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Original Research Article

# Effects of Foliar Application of Various Antioxidants on Growth, Physiological, Biochemical and Yield Attributes of Maize Grown Under Saline Conditions

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## Abstract

Maize crop is cultivated for the purpose to obtain high production of grains. Term maize is derived from Latin language, meaning life benefactor. Its grains possess high nutritional values. Because of high importance of maize grains, a study was performed according to CRD by three replications to examine the mitigating effects of antioxidants such as citric acid, ascorbic acid and salicylic acid to salt toxicity. Foliar application of these three antioxidants were done at 200 ppm concentration and one treatment as mixture of 100 ppm of all three antioxidants. Data was noted for different growth, physiological and yield attributes. Analysis of data revealed that 100 ppm concentration mixture of all three antioxidants provided highest results for all attributes. The 100 ppm antioxidant mixture foliar application caused 8% increase in plant height and 29% increase in ear length as compared to controlled plants sprayed with water. It also caused significant increase in concentration of all photosynthetic pigments such as Chl a, Chl b and carotenoids. Foliar application with 100 ppm of antioxidant mixture also caused 21% increase in catalase activity and 27% increase in peroxidase activity. This treatments also helped plants to accumulate more potassium and provided lowest (0.44) Na+/K+ ratio. Hundred grain weight was observed highest (44.7) in plants sprayed with 100 ppm of antioxidant mixture. Harvest index of those plants were also highest (45.65). So, from these results it can be assumed that 100 ppm antioxidant mixture foliar application provided highest results followed by 200 ppm concentration of ascorbic acid.

**Keywords:** Salt Toxicity, Antioxidants, Ascorbic Acid, Salicylic Acid, Maize and Yield.



## 1- INTRODUCTION

After rice and wheat, maize is the 3rd most prominent crop globally. It belongs to Poaceae family. Term maize is derived from Latin language, meaning life benefactor [1]. Maize crop is cultivated for the purpose to obtain high production of grains. Nutritional values of these grains are very high. Plant growth and metabolism of maize are significantly affected by salt toxicity. Salt toxicity is a major environmental challenge, which is expanded up to 800 million hectares of land by climate change and poor agricultural practices [2]. This problem is typically found in arid and semi-arid regions, where transpiration rates are high but annual rainfall is low. Salt tolerant plants (Halophytes) can grow well in these conditions, but maize and other crop species are not much resistant to salt toxicity. High concentrations of salt can cause significant decrease in growth and yield of maize [3].

Salt toxicity is a major threat to agricultural crops. It causes accumulation of Na+ and Cl- ions, which cause injuries to plants by causing nutrient imbalance [4]. K+ and Na+ show a totally opposite relation in soil, higher accumulation of Na+ causes reduction in accumulation of K+. Na+ accumulation is increased in roots and shoots of plants under salt stress which cause reduction in K+ accumulation [5]. Metabolic and physiological processes are also significantly affected by salt toxicity. Intensity and duration of salt toxicity determine its harmful effects [6]. Salt toxicity cause nutritional imbalance, ion toxicity, reduction in osmotic water availability and reduction in photosynthetic rate by reducing enzymes activities, which adversely effects on growth and yield of maize. Reactive oxygen species (ROS) are also generated by salt stress, which cause oxidative stress and destroy components of membranes [7]. These ROS caused oxidative stresses also effect proteins, DNA, nutritional balance and photosynthetic activities [8]. Salt toxicity also effects photosynthetic pigments and chloroplast structure, which leads to reduction in photosynthetic rate and in severe cases it may cause death of plants [9].

Antioxidative system consists of salicylic acid, citric acid and ascorbic acid. Main role of these antioxidants is to scavenge ROS (reactive oxygen species) produced in plants under sal toxicity and to help plants to overcome these stress conditions [10]. A non enzymatic antioxidant citric acid (CA) helps plants to remove free radicals produced in plants under stress conditions. Citric acid also helps plants to develop defense mechanisms by enhancing antioxidant enzymes activities [11]. Citric acid is also an important substrate in Krebs cycle and help plants in breakdown of proteins, fats and carbohydrates [12].

Ascorbic acid (ASA) is considered as the most important growth regulator to mitigate abiotic stresses [13]. Various biological defense mechanisms are activated by ASA to mitigate abiotic stresses [14]. ASA also reduces the production of ROS by providing electron donors to plants. Additionally, ASA also possesses antioxidant properties, which help plants in biochemical, physiological and other growth processes. ASA also helps plants to enhance cell division and other cellular processes such as cell differentiation [15]. ASA also works as a cofactor for many phytohormones and enzymes. Additionally, exogeneously applied ASA decreases oxidative damages caused by various abiotic stresses, it also improves root growth for nutrient uptake to overcome water deficit conditions [16].

Salicylic acid (SA) is also considered as a very important compound for promoting plant growth. SA has the ability to increase crop yield under very difficult soil conditions [17]. Moreover, SA also serves as a signalling molecule in plant defense mechanisms against different stresses [18]. SA perform key roles in photosynthesis regulation, it also perform role in nitrogen metabolism by acting as a precursor [19]. Additionally, exogenous application of SA improves antioxidant defense, membrane stability and the production of osmoprotectants [20]. SA also regulates gene expression related to ROS scavenging and for other defense mechanisms [21].

It is important to examine the effects of antioxidants on growth, physiological and yield attributes of maize, as salt toxicity is increasing continuously. Main aim of this experiment is to analyze the potential of CA, ASA and SA and to covers knowledge gap about the optimum application of these antioxidants. It is hypothesized that foliar application of these antioxidants will help plants to alleviate the harmful effects caused by salt toxicity and will enhance maize growth and yield.

## 2- MATERIALS AND METHODS

### 2.1. Experimental Details

Citric acid (CA), ascorbic acid (ASA) and salicylic acid (SA) foliar application were examined to mitigate salt toxicity in maize. A study was performed according to CRD by using three replications in botanical garden. Seeds of maize and clay pots were obtained from market. Compost, clay, silt and sand were mixed in equal proportions and pots were filled with this mixture. Then five seeds were cultivated in each pot. Then plants were thinned to one plant in each pot after 15 days of emergence and 140 mM NaCl treatment was applied. Then pots were irrigated with fresh water after application of salt toxicity. While first replication of foliar application of respective antioxidants of each pot were done after seven days of salt toxicity application. Normal irrigation of pots was done throughout the experimental period. Following treatments of antioxidants were applied in this experiment:

1. Foliar application of water (Controlled)
2. Foliar application of 200 ppm Citric Acid (T1)
3. Foliar application of 200 ppm Ascorbic Acid (T2)
4. Foliar application of 200 ppm Salicylic Acid (T3)
5. Foliar application of 100 ppm Citric Acid, Ascorbic Acid and Salicylic Acid (T4)

### 2.2. Biochemical, Mineral, and Antioxidant Enzyme Activity Measurement

Data was noted for different growth, physiological, biochemical and yield attributes of maize. Arnon method was used to measure Chl a, b and carotenoids [22]. In this method, after extraction of leaves in 80% acetone solution, Chl a, b and carotenoids were measured with spectrophotometer by using the following formulas:

Chlorophyll a = [12.7 (OD 663) − 2.69 (OD 645) × V/1000 × W

Chlorophyll b = [12.7 (OD 645) − 4.68 (OD 663) × V/1000 × W

Carotenoids = [OD 470 + 0.114 (OD 663) − 0.638 (OD 645)/2500] × 1000

Where V is the extract volume and W is the fresh leaf weight. With the help of flame photometer Na+ and K+ concentrations were measured by standard procedure of USDA Laboratory Staff [23]. While catalase (CAT) and peroxidase (POD) activities readings were noted on a spectrophotometer according to Chance and Maehly [24].

**2.3. Growth and Yield Attributes**

Growth attributes like plant height and spike length were measured with the help of measuring tape. From the top of plant to the level of soil was considered as height of plant. At the end of experiment grains of plants were harvested and were collected in separate bags. Then total grains yied and 100 grains weight were measured with the help of weight balance. Harvest index was also calculated for each treatment.

**2.4. Statistical Analysis**

This experiment was performed according to CRD to examine the effects of antioxidants on growth, biochemical, physiological and yield attributes of maize. Three replications of each antioxidant was applied on their respective pots. ANOVA of recorded data was done by using Statistix 8.1 software. To check the significant and non significant differences among mean values of each treatment, LSD all pairwise means comparison test was applied.

## 3- RESULTS AND DISCUSSIONS

**3.1. Growth Attributes**

Figure-1 shows the growth attributes such as plant height and ear length of maize under foliar application of various antioxidants. Salinity caused significant reduction in plant height and ear length. Foliar application of all antioxidants (citric acid, ascorbic acid, salicylic acid and mixture of all) caused significant increase in plant height and ear length. Highest value of ear length (27.347) and highest value of plant height (285.92) was observed in maize plants under foliar application of 100 ppm citric acid, ascorbic acid and salicylic acid followed by 200 ppm foliar application of ascorbic acid. Foliar application of all antioxidants (citric acid, ascorbic acid and salicylic acid) separately also significantly mitigated adverse effects of salt toxicity and caused significant increase in plant height and ear length. But their mixture at 100 ppm of all three antioxidants performed better as compared to separate application of each antioxidant. The observed growth improvement in maize plants during the study could be attributed to the role of antioxidants, which may enhance growth by regulating cell growth and division, likely through promoting the cell development cycle (Smirnoff and Wheeler, 2000). These findings align with those reported by [25, 26].

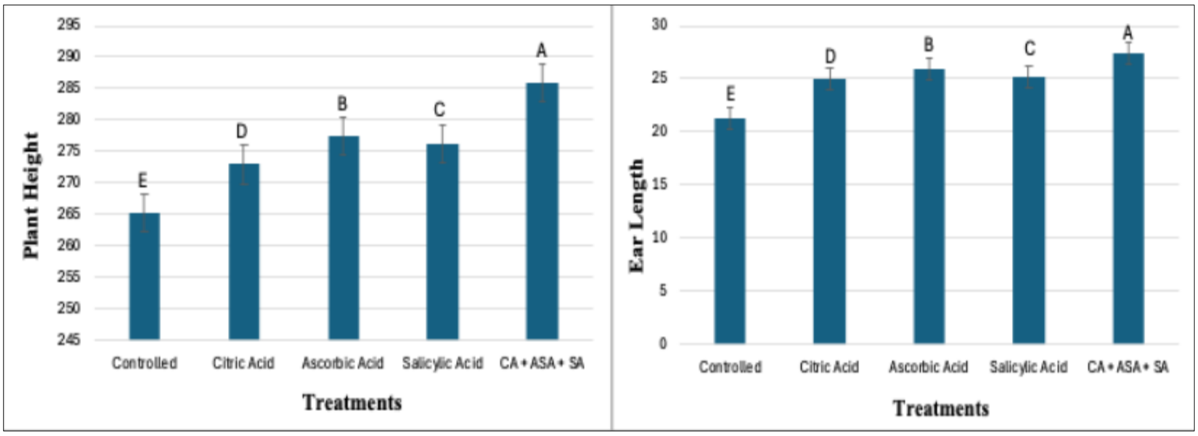


**Figure 1: Graphical representation of LSD test ± SE for Plant Height and Ear Length**

**3.2. Photosynthetic Pigments**

Figure-2 indicate that salinity significantly reduced chlorophyll a and chlorophyll b content in maize plants. Lowest levels of chlorophyll a and chlorophyll b were observed in those plants who didn't receive foliar application of any antioxidant. Foliar application of antioxidants separately also caused significant increase in chlorophyll a and chlorophyll b content. Among foliar application of each antioxidant separately, ascorbic acid provided highest values of chlorophyll a and chlorophyll b. While overall highest chlorophyll a (1.245) and chlorophyll b levels (0.715) were observed in plants who received foliar application of 100 ppm mixture of all antioxidants.

Similar trends were also observed for carotenoids content as shown in Figure-3. Lowest levels of carotenoids observed in those plants who didn't receive foliar application of any antioxidant. Foliar application of antioxidants separately also caused significant increase in carotenoids content. All antioxidants provided almost same values of carotenoids content as shown in Figure-3. While highest values of carotenoids (0.17) were observed in plants who received foliar application of 100 ppm mixture of all antioxidants.

In saline soil conditions, as observed in the control treatment, the levels of chlorophyll a and b, along with carotenoids, decreased in maize leaves. This reduction in chlorophyll content might be attributed to the degradation of chlorophyll pigments, likely caused by the activation of proteolytic enzymes such as chlorophyllase [27]. The efficiency of chloroplast senescence under abiotic stress is closely associated with chlorophyll degradation. Chlorophyll loss is often linked to the rapid accumulation of hydrogen peroxide ($H_2O_2$), a reactive oxygen species (ROS). Additionally, salinity stress has been reported to increase the activity of the chlorophyll-degrading enzyme chlorophyllase while also negatively affecting the biochemical synthesis of proteins that bind to chlorophyll molecules [28]. However, several studies have shown that the application of foliar antioxidants can enhance photosynthetic pigments [29].

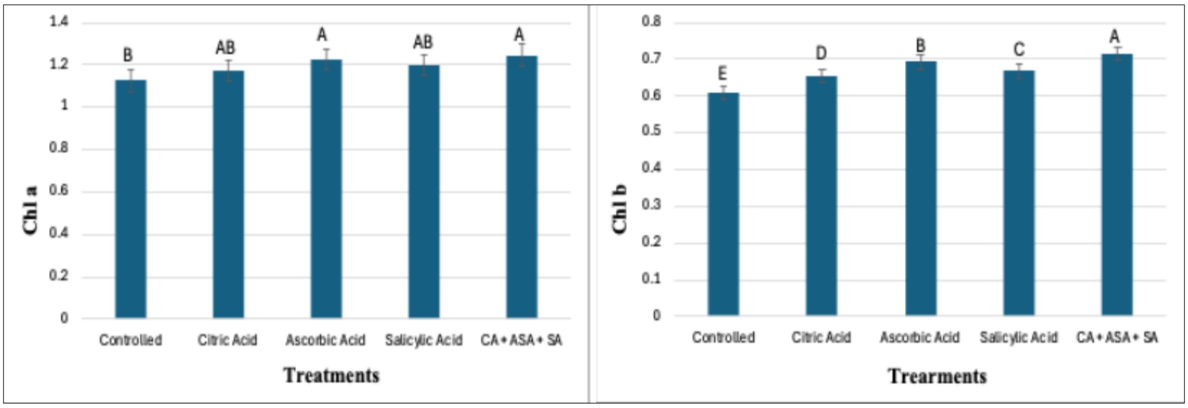


**Figure 2: Graphical representation of LSD test ± SE for Chlorophyll a and Chlorophyll b**

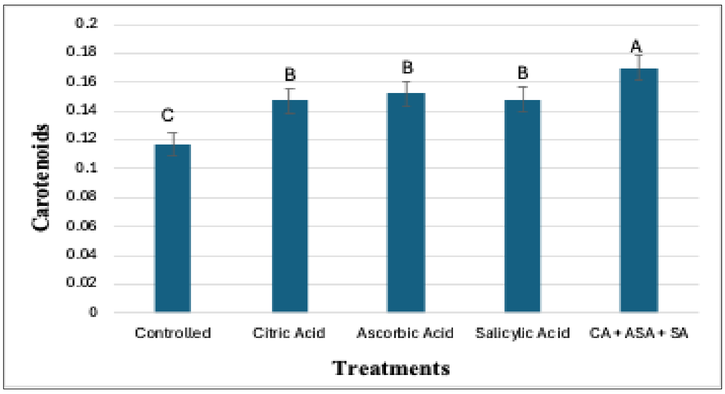


**Figure 3: Graphical representation of LSD test ± SE for Carotenoids Content**

### 3.3. Catalase and Peroxidase Activity

Catalase and peroxidase activity were also significantly reduced under salt stress. But when antioxidants were applied exogenously, catalse and peroxidase activity were significantly enhanced (Figure-4). Notably, maize plants exhibited highest catalase and peroxidase activity under foliar application of 100 ppm mixture of all three antioxidants. The effects of foliar application of antioxidants were evident in maize plants, providing noteworth results. Mixture of antioxidants at 100 ppm caused 22% increase in catalase activity and 27% increase in peroxidase activity. The data we obtained on enzymatic antioxidant activities following the application of antioxidants clearly highlight the significant role of ascorbic, citric, and salicylic acids in protecting maize plants from oxidative damage caused by salinity stress. These findings are consistent with the results reported by [30], demonstrating that exogenous antioxidants play a protective role by enhancing antioxidant capacity, which may boost the photosynthetic process and reduce the harmful effects of salinity stress on maize plants.

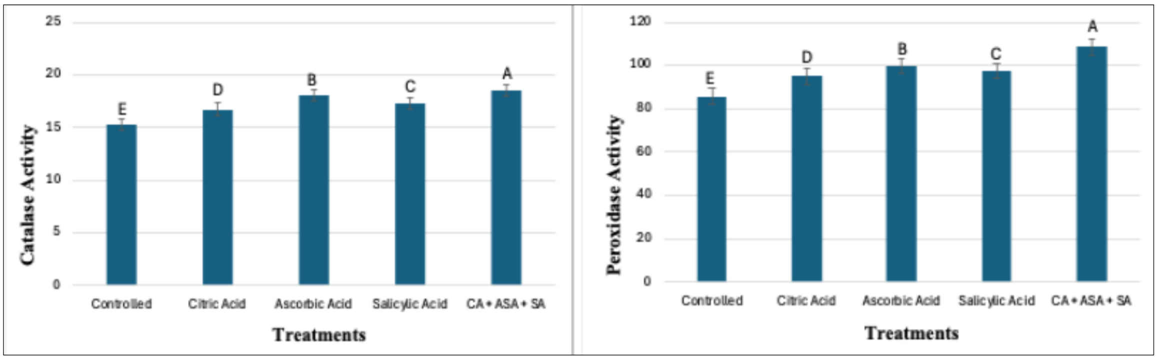


**Figure 4: Graphical representation of LSD test ± SE for Catalase and Peroxidase Activity**

### 3.4. Accumulation of K+, Na+ and Na+/K+ Ratio

An opposite relation is found between accumulation of Na+ and K+ as shown in Figure-5. Salt toxicity cause significant reduction in K+ concentration, while it enhances Na+ concentration. Antioxidants help plants to restore required concentration of K+ under salt toxicity. Foliar application of all antioxidants (citric acid, ascorbic acid and salicylic acid) separately also significantly mitigated adverse effects of salt toxicity and caused significant increase in K+ accumulation and reduced Na+ accumulation. They also caused significant reduction in Na+/K+ ratio as shown in Figure-5. But their mixture at 100 ppm of all three antioxidants performed better as compared to separate application of each antioxidant. This mixture provided highest values of K+ concentration (537.92) and lowest values of Na+ concentration (234.69). Mixture of antioxidants at 100 ppm also provided lowest Na+/ K+ ratio (0.44).

When plants are subjected to excessive salinity stress, some species tolerate this condition by inducing and accumulating organic compounds in the cytoplasm to lower osmotic potential. Proline is one such organic solute that acts as an osmoregulatory compound, helping to adjust the osmotic potential of plant cell cytoplasm. Ascorbic, citric, and salicylic acids have been reported to be effective in increasing potassium content and reducing sodium accumulation in the leaves of salt-stressed maize plants [31], observed that potassium levels in wheat leaves significantly declined under salinity stress, but the application of ascorbic acid to the foliage induced the accumulation of potassium ions in salt-stressed plants. Additionally, the exogenous application of antioxidants to the foliage significantly decreased the Na+/K+ ratio in the leaves of maize and barley plants [32].

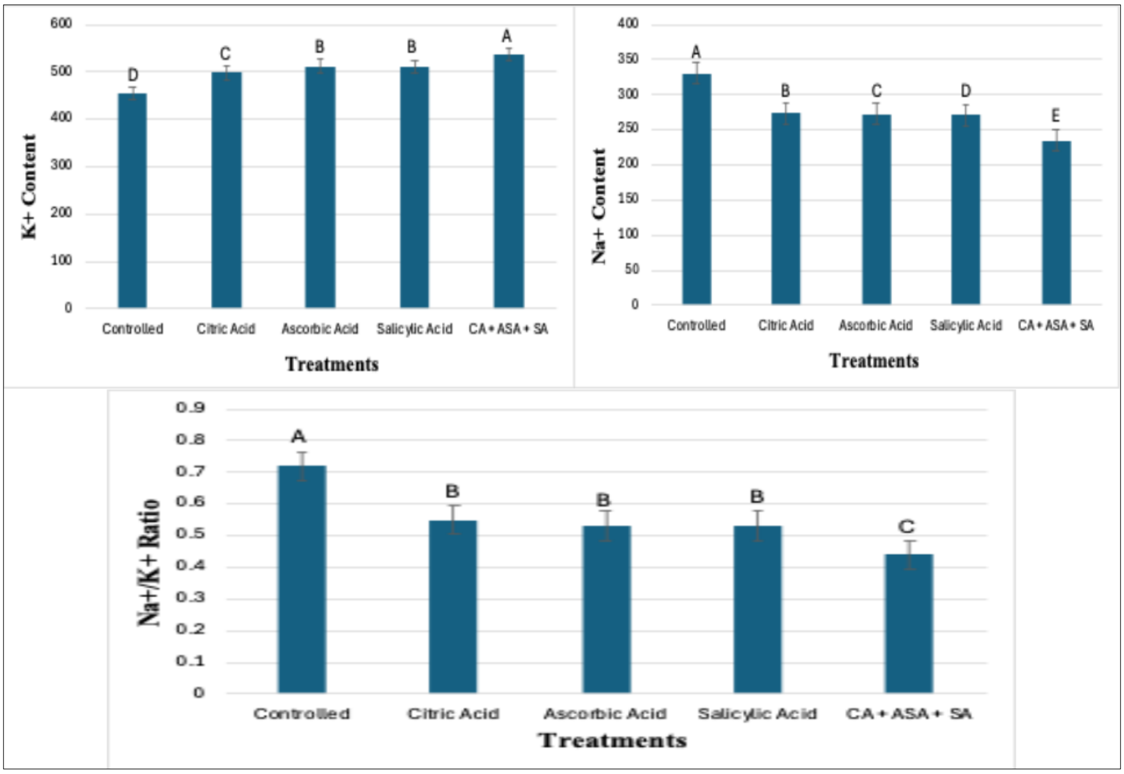


**Figure 5: Graphical representation of LSD test ± SE for K+ Content, Na+ Content and Na+/K+ Ratio**

### 3.5. Yield Attributes

Like all other attributes of maize, yield attributes were also significantly effected by salinity. Lowest hundred grain weight and lowest harvest index were observed in those plants, who didn't receive foliar application of any antioxidant. While all the antioxidants cause significant increase in yield attributes when applied separtely or in mixture as shown in Figure-6. Foliar application of citric acid (CA) caused 5.43% increase in hundred grain weight and 5.6% increase in harvest index as compared to controlled plants. Ascorbic acid (ASA) caused 10.77% increase in hundred grain weight and 7.21% increase in harvest index as compared to controlled plants. Similarly, salicylic acid (SA) caused 9.93% increase in hundred grain weight and 7.61% increase in harvest index as compared to controlled plants. While highest increase in hundred grain weight (16.11%) and harvest index (13.45%) were observed in plants who received foliar application of 100 ppm mixture of all three antioxidants.

The application of ascorbic acid (AA), citric acid (CA), and salicylic acid (SA) to the foliage improved growth parameters, leading to increased ear length, grain weight, 100-grain weight, and harvest index. The growth and yield improvements observed under saline soil conditions in response to non-enzymatic antioxidants may be due to their protective effect on cell membranes, which enhances plant tolerance to salinity

 

[34]. Several studies have highlighted the role of antioxidants in regulating maize plant responses to salt stress and suggested that antioxidants could serve as vital growth regulators, enhancing plant growth and nutrient uptake to mitigate the effects of salinity stress [35].

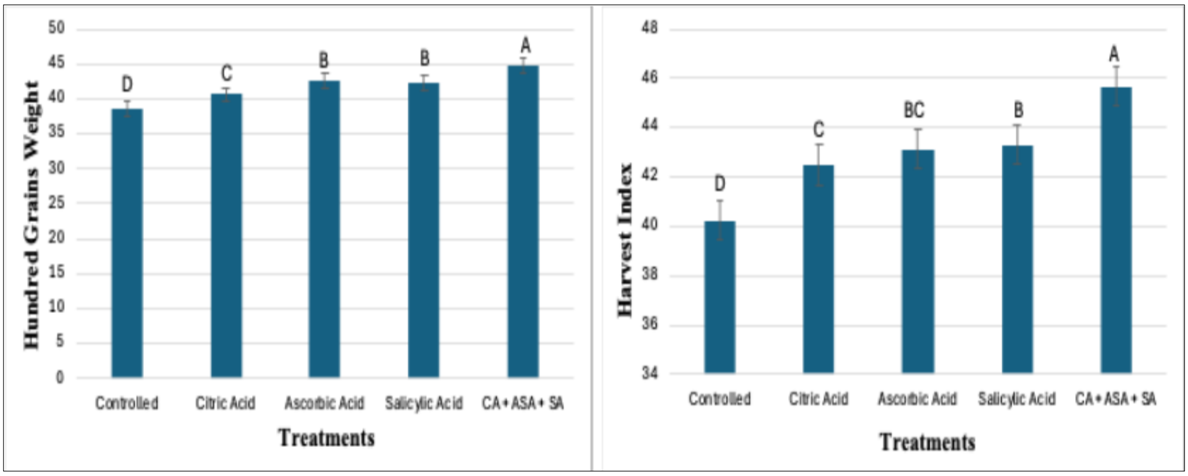


**Figure 6: Graphical representation of LSD test ± SE for Hundred Grain Weight and Harvest Index**

## CONCLUSIONS

From these results, it can be concluded that foliar spraying of antioxidants such as ascorbic, citric, and salicylic acids significantly helped plants to overcome salt toxicity and provided improved results for growth, photosynthetic and yield attributes of maize. But foliar spray with 100 ppm of ascorbic, citric, and salicylic acids mixture provided highest results for growth and yield of maize. So, it can be suggested to use mixture of antioxidants for better growth and yield of maize under saline conditions instead of use of single antioxidant.

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