



# Elucidating the Effects of Combined Treatments of Salicylic Acid and L-Proline on Greenhouse-Grown Cucumber Under Saline Drip Irrigation

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## Abstract

Salinity is one of the major abiotic stress factors that threaten crop development and sustainable food production. As a mitigation strategy, several plant growth regulators and osmoprotectants have been applied to ameliorate the negative effects of salinity stress in plants. Therefore, the current study aimed to investigate the effect of foliar applications of different concentrations of salicylic acid and proline on the growth, yield, fruit quality, and nutritional composition of cucumber crops grown under saline conditions. The three main irrigation salinity variations included electrical conductivity (EC) of 0.5 dS/m (control), EC 6.0 dS/m, and EC 12.0 dS/m. Foliar spray treatments were as follows: T1 (distilled water), T2 (1.0 mM salicylic acid), T3 (1.0 mM salicylic acid + 5.0 mM proline), and T4 (1.0 mM salicylic acid + 10 mM proline). Our results showed that foliar application of salicylic acid alone or in combination with proline under non-saline conditions improved the growth and yield of cucumber, with T4 recording the highest values. Irrigating plants with saline water (EC 6.0 and 12.0 dS/m) severely compromised cucumber's growth performance and yield, with the lowest values recorded at EC 12.0 dS/m. However, under EC 6.0 dS/m, T2 and T3 slightly ameliorated salinity stress effects regarding fruit yield, for T2, and nutritive composition of fruits, for T2 and T3. Overall, this study demonstrated that cucumber (*Cucumis sativa L.*) could tolerate irrigation salinity levels of up to EC 6.0 dS/m without significant detrimental effects on the growth performance, yield, and nutritional composition of fruits.

**Keywords** Saline drip irrigation · Cucumber · Osmoprotectants · Salicylic acid · Proline

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## Introduction

Cucumber (*Cucumis sativus L.*), belonging to the family *Cucurbitaceae*, is one of the most important greenhouse vegetables worldwide and the second most widely cultivated greenhouse vegetable crop in Egypt (Youssef et al. 2018). According to FAOSTAT (2020), Egypt's cucumber and gherkins harvested area was 16,104 ha with a total production of 364,571 tons and an average yield of 226,385 kg/ha by average in 2019. And as such, the high demand for cucumber is attributed to its high nutritional value. The fruits are rich in conventional antioxidants, vitamins, minerals, and other phytonutrients, vital for human health (Yildirim et al. 2008; Huang et al. 2009b; Imaizumi et al. 2018). However, cucumber production is constrained by several abiotic factors such as salinity, one of the major factors limiting crop production in arid, semi-arid, and Mediterranean regions (Aragüés et al. 2011). Agronomic practices in these regions mainly involve the heavy application of agrochemicals

combined with irrigation to maximize crop productivity without paying attention to the risks associated with soil salinization (Shaddad et al. 2020). According to FAOSTAT, over 6% of the world's cultivated arable land is salt-affected (FAOSTAT 2020). Moreover, 20% (45 million hectares) of the world's irrigated lands are affected by salinity (Metternicht and Zinck 2003; Wondim et al. 2020). Irrigation with saline water leads to the accumulation of salts in the soil profile, which in turn negatively affects several soil properties as well as the general physiology, morphology, and productivity of several crops (Khan et al. 2013; Haj-Amor et al. 2018; Youssef et al. 2018; Chen et al. 2020; Shaddad et al. 2020). For instance, the accumulation of salts in the root zone (i.e., build-up of sodium ions in the exchange complex of soil) results in changes in several soil properties such as soil porosity, water retention, permeability, swelling, compaction, and sealing (Shaddad et al. 2020). The rate of infiltration of irrigation water into the soil profile is reduced hence hindering plant-water uptake (Haj-Amor et al. 2018). Furthermore, saline irrigation leads to increased accumulation of toxic ions (i.e., sodium, chloride, and boron) in plant tissues which often results in ionic stress (Trajkova et al. 2006; Youssef et al. 2018; Shaddad et al. 2020).

Despite all these effects, plants have evolved to counteract salinity effects through several biochemical pathways to protect their cells from oxidative and ionic stress damage. Among these pathways are the expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Vighi et al. 2017; Moghaddam et al. 2020), exclusion of toxic sodium and chloride ions while accumulating calcium and potassium ions in tissues (Martin et al. 2020; Saddiq et al. 2020; Youssef et al. 2018; Hamaiel et al. 2020), and most interestingly, synthesis and accumulation of osmoprotectants (i.e., glycine betaine, proline, mannitol, sorbitol, fructans, and trehalose) and plant growth regulators (i.e., salicylic acid and jasmonic acid) in plant tissues (Singh et al. 2015).

Osmoprotectants are low molecular weight organic compounds, electrically neutral, highly soluble, and non-toxic to cells at high concentrations (Singh et al. 2015; Sofy et al. 2020). Several studies have shown that the exogenous application of osmoprotectants enhances the plants' tolerance against abiotic stress (Xing and Rajashekar 1999; Rezaei et al. 2012; Gholami Zali and Ehsanzadeh 2018; Estaji et al. 2019; Tonhati et al. 2020). This is achieved through the stabilization of proteins and membranes as well as reducing the osmotic potential of membranes to prevent tissue dehydration. Furthermore, they accumulate in the cells to maintain both the turgor pressure and osmotic pressure of the cells and scavenging for reactive oxygen species (ROS) (Huang et al., 2009a, b; Singh et al., 2015). Proline (Pro) is one of the most commonly studied and exogenously applied osmoprotectant under abiotic stress and has shown the potential

to ameliorate the effects of abiotic stress in several plant species (Huang et al. 2009a; Gholami Zali and Ehsanzadeh 2018; Merwad et al. 2018; El-beltagi et al. 2020; Tonhati et al. 2020; Hanif et al. 2021). It is an essential and multifunctional water-soluble amino acid commonly found in all plants and builds up at high concentrations under salinity stress (Su and Bai 2008; Banerjee et al. 2019; Ami et al. 2020; Liu et al. 2020; Mattioli et al. 2020).

On the other hand, plant growth regulators (PGRs) are naturally occurring plant hormones that play diverse roles in plant development and protection. Among the widely studied PGRs is salicylic acid (SA), which is involved in several physiological and biochemical processes such as growth and development, stomatal opening, photosynthesis, membrane permeability, ion uptake, enzymatic activity, and flower induction. Previous studies have shown that exogenous application of SA enhances the plants' tolerance against salinity stress (Yildirim et al. 2008; Karlidag et al. 2009; Faghih et al. 2017; Garg and Bharti 2018; Tahjib-UI-Arif et al. 2018). Moreover, exogenous application of SA triggers the synthesis and accumulation of osmoprotectants, including Pro, in plants cells, thus conferring protection against abiotic stress (Youssef et al. 2018; Sharma et al. 2019; Elhakem 2020).

To the best of our knowledge, no studies have been conducted on SA and Pro treatment combinations to ameliorate salinity stress effects in cucumber crops. Therefore, this study aimed to investigate the influence of the foliar application of SA alone or in combination with different concentrations of Pro on growth, yield, fruit quality, and nutritive composition of cucumber (Barracuda F1 Hybrid) under different concentrations levels of irrigation salinity.

## Materials and Methods

### Materials

Fungicide-treated cucumber seeds (*Cucumis sativus L.*, cv. *Barracuda F1*) produced by Seminis Company were obtained from the local distributor in Egypt. SA and Pro were purchased from Alpha chemika company.

### Experimental Procedure

The greenhouse experiment was conducted during the winter season of 2020/2021 at the Center for Applied Research on the Environment and Sustainability, the American University in Cairo (AUC), New Cairo Egypt (30° 01' 11.7" N31° 29' 59.8" E).

Seeds were sown on December 13, 2020, into 84-cell foam trays filled with a mix of vermiculite and peat moss (1:1) and irrigated with a commercial hydroponic mix

solution A and B from Yara company. Homogeneous healthy seedlings were transplanted at a 4-true leaf stage on December 27, 2020, and sown in each pot (20 cm diameter) containing a mixture of 1:1.5 perlite and coco peat, respectively. Spacing between plants was 30 cm along drip irrigation lines. The experiment was arranged in a 3 × 4 randomized completely block design (RCBD), which consisted of three main treatments levels and four subtreatments. Each subtreatment randomly had six replicates (Fig. 1). The fertilization program was as follows. Two weeks after transplanting, N P K (19:19:19) was added once a week at the rate of 5 g/L. Then, a mixture of 33%  $\text{NH}_4\text{NO}_3$  (2 g/L),  $\text{K}_2\text{SO}_4$  (0:0:50) at the rate of 2 g/L, 80%  $\text{HPO}_4$  (1:1000; v/v), and  $\text{MgSO}_4$  at the rate of 0.5 g/L was applied until the end of the experiment. Salinity treatments (main treatments) were initiated three weeks after transplanting by gradually adding salts to reach EC 6.0 dS/m and 12 dS/m salinity. Regular tap water (EC 0.5 dS/m) was used as a negative control of the main treatments. Table 1 shows the chemical properties of the salt used in our study.

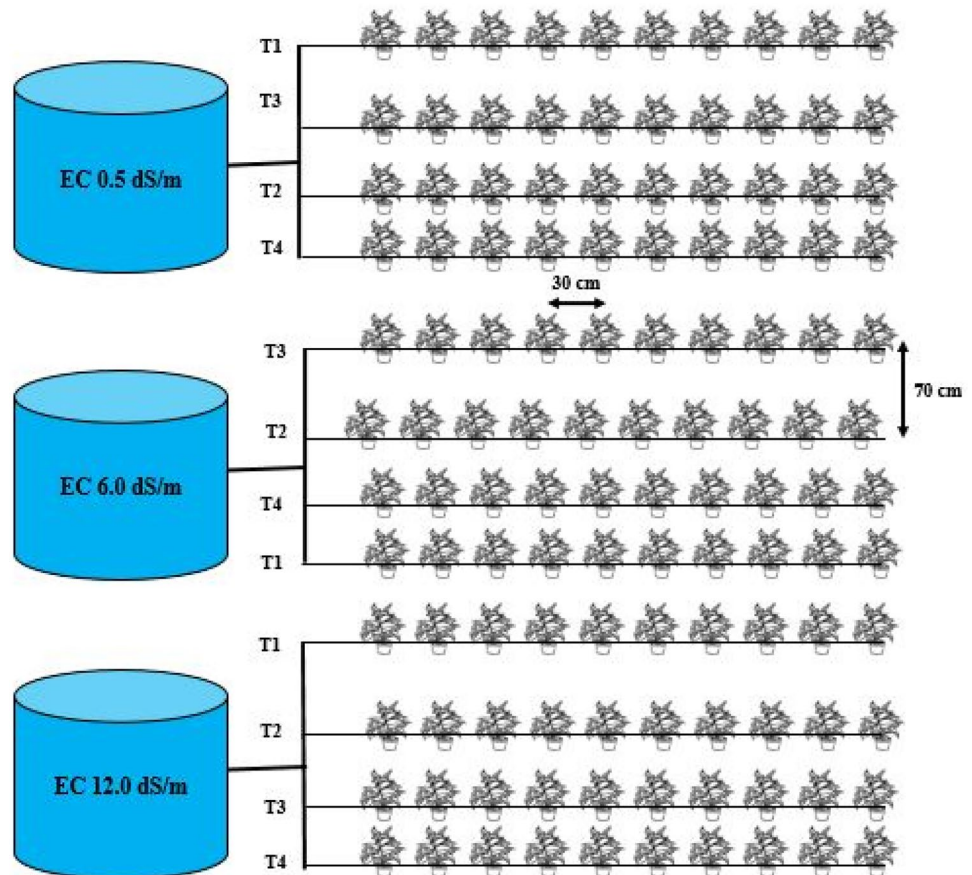
For subtreatments, concentrations of 1.0 mM SA, 5.0 mM Pro, and 10.0 mM Pro were prepared and pH adjusted to 7.0. A surfactant tween 20 (0.5%) was added to the solutions as a wetting agent. The selection of SA

**Table 1** Chemical properties of the salt used in the study

Elements in the dry salt sample			
Sodium chloride	98.5%	Bicarbonate	$4 \times 10^{-3}\%$
Moisture	0.23%	Iron	$3 \times 10^{-6}\%$
Insoluble matter	0.02%	Copper	$2 \times 10^{-6}\%$
Soluble matters	0.57%	Arsenate	$2 \times 10^{-5}\%$
Calcium	0.0721%	Lead	$2 \times 10^{-5}\%$
Magnesium	0.0722%	Mercury	$5 \times 10^{-6}\%$
Sulfate	0.313%	$\text{KIO}_3$	$5.3 \times 10^{-3}\%$
Potassium	0.02%	Cadmium	$8 \times 10^{-7}\%$

and Pro concentrations was based on previous studies, and treatment combinations (subtreatments) were as follows. T1: distilled water (control), T2: 1.0 mM SA + 0 mM Pro, T3: 1.0 mM SA + 5 mM Pro, and T4: 1.0 mM SA + 10 mM Pro. Foliar sprays were performed twice in the early morning using a hand-held atomizer spray bottle, and leaves were sprayed to complete wetness. The initial foliar sprays were conducted 2 weeks after transplanting when the plants had 3–4 true leaves. In contrast, the second foliar sprays were conducted 14 days after the initial foliar spray

**Fig. 1** Experimental design layout



treatments with a barrier between subtreatments rows to prevent spray drifts to other different subtreatment groups.

Agricultural practices (irrigation, disease, and pest control) for the greenhouse production of cucumber were conducted according to the recommendations of the Egyptian Ministry of Agriculture.

### Growth Parameter Measurements

Six uniformly growing plants from each subtreatment were tagged for sampling. Growth parameter measurements (vine length, leaf number, node number, and soil–plant analysis development (SPAD)) were taken every after 15 days. Briefly, vine length was taken from the base of the plant on the soil surface to the terminal growing point using a meter scale; leaf number was obtained by counting the number of fully expanded healthy leaves per plant and averages determined, Node number was obtained by counting the number of nodes per plant and averages determined, and SPAD was measured using a leaf chlorophyll meter apogee® instruments MC-100.

### Yield Parameters

A total of eight harvests were made, and fruits were harvested when their average length was approximately 15 cm, cumulatively counted to record the number of fruits plant per plant. Three fruits per subtreatment were obtained at each harvest measured and weighed to obtain the average length, diameter, fresh weight, and yield.

### Leaves and Fruit Nutritive Composition

Ten healthy leaves from six randomly tagged plants in each subtreatment were collected and pooled for nutritive composition analysis. Three fruits from six randomly tagged plants in each subtreatment were collected and pooled for nutritive composition analysis for cucumber fruits. All samples were stored at  $-20\text{ }^{\circ}\text{C}$  until analysis was performed. Nutritive composition, proline content, total phenolics content, total flavonoids content, vitamin C content, and sodium ion ( $\text{Na}^+$ ) content were analyzed at the Agricultural Research Center, Giza, Egypt, as follows.

Nitrogen was determined according to the procedures described by Plummer (1971), where 5 ml of the digestive solution was distilled with 10 ml of sodium hydroxide (NaOH) for 10 min to obtain ammonia. Back titration was then used to determine the amount of nitrogen present in ammonia. Phosphorus content was determined calorimetrically (660 nm) according to the procedures described by Jackson (1959). Potassium, calcium, and sodium were determined against a standard using a flame photometer (JEN way flame photometer) as described by Pipher (1950).

Magnesium, copper, manganese, zinc, and iron contents were determined using Atomic Absorption Spectrophotometer, Pyeunican SP1900, according to methods described by Brandifeld and Spincer (1965).

For determination of free proline, 25 mg of ground leaf samples was dissolved in 2 ml of 3% (w/v) aqueous 5-sulfosalicylic acid solution and centrifuged at  $6026\times g$  for 20 min. One ml of the supernatant was then mixed with 2 ml of acidic ninhydrin reagent (2.5 g ninhydrin/100 ml of a solution containing glacial acetic acid, distilled water, and 85% orthophosphoric acid at a ratio of 6:3:1) and boiled in a water bath for one h followed by cooling on ice. Two ml of toluene was added to the mixture and vortexed for 20 s. The colored toluene layer was decanted from the aqueous phase and left to stand at room temperature. Absorbance was read at 520 nm using a spectrophotometer with toluene as a blank. Free proline content was determined from the standard curve according to the method described by Bates et al. (1973). The proline concentration was calculated as a fresh weight basis (mg/g FW).

Total hydrolyzable carbohydrates were determined as glucose using phenol–sulfuric acid reagent described by Dubois et al. (1956), whereas total soluble solutes (TSS) were determined using a hand-held refractometer.

Fruit vitamin C content was determined using dichlorophenol indophenol reagent. As such, 10 g of fresh fruit tissues, including the skin, was crushed using a motor and pestle in the presence of 10 ml metaphosphoric acid 6% (Merck). This was followed by centrifugation at  $4000\times g$  for 5 min at  $4\text{ }^{\circ}\text{C}$ . Five ml of the supernatant was transferred into an Erlenmeyer flask, and 20 ml of 3% metaphosphoric acid was added. The extract was titrated by dichlorophenol indophenol (Sigma-Aldrich) until a rose color was observed. Vitamin C (mg/100 g FW) was then calculated and based on the standard curve of L-Ascorbic acid (Merck) concentrations.

Total phenolics content was determined by the Folin–Ciocalteu method as described by Singleton et al. (1999). Briefly, 1 ml of fruit extract and or different concentrations of gallic acid (standard) were mixed with 1 ml of Folin reagent. This was followed by the addition of 1 ml of 10% (w/v) sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution. The mixture was allowed to stand at room temperature for one h and the absorbance was measured at 700 nm using a spectrophotometer. Total phenolic content was expressed (mg) as gallic acid equivalent  $\text{g}^{-1}$  dry weight.

Total flavonoids content was determined calorimetrically using aluminum chloride as Zhishen et al. (1999) described. Briefly, 1 ml of fruit extract and or different concentrations of quercetin standard solution was mixed with 0.3 ml of 5% (w/v) sodium nitrite ( $\text{NaNO}_2$ ) solution. After 6 min, 0.3 ml of 10% (w/v) aluminum chloride ( $\text{Al}(\text{Cl})_3$ ) was added, and the mixture was allowed to stand at room temperature for 6 min.

This was followed by the addition of 0.4 ml of 1 M sodium hydroxide (NaOH). The mixture was then allowed to stand for 12 min at room temperature, and the absorbance was measured at 510 nm using a spectrophotometer. Total flavonoid content was expressed as mg quercetin equivalent  $\text{g}^{-1}$  dry weight.

Sodium-ion ( $\text{Na}^+$ ) concentration in leaf tissues was determined by grinding 100 mg of dry leaf samples, and these were ashed at 500 °C in a furnace. The ashed samples were dissolved in 3 ml nitric acid (1 M  $\text{HNO}_3$ ) and then in 12 ml of distilled water. The resulting solution was analyzed using an Atomic Absorption Spectrophotometer, Pyeunican SP1900 (Chen et al. 2020).

### Growth Media Analysis and Irrigation Water Use Efficiency

At the end of the growth cycle, 50 g of growth media was randomly obtained from three pots from each subtreatment within the main treatment, and samples were pooled for total dissolved solids (TDS) and pH determination. Growth media extracts were obtained by mixing equal volumes of growth media with distilled water for determination of TDS and pH using TDS and pH meter, from Thermos scientific instrument.

### Irrigation Water Use Efficiency

According to Howell et al. (1990), the water use efficiency was calculated using the following equation.

$$\text{IWUE} = (Y/I)$$

where

IWUE = irrigation water use efficiency ( $\text{kg}/\text{m}^3$ ).

$Y$  = yield ( $\text{kg}/\text{ha}$ ).

$I$  = applied amount of water ( $\text{m}^3$ ).

### Statistical Analysis

All data collected were analyzed using IBM-SPSS Statistical Tool (Version 22) and expressed as Mean  $\pm$  SE. These data were subjected to a Leven's test before analysis of variance (ANOVA) was conducted. ANOVA (both one and two-way ANOVA) was performed to detect significant differences in all the measured parameters, and the difference in means was analyzed by Duncan multiple range test (DMT) at  $\alpha = 0.05$ .

## Results

### Growth Parameters

As shown in Table 2, an increase in salinity stress generally and significantly reduced the growth parameters (vine length, leaf number, node number, and SPAD value) of cucumber plants ( $P < 0.05$ ) to reach their lowest values at a salinity level of EC 12.0 dS/m compared to those under non-saline conditions (EC 0.5 dS/m).

Data on vine length at 30 DAT indicated that T4 significantly recorded the highest vine length (30.33 cm) compared to T2 (28.56 cm) and T3 (28.50 cm) ( $P < 0.05$ ). Under saline irrigation (EC 6.0 dS/m) conditions, foliar application of T1, T2, T3, and T4 increased vine length (5.02, 6.88, 9.70, and 3.19%, respectively). Likewise, irrigating plants with highly saline water (EC 12.0 dS/m) resulted in an increase in vine length in T2 (6.37%) and T3 (5.35%), with T1 significantly recording the lowest vine length compared to other subtreatments ( $P < 0.05$ ). At 45 DAT, highly saline conditions (EC 12.0 dS/m) suppressed the average vine length of plants (T1: 12.52%, T2: 6.25%; T3: 7.91; T4: 9.55), with T2 significantly recording the highest vine length compared to other subtreatments ( $P < 0.05$ ). Similarly, highly saline conditions (EC 12.0 dS/m) at 60 DAT resulted in a percentage decline in vine length across all subtreatments (T1: 26.94%; T2: 22.95%; T3: 23.16%; T4: 29.11%) with T2 recording a higher vine length compared to other subtreatments, but this was not statistically significant. Data at 75 DAT also indicated a decline in vine length across all subtreatments (T1: 38.89%; T2: 36.20%; T3: 36.60%; T4: 42.21%) under highly saline conditions (EC 12.0 dS/m). Overall, T2 significantly ameliorated salinity stress effects on vine length compared to T4 ( $P < 0.05$ ) but not T3 and T1. There was a significant interaction between subtreatments and main treatments ( $P < 0.0001$ ).

The lowest values for the average leaf number and node number per plant were obtained under highly saline conditions (EC 12.0 dS/m) at 75 DAT. Generally, the average leaf number declined by 64.74, 77.92, 76.93, and 69.60% in T1, T2, T3, and T4, respectively, with T1 significantly recording higher values for the average leaf number per plant compared to other subtreatments ( $P < 0.05$ ). Likewise, the average node number per plant declined by 46.81, 49.72, 50.70, and 55.23% in T1, T2, T3, and T4,

**Table 2** Effect of foliar application of SA alone or in combination with Pro on growth parameters of cucumber under non-saline and saline conditions

Treatments	Vine length (cm)	Leaf no. /plant	Node no. /plant	SPAD value
30 DAT				
0.5 dS/m				
T1	29.50 <sup>abB</sup> ± 0.49	5.39 <sup>aA</sup> ± 0.11	5.83 <sup>aA</sup> ± 0.20	28.67 <sup>aA</sup> ± 0.55
T2	28.56 <sup>bB</sup> ± 0.49	5.72 <sup>aA</sup> ± 0.11	5.78 <sup>aA</sup> ± 0.20	28.74 <sup>aA</sup> ± 0.55
T3	28.50 <sup>bC</sup> ± 0.49	5.72 <sup>aA</sup> ± 0.11	5.78 <sup>aA</sup> ± 0.20	28.68 <sup>aA</sup> ± 0.55
T4	30.33 <sup>aA</sup> ± 0.49	5.39 <sup>aA</sup> ± 0.11	5.56 <sup>aA</sup> ± 0.20	28.08 <sup>aA</sup> ± 0.55
12.0 dS/m				
T1	31.06 <sup>aA</sup> ± 0.47	5.67 <sup>aA</sup> ± 0.11	5.83 <sup>aA</sup> ± 0.17	27.17 <sup>aA</sup> ± 0.55
T2	30.67 <sup>aA</sup> ± 0.47	5.67 <sup>aA</sup> ± 0.11	5.44 <sup>aA</sup> ± 0.17	27.14 <sup>ab</sup> ± 0.55
T3	31.56 <sup>aA</sup> ± 0.47	5.61 <sup>abA</sup> ± 0.11	6.06 <sup>aA</sup> ± 0.17	27.06 <sup>ab</sup> ± 0.55
T4	31.33 <sup>aA</sup> ± 0.47	5.11 <sup>bA</sup> ± 0.11	5.67 <sup>aA</sup> ± 0.17	27.39 <sup>aA</sup> ± 0.55
45 DAT				
0.5 dS/m				
T1	54.94 <sup>ab</sup> ± 1.02	10.33 <sup>aA</sup> ± 0.29	7.83 <sup>aA</sup> ± 0.31	46.39 <sup>aA</sup> ± 0.70
T2	55.17 <sup>aA</sup> ± 1.02	7.00 <sup>cA</sup> ± 0.29	7.22 <sup>aA</sup> ± 0.31	44.54 <sup>ab</sup> ± 0.70
T3	51.94 <sup>ab</sup> ± 1.02	6.11 <sup>dA</sup> ± 0.29	6.00 <sup>bA</sup> ± 0.31	45.50 <sup>aA</sup> ± 0.70
T4	53.50 <sup>aA</sup> ± 1.02	8.39 <sup>bA</sup> ± 0.29	7.17 <sup>aA</sup> ± 0.31	46.29 <sup>aA</sup> ± 0.70
6.0 dS/m				
T1	57.50 <sup>aA</sup> ± 0.93	6.00 <sup>bB</sup> ± 0.20	6.50 <sup>ab</sup> ± 0.25	45.62 <sup>aA</sup> ± 0.59
T2	53.39 <sup>ab</sup> ± 0.93	5.67 <sup>bB</sup> ± 0.20	6.94 <sup>aA</sup> ± 0.25	46.50 <sup>aA</sup> ± 0.59
T3	56.44 <sup>aA</sup> ± 0.93	5.56 <sup>bA</sup> ± 0.20	6.50 <sup>aA</sup> ± 0.25	45.99 <sup>aA</sup> ± 0.59
T4	54.78 <sup>abA</sup> ± 0.93	6.56 <sup>ab</sup> ± 0.20	6.78 <sup>aA</sup> ± 0.25	45.79 <sup>aA</sup> ± 0.59
12.0 dS/m				
T1	48.06 <sup>bC</sup> ± 0.93	4.67 <sup>aC</sup> ± 0.22	6.50 <sup>ab</sup> ± 0.30	45.19 <sup>aA</sup> ± 0.72
T2	51.72 <sup>ab</sup> ± 0.93	4.28 <sup>aC</sup> ± 0.22	6.17 <sup>ab</sup> ± 0.30	42.56 <sup>bC</sup> ± 0.72
T3	47.83 <sup>bC</sup> ± 0.93	3.83 <sup>ab</sup> ± 0.22	5.17 <sup>bB</sup> ± 0.30	45.31 <sup>aA</sup> ± 0.72
T4	48.39 <sup>bB</sup> ± 0.93	4.06 <sup>aC</sup> ± 0.22	5.28 <sup>bB</sup> ± 0.30	43.41 <sup>abB</sup> ± 0.72
60 DAT				
0.5 dS/m				
T1	86.61 <sup>aA</sup> ± 1.84	10.44 <sup>aA</sup> ± 0.37	8.56 <sup>aA</sup> ± 0.37	49.07 <sup>aA</sup> ± 0.85
T2	85.94 <sup>aA</sup> ± 1.84	8.00 <sup>bA</sup> ± 0.37	6.28 <sup>bA</sup> ± 0.37	47.64 <sup>aA</sup> ± 0.85
T3	82.72 <sup>aA</sup> ± 1.84	8.06 <sup>bA</sup> ± 0.37	6.00 <sup>cA</sup> ± 0.37	50.71 <sup>aA</sup> ± 0.85
T4	84.72 <sup>aA</sup> ± 1.84	9.00 <sup>bA</sup> ± 0.37	7.17 <sup>bA</sup> ± 0.37	49.35 <sup>aA</sup> ± 0.85
6.0 dS/m				
T1	88.06 <sup>aA</sup> ± 1.24	6.50 <sup>ab</sup> ± 0.24	5.67 <sup>ab</sup> ± 0.29	46.85 <sup>aAB</sup> ± 12.69
T2	76.61 <sup>cB</sup> ± 1.24	6.00 <sup>ab</sup> ± 0.24	5.56 <sup>aA</sup> ± 0.29	74.35 <sup>aA</sup> ± 12.69
T3	81.67 <sup>bA</sup> ± 1.24	5.83 <sup>ab</sup> ± 0.24	5.33 <sup>aAB</sup> ± 0.29	47.39 <sup>ab</sup> ± 12.69
T4	78.89 <sup>bCB</sup> ± 1.24	5.67 <sup>ab</sup> ± 0.24	5.06 <sup>ab</sup> ± 0.29	48.61 <sup>aA</sup> ± 12.69
12.0 dS/m				
T1	63.28 <sup>aA</sup> ± 1.51	4.61 <sup>aC</sup> ± 0.32	4.89 <sup>ab</sup> ± 0.28	44.76 <sup>ab</sup> ± 0.73
T2	66.22 <sup>aC</sup> ± 1.51	4.44 <sup>aC</sup> ± 0.32	5.17 <sup>aA</sup> ± 0.28	44.76 <sup>aA</sup> ± 0.73
T3	63.56 <sup>ab</sup> ± 1.51	3.11 <sup>bC</sup> ± 0.32	4.61 <sup>ab</sup> ± 0.28	44.01 <sup>aC</sup> ± 0.73
T4	60.06 <sup>aC</sup> ± 1.51	2.56 <sup>bC</sup> ± 0.32	3.33 <sup>bC</sup> ± 0.28	45.33 <sup>ab</sup> ± 0.73
75 DAT				
0.5 dS/m				
T1	119.27 <sup>aA</sup> ± 2.82	12.28 <sup>aA</sup> ± 0.47	8.78 <sup>aA</sup> ± 0.36	49.76 <sup>aA</sup> ± 0.82

**Table 2** (continued)

Treatments	Vine length (cm)	Leaf no. /plant	Node no. /plant	SPAD value
T2	123.22 <sup>aA</sup> ± 2.82	9.06 <sup>bA</sup> ± 0.47	8.83 <sup>aA</sup> ± 0.36	49.76 <sup>aA</sup> ± 0.82
T3	117.33 <sup>aA</sup> ± 2.82	8.67 <sup>bA</sup> ± 0.47	8.56 <sup>aA</sup> ± 0.36	51.16 <sup>aA</sup> ± 0.82
T4	120.56 <sup>aA</sup> ± 2.82	9.67 <sup>bA</sup> ± 0.47	9.56 <sup>aA</sup> ± 0.36	50.95 <sup>aA</sup> ± 0.82
6.0 dS/m				
T1	121.28 <sup>aA</sup> ± 2.20	7.94 <sup>aB</sup> ± 0.21	7.83 <sup>aB</sup> ± 0.36	41.57 <sup>aB</sup> ± 0.87
T2	107.61 <sup>bB</sup> ± 2.20	7.28 <sup>bB</sup> ± 0.21	7.17 <sup>aB</sup> ± 0.36	41.78 <sup>aB</sup> ± 0.87
T3	108.11 <sup>bB</sup> ± 2.20	7.89 <sup>abA</sup> ± 0.21	6.78 <sup>aB</sup> ± 0.36	42.96 <sup>aB</sup> ± 0.87
T4	107.44 <sup>bB</sup> ± 2.20	7.33 <sup>abB</sup> ± 0.21	7.39 <sup>aB</sup> ± 0.36	43.34 <sup>aB</sup> ± 0.87
12.0 dS/m				
T1	72.89 <sup>abB</sup> ± 1.93	4.33 <sup>aC</sup> ± 0.35	4.67 <sup>aC</sup> ± 0.33	33.14 <sup>aC</sup> ± 0.95
T2	78.61 <sup>aC</sup> ± 1.93	2.00 <sup>bC</sup> ± 0.35	4.44 <sup>aC</sup> ± 0.33	34.63 <sup>aC</sup> ± 0.95
T3	74.39 <sup>abC</sup> ± 1.93	2.00 <sup>bB</sup> ± 0.35	4.22 <sup>aC</sup> ± 0.33	36.52 <sup>aC</sup> ± 0.95
T4	69.67 <sup>bC</sup> ± 1.93	2.94 <sup>bC</sup> ± 0.35	4.28 <sup>aC</sup> ± 0.33	35.89 <sup>aC</sup> ± 0.95

Data are expressed as means ± standard error (SE). Different lower superscript letters within each main treatment indicate a significant difference at  $P < 0.05$  for each parameter (Duncan multiple range test). Different upper superscript letters within a subtreatment indicate a significant difference at  $P < 0.05$  (Duncan multiple range test). Subtreatments: T1: 0 mM salicylic acid + 0 mM proline; T2: 1.0 mM salicylic acid + 0 mM proline; T3: 1.0 mM salicylic acid + 5 mM proline; T4: 1.0 mM salicylic acid + 10 mM proline

respectively. Overall, a highly significant interaction was noted between the subtreatments and main treatments ( $P < 0.0001$ ).

For SPAD, results show no significant effect of all subtreatment groups on SPAD values under EC 0.5 dS/m and

6.0 dS/m at 30, 45, 60, and 75 DAT. At high salinity (EC 12.0 dS/m), there was a variation in SPAD values among different subtreatments at 30 and 45 DAT. In contrast, no significant effects in all the subtreatments groups were

**Table 3** Effect of foliar application of SA alone or in combination with Pro on Fruit quality and yield of cucumber grown under non-saline and saline conditions

Treatments	Fruit no. /plant	Fruit length (cm)	Fruit diameter (mm)	Fruit fresh weight (kg)	Fruit yield (ton. /ha)
0.5 dS/m					
T1	18.89 <sup>aA</sup> ± 0.49	15.85 <sup>aA</sup> ± 0.13	35.64 <sup>aA</sup> ± 0.32	0.12 <sup>aA</sup> ± 0.35	93.48 <sup>aA</sup> ± 3.81
T2	20.11 <sup>aA</sup> ± 0.49	15.60 <sup>aA</sup> ± 0.13	35.24 <sup>abA</sup> ± 0.32	0.12 <sup>aA</sup> ± 0.35	100.52 <sup>aA</sup> ± 2.82
T3	19.67 <sup>aA</sup> ± 0.49	15.93 <sup>aA</sup> ± 0.13	35.80 <sup>aA</sup> ± 0.32	0.13 <sup>aA</sup> ± 0.35	73.91 <sup>aA</sup> ± 1.89
T4	19.34 <sup>aA</sup> ± 0.49	15.23 <sup>bA</sup> ± 0.13	34.45 <sup>bA</sup> ± 0.32	0.19 <sup>aA</sup> ± 0.35	146.69 <sup>aA</sup> ± 52.72
6.0 dS/m					
T1	16.94 <sup>abB</sup> ± 0.36	16.47 <sup>aA</sup> ± 0.71	33.64 <sup>abB</sup> ± 0.29	0.12 <sup>aA</sup> ± 0.00	77.48 <sup>abB</sup> ± 2.43
T2	16.22 <sup>abB</sup> ± 0.36	14.95 <sup>abB</sup> ± 0.71	33.28 <sup>abB</sup> ± 0.29	0.11 <sup>bB</sup> ± 0.00	69.86 <sup>bbB</sup> ± 2.49
T3	15.72 <sup>bbB</sup> ± 0.36	14.95 <sup>abB</sup> ± 0.71	32.99 <sup>abB</sup> ± 0.29	0.11 <sup>bB</sup> ± 0.00	66.18 <sup>bcB</sup> ± 1.60
T4	15.28 <sup>bbB</sup> ± 0.36	14.63 <sup>abB</sup> ± 0.71	33.07 <sup>abB</sup> ± 0.29	0.10 <sup>bA</sup> ± 0.00	62.88 <sup>cAB</sup> ± 1.84
12.0 dS/m					
T1	10.35 <sup>aC</sup> ± 0.29	13.02 <sup>abB</sup> ± 0.17	30.48 <sup>aC</sup> ± 0.31	0.08 <sup>abB</sup> ± 0.00	32.67 <sup>aC</sup> ± 1.59
T2	8.83 <sup>bC</sup> ± 0.29	12.73 <sup>abC</sup> ± 0.17	29.86 <sup>aC</sup> ± 0.31	0.07 <sup>aC</sup> ± 0.00	25.90 <sup>bC</sup> ± 1.35
T3	9.11 <sup>bC</sup> ± 0.29	12.35 <sup>bcB</sup> ± 0.17	28.97 <sup>bC</sup> ± 0.31	0.07 <sup>aC</sup> ± 0.00	26.38 <sup>bC</sup> ± 0.74
T4	8.39 <sup>bC</sup> ± 0.29	12.86 <sup>aC</sup> ± 0.17	30.44 <sup>aC</sup> ± 0.31	0.08 <sup>aA</sup> ± 0.00	26.11 <sup>bbB</sup> ± 1.03

Data is expressed as mean ± SE. Different lower superscript letters within each main treatment indicate a significant difference at  $P < 0.05$  (Duncan multiple range test). Different upper superscript letters within a subtreatment indicate a significant difference at  $P < 0.05$  (Duncan multiple range test). Subtreatments: T1: 0 mM salicylic acid + 0 mM proline; T2: 1.0 mM salicylic acid + 0 mM proline; T3: 1.0 mM salicylic acid + 5 mM proline; T4: 1.0 mM salicylic acid + 10 mM proline

recorded at 60 and 75 DAT. No significant interaction was noted between subtreatments and main treatments.

### Fruit Quality and Yield

Results on the effect of foliar application of T1, T2, T3, and T4 on fruit quality and yield are presented in Table 3. Data on the average fruit number per plant shows no significant differences among all subtreatments under non-saline conditions (EC 0.5 dS/m). At EC 6.0 dS/m, a percentage decline in the average fruit number per plant was noted among all subtreatments (T1: 10.32%; T2: 19.34%; T3: 20.08%; T4: 20.99%) with T3 and T4 significantly recording the lowest fruit number per plant compared to other subtreatments ( $P < 0.05$ ). Likewise, a percentage decline in the average fruit number per plant was noted across all subtreatments (T1: 45.21%; T2: 56.09%; T3: 53.69%; T4: 56.62%) under highly saline conditions (EC 12.0 dS/m) with T1 significantly recording higher values for the average fruit number per plant compared to other subtreatments ( $P < 0.05$ ).

Data on the average fruit length indicated no significant differences among T1, T2, and T3 under non-saline conditions (EC 0.5 dS/m). However, irrigating plants with saline water (EC 6.0 dS/m) resulted in a percentage decline in the average fruit length in T2, T3, and T4 (4.17, 6.15, and 3.94% respectively). Under highly saline conditions (EC 12.0 dS/m), foliar application of T1, T2, T3, and T4 resulted in a percentage decline in the average fruit length (17.85, 18.40, 22.47, and 15.56%, respectively).

Results on the fruit diameter showed that T4 significantly recorded the smallest fruit diameter (34.45 mm) compared to T1 (35.64 mm), T2 (35.24 mm), and T3 (35.80 mm) ( $P < 0.05$ ) under non-saline conditions (EC 0.5 dS/m). However, irrigating plants with saline water (EC 6.0 dS/m) resulted in a percentage decline of fruit diameter across all subtreatments (T1: 5.61%, T2: 5.56%; T3: 7.85%; T4: 4.01%). Similarly, foliar application of T1, T2, T3, and T4 resulted in a percentage decline in fruit diameter (14.48, 15.27, 19.08, and 11.64%, respectively) under highly saline conditions (EC 12.0 dS/m), with T3 significantly recording the smallest fruit diameter compared to other subtreatments ( $P < 0.05$ ).

For fresh fruit weight, no significant differences were noted among all subtreatments under non-saline conditions (EC 0.5 dS/m). However, irrigating plants with saline water (EC 6.0 dS/m) resulted in a percentage decline in fresh fruit weight by 8.33, 15.38, and 47.36% in T2, T3, and T4, respectively, with T1 significantly recording higher values for fresh fruit weight compared to other subtreatments ( $P < 0.05$ ). Likewise, the percentage fruit fresh weight declined across all subtreatments (T1: 33.33%; T2: 41.67%; T3: 46.15%; T4: 57.89%) under highly saline conditions (EC 12.0 dS/m).

Results on the fruit yield indicated no significant differences in yield under non-saline conditions (EC 0.5 dS/m) across all subtreatments. However, irrigating plants with saline water (EC 6.0 dS/m) resulted in a percentage decline in yield by 17.12, 30.50, 10.56, and 57.13% in T1, T2, T3, and T4, respectively, with T1 recording significantly higher values for fruit yield compared to other subtreatments under similar conditions ( $P < 0.05$ ). Similarly, the percentage fruit yield under highly saline conditions (EC 12.0 dS/m) declined by 65.05, 74.23, 64.30, and 82.20% in T1, T2, T3, and T4, respectively, with T1 recording significantly higher values for fruit yield compared to other subtreatments ( $P < 0.05$ ).

### Leaves Nutritive, Sodium Ions, and Free Proline Composition

Results on the influence of the foliar application of SA alone or in combination with Pro on the nutritive composition of leaves under non-saline and saline conditions are presented in Table 4.

Foliar application of T1, T2, and T4 under saline conditions (EC 6.0 dS/m) resulted in a percentage increase of nitrogen (N) composition (33.16, 13.48, and 30.81%, respectively) in leaves. Similarly, irrigating plants with saline water at EC 12.0 dS/m increased the percentage nitrogen composition (T1: 26.82%; T2: 20.26%; T4: 30.81%) of leaves. However, foliar application of T3 resulted in a significant percentage increase in phosphorus (P) (9.80%) content, potassium (K) (2.19%), and magnesium (Mg) (29.35%) compared to other subtreatments under similar conditions ( $P < 0.05$ ). Results on the calcium (Ca) composition of leaves indicated that foliar application of T1 at EC 6.0 dS/m and 12.0 dS/m resulted in a significant percentage increase of Ca (38.49 and 41.70%, respectively) composition in leaves compared to other subtreatments ( $P < 0.05$ ).

Data on the nutritive composition of micro-elements in leaves under highly saline conditions (EC 12.0 dS/m) showed a percentage increase in the iron (Fe) content (T1: 56.42%; T2: 32.79%; T3: 32.67%), zinc (Zn) content (T1: 56.31%; T2: 18.58%; T3: 65.98%; T4: 43.28%) and manganese (Mn) content (T1: 42.09%; T2: 5.12%; T3: 21.53%; T4: 25.94%) upon foliar application of the studied subtreatments. The composition of Cu varied across different subtreatments, with T2 and T4 recording a percentage decrease (4.20 and 9.22%, respectively) in Cu composition under similar conditions. Overall, a highly significant interaction between subtreatments and main treatments was noted across all the leaves' nutritive composition parameters ( $P < 0.0001$ ).

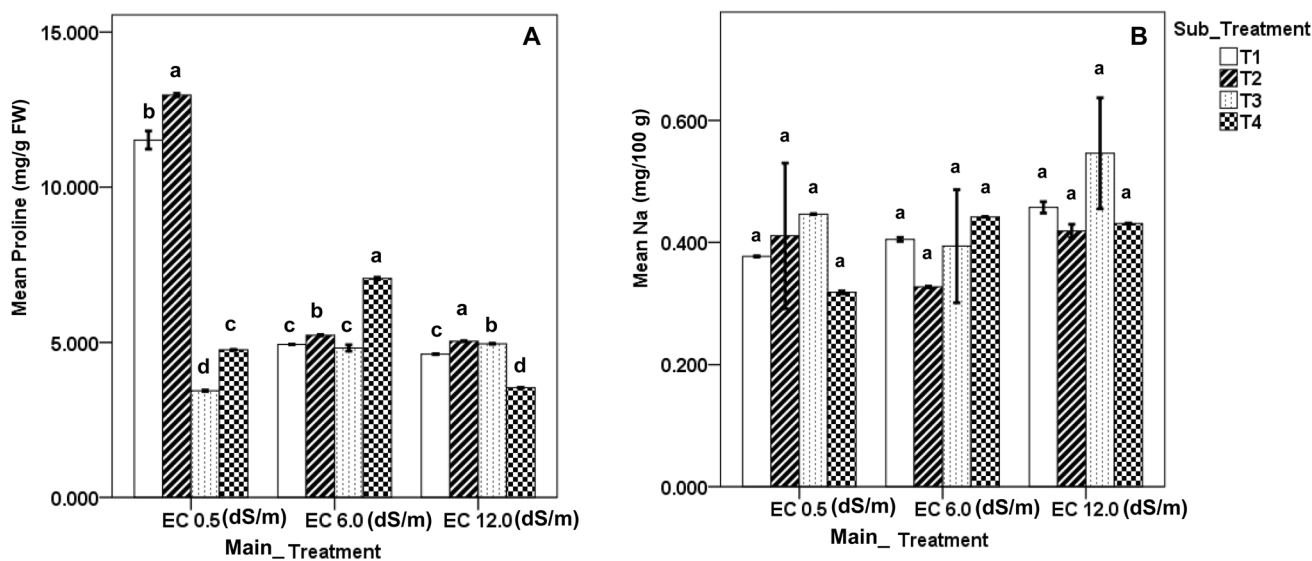
Results on free proline and sodium ions ( $\text{Na}^+$ ) content in leaf tissues are presented in Fig. 2. Generally, the proline content in leaves significantly decreased with increasing salinity ( $P < 0.05$ ). Under non-saline conditions (EC 0.5 dS/m; Fig. 2A), T1 and T2 significantly recorded the



**Table 4** Effect of foliar application of SA alone or in combination with Pro on the nutritive composition of leaves

Treatments	N%	P%	K%	Mg mg/100 g	Ca mg/100 g	Mn mg/100 g	Zn mg/100 g	Cu mg/100 g	Fe mg/100 g
0.5 dS/m									
T1	1.31 <sup>b</sup> ±0.01	0.86 <sup>b</sup> ±0.00	1.58 <sup>d</sup> ±0.00	550.79 <sup>c</sup> ±0.64	302.10 <sup>d</sup> ±0.15	26.49 <sup>c</sup> ±0.26	40.89 <sup>d</sup> ±0.07	1.75 <sup>c</sup> ±0.09	85.90 <sup>b</sup> ±0.01
T2	1.22 <sup>d</sup> ±0.00	0.94 <sup>a</sup> ±0.03	1.82 <sup>a</sup> ±0.00	541.63 <sup>d</sup> ±0.12	598.52 <sup>a</sup> ±0.10	42.62 <sup>a</sup> ±0.15	77.72 <sup>a</sup> ±1.06	2.62 <sup>a</sup> ±0.19	122.37 <sup>a</sup> ±0.02
T3	1.98 <sup>a</sup> ±0.02	0.92 <sup>a</sup> ±0.00	1.79 <sup>b</sup> ±0.00	670.54 <sup>b</sup> ±0.25	382.01 <sup>c</sup> ±0.67	42.50 <sup>a</sup> ±1.72	73.66 <sup>b</sup> ±0.11	2.04 <sup>b</sup> ±0.00	73.11 <sup>c</sup> ±0.01
T4	1.28 <sup>c</sup> ±0.00	0.69 <sup>c</sup> ±0.00	1.68 <sup>c</sup> ±0.01	691.54 <sup>a</sup> ±0.37	482.15 <sup>b</sup> ±0.03	38.88 <sup>b</sup> ±0.01	51.46 <sup>c</sup> ±0.70	2.17 <sup>b</sup> ±0.00	69.44 <sup>d</sup> ±0.02
6.0 dS/m									
T1	1.96 <sup>a</sup> ±0.03	0.62 <sup>ab</sup> ±0.08	1.72 <sup>a</sup> ±0.00	599.43 <sup>b</sup> ±0.39	491.13 <sup>a</sup> ±0.07	48.86 <sup>a</sup> ±0.03	79.91 <sup>a</sup> ±1.25	2.26 <sup>b</sup> ±0.01	99.75 <sup>b</sup> ±0.64
T2	1.41 <sup>d</sup> ±0.00	0.52 <sup>b</sup> ±0.00	1.63 <sup>c</sup> ±0.00	520.59 <sup>d</sup> ±0.17	361.83 <sup>c</sup> ±0.65	41.42 <sup>b</sup> ±0.05	77.12 <sup>b</sup> ±0.43	2.15 <sup>c</sup> ±0.01	139.03 <sup>a</sup> ±0.02
T3	1.73 <sup>c</sup> ±0.00	0.66 <sup>a</sup> ±0.00	1.44 <sup>d</sup> ±0.00	606.41 <sup>a</sup> ±0.39	337.59 <sup>d</sup> ±0.25	43.27 <sup>b</sup> ±1.34	60.90 <sup>d</sup> ±0.29	2.43 <sup>a</sup> ±0.00	68.74 <sup>c</sup> ±0.84
T4	1.85 <sup>b</sup> ±0.03	0.73 <sup>a</sup> ±0.00	1.67 <sup>b</sup> ±0.01	527.14 <sup>c</sup> ±0.02	447.39 <sup>b</sup> ±0.02	47.57 <sup>a</sup> ±1.95	69.63 <sup>c</sup> ±0.89	2.15 <sup>c</sup> ±0.03	70.32 <sup>c</sup> ±0.32
12.0 dS/m									
T1	1.79 <sup>a</sup> ±0.13	0.68 <sup>c</sup> ±0.01	1.48 <sup>d</sup> ±0.05	763.64 <sup>c</sup> ±0.33	518.15 <sup>a</sup> ±0.09	45.74 <sup>b</sup> ±1.28	93.60 <sup>b</sup> ±0.31	2.86 <sup>a</sup> ±0.03	197.12 <sup>a</sup> ±1.16
T2	1.53 <sup>a</sup> ±0.01	0.72 <sup>b</sup> ±0.00	1.60 <sup>c</sup> ±0.01	557.46 <sup>d</sup> ±0.93	398.64 <sup>c</sup> ±0.13	44.92 <sup>b</sup> ±0.03	95.46 <sup>b</sup> ±0.01	2.51 <sup>a</sup> ±0.06	182.06 <sup>b</sup> ±0.58
T3	1.65 <sup>a</sup> ±0.00	1.02 <sup>a</sup> ±0.01	1.83 <sup>a</sup> ±0.00	949.06 <sup>a</sup> ±0.16	482.04 <sup>b</sup> ±0.64	54.16 <sup>a</sup> ±0.03	216.50 <sup>a</sup> ±3.74	2.05 <sup>b</sup> ±0.05	108.58 <sup>c</sup> ±0.93
T4	1.85 <sup>a</sup> ±0.17	0.68 <sup>c</sup> ±0.00	1.74 <sup>b</sup> ±0.00	857.11 <sup>b</sup> ±0.19	341.41 <sup>d</sup> ±0.18	52.50 <sup>a</sup> ±0.28	90.73 <sup>b</sup> ±0.14	1.97 <sup>b</sup> ±0.25	92.80 <sup>d</sup> ±0.02

Data expressed as Mean ± SE. Different lower superscript letters within each main treatment indicate a significant difference at  $P < 0.05$  (Duncan multiple range test). Subtreatments: T1: 0 mM salicylic acid + 0 mM proline; T2: 1.0 mM salicylic acid + 0 mM proline; T3: 1.0 mM salicylic acid + 5 mM Proline; T4: 1.0 mM salicylic acid + 10 mM proline. *N* nitrogen, *P* phosphorus, *K* potassium, *Mg* magnesium, *Ca* calcium, *Mn* manganese, *Zn* zinc, *Cu* copper, *Fe* iron



**Fig. 2** Mean proline content (A) and sodium ion concentration (B) contained in leaves. Data presented as mean ± SE. Error bars represent the standard error of the mean. Bar columns within the same

main treatment having different letters are significantly different at  $P < 0.05$  (Duncan multiple range test)

highest proline content in leaves compared to other subtreatments ( $P < 0.05$ ). Under saline conditions (EC 6.0 dS/m), however, T3 and T4 significantly recorded a higher percentage increase in proline content (28.48 and 32.48%, respectively) compared to T1 and T2 ( $P < 0.05$ ). At EC 12.0 dS/m, foliar application of T1, T2, and T4 resulted in a decline in the percentage proline content (59.86, 61.14, and 25.63%, respectively) of leaves. Data on  $\text{Na}^+$  ions content of leaves

indicated no significant differences across all subtreatments and salinity levels (Fig. 2B).

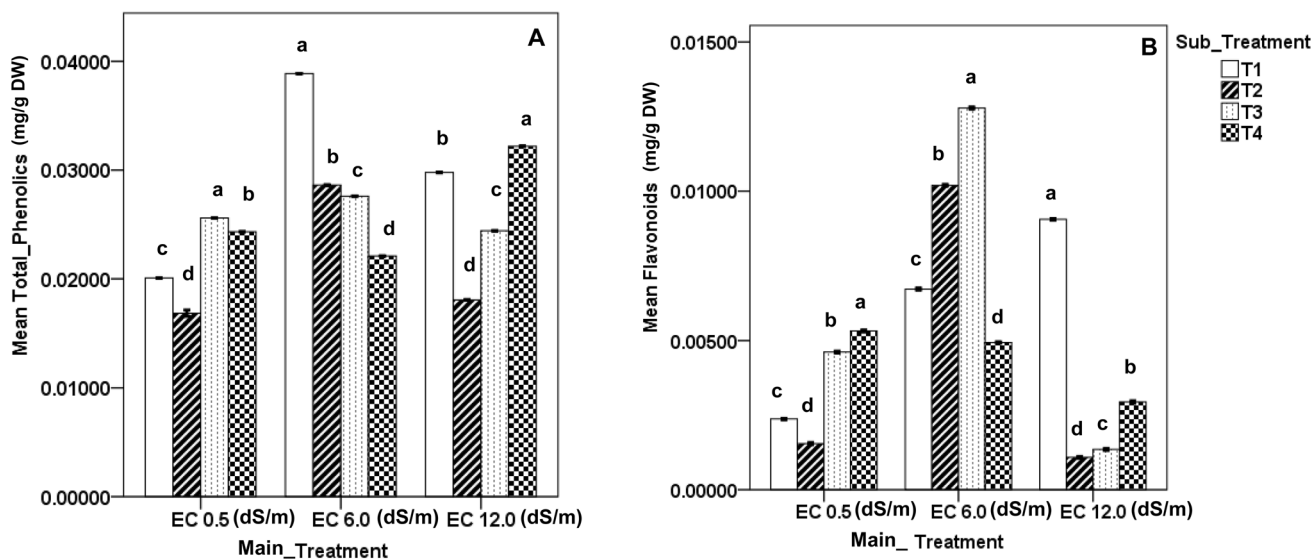
### Fruit Nutritive and Phyto-Chemical Composition

Results on the nutritive composition of fruits are presented in Table 5; Figs. 3 and 4. The total carbohydrate composition of fruits generally decreased with increasing irrigation

**Table 5** Effect of foliar application of SA alone or in combination with Pro on the nutritive composition of fruits

Treatments	Total Carbs % DW	TSS	N%	P%	K%	Mg mg/100 g	Mn mg/100 g	Zn mg/100 g	Fe mg/100 g
0.5 dS/m									
T1	81.12 <sup>a</sup> ± 4.20	4.23 <sup>b</sup> ± 0.03	1.81 <sup>b</sup> ± 0.00	0.59 <sup>a</sup> ± 0.01	1.81 <sup>a</sup> ± 0.00	198.72 <sup>c</sup> ± 1.00	9.53 <sup>b</sup> ± 0.00	20.43 <sup>a</sup> ± 0.89	54.45 <sup>b</sup> ± 0.00
T2	79.31 <sup>a</sup> ± 0.92	4.80 <sup>a</sup> ± 0.12	1.53 <sup>c</sup> ± 0.00	0.57 <sup>b</sup> ± 0.00	1.62 <sup>c</sup> ± 0.00	210.40 <sup>b</sup> ± 0.14	6.78 <sup>d</sup> ± 0.00	16.24 <sup>b</sup> ± 0.00	44.15 <sup>c</sup> ± 0.29
T3	61.68 <sup>b</sup> ± 0.29	3.60 <sup>c</sup> ± 0.23	1.90 <sup>a</sup> ± 0.00	0.60 <sup>a</sup> ± 0.00	1.74 <sup>b</sup> ± 0.00	219.79 <sup>a</sup> ± 0.57	10.18 <sup>a</sup> ± 0.00	21.11 <sup>a</sup> ± 0.00	64.72 <sup>a</sup> ± 0.00
T4	65.35 <sup>b</sup> ± 0.33	4.70 <sup>a</sup> ± 0.06	1.35 <sup>d</sup> ± 0.00	0.53 <sup>c</sup> ± 0.00	1.55 <sup>d</sup> ± 0.00	196.49 <sup>d</sup> ± 0.38	8.06 <sup>c</sup> ± 0.00	17.46 <sup>b</sup> ± 0.00	54.31 <sup>b</sup> ± 0.01
6.0 dS/m									
T1	62.27 <sup>c</sup> ± 0.27	4.57 <sup>a</sup> ± 0.03	1.49 <sup>b</sup> ± 0.02	0.59 <sup>a</sup> ± 0.00	1.66 <sup>d</sup> ± 0.00	230.70 <sup>a</sup> ± 0.57	7.89 <sup>d</sup> ± 0.01	17.64 <sup>a</sup> ± 1.15	53.42 <sup>d</sup> ± 0.00
T2	75.22 <sup>b</sup> ± 0.33	3.53 <sup>b</sup> ± 0.09	1.51 <sup>b</sup> ± 0.00	0.58 <sup>a</sup> ± 0.01	1.66 <sup>c</sup> ± 0.00	182.36 <sup>d</sup> ± 2.48	9.55 <sup>a</sup> ± 0.00	18.84 <sup>a</sup> ± 0.00	63.48 <sup>b</sup> ± 0.00
T3	79.09 <sup>a</sup> ± 1.37	3.10 <sup>c</sup> ± 0.06	1.52 <sup>b</sup> ± 0.00	0.56 <sup>a</sup> ± 0.00	1.74 <sup>b</sup> ± 0.00	199.21 <sup>b</sup> ± 0.00	8.82 <sup>b</sup> ± 0.01	19.14 <sup>a</sup> ± 0.00	55.55 <sup>c</sup> ± 0.00
T4	73.74 <sup>b</sup> ± 0.07	3.40 <sup>b</sup> ± 0.06	1.84 <sup>a</sup> ± 0.00	0.56 <sup>a</sup> ± 0.00	1.79 <sup>a</sup> ± 0.00	186.22 <sup>c</sup> ± 0.00	8.33 <sup>c</sup> ± 0.00	19.62 <sup>a</sup> ± 1.16	67.63 <sup>a</sup> ± 0.00
12.0 dS/m									
T1	71.53 <sup>a</sup> ± 1.73	3.40 <sup>a</sup> ± 0.06	1.83 <sup>a</sup> ± 0.00	0.52 <sup>c</sup> ± 0.00	1.60 <sup>c</sup> ± 0.00	177.02 <sup>c</sup> ± 1.73	10.63 <sup>a</sup> ± 0.00	18.36 <sup>a</sup> ± 0.00	69.99 <sup>a</sup> ± 0.00
T2	65.88 <sup>b</sup> ± 0.73	3.40 <sup>a</sup> ± 0.17	1.69 <sup>c</sup> ± 0.00	0.55 <sup>b</sup> ± 0.00	1.84 <sup>a</sup> ± 0.00	173.53 <sup>d</sup> ± 0.94	7.67 <sup>c</sup> ± 0.00	17.05 <sup>b</sup> ± 0.58	48.64 <sup>c</sup> ± 0.61
T3	64.75 <sup>b</sup> ± 1.78	3.57 <sup>a</sup> ± 0.03	1.82 <sup>b</sup> ± 0.00	0.47 <sup>d</sup> ± 0.00	1.74 <sup>b</sup> ± 0.03	182.36 <sup>b</sup> ± 0.32	9.51 <sup>b</sup> ± 0.03	12.90 <sup>c</sup> ± 0.00	53.18 <sup>b</sup> ± 0.00
T4	59.65 <sup>c</sup> ± 0.06	3.50 <sup>a</sup> ± 0.12	1.52 <sup>d</sup> ± 0.00	0.63 <sup>a</sup> ± 0.01	1.72 <sup>b</sup> ± 0.00	190.77 <sup>a</sup> ± 0.65	4.78 <sup>d</sup> ± 0.00	10.93 <sup>d</sup> ± 0.00	45.36 <sup>d</sup> ± 0.00

Data expressed as Mean ± SE. Different lower superscript letters within each main treatment indicate a significant difference at  $P < 0.05$  (Duncan multiple range test). Subtreatments: T1: 0 mM salicylic acid + 0 mM proline; T2: 1.0 mM salicylic acid + 0 mM proline; T3: 1.0 mM salicylic acid + 5 mM proline; T4: 1.0 mM salicylic acid + 10 mM proline. TSS total soluble solutes, N nitrogen, P phosphorus, K potassium, Mg magnesium, Mn manganese, Zn zinc, Fe iron

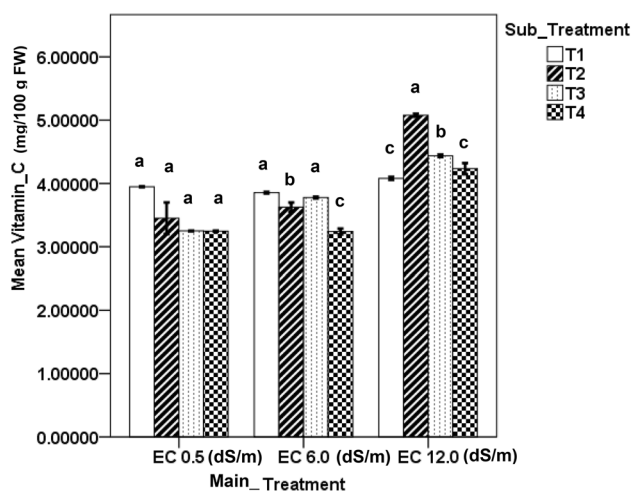


**Fig. 3** Total phenolics (A) and flavonoids (B) content in fruits. Data presented as mean ± SE. Error bars represent the standard error of means. Bar columns within the same main treatment having different letters are significantly different at  $P < 0.05$  (Duncan multiple range test)

salinity (Table 5). Results indicated that at EC 6.0 dS/m, foliar application of T1 and T2 led to a significant decline in the percentage total carbohydrate composition (23.24% and 5.16%, respectively) of fruits with T3 significantly recording higher values for total carbohydrate composition compared to other subtreatments ( $P < 0.05$ ). Under highly saline conditions (EC 12.0 dS/m), the total carbohydrate composition of fruits declined by 11.82, 16.93, and 8.72% in T1, T2, and T4,

respectively, with T3 recording a significant increase in the percentage total carbohydrate composition of fruits (4.98%) compared to other subtreatments ( $P < 0.05$ ).

For total soluble solutes (TSS), irrigating plants with saline water (EC 6.0 dS/m) resulted in a decline in TSS by 26.46, 13.89, and 27.66% in T2, T3, and T4, respectively, with T1 significantly recording the highest TSS compared to other subtreatments ( $P < 0.05$ ). No significant difference in



**Fig. 4** Vitamin C content in fruits. Data presented as mean  $\pm$  SE. Error bars represent the standard error of the mean. Bar columns within the same main treatment having different letters are significantly different at  $P < 0.05$  (Duncan multiple range test)

TSS was noted among all subtreatments under highly saline conditions (EC 12.0 dS/m), Table 5.

Data on macro-elements in fruits (Table 5) indicated that foliar application of T1, T2, and T3 resulted in a decrease in the percentage composition of N (16.93, 1.31, and 20%, respectively) except for T4 under saline conditions (EC 6.0 dS/m). However, at EC 12.0 dS/m, the percentage composition of N increased by 1.09, 9.47, and 11.18% in T1, T2, and T4, respectively. Results on the P composition of fruits indicated a percentage decline in P composition (11.86, 3.51, and 21.67%) in T1, T2, and T3, respectively, under highly saline conditions (EC 12.0 dS/m). However, the fruit composition of K increased by 2.41 and 13.41% in T2 and T4 at EC 6.0 dS/m. Under highly saline conditions (EC 12.0 dS/m), foliar application of T2 and T4 increased the K composition by 11.96 and 9.88%, respectively. Data on the microelement composition of fruits (Table 5) indicated that foliar application of T1 and T2 at EC 12.0 dS/m resulted in an increase in the Mn (10.35 and 11.60%) and Fe (22.20 and 9.23%) composition of fruits. However, T4 and T3 exhibited a decline in the Mn (40.69 and 6.58%) and Fe (16.48 and 17.83%) composition of fruits under similar conditions. Furthermore, the composition of Zn in fruits declined by 10.13, 38.89, and 37.40% in T1, T3, and T4, respectively, under highly saline conditions (EC 12.0 dS/m). Overall, a highly significant interaction between subtreatments and main treatments was noted across all the fruit nutritive composition parameters ( $P < 0.0001$ ).

Data on the total phenolics content of fruits is presented in Fig. 3A. Results indicated an increase in the percentage

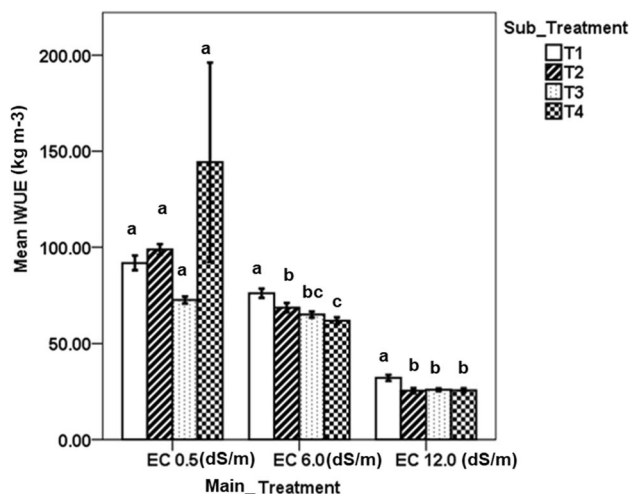
total phenolics content of fruits across all subtreatments (T1: 20%, T2: 69.23%, T3: 7.81%, T4: 1.84%) under saline conditions of EC 6.0 dS/m. At EC 12.0 dS/m, T4 significantly recorded higher values for total phenolics compared to other subtreatments ( $P < 0.05$ ).

Data on the total flavonoids content are presented in (Fig. 3B). The results indicated that foliar applications of T1, T2, and T3 led to a percentage increase in the total flavonoids contents (64, 98.53, and 96.40% respectively,  $P < 0.05$ ) under saline conditions of EC 6.0 dS/m. However, at EC 12.0 dS/m, foliar applications of T2, T3, and T4 significantly recorded the highest percentage decrease in total flavonoids content (99.02%, 99.22%, and 40%, respectively) compared to the control (T1) ( $P < 0.05$ ).

Results on vitamin C content indicated no significant difference among all the subtreatments under non-saline conditions (Fig. 4). However, an increase in the percentage content of vitamin C was noted among all subtreatments (i.e., T1: 3.27%, T2: 32.04%, T3: 26.75%, T4: 27.15%) under highly saline conditions (EC 12.0 dS/m) with T2 significantly having the highest vitamin C content compared to other sub-treatments ( $P < 0.05$ ).

### Irrigation Water Use Efficiency

Irrigation water use efficiency (IWUE) decreased with an increase in salinity levels. At EC 0.5 dS/m, no significant difference was noted among different subtreatment groups. At EC 6.0 and 12.0 dS/m, T1 significantly had a higher IWUE compared to other subtreatments ( $P < 0.05$ ) (Fig. 5).



**Fig. 5** Irrigation water use efficiency of cucumber under different irrigation salinity levels. Data expressed as mean  $\pm$  SE. Error bars represent the Standard Error. Bar columns within the same main treatment having different letters are significantly different at  $P < 0.05$  (Duncan multiple range test)

## Discussion

Saline irrigation and soil salinity have been recognized as one of the leading threats to vegetable crop production worldwide as saline conditions are known to suppress plant growth (Yildirim et al. 2008; Tahjib-Ul-Arif et al. 2018). Several approaches, including the foliar application of plant growth regulators (PGRs) and osmoprotectants, have shown promising results in the mitigation of salinity stress effects in several crops such as tomatoes (Heuer 2003; Rezaei et al. 2012; Kahlaoui et al. 2014; Elkhatib et al. 2017), cucumber (Yildirim et al. 2008; Youssef et al. 2018; Estaji et al. 2019), pepper (Elwan and El-Hamahmy 2009; Altae 2018; Abdelaal et al. 2020), strawberries (Karlidag et al. 2009; Faghih et al. 2017), and corn (Tahjib-Ul-Arif et al. 2018). SA and Pro, a vital PGR and osmoprotectant, have attracted attention among the naturally produced plant stress mitigating chemicals in the past decades. In this study, we have evaluated the effect of foliar application of SA alone or in combination with Pro on the growth, yield, fruit quality, and nutritional composition of cucumber under different levels of irrigation salinity.

Generally, exposure of plants to saline conditions induces ionic toxicity, which triggers both oxidative and osmotic stress, all of which suppress plant growth, development, and production (Yildirim et al. 2008; Youssef et al. 2018). In the present study, exposure of cucumber plants to prolonged irrigation salinity negatively and significantly impacted their growth (Table 2), fruit quality, and yield (Table 3) as well as in some phytochemical and mineral elements of fruits and leaves (Fig. 3; Tables 4 and 5). Similar results in cucumber have been previously reported (Yildirim et al. 2008; Wan et al. 2010; Kere et al. 2016; Youssef et al. 2018). Such effects could be attributed to the high salt concentration-mediated disturbance of several biochemical and physiological attributes including water uptake, photosynthetic capacity, stomatal conductance, oxidative stress, hormonal signaling, osmoprotectant accumulation, and mineral nutrient homeostasis (Yildirim et al. 2008; Huang et al. 2011; Khan et al. 2012; Kere et al. 2016). However, several studies have shown that foliar application of SA alone and or Pro ameliorates salinity stress effects in plants (Yildirim et al. 2008; Faghih et al. 2017; Garg and Bharti 2018; Youssef et al. 2018; Abdelaal et al. 2020). However, to the best of our knowledge, no study has been conducted to address the question of whether combined foliar applications of SA and Pro ameliorate the negative effects of salinity stress in cucumber. In this study, foliar application of SA alone or in combination with Pro did not positively influence the growth of cucumber under saline conditions. The results of this study contradict those of Yildirim et al. (2008), Youssef et al. (2018), and Huang et al. (2009a). The discrepancy

in results could be attributed to the difference in experimental conditions. Irrigating with saline water fertigated with ammonium-based fertilizers led to pH fluctuations in the growth media, affecting plant nutrient uptake and thus reducing plant growth. Ammonium-based nitrogen fertilizers have previously been reported to lower the soil pH, thus increasing soil acidity (Cheng et al. 2017; Wang et al. 2020). For normal plant growth and development, the pH should be in the range of 6.0 to 7.0 (USDA 1998) since, under this pH, both macro- and micro-mineral elements are available for plant uptake. In this study, the pH decreased to 5.5 and 4.9 under saline conditions of EC 6.0 and 12.0 dS/m, respectively, hindering the uptake of certain mineral elements required for plant growth. Furthermore, our experimental conditions such as the irrigation salinity levels (EC 6.0 dS/m, EC 12.0 dS/m), plant variety (*Cucumis sativus L., cv. Barracuda F1*), and frequency of foliar applications were different from those of earlier studies. IWUE is an important parameter for estimating water consumption by plants. Irrigating plants with saline water often decreases plant-water uptake and thus a decrease in IWUE. Results of this study showed a decline in the IWUE of plants under salinity stress. Allen et al. (1998) reported that saline conditions reduce plant-water uptake and evapotranspiration, which causes plants to use more energy in obtaining water from the soil.

This study also showed that high salinity levels negatively impact the SPAD reading values of leaves. Similar results have been obtained in cucumber (Yildirim et al. 2008; Youssef et al. 2018), tomato (Moniruzzaman et al. 2018; Ullah et al. 2020), and sweet pepper (Altae 2018; Abdelaal et al. 2020). The reduction in SPAD reading values could be attributed to stomatal closure, increased chlorophyllase enzyme activity which breaks down chlorophyll, and inhibition of chlorophyll synthesis (Parvaneh et al. 2012). Mittler (2002) reported that salt-induced oxidative damage or direct Na<sup>+</sup> toxicity could degrade the ultrastructure of chloroplasts, thus leading to a reduction in SPAD reading values of leaves. Consequently, carbon fixation is reduced, which results in the reduction of plant growth. Therefore, the decrease in SPAD reading values of leaves under salinity stress is in agreement with the decrease in plant growth parameters, most especially at 70 DAT (Table 2). Foliar application of SA in combination with Pro did not, however, show any effect on the SPAD reading values of leaves in our study. We anticipate that the protective role of SA in combination with Pro under our experimental conditions could be concentration-dependent since high concentrations could cause a toxic effect in plants. For example, although proline has been previously reported to induce plant tolerance toward salinity stress, it can as well suppress plant growth if exogenously applied in high concentrations (El Moukhtari et al. 2020). Rodriguez and Heyser (1988) found that foliar application of 10 mM Pro inhibited the normal

growth of *Distichlis* suspension cultures under 260 mM of salt stress. Similarly, Heuer (2003) demonstrated that external supplementation of 10 mM Pro to salt stressed *Solanum lycopersicum* not only decreased root and leaf fresh weights but was also lethal to plants.

Furthermore, to have more insights on the effect of such foliar applications, we investigated their impact on fruit quality and yield under saline conditions (Table 3). Saline conditions significantly and negatively affected several fruit quality parameters and yield. Similar results have been previously reported in cucumber (Youssef et al. 2018), tomato (Magán et al. 2008; Zhang et al. 2016, 2017), and pepper (Chartzoulakis and Klapaki 2000; Navarro et al. 2010). However, foliar applications of SA alone or in combination with Pro did not improve fruit quality and yield. We attribute this observation to the concentrations used in this study which suppressed plant productivity under our experimental conditions.

The current study also indicated that saline irrigation influences the nutritional content of leaves and fruits (Tables 4 and 5). In leaves, the percentage composition of N, Zn, Fe, and Mn increased in saline conditions (EC 6.0 dS/m) compared to non-saline conditions. Similarly, in fruits, the percentage composition of N and K increased in saline conditions of EC 6.0 dS/m and EC 12.0 dS/m, respectively, compared to non-saline conditions. The lowest values of several mineral elements were recorded at EC 12.0 ds/m, indicating that the studied variety has a salinity tolerance of up to EC 6.0 dS/m, probably due to high relative water content (RWC) under such conditions. Likewise, several studies have shown increased tolerance of plants to salinity stress with foliar applications or increase in concentrations of Zn, Cu, and Mn content in plant tissues (Chrysargyris et al., 2018; El-fouly et al., 2011; Hassanpouraghdam et al., 2011; Iqbal et al., 2018; Jabeen & Ahmad, 2011; Jan & Hadi, 2015; Mehrabani et al., 2018; Pérez-Labrada et al., 2019; Shahi & Srivastava, 2018; Adhikari et al., 2020; Çimrin et al., 2010; Khan et al., 2013). N is also an important nutrient that has been shown to ameliorate salinity stress effects in plants. For example, Iqbal et al. (2015) found that N regulates Pro and ethylene biosynthesis under salinity stress. In another study, Akram et al. (2011) observed that N application in salt stressed maize hybrids improved plants' net photosynthetic rate, stomatal conductance, and transpiration rate. T3 enhanced the nutrient content of fruits under non-saline stress. Previous studies have shown that the application of SA or Pro under normal conditions can also improve the nutritive composition of fruits (Elwan and El-Hamahmy 2009; El Sayed et al. 2014; Garde-Cerdán et al. 2015; Mohamed et al. 2018; García-Pastor et al. 2020). Under saline conditions, however, T2, T4, and T3 improved some of the mineral elements, flavonoids, total phenolics, vitamin

C, and total carbohydrates. T4 seemed to have a more positive impact on the nutritive composition of fruits under extremely saline conditions (EC 12.0 dS/m). Indeed, foliar application of SA and Pro have previously been reported to improve the nutritive composition of fruits under saline conditions (Kahlaoui et al. 2014; Butt et al. 2016; Elkhatib et al. 2017; Awad-Allah et al. 2020).

Vitamin C (Ascorbic acid) increased under saline conditions (Fig. 4). Accumulation of vitamin C in plant tissues under saline conditions is a defensive mechanism in plants aimed at building up tolerance toward abiotic stress. Several studies have indicated that exogenous application of ascorbic acid alone or in combination with other PGRs improves the plant's tolerance against abiotic stress (Faisalabad et al. 2006; Dolatabadian and Jouneghani 2009; Sadak et al. 2014; Billah et al. 2017). Ascorbic acid is an important antioxidant that can non-enzymatically scavenge hydrogen peroxide ( $H_2O_2$ ) and reactive oxygen species (ROS) and is also involved in ascorbate peroxidase mediated scavenging of  $H_2O_2$ . Likewise, ascorbic acid takes part in the regeneration of  $\alpha$ -tocopherol, which is a vital non-enzymatic antioxidant (Sairam et al. 2005).

An increase in total phenolics and flavonoids was noted in plants exposed to saline conditions (Fig. 3). Phenolics are a group of secondary metabolites with antioxidant capabilities whose build-up in plant tissues occurs under conditions of abiotic stress (Minh et al. 2016; Šamec et al. 2021). Minh et al. (2016) observed an increase in phenolic and flavonoid compounds in rice varieties (OM4900 and BC15TB) under saline conditions. The authors suggested that the observed increment in these antioxidants could be a defensive mechanism in plants against salinity stress. In this experiment, T4 seemed to be effective in increasing the total phenolics content under extremely saline conditions (EC 12.0 dS/m). Similarly, T2 and T3 also seemed to be effective in increasing the total flavonoids content under salinity stress (EC 6.0 ds/m). Therefore, our results are in agreement with previous studies in which foliar applications of SA and Pro increased the total phenolics and flavonoids content in plant tissues under abiotic stress (Ali et al. 2013; Khalil et al. 2018; Alkahtani et al. 2021).

## Conclusion

In conclusion, this study demonstrated that cucumber can tolerate irrigation salinity levels up to EC 6.0 dS/m and that foliar application of T2 and T3 can slightly ameliorate salinity stress effects with regard to fruit number per plant for T2, and nutritive composition of fruits, for T2 and T3. Their effects are generally suppressed under extreme saline conditions above the EC 6.0 dS/m threshold. However,

T4 seemed to perform better with regard to nutritive composition of fruits under extremely saline conditions (EC 12.0 dS/m). For maximum yield, foliar application of these osmoprotectants and PGRs under saline conditions is not recommended. Likewise, cultivation of this cucumber variety under saline conditions should not exceed salinities EC 6.0 dS/m.

**Author Contributions** HS involved in conceptualization; FK, HS, and MM contributed to Design; FK, HS, MM, and MD contributed to writing-reviewing & editing; FK and MM contributed to methodology, investigation, and data collection. MM contributed to software; FK, MM, and MD contributed to writing- original draft preparation; and MD and HS contributed to supervision.

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**Data Availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Code Availability** Not applicable.

## Declarations

**Conflict of interest** The authors have no conflict of interest to declare that are relevant to the content of this article.

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