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Fouzi A Ibrahim
Department of Biological Sciences Libyan Center for Studies and Researches in Environmental Science and Technology (SHUATS) Prayagraj, Uttar Pradesh. India

Reyad Mohialdeen
Department of Biological Sciences Libyan Center for Studies and Researches in Environmental Science and Technology (SHUATS) Prayagraj, Uttar Pradesh. India

Mohamed Dow Oshah
Department of Biological Sciences Libyan Center for Studies and Researches in Environmental Science and Technology (SHUATS) Prayagraj, Uttar Pradesh. India

Ahmed Al-Madhoni
Department of Biological Sciences Libyan Center for Studies and Researches in Environmental Science and Technology (SHUATS) Prayagraj, Uttar Pradesh. India

Corresponding Author:
Fouzi A Ibrahim
Department of Biological Sciences Libyan Center for Studies and Researches in Environmental Science and Technology (SHUATS) Prayagraj, Uttar Pradesh. India

Bio-chemical changes in tomato (*Lycopersicon esculentum* Mill) at different levels of salinity stresses

Fouzi A Ibrahim, Reyad Mohialdeen, Mohamed Dow Oshah and Ahmed Al-Madhoni

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Abstract
The present investigation was carried out at Department of Biological Science, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Pryagraj Uttar Pradesh. India. One of the most significant vegetable crops grown worldwide for its succulent fruits is the tomato (*Lycopersicon esculentum*). The Solanaceae family includes it. Bio-chemical parameters were carried out viz. Amount of chlorophyll a, b, Lycopene, Carotenoids, Total Soluble Solids. Observations have been made on biochemical parameters. According to the current study, T_6 ($CaCl_2$ 2400 ppm) was the most effective, followed by T_5 ($CaCl_2$ 1800 ppm). Out of all the treatments, T_3 (NaCl 300 ppm) had the greatest detrimental effects on tomato metrics. The study demonstrated the potential of calcium chloride to lessen the negative effects of sodium chloride on tomato.

Keywords: Chlorophyll a, b, lycopene, carotenoids, total soluble solids, $CaCl_2$, NaCl and tomato

Introduction

Tomato (*Lycopersicon esculentum*) is one of the most important vegetable crops cultivated all over the world for its fleshy fruits. It belongs to the family Solanaceae. The cultivated tomatoes originated as wild forms in the Peru-Ecuador-Bolivia area of South America .Earlier, tomatoes were thought to be poisonous and long before it was considered fit to eat, and it was grown only as an ornamental garden plant. Today, it is recognized as one of the important commercial and dietary vegetable crops (Bauer *et al.*, 2004) [3]. Importantly, calcium chloride is involved in the regulatory mechanisms that plants activate to adjust to adverse environmental conditions of salt (Zehra *et al*., 2012) [11]. Calcium chloride has been shown to ameliorate the adverse effects of stress on many plants (Xu *et al.*, 2013) [10]. Calcium appears to play a central role in many defense mechanisms that are induced by stress and Ca^{2+} signaling is required for the acquisition of stress tolerance or resistance (Cousson, 2009) [4]. Importantly, calcium chloride is involved in the regulatory mechanisms that plants activate to adjust to adverse environmental conditions of salt (Zehra *et al*., 2012) [11]. Calcium chloride has been shown to ameliorate the adverse effects of stress on many plants (Xu *et al.*, 2013) [10]. Calcium appears to play a central role in many defense mechanisms that are induced by stress and Ca2+signaling is required for the acquisition of stress tolerance or resistance (Cousson, 2009) [4]. Calcium chloride plays an active role on growth and photosynthetic pigments of tomato under salinity stress conditions (Ahlam and Babu 2015) [2]. Calcium chloride not only alleviates stress effects in potted ornamentals salvia splendens and Ageratum houstonianum but also improves all the growth and yield parameters under stress condition (Agata *et al*., 2016) [1]

Materials and Methods

The present investigation was carried out at Department of Biological Science, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Pryagraj Uttar Pradesh. India.

Bio-chemical parameters

Chlorophyll

Chlorophyll was determined according to 1 gram leaves sample was weighed and crushed with 80% acetone made the volume to 10 ml with 80% acetone, centrifuged at 800 ppm for 5 minute. The supernatant was read under 663, 645 nanometer. The readings were fed in the following formula and results were determined under spectrophotometer.

Amount of chlorophyll a, b and total chlorophyll are calculate as fallow –

Chlorophyll a (mg/g) = 12.9 × A (663 nm) –2.69 × A (645 nm) Chlorophyll b (mg/g) = 41.50 × A (645 nm) –5.10 × A (663 nm)

Lycopene

Extraction was performed according to (Fish *et al*. 2002) [5]. Samples were first chopped and homogenized in a laboratory homogenizer. Approximately 0.3-0.6 g samples were weighed and 5 mL of 0.05% (w/v) BHT in acetone, 5 mL of ethanol and J10 mL of hexane were added. The absorbance of the hexane layer (upper layer) was measured at absorbance 503 nm spectrophotometry using there-determined extinction coefficients (Perkins *et al.*, 2004) [5].

Carotenoids

Carotenoid was estimated according to method. The leaves were collected for the carotenoid content estimation. Fresh leaves weighing of 0.5 gm was taken and homogenized in 10 ml of acetone (80% acetone) and centrifuged at 3000rpm at 10 min. The absorbance was recorded at 470 nm.

It was calculated by using formula:

Total carotenoids = [1000 A470 – (2.270 Chl-a -81.4 Chl-b)]/227

Total Soluble Solids

The percentage of total soluble solids of the fruit was determined with the help of Portable Hand Refracto meter. The sample of juice for this purpose was taken from the strained juice. The observed value of T.S.S. was recorded from the scale of the instrument (0-32 range).

Results and Discussion

Effect of NaCl and $CaCl_2$ salinity on the Chlorophyll a & Chlorophyll b content (mg/g) of tomato leaves

The maximum chlorophyll a content was recorded (Table- 1 and fig. 1) in tomato leaves at T_6- $CaCl_2$ 2400 ppm (2.40 mg/g) followed by T_5-$CaCl_2$ 1800 ppm (1.81 mg/g), T_4- $CaCl_2$ 1200 ppm (1.68 mg/g), T_9- NaCl 100 ppm + $CaCl_2$ 2400 ppm (1.58 mg/g), T_8- NaCl 100 ppm + $CaCl_2$ 1800 ppm (1.43 mg/g), T_7- NaCl 100 ppm + $CaCl_2$ 1200 ppm (1.38 mg/g), T_{12}- NaCl 200 ppm + $CaCl_2$ 2400 ppm (1.34 mg/g), T_{11}- NaCl 200 ppm + $CaCl_2$ 1800 ppm (1.29 mg/g), T_{14}- NaCl 300 ppm + $CaCl_2$ 1800 ppm (1.27 mg/g), T_{10}- NaCl 200 ppm + $CaCl_2$ 1200 ppm, T_{13}- NaCl 300 ppm + $CaCl_2$ 1200 ppm (1.22 mg/g), T_1- NaCl 100 ppm (1.20 mg/g), T_2- NaCl 200 ppm and T_{15} T NaCl 300 ppm + $CaCl_2$ 2400 ppm (1.13 mg/g) respectively as compared to T_0- (1.55 mg/g) control. The minimum chlorophyll a content was recorded in treatment T_3- NaCl 300 ppm (0.97 mg/g). Individually Calcium is known to play a special role in tolerance under salinity .Calcium with magnesium increasing the chlorophyll contents, membrane stability under stress conditions. The same report was reported by (Pan *et al.*, 1995; Sagi, 2005) [6, 7]. The maximum chlorophyll a content was recorded (Table- 1 and fig. 2) in tomato leaves at T_6- $CaCl_2$ 2400 ppm (1.080 mg/g) followed by T_5-$CaCl_2$ 1800 ppm (0.975 mg/g), T_4- $CaCl_2$ 1200 ppm (0.950 mg/g), T_9- NaCl 100 ppm + $CaCl_2$ 2400 ppm (0.855 mg/g), T_8- NaCl 100 ppm + $CaCl_2$ 1800 ppm (0.795 mg/g), T_7- NaCl 100 ppm + $CaCl_2$ 1200 ppm (0.770 mg/g), T_{12}- NaCl 200 ppm + $CaCl_2$ 2400 ppm (0.765 mg/g), T_{11}- NaCl 200 ppm + $CaCl_2$ 1800 ppm (0.735 mg/g), T_{14}- NaCl 300 ppm + $CaCl_2$ 1800 ppm, T_{13}- NaCl 300 ppm + $CaCl_2$ 1200 ppm (0.720 mg/g), T_{10}- NaCl 200 ppm + $CaCl_2$ 1200 ppm (0.710 mg/g), T_1- NaCl 100 ppm (0.580 mg/g), T_2- NaCl 200 ppm (0.525 mg/g), and T_{15} T NaCl 300 ppm + $CaCl_2$ 2400 ppm (0.685 mg/g) respectively as compared to T_0- (0.900 mg/g) control. The minimum chlorophyll a content was recorded in treatment T_3- NaCl 300 ppm (0.465 mg/g). The phenomena is same as reported in chlorophyll a and the similar results was reported by (Pan *et al.*, 1995; Sagi, 2005) [6, 7].

Effect of NaCl and $CaCl_2$ salinity on the Lycopene content (mg/g fresh weight) of tomato leaves

The maximum lycopene content was recorded (Table- 1 and fig. 3) in tomato leaves at T_6- $CaCl_2$ 2400 ppm (3.13 mg/g) followed by T_4- $CaCl_2$ 1200 ppm (3.08 mg/g), T_{13}- NaCl 300 ppm + $CaCl_2$ 1200 ppm (2.96 mg/g), T_7- NaCl 100 ppm + $CaCl_2$ 1200 ppm, T_2- NaCl 200 ppm (2.94 mg/g), T_5-$CaCl_2$ 1800 ppm (2.92 mg/g), T_{11}- NaCl 200 ppm + $CaCl_2$ 1800 ppm (2.91 mg/g), T_{15} T NaCl 300 ppm + $CaCl_2$ 2400 ppm (2.80 mg/g), T_9- NaCl 100 ppm + $CaCl_2$ 2400 ppm, T_{10}- NaCl 200 ppm + $CaCl_2$ 1200 ppm (2.77 mg/g), T_{12}- NaCl 200 ppm + $CaCl_2$ 2400 ppm (2.75 mg/g), T_1- NaCl 100 ppm (2.58 mg/g), T_{14}- NaCl 300 ppm + $CaCl_2$ 1800 ppm (2.53 mg/g) and T_8- NaCl 100 ppm + $CaCl_2$ 1800 ppm (2.44 mg/g) respectively as compared to T_0- (2.31 mg/g) control. The minimum lycopene content was recorded in treatment T_3- NaCl 300 ppm (2.17 mg/g). As already mentioned that the application of $CaCl_2$ was found better to improve the carotenoid and lycopene, index of tomato fruits under stress conditions. The same report was reported by (Subiah and Perumal, 1990) [8].

Effect of NaCl and $CaCl_2$ salinity on the Carotenoids (mg/g fresh weight) in tomato leaves

The maximum lycopene content was recorded (Table- 1 and fig. 4) in tomato leaves at T_6- $CaCl_2$ 2400 ppm (4.40 mg/g) followed by T_4- $CaCl_2$ 1200 ppm (4.36 mg/g), T_{13}- NaCl 300 ppm + $CaCl_2$ 1200 ppm (4.23 mg/g), T_2- NaCl 200 ppm (4.21 mg/g), T_7- NaCl 100 ppm + $CaCl_2$ 1200 ppm, T_{11}- NaCl 200 ppm + $CaCl_2$ 1800 ppm (4.16 mg/g), T_5-$CaCl_2$ 1800 ppm (4.12 mg/g), T_{10}- NaCl 200 ppm + $CaCl_2$ 1200 ppm (4.02 mg/g), T_{14}- NaCl 300 ppm + $CaCl_2$ 1800 ppm (3.99 mg/g), T_9- NaCl 100 ppm + $CaCl_2$ 2400 ppm (3.96 mg/g), T_{12}- NaCl 200 ppm + $CaCl_2$ 2400 ppm (3.93 mg/g), T_{15} T NaCl 300 ppm + $CaCl_2$ 2400 ppm (3.92 mg/g), T_1- NaCl 100 ppm (3.69 mg/g), and T_8- NaCl 100 ppm + $CaCl_2$ 1800 ppm (3.48 mg/g) respectively as compared to T_0- (3.31 mg/g) control. The minimum lycopene content was recorded in treatment T_3- NaCl 300 ppm (3.11 mg/g). The application of $CaCl_2$ was found to be better to improve carotenoid and lycopene,index of tomato fruits under stress conditions. The similar report was reported by (Subiah and Perumal, 1990) [8]. (22.78^{0}Brix)

Effect of NaCl and $CaCl_2$ salinity on the Total soluble Solids (^{0}Brix) of tomato fruits

The maximum Total soluble Solids (^{0}Brix) was recorded (Table- 1 and fig. 4) in tomato leaves at T_6- $CaCl_2$ 2400 ppm (22.96 ^{0}Brix) followed by T_5-$CaCl_2$ 1800 ppm (20.44 ^{0}Brix), T_4- $CaCl_2$ 1200 ppm (20.24 ^{0}Brix), T_9- NaCl 100 ppm + $CaCl_2$ 2400 ppm (17.81 ^{0}Brix), T_8- NaCl 100 ppm + $CaCl_2$ 1800 ppm (17.62 ^{0}Brix), T_7- NaCl 100 ppm + $CaCl_2$ 1200 ppm (17.17 ^{0}Brix), T_{12}- NaCl 200 ppm + $CaCl_2$ 2400 ppm (17.20 ^{0}Brix), T_{11}- NaCl 200 ppm + $CaCl_2$ 1800 ppm (16.73 ^{0}Brix), T_{14}- NaCl 300 ppm + $CaCl_2$ 1800 ppm (16.62 ^{0}Brix), T_{10}- NaCl 200 ppm + $CaCl_2$ 1200 ppm (16.57 ^{0}Brix), T_{13}- NaCl 300 ppm + $CaCl_2$ 1200 ppm (16.40 ^{0}Brix), T_1- NaCl 100 ppm (16.33 ^{0}Brix), T_2- NaCl 200 ppm (16.08 ^{0}Brix) and T_{15} T NaCl 300 ppm + $CaCl_2$ 2400 ppm (16.06 ^{0}Brix) respectively as compared to T_0- (19.06 ^{0}Brix) control. The minimum Total soluble Solids (^{0}Brix) was recorded in treatment T_3- NaCl 300 ppm (15.22 ^{0}Brix). The application of $CaCl_2$ was found better to increase the total soluble solids even in presence of stress conditions in tomato crop. The similar report was seen by (Yaseen *et al.*, 2006) [9].

Table 1: Effect of NaCl and $CaCl_2$ salinity on the Chlorophyll a content (mg/g) of tomato leaves & fruits

Treatments	Details	Chlorophyll		Lycopene (mg/g)	Carotenoids (mg/g)	Total soluble Solids (^{0}Brix)
		Chlorophyll a (mg/g)	Chlorophyll b (mg/g)			
T_1	NaCl 100 ppm	1.20	0.580	2.58	3.69	16.33
T_2	NaCl 200 ppm	1.13	0.525	2.94	4.21	16.08
T_3	NaCl 300 ppm	0.97	0.465	2.17	3.11	15.22
T_4	$CaCl_2$ 1200 ppm	1.68	0.950	3.08	4.36	20.24
T_5	$CaCl_2$ 1800 ppm	1.81	0.975	2.92	4.12	20.44
T_6	$CaCl_2$ 2400 ppm	2.40	1.080	3.13	4.40	22.96
T_7	NaCl 100 ppm + $CaCl_2$ 1200 ppm	1.38	0.770	2.94	4.16	17.17
T_8	NaCl 100 ppm + $CaCl_2$ 1800 ppm	1.43	0.795	2.44	3.48	17.62
T_9	NaCl 100 ppm + $CaCl_2$ 2400 ppm	1.58	0.855	2.77	3.96	17.81
T_{10}	NaCl 200 ppm + $CaCl_2$ 1200 ppm	1.22	0.710	2.77	4.02	16.57
T_{11}	NaCl 200 ppm + $CaCl_2$ 1800 ppm	1.29	0.735	2.91	4.16	16.73
T_{12}	NaCl 200 ppm + $CaCl_2$ 2400 ppm	1.34	0.765	2.75	3.93	17.2
T_{13}	NaCl 300 ppm + $CaCl_2$ 1200 ppm	1.22	0.720	2.96	4.23	16.4
T_{14}	NaCl 300 ppm + $CaCl_2$ 1800 ppm	1.27	0.720	2.53	3.99	16.62
T_{15}	NaCl 300 ppm + $CaCl_2$ 2400 ppm	1.13	0.685	2.80	3.92	16.06
T_0	Control	1.55	0.900	2.31	3.31	19.06
	CD at 5%	0.22	0.07	0.19	0.34	0.53
	S(E)m±1	0.07	0.02	0.06	0.11	0.17

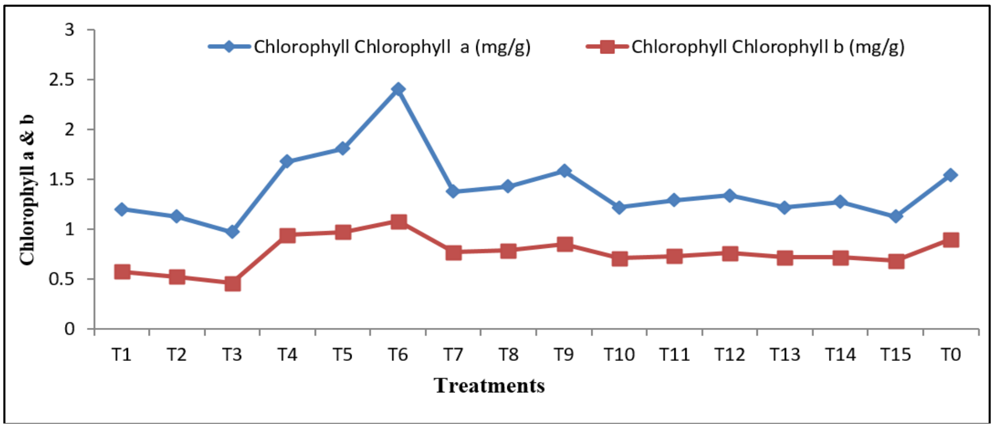

Fig 1: Effect of NaCl and $CaCl_2$ salinity on the Chlorophyll a & Chlorophyll b content (mg/g) of tomato leaves

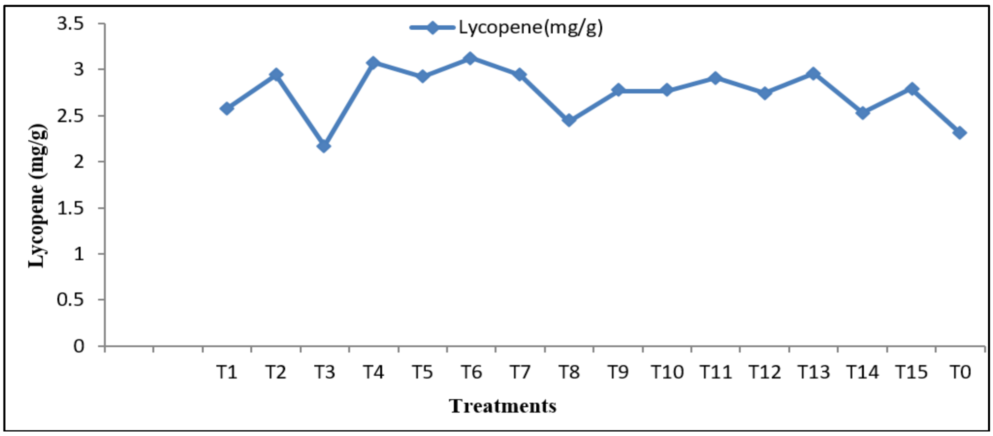

Fig 2: Effect of NaCl and $CaCl_2$ salinity on the Lycopene content (mg/g fresh weight) of tomato leaves

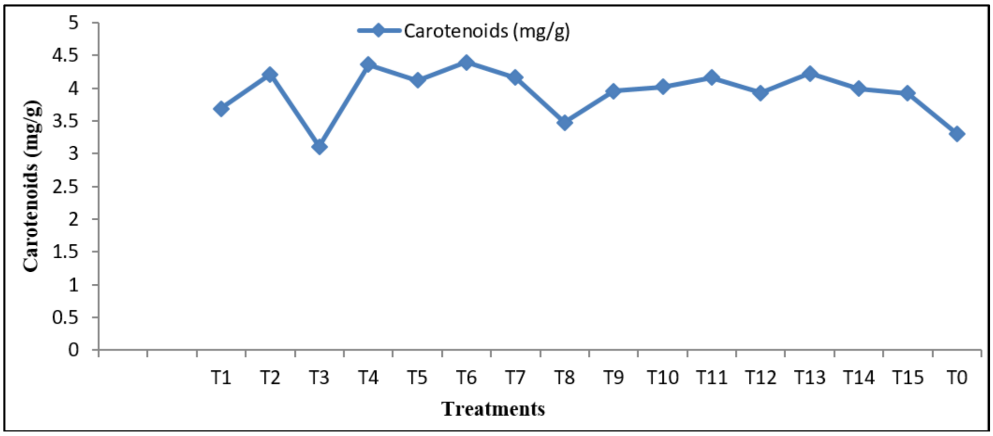

Fig 3: Effect of NaCl and $CaCl_2$ salinity on the Carotenoids (mg/g fresh weight) in tomato leaves

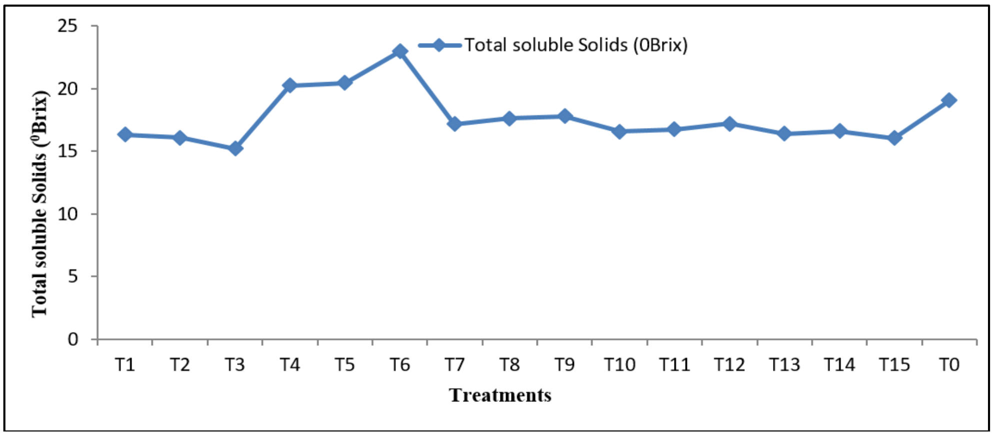

Fig 4: Effect of NaCl and $CaCl_2$ salinity on the Total soluble Solids (^{0}Brix) of tomato fruits

Conclusion

Results have been observed for biochemical parameters. From the present study it was concluded that T_6 ($CaCl_2$ 2400 ppm) was the most effective followed by T_5- ($CaCl_2$ 1800 ppm). Amongst all the treatments T_3- (NaCl 300 ppm) showed most deleterious effect on different parameters of tomato .It was clear from the study that calcium chloride could be used to mitigate the stress of sodium chloride on tomato.

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