## T.R. VAN YUZUNCU YIL UNIVERSITY INSTITUTE OF NATURAL AND APPLIED SCIENCES DEPARTMENT OF FIELD CROPS

### EFFECT OF HUMIC DOZES APPLICATIONS ON PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF SOYBEAN (*GLYCINE MAX* L.) GROWN UNDER SALT STRESS CONDITIONS

M. Sc. THESIS

PREPARED BY: Noor Maiwan BAHJAT SUPERVISOR: Prof. Dr. Murat TUNÇTÜRK VAN-2020



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### ACCEPTANCE and APPROVAL PAGE

This thesis entitled "**Humic Asit Uygulamalarının Tuz Stresi Koşullarında Yetiştirilen Soya (***Glycine max***L.)**" **nın Fizyolojik ve Biyokimyasal Özellikleri Üzerine Etkisi**" **presented by** Noor Maiwan BAHJAT under supervisor Prof. Dr. Murat TUNÇTÜRK in the Department of Field Crops has been accepted as a M. Sc. Thesis according to Legislations of Graduate Higher Education on 14/05/2020 with unanimity of votes of members of jury.

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All information presented in the thesis obtained in the frame of ethical behavior and academic rules. In addition, all kinds of information that does not belong to me have been cited appropriately in the thesis prepared by the thesis writing rules.

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### ABSTRACT

### EFFECT OF HUMIC ACID APPLICATIONS ON PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF SOYBEAN (*GLYCINE MAX* L.) GROWN UNDER SALT STRESS CONDITIONS

BAHJAT, Noor Maiwan M. Sc. Thesis, Department of Field Crop Supervisor: Prof. Dr. Murat TUNÇTÜRK June, 2020, 57 Pages

In this study, soybean (Glycine max L.), which has high economic value and importance, was used as plant material. This study was conducted to determine the tolerance level of the plant against salt stress by applying Humic acid to the soybean plant and to observe the physical and chemical changes in the plant. The study was carried out in the climate room of Van Yüzüncü Yıl University Faculty of Agriculture, Department of Field Crops in 2019. In the research, Glksoy soybean variety seeds was used as material. The experiment was carried out in 4 factorial orders according to the Factorial 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 in leaf tissues were determined. Properties such as index were also examined. As a result of the study, the longest root was 38 cm and plant height was 30.5 cm for the control plots that salt and humic acid didn't apply to the plants. The highest root fresh weight was 2.082 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.

Keywords: Glycine max L., Humic acid, Salt stress, Soybean.



### ÖZET

## HUMİK ASİT UYGULAMALARININ TUZ STRESİ KOŞULLARINDA YETİŞTİRİLEN SOYA (*GLYCINE MAX* L.)' NIN FİZYOLOJİK VE BİYOKİMYASAL ÖZELLİKLERİ ÜZERİNE ETKİSİ

BAHJAT, Noor Maiwan Yüksek Lisans Tezi, Tarla Bitkileri Anabilim Dalı Tez DanıĢmanı: Prof. Dr. Murat TUNÇTÜRK Haziran, 2020, 57 Sayfa

Bu calıGmada ekonomik değeri ve önemi yüksek olan soya (Glycine max L.) bitkisi materyal olarak kullanılmıGtır. Bu çalıGma, soya bitkisine Humic asit uygulanarak bitkinin tuz stresine karGı tolerans seviyesini belirlemek ve bitkide oluGan morfolojik ve biyokimyasal değiGimleri gözlemlemek amacıyla yapılmıGtır. ÇalıGma, 2019 yılında Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü' ne ait iklim odasında yürütülmüÇtür. AraĢtırmada tohumluk materyali olarak Glksoy soya çeĢidi kullanılmıGtır. Deneme, Tesadüf Parselleri Deneme Deseni' ne göre faktöriyel düzende 4 tekrarlamalı olarak yürütülmüGtür. AraGtırmada, dört farklı Humic asit dozu (0, 500, 1000 ve 2000 ppm) ve 3 farklı NaCl tuz dozu (0, 125 ve 250 mM) kullanılmıGtır. ÇalıĢmada Soyada kök uzunluğu, gövde uzunluğu, kök yaĢ ağırlığı, gövde yaĢ ağırlığı, kök kuru ağırlığı, gövde kuru ağırlığı, yaprak alanı, klorofil miktarı, yaprak dokularında iyon sızıntısı, lipid peroksidasyon düzeyi (MDA), yaprak dokularında bağıl su içeriği ve membran dayanıklılık indeksi gibi özellikler incelenmiGtir. ÇalıGma sonucunda en uzun kök (38 cm) ve bitki boyu (30 cm) bitkilere tuz ve humic asit uygulanmayan kontrol parsellerinden, en yüksek kök (2.082 g) ve gövde yaG ağırlığı (1.87 g) tuz uygulamasının yapılmadığı 500 ppm humic asit dozu uygulanan parsellerden alınmıÇtır. Ayrıca en yüksek klorofil oranı 51.05 ile 250 mM tuz uygulanan ve humic asit dozu

uygulamasının yapılmadığı bitkilerden elde edilmi Gtir-

Anahtar kelimeler: *Glycine max* L., Humic asit, Tuz stresi, Soya.



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May 2020

Noor Maiwan BAHJAT

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# SYMBOLS AND ABBREVIATIONS

Some symbols and abbreviations used in this study are presented below, along with descriptions.

Symbols	Description		
kg	Kilogram		
cm	Centimeter		
g	Gram		
mg	Milligram		
Ν	Nitrogen		
% HA	Percentage Humic acid		
NaCl	Sodium Chloride	dS/m	
deciSiemens per meter			
MDA	Determination of Lipid Peroxidation Levels		
RWC	Relative Water Content in Leaf Tissues		



### **1. INTRODUCTION**

Soybean (*Glycine max* L.) is the world's leading economic oilseed crop. Soybean (*Glycine max* L.) are one of the most valuable crops in the world not only as an oil seed crop and feed for livestock and aquaculture, but also as a good source of protein for the human diet and as a biofuel feedstock. Rapid soybean demand increases in the last decade challenge the reliability of supply, stock levels, and reasonable pricing (Mwenye et al., 2018).

Soybean development is a continuous process that begins when a seed germinates and is completed where a mature seed is ready for harvest. During its life, the soybean plant is exposed to many factors that may encourage or retard its development and productivity. Some factors are controlled by nature, such as wind, rain, hail, and frost. But farmers also influence soybean development and productivity by application of pesticides and fertilizers or by the timing and methods of planting, cultivation, and other cultural practices. A soybean plant's response to the conditions that it encounters depends on its stage of development.

Legumes are an important part of world agriculture as they fix atmospheric nitrogen by intimate symbioses with microorganisms. The soybean in particular is important worldwide as a predominant plant source of both animal feed protein and cooking oil (Fehr and Caviness, 1977).

Economic and social impact of soybean crop is undoubtedly of a great economic and social importance on the worldwide. Soybean provides about 64 percent of the world's oilseed meal supply and is the major source of oil, accounting for about 28 percent of total production (Islas-Rubio and higuera, 2002; Masuda and Goldsmith, 2009). A saline soil is defined as having a high concentration of soluble salts, high enough to affect plant growth. Salt concentration in a soil is measured in terms of its electrical conductivity.

Soybean (*Glycine max* L.) is one of the most important world crops and has been cultivated for oil and protein. Present world production is around 176.6 million tons of beans over 75.5 million ha. The crop is mainly grown under rainfed conditions but irrigation, specifically supplemental irrigatio, is increasingly used. The crop is grown under warm conditions in the tropics, subtropics and temperate climates. Soybean is

relatively resistant to low and very high temperatures, but growth rates decrease above 35°C and below 18°C. In some varieties, flowering could occur at temperatures below 24°C. Minimum temperatures for growth are about 10°C and for crop production about 15°C. Only 25 to 30 percent of the flowers set pods, the final number depending on the plant vigor during the flowering period. Year to year temperature variations can lead to differences in flowering (FAO, 2019).

Soybean is basically a short-day plant, but response to daylength varies with variety and temperature and developed varieties are adapted only to rather narrow latitude differences. Daylength has an influence on the rate of development of the crop; in short-day types, increased daylength may result in the delay of flowering and taller plants with more nodes. Short days hasten flowering, particularly for late-maturing varieties. Vegetative growth normally ceases during yield formation. The length of the total growing period is 100 to 130 days or more. Soybean is often grown as a rotation crop in combination with cotton, maize and sorghum. Row spacing varies from 0.4 to 0.6 m with 30 to 40 seeds per meter of row (FAO, 2019).

The crop can be grown on a wide range of soils except those which are very sandy. Optimum soil pH is 6 to 6.5. The fertilizer requirements are 15 to 30 kg/ha P and 25 to 60 kg/ha K. Soybean is capable of fixing atmospheric nitrogen which meets its requirements for high yields. However, a starter dose of 10 to 20 kg N/ha is beneficial for good early growth (FAO, 2019).

A shallow water table, particularly during the early growth period can adversely affect the yields. The plant is sensitive to waterlogging, but moderately tolerant to soil salinity. Yield decrease due to soil salinity is: 0% at ECe 5 mmhos/cm, 10% at 5.5, 25% at 6.2, 50% at 7.5 and 100% at ECe 10 mmhos /cm (FAO, 2019).

The graph below depicts the crop stages of soybean, and the table summarizes the main crop coefficients used for water management (FAO, 2019). The U.S. Department of Agriculture is estimating world soybean production in the 2017/2018 market year will be 346.02 million metric tons. Three countries, Brazil, Argentina and the U.S., are projected to produce over 82% of the world's soybeans. The United States soybean production estimate is 119.52 million metric tons, or 4,382 million bushels (USDA, 2018).

The world growth of soybean has been impressive; growth has increased by about 350% since 1987. The commercial growth of livestock and poultry is probably closely correlated with this growth (USDA, 2018). Soybeans are supplying the world a needed source of protein and oil required for growth (Rahman et al., 2019).

Salt stress is the accumulation of excessive salt contents in the soil which eventually results in the inhibition of crop growth and leads to crop death. On a global scale, no other toxic substance is as dangerous to crop growth as salt is. Salt stress is considered an alarming condition as it decreases the agricultural productivity of soil and results in reduced crop yields. It is assumed that 20% of all cultivated land and almost half of all irrigated land are affected by salt stress, decreasing production below the genetic potential. It is suspected that the rise in soil salinity is due to poor irrigation water, its quality, and the use of brackish. High-salt stress affects plants in multiple ways, such as ion toxicity, nutritional disorders, alteration of metabolic processes, oxidative stress, genotoxicity, membrane disorganization, reduction of cell division and expansion as well as water stress (Rahman et al., 2019).

According to the over 6% of the world's land is affected by either salinity or sodicity (Table 1). The term salt-affected refers to soils that are saline or sodic, and these cover over 400 million hectares, which is over 6% of the world land area (Table 1). Much of the world's land is not cultivated, but a significant proportion of cultivated land is salt-affected. Of the current 230 million ha of irrigated land, 45 million ha are salt-affected (19.5 percent) and of the 1,500 million ha under dryland agriculture, 32 million

Regions	Total	Saline soils		Sodic soil	
	areaMha				
		Mhe		Mhe	
Africa	1,899	39	2.0	34	1.8
Asia, the pacific and Australia	3,107	195	6.3	249	8.0
Europe	2,011	7	0.3	73	3.6
Latin America	2,039	61	3.0	51	2.5
Near East	1,802	92	5.1	14	0.8
North America	1,924	5	0.2	15	0.8
Total	12,781	397	3.1%	434	3.4%

Table1. Regional distribution of salt-affected soil, in million hectares

are salt-affected to varying degrees.

Salinity occurs through natural or human-induced processes that result in the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth. Sodicity is a secondary result of salinity in clay soils, where leaching through either natural or human-induced processes has washed soluble salts into the subsoil and left sodium bound to the negative charges of the clay (Rana, 2002).

A saline soil is defined as having a high concentration of soluble salts, high enough to affect plant growth. Salt concentration in a soil is measured in terms of its electrical conductivity, as described in the section below on measurements. Definition of a saline soil is having an ECe of 4 dS/m or more. ECe is the electrical conductivity of the 'saturated paste extract', that is, of the solution extracted from a soil sample after being mixed with sufficient water to produce a saturated paste. Howevercrops are affected by soil with an ECe less than 4 dS/m. The moisture content of a drained soil at field capacity may be much lower than the water content of its saturated paste. Further, under dryland agriculture, the soil water content might drop to half of field capacity during the life of the crop. The actual salinity of a rain-fed field whose soil had an ECe of 4 dS/m could be 8-12 dS/m. As described below, this would severely limit yield of most crops (Rana, 2002). The salinity of soil is among the most important abiotic stresses which limit agricultural productivity worldwide. The effects of salinity on growth, nutrient partitioning, chlorophyll, leaf relative water content, osmolytes accumulation and antioxidant compounds of pepper (Capsicum annuum L.) cultivars ('Granada', 'Goliath' and 'Nobili'), widely used in Cameroon, were investigated. Plants were subjected to four levels of NaCl (0, 50, 100 and 200 mM) at early seedling growth stage of plant development. Application of NaCl treatment led to a significant increase in total soluble sugars, proline, related to its induce of antioxidative enzyme system more efficiently, resulting in higher osmolytes accumulation under salinity. 'Granada' was more tolerant and stable in physiological and biochemical traits suggesting that it could be grown in salt-affected soils (Rana, 2002).

The salinity of soils formed out of saline parent materials, such as some old lake beds, may be impossible to change. The minerals in the soil are inherently high in salts, and as the minerals weather and are leached with water, they will continue to release salts into the soil solution. However, for soils that have become saline over time due to reasons stated above, such as improper irrigation, reclamation is feasible. (Miyamoto et al., 2004). Most reclamation approaches to treating saline soils involve leaching (flushing) of the soil with clean/relatively pure water. Sufficient water must be applied to dissolve the excess salts that have accumulated and cause them flow out of the soil profile, particularly the root zone. To accomplish this leaching of salts, adequate drainage is requisite. Once good drainage is assured, the soil can be irrigated with clean water. Runoff should be avoided to prevent erosion (Miyamoto et al., 2004).

The rate of infiltration or flow of water into the soil will determine how quickly water can be applied. The rate will be dependent on the type of soil. Fine-textured soils, such as clayey soils, will have slower infiltration rates than coarse-textured soils. Any restrictive layer, such as a plow pan, will slow the flow of water down through the soil. In all limiting cases, measures must be taken to improve drainage. The rate of infiltration will be faster initially, but will reach a constant rate. Observation and monitoring will be required to achieve leaching of salts while avoiding run-off. As a starting point, apply 6 inches of water to reduce salinity by 50% and 12 inches of water to reduce salinity levels by 80%. 24 inches of water may need to be applied to reduce salinity levels by 90%. Irrigation via sprinklers is best for sloped areas, but if necessary, flood irrigation may be used on level areas if berms or basins are used to contain the water (Miyamoto et al., 2004).

Testing initial soil salinity levels will enable determination of how much water should be applied to reduce salt concentrations to acceptable levels. Post-leaching soil salinity tests will ensure that saline-soil reclamation has been successful (Miyamoto et al., 2004).

### **Humic Acid**

Humic acid is a group of molecules that bind to, and help plant roots receive, water and nutrients. High humic acid levels can dramatically increase yields. (SoilBiotics, 2019)

Humic and Fulvic acids are the final break-down constituents of the natural decay of plant and animal materials. These organic acids are found in pre-historic deposits. Humic matter is formed through the chemical and biological humification of plant and animal matter and through the biological activities of micro-organisms. Humic acids are complex molecules that exist naturally in soils, peats, oceans and fresh waters. The one source of humic acids is the sedimentation layers referred to as Leonardite.

These layers were originally deep in the earth's crust, but over many years have been exhumed to near-surface location. Humic acids are found in high concentration in these layers. Leonardite is organic matter, which has not reached the state of coal and differs from soft brown coal by its high oxidation degree, a result of the process of coal formation, and has no value as fuel. The decomposition of concentrated organic acids is a lengthy process taking millions of years in the natural environment. Imagine, if you will, a prehistoric marsh or peat bog. Plants are harvesting carbon dioxide from the atmosphere and using the sun's energy to build plant biomass. These plants feed insects and vertebrates. As plants and animals die, they contribute their carbon back to the bottom of the bog. Over millions of years this cycle of organic matter is concentrated and compressed into layers in the earth (SoilBiotics, 2019).

What is it used for in agriculture? Leonardite is not a fertilizer. It acts as a conditioner for the soil and as a bio-catalyst and bio-stimulant for the plant. Humic acids are an excellent natural and organic way to provide plants and soil with a concentrated dose of essential nutrients, vitamins and trace elements. Compared to other organic products, Leonardite enhances plant growth (biomass production) and fertility of the soil. Another advantage of Leonardite is its long-term effectiveness, as it does not get consumed as quickly as animal manure, compost or peat. Leonardite decomposes completely; therefore, it does not enter into nutritional competition with plants for nutrients like nitrogen. This is not the case with partially decomposed compost, whereby the organic substances in soil are rapidly consumed by microorganisms and mineralized entirely without humus formation (SoilBiotics, 2019).

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 ameliorate 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).

The objectives of this study were:

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- 1- To investigate the integrated effects of humic acid fertilizer on soybean growth and nutrient uptake.
- 2- To understand the mechanism underlying soybean salt tolerance
- 3- 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.
- 4- To study Plant growth parameters and physiological and biochemical changes in soybean seedlings under salt stress.





#### 2. LITERATURE REVIEW

Streeter et al., (2001), Study indicate that methylated cyclitols are potentially important osmolytes in plants. In a search for genetic diversity for pinitol (D-3-Omethyl-chiro-inositol) accumulation in soybean, found that genotypes that accumulated high concentrations of pinitol, when grown under well-watered conditions, had been selected for performance in regions of China having low rainfall, A detailed study of pinitol accumulation in the soybean plant showed two- to three-fold gradients in pinitol concentration from the bottom to the top of the plant.

Kondetti et al., (2012), The effect of salinity stress on eleven (Co-1, CoSoy-2, DS-40, GujratSoy-1, JS-80-21, MACS-13, MAUS-2, NRC-2, PalamSoy, Pusa-16 and Shilageet) Indian soybean varieties were analyzed under increasing salinity levels (0, 120, 180, 240 and 300 mM) of NaCl. Salinity had adverse effects on germination and all the physiological parameters (root length, shoot length, root/shoot ratio, dry matter production in root and shoot, moisture content in root and shoot) for early seedling growth. The results revealed that varietal difference was present for all the parameters. The varietal difference was pronounced at high (240 and 300 mM) salt concentrations of NaCl. Co-1, GujratSoy-1 and NRC-2 varieties were salt sensitive and CoSoy-2, DS40, PalamSoy, Pusa-16 varieties were salt tolerant, and rest varieties were moderate in their response towards salt.

Çimrin et al., (2010), The objective of the study was to determine the effect of humic acids and phosphorus on growth and nutrient content of pepper seedlings (cv. Demre) grown under moderate salt stress in growth chamber conditions. Also, N, P, K, Ca, S, Fe, Mn, Zn and Cu contents of root were increased with humic acid application. Na contents of both shoot and root of pepper decreased with increased humic acid doses. It was concluded that high humic acid doses has positive effects on salt tolerance based on the plant growth parameters and nutrient contents.

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Abdel-Monaim et al., (2011), The ability of benzothiadiazole (BTH), humic acid (HA) and their combination when used as seed soaking to induce systemic resistance against a pathogenic strain of Fusarium oxysporum was examined in four soybean cultivars under greenhouse conditions. Similar results were obtained in the case of total phenol but HA increased the total phenol more than BTH in all tested cultivars.

Hanafy et al., (2013), This study was carried out to determine the effects of putrescine (Put) and humic acid (HA) foliar applications on growth, yield and chemical composition of Egyptian cotton (*Gossypium barbadense* 90) plants grown under saline soil condition. As a result of promoting growth induced by previous foliar applications, yield components e.g.; number of totals, open and closed bolls, seed cotton yield/plant, lint percentage and seed index were increased. Application of 2 ppm Put and 1% HA recorded the highest values of growth and yield characters.

Agarwal et al., (2015), Studied the effect of salinity on germination and seedling growth of soybean. For this, 15 soybean genotypes were tested in sand culture experiment. The seeds were irrigated with saline waters of different EC levels (0, 3, 6, 7.2, 10, 12, 14 dSm<sup>-1</sup>). Length and dry weight of root and shoot as well as PR were evaluated under salinity at 7 DAS. The results showed that shoot growth was affected more adversely than root growth. Cultivars showed a wide range of variation in their salinity tolerance as mediated by, PR (percent reduction in seedling dry weight over control) and SSI (salinity susceptibility index). PK 1029 and PK 416 exhibited higher levels of tolerance to salinity compared to the other cultivars.

ÇavuĢoğlu (2015), The effects of humic acid (HA) pretreatment on the seed germination, seedling growth and leaf anatomy of barley under both normal and saline conditions were studied. The results indicated that salinity of the medium caused changes in the leaf anatomy of seedlings. HA affected in different degrees the various parameters of leaf anatomy of barley seedlings grown in both normal and saline conditions, and this difference were statistically significant.

Kumari et al., (2015), Explored several features related to salt tolerance in soybean plants through plant growth-promoting rhizobacteria. They were investigated the leaf water content, osmolyte accumulation, and activities of stress-responsive enzymes in the absence and presence of salt stress. Gawlik et al., (2016), Carried out a laboratory research to examine the impact of humic acids (HA) on swelling and germination of 'NawiMSE' and 'Progres' soybean seeds under salt stress. The results showed that HA mitigate the negative impact of salinity and water deficit on swelling and germination of soybean seeds.

Said-Al Ahl et al., (2016), Studied the behavior of this plant and its cultivation under the conditions of soil salinity in El-Tinaplain area as a step towards the development of Sinai Peninsula. In 2010/2011 and 2011/2012, a field experiment was conducted in Egypt to evaluate the effect of Humic Acid (0 and 400 ppm), Indole Acetic Acid (0 and 400 ppm) and region (Nile Valley and Delta, Giza governorate) and (SinaiPeninsula, North Sinai governorate) on dill productivity, oil content and its composition. Results demonstrated that dill straw can be explored as a new source of essential oil. Generally found that the cultivation of dill in Giza gave the best results from cultivation in the North Sinai.

Pi et al., (2018), Salinity causes osmotic stress to crops and limits their productivity. To understand the mechanism underlying soybean salt tolerance, proteomics approach was used to identify phosphoproteins altered by NaCl treatment. Results revealed that 412 of the 4698 quantitatively analyzed phosphopeptides were significantly up-regulated on salt treatment.

Akladious and Mohamed, (2018), Work was carried out in order to determine the effects of calcium nitrate and humic acid applications either separately or in combination on the growth and fruit yield quality of pepper plants under salt stress condition. The combined treatment of calcium nitrate and humic acid applications:1500 mg/kg soil (HA2) was the most effective one on the previous criteria under salt stress conditions. Therefore, the usage of humic acid and calcium could be suggested to improve the soil properties, growth and antioxidant capacity of pepper plants and to mitigate the damage caused by salt stress.

Ghassemi-Golezani and Farhangi-Abriz, (2018), Conducted a greenhouse experiment with factorial arrangement based on randomized complete block design with four replications was conducted in 2015 to evaluate the effects of salicylic acid (SA) (1 mM) and jasmonic acid (JA) (0.5 mM) on oil accumulation and fatty acid composition of soybean oil (*Glycine max* L.) under salt stress. They concluded that oil percentage of soybean seeds increased, but seed and oil yields decreased with increasing salinity.

Abdulameer and Ahmed, (2019), A field experiment was carried out to study the role of humic acid in improving some growth characters of corn (*Zea mays* L.). The effect of interaction between two variables was significant for all the studied traits except the number of days to tasseling and number of leaves plant-1. It was concluded that in the case of phosphopeptides irrigation water, it could be irrigation with 60 % of the available water with 80 Kg ha-1 humic acid is practiced.

Akcin and Akcin, (2019), Conducted a study to investigate the effects of humic acid (HA) on photosynthetic pigment and malondialdehyde content (MDA) against chromium stress in *Triticum aestivum* L. It was concluded that HA application eliminated the toxicity of Cr stress by modulating the photosynthetic activities in wheat.

Baldotto et al., (2019), Theit study aimed to evaluate the response of high-yield corn to humic acids, isolated from organic manure compost, with or without the application of lime and fertilizer. Biomass productivity was evaluated at harvest time. Humic acids, whether applied as a seed coating, increased yield by ~15% higher than conventional farm cultivation, and this difference was statistically significant. Therefore, the use of humic acids-based plant regulators is positive and complementary to the inputs generally used for corn yield.

Belal et al., (2019), During the 2017 and 2018 seasons, two field experiments were conducted on newly reclaimed saline calcareous soil (7.13 dS m<sup>-1</sup>, 16.9% CaCO3) in the experimental farm of the Faculty of Agriculture, Demo, Fayoum Governorate, Egypt. The current work aimed at identifying the potential positive effects of applied humic acid (HA) and elemental sulfur (S) on some soil properties and barley plant performance. The results showed that the application of HA and/or S at different rates ameliorated the adverse effects of saline calcareous soil conditions and significantly reduced some chemical properties of the soil.

Bezuglova et al., (2019), Established a project to study the effect of humic preparation on the yielding capacity of winter wheat, the dynamics of mineral nutrients in the rhizosphere, and the activity of rhizosphere microbial community, as well as the protective properties of humate treatment under the stress caused by the application of a sulfonylurea herbicide. The results of production experiment showed that the application of sulfonylurea herbicide induced a chemical stress on winter wheat plants, but the use of humic preparation reduced this effect and increased the availability of phosphorus compounds. The treatment of plants with pesticides caused the general decrease in abundance of bacteria in the rhizosphere. The effect on quickly growing bacteria is more pronounced, while slowly growing bacteria and fungi are more resistant to this impact.

Dawood et al., (2019), Two field experiments were conducted at the Research and Production Station, Egypt, during the two successive winter seasons of 2014/2015 and 2015/2016. This work aimed to study the enhancement effect of foliar application of nicotinamide at 5, 10, and 20 mg/L and/or humic acid at 5% on quality and quantity of faba bean plants grown under sandy soil conditions. Data show that nicotinamide at 5, 10, and 20 mg/L and/or humic acid at 5% had a positive effect on growth parameters, photosynthetic pigments, seed yield.

Ekin (2019), In sustainable agriculture, seeking eco-friendly methods to promote plant growth and improve crop productivity is a priority. Humic acid (HA) and plant growth promoting rhizobacteria (PGPR) are among the most effective methods that utilize natural biologically-active substances. The aim of the theit study was to analyze the effect of the presence of HA on potato (*Solanum tuberosum* L.). The results demonstrated that this integrated approach has the potential to accelerate the transformation from conventional to sustainable potato production.

Kataria et al., (2019), Field experiment was conducted to study the influence of magneto priming with static magnetic field (SMF of 200 mT for 1 h) on growth, nitrogen fixation, photosynthesis, antioxidative system and yield of soybean under salt stress. The results revealed the adverse effect of salinity on growth, photosynthesis, nitrogenase activity and yield. Salt stress significantly elevated the level of hydrogen peroxide (H2O2) and ascorbic acid (ASA) and the activities of superoxide dismutase (SOD), ascorbic acid peroxidase (APX), glutathione reductase (GR) and guaiacol peroxidase (POD) in leaves of soybean seedlings emerged from unprimed seeds. Liu et al., (2019), The objective was to investigate the integrated effects of humic acid fertilizer and vermicompost on maize growth and nutrient uptake in coastal saline soil. the result important role in increasing the maize yield in coastal saline soil. Therefore, the application of humic acid fertilizer and vermicompost can be integrated as a practice for improving coastal saline soil.

Marenych et al., (2019), The aim of the research was to establish the effectiveness of preparations, made on the basis of humic and fulvic acids on the yields of crops in case of different methods and amounts used. The experiments were held with varieties and hybrids of winter wheat, soya, corn, and sunflower. Based on the obtained results of investigation during the period of 2015–2017, proceeding from the results of the research, the using of growth stimulators based on humic and fulvic acids, which contain high concentrations of these substances, can be recommended as an expedient and efficient measure of raising the productivity and improving qualitative indicators of corn, sunflower, soya, and winter wheat yields.

SoilBiotics, (2019), Conducted a field study in Iowa over four environments from 2014 examining productivity of soybean (*Glycine max* L.). The environments where humic product application positively influenced yield and seed quality, had greater rainfall deficits and air temperatures above the long-term average.

Pinos et al., (2019) The study aimed to characterize humic substances (HS) different origins and to evaluate the effects on germination and protective effects against salt stress in corn. The results were important for agriculture because maintaining HS with adequate structural quality in soils may protect plants from eventual periods of high salinity.

Rosa et al., (2019), To investigate the integrated effects of humic acid fertilizer on soybean growth and nutrient uptake. Humic acid (HA) use can improve phosphorus (P) availability in soils with high P fixation capacity. The aim of this study was to evaluate soil P availability and soybean growth in both medium-texture (MT) and clayey (CL) Oxisols under humic acidresidual resin-P in MT fertilized with Araxá phosphate rock (APR). Effects of HA on soil attributes, soybean growth and nutritional status rely on HA concentration, soil texture and P source used.

### **3. MATERIALS AND METHODS**

#### **3.1 Materials**

In the present study, Glksoy soybean variety obtained from Trakya Agricultural Research Institute was used as seed material in the experiments.

The experiment was carried out in 48 plastic pots with 500 cc capacity. The experimental design was Factorial 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 nitrogen, 45 mg / kg phosphorus and 75 mg / kg 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.

#### **3.2. Methods**

Humic acid doses was mixed into the soil before planting, and 300 mg / kg nitrogen, 150 mg / kg phosphorus and 200 mg / kg 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.



Figure 3.1. Soybean plant in pots.

## **3.2.1.** Growth parameters

**3.2.1.1 Root length (cm):** From the most extreme part of the root part of the plants up to the root neck was found by measuring.



Figure 3.2. Root length of soy plant.

**3.2.1.2 Plant height (cm):** The height of the plants was measured from the soil level to the highest point of the plant.



Figure 3.3 Stem length of soybean plant.

**3.2.1.3 Root fresh weight (g):** After separating the root part of the plants representing the applications, root fresh weight was determined using a sensitive balance.

**3.2.1.4 Plant fresh weight (g)**: After the plants representing the applications were cut at soil level, the fresh plant weight was determined using a sensitive balance.

**3.2.1.5 Root dry weight (g):** After the harvest, plant samples were kept in the oven at 70 °C for 48 hours and the root dry weight was calculated.

**3.2.1.6 Plant dry weight (g):** After the harvest, plant samples were stored in the oven at 70 °C for 48 hours and the root dry weight was calculated.

# 3.2.2 Physiological and biochemical changes in the plant

**3.2.2.1 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 25°C for

2 hours, and turgor weights were weighed. The samples were then dried at  $110^{\circ}$  C for 24 hours to record their weight. Arora et al. (1998) equation was used for calculating. RWC (%) = [(fresh weight-oven dry weight)/(turgor weight - oven dry weight)] x100



Figure 3.4. Water content due to leaf tissues.

**3.2.2.2. 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] 106



Figure 3.5. Determination of lipid peroxidation levels.

**3.2.2.3. 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). Ion Leakage in Leaf Tissues = (C1 / C2) x100



Figure 3.6. Determination of ion leakage in leaf tissues.

**3.2.2.4. 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):

Membrane Endurance Index in Leaf Tissues (%) =  $[1 - (C1 / C2)] \times 100$ .

**3.2.2.5. 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.

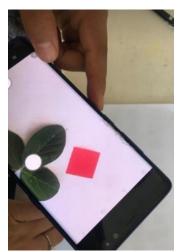


Figure 3.7. Determination of leaf surface area.

**3.2.2.6. 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.



Figure 3.8. Determination of total chlorophyll ratio.

# 3.2.3. Statistical analysis

The data obtained from the study was analyzed by using the Düz Costat statistical package program and the averages of the applications with significant impacts was grouped according to the Duncan multiple comparison test.

#### 4. RESULTS 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.

#### **4.1 Root Length (cm)**

Variance analysis results of the root lengths in soybean grown under salt stress in Humic Acid applications are given in Table 4.1. According to the results, there was a 5% statistical difference between HA dose applications in term of root length, while the effect of salt doses was non-significant. In addition, Salt x HA interaction was statistically significant at the 1% level.

Acid applicat	ions		
Source	DF	Mean Square	F Value
Salt (S)	2	3.770	0.446 <sup>n.s</sup>
Humic Acid (HA)	3	29.243	3.460*
S x HA	6	104.743	12.393**
Error	36	8.451	
Total	47		

Table 4.1 Analysis of variance of root length values for different salt stress and Humic Acid applications

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

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 (Table 4.2.). 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

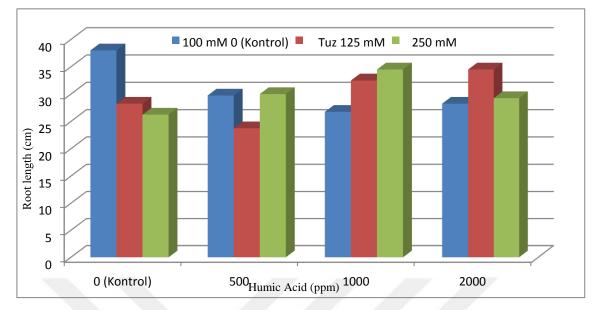
increased salt concentrations had a negative effect on the root length values of the plant. Kondetti et al. (2012), found that root seedling decreased linearly when the salt concentrations increased. The reduction of growth is a common phenomenon of many crop plants grown under saline conditions and our findings are in accompany with the earlier reports.

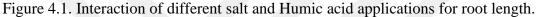
The effect of different dosage of Humic Acid application on the average soybean plant root length grown under salt stress varied between 27.8 and 31.3 cm (Table 4.2.). In this study, the longest root length (31.3 cm) was obtained from 1000 g Humic Acid application, and the lowest (27.8 cm) was determined in 500 g HA 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, BaGalma (2014), Malik and Azam (1985) reported that application to Humic Acids to wheat increases root length.

The interaction of both treatments was highly significant (Table 4.1.). However, the control treatment (0 mM NaCl and 0 g HA) produced plants with 38.0 cm roots, and 23.7 cm was the root length of plants grown under salt stress of 125 mM and 500 g HA, respectively (Figure 4.1).

Humic Acid Doses (ppm)								
		0 (Control)	500	1000	<u>2000</u>	Average		
	0 (Control	l) 38.0 a	29.7 а-с	26.7 bc	28.3 a-c	30.7		
Salt	125 mM	28.3 bc	23.7 d	32.5 ab	34.5 ab	29.7		
Doses	250 mM	26.3 b-d	30.0 a-c	34.5 ab	29.3 а-с	30.0		
	Average	<u>30.8 A</u>	<u>27.8 B</u>	31.3 A	30.7 A			
LSD (%5): Salt: 2.40; HA: 2.08, SXHA; 2.71								
C.V (%	): 9.579							

Table 4.2. Average root length values (cm) for different salt and Humic Acid doses
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#### 4.2 Plant Height (cm)

Variance analysis results of the root lengths in soybean grown under salt stress in Humic Acid applications are given in Table 4.3. According to the results of the analysis, there was a 1% statistical difference between HA and salt doses in term of plant height, while the Salt x HA interaction was non-significant.

Table 4.3. Analysis of variance of plant height values for different salt stress and Humic Acid applications

Source	DF	Mean Square	F Value
Salt (S)	2	61.75	11.087**
Humic Acid (HA)	3	63.916	11.476**
S X HA	6	10.25	1.840 <sup>n.s</sup>
Error	36	5.569	
Total	47		

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

The effect of salt stress was significantly high on the soybean plants and control application (0 mM NaCl) produced taller plants. Average plant 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 plant height.

The results from Table 4.4 shows that HA dose 0 g (control) produced plants with the highest value of plant height as 28.3 cm, while all the other application doses (500, 1000 and 2000 g) were in the same comparison group. Furhermore, 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 plant 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 plant height was reduced with the increase of HA doses.

The interaction of S x HA was non-significant. However, the highest plant height value (30.5 cm) was obtained from 0 mM NaCl (control) with 0 g HA (control), and the lowest (20.7 cm) value was found in 250 mM NaCl with 500 g HA (Figure 4.2).

	ę	1 0	`	·				
Humic Acid Doses (ppm)								
		<u>0 (Con</u>	trol) 50	0	1000	2000	Average	
	0 (Cont	trol) 30.5	27	7.3	26.5	23.7	27.0 A	
Salt	125 mM	26.3 23.	5 23.5	24.7	24.5 AB	C Doses	<b>250 mM</b> 28.0 20.7	
23.0 2	23.0 20.7 23.1 C Average 28.3 A 23.8 B 24.3 B 23.1 B							
LSD	(%5): Salt: 1.9	5; HA: 1.69						
<b>C.V</b> (	%): 9.469			-				

Table 4.4. Average plant height values (cm) for different salt and Humic Acid doses

\* Values belonging to the same letter group are not important according to Duncan 5%.

#### 4.3 Root Fresh Weight

The analysis of variance results of the soybean plants for fresh root weights are given in Table 4.5. According to the results, the salt doses had a significant effect at the 5% level, and the interaction of S x HA had a significant effect on the roots fresh weight at the 1% level, while the effect of the HA was non-significant.

Tunne Acia	applications		
Source	DF	Mean Square	F Value
Salt (S)	2	0.266	3.975*
Humic Acid (HA)	3	0.019	0.285n.s
S X HA	6	0.623	9.298**
Error	36	0.067	
Total	47		

Table 4.5. Analysis of variance of root fresh weight values for different salt stress and Humic Acid applications

\* P <0.05 significant. \*\* P <0.01 high significant, <sup>n.s</sup>: non-significant.

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 (Table 4.6.).

The highest value of root fresh weight for the HA treatment was 1.61 g obtained from the application of 2000 g HA, and the lowest value was 1.52 g obtained from 1000 g HA. However, the effect of the HA different doses was statistically non-significant on root fresh weight.

BaĢalma (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. Dincer and 180 g of Humic Acids treatment.

The S x HA interaction gave the highest value of rot fresh weight (2.082 g) under 0 mM NaCl with 500 g HA treatment. The lowest value was 1.121 g obtained from the 125 mM NaCl with 1000 g HA (Figure 4.2).

Table 4.6. Average root fresh weight values (g) for different salt and Humic Acid doses

	0 (Control)	500	1000		
		500	1000	2000	Average
Control)	1.28 bcd	2.08 a	1.56 a-d	1.23 cd	1.54 AB
5 mM	1.78 ab	1.18 d	1.12 d	1.78 ab	1.46 B
0 mM	1.72 abc	1.4 6 bcd	1.87 a	1.81 a	1.72 A
verage	1.59	1.57	1.52	1.61	
(	5 mM 0 mM verage	5 mM       1.78 ab         0 mM       1.72 abc         verage       1.59	5 mM       1.78 ab       1.18 d         0 mM       1.72 abc       1.4 6 bcd         verage       1.59       1.57	5 mM1.78 ab1.18 d1.12 d0 mM1.72 abc1.4 6 bcd1.87 a	5 mM1.78 ab1.18 d1.12 d1.78 ab0 mM1.72 abc1.4 6 bcd1.87 a1.81 arerage1.591.571.521.61

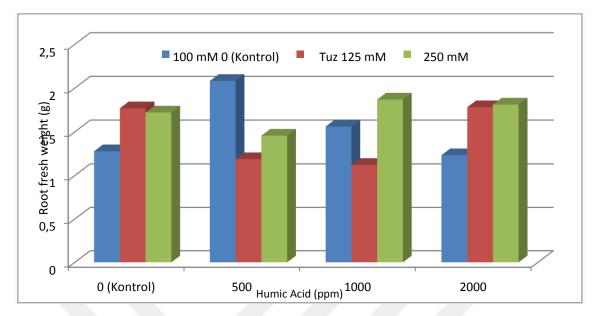


Figure 4.2. Interaction of different salt and Humic acid applications for root fresh weight.

### 4.4. Plant Fresh Weight

The analysis of variance results of the soybean plant fresh weight is given in Table 4.7. According to the results, the different salt doses, Humic Acid and their interaction a significant affect 1% on the roots fresh weight.

Table 4.7. Analysis of variance of plant fr	esh weight values for differen	t salt stress and
Humic Acid applications		

Source	DF	Mean Square	F Value
Salt (S)	2	0.440	9.558**
Humic Acid (HA)	3	0.486	10.571**
S X HA	6	0.217	4.719**
Error	36	0.046	
Total	47		

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

The different salt concentrations had a significant effect on the plant fresh weight. The highest weight was 1.41 g obtained from the control treatment 0 mM NaCl, while the lowest plant fresh weight was 1.07 g obtained from application of 125 mM

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NaCl. In the study, different salt concentration applications are adversely affected by the plant fresh weight values compared to the control application (Table 4.8.). Tunçturk and colleagues, 2009 reported that salt stress was detrimental to plant 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., 2011, suggested the same findings but on several canola (*Brassica napus* L.) cultivars.

The effect of HA doses was significant on the plant fresh weight. The highest fresh weight was 1.37 g obtained from applying 1000 g HA, and the lowest value was 0.93 g from the 2000 g HA dose. However, the control and 500 g HA applications were in the same group with the 1000 g HA, and the value of the plant fresh weight was 1.31 and 1.31 g respectively.

In terms of S x HA interaction, the plants which received 500 g HA with 0 mM NaCl, gave the highest value of plant fresh weight 1.87 g, and the lowest value was 0.86 g from 2000 g HA with 125 mM NaCl (Figure 4.3).

These findings are similar to Dawood et al. 2019 suggestions for faba bean plants. Humic Acid application caused increases in fresh weight plant.

Humic Acid Doses (ppm)						
		0 (Control)	500	1000	<u>2000</u>	Average
	0 (Control)	1.37 ab	1.87 a	1.45 ab	0.93 bc	1.41 A
Salt	125 mM	1.28 b	0.87 c	1.28 b	0.86 c	1.07 B
Doses	250 mM	1.27 b	1.19 b	1.39 ab	0.99 b	1.21 B
	Average	1.31 A	1.31 A	1.37 A	0.93 B	
<b>LSD</b> (%5)	: Salt: 0.17; H	A: 0.15; S XH				
CV (%):	17.497					

Table 4.8. Average plant fresh weight values (g) for different salt and Humic Acid doses

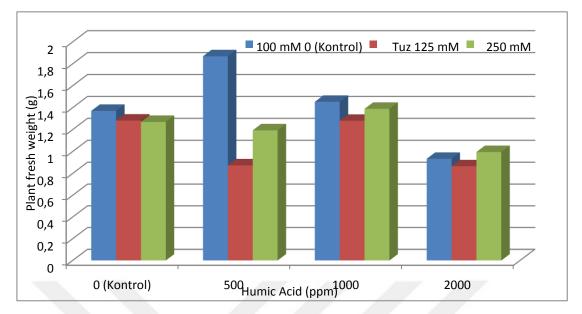


Figure 4.3. Interaction of different salt and Humic acid applications for plant fresh weight.

# 4.5. Root Dry Weight

The analysis of variance results of the soybean root dry weights is given in Table 4.9. According to the analysis, the different salt doses and Humic Acid had a significant effect at level of 1% on the roots dry weight; their interaction was significant at level of 5%.

Table 4.9. Analysis of variance of root dry weight values for different salt stress and Humic Acid applications

Source	DF	Mean Square	F Value
Salt (S)	2	0.0099	9.120**
Humic Acid (HA)	3	0.0053	4.932**
S X HA	6	0.0033	3.041*
Error	33	0.0010	
Total	47		

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

The different salt concentrations had a significant effect on the plant fresh weight. The highest 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. Tuncturk et al. (2008, 2011b) findings were similar, they suggested that salt stress affects negatively on soybean plant dry weight.

In terms of HA, the highest root dry weight was 0.28 g obtained from the application of 2000 g HA, and it was with same group with 1000 g HA with 0.26 g dry root weight. The lowest value was from the control with 0.23 g root dry weight.

BaGalma (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 poroduced plants with lower root dry weight and the highest value was from higer 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 1000 g had the highest value of root dry weight, 0.307 g, and those received 0 mM NaCl

(control) with 0 g (control) had the lowest value of root dry weight, 0.188 g (Figure 4.4).

Humic Acid Doses (ppm)						
		0 (Control)	500	1000	<u>2000</u>	Average
	0 (Control)	0.18 b	0.24 ab	0.22 ab	0.27 ab	0.23 B
Salt	125 mM	0.27 ab	0.25 ab	0.27 ab	0.29 a	0.27 A
Doses	250 mM	0.23 ab	0.28 ab	0.31 a	0.27 ab	0.27 A
	Average	<u>0.23 B</u>	<u>0.26 AB</u>	0.26 A	0.28 A	
<b>LSD</b> (%5)	: Salt: 0.027; H	IA: 0.023; S X				
<b>CV: 13.1</b>	96					

Table 4.10. Average root dry weight values (g) for different salt and Humic Acid doses

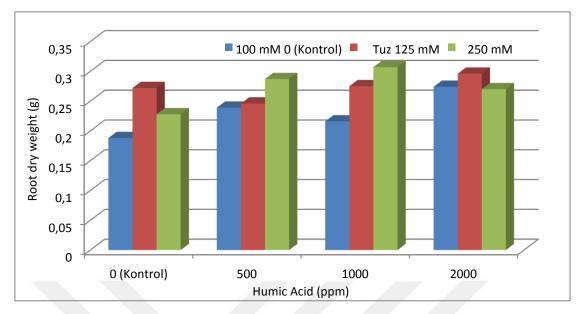


Figure 4.4. Interaction of different salt and Humic acid applications for root dry weight.

#### 4. 6 Plant Dry Weight

The analysis of variance results of the soybean plant dry weights is given in Table 4.11. According to the analysis, the different salt doses had a significant effect 5% on the plant dry weight, while the Humic Acid and the S x HA interaction was significant at level of 1%.

Table 4.11. Analysis of variance of plant dry weight values for different salt stress and Humic Acid applications

Source	DF	Mean Square	F Value
Salt (S)	2	0.0113	4.705*
Humic Acid (HA)	3	0.4085	16.897**
S X HA	6	0.0183	7.596**
Error	36	0.0024	
Total	47		

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

In this study, salt applications negatively affected plant dry weight averages. The highest plant dry weight was 0.31 g obtained from 0 mM NaCl (control) applications, while the lowest plant dry weight was 0.26 g obtained from the 125 mM NaCl application (Table 4.12).

The HA had a significant effect on plant dry weight. The highest value was 0.34 g obtained from the control and the lowest plant dry weight value was 0.22 g from the 1000 g HA dose.

For the interaction of S x HA, the highest plant dry weight value was 0.31 g obtained from the 250 mM NaCl with 1000 g HA, and the lowest value was 0.18 g obtained from control 0 mM NaCl with 0 g HA.

This result is similar to the findings of Tunçtürk et al. 2011b on Canola, salt stress caused a significant decrease in the plant dry weights. Furthermore, Kondetti et al., 2012 studied Phaseolus mungo under salt and observed that dry weight of the seedling decreased with increasing NaCl (Figure 4.5).

Table 4.12. Average plant dry weight values (g) for different salt and Humic Acid doses

	Humic Acid Doses (ppm)						
		0 (Control)	<u>500</u> 1000	2000	Average		
S-14	0 (Control)	0.18 b	0.24 ab 0.22 ab	0.27 ab	0.31 A		
Salt Degeg	125 mM	0.27 ab	0.25 ab 0,27 ab	0.29 a	0,26 B		
Doses	250 mM	0.23 ab	0.28 ab 0,31 a	0.27 ab	0.27 Ab		
	Average	0.34 A	0.33 A 0,22 B	0.23 B			
LSD (%5): Salt: 0.04; HA: 0.03; S X HA: 0.05							
<b>CV (%):</b>	17.208						

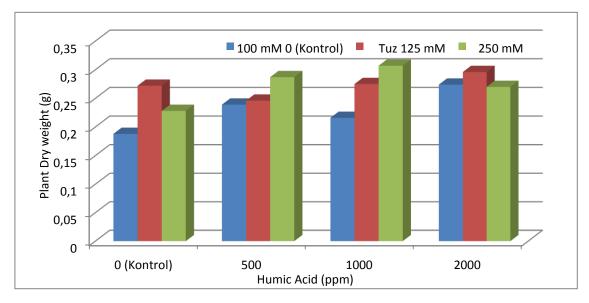


Figure 4.5. Interaction of different salt and Humic acid applications for plant dry weight.

#### 4.7. Relative Water Content in Leaf Tissues

Relative water content in leaf tissues data were summarized in Table 4.13. According to the results of the data analysis, there were no significant differences among salt and S X HA interaction on the plants for this trait. But there was a 5% statistical difference between HA dose applications in term of RWC (%).

applicatio	ns			
Source	DF	Mean Square	F Value	
Salt (S)	2	269.71	0.902 <sup>n.s</sup>	
Humic Acid (HA)	3	394.83	1.320 *	
S X HA	6	351.40	1.175 <sup>n.s</sup>	
Error	36	298.98		
Total	47			

Table 4.13. Analysis of variance of RWC values for different salt stress and Humic Acid applications

\* P <0.05 significant. \*\* P <0.01 high significant, <sup>n.s</sup>: non-significant.

In terms of salt doses (Table 4.14.), the highest value of the RWC was 71.85% obtained from 250 mM NaCl application, and the lowest value was 63.86% obtained from the control (0) mM NaCl (Table 4.14).

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 doses 500 and 2000 g respectively.

The highest value of RWC for the S x HA interaction was 87.58% obtained from 250 mM NaCl with 500 g HA, and the lowest RWC value was 46.27% obtained from 0 mM NaCl (control) with 0 g HA (control).

Leaf relative water content Leaf RWC 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. Similar results were obtained by Salwa et al. (2010) with peanut cultivars.

On the contrary, a significant decrease in RWC was found at high salinity level

(200 mM) in all cultivars. These results may be attributed to the accumulation of toxic ions such as Na+ and Cl-, reducing leaf expansion and stomata closure leading to a reduction in intracellular CO2 partial pressure (Hasegawa et al., 2000). According to Munns (2002) studies, salinity reduces the ability of plants to take up water, and this quickly causes reductions in growth rate, along a suite of metabolic changes identical to those causes by water stress.

Humic Acid Doses (ppm) 2000 Average 0 (Control) 500 1000 0 (Control) 65.49 63.86 74.92 68.78 46.27 Salt 125 mM 61.99 67.36 66.22 68.35 67.18 Doses 250 mM 66.40 65.38 87.58 65.26 69.20 71.85 Average Ab 67.13 AB 60.88 B 74.83 A LSD (%5): Salt: 14.31; HA: 12.39 C.V (%): 13.26

Table 4.14. Mean of RWC (%) values for salt stress and Humic Acid applications

\* Values belonging to the same letter group are not important according to Duncan 5%.

#### 4.8. Lipid Peroxidation Levels Determination

The results of the analysis of variance of the effects of Humic Acid doses on MDA of soybean plants grown under salt stress are given in Table 4.15.

Table 4.15. Analysis of variance of MDA values for different salt stress and Humic Acid applications

Source	DF	Mean Square	F Value
Salt (S)	2	0.1547	61.7**
Humic Acid (HA)	3	0.0118	4.7**
S X HA	6	0.0032	1.2 <sup>n.s</sup>
Error	36	0.0025	
Total	47		

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

Mean values of MDA and Duncan groups are given in Table 4.16. According to the statistical analysis using ANOVA, the effect of Salt and HA significant at the level of 1%, and the S x HA interaction was salt doses were non-significant for this trait.

Table 4.16. Average MDA values (nmol g <sup>-1</sup> F.W) for different salt and Humic Acid dose	20
Table 4.10. Average MDA values (innor g F. w) for different sait and Humic Acid dose	38

Humic Acid Doses (ppm)					
	0 (Control)	500	1000	2000	Average
0 (Contr	<b>•ol</b> ) 0.63	0.53	0.61	0.56	0.58 c
Salt Doses					
125 mM	0.75	0.68	0.67	0.64	0.68 b
250 mM (	0.81 0.78 0.75 0.7	'8 <b>0.78 a</b>	Average	0.73 a 0.66 b 0	.67 ab 0.66 b
LSD (%5): Salt: 0.02; HA	A: 0.01				
C.V (%): 9.96					

\* Values belonging to the same letter group are not important according to Duncan 5%.

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) (Table 4.16).

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.

HA had a significant effect on the soybean plants for MDA. The 0 g HA (control) had the highest MDA value 0.73 nmol  $g^{-1}$  F.W, and the MDA content in the 2000 g HA application was the lowest 0.66 nmol  $g^{-1}$  F.W.

Similar results was discovered by Chen et al. 1990 and Kıran et al. 2019, they documented that the application of HA on plants under stress reduces the MDA significantly.

#### 4.9 Determination of Ion Leakage in Leaf Tissues

The results of the analysis of variance of the effects of Humic Acid doses on ion leakage in leaf tissues of soybean plants grown under salt stress are given in Table 4.17. Mean values of ion leakage in leaf tissues and Duncan groups are given in Table 4.18. According to the statistical analysis using ANOVA, the effect of HA and the salt doses was significant at the level of 1% and 0.5 % and the S x HA interaction were nonsignificant for this trait.

Source	DF	Mean Square	F Value
Salt (S)	2	14.257	1.338 *
Humic Acid (HA)	3	23.759	2.23 **
S X HA	6	8.106	0.761 <sup>n.s</sup>
Error	36	10.650	
Total	47		

Table 4.17. Analysis of variance of ion leakage in leaf tissue values for different salt stress and Humic Acid applications

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

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 (Table 4.18).

In terms of HA doses, the highest value of this parameter was 5.26 % obtained from the 1000 g HA application, and the lowest value was 2.08 % obtained from the application of 0 g HA (control).

Humic A <u>cid Doses (ppm)</u>						
		0 (Control)	500	1000	<u>2000</u>	Average
	0 (Control)	2.40	2.11	3.49	3.69	<b>2.93</b> b
Salt	125 mM	1.88	4.44	5.67	7.03	<b>4.75</b> a
Doses	250 mM	1.96	2.65	6.61	2.49	<b>3.43</b> ab
	Average	2.08 c	<b>3.07</b> b	5.26 a	<b>4.41</b> ab	
LSD (%5)	: Salt: 2.70; HA: 2	.34				
CV (%)	: 11.21					

 Table 4.18.
 Average ion leakage in leaf tissue values (%) for different salt and Humic

 Acid doses

#### 4.10 Membrane Resistance Index of Leaf Tissues

The results of the analysis of variance of the effects of Humic Acid doses on the leaf tissues membrane resistance index of soybean plants grown under salt stress are given in Table 4.19. Mean values of ion leakage in leaf tissues are given in Table 4.20.

According to the statistical analysis using ANOVA, there were no significant differences among salt, HA and their interaction on the plants for this trait.

Table 4.19. Analysis of variance of membrane resistance index values for different salt stress and Humic Acid applications

Source	DF	Mean Square	F Value
Salt (S)	2	26.217	0.711 <sup>n.s</sup>
Humic Acid (HA)	3	11.897	0.322 <sup>n.s</sup>
S X HA	6	32.707	0.887 <sup>n.s</sup>
Error	36	36.841	
Total	47		

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

No significant effect was found for the application of the different salt and HA doses on the soybean plants for this parameter.

Membrane resistance index of leaf tissues obtained as a result of different salt applications varied between 87.90-90.42 %. According to the salt sources, the highest value was 90.42 % obtained from 250 mM NaCl, and the lowest value was 87.90 % 125 mM NaCl (Table 4.20).

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 highest value was 90.31 % obtained from 2000 g HA application on soybean plants, and the lowest value was 87.96 % obtained from control. 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 (Table 4.20).

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.

Humic Acid Doses (ppm)						
		0 (Control)	500	1000	2000	Average
Sal4	0 (Control)	88.51	85.66	89.59	91.38	88.78
Salt	125 mM	90.27	86.75	89.08	85.51	87.90
Doses	250 mM	87.15	91.47	89.04	94.04	90.42
	Average	87.96	89.24	89.24	90.31	
LSD (%5)	: ns					
CV (%):	8.99					

 Table 4.20. Average membrane resistance index values (%) for different salt and Humic

 Acid doses

\* Values belonging to the same letter group are not important according to Duncan 5%.

#### 4.11 Leaf Surface Area

Variance analysis results of the leaf surface area in soybean grown under salt stress in Humic Acid applications are given in Table 4.21. According to the results, there was a 1% statistical difference between HA dose applications in term of leaf surface area, while the effect of salt doses was non-significant. In addition, Salt x HA interaction was statistically significant at the 1% level.

Table 4.21. Analysis of variance of leaf area values for different salt stress and Humic Acid applications

i ieia appile	ations				
Source	DF	Mean Square	F Value		
Salt (S)	2	5.095	2.30 <sup>n.s</sup>		
Humic Acid (HA)	3	59.796	26.997**		
S X HA	6	11.739	5.30**		
Error	33	2.214			
Total	47				

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

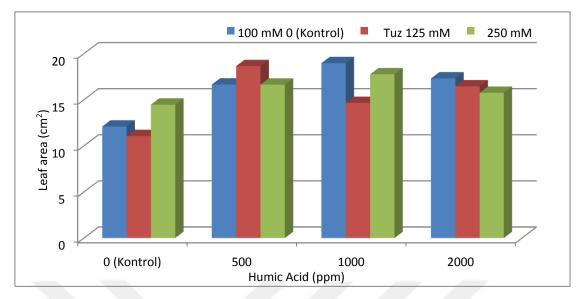
The highest value of leaf surface area was  $16.21 \text{ cm}^2$  obtained from the control applications, and the lowest value was  $15.20 \text{ cm}^2$  obtained from the 125 mM NaCl application. However, there was no significant differences when the data were statistically analyzed (Table 4.22). The effect of HA was significant, the highest value of leaf surface area was 17.25 cm<sup>2</sup> obtained from the 500 g HA applications, and the lowest value 12.54 cm<sup>2</sup> was obtained from the control (Table 4.22).

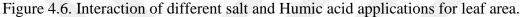
The interaction of S x HA was significant; the highest value of leaf surface area was 18.97 cm<sup>2</sup> obtained from the 0 mM NaCl with 1000 g HA applications. However, this treatment was with the same group with 125 mM NaCl with 500 g HA and 250 mM NaCl with 1000 g HA applications with values of 18.65 and 17.76 cm<sup>2</sup> respectively. The lowest value was 11.06 cm<sup>2</sup> obtained from 125 mM NaCl and control HA applications (Figure 6).

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.

Humic Acid Doses (ppm)						
		0 (Control)	<u>500</u>	<u>1000</u>	<u>2000</u>	Average
Salt	0 (Control)	12.10 cd	16.65 ab	18.97 a	17.31ab	16.21
Salt	125 mM	11.06 d	18.65 a	14.66 bc	16.45ab	15.20
Doses	250 mM	14.47 bc	16.64 ab	17.76 a	15.75b	16.15
	Average	12.54 B	17.25 A	17.13 A	16.49 A	
LSD (%5): Salt: 1.23; HA: 1.06; SXHA; 1.45						
CV (%): 8.894						

Table 4.22. Average leaf area values (cm<sup>2</sup>) for different salt and Humic Acid doses.





#### **4.12 Total Chlorophyll Content (SPAD)**

The results of the analysis of variance of the effects of Humic Acid doses on the total chlorophyll ratio of soybean plants grown under salt stress are given in Table 4.23. According to the statistical analysis using ANOVA, the effect of HA and the S x HA interaction was significant at the level of 1%, and the salt doses were non-significant for this trait.

Table 4.23. Analysis of variance of chlorophyll content values for different salt stress and Humic Acid applications

Source	DF	Mean Square	<b>F Value</b> 1.418 <sup>n.s</sup>	
Salt (S)	2	7.625		
Humic Acid (HA)	3	61.812	11.495**	
S X HA	6	39.605	7.365**	
Error	36	5.377		
Total	47			

\* P <0.05 significant. \*\* P <0.01 high significant, n.s: non-significant.

The highest value of total chlorophyll ratio was 45.63 obtained from the 250 mM NaCl applications, and the lowest value was 44.26 obtained from the control application. However, there was no significant differences when the data were statistically analyzed (Table 4.24).

The effect of HA was significant, the highest value of total chlorophyll ratio was 47.575 obtained from the control HA applications, and the lowest value 42.691 was obtained from the 2000 g HA (Table 4.24).

The S x HA interaction showed significant effect. The highest value was 51.05 from the 250 mM NaCl with 0 g HA, and the lowest value was 40.83 for both 0 mM NaCl with 2000 g HA and 250 mM NaCl with 1000 g HA (Figure 4.7).

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 et al. (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.

Humic Acid Doses (ppm)							
		0 (Control)	500	1000	2000	<b>Average</b>	
Salt Doses	0 (Control)	43.55 с-е	46.45 a-d	46.23 b-d	l 40.83 de 43	.65 <b>44.26</b>	
	125 mM	48.13 a-c	43.75 cd	cd 45	5.03 cd	45.14	
	250 mM	51.05 a	48.40 ab	40.83 e	42.23 de	45.63	
	Average	47.57 A	46.2 A	43.56 B	42.69 B		
LSD (%5): Salt: 1.91; HA: 1.66; SXHA; 1.85							
CV (%):	5.186						

Table 4.24. Average chlorophyll ratio values for different salt and Humic Acid doses

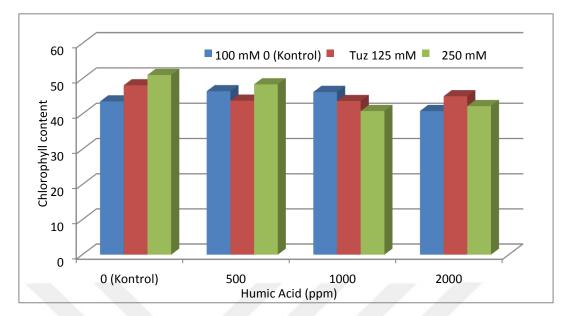


Figure 4.7. Interaction of different salt and Humic acid applications for chlorophyll content.



#### **5. 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, stem dry weight and leaf area) and some biochemical properties (RWC, MDA, membrane resistance index in leaf tissues, ion leakage in leaf tissues and total chlorophyll content) were determined.

The results of the experiment showed that; root fresh and dry weight, plant 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.



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# GENİŞLETİLMİŞ TÜRKÇE ÖZET (EXTENDED TURKISH SUMMARY)

# HUMİK ASİT UYGULAMALARININ TUZ STRESİ KOŞULLARINDA YETİŞTİRİLEN SOYA (*GLYCINE MAX* L.)' NIN FİZYOLOJİK VE BİYOKİMYASAL ÖZELLİKLERİ ÜZERİNE ETKİSİ

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1. ÖZ

Bu çalıĢmada ekonomik değeri ve önemi yüksek olan soya (*Glycine max* L.) bitkisi materyal olarak belirlenmiĢtir. Bu çalıĢma, soya bitkisine Humik asit uygulanarak bitkinin tuz stresine karĢı tolerans seviyesini belirlemek, bitkide oluĢan morfolojik ve biyokimyasal değiĢimleri gözlemlemek amacıyla yapılmıĢtır. ÇalıĢma, 2019 yılında Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü'ne ait iklim odasında yürütülmüĢtür. AraĢtırmada tohumluk materyali olarak Ġlksoy soya çeĢidi kullanılmıĢtır. Deneme, ne göre faktöriyel düzende 4 tekrarlamalı olarak yürütülmüĢtür. AraĢtırmada, dört farklı Humik asit dozu (0, 500, 1000 ve 2000 ppm) ve 3 farklı NaCl tuz dozu (0, 125 ve 250 mM) kullanılmıĢtır.

ÇalıĢmada Soyada kök uzunluğu, gövde uzunluğu, kök yaĢ ağırlığı, gövde yaĢ ağırlığı, kök kuru ağırlığı, gövde kuru ağırlığı, yaprak alanı, klorofil miktarı, yaprak dokularında iyon sızıntısı, lipid peroksidasyon düzeyi (MDA), yaprak dokularında bağıl su içeriği ve membran dayanıklılık indeksi gibi özellikler incelenmiĢtir.

ÇalıĢma sonucunda; en uzun kök (38 cm) ve bitki boyu (30 cm), bitkilere tuz ve humik asit uygulanmayan kontrol parsellerinden, en yüksek kök (2.082 g) ve gövde yaĢ ağırlığı (1.87 g) ise tuz uygulamasının yapılmadığı 500 ppm humik asit dozu uygulanan parsellerden alınmıĢtır. Ayrıca, en yüksek klorofil oranı 51.05 ile 250 mM tuz uygulanan ve humik asit dozu uygulamasının yapılmadığı bitkilerden elde edilmiĢtir.

# Anahtar kelimeler: *Glycine max* L., Humik Asit, Tuz stresi, Soya. 2. GİRİŞ

Önsanoğlu geçmi Gten günümüze temel gıda ihtiyaçlarını kar Gılamak için tarımla ilgilenmi Gtir. Bu ilgi ilerleyen zamanlarda üretimin arttırılması yönünde gerçekle Gmi Gtir. Toprakların verimliliğini önemli ölçüde azaltan tuzluluk problemi, özellikle yağı Gı yetersiz olan kurak ve yarı kurak iklim bölgelerinde sık kar Gıla Gılan bir sorundur. Bu stres faktörü, bitkinin cinsine, maruz kaldığı tuzun çe Gidine, stresin Giddetine ve etkili olduğu süreye bağlı olarak deği Gmektedir. Ekonomik değere sahip bitkilerin çoğu toprak tuzluluğuna kar Gı hassasiyet gösterir. Bu noktada, duyarlı bitkilerin tuz stresine kar Gı hassasiyetini gidermek tolerans mekanizmalarını arttırmak amacıyla birçok yöntem denenmektedir. Son yıllarda, Humik asidin bitkide sistematik direnci

arttırmasında ve abiyotik stres koÇullarında bitkide tolerans seviyesini arttırdığı ve koruyucu etkisinin olduğu gözlemlenmiÇtir.

Toprak yarayıĞlılığını büyük oranda etkileyen sorunların baĞında tuzluluk gelir. NaCl kaynaklı tuz stresi özellikle yağıĞın yetersiz olduğu kurak ve yarı kurak bölgelerde verimi çok ciddi bir Ģekilde etkilemektedir. Dünya genelinde tuzlu toprakların artıĞı her geçen gün artarak bitkisel üretimi sınırlandırmakta, verim azalmakta ve bazı alanlar aĞırı tuzlanma nedeniyle tamamen üretim dıĞı kalmaktadır. Bitkinin büyüme ve geliĞme aĞamalarını büyük oranda etkileyen sorunlardan birisi olan tuzluluk; tarım yapılan alanlarda toprağın yapısı bozup tuzluluk seviyesini arttırmakta, bitkilerin verimlilik ve ürün kalitesini önemli ölçüde engellemektedir.

Bitki bünyesinde osmotik ve iyon stresine neden olan büyüme ve geliĢmeye olumsuz etki eden tuz stresinin etkileri; tuzun çeĢidine, stresin seviyesine ve etkili olduğu süreye, stresle karĢılaĢan bitkinin genotipine ve geliĢim dönemlerine göre değiĢiklik gösterir. Tuz stresine maruz kalan bitkilerde farklı metabolik olayların ortaya çıkması ve özellikle de fotosentetik aktivitelerin bu stresten etkilenmesi bitkilerin yaĢamsal faaliyetlerini azaltabilmektedir. Bazı bitkiler tuzluluğa karĢı hassasiyet gösterirken bazıları farklı fizyolojik, biyokimyasal ve moleküler yanıtlar vererek indüklenen tolerans mekanizmaları ile yaĢamsal faaliyetlerini devam ettirirler.

Soya bitkisi, kolesterol ve doymuĢ yağlar içermediğinden dolayı, protein içeriği yüksek kalitede olup kullanım alanları çok yönlü olan bitkisel gıda maddesidir. Soya fasulyesinin preslenmesiyle tedarik edilen soya yağı, soya lesitini, soya sosu, soya unu, soya eti ve kıyması, soya sütü gibi ürünler gıda piyasalarında birçok ürünün elde edilmesinde kullanılmaktadır. Dünyada en fazla üretilen ve tüketilen soya yağında doymamıĢ yağ oranı yüksektir, Hayvansal yağlarda oranı oldukça yüksek olan kolesterol, soya yağında sıfirdır. Soya yağı B, E vitaminleri ile demir, çinko, magnezyum oranları açısından oldukça zengindir. Laktoz adıyla bilenen süt Ģekerini bulundurmaz. Bitkisel bir ürün olduğundan dolayı Laktoz intoleransı olan kiĢilerde rahatlıkla kullanılabilir (Anonim,2017).

Soya, hayvan gıdası olarak kullanıldığında yüksek oranda protein içermesi istenirken, bitkisel yağ üretiminde ise yağ oranının yüksek olması arzu edilir (Wilcox ve Guodong, 1997). Gıda sektörü, yem bitkileri ve sanayide de ham madde olarak kullanıldığından dolayı ülke tarımına büyük katkılar sağlayarak geniĢ bir kullanım alanı bulmaktadır. Gıda maddesi olarak unlu mamuller, bebek mamaları, Ģeker ürünleri, alerjik etkisi olmayan süt ve süt ürünleri, diyet ürünleri, yapay et ürünleri, kuru/soğuk hazır yemek karıĢımları eldesinde kullanılmaktadır. Endüstriyel madde olarak tutkal, mürekkep, sabun, benzin, böcek ilacı, alkol, plastik, lastik, vb. ürünlerin elde edilmesinde kullanılmaktadır. Yağı alındıktan sonra arta kalan küspe yüksek oranda protein ihtiva ettiğinden, iyi bir hayvan yemi olarak değerlendirilir. Özellikle kanatlı yem rasyonlarında bol miktarda kullanılmaktadır. Bunların yanı sıra soya yeĢil gübre olarak da kullanılmaktadır (Sepetoğlu, 1978).

Bu çalıĢmanın amacı soya bitkisine uygulanan farklı tuz konsantrasyonları ve farklı dozlardaki Humik asit uygulamalarının sota bitkisinin geliĢimine etkisi, bitki tuz stresi altındayken Humic asidin tolerans mekanizmalarını ne derece etkilediği ve nasıl sonuçlar doğuracağı, yaprak, sap, gövde ve köklerin geliĢimlerindeki değiĢimlerin gözlenmesidir.

## 3. MATERYAL VE YÖNTEM

Bu çalıĢmada ekonomik değeri ve önemi yüksek olan soya (*Glycine max* L.) bitkisi materyal olarak belirlenmiĢtir. Bu çalıĢma soya bitkisine Humik asit uygulanarak bitkinin tuz stresine karĢı tolerans seviyesini belirlemek ve bitkide oluĢan morfolojik ve kimyasal değiĢimleri gözlemlemek amacıyla yapılmıĢtır. ÇalıĢma, 2019 yılında Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü' ne ait iklim odasında yürütülmüĢtür. AraĢtırmada tohumluk materyali olarak Trakya Tarımsal AraĢtırma Enstitüsünden temin edilen Glksoy soya çeĢidi kullanılmıĢtır.

Deneme, 500 cc' lik plastik saksılarda, Tesadüf Parselleri Deneme Deseni' ne göre faktöriyel düzende 4 tekrarlamalı olarak yürütülmüĢtür. AraĢtırmada, dört farklı Humik asit dozu (0, 500, 1000 ve 2000 ppm) ve 3 farklı NaCl tuz dozu (0, 125 ve 250 mM) kullanılmıĢtır. Farklı tuz dozu uygulamaları ile bitkiler üzerinde yaratılan bu stresin Humik asit tarafından ne ölçüde önlenebildiği ve etkileri gözlemlenmeye çalıĢılmıĢtır.

ÇalıĢmada her saksıya 3 tohum ekilmiĢ, çıkıĢtan sonra en iyi durumdaki fide bırakılarak diğer fideler uzaklaĢtırılmıĢtır.

ÇalıĢma 1/3 perlit ve 2/3 toprak karıĢımı olarak hazırlanmıĢ ve saksılara doldurulmuĢ ortamda ekilmiĢtir. Ekimden sonra saksılar 16/8 saatlik aydınlık/karanlık fotoperiyotta, 25°C sıcaklık % 65 neme sahip iklim odasına yerleĢtirilmiĢtir. Bitkiler belirli bir olgunluğa geldiklerinde (yaklaĢık 1 ay sonra) tuz stresi uygulamalarına baĢlanmıĢtır. Bitkilerde fizyolojik sorunlar belirdiğinde (ekimden sonra yaklaĢık 7-8 hafta) gerekli analizler için hasat yapılarak deneme sonlandırılmıĢtır.

# 3.1. İncelenen Özellikler

- a) Kök Uzunluğu (cm)
- b) Gövde Uzunluğu (cm)
- c) Kök YaĢ Ağırlığı (g)
- d) Gövde YaĢ Ağırlığı (g)
- e) Kök Kuru Ağırlığı (g)
- f) Gövde Kuru Ağırlığı (g)
- g) Yaprak Dokularında Bağıl Su Ġçeriği (RWC) (%)
- h) Lipid Peroksidasyon Seviyelerinin Belirlenmesi (MDA nmolg<sup>-1</sup>T.A)
- i) Yaprak Dokularında Gyon Sızıntısının Belirlenmesi (%)
- j) Yaprak Dokularında Membran Dayanıklılık Öndeksi (%)
- k) Yaprak Alanı (cm<sup>2</sup>)
- l) Toplam Klorofil (SPAD)

#### 4. BULGULAR VE TARTIŞMA

ÇalıĢmada Soyada kök uzunluğu, gövde uzunluğu, kök yaĢ ağırlığı, gövde yaĢ ağırlığı, kök kuru ağırlığı, gövde kuru ağırlığı, yaprak alanı, klorofil miktarı, yaprak

dokularında iyon sızıntısı, lipid peroksidasyon düzeyi (MDA), yaprak dokularında bağıl su içeriği ve membran dayanıklılık indeksi gibi özellikler incelenmiÇtir.

AraĢtırma sonucunda; Tuz dozu uygulamalarının bitkinin kök yaĢ ve kuru ağırlığı, gövde yaĢ ve kuru ağırlığı, gövde uzunluğu ve lipid peroksidasyon düzeyi (MDA), üzerine istatistiki olarak önemli oranda etkili olduğu, Humik asit dozu uygulamalarının kök kuru ağırlığı, gövde yaĢ ve kuru ağırlığı, kök ve gövde uzunluğu, yaprak alanını ve klorofil miktarı üzerine etkisinin istatistiki olarak önemli olduğu belirlenmiĢtir. ÇalıĢmada uygulanan Tuz ve Humik asit dozlarının yaprak dokularında bağıl su içeriği, membran dayanıklılık indeksi ve yaprak dokularında iyon sızıntısı özellikleri üzerine etkisi istatistiki olarak önemli bulunmamıĢtır.

ÇalıĢma sonucunda en uzun kök (38 cm) ve bitki boyu (30 cm) bitkilere tuz ve humik asit uygulanmayan kontrol parsellerinden, en yüksek kök (2.082 g) ve gövde yaĢ ağırlığı (1.87 g) tuz uygulamasının yapılmadığı 500 ppm humik asit dozu uygulanan parsellerden alınmıĢtır. Ayrıca en yüksek yaprak alanı 18.97 cm ile tuz uygulamasının yapılmadığı 1000 ppm humik asit dozu uygulanan bitkilerden, en yüksek klorofil oranı 51.05 ile 250 mM tuz uygulanan ve humik asit dozu uygulamasının yapılmadığı bitkilerden alınmıĢtır.

ÇalıĢma sonucunda en yüksek Yaprak Dokularında Bağıl Su Ġçeriği (RWC) (%) (% 74.83 – 71.85) bitkilere 500 ppm humic asit dozu ve 250 Mm tuz uygulanan parsellerden alınmıĢtır. Ayrıca en yüksek Lipid Peroksidasyon Seviyesi (0.73 -0.78 nmolg<sup>-1</sup>T.A) ile Humic asit uygulanmayan ve 250 Mm tuz uygulamasının yapıldığı bitkilerden alınmıĢtır.

ÇalıĢmadan elde edilen bulgulara göre; Humik asit uygulamalarının bitkide tuz stresinden kaynaklanan zararlanmaları minimize ederek bitkinin yaĢam faaliyetlerini sürdürebilmesi açısından olumlu etkiler bıraktığı söylenebilir. Buna bağlı olarak özellikle ülkemiz topraklarında tuz stresinin tarımı büyük ölçüde etkilediği bu etkinin de azaltılması yönünden Humik asit uygulamalarının kullanılabileceği tavsiye edilebilir.

# **CURRICULUM VITAE**

Noor Maiwan BAHJAT was born in 1994, in Nainava province, Iraq. She completed primary school in Jordan, secondary and high school in Duhok province. She graduated from Field Crops Department, College of Agriculture College and University of Dohuk in 2017. She started her MSc. In The department in of Field Crops, The Institute of Natural and Applied Sciences, Van Yuzuncu Yil University in Van -Turkey on may 2020.



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