

## Reinforcing Pepper (*Capsicum annuum*) Growth by Gypsum and Salicylic Acid under Salinity Stress

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### ABSTRACT

**Purpose:** Salinity stress negatively affects plant growth, which leads to a decline in the food source. Low rainfall and high water evaporation rates in some regions are serious problems in the field of agriculture. In saline soil, plants are unable to absorb water. The aim was to find some amendments to reduce the toxic effects of saline soil.

**Research Method:** In this study, a pot experiment was performed to study the ameliorative role of gypsum (G; 0.38 g per 200 g of soil) and salicylic acid (SA was sprayed on the leaves at two different times) on pepper growth. Two concentrations of NaCl (50mM and 100mM) were used for irrigation every two days. After 18 days, some growth characteristics, including shoot and root length, dry and fresh weight, salt tolerance index, leaf surface area, and chlorophyll content, were evaluated.

**Findings:** The results showed that the application of gypsum alone and with foliar spray of SA was effective in reinforcing the pepper seedlings under salinity stress, regardless of salt concentration.

**Research Limitations:** Limited availability of some advanced instruments to collect more data.

**Originality/ Value:** The investigated ameliorator can be used to improve plant growth under salinity stress.

**Keywords:** *Capsicum annuum*, gypsum, salicylic acid, salinity stress

### INTRODUCTION

Soil salinity is a serious problem (Qadir *et al.*, 2008), as it limits plant development and growth and also limits soil fertility (Silveira *et al.*, 2001). It has a negative effect on plant production, particularly crop production (Isayenkov, 2012). Salt-affected soils are defined by an excessively high degree of water-soluble salts, including sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>), magnesium chloride (MgCl<sub>2</sub>), sodium chloride (NaCl), and calcium chloride (CaCl<sub>2</sub>), among others (Tanji, 2002). In inland salinity, NaCl could be a major pollutant in the soil; it has a particular size, and when oxidized by water, it produces sodium ions (Na<sup>+</sup>) and chloride ions (Cl<sup>-</sup>) which are absorbed by the roots of the plants and distributed to the whole plant cells (Rodriguez *et al.*, 2006; Tester and

Davenport, 2003). These poisonous particles cause osmotic stress at the cellular level of the plants (Chinnusamy *et al.*, 2005). Water stress is the main cause of a reduction in plant growth and development in saline soils. High uptake of sodium and chloride leads to cytotoxicity and nutritional imbalance. The production of reactive oxygen species is another problem with salinity stress (Isayenkov, 2012). There are two main stages in the response of plants to salinity: short-

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term and long-term responses (Isayenkov and Maathuis, 2019). The first response takes place within minutes to a day, and that includes stomatal closure and the prevention of cell development. A second response occurs over days or even weeks in which metabolic processes decrease and finally cause senescence and cell death (Roy and Chakraborty 2014). Chemical remediation, water leaching, and phytoremediation are some procedures that can be used to reduce and improve the effect of salt on the soil (Feizi *et al.*, 2010). A low-cost, effective, and easy procedure is using chelating agents such as gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ), calcium chloride ( $\text{CaCl}_2$ ), Calcite ( $\text{CaCO}_3$ ), and organic matter (green manure, farmyard manure, municipal solid waste, and organic amendment) (Makoi and Verplancke, 2010; Ghafoor *et al.*, 2001).

The most common amendment for reducing salt stress in the soil is the application of gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ). Actually, sodium ions in saline soils can be replaced by calcium ions in gypsum (Mahmoodabadi *et al.*, 2013). Gypsum, in addition to being used as an amendment, can also provide ions like sulphur and calcium to plants, which are used in developing countries (Yildiz *et al.*, 2017).

Salicylic acid is a chemical messenger or signal molecule with an important role in the defence mechanism and is well established in plants

(Syed and Khan, 2010). It plays a significant role in salt tolerance in many plants (Gautam and Singh, 2009). Exogenous application of salicylic acid has a great influence on the many diverse processes in plants, including stomatal closure, seed germination, membrane permeability, secondary metabolites, transportation, photosynthesis, ion uptake, plant development, and growth rate (Aftab *et al.*, 2010).

The aim of this study was to detect the application of gypsum and salicylic acid in improving the growth of pepper (*Capsicum annuum*) under salt stress conditions.

## MATERIALS AND METHODS

### Experiment Design

The study was carried out under greenhouse conditions during the spring of 2021 at Soran University of Erbil, Iraq. The *Capsicum annuum* seeds were sown in clay soils at a depth of 1.5–2.0 cm. When the seedlings developed four leaves, they were transplanted to the experiment pots. All pots (14 cm high × 7 cm diameter) were filled with 200 g homogeneous mixed clay soil. Then, each pot was prepared according to the experimental design shown in Table 01.

**Table 01:** Experiment Design. G: gypsum; S.W: Saline water; S.A: Salicylic acid.

Pot Composition	Irrigation	Treatment added
Soil (Control)	D.W	No treatment
Soil	50 ml S.W	S.A (one time)
Soil	100 ml S.W	S.A (one time)
Soil + G	50 ml S.W	S.A (one time)
Soil + G	100 ml S.W	S.A (one time)
Soil + G	50 ml S.W	S.A (two times)
Soil + G	100 ml S.W	S.A (two times)

In treatments 1 to 4, the gypsum wasn't added to the soil, but treatments 5 to 8 contained the gypsum (0.38 g /200g soil). In the irrigation of the treatments, treatments 1 and 2 were irrigated with distilled water (Depending on the plant's needs but, it was the same for all treatments). Nothing was added to treatment 1 except clay soil (control), and salicylic acid (S.A) was added to treatments 1 to 6 one time and added to treatments 7 and 8 two different times. The treatments were replicated three times, and each pot contained a single seedling. Pots were arranged in a completely randomized design. Irrigation with distilled water was done 4 days after transplanting the seedlings to the pots to ensure seedling survival, then every two days with saline water (same volume of distilled water) till 14 days (Aftab *et al.*, 2011; Cha-Um *et al.*, 2011; Hoagland and Arnon, 1950; Aftab *et al.*, 2010).

Salicylic acid solution (0.01 g / 100 ml distilled water) was supplied to plant leaves at two different times by foliar spray techniques, which was a strategy to increase plant resistance (Both sides of the leaf were considered to be covered, and the number of sprays was the same for all treatments). For the first time, S.A. was sprayed one day before transplanting seedlings, but in the second time, it was sprayed into 2 pots from the 7 pots, 2 days after plant transplantation and 2 days before salinity application (irrigation with saline water was started 4 days after plant transplantation). For all plants except control, salinity was applied 4 days after transplantation (Souri and Tohidloo, 2019). To prevent osmotic shock, the concentration of NaCl was gradually increased; at first, NaCl (25mM) was applied every two days, then increased to 50mM and 100 mM (Aftab *et al.*, 2010).

### **Plant Growth Parameters**

**Shoot and root length:** To determine the shoot and root length, initially, the plant was removed from the soil, then the shoot and root were cut, and by using a ruler, the lengths were measured.

**Fresh and dry weight:** Initially, the shoot and root were cut from the plant and immediately weighted, which was referred to as fresh weight. To measure the dry-weight biomass, the root and shoot were put in an oven at 75°C for 24h, to remove the water content. After 24h, the dry weight was measured. To estimate the reduction percentage of organ weight in comparison to control, the FWPR and DWPR were determined using the following formula as given by El-Goumi *et al.* (2014):

$$\text{FWPR} = 100 \times [1 - (\text{fresh weight salt stress} / \text{fresh weight control})]$$

$$\text{DWPR} = 100 \times [1 - (\text{dry weight salt stress} / \text{dry weight control})]$$

**Leaf surface area:** At first, the leaves were separated from the plant, then the leaves were laid on the 1-cm grid paper and their outlines were traced. After that, the number of square centimetres covered by leaves was counted. Petiole was not included in the calculations (Dey *et al.*, 2019).

**Chlorophyll a content:** The leaves were collected from the plant and cut into small, homogenous leaf particles. The sample leaf particles (0.5 g) were mixed with 10 mL of acetone, which is a common solvent used to extract chlorophyll from the leaves. Then, centrifugation of the solution at 8000rpm was done in 15 min. After that, the absorption of the solution was measured by a spectrophotometer at 663.2 and 646.8nm wavelengths. The concentration of chlorophyll a (mg/g) was calculated using the following formula (Gitelson *et al.*, 2003).

$$\text{Ch a (mg/g LFW)} = (12.25 A_{663.2} - 2.79 A_{646.8}) / 1000 W$$

Which V is volume of acetone and W is weight of plant sample.

**Salt tolerance index:** The salt tolerance index (STI) is the ratio of the seedling dry weight of the salt treatment to the seedling dry weight of the control. The STI was calculated from the

following relationship (El Goumi et al., 2014):

$$STI\% = 100 \times (\text{Total DW salt stress} / \text{Total DW control})$$

### Statistical Analysis

Totally the collected data were subjected to analysis of variance, and treatment means were compared via the LSD test at a significant level  $p \leq 0.01$ . In addition, the chart for each sample was drawn by Excel software.

## RESULTS AND DISCUSSION

The shoot length was reduced under salinity conditions compared to control (Figure 01). The shoot length of the gypsum (G) treatment irrigated with 50mM saline water, had no significant difference compared to the sample just irrigated with 50mM saline water, while the soil-added G irrigated with 100mM NaCl had a significant difference compared to the sample just irrigated with saline water (100mM NaCl).

### Shoot Length and Root Length

The sample of G+S.A irrigated with 50mM saline water had no significant difference compared to a treatment without any treatments irrigated with 50mM S.W. While, the difference between the G+S.A treatments irrigated with 100mM saline water in a pot without any treatment and irrigated with 100mM saline water was significantly different.

The root length of the plant under salinity stress was reduced compared to the control. Both samples of G and G+S.A irrigated with 50 and 100mM saline water had a significant difference compared to a sample without any treatment and irrigated with 50 and 100mM saline water, respectively. The mean difference between G and G+S.A in both states of irrigation (50 and 100mM saline water) was significant. The highest amount of shoot and root length between the treatments belonged to treatment G+S.A irrigated with 50mM saline water (6.24cm) which was like control (6.23cm). Meanwhile, the lowest amount for both organs belonged to a sample that didn't receive any treatment and was irrigated with 100mM saline water.

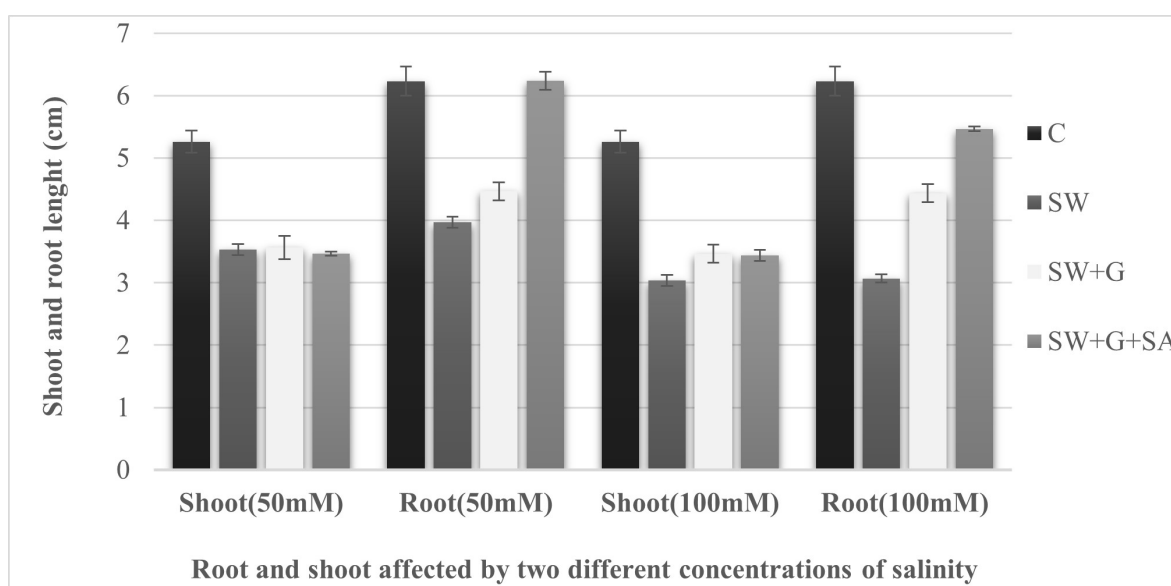


Figure 01: Shoot and root length of *Capsicum annuum* under different treatments; C: Control; SW: Saline water; G: Gypsum; S.A: Salicylic acid, Mean  $\pm$  SE.

However, because of the reduction in osmotic potential, which causes a decline in the absorption of water by roots, the roots grow into deep soil to find a sufficient amount of water. For this reason, the growth of the roots was better than that of the shoot. Increased root and shoot length in the untreated control was possibly due to the availability of sufficient moisture, which helped in the rapid growth of the root and shoot. Ahmad *et al.*, (2005) reported that the root and shoot length of the carrot were higher with a higher amount of water but reduced under salinity conditions.

High levels of salinity limit water absorption by the root because the water balance is affected due to the availability of more ions in the soil. Ashraf *et al.* (2010) reported that salicylic acid reduces the harmful effects of saline water and improves plant growth by increasing the activity of some enzymes. They expressed that it causes redox balance under salt stress. Up-regulation of proline biosynthesis enzymes and down-regulation of proline oxidase activity is another factor, reported by Misra and Saxena that can enhance cell turgor under salinity stress (Misra and Saxena, 2009). Husen *et al.* (2018) reported an increase in root length after applying salicylic acid to Ethiopian mustard under three doses

of salinity (50, 100, 150 mM NaCl). Clemente *et al.* (2017) investigated the root growth and yield of sugarcane as a function of increasing gypsum doses. They found that it improved the distribution of the root system along the soil profile.

### Fresh Weight and Dry Weight

The results as given in Figure 02 show that the fresh weight of shoots in plants without any treatment and irrigated with SW was reduced, with a percentage reduction of 88.12 for samples irrigated with 50mM and 72.49% for samples irrigated with 100mM as compared to control. In the G treatment that was irrigated with SW (50mM), the reduction percentage was 68.73%, which was less than the sample that was irrigated with S.W (50mM) without any treatment (88.12%). The lower amount of FWPR means a lower negative effect of salinity stress. The G+SA treatment irrigated with SW (50mM) had a significant difference with the G treatment compared to the sample irrigated with S.W (50mM) without any treatment. FWR was 13.49% lower in the G+SA treatment in comparison to the G treatment.

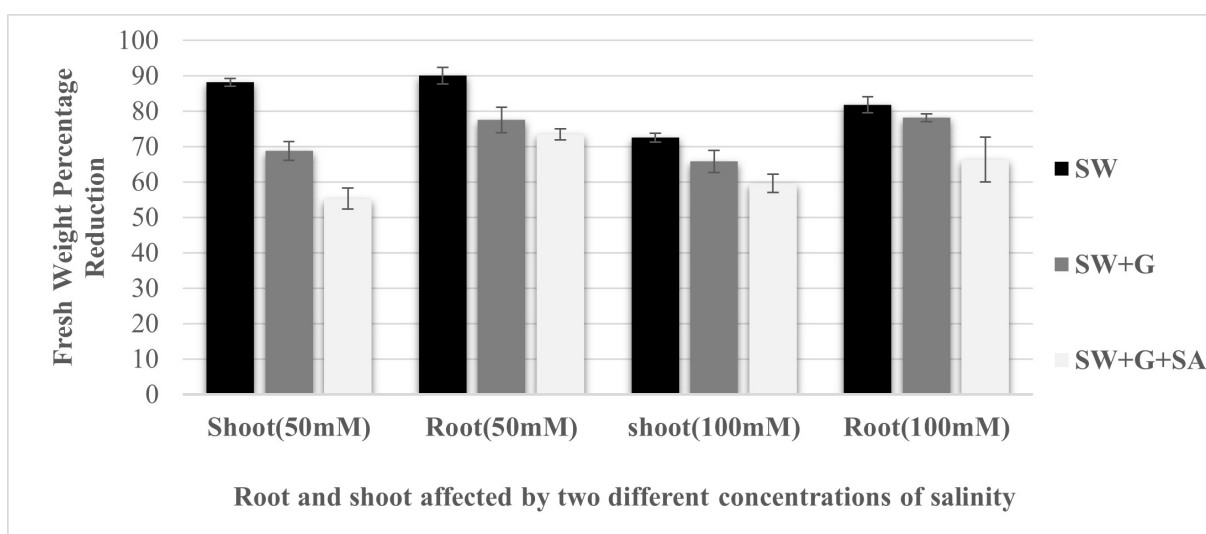


Figure 02: Fresh weight percentage reduction of *Capsicum annum* under different treatments; SW: Saline water; G: Gypsum; S.A: Salicylic acid, Mean $\pm$  SE.

The mean difference between the G treatment irrigated with S.W (100mM) and the sample irrigated with S.W (100mM) without any treatment was significant, and the reduction percentage was lower in the G treatment. Also, the difference between G+SA and G treatments irrigated with S.W (100mM) was significant, and the FWR was 6.17% lower in the G+SA treatment in comparison to the G treatment. G+SA treatment irrigated with SW (50mM) led to a lower reduction of FW as compared to other samples.

The fresh weight of root organs in samples without any treatment and irrigated with SW was reduced like shoots; the reduction percentage compared to control for samples irrigated with 50mM was 89.99% and for samples irrigated with 100mM was 81.80%. In G and G+SA treatments, the reduction percentage of FW was lower than in samples without any treatments and the difference was significant. However, in the comparison between G and G+SA, the difference just in samples irrigated with SW (100mM) was significant. Generally, the reduction percentage of FW was higher in the root than in the shoot.

The results given in Figure 03 show that dry weight has decreased under salinity conditions. The dry weight of the shoot in the samples without any treatment and irrigated with SW (50mM and 100mM) decreased compared to the control, and the difference was significant. Samples of G and G+S.A had the lowest reduction compared to samples without any treatment under salinity

stress, and the difference was significant. While the difference between treatments G and G+SA in the samples irrigated with both concentrations of salt was not significant.

The dry weight of the root organ in samples without any treatment was reduced under salinity stress compared to the control, like in the shoot, but the difference between two concentrations of salt was not significant. Also, the reduction percentage of the dry weight in the treatments of G and G +SA was lower than the sample without treatment under salinity stress, and the difference was significant. Meanwhile, the difference between G and G + SA in both concentrations of salt was not significant, like in the shoot. The lowest reduction percentage (66.14) belonged to the G+SA treatment irrigated with SW (100mM). In comparison, the reduction percentage of DW between root and shoot under salinity stress was highest in the shoot irrigated with SW (50mM) (88.84%) and the root irrigated with SW (100mM) (88.27%).

Generally, the weight of pepper was significantly reduced by irrigation with 100mM NaCl compared to 50mM that our result was similar to the result that Hassan *et al* (2019) reported. A reduction in plant growth and production biomass is a common physiological impact of NaCl salinity. The fresh weight was significantly reduced when irrigated with SW without any treatment, compared to G and G+SA treatments, which had the best growth and increased weight.

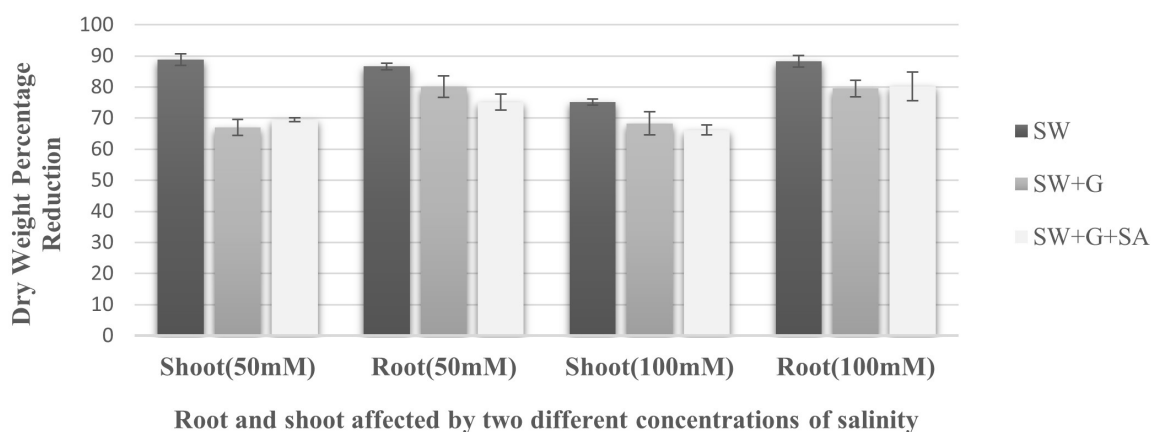


Figure 03: Dry weight percentage reduction of *Capsicum annuum* under different treatments; SW: Saline water; G: Gypsum; S.A: Salicylic acid, Mean± SE.

The salt tolerance index of samples with and without treatment and irrigated with SW (50 and 100mM) has been shown in Figure 04. The STI of samples without treatment and irrigated with SW (50mM) was lower than that of samples irrigated with SW (100mM) and the mean difference was significant. In the treatment of G, the STI was higher than in samples without treatment in both concentrations of salt, and the difference was significant. In this treatment, the difference between samples irrigated with SW (50mM) and samples irrigated with SW (100mM) was not significant. As the figure shows, the G+SA treatment had a higher STI compared to samples without treatment under salinity stress, and the difference was significant. Like treatment G, in treatment G+SA, there was no significant difference between samples irrigated with SW (50mM) and samples irrigated with SW (100mM). Also in the comparison of STI between the G treatment and the G+SA treatment, there was no significant difference in both concentrations of salt.

### **Salt Tolerance Index**

Salt tolerance is an index that shows the ability of plants to grow under salinity conditions, which, depending on the plant type, could be different. This diversity is due to the different mechanisms plants use adapt to salinity stress (Yeo, 1983). According to the findings of Azooz *et al.*, (2004), the K<sup>+</sup>/Na<sup>+</sup> ratio has an important role in salinity resistance. Thus, the high K<sup>+</sup> content might have a role in osmoregulation, which causes water uptake and resultantly increases the growth of leaves. Thus, it would be possible that the decreased level of Na with the help of treatments plays an important role in enhancing the salt tolerance of a plant. As in this study, the salt tolerance of the treated samples was higher than that of the untreated samples.

The leaf surface area of the samples under salinity stress has been shown in Figure 05. The LSA of samples without treatment decreased as compared to controls, and the difference was

significant. The LSA of control was 7.99 cm<sup>2</sup> while in samples without treatment and irrigated with SW (50mM) was 2.44cm<sup>2</sup>, and in samples irrigated with SW (100mM) it was 1.55cm<sup>2</sup>. The G treatments had a higher LSA compared to samples without G, but the mean difference between them as they were irrigated with SW (50mM) was not significant, while samples irrigated with SW (100mM) were significant. The highest value of LSA belonged to the G+SA treatment irrigated with SW (50mM).

### **Leaf Area**

Cha-um *et al.* (2011) reported an increase in leaf area of Thai jasmine rice cultivated in a saline field treated with gypsum. A similar finding was reported by Aslam *et al.* (2021), which mentioned the valuable effect of salicylic acid on plants grown under saline conditions. They have mentioned that exogenous application of SA spray at a concentration of 100 mM reduced the salinity stress effects. As Marcelis (1999) reported, the decrease in growth of radish (*Raphanus sativus L.*) at high salinity levels could be related to a decline in leaf area expansion (Marcelis and Hooijdonk, 1999).

### **Chlorophyll Content**

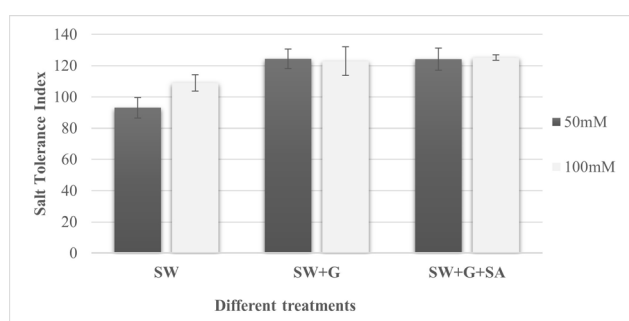
As Figure 06 Shows, the concentration of chlorophyll a in samples without treatment decreased under both concentrations of salinity as compared to control, and the difference was significant. The value of control was 0.4 mg/g, while in samples without treatment irrigated with SW (50mM) it was 0.33 mg/g, and in samples irrigated with SW (100mM) it was 0.31 mg/g. The difference between 50 mM irrigation and 100mM irrigation was not significant. In the G treatment, the concentration was higher than in samples without treatment, but the difference was not significant at both concentrations of salinity. Also, the difference between the G treatment irrigated with SW (50mM) and the G treatment

irrigated with SW (100mM) was not significant. In G+SA treatment, the concentration of chlorophyll a was higher than in samples without treatment, and the difference was significant at both concentrations of salinity. The value of chlorophyll a in G+SA treatment irrigated with SW (50mM) was 0.41 mg/g, while in samples without treatment and irrigated with SW (50mM), it was 0.33 mg/g. The value in G+SA treatment irrigated with SW (100mM) was 0.43 mg/g, while in samples without treatment and irrigated with SW (100mM) it was 0.31 mg/g. In the comparison of chlorophyll concentrations between samples treated with G and samples treated with G and SA, there was a significant difference under both concentrations of salinity.

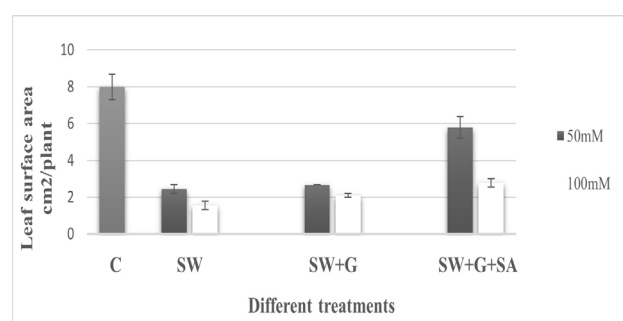
In the present study, chlorophyll content in leaves was also decreased by salinity, probably because of ROS formation (Kato and Shimizu, 1985). The effects of salinity on chlorophyll content in sensitive and salt-tolerant plants are different, and it will be increased in salt-tolerant plants.

Saline water may lead to reduced germination percentage, growth, and crop productivity of the plants after continuous application, and its effect depends on plant species and is very different. Mahmoud *et al.* (1983) reported that seed germination reached only 10% at 170mM NaCl; however, in other studies, higher germination was reached (Mahmoud *et al.*, 1983; De Pascale *et al.*, 2015; Alharby *et al.*, 2018)

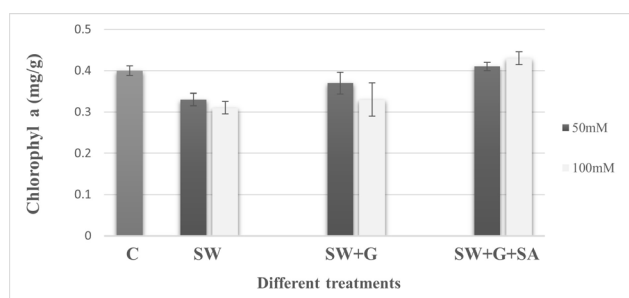
In the present study, both gypsum and salicylic acid had the ability to reduce salt stress and improve the growth of pepper seedlings. Moreover, the application of gypsum with salicylic acid was more efficient than gypsum alone under saline water irrigation. Na<sup>+</sup> and Cl<sup>-</sup> are the two salt ions in saline water that may induce ion toxicity for plants (Cao *et al.*, 2016). As some researchers have mentioned in their research, NaSO<sub>4</sub> will be produced from the reaction of gypsum (CaSO<sub>4</sub>) with NaCl, which can leach from soil easily (Mao *et al.*, 2014; Yu *et al.*, 2014).



**Figure 04:** Salt tolerance index of *Capsicum annuum* under different treatments; C: Control; SW: Saline water; G: Gypsum; S.A: Salicylic acid, Mean± SE.



**Figure 05:** Leaf surface area of *Capsicum annuum* under different treatments; C: Control; SW: Saline water; G: Gypsum; S.A: Salicylic acid, Mean± SE.



**Figure 06:** Chlorophyll a content of *Capsicum annuum* under different treatments; C: Control; SW: Saline water; G: Gypsum; S.A: Salicylic acid, Mean± SE.



The application of SA could moderate the adverse effects of salinity and has the potential to increase some growth characteristics by improving ion uptake, water relations, photosynthesis, membrane stabilization, and stomatal regulation (Said-Al Ahl *et al.*, 2014). More studies have reported that SA plays an effective role in removing reactive oxygen species (ROS) (Mittler, 2002). However, data obtained showed that spraying SA on seedlings grown in soil mixed with gypsum had a more significant effect on pepper growth compared to using them alone. Ahmed *et al.*, (2021) reported similar results for wheat seedlings in the treatment of SA, L-tryptophan, and gypsum in the saline field (Ahmed *et al.*, 2021). The novelty of this study is the combination of these two treatments in pepper seedlings.

## CONCLUSIONS

Generally, the growth of plants was significantly influenced by irrigation with different levels of saline water. The results showed that salinity stress significantly reduced all studied parameters at 50 mM and 100 mM of NaCl as compared to control. It was concluded from our study that the application of gypsum and salicylic acid under salinity stress helps pepper seedlings tolerate the unfavourable conditions. The study showed that mixing gypsum with soil was effective in reinforcing pepper seedlings under salinity stress. Meanwhile, foliar spraying of salicylic acid with the application of gypsum could be more effective to improve the investigated growth parameters.

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