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Response Of Maize To Green Walnut Husk Compost Application Under Salt And Drought Stress

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***Abstract*— The two most exposed challenges in agricultural production are drought and salt stress. Given that the world's resources are running out daily and that the population is growing and requiring more food, it is obvious how dangerous yield losses due to drought and salt stress are. It is well known that certain external treatments applied to plants might make them more stress tolerant. This study aimed at how compost, which is good for plants, the environment and people, affected maize development when it stressed by salt, drought, or both. In the experiment, the plants were subjected to three different levels of stress: 50 mM NaCl for salt stress, -0.5 Mpa PEG 6000 for drought stress, and a combination of 50 mM NaCl and -0.5 Mpa PEG 6000 for the interaction of the two stresses. Compost made from green walnut husks added to the pots at weight rates of 0%, 1%, and 2%. The plant samples' proline content, ascorbic acid (Vitamin C) content, phenol content and SH content were measured after harvest. Measurements showed that compost made from green walnut husks reduced the plants damage under all stress scenarios. Based on the results, it can be said that applying compost to a plant under stress may lessen its negative effects.**

Keywords— Compost; Walnut; Drought; Salt Stress; Maize

1 INTRODUCTION

According to the United Nations, desertification and drought affect more than 4 billion hectares of land in the world and directly threaten the lives of 1.2 billion people. Salinity affects more than 800 million hectares of land in the world, especially in arid and semi-arid climate regions [1]. The salinity brought by drought due to the changing rainfall regime significantly affects agriculture and limits crop production. Studies emphasize that the negative effects of stress factors affecting crop production in the world will increase with the increase in climate change and extreme climatic conditions [2].

Osmotic stress occurs in plants exposed to stress factors such as drought and salinity [3]. Osmotic stress affects plant growth in a negative manner, development and reproduction through morphological, physiological, biochemical and molecular changes, causing significant yield losses. Although drought and salt stress are two separate stress factors that affect plant development. These two stress factors can sometimes coexist and affect each other. Drought can restrict plants access ability to water resources, causing plants to be affected by salt stress increasingly. While drought makes it difficult for plants to draw water from the soil, salt stress prevents the movement of water from the roots through the plant. Restriction of water due to drought causes salts to accumulate in the soil and that's how plants exposed to salt stress. Both drought and salt stress increase the oxidative stress in plants. Therefore, plants under both drought and salt stress become more sensitive to oxidative stress. The coexistence of drought and salt stress creates a more complex environmental stress condition for plants. Therefore, it is extremely important to understand the interaction of these two stress factors and develop appropriate strategies.

In plants that can tolerate stress, osmotic protectors, antioxidant and hormonal systems works as defense mechanisms and ensures the plant to survive until generative period [4, 5, 6, 7, 8, 9]. Increasing drought problems due to global warming have made it necessary to retain water in the soil and increase its usability by plants, especially in agricultural lands. It has become very important to make practices that will increase the water retention capacity of the soil. One of the easiest and natural method that can be applied for this purpose is compost application. Compost enhances the soil's structural integrity [10], porosity and hydraulic conductivity [11] and water retention capacity [12] by raising the amount of organic matter in the soil. As a result of this application, plants can use soil water more effectively and for a longer period. Researcher reported that the compost applications increased seed germination by increasing soil water content at a depth of 15-30 cm [13]. According to earlier research, soils harmed by salt can be restored with organic fertilizers [14, 15]. Applications of compost improved plant development under salt stress and reduced plant stress in onion [16] and alfalfa [17].

Walnut is an important agricultural product and approximately 50% of the walnut produced turns into green waste as walnut husk [18]. Green walnut husk has limited usage area due to the lack of many studies on it. It is very important to conduct new studies to reveal the potential of this waste and increase its value. Walnut leaves contain compounds such as tannin and juglone that inhibit plant growth, but the harmful effects of these compounds disappear after the composting process [19, 20, 21]. For this reason, it is very important to use green walnut husk compost both to utilize the large amount of green waste and to increase the soil's capacity to hold water, which is the aim in this study. The aim of this study is to prevent maize as it's an important agricultural product from being damaged by drought and salt stress and to reduce yield losses by applying compost.

2 METHODOLOGY

In the experiment, soil with a loamy structure, pH value of 7.7 and electrical conductivity of 0.55 mS cm^{-1}, containing 6.8% calcium carbonate and 0.4% organic matter was used. After the soil was air dried, it was sieved through a 4 mm mesh and filled into pots. One seed was planted in each pot and the seeds were husk sterilized before planting. Plants were grown in the climate chamber under controlled conditions (16/8 hours day/night, 25/15 °C, 60-70% humidity). According to the experiment plan, 1% and 2% green walnut husk compost added into pots. Before application, green walnut husk compost was dried, milled and sieved. -0.5 Mpa PEG 6000 was applied to the plants for drought stress, 50 mM NaCl for salt stress, and -0.5 Mpa PEG 6000 and 50 mM NaCl were applied simultaneously for the interaction of two stress factors. Plants were grown by adding Hoagland nutrient solution to soil. The following characteristics of the plants were examined.

Proline Content

200 mg of plant leaves were sampled, crushed, and placed in a porcelain mortar. One milliliter of 3% sulfosalicylic acid was then added. Samples were centrifuged in Eppendorf tubes for five minutes at 14,000 rpm at 4 °C. Next, 0.2 ml of 96% acetic acid, 0.1 ml of 3% sulfosalicylic acid, 0.1 ml of supernatant sample, and 0.2 ml of ninhydrin acid (0.31 g ninhydrin, 7.5 ml of acetic acid, and 5 ml of 6 M phosphoric acid) were added to the tubes. After one hour at 96 °C in a hot water bath, the tubes were placed in an ice bath to halt the reaction. One milliliter of toluene was added to vortex them. After five minutes of centrifuging the samples at 14,000 rpm, the top (reddish pink) phase was reached at room temperature. The samples were measured at 520 nm in a UV/Vis spectrophotometer. Between 5 and 500 µm, proline standards were developed [22].

Ascorbic Acid (Vitamin C) Content

1000 mg of plant leaves were sampled and 10 ml of 5% metaphosphoric acid was used to smash the samples in a porcelain mortar. The samples were centrifuged for 15 minutes at 14,000 rpm at 4 °C. The tubes were filled with a 0.4 ml sample, 1 ml of 150 mM phosphorous buffer (pH 7.4) with 5 mM EDTA, and 0.1 ml of 10 mM DTT (1,4-dithiothreitol). The tubes were then kept at room temperature for 15 minutes. By the conclusion of this time, the tubes contained 0.4 ml of 3% FeCl3, 0.8 ml of 10% TCA (trichloroacetic acid), 0.8 ml of 44% orthophosphoric acid, 0.8 ml of 5% NEM (N-ethylmaleimide), and 0.8 ml of 42% 2,2-bipyridine (made in 70% ethyl alcohol). The samples were incubated for 50 minutes at 40 °C. The samples were measured at a wavelength of 525 nm using a UV/Vis spectrophotometer. Standards of ascorbic acid ranging from 0 to 100 µg ml^{-1} were produced [23].

Phenol Content

200 mg samples were collected from plant leaves to ground. The grounded samples were placed in glass tubes, filled with 5 milliliters of 80% methyl alcohol, and refrigerated for 48 hours. The homogenates were centrifuged at 4 °C for 20 min at 4000 rpm by the end of this period. The reaction mixture was vortexed after an hour of room temperature incubation. The supernatant of 1000 µl, 5 ml of distilled water, 400 µL 50% Folin–Ciocalteu's reagent (FCR), and 1000 µl 5% sodium carbonate (Na2 CO3) were added to the tubes. The samples were measured at a wavelength of 725 nm using a UV/Vis spectrophotometer. Gallic acid-prepared standard pictures were used to calculate the total phenol concentration in the leaf tissues [24].

SH Content

200 mg of plant leaves were sampled and crushed by mixing 5% metaphosphoric acid in a porcelain mortar. The materials were centrifuged for 15 minutes at 14,000 rpm at 4 °C. The tubes were filled with a 0.5 ml sample, 2.5 ml of 150 mM phosphorous buffer (pH 7.4) with 5 mM EDTA, and 0.5 ml of 6 mM DTNB. The tubes were then kept at room temperature for 20 minutes. At a wavelength of 412 nm, the samples were measured in a UV/Vis spectrophotometer. According to Cakmak and Marschner (1992), standards were made with 5% metaphosphoric acid and reduced glutathione in the range of 0–100 µg ml^{-1}.

A statistical package program was utilized to do an analysis of variance (ANOVA) on the experiment data, utilizing the generalized linear model (GLM).

3. RESULTS AND DISCUSSION

Data of ascorbic acid (Vitamin C) content are given in Table 1. Application of green walnut husk compost statistically changed the Vitamin C content both under salt and drought stress conditions and under condition of combined salt and drought stress.

Table 1. Ascorbic Acid (Vitamin C) Content (µg/ml/g)

	Ascorbic Acid (Vitamin C)			
	C0	**C%1**	**C%2**	**Mean**
Control	111.28 J	105.03 K	101.15 K	105.82 d
Salt	193.52 E	141.22 G	126.68 I	153.81 c
Drought	203.22 D	160.35 F	136.40 H	166.66 b
SaltxDrought	248.08 A	237.31 B	221.49 C	235.63 a
Mean	189.02 a	160.98 b	146.43 c	

Averages shown with the same letter are statistically in the same group.

One of the most researched antioxidants, ascorbic acid (Vitamin C) is a potent antioxidant that protects plants from harm when they are subjected to abiotic conditions [25, 26, 27]. Vitamin C content in plants which are grown under stress is higher than in plants that are not stressed. When we examine the results (Table 1), Vitamin C content increases under salt and drought stress and is higher in conditions where both stresses coexist. In addition, when Table 1 is examined, it is seen that green walnut husk compost applications reduced the Vitamin C content, making plants less affected by stress. The highest Vitamin C content (248.08 µg/ml/g) was obtained in plants where salt and drought stress were combined, and no compost was applied. While the lowest Vitamin C content (101.15 µg/ml/g) was obtained in plants that were not under stress and where 2% compost was applied. Research findings are not compatible with previous studies showing that compost application increases Vitamin C [28, 29].

Data of SH contents are given in Table 2. Green walnut husk compost application statistically changed the SH contents both under salt and drought stress conditions and under salt and drought stress conditions simultaneously.

Table 2. SH content (µg/g)

	Ascorbic Acid (Vitamin C)			
	C0	**C%1**	**C%2**	**Mean**
Control	44.81 F	40.67 G	34.19 H	39.89 c
Salt	57.63 D	54.00 E	53.57 E	54.31 b
Drought	61.39 C	56.27 D	45.28 F	55.07 b
SaltxDrought	70.24 A	64.51 B	60.39 C	65.04 a
Mean	58.51 a	53.86 b	48.36 c	

Averages shown with the same letter are statistically in the same group.

Oxidative stress is indicated by the oxidation of SH compounds from non-enzymatic antioxidants, and reactive oxygen species are necessary for the reduction of SH compound content [30]. According to some research, drought can cause the production of hydrogen peroxide and superoxide radicals, which can damage membrane lipids and deactivate enzymes that contain Sulphur [31]. When stress conditions were evaluated, plants were affected more by stress under conditions where salt and drought stress were combined, but compost application caused less damage to the plant under conditions where salt and drought stress were combined. The highest SH content was obtained in plants where salt and drought stress were combined and no compost was applied (70.24 µg/g), while the lowest SH content was obtained in plants which are not in stress conditions and where 2% green walnut husk compost was applied (34.19 µg/g). Green walnut husk compost applications reduced the damage to the plant in parallel with the increasing application dose and made the plant less affected by stress (Table 2). Our results are similar with previous studies showing that SH content increases with drought stress [32].

Data of phenol content are given in Table 3. Application of green walnut husk compost statistically changed the phenol content both under salt and drought stress conditions and under salt and drought stress conditions together.

Table 3. Phenol content (µg/g)

	Ascorbic Acid (Vitamin C)			
	C0	**C%1**	**C%2**	**Mean**
Control	213.76 G	207.17 H	204.85 H	208.59 c
Salt	253.88 D	229.27 E	215.31 G	232.82 b
Drought	258.72 C	223.07 F	216.86 G	232.88 b
SaltxDrought	283.72 A	273.45 B	270.55 B	275.91 a
Mean	252.52 a	233.24 b	226.89 c	

Averages shown with the same letter are statistically in the same group.

According to previous researches, phenols can function as antioxidants to lower free radicals and protect plants from the damaging effects of ROS brought on by abiotic stress [33]. Plants with higher phenol content have been linked to the systems required for stress survival [34]. Research findings showed that when drought stress increased, so did the phenol content. For a few plants (potato, wheat, and cucumber), similar findings were found in earlier researches [35, 36, 37]. When stress conditions were evaluated, plants were affected more by stress under conditions where salt and drought stress were combined, but compost application caused less damage to the plant under conditions where salt and drought stress were combined. The highest phenol content was obtained in plants where salt and drought stress were combined and no compost was applied (283.72 µg/g), while the lowest SH content was obtained in plants which are stress free and where 2% green walnut husk compost was applied (204.85 µg/g). Green walnut husk compost applications reduced the damage to the plant in parallel with the increasing application dose and made the plant less affected by stress (Table 3). Contrary to our study, Fiasconaro et al. (2019) stated that compost applied under drought conditions increased the proline and phenol content.

Data of proline content are given in Table 4. Application of green walnut husk compost statistically changed the proline content both under salt and drought stress conditions and under salt and drought stress conditions together.

Table 4. Proline content (µM/g)

	Ascorbic Acid (Vitamin C)			
	C0	**C%1**	**C%2**	**Mean**
Control	169.97 H	172.90 H	201.33 G	181.40 d
Salt	243.12 D	221.57 E	213.82 F	220.43 c
Drought	244.09 D	214.95 EF	202.26 G	226.17 b
SaltxDrought	371.77 A	287.27 B	263.46 C	307.50 a
Mean	257.24 a	224.17 b	220.22 c	

Averages shown with the same letter are statistically in the same group.

Low molecular mass substance proline reduces osmotic damage and controls osmotic pressure [38]. Plants respond to abiotic stressors by accumulating proline [34]. Proline buildup during drought stress is most likely related to osmotic pressure regulation systems [39]. Prior research yielded comparable findings, and during drought stress, the proline content of several plants—such as rice, sunflower, and wheat—increased [40, 41]. Increasing proline content has also been linked to higher salt concentrations in earlier researches [37, 42]. Proline content in plants grown under stress is higher than in plants that are stress free. According to research results (Table 4), the proline content increases under salt and drought stress and is higher in conditions where both stresses coexist. In addition, when Table 4 is examined, it is seen that green walnut husk compost applications reduce the proline content, ensuring that plants are less affected by stress. The highest proline content (371,776 µM/g) was obtained in plants where salt and drought stress were combined and no compost was applied, while the lowest proline content (169,976 µM/g) was obtained in plants that were stress free and without compost application. Compost is thought to be effective in alleviating the harmful effects caused by salinity by clearing H_2O_2 caused by oxidative stress [43]. In parallel with the study, it was also reported by other researchers that proline content increased with the addition of compost under salt stress [44].

4. CONCLUSION

With the applied stress conditions, the proline content, ascorbic acid (vitamin C) content, phenol content and SH content of the plants increased, but then decreased in parallel with the applied compost doses. Drought and salinity caused negative effects on the growth and physiology of the plant. In contrast, compost application reduced these effects. According to these results, compost addition may be an effective strategy to alleviate some of the negative effects of drought and salt stress on plants by improving the water retention capacity of the soil and slowing down ion uptake. Based on the data obtained from the study, further studies, especially field trials, are recommended to investigate possible mechanisms.

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