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Influence of Ascorbic Acid against Salt stress on the Germination, Biochemicals and Micronutrients Concentrations of *Solanum melongena* (L.)

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Abstract

The experiments were designed to know the function of ascorbic acid against salinity on germination, different biochemical and micronutrients concentrations of S*olanum melongena*. Ascorbic acid was applied as foliar spray on leaves and given in roots through irrigation at 100 and 200 ppm to the plants growed in non saline, 60mM and 100mM salt stress conditions. Different biochemical parameters i.e. chlorophyll, carotenoids, carbohydratrates, protein and micro nutrients concentrations of *Solanum melongena* were observed under different salt stress concentrations from control (non-saline), 60mM NaCl and 100mM NaCl solutions. The rates of different biochemical parameters and micro nutrients concentrations exhibited decrease in saline media in comparison with their respective control while ascorbic acid used as a foliar spray and through irrigation in roots at the concentration of 100 ppm and 200 ppm resulted promotion in non saline control as compared with salt concentrations media. It was determined that chlorophyll concentration was decreased considerably under salt stress. Amongst micro nutrients ionic composition of Na, K and Na/K ratio resulted that plants treated with different NaCl concentrations at 60mM NaCl and 100mM salt concentrations showed increase in Na and K ions and ascorbic acid were used as a foliar spray and in roots showed decrease in Na and K ions. Overall from the outcomes of the study it was observed that salinity stress significantly decrease vegetative and reproductive parameters that could be improved with the using of ascorbic acid. Best concentration found were 200 ppm ascorbic acid contributed towards an increase in growth irrespective of non-saline and saline conditions. Based on these findings, ascorbic acid treatment helps in alleviating the negative effects of salinity.

Key words: Ascorbic acid; Biochemicals; Micronutrients; *Solanum melongena*; Salinity

1. Introduction

Salinity means the presence of different kinds of salts in the soil in such amount that can interfere with the growth of plants. Salinity becomes dangerous for crops when an unnecessary amount of soluble salts occurs in the earth. Salinity is the main limiting factor affecting crops production across the world in the arid and semi arid areas [1]. Throughout the world sixty million hectares of cultivated land is badly affected due to salinity [2]. In Pakistan, salinity remains the main factor affecting crop production due to considerable rise in water table [3]. Higher salt absorption in the soil adversely affect seed germination, vegetative growth, fruit and flowering development resultantly decreasing crop productivity [4]. Stress causes increase level of reactive oxygen species includes oxide and superoxide radicals, hydroxyl and hydrogen peroxide (H_2O_2) [5]. Salinity reduces total leaf area of the plant by increasing its thickness [6]. NaCl accumulated in the chloroplasts of soybean showed decrease plant biomass due to reduced growth mostly related with hindrances in electron transport activity of the photosynthesis [7].

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Salinity adversely affects vegetative and reproductive parameters which lead to reduced yields [8, 9]. Salt stress also disturbs phytohormone levels which affect plant physiological activities at little concentration moreover in remote tissues to which they are elated or in the site of amalgamation [10].

Ascorbic acid serves as a growth regulator thereby providing protection against salinity by influencing a wide range of plant process such as seed germination, iron uptake & transport, stomata closure, membrane permeability, photosynthesis and growth rate [11]. *Solanum melongena* is one of the important species of Solanaceae cultivated throughout the world. Its stem is mostly spiny with white to purple flowers having five lobed corolla and yellow stamens, fruit is fleshy and botanically known as berry [11].

2. RESULTS

2.1 *Germination*

Seeds treated with 34 mM NaCl resulted germination percentage similar to control. Seeds treated with 60 mM, 100 mM and 136 mM NaCl level resulted prominent reduction in germination percentage as compared with control (Table 1).

Table: 1. Germination % of *Solanum melongena* seeds in distinct NaCl concentrations.

Treatment	Mean	SE
Control	60.0a	±5.773
34 mM NaCl	60.000a	±11.547
68 mM NaCl	33.333b	±6.666
100 mM NaCl	63.333b	±8.819
136 mM NaCl	23.333b	±12.018
$LSD_{0.05}$	25.408	

Seeds of set-I results increase in germination percentage of 60mM NaCl treatment as compared with control. Seeds of set-II resulted increase in germination percentage in 100mM NaCl treatment as compared with control. Seeds of set-III resulted increase in germination percentage in control as compared with salinity. Assessment of set-II and set-III, set-II resulted boost in germination percentage in saline media as compared with set-III. Assessment of set-II and III with set-I, Set-II resulted increased in 100mM NaCl and control, while set-III exhibited boost in control as compared with set-I (Table 2).

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Table 2. Germination % of *Solanum melongena* seeds in distinct NaCl and ascorbic acid concentrations.

Treatments	CONTROL	100 PPM AA	200 PPM AA
Control, Mean, SE	66.667 ±6.666	70.0 ±10.0	76.667 ±3.333
60 mM NaCl	73.333 ±3.333	70.0 ±10.0	63.333 ±6.666
100 Mm NaCl	63.333 ±3.333	83.333 ±3.333	46.667 12.019
$LSD_{0.05}$	20.15		

2.2 Shoot length

Seedlings of set-I resulted significant (P<0.01) decline in shoot length in 60mM NaCl treatment while resulted significant (P<0.01) boost in 100mM NaCl as compared with control. Seedlings of Set-II resulted significant (P<0.01) increase in shoot length in saline media as compare with control. Seedlings of set-III resulted increase in shoot length in saline media as compare with control. Assessment of set-II and set-III, set-II resulted boost in shoot length in all treatments as compared with set-III. Assessment of set-II and III with set-I, Set-II resulted increase in saline media, while set-III exhibited decrease in control as well as in saline media as compared with set-I (Fig. 1).

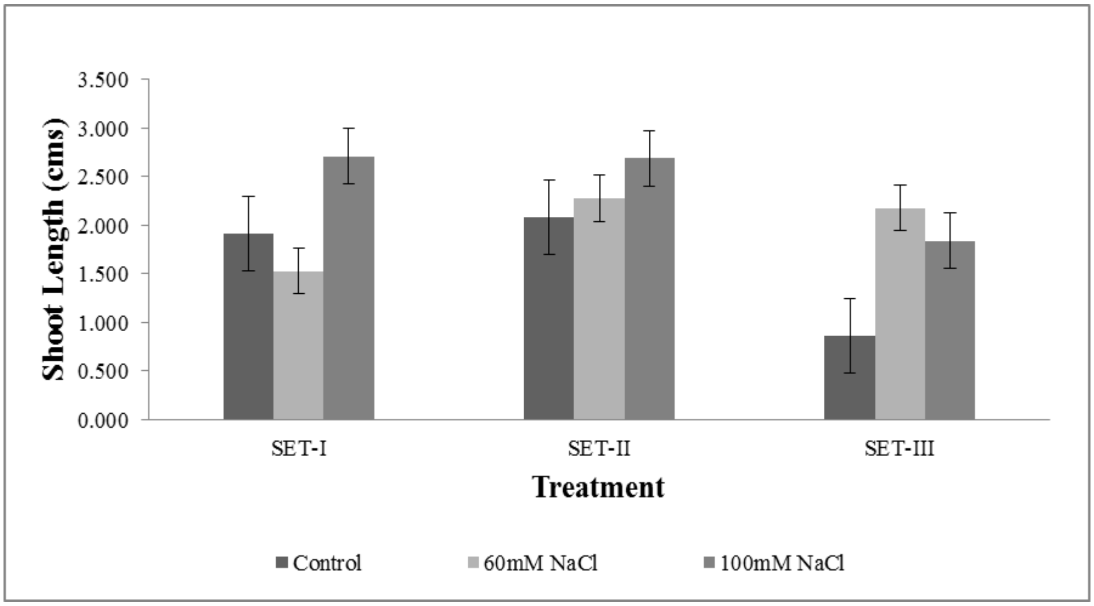

Fig.1. Effect of ascorbic acid and distinct NaCl meditation on shoot length (cms) of *Solanum melongena* seedlings. Set-I: Without ascorbic acid; Set-II: 100 PPM ascorbic acid with distinct meditation of NaCl; Set-III: 200 PPM ascorbic acid with distinct meditation of NaCl.

2.3 Root length

Plants of set-I resulted significant (P<0.05) decline in root length in saline media as compared with control. Plants of set-II and III showed significant (P<0.05) boost in root length at100 mM

NaCl level as compare to control. Assessment of set-II with set-III, set-II resulted increase in root length in all treatments as compare to set-III. Assessment of set-II and set-III with set-I, Set-II resulted boost in 100 mM salinity level while set-III exhibited overall decrease in all treatments as compare to set-I (Fig. 2).

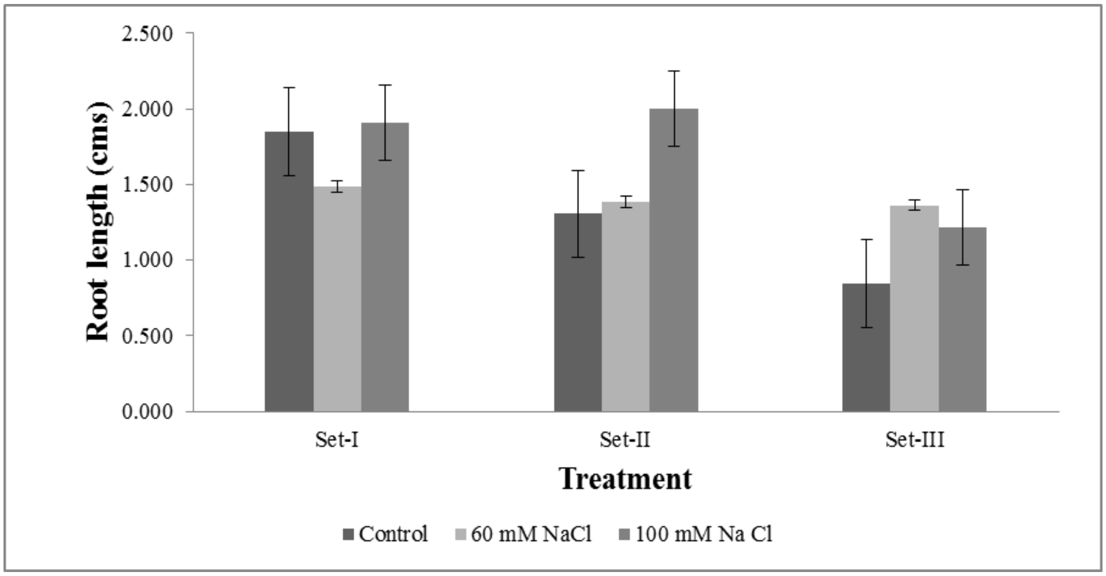

Fig.2. Effect of ascorbic acid and distinct NaCl meditation on root length (cms) of *Solanum melongena* seedlings. Set-I: Without ascorbic acid; Set-II: 100 PPM ascorbic acid with distinct meditation of NaCl; Set-III: 200 PPM ascorbic acid with distinct meditation of NaCl.

2.4 Fresh and dry biomass

Plants of set-I resulted non significant boost in fresh and dry weight in 100mM NaCl treatment as compared with control. Plants of set-II resulted non significant decline in fresh and dry weight in saline media as compared with control. Plants of set-III resulted little decline in fresh and dry weight in control. Comparison of set-II with set-III, set-II resulted boost in control and at 100mM NaCl as compared with set-III. Assessment of set-II and set-III with set-I, both sets resulted boost in control and at 60 mM NaCl as compared with set-I (Fig. 3 & 4).

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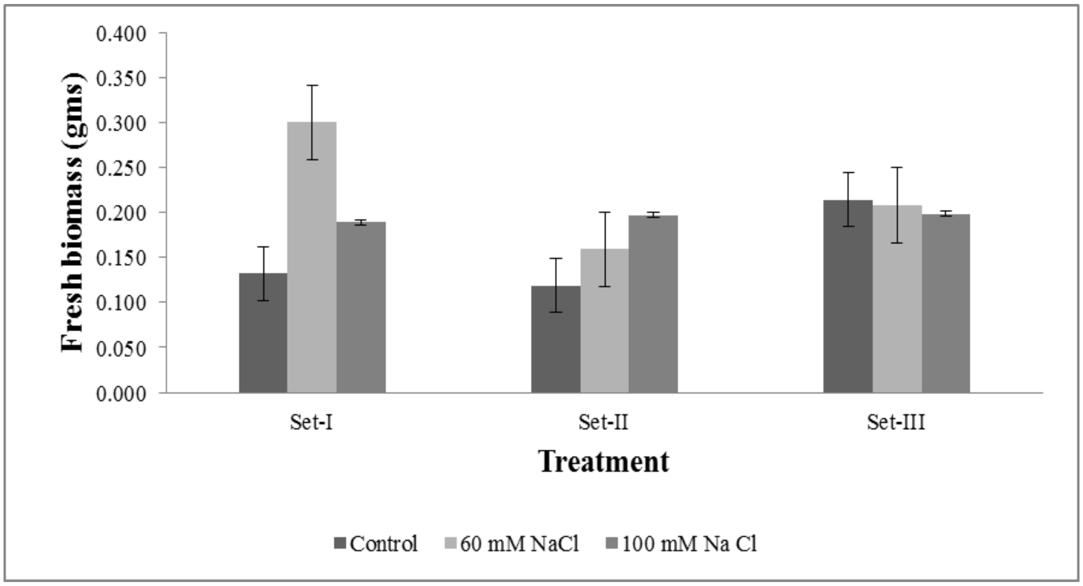

Fig.3. Effect of ascorbic acid and distinct NaCl meditation on fresh biomass (cms) of *Solanum melongena* seedlings. Set-I: Without ascorbic acid; Set-II: 100 PPM ascorbic acid with distinct meditation of NaCl; Set-III: 200 PPM ascorbic acid with distinct meditation of NaCl.

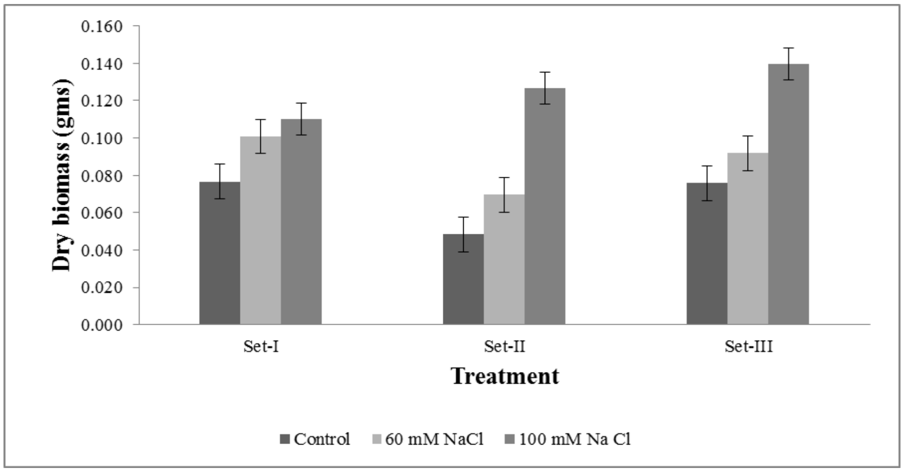

Fig.4. Effect of ascorbic acid and distinct NaCl meditation on dry biomass (cms) of *Solanum melongena* seedlings. Set-I: Without ascorbic acid; Set-II: 100 PPM ascorbic acid with distinct meditation of NaCl; Set-III: 200 PPM ascorbic acid with distinct meditation of NaCl.

2.5 Biochemicals analysis

2.5.1 Chlorophyll estimation

Plants of set-I and II resulted non Significance decline in chlororophyll a, total chlorophyll and a/b ratio in saline media as compared with control, while in set-I chlororophyll b resulted significant ($p<0.05$) boost in Saline media as compared with control. Plants of set-III and IV resulted boost in chlororophyll a, b, total chlorophyll and in chlorophyll a/b ratio in saline media as compared with control. Plants of set-V resulted non-significance boost in chlororophyll a, b, total chlorophyll and in chlorophyll a/b ratio in control and at 100mM NaCl level. Assessment of set-II and set-III, set-II resulted boost in chlororophyll a, b, total chlorophyll and chlorophyll a/b in control, while resulted decline at 100mM NaCl and boost in chlororophyll b and total chlorophyll at 60 mM NaCl as compared with set-III. Assessment of set-II and set-III with set-I, set-II resulted boost in chlororophyll a, b, total chlorophyll in control while chlorophyll a/b resulted reduction in control. Chlororophyll a, total chlorophyll and chlorophyll a/b ratio resulted boost at 60mM Nacl while set-III resulted boost in chlororophyll a, total chlorophyll and chlorophyll a/b ratio at 60mM NaCl as compared with set-I. Assessment of set-IV and set-V resulted that Set-IV showed reduction in chlororophyll a, b, total chlorophyll and in chlorophyll a/b ratio overall in all treatments except chlororophyll b that resulted a little boost at 60 mM NaCl while chlorophyll a/b ratio resulted boost at 100mM NaCl level as compared with set-V. Assessment of set-IV and set-V with set-I, set-IV resulted boost in chlororophyll- a, b, total chlorophyll and in chlorophyll a/b ratio in control, boost in chlororophyll a, total chlorophyll and in chlorophyll a/b ratio at 60mM NaCl, boost in chlorophyll a/b ratio at 100mM NaCl as compared with set-I (Table 3).

2.5.2 Carotenoids estimation

Plants of set-I and IV resulted non-significance boost in Carotenoids in saline media as compared with control. Plants of set-III resulted significant ($p<0.05$) boost in Carotenoid in saline media as compared with control. Plants grown in set-II exhibited non-significance boost in Carotenoid in 100mM NaCl treated plants as compared with control. Plants of set-V resulted non-significance decline in Carotenoids in 100mM NaCl as compared with control. Assessment of set-II and set-III, set-III resulted boost in Carotenoids in 60mM NaCl as compared with set-II. Assessment of set-II and set-III with set-I, both sets exhibited decline in Carotenoids in 60mM NaCl treated plants as compared with set-I. Assessment of set-IV and set-V; Set-IV resulted slight decline in Carotenoids in control and 60mM NaCl as compared with set-V. Comparison of set-IV and set-V with set-I, both sets resulted boost in Carotenoids in saline media as compared with set-I (Table 3).

2.5.3 Proteins estimation

Plants of set-II, III and IV exhibited decline in proteins at saline media as compared with control. Set-I and V resulted significant ($p<0.05$) reduction in proteins at 100mM NaCl level as compared with control. Assessment of set-II and set-III, set-II resulted increase in proteins in control as compared with set-III. Assessment of set-II and set-III with set-I, Set-II resulted boost in proteins in control and at 100mM and set-III resulted increase in saline media as compare with set-I. Set-IV and V also give same result by comparing with one another and with set-I (Table 3).

2.5.4 Carbohydrates estimation

Plants of set-II, III and IV resulted decline in carbohydrates in saline media as compare with control. Set-I and V resulted significant ($p<0.05$) reduction in carbohydrates at 60 mM NaCl. Assessment of set-II and set-III, set-II resulted increase in saline media as compared with set-III. Assessment of set-II and set-III with set-I, Set-II resulted boost in saline media and set-III resulted overall increase in all treatments as compared with Set-I. Assessment of set-IV and set-V, set-V resulted boost in carbohydrates in control and at 100mM NaCl as compared with set-IV. Assessment of set-IV and set-V with set-I, Set-IV resulted increase in saline media and set-V resulted increase in carbohydrates in control and at 100mM NaCl as compared with set-I (Table 3).

Table 3. Influence of ascorbic acid and different salt concentration on Chlorophyll a, b, total Chlorophyll, Chlorophyll a/b ratio, Carotenoids, Carbohydrates and Proteins concentrations of *Solanum melongena.*

Set-I*:* without ascorbic acid

Treatment	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll	Chlorophyll a/b Ratio	Carotenoids	Carbohydrates	Proteins
	(mg/g)	(mg/g)	(mg/g)	(mg/g)	(mg/g)	(mg/g)	(mg/g)
Control, Mean, SE	0.0528a ±0.00447	0.0239a ±0.0034	0.0767a ±0.0079	2.254a ±0.1506	0.179a ±0.0116	2.112a ±0.0808	1.69a ±0.0769
60mM NaCl, Mean, SE	0.0380a ±0.0055	0.0109a ±0.0086	0.0490a ±0.00602	1.3308a ±0.1288	0.1133a ±0.0027	0.2344a ±0.0135	0.268ab ±0.0072
% (+/-)	(-28.081)	(-54.078)	(-36.179)	(-40.960)	(-36.755)	(-88.90)	(-84.151)
100mM NaCl, Mean, SE	0.0321a ±0.0019	0.0182a ±0.0004	0.0339a ±0.0168	1.756a ±0.059	0.091a ±0.0072	0.202a ±0.006	0.126ab ±0.0103
% (+/-)	(-39.196)	(-23.625)	(-55.826)	(-22.06)	(-49.039)	(-90.40)	(-92.523)
$LSD_{0.05}$	0.0483	0.01637	0.0411	1.892	0.0661	0.532	0.493

Table 3……(Contd)

Set-II= 0.25 ppm ascorbic acid applied through roots

Treatment	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll	Chlorophyll a/b Ratio	Carotenoids	Carbohydrates	Proteins
	(mg/g)	*(mg/g)*	*(mg/g)*		*(mg/g)*	*(mg/g)*	*(mg/g)*
Control, Mean, SE	0.053a ±0.0087	0.030a ±0.005	0.0740a ±0.0148	1.7907a ±0.0813	0.167a ±0.0075	2.424a ±0.0587	2.134a ±0.0370
60mM NaCl, Mean, SE	0.0317b ±0.0017	0.0185a ±0.0009	0.0502a ±0.0026	1.709a ±0.0195	0.0707a ±0.0254	0.0453b ±0.0033	0.0230b ±0.0024

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% (+/-)	(-40.931)	(-38.243)	(-32.131)	(-4.558)	(-57.691)	(-98.13)	(-98.919)
100mM NaCl, Mean, SE	0.0235b ±0.0040	0.0265a ±0.0112	0.0501a ±0.0147	1.382a ±0.6377	0.0802a ±0.0051	0.0191b ±0.001	0.0244b ±0.0017
% (+/-)	(-56.092)	(-9.142)	(-31.321)	(-22.80)	(-52.049)	(-99.21)	(-98.853)
$LSD_{0.05}$	0.0197	0.021	0.0407	0.892	0.1499	0.542	0.476

Table 3……… (Contd)

Set-III= 0.5 ppm ascorbic acid applied through roots

Treatment	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll	Chlorophyll a/b Ratio	Carotenoids	Carbohydrates	Proteins
	(mg/g)	(mg/g)	(mg/g)		(mg/g)	(mg/g)	(mg/g)
Control, Mean, SE	0.0527a ±0.0077	0.0369a ±0.0065	0.089a ±0.0043	1.870a ±0.4651	0.198a ±0.0091	1.906a ±0.0841	0.985a ±0.0462
60mM NaCl, Mean, SE	0.0406b ±0.0013	0.0089ab ±0.0053	0.049a ±0.0040	1.849a ±0.0544	0.118ab ±0.0092	1.656a ±0.0352	0.674a ±0.0456
% (+/-)	(-22.909)	(-75.821)	(-44.708)	(-1.083)	(-40.059)	(-13.11)	(-45.223)
100mM NaCl, Mean, SE	0.0291b ±0.0080	0.023a ±0.01404	0.0529a ±0.01521	1.730a ±0.6752	0.105b ±0.0091	1.460a ±0.0341	1.288ab ±0.0815
% (+/-)	(-44.653)	(-35.750)	(-40.985)	(-7.479)	(-46.940)	(-23.39)	(+4.703)
$LSD_{0.05}$	0.029	0.0224	0.0374	0.8924	0.12	0.64	0.53

Table 3…….. (Contd)

Set-IV= 0.25 ppm ascorbic acid applied as a foliar spray

Treatment	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll	a/b Ratio	Carotenoids	Carbohydrates	Proteins
	(mg/g)	*(mg/g)*	*(mg/g)*		*(mg/g)*	*(mg/g)*	*(mg/g)*
Control, Mean, SE	0.05057a ±0.0114	0.01074a ±0.0040	0.0613a ±0.0079	7.226a ±3.412	0.159a ±0.0261	1.785a ±0.0929	1.655a ±0.0616
60mM	0.0461a	0.0188a	0.065a	4.282a	0.146a	1.016a	0.862b

NaCl, Mean, SE	±0.0102	±0.0086	±0.0127	±2.044	±0.0253	±0.0067	±0.032
% (+/-)	(-8.785)	(+75.879)	(+6.051)	(-40.73)	(-8.460)	(-43.06)	(-47.925)
100mM NaCl, Mean, SE	0.0269ab ±0.0090	0.0206a ±0.0073	0.0475a ±0.0029	2.010a ±1.0093	0.131a ±0.0223	0.964a ±0.0355	0.729b ±0.0241
% (+/-)	(-46.665)	(+91.902)	(-22.383)	(-72.183)	(-17.796)	(-45.977)	(-55.940)
$LSD_{0.05}$	0.0358	0.0295	0.0308	4.564	0.149	0.71	0.61

Table 3…….. (Contd)

Set-V: 0.5 ppm ascorbic acid applied as a foliar spray

Treatment	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll	a/b Ratio	Carotenoids	Carbohydrates	Proteins
	(mg/g)	(mg/g)	(mg/g)		(mg/g)	(mg/g)	(mg/g)
Control, Mean, SE	0.0583a ±0.0031	0.033a ±0.0073	0.091a ±0.0103	1.912a ±0.354	0.162a ±0.0191	2.056a ±0.0604	1.876a ±0.0390
60mM NaCl, Mean, SE	0.0313a ±0.0085	0.0202a ±0.0069	0.0515a ±0.0140	1.775a ±0.6440	0.0934a ±0.0467	1.809a ±0.0456	1.695a ±0.0362
% (+/-)	(-46.378)	(-39.006)	(-43.704)	(-7.163)	(-42.512)	(-12.04)	(-9.662)
100mM NaCl, Mean, SE	0.0330a ±0.009	0.0211a ±0.0055	0.0542a ±0.0127	1.619a ±0.49	0.080a ±0.0065	1.475a ±0.0292	1.472a ±0.0269
% (+/-)	(-43.318)	(-36.202)	(-40.737)	(-15.339)	(-50.552)	(-28.28)	(-21.56)
$LSD_{0.05}$	0.026	0.0232	0.044	1.778	0.102	0.62	0.59

2.6 Micronutrients composition

2.6.1 Sodium (Na+)

Plants grown in all five sets resulted significant($p<0.05$) boost in Na+ absorption in stem, roots and leaves as compared with K+ concentration resulted non-significant reduction in all sets except set-IV and V where stem and root resulted significant($p<0.05$) reduction as compared with control. Assessment of set-II and set-III, set-II resulted Na+ concentration in stem increased in 100mM NaCl, in roots and leaves increase in control and both NaCl concentration as compare

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with set-III. Assessment of set-II and set-III with set-I, Set-II resulted Na+ concentration in stem decrease in both salinity treatments and control in roots promotion at100mM NaCl level and in leaves reduction at100mM NaCl level while set-III resulted Na+ concentration in stem decrease in both salinity treatments and control in roots promotion in 60mM NaCl and in leaves reduction in both salinity treatments and control as compared with set-I. Assessment of set-IV and set-V, set-V resulted boost in control in stem and leaves and increase in control and 60mM Nacl in roots as compared with set-IV. Assessment of set-IV with set-I and V resulted that Na+ concentration in stem decrease in both salinity treatments and control in roots promotion in saline media and in leaves reduction in both salinity treatments and control, while set-V resulted that Na+ concentration in stem decrease in both salinity treatments and control in roots promotion in both salinity treatments and control and in leaves reduction in 100mM NaCl as compared with set-I (Table 4).

2.6.2 Potassium (K+)

Plants grown in set-I resulted that K^+ concentration, increase in stem in 60mM NaCl, and decline in root in saline media and increase in leaves in saline media as compared with control. Plants of set-II and set-III resulted that K+ concentration, decrease in stem at100mM NaCl level and increase in roots in saline media and decrease in leaves in saline media as compared with control. Plants of Set-IV resulted that K^+ concentration, decrease in stem and leaves in saline media, increase in roots in saline media as compared with control. Plants grown in Set-V resulted that K^+ concentration, decrease in stem, root and leaves in saline media, as compared with control. Comparison of set-II and set-III, set-II resulted that K^+ concentration, decrease in stem and roots in control and both NaCl treatment and in leaves in 100mM NaCl concentration as compared with set-III. Assessment of set-II and set-III with set-I, Set-II resulted that K^+ concentration, decrease in stem and leaves in both salinity treatments and control and promotion in roots in saline media, while set-III resulted that K^+ concentration, decreased in stem and leaves in both salinity treatments and control and decreased in roots in saline media as compared with set-I. Assessment of set-IV and set-V, set-V resulted that K^+ concentrations, increase in stem in control and 100mM NaCl, decline in roots in control and 60mM NaCl and in leaves increase both NaCl concentrations as compared with set-IV. Assessment of set-IV and set-V with set-I, Set-IV resulted that Na+ concentration, decrease in stem in both salinity treatments and control, promotion in roots in 100mM NaCl and in leaves reduction in both salinity treatments and control while set-V resulted that Na^+ concentration in stem decrease in both salinity treatments and control, in roots promotion in control and in leaves reduction in both salinity treatments and control as compared with set-I (Table 4).

2.6.3 Sodium Potassium ratio

Plants of set-I and V in stem, set-I, III, IV and V in roots and set-II, III and IV in leaves resulted non-significant decrease in salt concentrations as compared with their respective control. Plants of set-II, III, and IV in stem and set-I and V in leaves results non-significant boost in 60mM salt concentration as compared with their control, while plants of set-II in roots resulted non significant boost in 100mM salt concentration as compared with control and 60mM salt concentration. Assessment of plants of set-II with III plants of set-II exhibited boost in control and 60mM salt concentration in stem and leaves while showed increase in 100mM salt concentration in stem and leaves. Assessment of set-IV and V resulted that plants of set-IV

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resulted increase in all concentrations of root and leaves along with control in stem while decrease in 60 and 100mM salt concentrations in stem. Assessment of set-II, III, IV and V with set-I resulted that set-I exhibited boost in all concentrations of stem, roots and leaves except 100mM salt concentration in set-III, control in set-IV in stem and control in set-II and 60mM salt concentration in set-II and V showed decrease in roots (Table 4).

Table 4. Micro nutrients composition of *Solanum melongena* grown at different concentration of Ascorbic acid against salt stress

Set-I: Without ascorbic acid

Treatment		STEM			ROOT		LEAVES		
	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+
Control, Mean, SE	246.65a ±39.27	200.58a ±6.6	0.861a ±0.1468	294.78a ±43.61	143.26a ±30.07	0.48a ±0.04	153.26a ±23.3	307.32a ±36	2.088a ±0.387
60mM NaCl, Mean, SE	243.26a ±61.7	182.78a ±84.9	0.804a ±0.3	259.82a ±9.9	84.5a ±17.5	0.33a ±0.072	146.59a ±20.2	345.54a ±18.5	2.47a ±0.443
% (+/-)	(-1.337)	(-8.99)	(-6.62)	(-11.85)	(-41.01)	(-31.56)	(-4.35)	(+12.43)	(+18.31)
100mM NaCl, Mean, SE	371.91a ±36.6	111.28a ±11.6	0.31a ±0.057	710.47a ±74.09	163.93a ±48.1	0.242a ±0.086	329.74a ±43.2	378.82a ±88.9	1.167a ±0.264
% (+/-)	(+50.83)	(-44.5)	(-63.9)	(+141.01)	(+14.42)	(-49.6)	(+115.15)	(+23.26)	(-44.1)
$LSD_{0.05}$	163.5	171.7	0.984	198.82	233.8	0.34	212.76	38.034	0.7

Table 4……… (Contd)

Set-II: 100 ppm ascorbic acid applied through roots

Treatment		STEM			ROOT		LEAVES		
	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+
Control, Mean, SE	223.18a ±14.5	116.22a ±30.7	0.54a ±0.174	499.87a ±70.04	171.73a ±13.06	0.365a ±0.075	199.87a ±23.1	134.55a ±10.30	0.697a ±0.118
60mM NaCl, Mean, SE	210.57a ±38.4	124.45a ±39.6	0.61a ±0.23	590.78a ±104.09	185.45a ±10.9	0.245a ±0.0237	303.22a ±44.8	116.22a ±1.56	0.398ab ±0.049
% (+/-)	(+4.5)	(+7.15)	(+12.4)	(+27.99)	(-11.58)	(-32.79)	(+51.7)	(-13.6)	(-42.8)
100mM NaCl,	287.47a ±27.12	78.25a ±9.14	0.225a ±0.0087	590.31a ±129.13	240.58a ±52.95	0.470a ±0.075	209.9a ±9.9	124.64a ±10.4	0.59b ±0.024

Mean, SE									
% (+/-)	(+31.39)	(-43.06)	(-58.69)	(+33.49)	(+46.63)	(+5.68)	(+5.02)	(-7.4)	(-15.12)
$LSD_{0.05}$	97.81	101.64	0.6	373.6	112.63	0.22	102.7	29.5	0.262

Table 4…….(Contd)

Set-III: 200 ppm ascorbic acid applied through roots

Treatment		STEM			ROOT		LEAVES		
	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+
Control, Mean, SE	253.30a ±19.12	126.7a ±24.15	0.513a ±0.125	230.42a ±12.13	224.31a ±22.05	0.9622a ±0.089	203.25a ±3.37	140.34a ±10.33	0.6612a ±0.0439
60mM NaCl, Mean, SE	266.5a ±17.16	141.18a ±17.95	0.542 ±0.0967	428.57a ±73.1	218.58a ±40.2	0.524b ±0.0349	317.75b ±20.01	120.58a ±20.42	0.3904b ±0.082
% (+/-)	(+5.23)	(+11.95)	(+5.78)	(+89.17)	(+2.68)	(-45.525)	(+52.43)	(-11.78)	(-40.9)
100mM NaCl, Mean, SE	223.42a ±35.08	79.87a ±9.2	0.417a ±0.0855	495.87b ±0.01	261.54a ±49.2	0.51b ±0.096	500.10c ±23.2	120.14a ±12.63	0.236b ±0.016
% (+/-)	(-13.1)	(-31.64)	(-18.6)	(+125.07)	(+19.35)	(-47.05)	(+137.75)	(-14.8)	(-60.48)
$LSD_{0.05}$	85.99	63.4	0.4	148.021	134.41	0.28	61.7	81.8	0.19

Table 4……. (Contd)

Set-IV: 200 ppm ascorbic acid applied as a foliar spray

Treatment		STEM			ROOT		LEAVES		
	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+
Control, Mean, SE	117.92a ±14.02	169.39a ±20.02	1.48a ±0.234	236.6a ±87.4	97.89a ±4.3	0.525a ±0.162	186.5a ±27.36	204.4a ±25.1	1.107a ±0.064
60mM NaCl, Mean, SE	269.18ab ±39.3	59.67ab ±2.25	0.231b ±0.031	384.79a ±58.2	118.69b ±10.13	0.33a ±0.0667	239.9a ±61.1	147.81a ±16.8	0.774a ±0.32
% (+/-)	(+128.28)	(-64.7)	(-84.3)	(+62.63)	(+21.24)	(-37.8)	(+28.65)	(-27.6)	(-30.1)

100mM NaCl, Mean, SE	415.52b ±112.1	71.52b ±8.2	0.1911b ±0.056	701.73a ±69.58	219.32b ±27.86	0.332a ±0.038	319.57a ±48.09	140.75a ±18.7	0.433a ±0.068
% (+/-)	(+252.66)	(-60.1)	(-87.07)	(+187.29)	(+128.02)	(-36.7)	(+75.16)	(-32.5)	(-60.8)
$LSD_{0.05}$	238.98	43.5	0.5	209.57	59.9	0.36	164.74	71.04	0.67

Table 4…….. (Contd)

Set-V: 200 ppm ascorbic acid applied as a foliar spray

Treatment		STEM			ROOT		LEAVES		
	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+
Control, Mean, SE	189.9a ±35.11	119.47a ±10.01	0.69a ±0.15	443.21a ±69.33	250.77a ±115.4	0.52a ±0.168	199.87a ±37.83	131.17a ±13.61	0.684a ±0.0838
60mM NaCl, Mean, SE	176.5a ±36.6	83.07a ±6.63	0.5ab ±0.081	536.6b ±29.073	122.85a ±12.06	0.23a ±0.018	266.57a ±99.03	127.8a ±10.8	0.701a ±0.334
% (+/-)	(-7.064)	(-30.4)	(-27.1)	(+21.06)	(-51.01)	(-55.9)	(-33.37)	(-2.57)	(-2.47)
100mM NaCl, Mean, SE	238.28a ±31.9	65.52a ±13.7	0.271b ±0.036	643.85b ±65.2	159.38a ±15.2	0.25a ±0.0073	309.9b ±10.04	124.54a ±7.66	0.4044a ±0.0389
% (+/-)	(+25.47)	(-45.157)	(-60.544)	(+45.26)	(-36.4)	(-52.2)	55.044	(-5.05)	(-40.8)
$LSD_{0.05}$	125.622	39.049	0.135	198.829	233.029	0.343	145.068	117.264	0.882

3. DISCUSSION

Salinity significantly decreases the growth and development of crops [16]. Seed germination resulted increment at little level of salt stress (60 mM). Throughout seedling growth, it was witnessed that fresh and dry weight of the root and shoot was improved at reduced salinity's level but decrease at high level. The decrease in fresh and dry weight due to salt stress was previously reported by [17]. Plants that were grown under both salinity as well as ascorbic acid resulted boost in overall germination and seedlings growth as compared with the control. It was known that ascorbic acid cause reduction in harmful effects of salinity on germination and seedlings growth [18]. Plants treated with distinct NaCl concentration resulted decline in chlorophyll-a, total chlorophyll and chlorophyll a/b ratio. The high salinity instigated reduction in chlorophyll contents [19].

Ascorbic acid treatment (100 ppm) of the plants results in overall increase in total chlorophyll, chlorophyll a and a/b ratio in saline medium (60 mM NaCl) as well as in the control

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medium. Similarly, plant treated with 200ppm ascorbic acid also resulted increase in chlorophyll-a, total chlorophyll and chlorophyll a/b ratio on 60mM NaCl medium as compared with control. Ascorbic acid reduces the pessimistic effects of salinity on chlorophyll a and b concentration [20]. Our result showed that without salinity the foliar application of ascorbic acid led to increase biochemical concentration particularly at higher concentration. Increase in this regard, seems to be more obvious on chlorophyll a as compared to chlorophyll b and carotenoids [21]. Different concentrations of NaCl reduced Carbohydrates level in plants. Plants treated with 100 and 200ppm concentration of ascorbic acid exogenously applied though roots and as foliar spray showed decrease in carbohydrates content in saline media. Plants treated with different concentration of NaCl showed decrease in protein level and this reduction is more apparent at high salinity level. Similarly, plants treated with 100 and 200ppm ascorbic acid exogenously applied though roots and as foliar spray exhabited reduction in overall protein level in saline media. Plants treated with different NaCl concentration showed boost in Na+ level in root, stem and leaves, however K+ level and K+/Na+ ratio of stem and roots was reduced. Plants treated with ascorbic acid (100ppm) resulted boost in K+ level in root at both low along with high salinity stress (100mM).

4. **Materials and Methods**

4.1 Germination studies

Set-I

The study on germination of *Solanum melongena* was conducted on distinct salinity levels from Control, 34 mM NaCl (EC.4.5mS/cm), 68 mM NaCl (EC.7.4), 100 mM NaCl (EC.8.5) and 136 mM NaCl (EC.9.13) solutions. Seeds were sterilized on 75% ethanol and imbibed in distilled water for half an hour before keeping for germination. Imbibed seeds was put in sterilized Petri plates along with filter paper discs used to avoid any possible contamination. Triplicates were used for each treatment with ten seeds placed per Petri plate. Three ml of distilled water was pipette out to moisten each filter paper disc. The numbers of germinating seeds were recorded daily. Experiment was terminated after fifteen days.

Set-II

In the second set of experiment seeds of *Solanum melongena* was germinated by using distinct NaCl and different concentrations of ascorbic acid. Distinct sets for germination are given below.

Set-I=Control (non-saline), 60mM NaCl, 100 mM NaCl

Set-II=100 ppm ascorbic acid was used with different concentration of NaCl.

Set-III=200 ppm ascorbic acid was used with different concentration of NaCl.

4.2 Growth experiment

Each pot contained four Kilograms of soil, five sets of sixty pots were used and their detail is given below.

Set I: without Ascorbic Acid; non saline control and two NaCl treatments (60mM and 100mM.)

Set II: 100 ppm Ascorbic Acid plus (60mM and 100mM) NaCl applied in roots.

Set III: 200 ppm Ascorbic Acid plus (60mM and 100mM) NaCl given in roots.

Set IV:100 ppm Ascorbic Acid given as foliar spray plus (60mM and 100mM) NaCl

Set V: 200 ppm Ascorbic Acid given as foliar spray plus (60mM and 100mM) NaCl

Each set contain twelve pots, out of twelve pots in each set four replicates was used for each treatment i.e. control (E .C 0.05dS/m), 60 mM NaCl (E.C 5.76 dS/m) and 100 mM NaCl (E.C 7.47 dS/m). Three seedlings were transplanted in each pot and start irrigation with tape water. Seedlings were thinned to one/pot after establishment of seedlings and saline water irrigation started concentration of NaCl was slowly increase in irrigation water till it reach to desirable salinity. Each pot was irrigated with 1.5 Liter of tape water per NaCl solution two times a week. Doses of ascorbic acid were applied monthly in the soil by dissolving in irrigation water in set-II and set-III while applied as a foliar spray in set-IV and set-V.

4.3 Biochemical concentration

4.3.1 Chlorophyll Estimation

Chlorophyll was estimated in the leaf samples collected from non saline control treated plants by the method of [12].

4.3.2 Carbohydrates Estimation

The estimation of carbohydrate in the leaf samples collected from plants of different sets was conducted as outlined by [13].

4.3.3 Proteins estimation

The estimation of protein in the leaf samples collected from all treated plants was conducted as outlined by [14].

4.3.4 Micronutrients estimation

The estimation of different micronutrients in the leaf samples collected from all treated plants was conducted as outlined by [15].

4.4 Statistical analysis

Analysis of all the data was conducted by means of Costat 6.33 (Cohort, California, USA) software. The mean value and percent promotion (+) and reduction (-) were find out according to new duncan's multiple range test ($P < 0.05$).

5. Conclusions

Salinity has negative effects on seed germination, different biochemical concentrations and micronutrients that were significantly overcome by the usage of ascorbic acid as foliar spray and in roots through irrigation. Salt concentration resulted decrease in different biochemical constituents and micro nutrients while ascorbic acid applied through irrigation in roots and as a foliar spray at the concentration of 100 and 200 ppm resulted the enhancement of bio chemical concentration and micro nutrients as compared with their respective saline media. Salt stress had adverse effects on different bio chemicals concentrations and micro nutrients and they were significantly enhanced by the usage of ascorbic acid. It overcomes the dangerous effects of salt concentrations i.e. causes different metabolic changes in the plants which leads to boost up growth and development of plants. Ascorbic acid applications by both ways i.e. foliar spray and irrigation in roots mostly cause increased the concentrations of almost all the studied biochemical and micro nutrients concentrations. However, the application of ascorbic acid given through roots was more effective than application given as a foliar spray.

Author's contribution

MI compiled the data, GJ and FGJ designed the experiment and WM helped in writing and revising of manuscript.

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Conflict of Interest

The authors declare no conflict of interest amongst themselves.

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