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**NUTRIENT COMPOSITION, ANTIOXIDANT COMPONENTS
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FRUIT (*Capsicum annuum* L.) CULTIVARS GROWN UNDER
SALT STRESS**

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Abstract

Purpose: Salinity stress occurs due to the accumulation of high levels of salts in soil, which ultimately leads to the impairment of plant growth and crop loss. Stress tolerance-inducing compounds have a remarkable ability to improve growth and minimize the effects of salinity stress without negatively affecting the environment by controlling the physiological and molecular activities in plants.

Methodology: The pots were arranged in a complete randomized design with one plant per pot and four replicates per treatment and carried out in 2017 and 2018 to study the influence of four levels of NaCl (0, 50, 100 and 200 mM) on the antioxidant, ascorbic acid, organic and inorganic compounds of three pepper fruits cultivars ('Granada', 'Goliath' and 'Nobili') at mature stage.

Findings: The results obtained showed that salinity decreased the mineral content (Ca, K, Zn, Mg, Mn, Iron, S and P), relative water content, and agromorphological parameters of pepper fruit. This decrease was accompanied by a significant increase of Na, soluble proteins, proline content, soluble sugar (fructose, glucose) and antioxidants, including total phenolics and flavonoids, and reduced ascorbic acid and β -carotene content. It could be an indicator of salinity tolerance which explains the maintenance of a good water status among the studied plants. However, a varietal difference response to salt stress was observed between the studied varieties. Indeed, the varieties Granada and Nobili are characterized by their vigour in absence as in the presence of salt. Under the studied salinity level there was an enhancement of health-

promoting compounds (phenolic compounds, flavonoids, β -carotene and ascorbic acid) synthesis in pepper fruits, with significant changes in other quality parameters.

Unique contribution to theory, practice and policy: It is concluded that varieties ‘Granada’ and ‘Nobili’ is more tolerant to salinity compared to the variety ‘Goliath’. Hence, they have a significant role to play in agriculture, food, and economy.

Keywords: *Agromorphological parameters, antioxidant, ions distribution, organic compound, salinity.*

Abbreviations: Calcium-Ca; β -carotene-CA; chlorophyll-CHL; days after planting-DAP; flavonoids-FLA; magnesium-Mg; Ascorbic acid-ASA; fructose-FRU; glucose-GLU; number of ripe fruit per plant-NF; fruit length-FL; fruit diameter-FD; sodium-Na; zin-Zn; iron-Ir; Manganese-Mn; sulfate-S; phosphorus-P; potassium-K; proline-PRO; relative water content-RWC; fruit dry weight-FDW; fruit fresh weight-FFW; thickness of fruit-TF soluble proteins-SP; total free amino acids content-FAA; copper-Cu; iron-Fe; total phenolic-TP; total soluble sugars-SS.

1. INTRODUCTION

Salinity is one of the most important environmental factors that affect the distribution and abundance of plant species. Soil salinization occurs mainly in two ways: high evaporation relative to precipitation in association with weak leaching in soils, and salt accumulation as a result of the use of saline water (Singh, 2015). It is estimated that about 50 % of the world’s land will be saline by the middle of the 21st century (Mahajan and Tuteja, 2005). Globally, 20 % of irrigated land and 2.1% of dry land agriculture suffers from the salt problem and NaCl is the predominant salt causing salinization (Munns and Tester, 2008). Salinity adversely affects germination, growth, physiology and productivity by reducing the ability of plants to take up water causing foliage damage and even death of the plants, imbalance in osmotic potential; ionic equilibrium and nutrient uptake (Niu et al., 1995). Further, it facilitates severe ion toxicity by depositing high concentration of Na^+ which causes membrane disorganization, inhibition of cell division and expansion. The influence of salinity and mineral nutrient solution, on productivity, photosynthesis and growth has been studied in different plants (Hosseini and Thengane, 2007; Li et al., 2008; Taffouo et al., 2010). It stated that high levels of Na^+ inhibits K, Ca and Mg in leaves, which results in a K/Na antagonism and net photosynthesis is affected strongly by NaCl conditions, which is related directly to the closure of stomata as to low intercellular CO_2 levels (Al-Karaki, 2000; Turan et al., 2007). To develop saline zones and/or the zones having only brackish water resources, it is important to select tolerant varieties. Salinity imposes stress conditions on crop plants and affects growth and chemical contents and has been shown to limit pepper yield (Paridam and Das, 2005). Salt stress severely inhibits plant growth for two reasons: firstly due to an osmotic or water-deficit effect of salinity and secondly due to a salt-specific or ion excess effect of NaCl. Soils with high levels of salinity have a low water potential zone; consequently, it is difficult for the plant to absorb water and nutrients. In other words saline soils expose plants to osmotic stress (Agarwal and al., 2013). One of the most important consequences of osmotic stress on plants is the production of reactive oxygen species (ROS) in large amounts that followed by oxidative damages, the degradation of proteins, lipids, pigments, and DNA (Das and Roychoudhury, 2014). Plants growing on saline conditions take up harmful ions, especially Na^+ and Cl^- ions. Accumulation of Na^+ and Cl^- ions in large amounts is toxic for the cell, and compounds osmotic stress (Agarwal and al., 2013). These ions disrupt membrane integrity, cell metabolism, enzyme structure, cell growth, and

photosynthesis (Ghosh and Xu, 2014). Although plants have a variety of ways of withstanding the stress, significant loss of yield occurs (Deinlein and al., 2014). Salt stress is known to negatively affect plant growth at all developmental stages, but sensitivity varies greatly at different stages (Akinci and al., 2004). Crop production in saline areas largely depends on successful germination, seedling emergence and establishment and efficient reproductive phase (Akinci and al., 2004). Moreover, as environmental stress it may have a strong influence on the concentration of bioactive compounds of vegetables (Prasad and al., 2014). Meanwhile, there is an increasing need to produce enough food for the world's growing population (Deinlein and al., 2014; Singh, 2015). In order to address these challenges to the world's food security, the engineering of plants to create species that tolerate salinity has been considered as a promising strategy.

Pepper (*Capsicum annuum* L.) is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits; it is an excellent source of natural colors, vitamin C and antioxidant compounds important for human health (Howard and al., 2000). Pepper is moderately sensitive to salt stress (Lee, 2006), and it is grown under protected glasshouse conditions in temperate regions and in the open field under warm Mediterranean climates. Salinity imposes stress conditions on crop plants (Hajer and al., 2006) and affects growth and chemical contents and has been shown to limit pepper yield (Paridam and Das, 2005). Salt stress severely inhibits plant growth for two reasons: first by an osmotic or water-deficit effect of salinity and second by a salt-specific or ion excess effect of NaCl. Moreover, plants subject to salinity stress conditions produce cytotoxic activated oxygen that can seriously disrupt normal metabolism, through oxidative damage of lipids, proteins, and nucleic acids (Abbaspour, 2012). To defend against such oxidants, plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes that protect against the potentially-cytotoxic species of activated oxygen.

In case of high salinity, oxidative stress occurs due to closure of stomata, interruption of photosynthetic electron transport and disruption of cellular membrane integrity and antioxidative defense systems of plants start work against oxidative damage. The antioxidants include non-enzymatic ones, such as vitamin E, vitamin C, glutathione and carotenoid (β -carotene and zeaxanthin), and enzymes such as superoxide dismutase and catalase (Whittington and Smith, 1992).

The objective of this study was to investigate the effect of NaCl treatment on nutrient composition, antioxidant components, ascorbic acid content, and agromorphological parameters of three varieties of pepper fruit (*Capsicum annuum* L.) in order to better understand their differences on salt stress tolerance and select tolerant varieties which could be cultivated in arid, semi-arid and coastal saline soils.

The changes in some agromorphological parameters, minerals, antioxidants, organic compounds, carotenoids (β -carotene and lycopene), vitamin C, and secondary metabolites including individual phenolics compounds, total flavonoids and phenolics were followed.

2. MATERIALS AND METHODS

2.1. Materials

Plant materials:

Pepper (*Capsicum annum* L.) is especially productive in warm and dry climates than *C. frutescens* which can tolerate most climates. It also displays a greater resistance to disease and insects, especially to the tobacco mosaic virus. Capsaicinoids chemicals and antioxidants such as carotenoids provide the distinctive tastes in *C. annum* variants. The fruit are berries that may be green, yellow or red when ripe. The mature green stage is ideal to acquire maximum pungency due to capsaicinoids, whereas peppers at red ripe stage are best sources of ascorbic acid and dried fruits contain higher levels of total carotenoids (Iqbal et al., 2013). Hot peppers are used in medicine as well as food in Africa. Seeds of three pepper cultivars ('Granada', 'Goliath' and 'Nobili'), provided by the breeding program of the Agronomic Institute for Research and Development of Cameroon were used in the study. These varieties were chosen for their socio-economic rank and resistance at the tobacco mosaic virus.

2.2 Methods

Plant growth conditions and salt treatments

The present work was performed in the greenhouse of the Faculty of Science at University of Douala, Cameroon, from September 2017 to August 2018. The seeds were surface sterilized with 3% sodium hypochlorite for 20 min and washed four times with deionized water. One-month-old pepper seedlings were transplanted into 10-L plastic pots filled with 5 kg of sterilized sand. The pots were arranged in a complete randomized design with one plant per pot and four replicates per treatment. All plants were fertilized daily with a modified nutrient solution (in g L⁻¹): 150 g Ca(NO₃)₂, 70 g KNO₃, 15 g Fe-EDTA, 0.14 g KH₂PO₄, 1.60 g K₂SO₄, 11 g MgSO₄, 2.5 g CaSO₄, 1.18 g MnSO₄, 0.16 g ZnSO₄, 3.10 g H₃BO₄, 0.17 g CuSO₄ and 0.08 g MoO₃ (Hoagland and Arnon, 1950). The pH of the nutrient solution was adjusted to 7.0 by adding HNO₃ 0.1 mM. For the determination of physiological and biochemical responses of pepper cultivars to salt stress, each cultivar was subjected to 0 (control), 50, 100 and 200 mM NaCl. Plants were watered with deionized water every morning. The daily amounts of water added to the pots were the same for all treatments. Throughout the growth period, average day/night temperatures in the greenhouse were 26°C/20 °C and the relative air humidity averaged 68.5%.

Plant measurements

Plant samples were harvested after 4 months culture under salt stress, fruits were collected. The tissues (fruits) were dried for 24 h at 105 °C (Taffouo and al., 2008). The dry samples were weighted. Ninety days after sowing, samples from each treatment were collected to determine agromorphological characters (number of fruit per plant, fresh fruit weight of fruit, dry fruit weight, thickness of fruit, fruit length, fruit diameter), organic components (total soluble proteins, proline, fructose, glucose), inorganic components (Na, K, Ca, Mg, P, S, Zn, Cu, Mn, Fe content, K/Na, Ca/Na, Mg/Na), relative water content, antioxidant levels (total flavonoid content, total phenolic content, ascorbic acid, β-carotene content) in three pepper fruit cultivars.

Agromorphological characters

90 days after transplanting, the fresh fruit weight (FFW) and dry fruit weight (DFW) were determined. The number of fruit per plant, the fruit length and diameter were measured as described by Adedeji and al. (2006).

Organic components

Total soluble proteins

Protein content was determined by Bradford's method (Bradford, 1976). Briefly, appropriate volume (from 0-100 μ l) of sample was aliquoted into a tube and the total volume was adjusted to 100 μ l with distilled water. A 1 ml of Bradford working solution was added to each sample well. Then the mixture was thoroughly mixed by vortex mixer. After left for 2 min, the absorbance was read at 595 nm. The standard curve was established by replacing the sample portions in the tubes with proper serial dilutions of bovine serum albumin.

Free proline

Free proline were determined in 95% ethanol extracts from fruits. Samples of 0.5 g of tissues freshly harvested were crushed in 5 ml 95% (v/v) ethanol. The insoluble fraction of the extract was washed twice with 5 ml of 70% ethanol. All soluble fractions were centrifuged at $3500 \times g$ for 10 min. The supernatants were collected and stored at 4 °C for proline determination (Irigoyen and al., 1992). The free proline content was measured according to the method described by Paquin and Lechasseur (1979).

Total soluble sugar

Glucose and fructose were extracted from pepper fruit homogenate aliquots according to the protocol described in Hostettler and al. (2011), and then quantified enzymatically according to Thalmann and al. (2016).

Minerals

P, K, Ca, Mg, S and Na contents in the fruit tissue of the plants were evaluated in dry, ground, and digested samples in a CEM microwave oven (Abreu and al., 1995). P was determined by colorimetry; sodium and potassium by flame photometry; copper determination was carried out by means of mass spectrometry with source of plasma connected by induction (ICP-MS), by means of a Hewlett Packard 4500 series; calcium and magnesium by atomic absorption spectrometry and sulfur by turbidimetry of barium sulfate (Malavolta and al., 1997). Iron, zinc and manganese contents were determined by method reported in Pauwels and al. (1992). Fruit of pepper were dry ashed at 450°C for 2 hours and digested on heat cave with 10 ml HNO₃ 1 M. The solution was filtrated and adjusted at 100 ml with HNO₃ at 1/100 and analyzed with an atomic absorption spectrophotometer (Rayleigh, WFX-100).

Relative water content

The relative water content (RWC) in fruits was recorded according to the formula of Sanchez and al. (2004) as follows: $RWC = (FFW - FDW) / (TW - FDW) \times 100$, where FFW is fresh weight, FDW is dry weight, and TW is turgid weight.

Antioxidant compound

Total flavonoid content

FLA content of crude extract was determined by the aluminium chloride colorimetric method (Chang and al., 2002). 50 μ L of crude extract (1 mg/mL ethanol) were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5 % NaNO₂ solution ; 0.3 mL of 10 % AlCl₃ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand

for 15 min, and absorbance was recorded on spectrophotometer (Pharmaspec UV-1700 model) at 510 nm wavelength. FLA content was calculated from a rutin calibration curve, and the result was expressed as g rutin equivalent per g dry weight.

Total phenolic content

TP content of the extract was determined by the Folin Ciocalteu method (Marigo, 1973). Subsamples (1 g) of fresh fruits were ground at 4 °C in 3 mL of 0.1 N HCl. After incubation to 4 °C during 20 min, the homogenate was centrifuged at 6000 g during 40 min. The supernatant was collected, the pellet re-suspended in 3 mL of 0.1 N HCl and centrifuged as previously. The two supernatant are mixed and constitute the crude extract of soluble phenol. The reaction mixture containing 15 µL of extract, 100 µL Folin-Ciocalteu reagents, 0.5 mL of 20% Na₂CO₃ was incubated at 40°C for 20 min and absorbance read at 720 nm wavelength with a spectrophotometer (Pharmaspec UV-1700 model). A standard curve was established using chlorogenic acid. TP content was expressed as mg g⁻¹ fresh weight.

Ascorbic acid content

For estimation of ascorbic acid content (ASA), 1 g of frozen fruit tissues was homogenised in 5 mL of ice-cold 6 % m-phosphoric acid (pH 2.8) containing 1 mM EDTA (Gossett and al., 1994). The homogenate was centrifuged at 20,000 × g for 15 min at 4 °C. The supernatant was filtered through a 30-µm syringe filter, and 50 µL of the filtrate was analyzed using a HPLC system (PerkinElmer series 200LC and UV/VIS detector 200 LC, USA) equipped with a 5-µm column (Spheri-5 RP-18; 220 × 4.6 mm; Brownlee) and UV detection at 245 nm with 1.0 mL/min water (pH 2.2) as the mobile phase, run isocratically (Gahler and al., 2003)

β-carotene content

β-carotene was extracted by grinding fruit tissues in a solution of 100% acetone containing CaCO₃ (Jung, 2004). The extracts were centrifuged at 16,000 × g for 10 min, and 20 µL of the resulting supernatants were used for HPLC analysis, as described by Gilmore and Yamamoto (1991) using the previously mentioned HPLC system. Solvent A (acetonitrile, methanol, Tris-HCl buffer 0.1 M, pH 8.0, 72:8:3) was run isocratically from 0 to 4 min followed by a 2.5 min linear gradient to 100% solvent B (methanol, hexane, 4:1) at a flow rate of 2 mL/min. The detector was set at 440 nm for the integration of peak areas after calibration with the external standard.

Experimental design and statistical analysis

The experiment was conducted as a factorial completely randomized design with four NaCl treatments and three cultivars in five replications. Data are presented in term of mean (± standard deviation). All data were statistically analysed using Statistica (version 9, Tulsa, OK, USA) and first subjected to analyses of variance (ANOVA). Statistical differences between treatment means were established using the Fisher LSD test at $p < 0.05$.

3. RESULTS

3.1. Agromorphological parameters

According to our findings in Table 1, salt stress at 100 and 200 mM NaCl caused significant decreases in NF, FFW, FDW, TF, FL and FD. The lowest values of these traits were recorded with salt stressed plants at 200 mM concentration, followed by 100 mM. In ‘Granada’, ‘Goliath’ and ‘Nobili’ there was a gradual decrease in the fruit length and diameter per plant following the increase in NaCl salinity. The cultivar ‘Granada’ presented the higher FL 18.86 cm for

control and 14.84 cm at 200 mM NaCl and ‘Goliath’ present higher FD, 8.84 to 6.37 cm, when applying the highest of NaCl (200 mM) (Table 1.). The NF decreased linearly with the increase in the salinity, with significant effect on both cultivars (Table 1). The NF of the cultivar ‘Goliath’, 11.41 to 6.89, were higher than those of the cultivar ‘Nobili’, 10.28 to 7.68 and ‘Granada’, 9.81 to 7.23, when applying the highest of NaCl (200 mM). The cultivar ‘Granada’ showed higher FFW, varied within a range of 45.55 to 37.81 g respectively to control and 200 mM NaCl (Table 1). The FFW of the cultivars decreased 16.99% in ‘Granada’, 27.18% and 34.98% in ‘Goliath’ with the highest salinity (200 mM NaCl) compared with the control. These results were directly related to FDW, which decreased with increasing salinity (Table 1). The TF losses of the cultivars reached 30.06% in ‘Granada’, 32.46% in ‘Nobili’ and 28.02% in ‘Goliath’ under the highest NaCl (200 mM). Under salinity stress of 200 mM, the best results of NF, FFW, FDW, FL and FD were recorded with ‘Nobili’, followed by ‘Granada’ and ‘Goliath’.

3.2. Organic compounds

The presence of NaCl resulted in a significant increase in GLU, FRU, SP and PRO contents in fruit of all cultivars compared to untreated plants, thereby playing a major role as osmotic adjustment (Fig. 2 A, B, C and D). The concentration of free sugars is one of the components along with organic acids that determine fruit flavor attributed to sweetness of pepper fruit. Both FRU and GLU composition differed by level of NaCl and varieties. The level of fructose across all samples was higher than that of GLU (Fig. 2C and D). The contents of FRU and GLU across varieties were higher in pepper produced at 0 mM NaCl in ‘Granada’ and ‘Nobili’, at 200 mM ‘Granada’ than those of ‘Goliath’ and other level of NaCl. SP content in plants increased significantly under salt stress in all cultivars compared to untreated plants (Fig. 2B). These increases were 49.71% in ‘Granada’, 68.55% in ‘Nobili’ and 70.23% in ‘Goliath’ at 200 mM NaCl in comparison with the control plants. The proline content had markedly accumulated in sweet pepper fruits; the highest concentration was recorded with a salinity at 200 mM NaCl (47.64% in ‘Granada’, 86.29% in ‘Nobili’ and 100.18% in ‘Goliath’ in comparison to the control plants (Fig. 2A). The salt tolerant ‘Granada’ accumulated the highest amount of all osmolytes followed by the moderately tolerant ‘Nobili’ and the saltsensitive ‘Goliath’.

3.3. Antioxydant compound

NaCl effect on fruits TF, TP, CA and ASA concentrations is shown in figure 1. A significant increase ($p < 0.05$) was observed for TF and TP at 100 and 200 mM NaCl in ‘Granada’ and ‘Nobili’ and 50 mM NaCl in ‘Goliath’. These increases for TF were 30.72% in ‘Granada’, 46.59% in ‘Nobili’ and 71.06% in ‘Goliath’; and 42.89% in ‘Granada’, 52.74% in ‘Nobili’ and 63.13% in ‘Goliath’ for TP under the highest NaCl (200 mM) in comparison with the control (Fig. 1A and B). Salt effect resulted in a significant decrease for ASA and CA ($p < 0.05$). The decreases for ASA were 79.83% in ‘Granada’, 82.71% in ‘Nobili’ and 82.58% in ‘Goliath’; and 27.87% in ‘Granada’, 30.60% in ‘Nobili’ and 31.45% in ‘Goliath’ for CA under the highest NaCl (200 mM) in comparison with the control (Fig. 1C and D).

3.4. Fruit relative Water Content

Fruit RWC of pepper cultivars at different salinity levels is depicted in Fig. 3. There are significant differences between cultivars. A significant decrease in RWC was found at high salinity level (200 mM) in all cultivars compared with control.

3.5. Minerals

Pepper fruit contain many essential minerals. Most minerals showed variation among the 03 pepper varieties at the different levels of NaCl. Salt treatments differently affected the fruit contents of P, Na, Mg, Ca, S, Fe, Cu, Zn and Mn, depending on the genotype (Table 2 A and 2B). The main effect of NaCl on fruit Na concentrations of plants under salt stress showed significant increases as compared to control plants (Table 2A). The highest Na concentrations (11.22 mg on 100 g) were detected in ‘Goliath’ cultivar while the lowest (10.62 mg on 100 g) were recorded in ‘Granada’ at 200 mM NaCl (Table 2A). In this study, K, Ca, P, Mn, S, Cu, Fe, Zn and Mg concentrations were significantly reduced with increasing salinity in all cultivars (Table 2 A and 2B). K is the most abundant mineral, followed by P, Mg, Ca, S, Fe, Cu, Zn and Mn. The levels of each mineral across four levels of NaCl showed a high (significant) variation, indicating these compounds are strongly influence by salinity. The Ca, Mg, Na, Cu, Fe, P, S, Zn, Mn, K content decreased significantly between 100 and 200 mM NaCl in ‘Granada’ and ‘Nobili’ cultivars and 50 mM NaCl in ‘Goliath’ cultivar (Table 2A and B). According to the analysis of variance of the mineral nutrient contents in the sweet pepper fruits, the interaction of the NaCl with the cultivars affected their Ca, P, Zn, Fe, Mn, S, K, Na and Mg contents (Table 2 A and 2B). The cultivars factor had a significant effect for all evaluated nutrients. The fruit K/Na, Ca/Na and Mg/Na ratios were found to be significantly highest in ‘Granada’ and lowest in ‘Goliath’ (Table 2B).

Table 1. Effect of salinity on some agromorphological parameters of pepper fruit (FL, FD, NF, TF, FFW and FDW) at the mature stage (90 DAP).

Cultivar	Treatment (mM NaCl)	FL (cm)	FD (cm)	NF	FFW (g)	DFW (g)	TF (m)
Granada	0	18.86±0.14a	5.98±0.09b	12.82±0.18a	45.55±2.25a	4.03±0.07a	5.82±0.0
	50	16.89±0.18ab	4.54±0.11b	11.22±0.19a	43.81±2.30a	3.15±0.04a	5.78±0.0
	100	14.12±0.15b	3.77±0.08bc	8.38±0.15b	39.74±2.01b	1.88±0.03b	4.32±0.0
	200	14.84±0.11b	4.08±0.12b	7.93±0.21b	37.81±1.88b	1.61±0.04b	4.07±0.0
Nobili	0	13.68±0.09b	7.16±0.13a	11.62±0.22a	37.67±1.92b	3.74±0.05a	7.27±0.1
	50	12.16±0.08bc	6.87±0.15a	10.09±0.23a	32.32±1.89d	2.04±0.07ab	6.66±0.1
	100	10.64±0.07d	4.83±0.18b	8.63±0.21b	35.22±1.99c	1.05±0.08b	5.15±0.0
	200	9.88±0.10d	4.29±0.21b	7.68±0.24b	27.43±1.49e	0.92±0.04b	4.91±0.1
Goliath	0	10.77±0.11d	8.84±0.07a	11.41±0.2a	31.27±1.87d	2.38±0.07ab	7.78±0.1
	50	8.93±0.08de	6.96±0.09a	8.23±0.19b	23.89±1.66	0.81±0.02b	5.89±0.0
	100	6.77±0.07e	7.11±0.12a	7.81±0.18b	25.65±1.27e	0.93±0.01b	6.04±0.0
	200	6.88±0.06e	6.37±0.11a	6.89±0.20c	20.33±1.09f	0.73±0.02c	5.60±0.1
Two way ANOVA results							
Cultivar (C)		*	*	NS	**	*	NS
Salt treatment (S)		**	*	*	*	*	*
Interaction C X S		NS	NS	NS	*	NS	NS

Values shown are means (n=10) ± SD; within columns, means followed by different letter are significantly different (p < 0.05).

**, * significant at 1 and 5% probability levels, respectively, NS not significant

Table 2A. Effect of salt stress on ions concentrations (mg on 100 g of fresh weight) of pepper fruit cultivars at mature stage (90 DAP)

Cultivar	Treatment (mM NaCl)	Na	K	Ca	Mg	P	S
Granda	0	4.88±0.0 1bc	263.71±2. 43a	12.89±0.2 1a	16.81±0.3 4a	36.81±0.3 9a	12.39±0. 31a
	50	5.37±0.0 4b	257.57±2. 47b	13.97±0.2 3a	15.12±0.3 7a	32.34±0.4 1b	10.86±0. 29a
	100	8.14±0.0 7a	222.72±2. 49e	11.39±0.1 9a	13.99±0.2 4ab	29.33±0.4 4bc	11.53±0. 25a
	200	10.62±0. 08a	201.26±1. 83f	11.77±0.2 5a	11.30±0.4 2b	28.59±0.4 2c	8.63±0.2 3b
Nobili	0	5.39±0.0 3b	243.19±2. 39d	13.27±0.1 25a	15.57±0.3 9a	33.53±0.3 8ab	11.69±0. 19a
	50	6.46±0.0 5b	221.47±2. 42e	10.82±0.2 6ab	16.19±0.3 6a	34.80±0.3 6a	9.31±0.1 8ab
	100	8.80±0.0 6a	205.79±3. 44f	11.43±0.1 9a	12.82±0.4 1b	31.09±0.3 2b	7.62±0.2 1b
	200	10.88±0. 09a	190.15±2. 51g	9.93±0.22 c	10.91±0.3 9bc	27.15±0.3 8c	8.35±0.1 8b
Goliah	0	5.15±0.1 0b	251.81±2. 42c	12.81±0.1 9a	14.94±0.3 7a	30.26±0.4 2bc	11.90±0. 23a
	50	7.41±0.0 8ab	218.53±1. 44e	10.26±0.2 1a	11.92±0.2 9b	31.42±0.4 4b	8.92±0.2 2b
	100	9.64±0.0 7a	192.45±2. 47g	8.88±0.26 c	10.28±0.2 4bc	23.77±0.4 1d	7.41±0.2 1b
	200	11.22±0. 09a	187.68±1. 54h	8.27±0.23 c	8.12±0.28 c	18.20±0.3 9e	6.67±0.1 9bc
Two way ANOVA results							
Cultivar (C)		*	*	NS	*	*	NS
Salt treatment (S)		**	*	*	*	*	*
Interaction C X S		*	*	NS	NS	*	NS

Values shown are means (n=5) ± SD; within columns, means followed by different letter are significantly different (p < 0.05).
**, * significant at 1 and 5% probability levels, respectively, NS not significant

Table 2B. Effect of salt stress on ions concentrations ((μg on 100 g of fresh weight) of pepper fruit cultivars at mature stage (90 DAP)

Cultivar	Treatment (mM NaCl)	Mn	Cu	Fe	Zn	K/Na	Ca/Na	Mg/Na
Granda	0	201.1 \pm 4.2 1a	141.31 \pm 3. 28b	887.66 \pm 5. 27a	572.22 \pm 2. 99a	54.03 a	2.64 a	3.44a
	50	198.91 \pm 3. 49b	125.23 \pm 3. 09d	855.32 \pm 4. 88b	537.52 \pm 3. 02c	47.96 b	2.60 a	2.81a
	100	158.84 \pm 3. 38e	111.12 \pm 2. 89e	818.21 \pm 5. 52c	511.23 \pm 3. 33e	27.36 c	1.39 a	1.71a
	200	139.71 \pm 3. 66g	107.09 \pm 3. 02f	790.33 \pm 3. 29f	499.93 \pm 3. 71h	18.95 d	1.11 ab	1.06 b
Nobili	0	177.48 \pm 4. 09c	140.25 \pm 3. 13b	801.27 \pm 3. 67e	558.02 \pm 2. 96b	45.11 b	2.46 a	2.88a
	50	163.26 \pm 4. 27d	133.20 \pm 2. 33c	788.04 \pm 4. 21g	524.71 \pm 3. 02d	34.28 a	1.67 a	2.50a
	100	128.87 \pm 4. 11h	127.11 \pm 2. 67d	735.19 \pm 4. 44h	501.88 \pm 3. 66g	23.38 c	1.29 ab	1.45a
	200	120.73 \pm 3. 21i	110.08 \pm 3. 01e	709.81 \pm 3. 89j	488.05 \pm 3. 43i	17.47 d	0.91 b	1.01 b
Goliah	0	180.91 \pm 4. 55c	145.29 \pm 3. 29a	813.40 \pm 4. 10d	537.79 \pm 3. 61c	48.89 b	2.48 a	2.90a
	50	157.75 \pm 3. 89e	133.11 \pm 2. 58c	761.11 \pm 3. 77h	509.72 \pm 3. 80f	29.49 b	1.38 a	1.60a
	100	143.61 \pm 3. 30f	112.26 \pm 2. 17e	723.78 \pm 3. 22i	480.69 \pm 2. 76j	19.96 d	0.92 b	1.06 b
	200	129.71 \pm 2. 99h	101.07 \pm 2. 15g	694.25 \pm 3. 19k	444.58 \pm 2. 88k	16.72 de	0.73 bc	0.72 bc
Two way ANOVA results								
Cultivar (C)		*	*	*	*	*	NS	*
Salt treatment (S)		**	*	**	*	*	*	*
Interaction C X S		*	*	*	*	NS	NS	NS

Values shown are means (n=5) \pm SD; within columns, means followed by different letter are significantly different (p < 0.05).
**, * significant at 1 and 5% probability levels, respectively, NS not significant

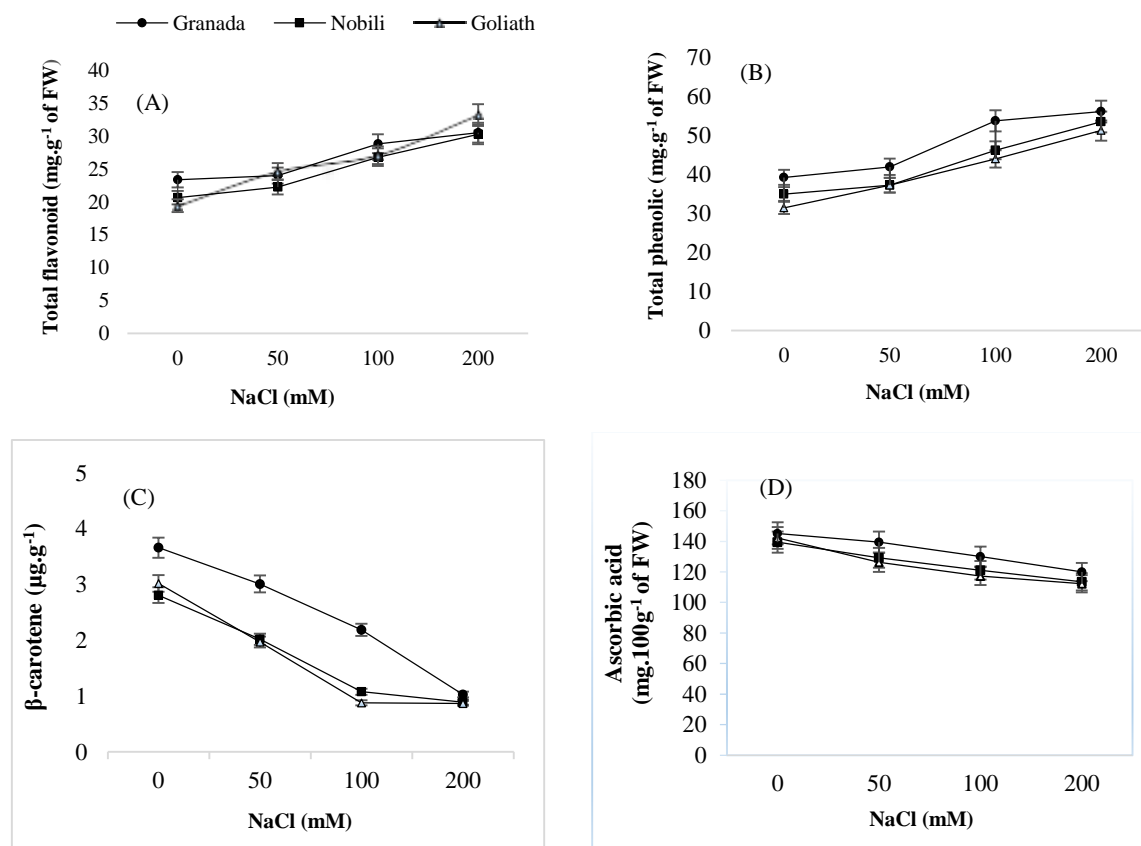
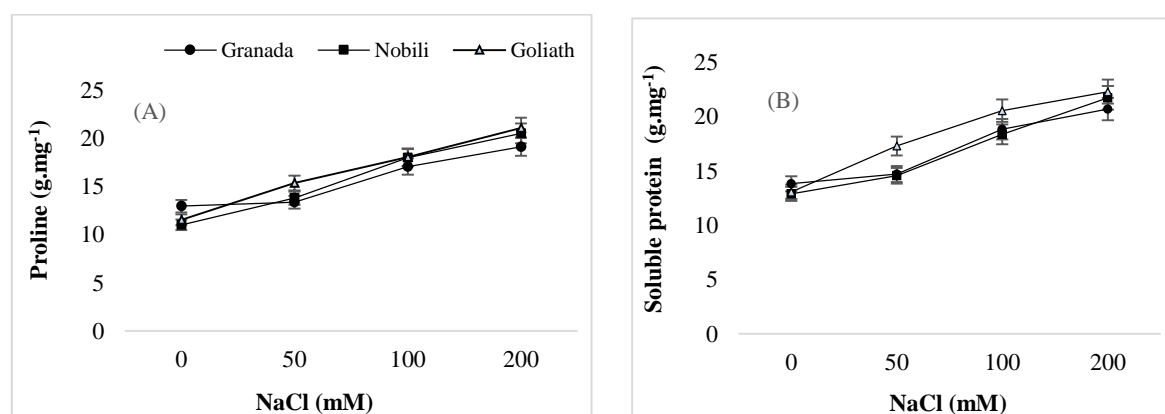


Fig. 1. Effect of salt on antioxidant compounds in pepper fruit cultivars at the mature stage (90 DAP). Total flavonoid content (A), total phenolic content (B), β-carotene content (C) and ascorbic acid content (D). Bars are means (n=5) ± SD. Means followed by different letter are significantly different (p < 0.05)



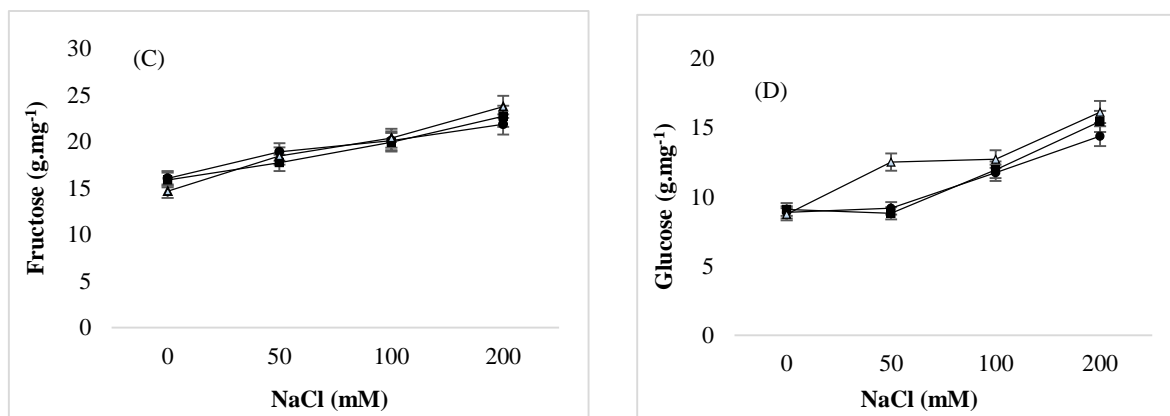


Fig. 3. Effect of salt on accumulation of organic compound in pepper cultivars (90 DAP). Proline content (A), soluble proteins (B), fructose content (C) and glucose content (D). Bars are means (n=5) \pm SD

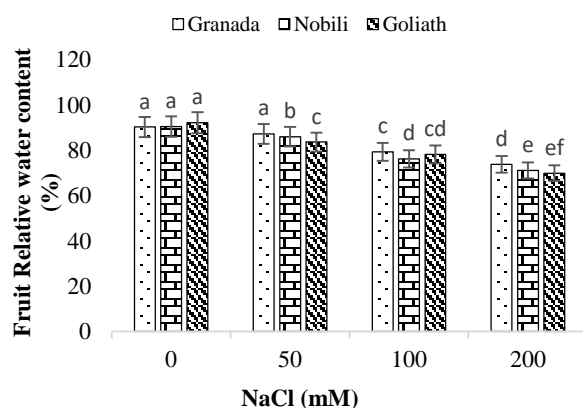


Fig 3. Effect of salt on fruit relative water content in pepper varieties at vegetative mature (90 DAP). Bars are means (n=5) \pm SD Means followed by different letter are significantly different (p < 0.05)

4. DISCUSSION

4.1. Agromorphological parameters

In the present study, NaCl significantly decreased NF, FFW, FDW, TF, FL and FD of all cultivar (Table 1). Similar results were also reported for tomato (Rahman and al., 2006; Saeed and Ahmad, 2009) and strawberry (Khayyat and al., 2007) grown in saline soil. In contrast, several authors reported that FDW significantly increased under saline conditions in a number of horticultural crop species including tomato (Krauss and al., 2006; Gautier and al., 2010) and cucumber (Chartzoulakis, 1992). The reductions of NF, FFW, FDW, TF, FL, FD under salt conditions are possibly due to the adverse impacts of salinity on the growth characteristics and physiological processes such as water uptake, photosynthesis, FLO, and FRU formation, which led to diminished yields. Accordingly, the highest level of salt (200 mM NaCl) was adversely more effective than the lowest one (50 mM NaCl). The same trends of salt stress were

previously described in faba bean (Abdul Qado, 2011) and strawberry plants (Yildirim and al., 2009). Pulp thickness and firmness of pepper fruit are an important variable affecting pepper quality, since it guarantees better postharvest conservation and prevents injury by manipulation. High salinity, above 200 mM NaCl, tended to decrease pulp thickness and fruit firmness. Firmness in pepper fruit has been related to the level of calcium in the fruit (Belakbir el al., 1998). Thus, salinity could reduce fruit pulp thickness and firmness by reducing the availability of calcium in the fruit.

4.2. Fruit relative water content

Additionally, fruit relative water content (RWC) was decreased under salt stress. This decrease may

be due to the reduction in water uptake (Parvin and al., 2019) and/or its harmful effect on cell wall structure (Abdelaal and al., 2018). The ameliorative effects of these treatments on RWC could be due to the increase in osmoregulators, as well as to osmotic adjustment in plant cells (Kasim and al., 2017; Hafez and al., 2019). So, increasing NaCl salinity concentration tended to reduced the absorption of water leading to a drop in water content, the inhibitory effect of NaCl on growth parameters could be attributed to the osmotic effect of NaCl salinity, in addition, the changes in water status under NaCl stress may cause a reduction in meristem activity as well as cell elongation (Salter et al., 2007; Chookhampaeng, 2011). These suggestions are in a good agreement with present results, which showed that the increase of RWC was associated with a decrease in transpiration rate.

4.3. Organic components

Results presented in figure 2 indicated that the effect of salinity concentration on pepper fruit marked significant increase in SP, FRU, GLU and PRO contents compared with control pepper plants. Kapoor and Srivastava (2010) observed an increase in protein content when increasing salt concentration. The plants under salinity condition change their metabolism to overcome the changed environmental condition. According to Ebrahimian and Bybordi (2012) the SP content decreased on account of salinity stress, one of the mechanisms affected by salt stress in plants was protein synthesis. It is known that SP content is an important indicator of physiological status of plants. Proline is a typical adaptive response in plants and it may be a part of stress signal (Maggio et al., 2002; Yang et al., 2009). Proline, which is an amino acid is one such organic solute that plays a major role in this osmotic adjustment (Chookhampaeng, 2011; Loukehaich et al., 2011). Likewise, in tomato salt tolerance was attributed to the degree of plant to accumulate osmoprotectants, like proline (Patel and Pandey, 2008; Dasgan et al., 2009). Proline is one of well-known osmoprotectants and its accumulation is widely observed in various organisms under salt stress. The amino-acid may play a role in protecting membranes and proteins against adverse effects of higher concentrations of inorganic ions and temperature extremes. Chookhampaeng (2011) noted that the salinity treatments caused the increased PRO content in pepper plant. One mechanisms utilized by the plants for overcoming the salt stress effects might be via accumulation of compatible osmolytes, such as proline and soluble sugar. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress is an adaptive response. Jarret and al. (2009) showed that great diversity of free sugars within the *Capsicum chinense* gene pool. Further, free sugar quantity

was affected by pepper cultivars, fruit maturation, and environment factor (Bhandari and al., 2013; Hwang and al., 2014).

Zhang et al. (2016) confirmed that tomato (*Lycopersicon esculentum*. Pepe) total fruit sugar content increased with increased salinity; Sucrose played a main role in the regulation of the root osmotic potential followed by K, GLU and Na this agree with the results (Eisa and Ali, 2003).

In contrary, other authors found FRU and GLU concentrations in pepper fruits decreased with increasing NaCl. A high respiration rate in pepper fruit was observed when the ionic strength of the nutrient solution was increased (Tadesse et al., 1999). The reduction of sugars content in pepper fruit with salinity could be due to this increase in fruit respiration. The accumulation of osmolyte compounds is often proposed as a solution to overcoming the negative consequences of water deficits in crop production which has been proposed as an adaptive mechanism for drought and salt tolerance. Indeed, osmolyte accumulation in plant cell results in a decrease of the cell osmotic potential and help in the maintenance of water absorption and cell turgor pressure, which might contribute to sustaining physiological processes, such as stomatal opening, photosynthesis and expansion growth (Blum, 1996).

4.4. Antioxidant compound

ASA (vitamin C) is an essential nutrient which occurs widely in crop foods products, especially in fresh fruits and green leafy vegetables (Ratnakar and Rai, 2013). It is a small, water soluble, antioxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide (Beltagi, 2008). Vitamin C also helps in absorption of dietary iron by keeping it in the reduced form (Ratnakar and Rai, 2013). Our results, the ascorbic acid content decreased significantly under NaCl stress. In other vegetables such as amaranth species leaves, Ratnakar and Rai (2013) observed a decrease of ascorbic acid content with increase of salt concentration. Navarro and al. (2006) reported also that salinity decreased the ASA content of pepper fruits, and this effect was dependent on the maturity stage. However, the contradictory results reported on the impact of salinity on ascorbic acid content in tomato fruit might be related to genetic differences in sensitivity to salinity stress, differences in the intensity of salinity applied to the plant, and interactions with other factors like ripening stage (Dumas and al., 2003). In addition, the possibility for a plant to limit salt accumulation within its tissues triggers differences in the intensity of salinity stress perceived by the plant. Furthermore, it is well known that ASA is an important component of several fruits (tomato, pepper, and strawberry) that reacts with singlet oxygen and other free radicals and suppresses peroxidation (Dorais and al., 2008). Whereas Wouyou et al. (2017) reported an opposite response in other amaranth cultivar. In tomato fruits, the increase of ascorbic acid contents under salt stress was reported (Stamatakis and al., 2003; Kim and al., 2008; Gautier and al., 2010). Under the prevailing experimental conditions, increased concentrations of ASA in pepper fruits grown under saline conditions was in agreement with data reported for several tomato varieties grown under similar conditions (Dumas and al., 2003; Dorais and al., 2008).

Among several horticultural crops, pepper has been reported to be the source of carotenoids, which play an important role in fruit colouring. In addition, CA are widely known as powerful natural antioxidants that act as the most efficient singlet oxygen quenchers in vitro among common carotenoids (Di Mascio and al., 1989). In the present study, salinity decreased CA contents of pepper fruits (Fig. 1C) In agreement with these data, Dorais and al. (2000) showed that CA in tomato fruit was significantly decreased under salt stress. In contrast, Krauss and al.

(2006) reported that moderate salinity enhances CA in fresh tomato fruit, although this was not confirmed by the results of Fernández-García and al. (2004). Thus, under the prevailing experimental conditions the decrease in CA contents may relate to the decrease in photosynthetic processes under salinity. A possible explanation would be that salinity may inhibit or upregulate the biosynthetic pathway of carotenoids via inhibition of the genes encoding enzymes related to β -carotene (Dumas and al., 2003). Recently, Babu and al. (2011) reported that salt stress caused an inhibition in the expression of the gene encoded for lycopene β -cyclase, the enzyme that converts lycopene to beta carotene. The results on phenol contents are in conformity with the findings in pepper (Navarro and al., 2006) and tomato fruits (Krauss and al., 2006), while it contrasts with those of Maggio and al. (2007) in other tomato varieties. In addition, Shi et al. (2002) reported that adding NaCl to the nutrient solution did not affect phytonutrients such as flavonoids (quercetin). It is well known that anthocyanins are members of the flavonoid class of plant secondary metabolites that are not usually synthesised in tomato fruits (Mes and al., 2008). The increased synthesis of TP, TF contents under saline conditions may reflect some kind of defense against stress conditions since salt stress was accompanied by increased production of reactive oxygen species (Rezazadeh and al., 2012).

4.5. Minerals

Data presented in Table 2A and B, indicated that the increasing NaCl salinity concentration tended to decrease the inorganic mineral elements (K, S, P, Mg, Fe, Ca, Mn and Zn) contents and increased significantly Na content compared with control plant. Also, Bassuony and al. (2008) found the content of K^+ , Ca^{2+} and Mg^{2+} in Zea mays plant decreased significantly under salinity stress, compared with control. The deleterious effects of salinity on plant growth are associated with low water potential of the root medium which causes a water deficit within the plant; toxic effects of ions mainly Na^+ and Cl^- ; nutritional imbalance caused by reduced nutrient (K, S, P, Mg, Cu, Fe, Ca, Mn and Zn) uptake and/or transport to the shoot. Salinity mainly causes both hyper-osmotic stress and hyper-ionic toxic effects and the consequence can be plant demise (Serrano and al., 1999; Hasegawa and al., 2000). The contents of K in the fruit tissues decreased with increasing salinity for all varieties, confirming the effect of salt stress by the high concentration of Na (Duman, 2012). Also, Khafagy and al. (2009) found that the significant decrease in K^+ concentration occurred with increasing salinity levels in sweet pepper plants. Potassium may play a role on the synthesis of endogenous plant hormones (Haeder and al., 1981). Despite its obvious importance, the low mobility of Ca^{2+} make the rates of its uptake and distribution limiting processes for many key plant functions. Furthermore, the general lack of recognition of the limiting role of Ca^{2+} is due in part to the fact that some important plant functions are controlled by changes in very small physiologically active pools of Ca^{2+} within the cytoplasm. As such, whole-leaf Ca^{2+} levels might not reflect any potential limitations (McLaughlin and Wimmer, 1999). Soltani Nezhad and al. (2011) found that the P content in tomato (*Lycopersicon peruvianum* L) plant decreased was increased NaCl at 150 mM. Decreased P contents due to increasing salinity were caused probably by the high levels of the Cl ion, which can have antagonistic interactions with phosphorus (Hajiaghahi-Kamrani and Hosseinniya, 2013), however, there is no clear evidence of the interaction between salinity and changes in P absorption (Duman, 2012). Salt stress significantly increased the sodium content of pepper fruit. In agreement with these data, several authors reported that salt stress induced the accumulation of Na^+ in pepper fruit, and this may also result in an enhancement of oxidative parameters (Gautier and al., 2010). In this study, K, Ca, Cu, Mn, Fe, Zn, S and Mg concentrations were significantly reduced with increasing salinity in all cultivars. It has been reported that salinity affects plant physiology through changes of water and ionic status in the

cells because of ionic imbalance due to excessive accumulation of Na and Cl and reduced uptake of other mineral nutrients, such as K, Ca and Mg (Hasegawa et al., 2000). According to Saghir et al. (2002), the ionic stress affects plant growth by increasing Na and Cl levels in cells in response to high concentrations of NaCl, and decreased Ca, K, and Mg concentrations. This could be also attributed to the competition of Na with the uptake K, Ca, Mg resulting in a K/Na, Ca/Na and Mg/Na antagonism (Hosseini and Thengane, 2007). The fruit K/Na, Ca/Na and Mg/Na ratios were found to be significantly highest in 'Granada' and lowest in 'Goliath' (Table 2B). The relationship between the degree to which plant tolerate salt stress and their capacity to maintain a high leaf ratio K/Na has been noted by several authors (Al-Karaki, 2000). The levels of each mineral across levels of NaCl showed a high (significant) variation, indicating these compounds are strongly influence by salinity. Previous studies have shown that amounts of minerals in pepper fruit depend on the ripening stage, agricultural practices, genotype and environment factors (Guil-Guerrero and al., 2006; Pérez-Lopez and al., 2007).

5. CONCLUSION

As a high consumption vegetable, agromorphological composant and fruit quality of pepper are of some importance in its cultivation. In most environmental conditions and pepper cultivars, the pepper begins to suffer inhibited growth, lose yield and fruit quality when nutrient solution EC is above 2.5-4 dS m⁻¹. Higher salinity negatively affects pepper organic and inorganic compounds, antioxidant activity and ASA content and yield, while improves fruit quality. Under salinity condition, pepper root is the first organ exposed to salinity stress, and salinity induced root zone water stress. This may led to less water intake by the root and decreased transportation to the shoot. Consequently, less water is available for normal growth and development. Result from this investigation show that mineral nutrient, agromorphological, osmolytes and antioxidant compound to salt stress response among pepper varieties exist. The results obtained show that, the increasing within twelve weeks of treatment doses of NaCl, inhibited all agromorphological parameters, acid ascorbic, β -carotene and inorganic compound (Cu, P, Mn, S, Fe, K, Zn, Ca and Mg) from 50 mM NaCl for Goliath variety and from 100 to 200 mM NaCl, for Granada and Nobili varieties and the total flavonoid, phenolic, fructose, glucose, proline, soluble protein and Na⁺ significant accumulation in the fruits. The good behaviour of Granada and Nobili varieties in the face of salinity can be considered for their use to better enhance the sahelian and coastal areas.

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Table 3. Effect of NaCl (mM) on antioxidant compounds (ASA, CA, TP and TF) of pepper fruit cultivars at mature stage (90 DAP)

Cultivar	Treatment (mM NaCl)	TF (mg.g ⁻¹ of FW)	TP (mg.g ⁻¹ of FW)	CA (µg.g ⁻¹)	ASA (mg.100g ⁻¹ of FW)
Granada	0	23.37±0.13d	39.29±0.33c	3.66±0.04a	145.22±3.34a
	50	24.02±0.16cd	41.99±0.35bc	3.01±0.03a	139.47±4.47b
	100	28.87±0.12b	53.78±0.25ab	2.19±0.02a	130.08±3.44c
	200	30.55±0.13a	56.14±0.23a	1.03±0.02ab	119.92±3.49e
Nobili	0	20.67±0.19e	35.02±0.27d	2.81±0.03a	139.47±4.09b
	50	22.25±0.20d	37.27±0.33c	2.02±0.04a	129.15±3.16c
	100	26.82±0.18c	46.21±0.38b	1.08±0.05ab	121.01±4.24e
	200	30.30±0.22a	53.49±0.31ab	0.89±0.02c	113.51±4.39g
Goliath	0	19.42±0.21e	31.44±0.29e	3.02±0.06a	142.25±3.77a
	50	24.67±0.19cd	37.28±0.32c	1.97±0.07a	126.32±3.44d
	100	27.11±0.25c	44.02±0.28b	0.88±0.06b	117.28±2.14f
	200	33.22±0.22a	51.29±0.31b	0.87±0.03b	112.22±2.38g
Two way ANOVA results					
Cultivar (C)		*	*	NS	*
Salt treatment (S)		*	*	*	*
Interaction C X S		NS	*	NS	*

Values shown are means (n=5) ± SD; within columns, means followed by different letter are significantly different (p < 0.05).
**, * significant at 1 and 5% probability levels, respectively, NS not significant

Table 4. Effect of NaCl (mM) on osmolytes (PRO, SP, FRU and GLU) of pepper fruit cultivars at mature stage (90 DAP)

Cultivar	Treatment (mM NaCl)	PRO (g.mg ⁻¹)	SP (g.mg ⁻¹)	FRU (g.mg ⁻¹)	GLU (g.mg ⁻¹)	RWC (%)
Granada	0	12.97±0.09 cd	13.82±0.11 c	16.04±0.18 bc	8.89±0.03c	90.31±0.45 a
	50	13.39±0.11 c	14.70±0.13 c	18.88±0.16 b	9.18±0.02b	87.28±0.59 b
	100	17.09±0.10 b	18.83±0.12 b	20.08±0.15 a	11.75±0.03 b	79.39±0.38 e
	200	19.15±0.12 a	20.69±0.15 a	21.83±0.19 a	14.39±0.04 a	73.88±0.33 f
Nobili	0	11.02±0.09 d	12.88±0.09 cd	15.86±0.09 c	9.10±0.02b	90.55±0.51 a
	50	13.80±0.08 c	14.55±0.11 c	17.71±0.11 b	8.81±0.11c	86.02±0.44 c
	100	18.01±0.13 ab	18.36±0.14 b	19.93±0.14 b	11.97±0.07 b	76.29±0.48 e
	200	20.53±0.14 a	21.71±0.12 a	22.69±0.13 a	15.46±0.12 a	71.16±0.19 f
Goliath	0	11.55±0.15 d	13.07±0.08 c	14.66±0.08 c	8.75±0.09c	92.22±0.45 a
	50	15.38±0.13 c	17.29±0.11 b	18.44±0.14 b	12.52±0.11 b	83.66±1.11 d
	100	18.06±0.09 ab	20.55±0.12 a	20.36±0.16 a	12.73±0.12 b	78.21±1.07 e
	200	21.12±0.07 a	22.29±0.13 a	23.73±0.18 a	16.14±0.08 a	69.93±0.68 g
Two way ANOVA results						
Cultivar (C)		*	*	*	NS	*
Salt treatment (S)		*	*	*	*	*
Interaction C X S		NS	NS	NS	NS	*

Values shown are means ($n=5$) \pm SD; within columns, means followed by different letter are significantly different (p < 0.05).

**, * significant at 1 and 5% probability levels, respectively, NS not significant