



# Alpha-tocopherol reinforce selenium efficiency to ameliorates salt stress in maize plants through carbon metabolism, enhanced photosynthetic pigments and ion uptake



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## ABSTRACT

Individually selenium and  $\alpha$ -tocopherol showed a pivotal role in combating abiotic stresses in plants. However, there is no report on how selenium behaves in the presence or absence of  $\alpha$ -tocopherol under salt stress in crop plants. With this view, the present study was undertaken to dissect the interaction between selenium and  $\alpha$ -tocopherol on growth performance, carbon metabolism, and uptake of different ions in maize plants grown under salt stress. This study was conducted with surface-sterilized seeds of maize that were soaked in deionized water (control), selenium (0.5  $\mu$ M), and/or  $\alpha$ -tocopherol (200 ppm) for 12 h before sowing and different salt levels (0, 100, 150, and 200 mM) in the form of NaCl was created in soil. A sample of maize plants from each treatment was collected 40 days after sowing. The results revealed that salinity lowered the growth performance, chlorophyll content, insoluble sugar, carbohydrate, phenolic, flavonoid content, and different ions uptake in concentration dependent manner whereas, soluble sugar,  $\alpha$ -amylase activity, and sodium ion increased in comparison to control plants. Moreover, selenium and/or  $\alpha$ -tocopherol treated plants without stress significantly amplified the growth performance, chlorophyll and carotenoid content, carbohydrate, phenolic, and flavonoid content, and improved the uptake of different concentrations ions ( $K^+$ ,  $Ca^{+2}$ ,  $K^+/Na^+$ ,  $Ca^{+2}/Na^+$ , and  $Mg^{+2}$ ) over the control plants. Interestingly, treatment of combined selenium and  $\alpha$ -tocopherol to the salt-stressed plants successfully recover the loss caused by increasing salt levels in maize plants through altered carbon metabolism and ions uptake reflected in improved growth performance and bigger growth leaf area of maize plants under salt stress. Selenium and  $\alpha$ -tocopherol in combination also enhance the antioxidative defensive mechanism through the production of phenolics and flavonoids content in maize plants under salt stress. This approach could be an effective measure to lower salt stress and enhance the crop productivity of maize plants.

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## 1. Introduction

Excess salinity is one of the significant problems of arid and semi-arid regions that limit crop productivity to reach its full genetic potential (Suo et al., 2017; Wan et al., 2018). As per the record of FAO Land and Plant Nutrition Management Services, it is believed that more than 6% of the total land is affected by salinity. The devastating effect can be seen in the morphological and biochemical responses of plants. It includes reduced seed germination, plant growth and development, and subsequently low yield (Liu et al., 2018). Salinity damages photosynthetic machinery and its related traits through reduced chlorophyll content and altered chloroplast structure

(Wungrampha et al., 2018; Pan et al., 2020;). Additionally, the availability of excess salt in soil drops soil-water potential and leaf-water potential and disturbs the plant's turgor, leading to osmotic stress (Navada et al., 2020). Uptake of salt from the soil via transporters induces ion toxicity and disturbs ion uptake and its homeostasis. Salinity leads to extensive accumulation of ions ( $Na^+$ ,  $Cl^-$ ) and inhibits  $K^+$  and  $Ca^{2+}$  uptake, and results in ionic stress (Isayenkov and Maathuis, 2019). Salinity also induces reactive oxygen species (ROS) in plant cells and generates oxidative stress that is reflected in increased lipid peroxidation, membrane deterioration, and protein damage (Bose et al., 2014; Habib et al., 2016). The hour needs to control salinity stress through sustainable agriculture practices and undertake present and future food demand globally (Zorb et al., 2019).

Selenium (Se), being a beneficial micronutrient, play a pivotal role in enhancing the physiological efficiency of plants under compromised

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environmental conditions. The findings of Hasanuzzaman et al. (2010) reported that the treatment of *Brassica juncea* with selenite improved the growth and yield. Chan Chi Chen and Jih Min Sung (2001) showed that seeds of bitter melon soaked in selenite showed higher percent germination under abiotic stress conditions. In addition to this, foliar spray of Se solutions enhanced the growth efficiency of ryegrass, lettuce, potato, and green tea leaves through enhanced starch accumulation and protection of cellular components (Hasanuzzaman et al., 2010). Se improved photoassimilate allocation for potato tuber growth and created a substantial carbohydrate sink in the young upper leaves, stolons, roots, and tubers (Turakainen 2007). A low concentration of selenium can increase the activities of antioxidant enzymes and non-antioxidant enzymes in plants (Ríos et al., 2009). It is also observed that Se application to stressed plants lowered the accumulation of H<sub>2</sub>O<sub>2</sub> content and lipid peroxidation (Banerjee et al., 2019). On the other hand, higher Se concentration proved to be toxic, and its concentration needs to be optimized to excel in agricultural benefits.

$\alpha$ -Tocopherol is only synthesized by all plants and act as a lipid-soluble antioxidant. They are naturally produced in green photosynthetic organisms (Falk and Munné-Bosch, 2010). The  $\alpha$ -tocopherol is present primarily in the plant membranes, such as photosynthetic membranes of plant leaves (Grilo et al., 2014). Its concentration varies under various environmental stress conditions (Kumar et al., 2012) and actively takes part in various metabolic activities (Hasegawa et al., 2000). It plays a pivotal role in ROS scavenging, membrane stabilization while interacting with the polyunsaturated acyl groups of lipids (Maeda and DellaPenna, 2007).  $\alpha$ -tocopherol was found to be effective in plants through endogenous synthesis or through the exogenous application under various abiotic stresses (Jamil et al., 2015; Sadiq et al., 2018). In sunflower, enhanced photosynthesis was found after tocopherol application that was associated with decreased ABA content (Hassanein et al., 2009). Its exogenous application also improved the growth and productivity of plants under environmental cues (Sadiq et al., 2018).

Maize is a vital cereal crop widely grown across the globe but normally submissive to salt stress. Its production is greatly affected by soil salinization. Therefore, there is a need to develop efficient strategies that could improve salinity tolerance in maize plants to avoid significant yield losses (Fukami et al., 2018; Geilfus et al., 2018; Hassanein et al., 2009; Zhang et al., 2018). As per the above reports, it is found that selenium and  $\alpha$ -tocopherol individually confer tolerance to various abiotic stresses in their capacity, but the relationship between both under salt stress is missing and needs to be explored through the physiological and biochemical approaches. Therefore, the present study was designed to unravel the relationship between selenium and  $\alpha$ -tocopherol on growth performance, carbon metabolism, and uptake of different ions in maize plants grown under salt stress and assess their ability to confer tolerance against salt stress in maize plants.

## 2. Material and methods

### 2.1. Procurement of materials

The pure strain of *Zea mays* L. (TWC. 321) seeds were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Sodium selenate (Na<sub>2</sub>SeO<sub>4</sub>) as source of selenium and  $\alpha$ -tocopherol (vitamin E) were obtained from Sigma Chemical Company, Egypt.

### 2.2. Experimental plan and treatment pattern

This experiment was conducted with 80 plastic pots (25 cm in diameter and 40 cm in depth) under a completely randomized block

design. Each pot filled with equal amounts of clay-sand soil mixture (2:1 v/v ratio). Phosphorus and potassium fertilizer was added before sowing, at a rate of 6 and 3 g/pot in the form of calcium superphosphate (15.5 % CaH<sub>4</sub>P<sub>2</sub>O<sub>8</sub>) and potassium sulfate (48 % K<sub>2</sub>SO<sub>4</sub>). Five pots (replicates) were assigned and in each pot, three plants were maintained throughout the experiment. Prior to sowing, surface-sterilized uniform-sized seeds were soaked in deionized water (control), Na<sub>2</sub>SeO<sub>4</sub> (0.5  $\mu$ M),  $\alpha$ -tocopherol (200 ppm), and Na<sub>2</sub>SeO<sub>4</sub> (0.5  $\mu$ M) +  $\alpha$ -tocopherol (200 ppm) solutions. Seeds were soaked individually for 12 h in deionized water, Na<sub>2</sub>SeO<sub>4</sub>, and  $\alpha$ -tocopherol and 6 hours each in Na<sub>2</sub>SeO<sub>4</sub> (0.5  $\mu$ M) +  $\alpha$ -tocopherol (200 ppm) solutions. These concentrations were based on a preliminary experiment of percent germination. After sowing, plants were allowed to grow till 20 days stage of growth under natural environmental conditions. At 21 days after sowing, plants were exposed with 300 ml of salinization levels (0, 100, 150, and 200 mM NaCl) through the soil. Plants were irrigated with water, and the water-holding capacity of each pot was kept at 80% until the end of the experimental period. At 40 days after sowing, maize plant samples were collected from each treatment and examined different growth traits, photosynthetic pigments, carbon metabolism traits, and ion uptake.

### 2.3. Extraction and determination of photosynthetic pigments

Chlorophyll a (Chl. a), chlorophyll b (Chl. b) and carotenoids in fresh leaves were assayed using the method described by Metzner et al. (1965) and described by Hassanein et al. (2009a).

$$\text{Chl.a} = 10.3A_{663} - 0.918A_{644}$$

$$\text{Chl.b} = 19.7A_{644} - 3.87A_{663}$$

$$\text{Carotenoids} = 4.2A_{452.5} - (0.0264\text{Chl.a} + 0.4260\text{Chl.b})$$

Finally, the pigment contents were expressed as mg pigment g<sup>-1</sup> fresh mass of leaves.

### 2.4. Extraction and estimation of carbohydrates

As per the method described by (Radwan et al., 2007), water-soluble sugars were extracted. A specified weight of the dry plant tissue was boiled in 10 ml of deionized water for one hour. The mixture was cooled and centrifuged at 5000 g for 10 minutes. One ml of supernatant was hydrolyzed with one ml of 2 N HCl in the water bath to obtain the total soluble reducing sugars.

### 2.5. Total carbohydrates

A known weight of dry powdered tissue was boiled in 10 ml of 6 N HCl on the water bath for 2 hours (Radwan et al., 2007). After cooling, the extract was centrifuged at 5000 g for 10 minutes. The supernatant was neutralized, completed up to known volume with distilled water, and the reducing value of the total carbohydrate was measured.

The reducing value of each sugar extract was determined according to the method (Clark and Switzer, 1977). One ml of each sugar extract was mixed with 1 ml of freshly prepared Nelson's alkaline copper reagent. (Nelson's A: B; 25:1) and heated in a boiling water bath for 20 min. After that, 1 ml of arsenomolybdate reagent was added with several shakings to dissolve Cu<sub>2</sub>O. When effervescence stopped, the mixture was made up to 10 ml with distilled water, and its color intensity was measured at wavelength 540 nm against water-reagent blank treated in the same manner as the sample. The content of reducing sugar was determined from the glucose standard curve and then calculated as mg sugar g<sup>-1</sup> dry weight. Subtracting the total carbohydrate content from the total soluble sugar content gave the insoluble carbohydrate fraction.

## 2.6. Amylase activity

Fresh leaves were homogenized in acetate buffer (pH 6.0), and the reaction mixture was prepared with 0.5 ml extract, 0.5% soluble starch prepared in 0.1 M of acetate buffer, pH 6.0, and 5 mM CaCl<sub>2</sub>. HgCl<sub>2</sub> terminated the reaction mixture after 30 min of incubation at 40°C. The resulting reducing sugars were estimated by Nelson's method (Plummer, 1978).

## 2.7. Extraction and determination of phenolic compounds

Phenolic compounds were extracted according to Sauvesty et al. (1992). A dry powder of plant tissue was extracted in 70% ethanol at 40°C overnight. One ml of extract was hydrolyzed with 1 ml of 2 N HCl in a boiling water bath for one h. The mixture was neutralized and filled to a known volume with distilled water, then used for the determination of total phenolics. The Folin–Ciocalteu phenol method (Lowe, 1993) was used for phenolic aglycone determination. One ml of phenolic extract was mixed with one ml of 10% Folin–Ciocalteu phenol reagent and one ml of 20% anhydrous sodium carbonate and then completed up to a known volume with distilled water. The absorbance of blue color was measured after 30 minutes at wavelength 650 nm against a water reagent blank. The phenolic content was obtained from the standard curve of catechol and then calculated as mg phenolic g<sup>-1</sup> dry weight. Subtraction of phenolic content after and before acid hydrolysis gave the content of phenolic glycosides.

## 2.8. Extraction and determination of flavonoids

Total flavonoid content was measured using aluminum chloride colorimetric assay (Sakanaka et al., 2005). Dry samples were extracted with methanol, and then 0.25 ml of the clear extract was mixed with 1.25 ml of distilled water in a test tube, followed by the addition of 75 µl of 5% (w/v) sodium nitrite solution. After 6 min of incubation, 150 µl of 10% (w/v) aluminum chloride solution was added, and the mixture was allowed standing for a further 5 min before adding 0.5 ml of 1 M sodium hydroxide. The mixture was completed up to 2.5 ml with distilled water. The absorbance was measured immediately at 510 nm, and the concentration of total flavonoids was calculated using a standard curve of myricetin, then expressed as mg g<sup>-1</sup> dry weight.

## 2.9. Estimation of certain minerals

The dried matter was digested according to the method of (Chapman and Pratt, 1962). A 250 ml digestion flask was filled with 0.2 g of grounded plant material. A mixture of concentrated sulphuric acid and perchloric acid in the ratio 5:1 (v/v) was added to the digestion flask. The samples were digested, and finally, the solution became clean and was about 2.5 ml. Samples were allowed to cool and diluted with deionized water and transferred into a 50 ml volumetric flask. The volumes were made up to volume with distilled water. Filtration was carried out using filter paper Whatman No.42. The solution was stored for potassium, calcium, sodium, magnesium, and selenium. Potassium (K) was measured by flame-photometer according to Ranganna (1977) and Ca, Na, Mg, and Se by atomic absorption according to the method described in Julshamn et al. (2005).

## 2.10. Statistical analysis

The results presented in the graphs and table are the means ± standard error of five replicates (n = 5). The results were statistically confirmed by analysis of variance (ANOVA) followed by Duncan's multiple range tests at  $P \leq 0.05$  using IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp. Mean that do not share a letter are significantly different at the  $P \leq 0.05$  significance level.

## 3. Results

### 3.1. Growth characteristics

It is evident from Fig. 1 and 2, growth parameters (shoot and root length; fresh and dry mass of shoot and root; leaf area per plant) decreased significantly in the presence of NaCl in a concentration-dependent manner. Maximum loss was observed under 200 mM of salt and reduced dry mass of root (52%) and shoot (48.7%) in comparison to control plants at 40 days after sowing. The application of selenium (0.5 µM) and α-tocopherol (200 ppm) either alone or in combination showed a significant increase in the growth parameters values (shoot and root length; the fresh and dry mass of shoot and root; and leaf area per plant. Treatments of 0.5 µM sodium selenate and 200 ppm α-tocopherol together proved best to enhance the growth performance of plants under NaCl stress as well stress-free conditions.

### 3.2. Photosynthetic pigment

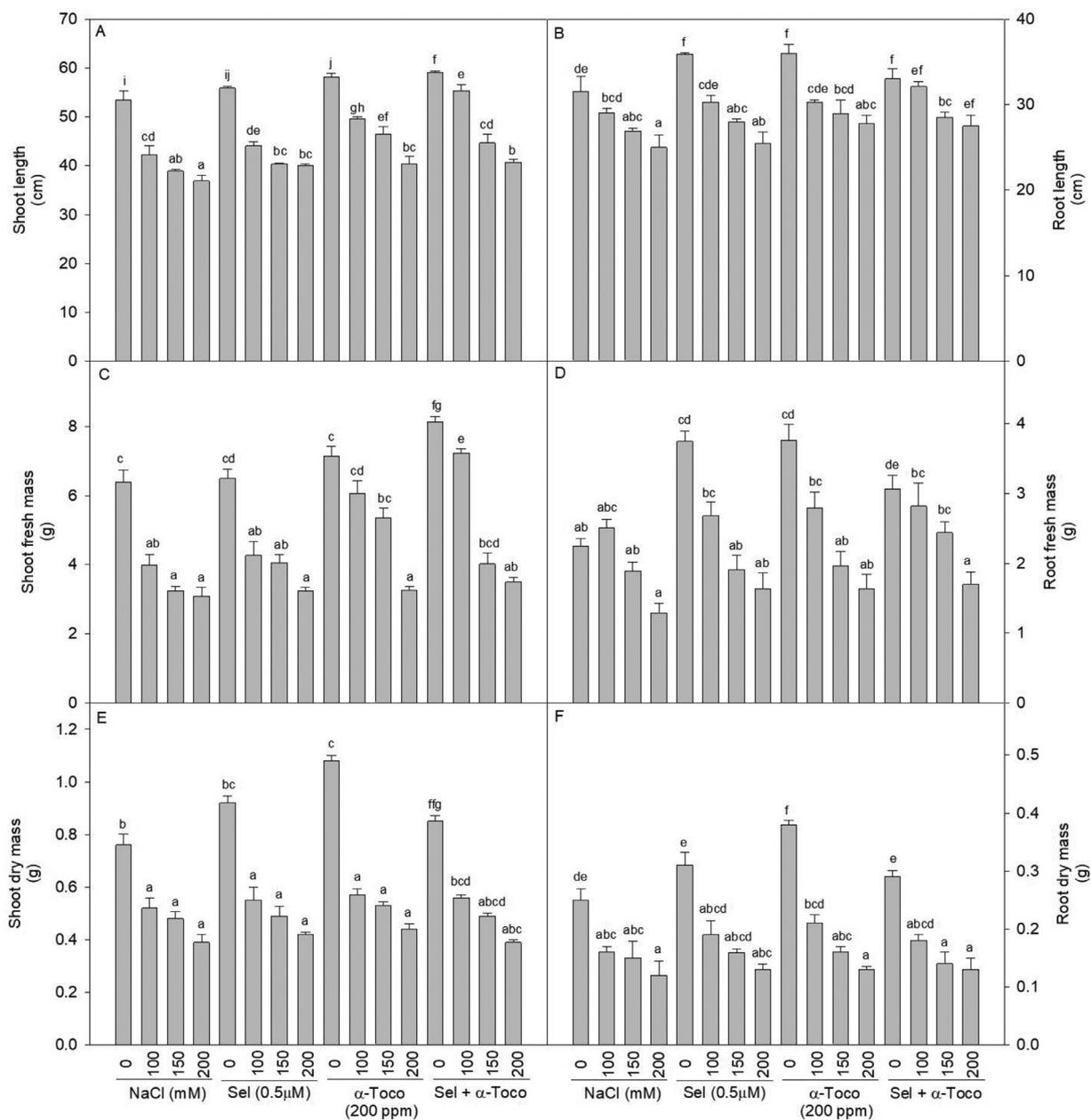
Chlorophyll a, Chlorophyll b, and Carotenoids contents of 40-days old maize plants decreased with the increasing concentration of salinity as compared with non-treated (control) plants (Fig 2). The maximum damage induced by salinity on chlorophyll a, chlorophyll b, and carotenoid were recorded at 200 mM NaCl by 75.3%, 75%, and 56.8%, respectively, in comparison to the control plant. However, sodium selenate, α-tocopherol alone or in combination significantly increased chlorophyll a, chlorophyll b, and carotenoids contents as compared with their untreated control plants. Additionally, the ameliorative effect of sodium selenate and α-tocopherol combination revealed and overcome the damage caused by salt stress in *Zea mays* plants.

### 3.3. Carbohydrates content

The pattern of changes in the amount of various carbohydrate fractions and amylases activity in shoots of maize plants subjected to salt stress in presence or absence of selenium, α-tocopherol, and their interaction are demonstrated in fig. 3. The figure clearly revealed that varying concentrations of NaCl caused a marked increase in total soluble sugar (23.22%) and amylases activity (57.9%) whereas decreased insoluble sugars (64.85%) and total carbohydrate contents (20.03%) in comparison to control plants. However, selenium, α-tocopherol alone or in combination showed a non-significant increase in the contents of total carbohydrates. It significantly increased insoluble sugars and a significant decrease in total soluble sugar and amylases activity in the shoot of maize plants. The maximum increasing value of total carbohydrate contents in the test plant was estimated at 2.88%, 6.05%, and 5.32%, and amylases activity decreased by 50%, 58.3%, and 70.8%, respectively, as compared with reference controls.

### 3.4. Phenolics and flavonoids content

It is depicted from fig. 4 that exposure of *Zea mays* plants to various concentrations (100, 150, and 200 mM) of NaCl significantly lowered total phenol and flavonoids contents as compared with untreated control plants. The maximum reduction was recorded in plants treated with 200 mM of NaCl and was estimated at 27.6% and 37.55% less total phenols and flavonoids content, respectively, in comparison to their control plants. Contrary to this, maize plants treated with sodium selenate and α-tocopherol alone or in combination showed a significantly increasing trend in terms of phenolic and flavonoid content. In contrast, the duo of sodium selenate and α-tocopherol treatment to the salt-stressed plants successfully encounter the damage generated by salinity in *Zea mays* plants.



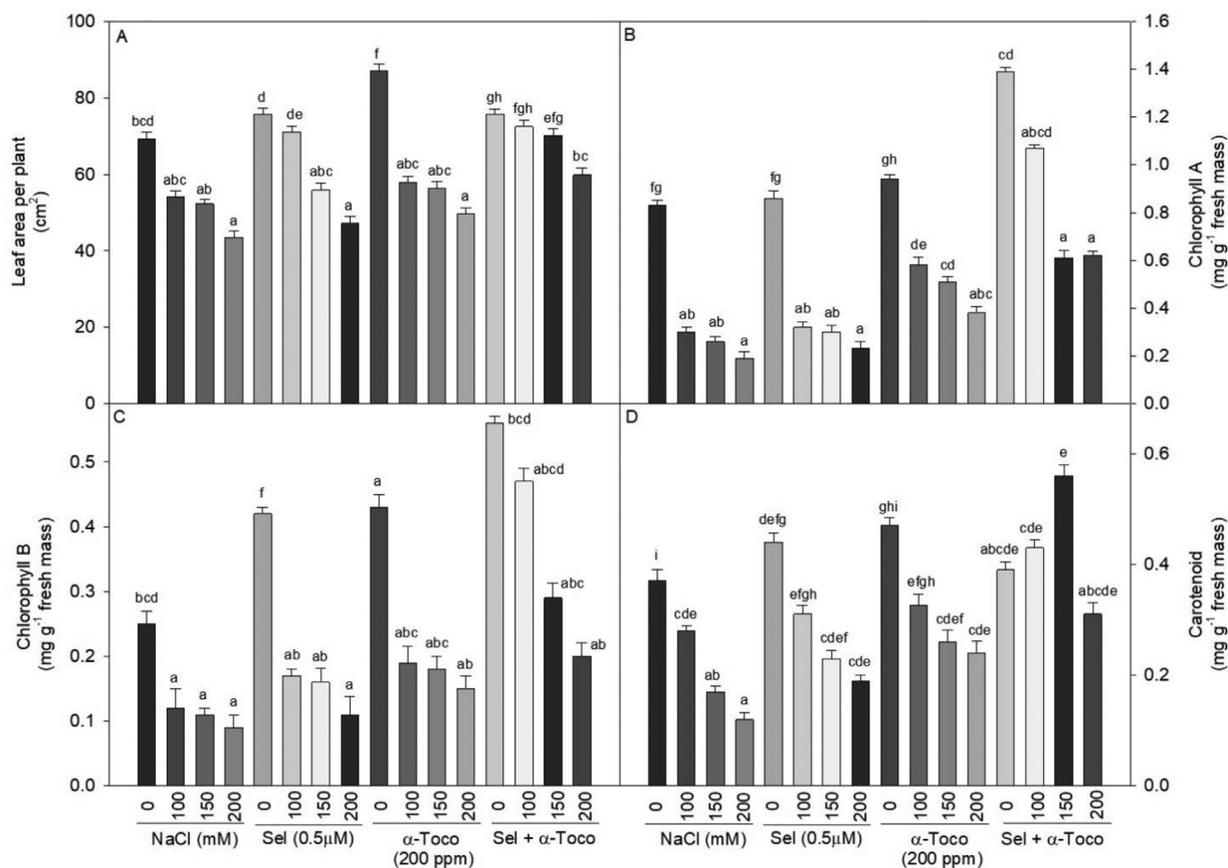
**Fig. 1.** Effect of sodium selenite (Sel) and/or  $\alpha$ -tocopherol ( $\alpha$ -Toco) induced changes in (A) shoot length (B) root length, (C) shoot fresh mass, (D) root fresh mass, (E) shoot dry mass, and (F) root dry mass under varied levels of salt stress in 40 days old *Zea mays* L. plants. Values are expressed as mean  $\pm$  standard error of 3 independent replicates. Different letters indicate a significant difference between control and treatment ( $P \leq 0.05$ ).

### 3.5. Inorganic cations content

Results obtained in table 1 showed a significant increase in sodium contents with the increasing salinity level over the non-treated control plants. At the highest salinity level, 200 mM of NaCl, the accumulation of  $\text{Na}^+$  and  $\text{Se}^{-2}$  were 33.3% and 23.6% as compared to the control plant. On the other hand, the contents of  $\text{K}^+$ ,  $\text{Ca}^{+2}$ ,  $\text{K}^+/\text{Na}^+$ ,  $\text{Ca}^{+2}/\text{Na}^+$ , and  $\text{Mg}^{+2}$  in maize shoots showed a significant decrease with the increased NaCl level. Minimum values were estimated by 9.25%, 9.79%, 27.20%, 27.63% and 10.77% under 200 mM-treated plants in comparison to control plants. Application of sodium selenate,  $\alpha$ -tocopherol, and both resulted either in a highly significant decrease in  $\text{Na}^+$  contents or a highly significant increase of  $\text{Se}^{-2}$ ,  $\text{K}^+$ ,  $\text{Ca}^{+2}$ ,  $\text{K}^+/\text{Na}^+$ ,  $\text{Ca}^{+2}/\text{Na}^+$ , and  $\text{Mg}^{+2}$ , as compared with reference control plants.

### 4. Discussion

The occurrence of salt stress causes characteristic changes in the plant from the time of occurrence until the maturity of plants (Munns, 2002). In the present study, plants grown under varying salinity levels showed deleterious effects on growth parameters such as root and shoot length, fresh and dry mass of root and shoot, and leaf area of the plant (Figs. 1 and 2). This happened due to the shrinkage and dehydration of plant cells exposed to salt stress. It also restricts the cell elongation and division that, in return, resulted in reduced root, shoot, and leaf growth rate. This response is due to changes in cell-water relation resultants of osmotic changes outside the root. The osmotic effect leads to a reduction in the capability of crops to absorb water (Munns, 2005). Our results align with the findings of (Ghoulam et al., 2002) that revealed salinity caused a



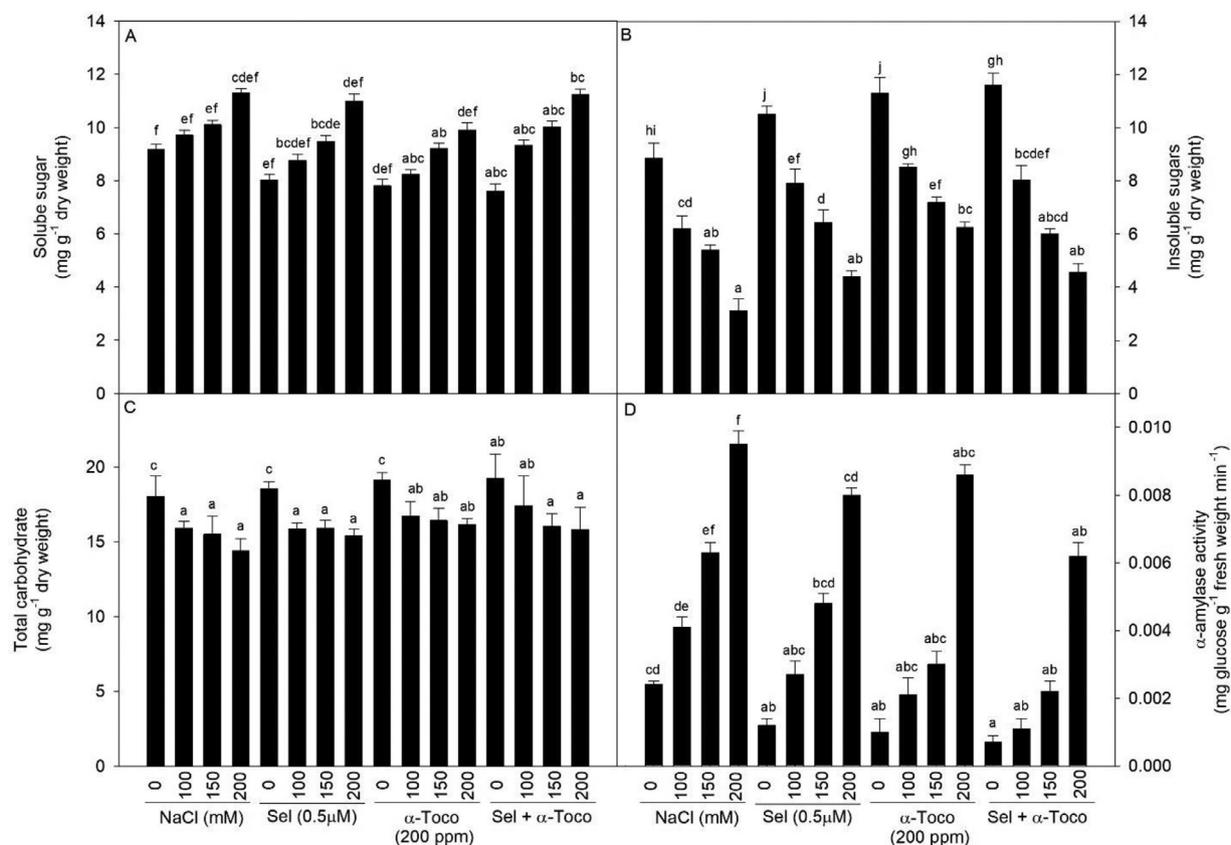
**Fig. 2.** Effect of sodium selenite (Sel) and/or  $\alpha$ -tocopherol ( $\alpha$ -Toco) induced changes in (A) leaf area per plant (B) chlorophyll a, (C) chlorophyll b, and (D) carotenoid content under varied levels of salt stress in 40 days old *Zea mays* L. plants. Values are expressed as mean  $\pm$  standard error of 3 independent replicates. Different letters indicate a significant difference between control and treatment ( $P \leq 0.05$ ).

noticeable decline in growth traits and with the study of (Kaya et al., 2018). Application of selenium,  $\alpha$ -tocopherol in salt-stressed plants alone or in combination improved the growth performance of *Zea mays* plants. Selenium plays a vital role in regulating plant growth through improved antioxidant systems and secondary metabolite metabolism (Jiang et al., 2017).

Additionally, the Se-induced reduction of Na<sup>+</sup>/K<sup>+</sup> ratio in plants under salt stress is reflected in the protection of some essential processes and balanced osmotic potential of plants (Astaneh et al., 2018). There are various reports that revealed the significance of selenium in the improvement of the growth performance of multiple crops such as rice (Subramanyam et al., 2019) and wheat (Elkelish et al., 2019) under both stressed and non-stressed conditions. Se application improved the growth and yield of eggplants under increasing levels of soil salinity (Butt et al., 2016). On the other hand,  $\alpha$ -tocopherol has the ability to regulate the growth performance through enhanced activities of both glutathione S-transferases (GST) and glutathione peroxidase (GPX) during stressed and stress-free environments and protect the plants' metabolism (Sadiq et al., 2019). A study by (Hussein et al., 2007) showed that exogenously applied  $\alpha$ -tocopherol improved the growth of cowpea plants, and (Mekki et al., 2015) revealed that exogenous application of  $\alpha$ -tocopherol improved the growth performance of cotton plants under abiotic stress conditions.

Chloroplasts are organelles that have the most significant effect on salinity. Salinity causes damage to the chloroplast structure and the instability of the colored protein compounds. Carotenoids are also affected due to salinity; the amount of chlorophyll is reduced due to more chlorophyllase activity under salt stress conditions (Mosavi et al., 2018). Reducing the amount of chlorophyll can be due

to the change in the nitrogen metabolism associated with the formation of compounds such as proline that are used in osmotic regulation. Salt irritates glutamine kinase activity, the first enzyme in the proline biosynthesis pathway (Bollivar and Beale, 1996; El-Bassiouny and Sadak, 2014). El-Sawy (2009) and (Hassanein et al., 2009) found that reduction in plant pigments concentrations may be due to a decrease in absorption of some ions as Mg<sup>2+</sup> and Fe<sup>3+</sup>, which were involved in chlorophyll biosynthesis under stress conditions. Salinity effectively altered the carbon assimilation, stomatal conductance, and photosynthetic electron transport efficiency (Sun et al., 2016). Accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in plant tissues is one of the characteristics markers of salt-induced changes in plants. During these changes, major physiological imbalances take place due to altered uptake of essential minerals from soil (James et al., 2011). An adequate presence of Se helps in improving photosynthetic efficiency through improved CO<sub>2</sub> assimilation and chlorophyll fluorescence under stress and stress-free conditions (Alyemeni et al., 2018). Moreover, treatment of stressed plants with Se showed a favorable response in terms of improved growth and higher content of photosynthetic pigments in *Melissa officinalis* L. (Habibi, 2017) whereas, at the same time, Se decreased Na<sup>+</sup> accumulation and increased K<sup>+</sup> in the roots and shoot as well (Shekari et al., 2017). Das and Roychoudhury (2014) found that the exogenous application of  $\alpha$ -tocopherol significantly improved photosynthetic pigment contents. The  $\alpha$ -tocopherol is found in photosynthetic membranes of plant leaves (Grilo et al., 2014). Being abundant in membranes, tocopherols scavenge these free radicals, counteract membranous lipid peroxidation, neutralize ROS, and play a vital role in heat dissipation in the chloroplast (Falk and Munne-Bosch, 2010). Kruk and Strzalka (2001) reported that  $\alpha$ -tocopherol protects chloroplasts by

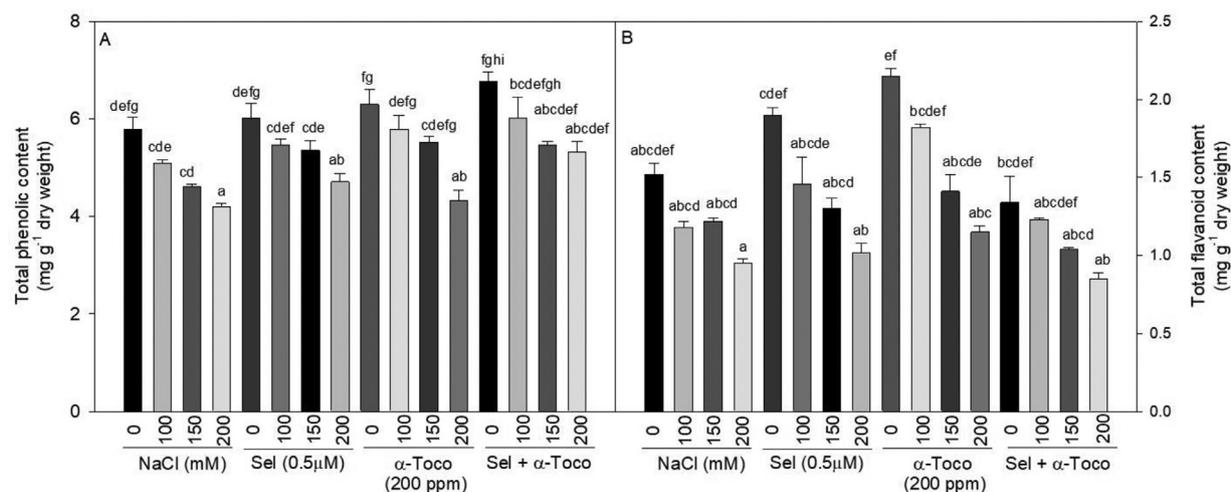


**Fig. 3.** Effect of sodium selenite (Sel) and/or  $\alpha$ -tocopherol ( $\alpha$ -Toco) induced changes in (A) soluble sugar (B) insoluble sugar (C) total carbohydrate and (D)  $\alpha$ -amylase activity under varied levels of salt stress in 40 days old *Zea mays* L. plants. Values are expressed as mean  $\pm$  standard error of 3 independent replicates. Different letters indicate a significant difference between control and treatment ( $P \leq 0.05$ ).

reducing cytochrome b559 during periodical electron flow through photosystem II. Semida et al. (2016) said that external use of  $\alpha$ -tocopherol improved photosynthetic performance, endogenous turgor status, enzymatic and non-enzymatic antioxidant activities/levels in salt-stressed onion plants. Our findings are in line with the above-mentioned reports of various researchers in different crops.

The decrease in dry weight of shoots by increasing the salinity level (Fig. 1) could be credited to a reduction in photosynthetic

output as indicated by the significant cuts in chlorophylls, and total carbohydrates in saline stressed *Zea mays* plants (Fig. 2). The decrease in carbohydrate contents, photosynthetic pigment contents, and all the estimated growth parameters was directly proportional to the applied concentration of NaCl (Figs. 1-3). However, Gul et al. (2017) concluded that plants treated with Se under salt stress significantly enhanced physiological performance that is reflected in increased chlorophyll contents, carbohydrates, proteins, and carotenoids,



**Fig. 4.** Effect of sodium selenite (Sel) and/or  $\alpha$ -tocopherol ( $\alpha$ -Toco) induced changes in (A) total phenolic content and (B) total flavanoid content under varied levels of salt stress in 40 days old *Zea mays* L. plants. Values are expressed as mean  $\pm$  standard error of 3 independent replicates. Different letters indicate a significant difference between control and treatment ( $P \leq 0.05$ ).

**Table 1**

Effect of sodium selenite (Sel) and/or  $\alpha$ -tocopherol ( $\alpha$ -Toco) compartmentalization of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+/\text{Na}^+$ ,  $\text{Ca}^{2+}/\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Se}^{2-}$  under varied levels of salt stress in 40 days old *Zea mays* L. plants. Values are expressed as mean  $\pm$  standard error of 3 independent replicates. Different letters indicate a significant difference between control and treatment ( $P \leq 0.05$ ).

Treatments	NaCl (mM)	$\text{Na}^+$ (%)	$\text{K}^+$ (%)	$\text{Ca}^{2+}$ (%)	$\text{K}^+/\text{Na}^+$ (%)	$\text{Ca}^{2+}/\text{Na}^+$ (%)	$\text{Mg}^{2+}$ (%)	$\text{Se}^{2-}$ (%)
Reference control	0	2.720 <sup>f</sup>	1.882 <sup>e</sup>	2.245 <sup>i</sup>	0.692 <sup>bcd</sup>	0.825 <sup>bc</sup>	1.958 <sup>bcd</sup>	0.0229 <sup>de</sup>
	100	2.805 <sup>ef</sup>	1.798 <sup>cd</sup>	2.237 <sup>h</sup>	0.640 <sup>cd</sup>	0.797 <sup>abc</sup>	1.871 <sup>bc</sup>	0.0235 <sup>e</sup>
	150	3.371 <sup>ef</sup>	1.694 <sup>ab</sup>	2.111 <sup>gh</sup>	0.502 <sup>a</sup>	0.626 <sup>a</sup>	1.801 <sup>abc</sup>	0.0264 <sup>ef</sup>
	200	3.387 <sup>def</sup>	1.708 <sup>a</sup>	2.025 <sup>fg</sup>	0.504 <sup>a</sup>	0.597 <sup>a</sup>	1.747 <sup>ab</sup>	0.0283 <sup>ef</sup>
Sodium selenite (0.5 $\mu\text{M}$ )	0	2.293 <sup>de</sup>	1.891 <sup>ef</sup>	2.746 <sup>e</sup>	0.824 <sup>bc</sup>	1.197 <sup>de</sup>	2.480 <sup>de</sup>	0.0220 <sup>e</sup>
	100	2.371 <sup>def</sup>	1.810 <sup>e</sup>	2.647 <sup>ef</sup>	0.763 <sup>ab</sup>	1.116 <sup>cd</sup>	2.410 <sup>cd</sup>	0.0169 <sup>d</sup>
	150	3.187 <sup>ef</sup>	1.756 <sup>d</sup>	2.511 <sup>ef</sup>	0.551 <sup>ab</sup>	0.787 <sup>abc</sup>	2.088 <sup>bc</sup>	0.0216 <sup>e</sup>
	200	3.347 <sup>ef</sup>	1.802 <sup>ab</sup>	2.187 <sup>fg</sup>	0.538 <sup>b</sup>	0.653 <sup>ab</sup>	1.781 <sup>ab</sup>	0.0243 <sup>e</sup>
$\alpha$ -tocopherol (200 ppm)	0	2.137 <sup>cd</sup>	2.265 <sup>i</sup>	3.163 <sup>d</sup>	1.059 <sup>abc</sup>	1.480 <sup>ef</sup>	3.242 <sup>f</sup>	0.0241 <sup>e</sup>
	100	2.200 <sup>cde</sup>	2.069 <sup>gh</sup>	3.035 <sup>de</sup>	0.940 <sup>bc</sup>	1.379 <sup>def</sup>	3.026 <sup>ef</sup>	0.0201 <sup>d</sup>
	150	3.060 <sup>ef</sup>	1.797 <sup>ab</sup>	2.776 <sup>e</sup>	0.587 <sup>b</sup>	0.907 <sup>d</sup>	2.433 <sup>de</sup>	0.0195 <sup>cd</sup>
	200	3.328 <sup>ef</sup>	1.932 <sup>bc</sup>	2.244 <sup>i</sup>	0.581 <sup>b</sup>	0.674 <sup>bc</sup>	2.086 <sup>bc</sup>	0.0188 <sup>e</sup>
Sodium selenite (0.5 $\mu\text{M}$ ) + $\alpha$ -tocopherol (200 ppm)	0	1.713 <sup>bc</sup>	2.473 <sup>ghi</sup>	3.222 <sup>cd</sup>	1.443 <sup>abcd</sup>	1.880 <sup>g</sup>	3.842 <sup>gh</sup>	0.0189 <sup>e</sup>
	100	1.743 <sup>bc</sup>	2.102 <sup>gh</sup>	3.185 <sup>d</sup>	1.205 <sup>abc</sup>	1.827 <sup>fg</sup>	3.319 <sup>f</sup>	0.0225 <sup>e</sup>
	150	2.438 <sup>def</sup>	1.874 <sup>bcd</sup>	2.787 <sup>e</sup>	0.768 <sup>ab</sup>	1.143 <sup>f</sup>	3.007 <sup>ef</sup>	0.0244 <sup>e</sup>
	200	2.716 <sup>f</sup>	1.969 <sup>ab</sup>	2.567 <sup>ef</sup>	0.725 <sup>bc</sup>	0.945 <sup>ef</sup>	2.745 <sup>e</sup>	0.0183 <sup>ab</sup>

which are required to regulate various metabolic processes such as photosynthesis in maize (*Zea mays* L.). Similarly, Se also had a positive effect on growth and improved the total chlorophyll content and carbohydrates content in lemon balm (*Melissa officinalis* L.), decreasing  $\text{Na}^+$  ions (Habibi, 2017). Additionally,  $\alpha$ -tocopherol could alleviate the harmful effect of ROS induced by salt stress through its powerful antioxidant properties (Bughdadi, 2013).  $\alpha$ -Tocopherol improved the synthesis of sugar in plants by protecting chloroplasts in the plastid membrane (Sharma et al., 2012; Spicher et al., 2016). El-Bassiouny and Sadak (2014) revealed that treated flax cultivars with  $\alpha$ -tocopherol as a foliar spray enhanced total carbohydrates, free amino acids, and nucleic acids but significantly reduced the activities of polyphenol oxidase under saline conditions.

Leaf phenolic contents are essential protective components of plant cells. The synthesis of phenolics is affected by different abiotic stresses, including salinity (Parida et al., 2004). Depression in phenolics synthesis is due to the inhibition in synthetics of PAL enzyme under stress conditions and is therefore involved in the biosynthesis of the polyphenol compounds such as flavonoids, phenylpropanoids, and lignin in plants (Fritz et al., 1976). Some studies revealed that phenolic compounds declined in salt-treated plants (Blasco et al., 2013; Wahid and Ghazanfar, 2006). However, other studies have indicated that NaCl-treated plants exhibited enhanced phenolic biosynthesis (Burchard et al., 2000; Mahmoudi et al., 2010). (Miladinova et al., 2013) observed leaves of the Paulownia clones and noticed an increase in total flavonoid content with increasing salt stress. However, supplementation of Se at lower dosages not only protects plants from ROS-induced oxidative damage by activating the antioxidative mechanisms (Shahzadi et al., 2017). The work of (Poldma et al., 2013) supported our results that observed total phenols increased in onion bulbs with selenium treatment. In tomatoes, total phenolic content was increased in the presence of selenium (Schiavon et al., 2013). The production of phenolics and flavonoids content is also linked with the antioxidative defensive mechanism that is of significant importance under stressful environmental conditions for the functioning of cellular membranes. Similarly, the application of  $\alpha$ -tocopherol mediated antioxidative defense mechanism in terms of increased activities of enzymatic antioxidant and the content of non-enzymatic compounds (Ali et al., 2019). The application of  $\alpha$ -tocopherol significantly increased total phenols concentration and antioxidant activity in mango trees (Orabi and Abdelhamid, 2016). (Hussein et al., 2007) reported that the application of  $\alpha$ -tocopherol increased total phenols and flavonoids in cotton plants. (Subedi et al., 2014) in extracts of some medicinal plants observed the greater content of both phenols and flavonoids, suggesting the

correlation between polyphenolic content and antioxidant activity.  $\alpha$ -tocopherol mitigated the negative impact of salt (NaCl) stress in sunflower (cultivars) by raising the level of non-enzymatic antioxidants (total phenolic content and ascorbic acid; Lalarukh and Shahbaz 2020).

The salt-induced delay in reserve mobilization has been associated with a decrease in the activity of reserve-degrading enzymes. Indeed, salinity reduces the activity of amylases in the cotyledons of germinating cotton seeds (Yasin Ashraf et al., 2002). It also decreases the activity of amylases in the cotyledons of cashew during late seedling establishment (Marques et al., 2013). (Mohamed Zeid et al., 2019) reported that soaking cowpea seeds in  $\text{Na}_2\text{SeO}_3$  solutions increased the activities of  $\alpha$ -amylase,  $\beta$ -amylase in cowpea seedlings. Enhanced accumulation of carbohydrates was found in maize plants under water stress by  $\alpha$ -tocopherol application with inhibition in amylase enzyme activity (Hassanein et al., 2009). (Abdallah et al., 2013) reported that an increase in the growth of sunflower plants was associated with the improvement in photosynthetic pigments and carbohydrate and protein accumulation with inhibition activities of some hydrolytic enzymes.

Availability of excess salt in the soil leads to uncontrolled uptake of  $\text{Na}^+$  ion in plants which is highly toxic due to its ability to interact with  $\text{K}^+$  ions. Interaction with  $\text{K}^+$  ion leads to the altered stomatal regulation. Consequently, it is obligatory to maintain equilibrium in the  $\text{K}^+/\text{Na}^+$  ratio that served as an effective strategy for conferring salt tolerance in plants (Abbasi et al., 2015; Shabala and Cuin, 2008). The cumulative effect of  $\text{K}^+$  ion absorption maintenance, the reduction of  $\text{K}^+$  efflux from cells, the prevention of  $\text{Na}^+$  uptake, and the enhancement of  $\text{Na}^+$  efflux from cells can retain the  $\text{K}^+/\text{Na}^+$  ratio in the cytosol (Wakeel et al., 2011). Also, a high concentration of NaCl in the growth medium reduced the uptake of  $\text{K}^+$  ions (Alpaslan and Gunes, 2001; Faheed, 2012; Noreen et al., 2017). However, Se supply reduced  $\text{Na}^+$  ions uptake under salt stress and improved root growth, and thereby, might have enhanced the water supply to shoots and sustained plant growth (Rietz and Haynes, 2003). Furthermore, Se plays a pivotal role in the improvisation of nutrient absorption and its compartmentalization in various crop plants, which eventually improves growth performance and yield (Shahzadi et al., 2017). (Astaneh et al., 2018; Shekari et al., 2017) observed that Se increased the uptake of  $\text{K}^+/\text{Na}^+$  ratio in salt-stressed plants by blocking the uptake of  $\text{Na}^+$  ions in various crops. Similarly, Se treatment to lemon balm and dill plants showed a significant increase in growth performance and photosynthetic efficiency along with total amino acid contents with increasing  $\text{K}^+$  concentrations in the roots and shoots of plants (Habibi, 2017; Shekari et al., 2017). Similarly, the

promotion in nutrient contents may be attributed to the role of anti-oxidants, including  $\alpha$ -tocopherol, in increasing osmo-tolerance and/or regulating various processes, including absorption of nutrients from the soil solution and improving membrane permeability (Orabi and Abdelhamid, 2016). The application of  $\alpha$ -tocopherol led to an increase in the contents of ions in the leaf through regulating various processes, including absorption of nutrients from soil solution (El-Bassiouny and Sadak, 2014). (Orabi and Abdelhamid, 2016) also found an essential role for  $\alpha$ -tocopherol in alleviating the deleterious effects of salinity on *Faba beans*. (El-Bassiouny and Sadak, 2014) saw that exogenous  $\alpha$ -tocopherol significantly declined  $\text{Na}^+$  and  $\text{Cl}^-$  levels coupled with enhanced  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ , and  $\text{K}^+$  levels in flax cultivars irrigated with saline water. Semida et al. (2016) approved that, application of  $\alpha$ -tocopherol reversed the status of these ions, since increased  $\text{K}^+$  and  $\text{Ca}^{+2}$  contents, reduced  $\text{Na}^+$  content, and increased their relations that are positively reflected in plant growth and yield.

## 5. Conclusions

In conclusion, selenium and  $\alpha$ -tocopherol alone or in combination can mitigate the adverse effects of salt-induced damages through protecting the photosynthetic pigments, increased carbohydrate contents, and nutrient management. Moreover, a combination of both selenium and  $\alpha$ -tocopherol proved to be best. Additionally, a combination of selenium and  $\alpha$ -tocopherol inhibits the  $\text{Na}^+$  uptake and enhanced transport of  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ , and  $\text{K}^+$  levels and also enhances the antioxidative defensive mechanism through the production of phenolics and flavonoids content in maize plants under salt stress.

## Author's contribution

RK and MY– Conceive and conceptualize the idea; FB and SH– Investigated and collected data; RK, MY, AG – Analyzed data and prepared graphs; MY and RK– Prepared original draft and revised, MY– Edited and content improvement

## Declaration of Competing Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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