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Article

Response of Duranta erecta L. Var. Variegata plants to the proline and ascorbic acid under irrigation by seawater

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Abstract

The study was carried out at the Faculty of Desert Agriculture and Environmental in Fouka, Matrouh University (Matrouh governorate) Egypt, during the two consecutive seasons of 2019/2020 and 2020/2021. The investigation aimed to study the response of *Duranta erecta* L. Var. Variegata plants to the proline concentrations (0.0, 40 and 80 ppm) and ascorbic acid concentrations (0.0, 100 and 200 ppm) under irrigation by seawater levels (0.0, 2000, 4000 and 6000 ppm) and their combinations on plant growth and some chemical constituents of *Duranta erecta* L. Var. Variegata plants. The study was designed in a split-plot design in random completely block design (RCBD) with three replications. In this experiment, the main plot was salt water levels and the sub-plot was spraying by proline and ascorbic acid treatments.

The obtained results showed that irrigation plants with salinity levels (2000, 4000 and 6000 ppm) significantly decreased growth parameters; number of branches, number of leaves per branch, leaves fresh weight per branch, stem diameter, leaf area, total leaf chlorophyll contents, total carotenoid contents, leaf relative water content, leaf proline content and some chemical constituents (N, P, Fe, Mn) compared to control. Moreover, the highest values were recorded by proline at 80 ppm followed by 200 ppm ascorbic acid in both seasons. This study showed that treated plants with proline and ascorbic acid alleviating the negative effects of salinity stress on duranta growth.

Keywords: Ascorbic acid, proline, Duranta, salinity stress

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1. Introduction

Durantaceae family includes *Duranta erecta* L. var. variegata **(Hiradate *et al*., 1999)**. Duranta is a little tree 1 to 3 m height **(Liogier, 1995)**. There are about 35 Duranta species with evergreen shrubs that can be found in tropical and subtropical areas **(Pipattanawong, *et al*., 2008))**. Salt stress is major abiotic stresses, its affect plant growth and crop production **(Mahajan and Tuteja, 2005)**.

Well-producing soil is a scarce resource in arid regions, especially in very dry desert environments. Mostly, these areas suffer from a scarcity of fresh water, so high-quality marginal water must be used for agriculture. Improper use of water of marginal quality as well as poor soil and water management leads to the degradation of prime lands in many countries through salinization of soils and reduced crop productivity. Salinity is one of the most severe environmental factors affecting plant growth **(Ramawat, 2010)**. Salt stress has a negative impact on plant species' morphological **(Nazar *et al*., 2011)**, and biochemical responses, so it is important to select plant varieties and species that are capable to tolerating high levels of salinity **(Hassanain *et al*., 2017)** or search for compounds that improve the growth of plants grown under saline conditions and determine the best concentrations**.** There are a lot of ways to improve tolerance to salinity in plants such as using ascorbic acid and amino acids **(Helaly *et al*., 2016)**. **Sharma *et al*. (2019)** investigated that ascorbic acid increase some biochemical constituents and productivity of many species of plants under salinity stress.

Ascorbic acid is a naturalist product; it relates with chloroplasts in the effects of oxidative stress of photosynthesis **(Latif *et al*., 2016)**. Moreover, ascorbic acid play role on cell division, protein modification and increased antioxidant enzyme activity in plant cells **(Rady, 2013)**.

Proline foliar spraying has been shown to help plants tolerant abiotic stress **(Ali *et al*., 2008)**. Proline plays a role in cell osmoregulation and protein protection during dehydration, as well as acting as an enzymatic regular under stress. Furthermore, under salinity stress, it is a prominent organic molecule responsible for osmotic adjustment mediation as well as stabilizing sub-cellular structures that might be called an energy bowl and a stress-related signal **(Rontein *et al*., 2002)**.

This work aimed to study the response of *Duranta erecta* L. Var. Variegata plants to the proline and ascorbic acid under irrigation by seawater**.**

2. Materials and Methods

This study was carried out during two successive seasons of (2019-2020) and (2020-2021) at the Faculty of Desert Agriculture and Environmental in Fouka, Matrouh University (Matrouh governorate) Egypt, to study the effects of ascorbic acid and proline on the growth, some chemical composition of *Duranta erecta* L Var. Variegata plants under salinity stress condition. Plants with 70cm height and 5 branches, plant were used. One year old plants were planted in a soil mixture of 50% sandy soil from Matrouh and 50% clay soil. Physical and chemical analysis of soil is shown in **Table (1)**.

Treatments and cultural aspects: four levels of seawater irrigation (tap water, 2000, 4000 and 6000 ppm), and two anti-stress compounds: proline at 40 and 80 ppm and ascorbic acid at 100 and 200 ppm were used. Plants sprayed with anti-stress compounds for four months only starting from the beginning of the experiment and reapeated every two weeks, plants sprayed with the ascorbic acid and proline until the run off point at the morning, plants irrigated twice a week with different levels of salinity until May, where irrigation rate increased to 3 time per week. Plants irrigated with tap water once every two weeks.

Average of temperature and relative humidity during the experiments presented in **Table (2)**.

Table 1: Physical and chemical analysis of the used soil

Physical properties	Chemical properties	
	pH	8.34
Clay: 21.78 %	E.C (ds/m)	8.20
Silt: 11.54 %	O.M %	1.5
Sand: 66.68 %	$CaCo_3$%	24.53
Soil type: Sandy clay loam.	Hco_3^- (meq L^{-1})	4.7
	Cl^- (meq L^{-1})	90.1
	$SO4^{--}$ (meq L^{-1})	38.5
	Ca^{++} (meq L^{-1})	36.8
	Na^+ (meq L^{-1})	65.2
	Mg^{++} (meq L^{-1})	28.4
	K^+ (meq L^{-1})	2.9

Estimated data

The data was collected twice, once in April after the completion of the spraying (proline and ascorbic acid) as first cut and once in August as second cut.

Number of branches per plant and **number of leaves per branch** were recorded.

Leaves fresh weight per branch (g) and **stem diameter (mm)** were measured.

Leaf area (cm^2) was determined as described by **Zidan (1962).**

Leaf relative water content (LRWC) was calculated according to the methods of **Yamasaki and Dillenburg (1999)**.

Leaf proline content was determined in the leaf according to the method of **Bates *et al.* (1973).**

Total leaf chlorophyll was determined according to **Moran (1982**)

Total carotenoid contents were extracted using the method described by **Guan *et al.* (2005)**.

Carotenoids (C_K) =4.7 A_{440} – 0.27 C_{A+B} = µg/mL.

Table 2: Average of monthly temperature (ºC) and relative humidity (R.H.) % measured during the growing seasons of *Duranta erecta* L Var. Variegata plants

Month	first season 2019-2020				second season 2020-2021			
	Max temp. (°C)	Min temp. (°C)	Max humidity (%)	Min humidity (%)	Max temp. °C)	Min temp. (°C)	Max humidity (%)	Min humidity (%)
Dec	24.09	11.85	74.32	36.74	21.98	13.17	77.57	37.83
Jan	17.01	9.59	75.97	40.55	21.11	11.69	84.20	38.23
Feb	19.44	11.57	80.59	39.79	22.06	11.63	86.56	34.39
Mar	22.90	13.75	77.03	32.84	23.81	12.04	73.27	30.40
Apr	26.69	16.15	77.87	24.47	28.97	12.82	72.31	20.03
May	32.22	19.86	71.19	20.00	35.24	20.91	68.20	19
Jun	34.22	22.07	71.17	22.00	33.91	21.88	77.03	25.93
Jul	34.70	24.14	81.45	30.19	36.52	24.09	79.07	26.93
Aug	35.58	24.86	80.55	26.35	38.17	27.17	82.9	23.5

Nitrogen percentage (%) was determined by modified micro Kieldahl method as described by **Evenhuis and Waard (1980)**.

Phosphorus content of leaves (%) was estimated as described by **Murphy and Riley (1962)**.

Manganese and iron content of leaves (%) were estimated as the method of **Benton (2001).**

Experimental Layout and Statistical analysis

The experiment was designed in a split-plot design in RCBD with three replications. Salt water levels as main plot and the sub-plot contained proline and ascorbic acid treatments. Plants treated with 4 levels of salt water (0. 2000, 4000 and 6000 ppm) and (control, 2 rates proline and 2 rates ascorbic acid) with three replicates. Total of 240 plants involved in this experiment (4 levels of salinity × 5 concentrations of anti-stress compounds) and 4 pots for each pot. The collected data from plants during the seasons (from December 2019 until August 2020 and December 2020 until August 2021) in this study were subjected to analysis of variance (ANOVA) using CoStat program. Least significant difference (LSD) was used at 0.05 level of probability to test differences between treatments. Data were analyzed according to **Gomez and Gomez (1984)**.

RESULTS:

The vegetative growth

Results presented in **Tables 3 , 4** showed that all studied vegetative growth traits; number of branches per plant, number of leaves per branch, leaf area, fresh weight of leaves per branch and stem diameter significantly decreased gradually with increasing salinity level in the absence of anti-stress compounds for both cuttings and seasons in most cases. 6000 ppm of seawater gave the lowest values and reduced all vegetative growth traits in the first cut by 27.2 and 34.8 % for number of branches per plant, 35.5 and 37.7 % for number of leaves per branch, 56.8 and 56.8 % for leaves fresh weight per branch, 45.9 and 49.5 % for leaf area per branch and 28.4 and 27.6 % for stem diameter. When the plant continued to be irrigated with salt water at 6000 ppm but spraying with ascorbic acid and proline were stoped, all plants died before second cut.

All anti-stress compounds (ascorbic acid and proline) concentrations increased significantly all vegetative growth in both seasons and cutttings. Plants sprayed with 80 ppm of proline gave the best results for all vegetative traits with significant effects compared to control for both seasons and cutting. Th second best treatment to stimulate plant growth was ascorbic acid at 200 ppm for both season and cutting. Proline at 80 ppm increased number of branches per plant as the mean effects by 28.4, 28.1, 59.3 and 59.5 % for first and second cut of first and second season respectively; it increased number of leaves per branch by 68.5, 64.2, 39.8 and 40.6 % for first and second cut of both seasons respectively.

With respect to leaves fresh weight per branch, the increamrnt reached to 99.6, 100.7, 181.1 and 183.1 % for first and second cut of both seasons respectively. At the same time, treatment with 80 ppm proline increased leaf area per branch as the mean effects by 109.6, 110.9, 103.8 and 104.67 % for first and second cut of both seasons respectively. The increased in stem diameter, reached 27.2, 29.2, 39.0 and 42.0 % in the first and second season of both seasons respectively.

Table 3: Effects of ascorbic acid and proline and their interaction treatments on number of branches per plant and number of leaves per branch of *Duranta erecta L Var*. Variegata plants under salinity stress condition at 2019/2020 and 2020/2021

	2019/2020 season						**2020/2021 season**					
Saline water level (ppm)	**Proline (P) and Ascorbic (As) concentration (ppm)**						**Proline (P) and Ascorbic (As) concentration (ppm)**					
	0.0	**As 100**	**As200**	**P40**	**P80**	**Means (S)**	**0.0**	**As 100**	**As200**	**P40**	**P80**	**Means (S)**
	Number of branches per plant											
	First cut						**First cut**					
0.0	7.33	8.00	8.00	8.33	9.67	8.27	7.67	8.33	8.00	8.33	9.67	8.40
2000	7.00	7.33	7.67	8.67	9.67	8.07	7.33	8.00	8.33	8.67	9.67	8.40
4000	7.33	7.67	7.33	8.00	9 00	7.87	7.33	7.67	7.67	8.33	9.33	8.07
6000	5.33	6.00	5.33	6.33	6.33	5.87	5.00	6.00	6.00	6.33	6.33	5.93
Means	6.75	7.25	7.08	7.83	8.67		6.83	7.50	7.50	7.92	8.75	
LSD at 5%	S= 0.45*** F=0.44*** (S) * (F) = 0.89^{ns}						S= 0.44*** F=0.45*** (S) * (F) = 0.90^{ns}					
	Second cut						**Second cut**					
0.0	11.67	13.00	14.67	14.33	16.00	14.13	11.33	13.00	15.33	14.67	15.33	13.93
2000	9.33	13.33	15.67	16.33	16.00	13.93	9.00	12.67	15.33	16.67	15.67	13.87
4000	9.33	13.00	15.67	12.67	16.33	13.40	9.33	13.33	16.00	12.00	16.33	13.40
Means	10.11	13.11	15.33	14.44	16.11		9.89	13.00	15.56	14.44	15.78	
LSD at 5%	S= 0.80^{ns} F= 1.06*** (S) * (F) = 1.83*						S= 0.67^{ns} F= 1.22*** (S) * (F) = 2.10**					
	Number of leaves per branch											
	First cut						**First cut**					
0.0	7.67	10.96	12.21	12.28	11.2	10.86	8.00	11.63	12.92	12.97	10.64	11.23
2000	7.28	10.73	11.43	10.52	12.84	10.56	7.08	10.81	12.02	10.90	13.21	10.80
4000	6.33	10.09	10.88	11.02	12.49	10.16	6.67	10.38	10.85	11.02	12.47	10.28
6000	4.94	6.25	7.01	8.26	7.71	6.84	4.98	6.23	6.96	8.27	7.56	6.80
Means	6.56	9.51	10.38	10.52	11.06		6.68	9.76	10.69	10.79	10.97	
LSD at 5%	S= 1.55** F=0.82*** (S) * (F) = 1.63^{ns}						S= 1.66** F=0.81*** (S) * (F) = 1.62**					
	Second cut						**Second cut**					
0.0	8.51	10.03	10.56	8.58	12.15	9.97	8.34	9.65	10.51	8.59	11.95	9.81
2000	8.47	11.23	11.22	11.28	12.10	10.86	8.57	11.43	11.03	11.23	11.97	10.85
4000	8.25	11.25	10.60	10.15	11.03	10.26	8.36	11.4	10.95	10.62	11.59	10.58
Means	8.41	10.84	10.79	10.01	11.76		8.42	10.83	10.83	10.15	11.84	
LSD at 5%	S= 0.91^{ns} F= 0.63*** (S) * (F) = 1.10*						S= 0.63* F= 0.81*** (S) * (F) = 1.40^{ns}					

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

Leave relative water content (LRWC) %

Leave relative water content decreased gradually with increasing salinity level to reach the minimum value at 6000 ppm for the first cut of first and second seasons as shown in **Table 5.** The values reached 29.1 and 28.4

% respectively in the absence of spraying with anti-stress salinity compounds.

Spraying ascorbic acid and proline, at all concentrations increased significantly LRWC% in both seasons and cuttings. Proline at 80 ppm gave the highest value as the main effects and as interaction with all the salinity levels, followed by ascorbic acid 200 ppm. The increase was estimated at 18.1, 18.0, 16.6 and 12.7 % as a mean effects for first and second seasons respectively.

Chemical constiuents

Leave proline contents was increased gradually with increasing salinity stress levels as shown in **Table (5).** Irrigation with 6000 ppm of seawater gave the highest proline content compared to control in the first and second seasons, it increased proline by 569.8 and 580.9 % respectively.

At the same time, all ascorbic acid and proline increased leaf proline content in both seasons and cuttings. 80 ppm proline gave the highest values followed by 200 ppm ascorbic acid as the mean effects and at the level of salinity stress as interaction effects.

As shown in **Table (6)** all salinity stress levels decreased significantly chlorophyll (a), chlorophyll (b), total chlorophyll and total carotenoids in both cuttings. 6000 ppm of seawter was more effective; it decreased these traits in the absence of anti-stress by 34.5, 44.6, 36.6 and 46.1% respectively.

All ascorbic acid and proline concentrations increased these traits in both cuttings. 6000 ppm of seawater was the best treatment, the increase in the first and second cut reached 28.4 and 46.1 for chlorophyll (a), chlorophyll (b) by 73.6 and 65.8, total chlorophyll by 36.9 and 31.2 and carotenoids by 56.6 and 69.6.

Nitrogen, phosphorus, manganese and iron content of leaves

In the absence of anti-stress all salinity levels decreased significantly nitrogen %, phosphorus %, manganes and iron of leaves content in the both cuttings of the two seasons. 6000 ppm seawater irrigation gave the lowest values. On the other hand, all ascorbic acid and proline concentrations increased significantly these traits.

80 ppm proline was the best treatments and increased the nitrogen of leaves % by 86.9, 93.0, 55.8 and 50 % for first and second cut of first and second seasons respectively. It increased phosphorus of leaves % by 106.6, 106.6, 70.5, 75 % for first and second cut of first and second seasons respectively. Along the same line it increased manganese of leaves contents by 52.0, 33.7, 44.7, and 50 % respectively, and the increase in iron of leaves reached 38.9, 31.0, 37.5 and 27.5 ppm respectively **(Tables 7, 8)**.

DISCUSSION

Results showed that all vegetative growth decreased by increasing salinity stress. Salinity inhibits plant growth by causing osmotic stress and then ion toxicity as supported by (**Rahnama *et al*., 2010).**

The increment in all growth parameters and chemical composition after proline and ascorbic acid treatment was found. This result agrees with the results of **Gadallah *et al*. (2020**) and **Salem (2021)**. The positive effect of proline can be explained by the fact that proline is an amino acid that plays a critical role in plants under salinity stress condition, besides that proline

an excellent osmolyte. Proline serves as a metal chelator, an antioxidative defence molecule, and a signalling molecule during times of stress as explained by **Shamsula *et al*. (2012)**.

Table 4: Effects of ascorbic acid and proline and their interaction treatments on leaves fresh weight per branch (g), leaf area per branch (cm^2) and stem diameter (mm) of *Duranta erecta L Var*. Variegata plants under salinity stress condition at 2019/2020 and 2020/2021

Saline water level (ppm)	2019/2020 season						2020/2021 season					
	Proline (P) and Ascorbic (As) concentration (ppm)						Proline (P) and Ascorbic (As) concentration (ppm)					
	0.0	As 100	As200	P40	P80	Means (S)	0.0	As 100	As200	P40	P80	Means (S)
	Leaves fresh weight per branch (g)											
	First cut						**First cut**					
0.0	16.65	23.05	23.18	22.92	23.35	21.83	16.33	22.40	23.85	23.21	23.68	21.89
2000	14.06	20.14	24.25	28.48	22.16	21.82	14.07	20.19	24.26	22.18	28.14	21.77
4000	11.73	20.45	20.30	23.97	29.16	21.12	11.37	19.76	23.35	20.29	28.49	20.65
6000	7.19	10.17	10.75	10.17	18.14	11.28	7.04	10.51	10.56	10.66	17.63	11.28
Means	12.41	18.45	19.47	19.95	24.78		12.20	18.22	19.74	19.85	24.49	
LSD at 5%	$S= 2.84^{***}$ $F=2.37^{***}$ $(S) * (F) = 4.75^{ns}$						$S= 2.37^{***}$ $F=2.29^{***}$ $(S) * (F) = 4.58^{ns}$					
	Second cut						**Second cut**					
0.0	18.33	25.42	34.96	28.33	38.80	29.17	18.16	24.83	27.64	34.71	38.16	28.70
2000	15.07	29.91	41.99	43.02	50.06	36.01	15.32	29.91	41.68	42.54	49.89	35.87
4000	14.46	26.60	42.92	31.46	45.66	32.22	13.76	26.64	41.54	30.79	45.63	31.67
Means	15.95	27.31	37.75	36.48	44.84		15.74	27.13	36.95	36.01	44.56	
LSD at 5%	$S= 1.32^{***}$ $F= 2.99^{***}$ $(S) * (F) = 5.18^{***}$						$S= 1.49^{***}$ $F= 3.10^{***}$ $(S) * (F) = 5.37^{***}$					
	Leaf area per branch (cm^2)											
	First cut						**First cut**					
0.0	14.54	17.37	24.11	17.18	21.64	18.97	14.88	18.11	24.08	17.84	21.99	19.38
2000	9.81	14.38	24.34	21.07	26.28	19.17	9.85	14.38	24.33	21.06	25.73	19.07
4000	8.96	17.30	17.04	17.89	24.81	17.20	8.59	17.35	17.21	17.89	25.12	17.23
6000	7.86	9.19	10.17	9.77	13.56	10.11	7.51	9.07	10.43	10.06	13.34	10.08
Means	10.29	14.56	18.91	16.48	21.57		10.21	14.72	19.01	16.71	21.54	
LSD at 5%	$S=1.81^{***}$ $F=1.50^{***}$ $(S) * (F) = 3.00^{***}$						$S= 2.13^{***}$ $F=1.67^{***}$ $(S) * (F) = 3.34^{***}$					
	Second cut						**Second cut**					
0.0	15.48	16.23	24.68	16.55	20.00	18.59	14.98	16.59	25.39	16.62	18.61	18.44
2000	9.23	13.75	22.81	19.56	25.76	18.23	8.87	13.65	23.14	19.50	25.47	18.13
4000	8.62	16.19	15.72	17.15	22.19	15.97	8.22	16.12	15.42	17.15	21.56	15.70
Means	11.11	15.39	21.07	17.75	22.65		10.69	15.45	21.32	17.76	21.88	
LSD at 5%	$S= 1.28^{**}$ $F= 1.88^{***}$ $(S) * (F) = 3.25^{***}$						$S= 1.43^{*}$ $F= 1.78^{***}$ $(S) * (F) = 3.08^{***}$					
	Stem diameter (mm)											
	First cut						**First cut**					
0.0	4.85	4.83	5.95	5.85	5.26	5.35	4.85	4.76	6.04	5.91	5.27	5.37
2000	3.91	5.31	5.09	5.42	5.63	5.07	3.91	5.51	5.30	5.38	5.70	5.16
4000	3.44	4.10	4.36	4.64	5.19	4.35	3.54	4.15	4.57	4.67	5.57	4.50
6000	3.47	3.44	3.51	3.54	3.89	3.57	3.51	3.53	3.55	3.62	3.91	3.62
Means	3.92	4.42	4.73	4.87	4.99		3.96	4.87	4.88	4.90	5.12	
LSD at 5%	$S= 0.37^{***}$ $F=0.22^{***}$ $(S) * (F) = 0.43^{***}$						$S= 0.42^{***}$ $F=0.31^{***}$ $(S) * (F) = 0.62^{***}$					
	Second cut						**Second cut**					
0.0	8.71	9.79	10.85	8.10	12.08	9.91	8.70	9.70	10.31	9.35	11.94	10.00
2000	8.12	11.59	9.89	11.28	12.37	10.65	7.84	11.80	9.72	11.42	12.22	10.60
4000	8.73	10.26	9.96	9.82	11.08	9.97	8.87	10.26	10.16	9.82	11.92	10.21
Means	8.52	10.55	10.24	9.73	11.85		8.47	10.59	10.07	10.20	12.03	
LSD at 5%	$S= 0.35^{**}$ $F= 0.42^{***}$ $(S) * (F) = 0.73^{***}$						$S= 0.47^{ns}$ $F= 0.64^{***}$ $(S) * (F) = 1.10^{**}$					

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

Table 5: Effects of ascorbic acid and proline and their interaction treatments on leaf proline content (ppm) of *Duranta erecta L Var*. Variegata plants under salinity stress condition at 2019/2020 and 2020/2021

	2019/2020 season						**2020/2021 season**					
Saline water level (ppm)	**Proline (P) and Ascorbic (As) concentration (ppm)**						**Proline (P) and Ascorbic (As) concentration (ppm)**					
	0.0	**As 100**	**As200**	**P40**	**P80**	**Means (S)**	**0.0**	**As 100**	**As200**	**P40**	**P80**	**Means (S)**
	Leaf relative water content (LRWC) %											
	First cut						**First cut**					
0.0	91.82	93.52	94.98	92.01	95.24	93.51	91.05	93.40	94.14	92.94	93.53	93.01
2000	79.44	83.60	90.23	84.28	92.87	86.09	78.88	82.49	90.72	84.29	91.31	85.54
4000	71.77	79.66	90.23	80.90	93.13	83.14	71.53	83.30	90.72	83.23	92.96	84.35
6000	65.04	70.93	81.76	71.73	82.67	74.42	65.14	70.87	80.80	71.74	84.20	74.55
Means	77.02	81.93	89.30	82.23	90.98		76.65	82.52	89.10	83.05	90.50	
LSD at 5%	S= 2.41*** F=1.79*** (S) * (F) = 3.95***						S= 3.34*** F=1.79*** (S) * (F) = 3.76***					
0.0	89.32	90.40	92.56	91.46	93.55	91.46	90.47	91.01	92.89	90.52	90.10	90.99
2000	75.85	80.95	89.13	83.16	91.01	84.02	75.95	81.86	88.70	80.91	88.73	83.23
4000	68.83	80.52	88.86	74.19	88.46	80.17	70.65	82.44	87.13	80.04	88.42	81.73
Means	78.00	83.96	90.19	82.93	91.00		79.03	85.10	89.57	83.82	89.08	
LSD at 5%	S= 0.78*** F= 2.84*** (S) * (F) = 4.91***						S= 2.59** F= 1.37*** (S) * (F) = 2.37***					
	Leaf proline content (ppm)											
	First cut						**First cut**					
0.0	1.29	2.29	4.46	2.57	4.55	3.03	1.31	2.31	4.46	3.30	4.53	3.18
2000	5.23	5.35	6.43	5.49	6.67	5.83	5.23	5.36	4.85	5.51	6.66	5.52
4000	7.20	7.21	8.46	7.65	8.67	7.84	7.19	7.20	8.45	7.66	8.69	7.84
6000	8.99	9.00	9.58	9.11	10.04	9.34	8.92	9.01	9.37	9.09	9.93	9.26
Means	5.68	5.97	7.23	6.21	7.48		5.67	5.97	6.78	6.39	7.45	
LSD at 5%	S= 0.28*** F=0.28*** (S) * (F) = 0.56***						S= 0.44*** F=0.45*** (S) * (F) = 0.89**					
	Second cut						**Second cut**					
0.0	1.50	2.43	4.59	2.56	5.03	3.22	2.56	2.46	4.58	2.82	5.02	3.49
2000	5.24	5.45	6.76	5.66	6.72	5.97	5.27	5.68	6.77	5.91	6.72	6.07
4000	7.49	7.47	8.52	7.70	8.82	8.00	7.51	7.78	8.51	8.02	8.77	8.12
Means	4.74	5.12	6.62	5.31	6.85		5.11	5.31	6.62	5.58	6.84	
LSD at 5%	S= 0.11*** F= 0.33*** (S) * (F) = 0.57***						S= 0.45*** F= 0.51*** (S) * (F) = 0.88^{ns}					

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

Table 6: Effects of ascorbic acid and proline and their interaction treatments on Chlorophyll a, Chlorophyll b, total chlorophyll and total carotenoids contents µg/ml of *Duranta erecta L Var*. Variegata plants under salinity stress condition at 2020/2021

	2020/2021 season						**2020/2021 season**					
Saline water level (ppm)	**Proline (P) and Ascorbic (As) concentration (ppm)**						**Proline (P) and Ascorbic (As) concentration (ppm)**					
	0.0	**As 100**	**As200**	**P40**	**P80**	**Means (S)**	**0.0**	**As 100**	**As200**	**P40**	**P80**	**Means (S)**
	Chlorophyll a (µg/ml)						**Chlorophyll b (µg/ml)**					
	First cut						**First cut**					
0.0	23.37	23.61	26.10	23.86	27.44	24.88	6.76	7.96	9.64	6.69	10.06	8.22
2000	21.94	21.80	26.57	22.29	28.18	24.15	4.91	6.57	7.82	5.92	8.86	6.82
4000	22.29	23.29	26.85	21.98	28.67	24.61	4.01	7.37	8.14	7.66	8.21	7.08
6000	15.34	17.00	19.90	14.30	22.21	17.75	3.74	3.73	5.95	4.34	6.54	4.86
Means	20.73	21.43	24.85	20.61	26.62		4.85	6.41	7.89	6.15	8.42	
LSD at 5%	$S= 0.65^{***}$ $F=0.58^{***}$ $(S) * (F) = 1.16^{***}$						$S= 1.07^{**}$ $F=0.61^{***}$ $(S) * (F) = 1.23^{**}$					
	Second cut						**Second cut**					
0.0	21.21	23.17	26.36	21.33	26.98	23.81	6.60	5.49	8.21	5.32	8.57	6.84
2000	21.05	19.64	24.10	21.23	26.47	22.50	4.42	6.07	7.66	5.95	9.33	6.69
4000	12.50	22.01	23.71	22.22	26.57	21.40	4.98	5.81	7.19	6.08	8.63	6.54
Means	18.25	21.61	24.72	21.59	26.67		5.33	5.79	7.69	5.78	8.84	
LSD at 5%	$S= 1.95^{ns}$ $F= 1.63^{***}$ $(S) * (F) = 2.82^{***}$						$S= 0.63^{***}$ $F= 0.45^{***}$ $(S) * (F) = 0.78^{***}$					
	Total chlorophyll (µg/ml)						**Total carotenoid (µg/ml)**					
	First cut						**First cut**					
0.0	30.12	31.58	35.74	30.55	37.50	33.10	5.96	6.20	6.76	6.48	7.84	6.65
2000	26.84	28.37	34.39	28.21	37.04	30.97	4.86	5.11	8.05	4.95	7.99	6.19
4000	26.30	30.66	34.99	29.64	36.88	31.69	3.14	5.03	7.47	5.14	7.96	5.75
6000	19.07	20.73	25.85	18.91	28.75	22.66	3.21	3.48	3.82	3.12	3.10	3.35
Means	25.59	27.84	32.74	26.83	35.04		4.29	4.96	6.53	4.92	6.72	
LSD at 5%	$S= 1.49^{***}$ $F=1.03^{***}$ $(S) * (F) = 2.06^{ns}$						$S= 0.48^{***}$ $F=0.29^{***}$ $(S) * (F) = 0.58^{***}$					
	Second cut						**Second cut**					
0.0	27.83	28.69	34.57	26.65	35.44	30.64	4.26	5.86	5.99	5.04	6.46	5.52
2000	25.47	25.71	31.63	27.42	35.47	29.14	4.10	4.12	7.22	3.85	6.09	5.07
4000	17.47	28.35	30.90	28.34	34.70	27.95	3.02	4.37	6.13	4.29	6.73	4.91
Means	23.59	27.58	32.37	27.47	35.20		3.79	4.78	6.45	4.39	6.43	
LSD at 5%	$S= 1.84^{ns}$ $F= 1.03^{***}$ $(S) * (F) = 1.78^{**}$						$S= 0.31^{*}$ $F= 0.50^{***}$ $(S) * (F) = 0.87^{**}$					

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

The anti-stress prevents and decreases the breakdown of protein in the cell and continues the vitality of the plant, as found by **Mattioli *et al.* (2009)**. Ascorbic acid increases indole acetic acid (IAA) content, which accelerates cell division and/or cell enlargement, which improves plant development. The increase in stem diameter can be attributed to changes in photosynthetic activity and water relationship properties, as well as decreased stem elongation **(Ben Ahmed *et al.*, 2011)**.

Proline ability to promote the synthesis of chloroplast pigments could be owing to its antioxidant properties, which make it one of the protective systems for chloroplast pigments **(El-Lethy *et al.*, 2013**).

Enhanced carotenoids, which may act as a free radical scavenger, may be responsible for the increased chlorophyll content and improved plant capacity to decrease the detrimental effects of ROS associated with proline treatment this agreement with **Abdallah *et al*. (2020)**.

According to **Khan *et al*. (2011)** foliar sprays of ascorbic acid stimulated chlorophyll synthesis, which resulted in an increase in photosynthetic metabolites, resulting in the accumulation of different fractions of soluble sugars and nitrogen content in plant tissues under saline conditions and possibly alleviating the inhibitory effects of salinity.

Table 7: Effects of ascorbic acid and proline and their interaction treatments on nitrogen of leaves (%) and phosphorus of leaves (%) of *Duranta erecta L Var*. Variegata plants under salinity stress condition at 2019/2020 and 2020/2021

	2019/2020 season						**2020/2021 season**					
Saline water level (ppm)	**Proline (P) and Ascorbic (As) concentration (ppm)**						**Proline (P) and Ascorbic (As) concentration (ppm)**					
	0.0	**As 100**	**As200**	**P40**	**P80**	**Means (S)**	**0.0**	**As 100**	**As200**	**P40**	**P80**	**Means (S)**
	Nitrogen of leaves (%)											
	First cut						**First cut**					
0.0	1.88	2.06	2.13	2.03	2.26	2.07	1.71	1.94	2.03	1.94	2.31	1.98
2000	1.18	1.68	1.96	1.60	2.17	1.72	1.36	1.79	1.99	1.70	2.27	1.82
4000	0.84	1.55	2.08	1.71	2.18	1.67	0.81	1.38	2.06	1.72	2.29	1.65
6000	0.70	1.17	2.06	1.54	2.01	1.50	0.73	1.46	2.35	1.53	2.02	1.62
Means	1.15	1.61	2.05	1.72	2.15		1.15	1.64	2.11	1.72	2.22	
LSD at 5%	$S= 0.12^{***}$ $F=0.10^{***}$ $(S) * (F) = 0.19^{***}$						$S= 0.25^{*}$ $F=0.20^{***}$ $(S) * (F) = 0.40^{*}$					
	Second cut						**Second cut**					
0.0	1.92	1.98	2.24	2.03	2.44	2.12	1.62	1.90	2.05	1.92	2.20	1.94
2000	1.42	1.80	2.20	1.89	2.07	1.88	1.76	1.82	2.26	2.01	2.11	1.99
4000	0.95	1.47	2.05	1.73	2.22	1.68	0.95	1.56	1.98	1.78	2.17	1.69
Means	1.43	1.75	2.16	1.88	2.24		1.44	1.76	2.10	1.90	2.16	
LSD at 5%	$S= 0.07^{***}$ $F= 0.16^{***}$ $(S) * (F) = 0.27^{**}$						$S= 0.30^{ns}$ $F= 0.14^{***}$ $(S) * (F) = 0.24^{**}$					
	Phosphorus of leaves (%)											
	First cut						**First cut**					
0.0	0.24	0.22	0.33	0.24	0.33	0.27	0.18	0.20	0.32	0.25	0.35	0.26
2000	0.12	0.21	0.31	0.22	0.31	0.23	0.17	0.24	0.32	0.21	0.31	0.25
4000	0.13	0.18	0.32	0.23	0.32	0.23	0.12	0.18	0.24	0.23	0.31	0.22
6000	0.10	0.12	0.18	0.18	0.29	0.17	0.13	0.15	0.24	0.21	0.26	0.20
Means	0.15	0.18	0.28	0.22	0.31		0.15	0.19	0.28	0.22	0.31	
LSD at 5%	$S= 0.03^{**}$ $F=0.03^{***}$ $(S) * (F) = 0.06^{ns}$						$S= 0.02^{**}$ $F=0.05^{***}$ $(S) * (F) = 0.10^{ns}$					
	Second cut						**Second cut**					
0.0	0.24	0.21	0.34	0.22	0.32	0.27	0.19	0.25	0.33	0.22	0.27	0.25
2000	0.12	0.20	0.30	0.24	0.27	0.22	0.17	0.18	0.24	0.24	0.29	0.22
4000	0.15	0.20	0.29	0.23	0.28	0.23	0.12	0.21	0.30	0.21	0.28	0.22
Means	0.17	0.20	0.31	0.23	0.29		0.16	0.21	0.29	0.22	0.28	
LSD at 5%	$S= 0.02^{**}$ $F= 0.04^{***}$ $(S) * (F) = 0.07^{ns}$						$S= 0.09^{ns}$ $F= 0.04^{***}$ $(S) * (F) = 0.07^{ns}$					

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

Proline enhancing osmotolerance and/or regulating numerous processes such as nutrient absorption from soil solution, spraying plants with proline led to an increase in the concentrations of ions in the leaf of Duranta, this supported by the finding of **Sadak and Dawood (2014)**.

Spraying ascorbic acid on plants works on biostimulation inside plants and overcoming abiotic stress due to the positive effect of ascorbic acid on root growth, which led to an increase in the absorption of nutrients such as manganese, which increases the efficiency of the process of photosynthesis in a plant **(Abdel-Hafeez *et al*., 2019**; **Hussein and Alva, 2014)**. About 140 enzymes use iron (Fe) as a cofactor to conduct specific biological reactions **(Brittenham, 1994)**. As a result, iron plays a key role in plant growth and development, such as thylakoid synthesis, chlorophyll production and chloroplast development **(Miller *et al*., 1995)**. The obtained results are confirmed with the results of **Abdelkader *et al*. (2019)**, **Nassar *et al*. (2019), Ibrahim *et al*. (2019)** and **Behairy *et al*. (2017).**

CONCLUSION

Spraying *Duranta erecta L Var*. *Variegata* plants with proline at 80 ppm improved, plant growth as morphological and chemical constituents under salinity stress condition

Table 8: Effects of ascorbic acid and proline and their interaction treatments on manganese of leaves (ppm) and iron of leaves (ppm) of *Duranta erecta L Var*. Variegata plants under salinity stress condition at 2019/2020 and 2020/2021

	2019/2020 season						**2020/2021 season**					
Saline water level (ppm)	**Proline (P) and Ascorbic (As) concentration (ppm)**						**Proline (P) and Ascorbic (As) concentration (ppm)**					
	0.0	**As 100**	**As200**	**P40**	**P80**	**Means (S)**	**0.0**	**As 100**	**As200**	**P40**	**P80**	**Means (S)**
	Manganese of leaves (ppm)											
	First cut						**First cut**					
0.0	33.87	39.64	48.03	40.73	50.77	42.61	33.52	39.66	46.67	41.06	49.40	42.06
2000	31.54	35.39	45.69	37.03	49.57	39.84	32.16	36.40	45.11	37.37	49.36	40.08
4000	29.42	33.29	44.84	32.99	49.63	38.03	28.93	33.60	43.84	34.01	49.28	37.93
6000	24.59	28.66	32.42	29.63	31.58	29.38	23.89	28.67	32.19	28.60	30.91	28.85
Means	29.85	34.25	42.75	35.09	45.39		29.63	34.58	41.95	35.26	44.74	
LSD at 5%	S= 0.54** F=1.08*** (S) * (F) =2.16***						S= 1.11** F=1.93*** (S) * (F) = 3.87**					
	Second cut						**Second cut**					
0.0	31.39	35.35	45.29	38.03	45.37	39.08	31.01	37.43	44.64	39.71	45.48	39.65
2000	30.25	33.68	43.46	34.67	42.35	36.88	29.51	36.34	43.46	36.37	44.02	37.94
4000	26.91	31.63	41.01	30.32	40.37	34.05	26.95	31.61	42.01	32.68	41.72	35.00
Means	29.51	33.55	43.25	34.34	42.69		29.16	35.13	43.37	36.25	43.74	
LSD at 5%	S= 2.25** F= 1.62*** (S) * (F) = 2.81^{ns}						S= 0.37*** F= 1.72*** (S) * (F) = 2.99^{ns}					
	Iron of leaves (ppm)											
	First cut						**First cut**					
0.0	109.81	116.66	140.69	116.50	147.22	126.18	108.50	120.24	141.45	119.54	149.67	127.88
2000	104.20	114.64	138.02	126.28	146.16	125.86	105.82	120.14	132.88	127.95	138.55	125.07
4000	94.91	116.37	132.56	115.45	147.02	121.26	102.28	115.77	132.62	119.14	132.37	120.44
6000	92.31	101.35	121.07	103.89	117.06	107.14	96.71	109.78	124.23	110.72	120.96	112.48
Means	100.31	112.26	133.08	115.53	139.37		103.33	116.48	132.80	119.34	135.39	
LSD at 5%	S= 1.77*** F=1.30*** (S) * (F) =2.61***						S= 4.89** F=4.63*** (S) * (F) = 9.25^{ns}					
	Second cut						**Second cut**					
0.0	106.02	117.95	134.88	120.13	144.06	124.61	107.49	119.64	131.43	121.84	139.75	124.03
2000	107.14	119.74	132.84	120.47	140.91	124.22	106.33	121.82	129.14	127.90	133.19	123.67
4000	94.80	117.39	127.47	117.15	138.72	119.11	99.64	113.21	130.22	117.20	127.03	117.46
Means	102.66	118.36	131.73	119.25	141.23		104.49	118.22	130.26	122.32	133.32	
LSD at 5%	S= 1.08*** F= 1.93*** (S) * (F) = 3.34**						S= 4.12* F= 4.29*** (S) * (F) = 7.43^{ns}					

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

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