development are generally negatively affecting plants under salt stress, and in some cases the plant dies as an effect of the salt effect (Erdal et al., 2000). In many similar studies (Turkmen et al., 2008; Tunçtürk et al., 2011a; Kalyoncu, 2013), it was reported that increased salt concentrations had a negative effect on the root length values of the plant. Kondetti et al. (2012), found that root seedling decreased linearly when the salt concentrations increased. The effect of different dosage of humic acid application on the average soybean plant root length grown under salt stress varied between 27.8 and 31.3 cm. In this study, the longest root length (31.3 cm) was obtained from HA_2 application. But, there is no statistical difference between control and HA_3 applications. The lowest (27.8 cm) was determined in HA_1 application. The results indicate that increasing humic acid dosage had a positive effect on the plant root length. Kalyoncu (2013) reported that increasing humic acid doses positively affects the root length of mung bean plants, which is similar to the findings of this study. Furthermore, Başalma (2014), Malik and Azam (1985) reported that application to humic acids to wheat increases root length.

Table 1. The effect of humic acid applications on some morphological parameters in salt stressed soybean.

Salt Doses (mM)	Humic Acid Doses (ppm)	Root Length (cm)	Stem Height (cm)	Root Fresh Weight (g)	Stem Fresh Weight (g)	Root Dry Weight (g)	Stem Dry Weight (g)
S_0	**HA_0**	38.0 ^{a}	30.5	1.28 ^{bcd}	1.37 ^{ab}	0.18 ^{b}	0.18 ^{b}
	HA_1	29.7 $^{a-c}$	27.3	2.08 ^{a}	1.87 ^{a}	0.24 ^{ab}	0.24 ^{ab}
	HA_2	26.7 ^{bc}	26.5	1.56 $^{a-d}$	1.45 ^{ab}	0.22 ^{ab}	0.22 ^{ab}
	HA_3	28.3 $^{a-c}$	23.7	1.23 ^{cd}	0.93 ^{bc}	0.27 ^{ab}	0.27 ^{ab}
S_0 Average		**30.7**	**27.0 A**	**1.54 AB**	**1.41 A**	**0.23 B**	**0.31 A**
S_1	**HA_0**	28.3 ^{bc}	26.3	1.78 ^{ab}	1.28 ^{b}	0.27 ^{ab}	0.27 ^{ab}
	HA_1	23.7 ^{d}	23.5	1.18 ^{d}	0.87 ^{c}	0.25 ^{ab}	0.25 ^{ab}
	HA_2	32.5 ^{ab}	23.5	1.12 ^{d}	1.28 ^{b}	0.27 ^{ab}	0,27 ^{ab}
	HA_3	34.5 ^{ab}	24.7	1.78 ^{ab}	0.86 ^{c}	0.29 ^{a}	0.29 ^{a}
S_1Average		**29.7**	**24.5 ABC**	**1.46 B**	**1.07 B**	**0.27 A**	**0.26 B**
S_2	**HA_0**	26.3 $^{b-d}$	28.0	1.72 ^{abc}	1.27 ^{b}	0.23 ^{ab}	0.23 ^{ab}
	HA_1	30.0 $^{a-c}$	20.7	1.46 ^{bcd}	1.19 b	0.28 ^{ab}	0.28 ^{ab}
	HA_2	34.5 ^{ab}	23.0	1.87 ^{a}	1.39 ^{ab}	0.31 ^{a}	0,31 ^{a}
	HA_3	29.3 $^{a-c}$	20.7	1.81 ^{a}	0.99 ^{b}	0.27 ^{ab}	0.27 ^{ab}
S_2Average		**30.0**	**23.1 C**	**1.72 A**	**1.21 B**	**0.27 A**	**0.27 AB**
Humic Acid Doses (ppm)	**HA_0**	30.8 A	28.3 A	1.59	1.31 A	0.23 B	0.34 A
	HA_1	27.8 B	23.8 B	1.57	1.31 A	0.26 AB	0.33 A
	HA_2	31.3 A	24.3 B	1.52	1.37 A	0.26 A	0.22 B
	HA_3	30.7 A	23.1 B	1.61	0.93 B	0.28 A	0.23 B
C.V (%)		**9.579**	**9.469**	**16.925**	**17.497**	**13.196**	**17.208**
S		ns	**	*	**	**	*
HA		*	**	ns	**	**	**
S x HA		**	ns	**	**	*	**

S: Salt Doses, HA: Humic asit, HA0: 0 (Control), HA1:500 ppm, HA2: 1000 ppm, HA3: 2000 ppm, S0: 0 (Control), S1: 125 mM, S2: 250 mM, * P <0.05; ** P <0.01 ns: non-significant.
There is nonsignificant difference between values with the same letter in the same column.

The effect of salt stress was significantly high on the soybean plants and control application (0 mM NaCl) produced taller plants. Average stem height was 27.0 cm, and the shortest plants were obtained from 250 mM salt application as 23.1 cm. However, the control and 125 mM salt concentration were within the same statistical mean group. Tunçturk et al. (2008 and 2011b) findings were similar, and they suggested that salt stress negatively affected on the stem height. The results from Table 1 shows that HA_0 (control) produced plants with the highest value of stem height as 28.3 cm, while all the other application doses (500, 1000 and 2000 g) were in the same comparison group. Furthermore, the plants were shorter than that of the control application, (23.8, 24.3 and 23.1 cm) respectively. Several previous researches support the results of this experiment's findings. El-Shafey and Zen El-Dein (2016), reported that the lowest values of stem height and ear height were recorded when maize intercropped with soybean and fertilizer by foliar humic acid in the two experimental seasons. Dawood et al. (2019), found that stem height was reduced with the increase of HA doses.

The average root fresh weight obtained from different salt applications varied between 1.46 g and 1.72 g. The highest root fresh weight (1.72 g) determined in the 250 mM NaCl applications, while the lowest root fresh weight (1.46 g) was obtained from 125 mM NaCl application. The highest value of root fresh weight for the HA treatment was 1.61 g obtained from the application of HA_3, and the lowest value was 1.52 g obtained from HA_2. However, the effect of the HA different doses was statistically non-significant on root fresh weight. Basalma (2014), studied safflower varieties and humic acids levels and found that there were no significant effect the HA in terms of fresh root weight among the varieties, as well as humic acids doses, the highest root weight was achieved 5.189 g and 5.179 g respectively, from cv. Dinçer and 180 g of humic acids treatment. The S x HA interaction gave the highest value of rot fresh weight (2.08 g) under 0 mM NaCl with HA_1 treatment. The lowest value was 1.121 g obtained from the 125 mM NaCl with HA_2.

The different salt concentrations had a significant effect on the stem fresh weight. The highest weight was 1.41 g obtained from the control treatment 0 mM NaCl, while the lowest stem fresh weight was 1.07 g obtained from application of 125 mM NaCl. It was same group with 250 mM NaCl applications. In the study, different salt concentration applications are adversely affected by the stem fresh weight values compared to the control application. Tunçturk and colleagues, (2009), reported that salt stress was detrimental to stem fresh weight in soybean, weight of plants under salt stress at final harvest were significantly reduced compared with those of plants in the control treatment. Another work by Tunçturk et al., (2011a), suggested the same findings but on several canola (*Brassica napus* L.) cultivars. The effect of HA doses was significant on the stem fresh weight. The highest stem fresh weight was 1.37 g obtained from applying HA_2, and the lowest value was 0.93 g from the HA_3 dose. However, the control and HA_1 applications were in the same group with the HA_2, and the value of the stem fresh weight was 1.31 and 1.31 g respectively. In terms of S x HA interaction, the plants which received HA_1 with 0 mM NaCl, gave the highest value of stem fresh weight 1.87 g, and the lowest value was 0.86 g from HA_3 with 125 mM NaCl. These findings are similar to Dawood et al. (2019) suggestions for faba bean plants. Humic acid application caused increases in stem fresh weight.

The different salt concentrations had a significant effect on the root dry weight. The highest root dry weight was 0.27 g obtained from 125 and 250 mM NaCl application, while the lowest value was 0.23 g from the control applications. These results are similar to what Kondetti et al. (2012) found. They reported that root dry weight production of *Phaseolus mungo* for all the studied varieties decreased from 12.10 mg to 0.55 mg as salt concentrations increased from 0-300 mM NaCl. Tunçturk et al. (2008, 2011b) findings were similar; they suggested that salt stress affects negatively on soybean stem dry weight. In terms of HA, the highest root dry weight was 0.28 g obtained from the application of HA_3, and it was same group with HA_1 and HA_2 with 0.26 g dry root weights. The lowest value was from the control with 0.23 g root dry weight. Basalma (2014), finding was close to these results. There was variation in safflower seedling root dry weight, different cultivars were grown under different HA dosages, and the control application produced plants with lower root dry weight and the highest value was from higher doses of HA. In another experiment by Boogar et al. (2014), the effect of humic acid on the measured traits of betonia hybrid root weight did not show a statistically significant difference between humic acid treatments, but there was significant statistical difference between HA and the control. They found that increase in fresh and dry weight of roots was observed with HA applications. The interaction of S x HA results showed that plants received 250 mM NaCl with HA_2 had the highest value of root dry weight, 0.31 g, and those received 0 mM NaCl (control) with HA_0 (control) HA had the lowest value of root dry weight, 0.18 g.

In this study, salt applications negatively affected stem dry weight averages. The highest stem dry weight was 0.31 g obtained from 0 mM NaCl (control) applications, while the lowest stem dry weight was 0.26 g obtained from the 125 mM NaCl application. It was same Duncan group with 250 mM NaCl applications. The HA had a significant effect on stem dry weight. The highest value was 0.34 g obtained from the control and the lowest stem dry weight value was 0.22 g from the HA_2. For the interaction of S x HA, the highest stem dry weight value was 0.31 g obtained from the 250 mM NaCl with HA_2, and the lowest value was 0.18 g obtained from control 0 mM NaCl with HA_0. This result is similar to the findings of Tunçtürk et al. (2011b) on Canola, salt stress caused a significant decrease in the stem dry weights. Furthermore, Kondetti et al., (2012) studied *Phaseolus mungo* under salt and observed that dry weight of the seedling decreased with increasing NaCl.

The Relative water content in leaf tissues (RWC) was not statistically affected by salt doses (Table 2), the RWC values determined between 63.86-71.85%. The results indicate that increasing humic acid dosage had a positive effect on the average RWC, the highest value of RWC was 74.83% and the lowest value was 60.88% obtained from HA_1 and HA_3 respectively. RWC in leaf tissues of pepper cultivars at different salinity levels was investigated by Hand et al., (2017). The increased RWC values in salt-tolerant cultivars suggest that, accumulation of osmolytes makes the surplus of water uptake possible.

The highest MDA value obtained from different salt applications was 0.78 nmol g^{-1} F.W obtained from 250 mM NaCl application, while the lowest value was 0.58 nmol g^{-1} F.W obtained from 0 mM NaCl application (control). In a conducted experiment on the effect of salt stress on soybean plant by Kumari et al., (2015), they found that MDA values increases with the increase of salt stress. The same result was discovered on other crops (Sairam and Srivastava, 2002; Porcel et al.,2003; Yildirim et al., 2004; Han and Lee, 2005; Shukla et al., 2012; Yolci et al. 2021). HA had a significant effect on the soybean plants for MDA. The HA_0 (control) had the highest MDA value 0.73 nmol g^{-1} F.W, and the MDA content in the HA_3 application was the lowest 0.66 nmol g^{-1} F.W. Similar results was discovered by Chen and Aviad (1990) and Kıran et al. (2019) , they documented that the application of HA on plants under stress reduces the MDA significantly.

Table 2. The effect of humic acid applications on some physiological parameters in salt stressed soybean

Salt Doses (Mm)	Humic Acid Doses (ppm)	Relative Water Content in Leaf Tissues (%)	MDA (nmol g^{-1} F.W)	Ion Leakage in Leaf Tissues (%)	Membrane Resistance Index of Leaf Tissues (%)	Leaf Area (cm^2)	Total Chlorophyll Content (SPAD)
S_0	**HA_0**	65.49	0.63	2.40	88.51	12.10 ^{cd}	43.55 $^{c-e}$
	HA_1	74.92	0.53	2.11	85.66	16.65 ^{ab}	46.45 $^{a-d}$
	HA_2	68.78	0.61	3.49	89.59	18.97 ^{a}	46.23 $^{b-d}$
	HA_3	46.27	0.56	3.69	91.38	17.31^{ab}	40.83 ^{de}
S_0 Average		**63.86**	**0.58 C**	**2.93 B**	**88.78**	**16.21**	**44.26**
S_1	**HA_0**	68.35	0.75	1.88	90.27	11.06 ^{d}	48.13 $^{a-c}$
	HA_1	61.99	0.68	4.44	86.75	18.65 ^{a}	43.75 ^{cd}
	HA_2	67.36	0.67	5.67	89.08	14.66 ^{bc}	43.65 ^{cd}
	HA_3	67.18	0.64	7.03	85.51	16.45^{ab}	45.03 ^{cd}
S_1 Average		**66.22**	**0.68 B**	**4.75 A**	**87.90**	**15.20**	**45.14**
S_2	**HA_0**	65.38	0.81	1.96	87.15	14.47 ^{bc}	51.05 ^{a}
	HA_1	87.58	0.78	2.65	91.47	16.64 ^{ab}	48.40 ^{ab}
	HA_2	65.26	0.75	6.61	89.04	17.76 ^{a}	40.83 ^{e}
	HA_3	69.20	0.78	2.49	94.04	15.75 ^{b}	42.23 ^{de}
S_2 Average		**71.85**	**0.78 A**	**3.43 AB**	**90.42**	**16.15**	**45.63**
Humic Acid Doses (ppm)	**HA_0**	66.40 AB	0.73 A	2.08 C	87.96	12.54 B	47.57 A
	HA_1	74.83 A	0.66 B	3.07 B	89.24	17.25 A	46.2 A
	HA_2	67.13 AB	0.67 AB	5.26 A	89.24	17.13 A	43.56 B
	HA_3	60.88 B	0.66 B	4.41 AB	90.31	16.49 A	42.69 B
C.V (%)		**13.26**	**9.96**	**11.21**	**8.99**	**8.894**	**5.186**
S		**ns**	**	*	ns	ns	ns
HA		*	**	**	ns	**	**
S x HA		**ns**	ns	ns	ns	**	**

S: Salt, HA: Humic asit, HA0: 0 (Control), HA1:500 ppm, HA2: 1000 ppm, HA3: 2000 ppm, S0: 0 (Control), S1: 125 mM, S2: 250 mM, ns: Non-significant. * P <0.05 significant. ** P <0.01 high significant, n.s: non-significant.
There is nonsignificant difference between values with the same letter in the same column.

The highest leakage in leaf tissues obtained from different salt treatments applied to soybean plant seedlings was 4.75 % obtained from 125 mM NaCl application, and the lowest value was obtained from control application with 2.93 %. At the end of the study, it was determined that the ion leakage in the leaf tissues increased in the plants applied salt source according to control applications. In terms of HA doses, the highest value of this parameter was 5.26 % obtained from the HA_2 application, and the lowest value was 2.08 % obtained from the application of HA_0 (control).

Membrane resistance index of leaf tissues obtained as a result of different salt applications varied between 87.90-90.42 %. The results of the application of HA on soybean, the mean membrane resistance index of plant leaf tissues varied between 87.96-90.31 %. The effect of soybean applications with HA on membrane resistance index of leaf tissues in plant was positive and the rate increased as the doses increased. Sairam and Srivastava (2002), in the study of the effects of salt stress on antioxidant properties of long-term salt applications in wheat plants in the study of salt membrane stability index of the study reported that the reduction of the membrane shows a parallel with this study.

The leaf area varied between 15.20 and 16.21 cm^2 in terms of salt doses. However, there was no significant differences when the data were statistically analyzed. The effect of HA was significant, the highest value of leaf area was 17.25 cm^2 obtained from the HA_1 applications, and the lowest value 12.54 cm^2 was obtained from the control. But, HA_1 application was same group with HA_2 and HA_3 applications. The interaction of S x HA was significant; the highest value of leaf area was 18.97 cm^2 obtained from the 0 mM NaCl with HA_2 applications. However, this treatment was with the same group with 125 mM NaCl with HA_1 and 250 mM NaCl with HA_2 applications with values of 18.65 and 17.76 cm^2 respectively. Yasar (2003), stomata of plants containing salt stress to close the leaf area is reported to be reduced by reducing transpiration rates. Our findings were in parallel to the results of these studies and the results of our research. El-Shafey and Zen El-Dein (2016) results on soybean plant experiment showed similar effect on leaf area.

In study, there was no significant differences when the data were statistically analyzed in terms of salt doses. It was obtained between 44.26-454.63 SPAD values. The effect of HA was significant, the highest value of total chlorophyll ratio was 47.57 obtained from the control HA applications. But, it was same group with HA_1 application. The lowest value 42.69 was obtained from the HA_3. There aren't differences statistically with HA_2 applications. The S x HA interaction showed significant effect. The highest value was obtained 51.05 from the 250 mM NaCl with HA_0, and the lowest value was obtained 40.83 from 250 mM NaCl with HA_2. Sairam et al. (2000), reported that chlorophyll content in plants was negatively affected as a result of salt applications. Sairam and Srivastava (2002) observed that salt stress in wheat genotypes reduced total chlorophyll content in leaf tissue. Turan and Aydin (2005), examined the effect of different salts on some physiological properties of corn plant in a study, determined that the plant growth and chlorophyll content decreased as the applied salt concentration increased. Turhan et al. (2006), salt stress due to the negative effects of chlorophyll in sunflower found. Turan (2007), salt stress in the lentil plant as a result of increased salt applications reported that the total chlorophyll content significantly decreased compared to control.

4. Conclusion

Soybean plant has become one of the most important plants in the world with the increasing usage areas in recent years. In the study, physiological and biochemical changes occurring in the plant under stress conditions were observed by applying different salt doses on soybean plants along with the application of different humic acid doses. In the research, by applying different humic acid doses and different salt doses to soybean plant, some growth parameters (root length, stem length, root fresh weight, stem fresh weight, root dry weight and stem dry weight) and some biochemical properties (RWC, MDA, membrane resistance index in leaf tissues, ion leakage in leaf tissues total chlorophyll content, and leaf area) were determined. The results of the experiment showed that; root fresh and dry weight, stem fresh and dry weight, stem length, and lipid peroxidation level (MDA), among the properties examined with salt applications, were statistically affected. The application of different humic acid doses, had statistically affected the root and stem length, leaf area and chlorophyll content. The effect of salt and humic acid doses applied in the study on relative water content, membrane resistance index and ion leakage properties in leaf tissues was not found statistically significant. According to the results obtained from the research; it can be recommended that humic acid applications is preferable in terms of minimizing the stress factors on plants that are adversely affected by salt stress conditions. In addition, it is thought that more positive results can be obtained on the physical and biochemical properties of the plant by applying humic acid applications before the stress effects are seen in the plant.

Ackonowledge

This study titled *"*Effect of Humic Doses Applications on Physiological and Biochemical Properties of Soybean (*Glycine max* L.) Grown Under Salt Stress Conditions*"* is the summary of Noor Maiwan BAHJAT master's thesis.

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