



Growth Characteristics and Fruit Quality of Chili Pepper under Higher Electrical Conductivity of Nutrient Solution Induced by Various Salts

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ABSTRACT

Salinity as a global challenge is a very complex issue in agricultural food production. In this study, the growth characteristics of chili pepper (*Capsicum annuum* var. crusader) were evaluated under higher Electrical Conductivity (EC) (5 dS/m) induced by NaCl, KCl, K₂SO₄ or “All Nutrients” of Hoagland nutrient solution. The standard Hoagland solution (with EC 1.8 dS/m) was used as the control. The results showed that EC5 induced by NaCl resulted in the most negative effects on growth, yield and fruit quality parameters. Application of KCl (to induce EC5) also resulted in some reduction in growth parameters. However, application of K₂SO₄ and particularly “All Nutrients” (to induce EC5) resulted in better growth characteristics compared to plants treated with NaCl or KCl salts. There was a significant reduction in growth and fruit quality traits including plant height, leaf SPAD value, fresh and dry weights of shoots and roots, fruit fresh yield, fruit total soluble solids (TSS) and vitamin C content by NaCl and to lesser extent by KCl. However, there was no significant reduction in these traits by “All Nutrients” and for some traits by K₂SO₄ application compared to control plants.

INTRODUCTION

Salinity stress is a complex issue, and plants can experience significant alteration in their morpho-physiological characteristics under saline stress and/or in order to acquire more tolerance to this stress. The climate conditions and human activities are the two major contributing factors to soil and water salinity (Munns & Gilliam, 2015; Negrão, Schmöckel, & Tester, 2017). Many agricultural and industrial activities generally contribute to an increase in the soil and water salinity. There is also a general decrease in the soil and water quality with industrial activity and modern life in many parts of the world (Ahmadi & Souri, 2018). During previous decades, rapid increase in the world population imposed great pressure on soil fertility and agricultural productivity. This was mainly due to high application rate of chemical fertilizers, expecting high yield, yet significantly contributed to soil salinity (Aslani & Souri, 2018).

The increase of EC of soil or irrigation water has important consequences on plant water

potential, since higher salinity generally reduces water uptake of plants and therefore reduces the leaf water potential (Cocozza et al., 2013; Negrão, Schmöckel, & Tester, 2017; Parihar, Singh, Singh, Singh, & Prasad, 2015). Plant growth and production are generally restricted by saline condition at root zone (Karjunita, Khumaida, & Ardie, 2019; Munns & Gilliam, 2015). On the other hand, the increasing world population and demand for higher food supply have resulted in the use of saline water and saline soils for agricultural food production in many parts of the world (Ahmadi & Souri, 2018; Malash, Flowers, & Ragab, 2008). Therefore, in such regions the future of agricultural production depends on cultivation of more tolerant plant species to salinity as well as on the increase in plant tolerance to salinity through biotechnology and genetic engineering approaches (Munns & Gilliam, 2015).

Over the last decades, huge scientific works were conducted to evaluate and improve the plant salinity tolerance in many important crops (Grewal,

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2010; Martínez-Ballesta, Martínez, & Carvajal, 2004). The most of these studies were conducted using NaCl induced salinity, that is only a major constitute (but not all) of salinity in the nature. However, natural soil or water salinity consisted of different anions and cations that can have different effects on plants than NaCl. In the present study, a given electrical conductivity of nutrient solution (EC5 dS/m) generated by various chemical salts were evaluated on growth and yield of chili pepper plants.

MATERIALS AND METHODS

The experiment was running from 10 March until 15 June 2017 under greenhouse conditions of $28 \pm 5^\circ\text{C}$ temperature, 75-85% relative humidity and 250-300 $\mu\text{mole/m}^2/\text{s}$ light intensity. This study was performed at Faculty of Agriculture, Tarbiat Modares University, Tehran-Iran. Seeds of chili pepper (*Capsicum annuum* var *Crusader*) were first disinfected with 1% hypochlorite for 10 minutes and after being washed with tap water, seeds were germinated in sand for two weeks at temperature of $25 \pm 3^\circ\text{C}$. The black plastic pots with five liter volume were filled with a mix of perlite and cocopeat (3:7 v/v), and the uniform seedlings at four-leaf stage were transplanted to this pot medium. The experiment was conducted under hydroponic culture, in which plants were regularly fed with nutrient solution. The Hoagland formula was used for preparation of nutrient solution, and the salts for generating the higher conductivity (EC5) were added to this nutrient solution. After transplanting, seedlings were irrigated with standard Hoagland nutrient solution for ten days, thereafter different chemical salts were applied to the plants via nutrient solution to induce higher EC5.

The experiment was arranged in a non-factorial completely randomized design with four replications in which each pot represented one replication containing two chili pepper plants. Treatments were various chemical salts including NaCl, KCl, K_2SO_4 and "All Nutrients" that were applied into the Hoagland nutrient solution to increase its basic EC1.8 dS/m to EC5 dS/m. The general EC of Hoagland solution (1.8 dS/m) was considered the control. The "All Nutrients" treatment was the concentrated Hoagland solution and represents all macro and micro nutrients of the Hoagland formula that were added to the solution (to generate EC 5 dS/m) without changes in nutrients proportion.

These were calcium nitrate, potassium nitrate, di-hydrogen potassium phosphate, magnesium sulfate, boric acid, zinc sulfate, manganese chloride, copper sulfate, ammonium molybdenum and iron EDTA chelate.

The chili plants were fed two times per day (in the morning and at the afternoon), with nutrient solution containing the mentioned EC treatments. The amount of applied nutrient solution was increased by plant size, from a basic of 80 ml to 400 ml per pot per day. Every three days, the pot medium was washed out with additional tap water to avoid salt accumulation in the medium.

Plants were harvested after four months. The average number of leaves, chlorotic leaves and lateral shoots for two plants per pot was counted and recorded. Plant height was measured by a ruler from plant collar till the terminal shoot point. Similarly, the average record of overall root length for two plants per pot was recorded using a ruler, after separation, cleaning and washing of roots from medium particles. The leaf SPAD value (The Soil and Plant Analysis Development) was recorded by average of 30 readings on different points of middle plant leaves in each pot (from two plants) using SPAD meter (Model SPAD-502 Plus). The whole plant leaf area per pot was measured by leaf area meter (Delta-T Devices Ltd, England) and calculated per single leaf. The plant stem diameter, peduncle length, fruit length and fruit diameter were measured using a caliper (model Mitutoyo Japan). The cumulative harvested fruits (light green stage) during growth period were recorded as fruit fresh yield. Shoot and root fresh weights were measured by a precise digital scale, after their washing with distilled water and blotting dry with tissue paper. Dry weights of shoots and roots were recorded after drying the materials in an oven (65°C for 48 hours). For determination of fruit total soluble solids (TSS), titrateable acidity (TA) and pH, 10 g fresh fruits were crushed in a mortar and a drop was used for TSS reading using a refractometer (Model GMK 703, G-WON HITECH, Guro-dong, South Korea). Thereafter, 10 ml distilled water was added to the crushing fruits that were further centrifuged for 5 minutes at $717 \times g$ at 4°C . The supernatant was used for TA and pH determination following Mardanluo, Souri, & Ahmadi (2018).

The L-ascorbic acid concentration (Vitamin C) of chili pepper fruits were determined using

2,6-dichloroindophenol, in which 10 g of fruits from final harvest (randomly selected from light green stage fruits) were gently washed, cut in small pieces and then crushed in a mortar in presence of 10 ml of 2% metaphosphoric acid. The mixture immediately was centrifuged at 9000 rpm (Eppendorf Centrifuge 5810R, Hamburg, Germany) for five minutes at 4°C. The supernatant was used for titration by 2,6-dichloro indophenols, and the amount of L-ascorbic acid (vitamin C) for 100 g fresh pod was calculated in relation to a standard curve of different concentrations of ascorbic acid of 0, 50, 100, 200 and 400 mg/l (Aslani & Souri, 2018).

Data were analyzed using ANOVA (SPSS 16) and comparison of means was performed using Duncan's multiple range test at 5% level.

RESULTS AND DISCUSSION

The plant height was reduced under higher conductivity (EC5 dS/m) generated by various salt treatments compared to control (EC1.8) except in the "All Nutrients" treatment (Table 1). The shortest plants were those treated with EC5 induced by NaCl or KCl, while application of K₂SO₄ to induce EC5 resulted in taller plants than those treated with NaCl or KCl. The plant stem diameter and leaf number were similar in control, K₂SO₄ and the "All nutrients" treatments. The plants treated with NaCl and KCl showed significantly thinner stem diameter and less leaf number than control plants.

The chili plants had more leaf under KCl compared to NaCl treatment (Table 1). The number of lateral shoots per plant showed less variation under higher EC5 induced by various salinity treatments compared to control plants. The plants under treatment of the "All Nutrients", however had

significantly more lateral shoots than those treated with NaCl or KCl. Leaf SPAD value was reduced by higher EC5 induced by NaCl or KCl application compared to control, whereas SPAD value of plants under "All Nutrients" and K₂SO₄ treatments showed negligible differences from those of control plants. The plant leaf area was significantly reduced only by NaCl, but those treated with KCl treatment showed insignificant differences than control plants. The plant leaf area in K₂SO₄ or in the "All Nutrients" treatment was almost similar to those of control.

Application of higher EC5 by all salts increased the number of chlorotic leaves, though under K₂SO₄ the values were not significant compared to control. The more chlorotic leaves were prominent under NaCl treatment than other treatments (Table 1). The root length of plants under NaCl was observed to be the longest, while the value of K₂SO₄ was the least. While the root lengths of other treatments were less varied compared to control.

The shoot fresh weight significantly under higher EC5 induced by NaCl or KCl salts. The values of K₂SO₄ or the "All Nutrients", however, were not different with the control (Fig. 1). Shoot dry weight of "All Nutrients" were observed higher than all induced EC5 salts treatments except K₂SO₄ yet the value was not significantly difference from control plants (Fig. 1). The plant root fresh weight was significantly reduced under higher EC5 induced by NaCl or KCl, whereas the plants treated with K₂SO₄ or the "All Nutrients" showed less variation than control plants (Fig. 2). Root dry weight was significantly reduced only in plants treated with KCl treatment, whereas plants treated with NaCl, K₂SO₄ or "All Nutrients" showed no significant difference than control plants (Fig. 2).

Table 1. Vegetative growth parameters of chili pepper plants under four months salinity of nutrient solution generated with different salts.

Treatment	Plant height (cm)	Stem diameter (mm)	Number of leaves	Number of lateral shoots	SPAD value	Leaf area (cm ²)	Number of chlorotic leaves	Overall root length (cm)
Control (EC1.8)	88±6.2a	9.1±1.2a	94±8a	7±0.8ab	53±4a	35±3.5a	4±0.8d	27±1.5bc
NaCl (EC5)	54±5.4c	7.1±0.6b	55±5c	6.1±0.9b	45±3b	25±2.3b	11±1.3a	32±1.6a
KCl (EC5)	61±5.4c	7.0±0.8b	71±4b	6.3±0.6b	41±6b	29±3ab	7±1.7b	29±1.4ab
K ₂ SO ₄ (EC5)	78±2.9b	9.1±0.8a	85±6a	7±0.8ab	48±5ab	33±3.9a	5±0.8cd	22±3.1d
All Nutrients (EC5)	83±7.1ab	9.4±0.7a	92±14a	8±0.7a	53±5a	34±3.7a	6±0.6bc	25±2.3cd

Remarks: Values in the same column followed by different letters differ significantly under DMRT 5%

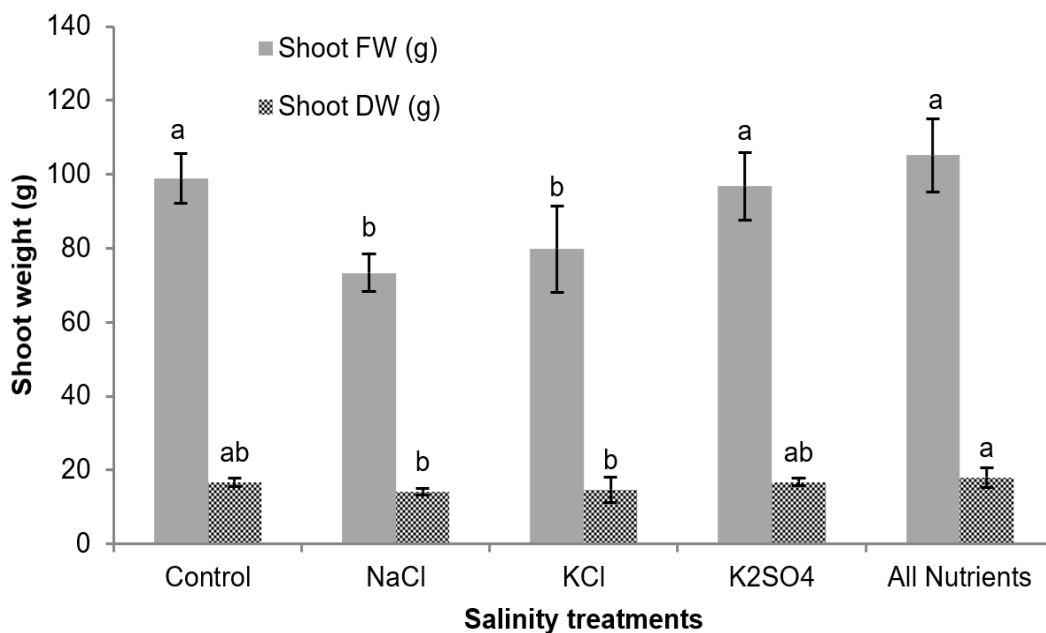


Fig. 1. Shoot fresh and dry weights of chili pepper plants under higher nutrient solution conductivity (EC5 dS/m) generated with different salts. Nutrient solution EC in control was 1.8 dS/m and in other treatments was 5 dS/m. Comparison of means was performed based on Duncan’s multiple range test at 95% level of confidence

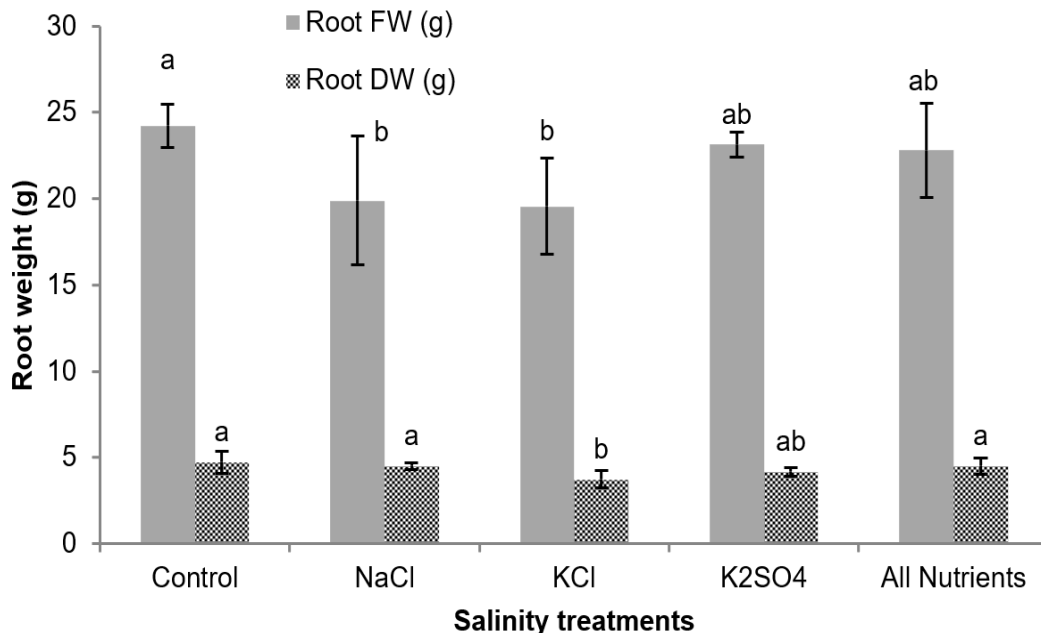


Fig. 2. Root fresh and dry weights of chili pepper plants under higher nutrient solution conductivity (EC5 dS/m) generated with different salts. Nutrient solution EC in control was 1.8 dS/m and in other treatments was 5 dS/m. Comparison of means was performed based on Duncan’s multiple range test at 95% level of confidence

The results showed that vegetative growth parameters of chili pepper were differently affected by various salts at a given higher electric conductivity (EC5 dS/m). Application of higher EC5 induced by NaCl, significantly reduced the shoot and root growth parameters (except root dry weight). These parameters were also reduced by KCl treatment (but to lesser extent), whereas the "All Nutrients" and to lesser extent K_2SO_4 treatments gave less decrease in growth parameters. The plants treated with KCl showed better records only in the case of plant leaf number and number of chlorotic leaves than NaCl treated plants. Nevertheless, NaCl and K_2SO_4 treated plants had the longer and shorter roots than control plants. Decrease in vegetative growth traits of many agricultural crops has been reported due to NaCl salinity in soil or in nutrient solution culture (Aini, Syekhfani, Yamika, Dyah P., & Setiawan, 2014; Grewal, 2010; Martínez-Ballesta, Martínez, & Carvajal, 2004). The reduction in photosynthesis rates of plants due to reduced leaf area, less chlorophyll content of leaves, impaired water conductivity and water balance of tissues and impaired photo-assimilation and protein biosynthesis have been reported under NaCl salinity conditions (Choudhury, Rivero, Blumwald, & Mittler, 2017; Marschner, 2012; Shabala et al., 2010). These conditions generally led to reduced plant biomass production and limited yield and qualities (Zörb,

Geilfus, & Dietz, 2019). In the present study, the differences in growth of treated plants seem to be caused by anion, mainly by chloride. In treatments where salts contain Cl, performance of the plants was generally reduced, and there was no significant difference in growth performance of the plants in the two chloride salts treatments, and the growth of the plants under these two treatments were generally less than the other treatments. Both chloride and sodium can adversely affect plant water conductivity and tissues water potential (Marschner, 2012; Martínez-Ballesta, Martínez, & Carvajal, 2004). The longer root length in NaCl treated plants can also be due to the negative effects of these two ions on water potential of plants and the plant physiological mechanism against this situation.

The fruit fresh yield of plant (Fig. 3) was significantly reduced under NaCl, KCl and K_2SO_4 treatments compared to control plants, whereas in the "All Nutrients" treatment the value was not significantly different. The lowest fruit fresh yield was achieved in plants treated with NaCl. Plants treated by KCl also produced more fruit fresh yield than those under NaCl treatment. Reduction in plant yield due to salinity is a common response of many plant species treated with degrees of salinity (Akhtar, Andersen, & Liu, 2015; Kosová, Vítámvás, Urban, & Prášil, 2013; Marschner, 2012).

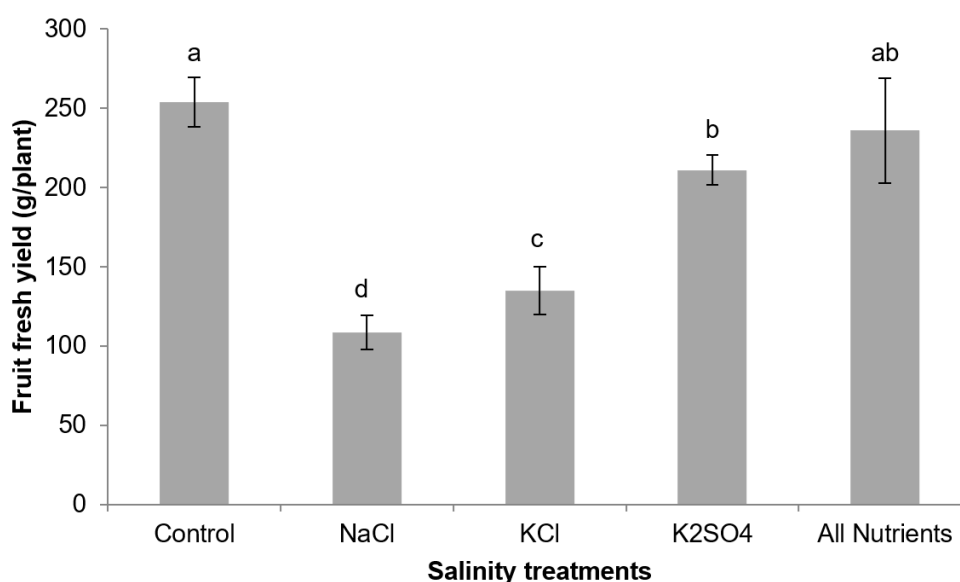


Fig. 3. Yield of fresh fruits of chili pepper under higher nutrient solution conductivity (EC5 dS/m) generated with different salts. Nutrient solution EC in control was 1.8 dS/m and in other treatments was 5 dS/m. Comparison of means was performed by Duncan's multiple range test at 5% level

Reduction of leaf photosynthesis has been suggested as the main reason behind the reduced growth, and yield losses under salinity or higher conductivity of root medium (Mardanluo, Souri, & Ahmadi, 2018; Marschner, 2012; Negrão, Schmöckel, & Tester, 2017). Nevertheless, impaired nutrient uptake and photo-assimilate allocation, and associate changes can also play key roles in reduced plant yield under salinity (Grewal, 2010; Neocleous, Koukounaras, Siomos, & Vasilakakis, 2014; Shabala et al., 2010). Similarly, better yield production of plants under KCl than NaCl treatment can be due to better leaf water status and better photosynthesis under KCl treatment, as this treatment also showed more leaf number and less chlorotic leaves than NaCl treated plants. Potassium is an important essential mineral element involved in all metabolic reactions and all physiological processes (Bose et al., 2014; Mardanluo, Souri, & Ahmadi, 2018; Marschner, 2012) that can mitigate the adverse effects of chloride or even Na on plants (Martínez-Ballesta, Martínez, & Carvajal, 2004; Shabala & Pottosin, 2014; Wang, Zheng, Shen, & Guo, 2013; Wu, Shabala, Barry, Zhou, & Shabala, 2013).

The fruit dry weight per plant (Table 2) was unchanged in the “All Nutrients” and K₂SO₄ treatments, whereas it was reduced in NaCl or KCl treated plants than control. Fruit length was also reduced by NaCl, KCl or the “All Nutrients” treatments, yet it has no significant reduction in K₂SO₄ treatment than control plants. Salinity treatments had no significant effects on fruit diameter (Table 2). Application of KCl or K₂SO₄ treatment resulted in higher peduncle length, whereas there was no

significant difference in plants treated with NaCl or the “All Nutrients” with control plants (Table 2).

Fruit biochemical characteristics were also affected by salinity treatments. The fruit total soluble solids (TSS) showed no significant changes than control plants; however, plants treated with the “All Nutrients” or K₂SO₄ treatment had significantly higher TSS than those plants treated with NaCl (Table 2). Fruit juice pH was not affected by salinity treatments, but fruit titrateable acidity (TA) was significantly reduced only in plants treated with NaCl or KCl at EC5 dS/m. Determination of fruit vitamin C content (Fig. 4) showed that only NaCl treatment significantly reduced fruit vitamin C content than control plants. There was slight increase in fruit vitamin C at EC5 dS/m induced by the “All Nutrients” and K₂SO₄ treatments; however, the increase was not significant compared to control plants. Moreover, despite KCl treatment reduced the fruit vitamin C, but the reduction was not significant compared to control plants. Salinity treatments have shown to reduce fruit biochemical traits (Galli et al., 2016; Hellal, Abdelhamid, Abo-Basha, & Zewainy, 2012). Changes in plant cell metabolism and biochemistry have been reported due to higher NaCl salinity treatment (Marschner, 2012; Pires, Negrão, Oliveira, & Purugganan, 2015). Salinity can change plant metabolism toward more production of protein, amino acids and sugars that play important role in plant stress tolerance (Marschner, 2012; Zhu, Zhou, Shabala, & Shabala, 2017). Reduction of vitamin C content in fruits as a water soluble vitamin by NaCl can be due to negative effects of sodium and chloride on water status of plant tissues (Ahmadi & Souri, 2018).

Table 2. Fruit physiochemical characteristics of chili pepper under nutrient solution salinity generated with different salts.

Treatment	Fruit dry weight (g/plant)	Fruit length (cm)	Fruit diameter (mm)	Peduncle length (cm)	Fruit TSS (%)	Fruit juice pH	Fruit TA (%)
Control (EC1.8)	26.2±1.4a	10.7±2.1a	8.5±0.2a	2.5±0.22b	5.1±0.2ab	6.4±0.3a	0.87±0.17a
NaCl (EC5)	15.3±0.8b	6.3±0.5c	7.8±0.7a	2.4±0.3b	4.0±0.5b	5.9±0.1a	0.57±0.12bc
KCl (EC5)	15.6±0.7b	6.7±0.8c	7.7±1.7a	3.1±0.35a	4.9±0.6ab	6.0±0.3a	0.52±0.10c
K ₂ SO ₄ (EC5)	24.8±1.8a	9.3±0.6ab	8.0±1.4a	3.1±0.13a	5.4±0.7a	6.0±0.1a	0.88±0.21a
All Nutrients (EC5)	26.6±2.8a	8.8±1.1b	8.5±0.8a	2.5±0.41b	5.6±0.5a	6.1±0.2a	0.85±0.20ab

Remarks: Values in the same column followed by different letters differ significantly under DMRT 5%; TSS = total soluble solids, TA = titrateable acidity.

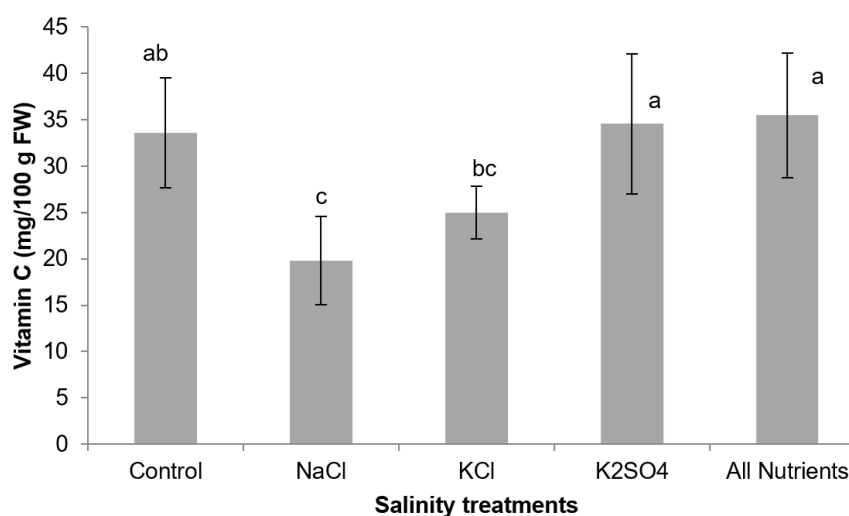


Fig. 4. Fruit vitamin C concentration of chili pepper under higher nutrient solution conductivity (EC5 dS/m) generated with different salts. Nutrient solution EC in control was 1.8 dS/m and in other treatments was 5 dS/m. Comparison of means was performed by Duncan's multiple range test at 5% level.

In the present study, application of other salts rather than NaCl to induce EC5 dS/m resulted in significantly less adverse effects on chili pepper growth and productivity. For many traits, there was no significant difference between plants treated with the "All Nutrients" and control plants. A similar response but to lesser extent was observed in K₂SO₄ treated plants. Application of calcium in different chemical forms has been shown to mitigate salinity effects on plant growth (Methenni et al., 2018; Roy, Tahjib-Ul-Arif, Polash, Hossen, & Hossain, 2019), while the calcium source still play a critical role in this aspect (Ahmadi & Souri, 2018). Nevertheless, it seems that addition of other nutrients to saline medium induced by NaCl, could have beneficial consequences for plant growth, indicating the ameliorating effect of some ions on negative effects of other ions.

Application of 100 mM NaCl to pepper plants showed a significant positive correlation between leaf symptoms and stem concentration of Na (Aktas, Abak, & Cakmak, 2006), but there was no correlation of K and Ca concentrations with leaf symptoms. However, the roles of potassium in plant stress responses have been well established (Ebrahimi, Souri, Ebrahimi, & Ahmadizadeh, 2012; Marschner, 2012; Wang, Zheng, Shen, & Guo, 2013). Supplementary potassium and calcium can have ameliorating effect on salinity induced damage in plants (Mardanluo, Souri, & Ahmadi, 2018; Marschner, 2012). However, it seems that the mitigation effects of potassium

on salinity depends on potassium salts, as in the present study potassium in form of K₂SO₄ treatment showed less negative effects on plants compared to KCl treatment. In addition, potassium may differently affect to ameliorate salinity effects than calcium supplementary. It has been shown that pepper plants benefit from higher potassium concentration in nutrient solution up to 350-500 mg/l (Ebrahimi, Souri, Ebrahimi, & Ahmadizadeh, 2012; Mardanluo, Souri, & Ahmadi, 2018).

In this study, various salts were used to increase Hoagland nutrient solution EC1.8 dS/m to EC5 dS/m, and plants were fed with this saline nutrient solution during their growth period. The response of chili pepper plants to these treatments were quite different, as the "All Nutrients" and K₂SO₄ treatments showed no or minor adverse effects on plant growth, whereas NaCl and KCl treatments showed the significant growth retardations. The two treatments of the "All Nutrients" and K₂SO₄ contain minerals (cations and anions) that are essential for plant growth, and their higher concentrations in root medium or within the plant tissues generally could not have adverse effect on plants. On the other hand, NaCl and KCl showed adverse effects on chili pepper growth, that in the case of NaCl it seems that both Na and Cl had cumulative deteriorating effects on plant growth. Chloride in higher concentrations has been shown to have toxicity effects on plant growth (Ahmadi & Souri, 2018; Marschner, 2012; Martínez-Ballesta, Martínez, & Carvajal, 2004).

However, the presence of K in KCl slightly reduced the chloride toxicity on chili pepper plants in the present study, as it recorded better for some traits than NaCl treatment. On the other hand, potassium has been shown to prevent or ameliorate many ion toxicity effects (Wang, Zheng, Shen, & Guo, 2013). Nevertheless, it has been reported that Na uptake avoidance is the major response of plant tolerance to salinity in pepper, and plants generally show significant genotype variability to NaCl induced salinity (Aktas, Abak, & Cakmak, 2006; Foti, Khah, & Pavli, 2019).

CONCLUSION

In the present study, the effects of NaCl or KCl chemical salts to induce higher conductivity of nutrient solution (EC5 dS/m) were worse than those of K₂SO₄ and the “All Nutrients” treatments, on growth, yield and fruit biochemical traits of chili pepper. Application of NaCl and to lesser extent KCl at EC5 dS/m significantly reduced chili pepper growth, whereas the “All Nutrients” and to lesser extent K₂SO₄ treatments showed no significant reduction in plant growth than control plants. Two salts of potassium namely KCl and K₂SO₄ showed quite different effects (K₂SO₄ had much better effects than KCl on chili pepper growth), probably due to negative effects of chloride anion (in KCl) on plant growth. The induced salinity by other forms from NaCl generates the higher conductivity, and plants less suffer from adverse effects of salinity and have higher conductivity in their root medium.

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