Ascorbic acid and Proline alleviat the adverse effects of salinity stress in *Duranta erecta* L. var. *Variegata* plants

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Abstract

The present study was carried out at the Faculty of Desert Agriculture and Environmental in Fouka, Matrouh University (Matrouh governorate) Egypt, during the two successive seasons of 2019/2020 and 2020/2021. This investigation aimed to study the effects of ascorbic acid (0.0, 100, 200 ppm) and proline (0.0, 40, 80 ppm) on alleviating the adverse effects of salinity stress at 0.0, 2000, 4000, 6000 ppm seawater in *Duranta erecta* L. var. *Variegata* plants. The experiment 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 results showed that treated plants with proline at 80 ppm and ascorbic acid at 200ppm enhanced plant height, leaves dry weight, leaf green color degree (SPAD units), plant survival percentage, stomata measurement, potassium of leaves and antioxidant such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), cytochrome b5, cytochrome P450 (CYP450), amidopyrine N-demethylase and aniline 4-hydroxylase activity. Moreover, treatments decreased thiobarbituric acid reactive substances (TBARS), sodium and chloride. Finally, proline and ascorbic acid alleviate the negative effects of salinity and increase *Duranta* plant growth and plant survival percentage by enhancing the antioxidant enzymes, potassium cations and photosynthetic system, decreasing DNA breakage %, harmful ions eg: sodium and chloride.

Introduction

Duranta erecta L. var. *Variegata*, belongs to the family Verbenaceae, is a small tree or a vigorous shrub and a sprawling shrub. It is planted frequently for the beauty of white and green leaves and blue tubular flowers appearances almost all year round (Huxley et al. 1992). It is grown in tropical and subtropical areas and used for accent plants (Pipattanawong et al. 2008).

Salinity reduces plant growth, exposure of plants for long periods to salinity or severe salinity leads to death of plants. Salinity is a major concern in arid and semi-arid regions, especially the region that have not enough amount of rain to wash the soil from salts, and is often exacerbated by climate change (Ramawat 2010). Salinity also modifies gene expression (Soussi et al. 2001) increased the levels of reactive oxygen species (ROS) (Apel and Hirt 2004), chlorophyll breakdown (Kato and Shimizu 1987) and many other effects such as leaf senescence (Allu et al. 2014). Gene expression leads to the emergence of new proteins that are not present before the stimulation process. It is known that in recent time's fresh water resources have been greatly depleted, so it is necessary to try to use different alternative sources, such as desalinated water or ground water that suffers from salinity. Salinity problems have become more prevalent in the past ten years, using of alternative non-potable water become a major contributor to irrigation (Marcum 2006). From the above, it is important to choose tolerant plant varieties and species or search about some substances can help plants to tolerate high levels of salinity (Hassanain et al. 2017). Several enzymes help detoxify reactive oxygen species. Superoxide dismutase is the first defense enzyme that converts superoxide into H₂O₂ (Sairam et al. 2005).

Ascorbic acid (C₆H₈O₆) is a small molecular soluble in water and insoluble in other solvents as chloroform, benzene and fats. Ascorbic acid acts as an antioxidant, working to remove enzymatic toxins such as H₂O₂ and other harmful factors that affect plant photosynthesis (Galal et al. 2000). It is a reductant and reacting with and/or scavenging many types of free radicals. Ascorbic acid reacts non-enzymatically with superoxide, hydrogen peroxide and singlet oxygen (Pourcel et al. 2007). Proline is an amino acid that preserves the plant cell under conditions of drought and salinity where it prevents and reduces the breakdown of protein in the cell and maintains the vitality of the plant. Proline acts as a visceral osmotic solute and as a source of energy and nitrogen, so proline has a role in reducing the harmful effects of salt stress (Venkatesan and Chellappan 1998).

This investigation aimed to study, the effects of ascorbic acid and proline on alleviating the adverse effects of salinity stress in *Duranta erecta* L. var. *Variegata* plants.

Material And Methods

The present 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 investigate the effects of proline and ascorbic acid on the growth, some chemical and biochemical composition of *Duranta erecta* L. Var. *Variegata* plants under salinity stress condition. One year old plants were obtained from commercial (private) nursery for ornamental plants in Marsa Matrouh city. The plants had a uniform sizes and shapes, with 5 branches / plant and 70 cm in height were used. One plant / pot was cultivated. The used soil is a mixture of 50% sandy soil (the available soil in the farm of Faculty of Desert Agriculture and Environmental in Fouka, Matrouh University and 50% clay soil. Physical and chemical soil analysis is shown in Table (1).

Treatments and cultural aspects: four concentrations of the seawater irrigation (tap water, 2000, 4000 and 6000 ppm), and two anti - salt stress materials: proline at 40 and 80 ppm and ascorbic acid at 100 and 200 ppm were used. Plants were sprayed with anti-salt stress materials and spraying with tap water as a control until run off points. *Duranta* plants were planted in pots of 30 cm diameter on 20th of December 2019 in the first season and 20th of December 2020 in the second season. Plants sprayed directly with anti-stress materials repeated every two weeks for 4 months. Irrigation was carried out twice a week with saline water and irrigated with tap water once every two weeks. Plants were irrigated three times a week from May to the end of the experiment (20 August in both seasons) with saline water and irrigation with tap water once every two weeks until 20th of August. On the other hand, spraying plants with anti-salt stress compounds was stopped after the first cut (four months from the beginning of the experiment) while irrigation with seawater concentration was continued to the end of the experiment.

Table 1
Physical and chemical analysis of the soil

Physical properties	Chemical properties
Clay: 21.78%	pH 8.34
Silt: 11.54%	E.C (ds/m) 8.20
Sand: 66.68%	O.M % 1.5
Soil type: Sandy clay loam.	CaCO ₃ % 24.53
	HCO ₃ ⁻ (meq L ⁻¹) 4.7
	Cl ⁻ (meq L ⁻¹) 90.1
	SO ₄ ⁻ (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

Recorded data

data was collected twice per season, after four and eight months for the first and second cut respectively.

Plant height (cm) was measured.

Leaves dry weight per branch (g) leaves dry weight was estimated after dried on oven at 70°C for 72 hours.

Plant survival was estimated as the percentage of plants survival (how many plants kept growing to that failed).

Leaf green color degree (SPAD units)

It was determined on the fresh leaves as indicator to chlorophyll content. The determination was done according to the method described by **Yadawa (1986)** using Minolta SPAD Chlorophyll Meter model-502.

Stomata measurements

Number, length and diameter of stomata were calculated as the methods of **Dore (1986)**. Individual leaves (4th leaf) were first removed from stem. Leaf a baxial epidermis cleaned using a degreased cotton ball and then carefully smeared with nail varnish in the mid-area between the central vein and leaf edge for approximately 20 min. Thin film was peeled off from the leaf surface, placed into a microscope slide after one drop of tap water was added then the cover glass closed. Under a light microscope, number of stomata was counted, as well as length and diameter of three stomata per leaf were measured magnifying by 100 × 10 (objective × oculars). Values obtained were multiplied by 1.25; a coefficient obtained by adjusting the ocular micrometer.

Potassium contents of leaves (%)

Potassium percentage was determined photometrically in the acid digested samples by using flam photometer as described by **Page et al. (1982)**.

Sodium contents of leaves (%)

Sodium percentage was determined according to **Piper. (1967)**.

Chloride contents of leaves (%)

Chloride percentage was determined according to **Piper. (1967)**.

Determination of antioxidant enzymes:

Enzyme extraction

Leaves were taken to estimate the enzymes. Once after 120 days and once again after 240 days from treatment. Sample preparation was done as described by **Mukherjee and Choudhuri (1983)**. A fresh sample (250 mg) was frozen in liquid nitrogen and finely ground by pestle in a chilled motor, the frozen powder was added to 10 ml of 100mM phosphate buffer (KH₂PO₄ /K₂HPO₄, pH 7.0) containing 0.1mM Na₂EDTA and 0.1 g of polyvinylpyrrolidone (PVP), the homogenate was filtered through cheese cloth then centrifuged at 15000 g for 10 min. The supernatant was recentrifuged at 18000 g for 10 min; the resulted supernatant was collected and stored at 4 °C for assay of superoxide dismutase (SOD), catalase (CAT), thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), cytochrome b5, cytochrome P450 (CYP450), amidopyrine N-demethylase and aniline 4-hydroxylase activity.

Thiobarbituric acid reactive substances(TBARS)were measured as described by **Tappel and Zalkin (1959)**.

Reduced glutathione(GSH) was determined according to the method described by **Ellman (1959)**.

Catalase (CAT; EC 1.11.1.6) was determined according to the method described by **Sinha (1972)**.

Superoxide dismutase (SOD, EC 1.15.1.1) was assayed in plant tissue extracts according to the method described by **Nishikimiet al. (1972)**.

Determination of oxidative stress and biomarkers

Assay of cytochrome b5 and cytochrome P450 were estimated by the method of **Omura and Sato (1964)**.

The activity of amidopyrine N-demethylase was measured according to **Nash (1953)**.

The activity of aniline 4-hydroxylase was measured according to **Kato and Gillette (1965)**.

The diphenylamine DNA fragmentation percentage assay is a very useful method for measuring apoptosis by determining the percentage of fragmentation of DNA into oligosomal-sized fragments. Plants DNA breakage was conducted using the procedure of **Wu et al. (2006)**.

Experimental layout and statistical analysis

The experiment was designed in a split-plot design in RCBD with three replications. In this experiment, salt water levels expressed as the main plot and the sub-plot was spraying by proline and ascorbic acid treatments. Each experiment was included 20 treatments, 4 levels of salt-water irrigation and 5 treatments of anti-stress compounds (control, 2 rates proline and 2 rates ascorbic acid). 4 pots for each treatment per replicate and each pot contained 1 plant). The collected data 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 (**Gomez and Gomez. 1984**).

Results

Vegetative traits

All salinity concentrations decreased significantly plant height and leaves dry weight per branch in both seasons and cuttings without using anti-stress compounds as shown in **Table (2)**. Plant irrigation with 6000 ppm treatment gave the lowest values and died befor second cut. While, addition of proline and ascorbic acid at all concentrations inhanced plant growth and increased significantly plant height and leves dry weight in both seasons and cuttings. The concentrations of 80 ppm proline gave the best results it increased plant height by 8.0, 18.0, 9.4 and 18.44% for the first and second cut of the first and second seasons respectively. In addition, it increased leaves dry weight per branch by 94.4, 131.8, 96.3 and 131.3% for first and second cut of the first and second seasons respectively.

Chemical composition

Seawater irrigation at all levels of salinity dereased significantly total chlorophyll content (SPAD unit) and potassium of leaves % in the absence of proine and ascorbic acid in the both cutting and seasons as shown in **Table (3)**. Seawater at 6000 ppm gave the lowest vauaes in the first cut of both seasons. On the other hand, all concentrations of ascorbic acid and proline increased significantly total chlorophyll content and potassium of leaves % in both cuttings of both seasons. Spraying plants with proline at 80 ppm gave the highest values followed by ascorbic acid at 200 ppm compared to untreated plants as the mean effect for total chlorophyll content. This increment valued by 23.4, 29.5, 26.92 and 12.9% in the first and second cut of the first and second season. For potassium of leaves % the increment valued by 98.3, 56.68, 85.7 and 107.1% for the first and second cut of the first and second seasons respectively compared with untreated plants.

As the contrary effects, all salinity levels increased significantly sodium and chlorid of leaves in both seasons and cuttings as shown in **Table (4)**. The concentration of 6000 ppm gave the highest values in the first cut of both seasons.

Table 2

Effects of ascorbic acid and proline and their interaction treatments on plant height (cm) and leaves dry weight (g) 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)
	Plant height											
	First cut						First cut					
0.0	93.03	96.10	92.37	96.10	93.00	92.37	93.73	88.03	93.07	96.10	96.67	93.52
2000	83.57	87.10	92.43	94.03	94.33	90.29	83.37	87.10	92.93	94.07	95.00	90.49
4000	84.60	96.53	90.57	93.37	92.23	91.46	84.90	97.23	89.63	91.93	91.67	91.07
6000	73.33	78.03	73.23	80.3	81.87	77.35	71.60	77.73	73.30	79.78	81.70	76.82
Means	83.63	87.25	87.15	90.95	90.36		83.40	87.52	87.23	90.47	91.26	
LSD at 5%	S = 3.26 ** F = 3.51*** (S) * (F) = 7.02***						S = 2.43*** F = 3.14*** (S) * (F) = 6.27*					
	Second cut						Second cut					
0.0	117.1	100.87	107.23	123.33	124.5	114.61	116.83	102.23	108.90	123.77	124.83	115.31
2000	89.83	99.47	106.30	110.77	122.57	105.78	89.87	99.07	105.57	113.47	122.57	106.11
4000	100.9	106.6	109.50	107.37	116.20	108.11	100.30	106.27	109.33	108.07	116.20	108.03
Means	102.61	102.31	107.68	113.82	121.09		102.33	102.52	107.93	115.10	121.20	
LSD at 5%	S = 2.62 ^{ns} F = 3.65* (S) * (F) = 2.06 ^{ns}						S = 2.23*** F = 3.68*** (S) * (F) = 6.37***					
Leaves dry weight per branch												
	First cut						First cut					
0.0	4.84	6.45	6.48	6.41	6.36	6.11	4.77	6.37	6.52	6.28	6.37	6.06
2000	4.03	5.76	6.93	6.23	8.38	6.26	4.03	5.79	6.99	6.09	8.36	6.25
4000	3.39	5.80	5.88	6.10	8.09	5.85	3.23	5.62	5.89	6.05	7.93	5.74
6000	2.08	2.84	2.85	3.07	5.09	3.19	2.05	2.75	3.00	2.95	5.00	3.15
Means	3.59	5.21	5.53	5.45	6.98		3.52	5.13	5.60	5.34	6.91	
LSD at 5%	S = 0.79*** F = 0.64*** (S) * (F) = 1.29*						S = 0.76*** F = 0.59*** (S) * (F) = 1.19*					
	Second cut						Second cut					
0.0	5.28	6.98	7.76	6.67	10.50	7.44	5.26	6.84	7.61	6.64	10.27	7.32
2000	4.14	7.97	8.96	9.58	11.23	8.38	4.14	8	8.74	9.69	10.97	8.31
4000	3.97	7.57	7.91	7.05	9.29	7.16	3.81	7.39	7.80	6.91	9.31	7.04
Means	4.46	7.51	8.21	7.77	10.34		4.40	7.41	8.05	7.75	10.18	
LSD at 5%	S = 0.33** F = 0.61*** (S) * (F) = 1.05***						S = 0.32*** F = 0.66*** (S) * (F) = 1.14**					
Values in the same column not followed by the same letter are significantly different at the 5% level of probability.												

Spraying plants with all concentrations of ascorbic acid and proline decreased significantly sodium and chloride of leaves in both seasons and cuttings. Proline at 80 ppm gave the best results. When plants irrigated with seawater at 6000 ppm and sprayed with 80 ppm proline, sodium of leaves decreased by 25.0 and 12.7% compared with unsprayed plants in the first and second seasons of the first cut. In addition, chloride of leaves decreased by 10.5, 15%.

Number, length and width of stomata:

Table (5) showed that number, length and width of stomata were decreased significantly with increasing salinity level in both cuttings in most cases in the absence of anti-stress compounds. The level of 6000 ppm seawater gave the lowest values in the first cut and plants died before second cut when the foliar spray with ascorbic acid or proline was stopped. Spraying plants with proline and ascorbic acids increased significantly all stomata parameter. The concentration of 80ppm proline gave the best results 12.88, 29.12 and 24.11 for number, length and width of stomata in the first cut compared to control(9.25, 25.47 and 20.83 respectively) followed by 200 ppm ascorbic acid.

Antioxidant enzyme

No significant differences were found for salinity stress on the antioxidant enzymes in the both seasons and cuttings except for superoxide dismutase activity and aniline 4-hydroxylase activity in the absence of proline and ascorbic acid in the first cut of the first season as shown in **Tables (6, 7, 8, 9 and 10)**. All concentrations of proline and ascorbic acid increased significantly most enzymatic and non enzymatic antioxidants; increased reduced glutathione (GSH), catalase activity, superoxide dismutase activity (SOD), cytochrome b5, cytochrome p₄₅₀, amidopyrine N-demethylase and aniline 4-hydroxylase activity

Proline at 80 ppm followed by ascorbic acid at 200 ppm, were the most effective treatments to increase the antioxidant parameter. On the other hand, thiobarbituric acid reactive substances were decreased by proline and ascorbic acid addition. Catalase activity increased slightly with increasing salinity levels.

Table 3
Effects of ascorbic acid and proline and their interaction treatments on leaf green color degree (SPAD units) and potassium of leaves (%) of *Duranta erecta* L. Var. *Variegata* plants under salinity stress condition at 2019/2020

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 green color degree (SPAD units)												
	First cut						First cut						
0.0	31.33	32.77	35.63	32.94	36.87	33.91	30.76	31.38	35.57	32.05	36.84	33.32	
2000	29.55	32.18	36.40	31.14	36.96	33.25	29.97	31.74	36.17	29.68	35.37	32.58	
4000	27.61	32.20	35.91	30.58	36.69	32.60	26.77	32.49	36.05	29.49	36.73	32.31	
6000	21.82	23.54	26.87	22.19	25.64	24.01	19.89	22.08	25.72	20.28	27.36	23.07	
Means	27.58	30.17	33.70	29.21	34.04		26.85	29.42	33.38	27.87	34.08		
LSD at 5%	S = 2.73 ^{***} F = 1.86 ^{***} (S) * (F) = 3.71 ^{ns}						S = 2.22 ^{***} F = 1.73 ^{***} (S) * (F) = 3.46 ^{ns}						
	Second cut						Second cut						
0.0	27.84	30.33	34.29	30.52	34.66	31.53	28.71	28.66	31.08	28.33	30.98	29.55	
2000	27.08	29.55	33.57	28.84	34.94	30.80	27.55	27.72	30.36	26.55	28.82	28.20	
4000	25.47	26.96	34.00	28.29	34.56	29.85	24.07	25.88	32.22	27.46	30.99	28.12	
Means	26.80	28.95	33.95	29.22	34.72		26.78	27.42	31.22	27.45	30.26		
LSD at 5%	S = 1.53 ^{ns} F = 1.86 ^{***} (S) * (F) = 3.23 ^{ns}						S = 1.30 ^{ns} F = 1.34 ^{***} (S) * (F) = 2.33 [*]						
Potassium of leaves (%)													
	First cut						First cut						
0.0	1.53	1.49	2.55	1.49	2.70	1.95	1.69	1.81	2.62	1.57	2.71	2.08	
2000	1.16	1.47	2.53	1.57	2.59	1.86	1.23	1.62	2.60	1.14	2.61	1.84	
4000	1.02	1.44	2.46	1.79	2.53	1.85	1.08	1.43	2.27	1.75	2.53	1.81	
6000	1.00	1.26	1.56	1.31	1.52	1.33	1.03	1.29	1.55	1.60	1.52	1.40	
Means	1.18	1.42	2.28	1.54	2.34		1.26	1.54	2.26	1.51	2.34		
LSD at 5%	S = 0.17 ^{***} F = 0.22 ^{***} (S) * (F) = 0.45 [*]						S = 0.24 ^{**} F = 0.30 ^{***} (S) * (F) = 0.61 ^{ns}						
	Second cut						Second cut						
0.0	2.32	2.62	2.71	2.18	2.54	2.47	2.06	2.13	2.54	2.09	2.73	2.31	
2000	1.27	1.89	2.60	1.40	2.43	1.92	1.17	3.61	2.34	2.26	2.60	2.40	
4000	1.13	1.48	2.74	2.11	2.40	1.97	1.08	1.45	2.55	1.71	2.51	1.86	
Means	1.57	1.99	2.69	1.90	2.46		1.44	2.40	2.48	2.02	2.61		
LSD at 5%	S = 0.18 ^{**} F = 0.17 ^{***} (S) * (F) = 0.30 ^{***}						S = 0.45 ^{ns} F = 0.47 ^{***} (S) * (F) = 0.82 ^{**}						
Values in the same column not followed by the same letter are significantly different at the 5% level of probability.													

Table 4
Effects of ascorbic acid and proline and their interaction treatments on sodium of leaves (ppm) and chloride 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)
	Sodium of leaves (ppm)											
	First cut						First cut					
0.0	158.98	156.10	155.67	155.52	151.63	155.58	159.63	151.73	148.97	149.10	151.87	152.26
2000	306.57	289.54	256.84	300.64	235.42	277.80	323.33	287.15	266.41	292.84	250.51	284.05
4000	370.56	300.42	252.44	316.97	257.67	299.61	357.52	302.39	264.03	319.85	278.26	304.41
6000	393.00	324.44	384.36	377.90	314.29	358.80	393.58	334.87	384.39	372.63	349.14	366.92
Means	307.28	267.63	262.33	287.76	239.75		308.51	269.04	265.95	283.60	257.45	
LSD at 5%	S = 10.37*** F = 13.94*** (S) * (F) = 27.88***						S = 14.39*** F = 13.88*** (S) * (F) = 27.75***					
	Second cut						Second cut					
0.0	166.77	141.58	151.74	143.44	142.64	149.23	168.06	140.48	152.24	147.77	148.21	151.35
2000	333.93	258.83	284.27	239.92	263.65	276.12	346.58	293.71	349.51	304.19	255.01	309.80
4000	372.22	307.00	249.48	317.53	272.25	303.69	374.07	311.34	265.72	327.30	290.56	313.80
Means	290.98	235.80	228.50	233.63	226.18		296.24	248.51	255.82	259.75	231.26	
LSD at 5%	S = 4.08*** F = 16.81*** (S) * (F) = 29.12***						S = 6.35*** F = 18.37*** (S) * (F) = 31.82***					
	Chloride of leaves (%)											
	First cut						First cut					
0.0	0.05	0.06	0.05	0.05	0.04	0.05	0.05	0.05	0.04	0.05	0.03	0.05
2000	0.14	0.13	0.09	0.14	0.09	0.12	0.16	0.14	0.10	0.15	0.09	0.13
4000	0.18	0.17	0.10	0.16	0.08	0.14	0.20	0.17	0.10	0.17	0.09	0.15
6000	0.19	0.17	0.17	0.17	0.17	0.17	0.20	0.18	0.18	0.17	0.17	0.18
Means	0.14	0.13	0.10	0.13	0.10		0.15	0.14	0.10	0.14	0.10	
LSD at 5%	S = 0.01*** F = 0.01*** (S) * (F) = 0.03**						S = 0.01*** F = 0.01*** (S) * (F) = 0.02***					
	Second cut						Second cut					
0.0	0.04	0.03	0.03	0.04	0.03	0.03	0.05	0.06	0.06	0.06	0.04	0.06
2000	0.16	0.14	0.15	0.15	0.14	0.15	0.16	0.15	0.10	0.16	0.10	0.14
4000	0.18	0.17	0.16	0.17	0.16	0.17	0.20	0.18	0.13	0.18	0.12	0.16
Means	0.13	0.12	0.12	0.12	0.11		0.14	0.13	0.10	0.13	0.09	
LSD at 5%	S = 0.01*** F = 0.01 ^{ns} (S) * (F) = 0.02 ^{ns}						S = 0.01*** F = 0.01*** (S) * (F) = 0.02***					
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 number, length and width of stomata of *Duranta erecta* L. Var. *Variegata* plants under salinity stress condition at 2020/2021

Condition at 2020/2021																
Number of stomata							Length of stomata (µm)						Width of stomata (µm)			
2020/2021 season							2020/2021 season						2020/2021 season			
Saline water level (ppm)	Proline (P) and Ascorbic (As) concentration (ppm)						Proline (P) and Ascorbic (As) concentration (ppm)						Proline (P) and Ascorbic (As) con			
	0.0	As 100	As200	P40	P80	Means (S)	0.0	As 100	As200	P40	P80	Means (S)	0.0	As 100	As200	P40
	First cut						First cut						First cut			
0.0	10.50	11.00	13.50	11.00	14.00	12.00	29.38	24.38	30.00	29.80	30.83	28.88	23.54	23.13	25.83	24.59
2000	10.00	10.50	13.00	11.00	12.00	11.30	26.46	24.79	27.71	26.67	29.80	27.08	21.46	21.04	24.79	20.63
4000	8.50	10.00	10.50	10.00	13.50	10.50	23.33	24.59	29.79	26.04	30.63	26.87	19.58	20.00	26.05	20.63
6000	8.00	9.00	11.00	9.00	12.00	9.80	22.71	23.34	25.00	24.79	25.21	24.21	18.75	19.59	21.05	20.21
Means	9.25	10.13	12.00	10.25	12.88		25.47	24.27	28.13	26.83	29.12		20.83	20.94	24.43	21.51
LSD at 5%	S = 2.28 ^{ns} F = 0.93 ^{***} (S) * (F) = 1.87 ^{ns}						S = 1.80 [*] F = 2.03 ^{***} (S) * (F) = 4.06 ^{ns}						S = 1.60 [*] F = 1.45 ^{***} (S) * (F) = 2.91 ^{ns}			
	Second cut						Second cut						Second cut			
0.0	11.00	10.50	12.50	11.00	11.50	11.30	22.92	25.63	25.42	23.96	24.59	24.50	19.80	21.67	21.04	20.63
2000	7.50	7.50	11.50	10.00	12.00	9.70	24.59	24.38	25.21	26.46	25.42	25.21	19.79	21.04	21.88	21.46
4000	8.00	8.50	10.00	10.50	13.00	10.00	23.96	25.21	25.00	25.63	27.92	25.54	21.25	22.71	22.71	21.88
Means	8.83	8.83	11.33	10.50	12.17		23.82	25.07	25.21	25.35	25.97		20.28	21.81	21.88	21.32
LSD at 5%	S = 1.05 [*] F = 2.12 [*] (S) * (F) = 3.68 ^{ns}						S = 3.43 ^{ns} F = 1.75 ^{ns} (S) * (F) = 3.04 ^{ns}						S = 2.80 ^{ns} F = 1.36 ^{ns} (S) * (F) = 2.33 ^{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, proline and their interaction treatments on thiobarbituric acid reactive substances (TBARS) nmole/g of *Duranta erecta* L. Var. *Variegata* plants under salinity stress condition at 2019/2020

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)	
	First cut						First cut						
0.0	9.26	8.94	9.08	8.80	9.22	9.06	9.30	9.08	9.03	8.91	9.27	9.12	
2000	9.73	8.65	9.08	9.04	7.62	8.82	9.80	9.85	9.16	9.08	7.54	9.08	
4000	9.93	8.89	8.84	8.93	7.36	8.79	9.97	8.92	8.90	9.01	7.35	8.83	
6000	9.54	8.70	8.83	8.72	9.23	9.00	9.39	8.60	8.75	8.66	9.26	8.93	
Means	9.61	8.79	8.96	8.87	8.36		9.61	9.11	8.96	8.91	8.35		
LSD at 5%	S = 1.17 ^{ns} F = 1.65 ^{ns} (S) * (F) = 2.68 ^{ns}						S = 1.46 ^{ns} F = 2.07 ^{ns} (S) * (F) = 2.19 ^{ns}						
	Second cut						Second cut						
0.0	9.27	9.14	9.02	8.97	9.32	9.14	9.20	9.00	8.98	8.82	9.34	9.07	
2000	9.82	8.66	9.19	9.06	6.32	8.61	9.85	8.61	8.87	8.97	7.59	8.78	
4000	10.02	8.93	9.05	8.87	7.50	8.61	10.08	8.92	8.85	8.94	7.35	8.83	
Means	9.70	8.91	9.09	8.97	7.71		9.71	8.84	8.90	8.91	8.09		
LSD at 5%	S = 1.89 ^{ns} F = 2.31 [*] (S) * (F) = 2.06 ^{ns}						S S = 1.57 ^{ns} F = 1.92 ^{ns} (S) * (F) = 2.23 ^{ns}						
Values in the same column not followed by the same letter are significantly different at the 5% level of probability.													

Table 7

Effects of ascorbic acid and proline and their interaction treatments on reduced glutathione (GSH) mg/g tissue and catalase activity (IU/g tissue) 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)	
	Reduced glutathione (GSH) mg/g												
	First cut						First cut						
0.0	21.11	28.80	39.33	34.18	43.46	33.37	20.53	28.31	38.66	33.60	42.80	32.78	
2000	20.40	30.80	40.93	33.78	42.66	33.71	19.86	30.71	40.80	34.31	43.20	33.77	
4000	21.51	30.53	39.78	34.49	42.71	33.80	21.20	30.00	38.81	34.80	42.58	33.47	
6000	20.00	26.40	39.20	27.20	39.64	30.48	19.69	26.49	38.58	27.51	38.93	30.24	
Means	20.75	29.13	39.81	32.41	42.12		20.32	28.88	39.21	32.55	41.87		
LSD at 5%	S = 6.30 ^{ns} F = 4.01 ^{***} (S) * (F) = 8.02 ^{ns}						S = 6.32 ^{ns} F = 3.96 ^{***} (S) * (F) = 7.92 ^{ns}						
	Second cut						Second cut						
0.0	21.38	29.20	39.60	34.40	43.73	33.66	21.38	29.38	39.99	35.24	43.78	33.95	
2000	20.71	30.80	41.20	34.00	43.11	34.00	20.66	31.06	41.24	34.00	43.64	34.12	
4000	21.78	31.06	40.31	34.80	42.26	34.04	21.60	30.98	39.46	34.40	42.13	33.71	
Means	21.29	30.41	40.37 a	34.40	43.03		21.21	30.47	40.23	34.54	43.18		
LSD at 5%	S = 10.52 ^{ns} F = 5.40 ^{***} (S) * (F) = 9.35 ^{ns}						S = 9.66 ^{ns} F = 5.11 ^{***} (S) * (F) = 8.86 ^{ns}						
	Catalase activity (IU/g tissue)												
	First cut						First cut						
0.0	8.20	11.84	17.60	17.99	19.14	14.95	8.51	12.91	18.72	18.96	20.70	15.96	
2000	8.60	11.29	16.31	16.00	17.08	13.85	9.39	11.80	17.27	17.38	18.38	14.84	
4000	9.40	10.34	16.99	16.28	19.74	14.55	11.13	11.27	17.95	17.24	20.35	15.59	
6000	11.57	12.80	13.07	11.84	15.58	12.97	12.37	13.87	14.15	13.45	16.81	14.13	
Means	9.44	11.57	15.99	15.52	17.88		10.35	12.46	17.02	16.75	19.06		
LSD at 5%	S = 1.70 ^{ns} F = 1.90 ^{***} (S) * (F) = 3.80 ^{ns}						S = 1.56 ^{ns} F = 1.96 ^{***} (S) * (F) = 3.92 ^{ns}						
	Second cut						Second cut						
0.0	8.04	11.92	16.96	17.63	18.49	14.60	8.48	9.85	16.32	18.85	19.74	14.65	
2000	9.01	11.38	16.80	15.65	17.06	13.98	8.12	9.85	17.19	12.53	17.69	13.07	
4000	9.28	11.02	17.20	17.39	19.95	14.97	8.85	12.46	15.84	17.19	19.55	14.78	
Means	8.77	11.44	16.98	16.89	18.50		8.48	10.72	16.45	16.19	18.99		
LSD at 5%	S = 3.29 ^{ns} F = 2.24 ^{***} (S) * (F) = 3.88 ^{ns}						S = 3.99 ^{ns} F = 1.54 ^{***} (S) * (F) = 2.66 [*]						
Values in the same column not followed by the same letter are significantly different at the 5% level of probability.													

Table 8

Effects of ascorbic acid, proline and their interaction treatments on superoxide dismutase activity (SOD) IU/g tissue 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)
	First cut						First cut					
0.0	1412.11	1423.83	1453.13	1435.55	1458.99	1436.72	1400.39	1417.97	1435.55	1429.69	1453.13	1427.30
2000	1417.97	1429.69	1453.13	1447.27	1453.13	1440.24	1406.25	1423.83	1406.26	1429.69	1447.27	1422.60
4000	1400.39	1429.69	1458.99	1435.55	1464.85	1437.89	1406.25	1376.96	1458.99	1441.41	1476.41	1432.00
6000	1388.67	1412.11	1423.82	1417.97	1435.54	1415.62	1347.66	1371.10	1412.11	1435.55	1423.82	1398.00
Means	1404.79	1423.83	1447.27	1434.09	1453.12		1390.14	1397.46	1428.23	1434.09	1450.15	
LSD at 5%	S = 6.81** F = 12.18*** (S) * (F) = 24.37 ^{ns}						S = 72.37 ^{ns} F = 26.49*** (S) * (F) = 52.98 ^{ns}					
	Second cut						Second cut					
0.0	1394.53	1417.97	1441.41	1423.83	1441.41	1423.83	1406.25	1423.83	1458.99	1429.69	1447.27	1433.20
2000	1394.53	1412.11	1400.40	1406.25	1441.41	1410.94	1406.25	1417.97	1429.69	1423.83	1447.27	1425.00
4000	1353.52	1388.68	1447.27	1453.13	1476.56	1423.83	1347.66	1388.68	1447.27	1447.27	1464.85	1419.10
Means	1380.86	1406.25	1429.69	1427.74	1453.13		1386.72	1410.16	1445.31	1433.60	1453.13	
LSD at 5%	S = 17.94 ^{ns} F = 45.91* (S) * (F) = 79.52 ^{ns}						S = 46.39 ^{ns} F = 42.34* (S) * (F) = 73.34 ^{ns}					
Values in the same column not followed by the same letter are significantly different at the 5% level of probability.												

Table 9

Effects of ascorbic acid and proline and their interaction treatments on cytochrome b5 and cytochrome P450 (CYP450) nmol cytochrome/mg protein of *Duranta erecta* L. Var. *Variegata* plants under salinity stress condition at 2019/2020 and 2020/2021

protein of <i>Duranta erecta</i> L. var. <i>vaiegata</i> 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)
	Cytochrome b5 nmol cytochrome/mg protein											
	First cut						First cut					
0.0	0.70	1.04	1.34	1.39	1.42	1.18	0.60	1.02	1.70	1.35	1.40	1.21
2000	0.60	1.08	1.37	1.40	1.41	1.17	0.58	1.04	1.60	1.38	1.55	1.20
4000	0.63	1.17	1.57	1.40	1.43	1.24	0.61	0.97	1.55	1.22	1.60	1.16
6000	0.56	1.22	1.42	1.38	1.45	1.20	0.64	1.12	1.48	1.37	1.38	1.20
Means	0.62	1.12	1.42	1.39	1.43		0.61	1.04	1.58	1.33	1.40	
LSD at 5%	S = 0.47 ^{ns} F = 0.24 ^{***} (S) * (F) = 0.49 ^{ns}						S = 0.18 ^{ns} F = 0.26 ^{***} (S) * (F) = 0.53 ^{ns}					
	Second cut						Second cut					
0.0	0.70	1.06	1.38	1.38	1.42	1.18	0.61	1.06	1.41	0.95	1.89	1.18
2000	0.54	1.09	1.45	1.42	1.39	1.18	0.57	1.02	1.47	1.24	1.90	1.24
4000	0.63	1.13	1.49	1.10	1.40	1.15	0.59	1.17	1.56	1.32	1.90	1.31
Means	0.62	1.09	1.44	1.30	1.40		0.59	1.08	1.48	1.17	1.90	
LSD at 5%	S = 0.92 ^{ns} F = 0.23 ^{***} (S) * (F) = 0.41 ^{ns}						S = 0.37 ^{ns} F = 0.17 ^{***} (S) * (F) = 0.30 ^{ns}					
Cytochrome P450 (CYP450) nmol cytochrome/mg protein												
	First cut						First cut					
0.0	1.62	1.83	2.27	1.94	2.66	2.06	1.51	1.71	2.35	2.03	2.38	1.99
2000	1.53	2.03	2.33	1.95	2.61	2.09	1.44	1.75	2.30	2.11	2.52	2.02
4000	1.42	1.60	2.22	1.64	2.53	1.88	1.33	1.64	2.34	1.78	2.74	1.96
6000	1.43	1.42	2.30	2.08	2.39	1.92	1.50	1.72	2.43	2.18	2.47	2.06
Means	1.50	1.72	2.28	1.90	2.55		1.44	1.70	2.35	2.02	2.53	
LSD at 5%	S = 0.33 ^{ns} F = 0.37 ^{***} (S) * (F) = 0.74 ^{ns}						S = 0.39 ^{ns} F = 0.36 ^{***} (S) * (F) = 0.72 ^{ns}					
	Second cut						Second cut					
0.0	1.39	1.75	2.23	1.37	2.52	1.85	1.16	1.53	1.93	1.91	2.44	1.80
2000	1.54	1.94	2.23	2.12	2.55	2.07	1.38	1.32	2.16	2.11	2.22	1.84
4000	1.12	1.34	2.17	1.23	2.74	1.72	1.38	1.92	2.26	1.91	2.54	2.00
Means	1.35	1.67	2.21	1.57	2.60		1.31	1.59	2.11	1.98	2.40	
LSD at 5%	S = 1.12 ^{ns} F = 0.41 ^{***} (S) * (F) = 0.70 ^{ns}						S = 0.33 ^{ns} F = 0.35 ^{***} (S) * (F) = 0.60 ^{ns}					
Values in the same column not followed by the same letter are significantly different at the 5% level of probability.												

Table 10

Effects of ascorbic acid and proline and their interaction treatments on amidopyrine N-demethylase $\mu\text{mol}/\text{min} \times \text{kg}$ (plant tissue) and aniline 4-hydroxylase activity $\mu\text{mol}/\text{min} / \text{mg}$ protein 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)
	Amidopyrine N-demethylase $\mu\text{mol}/\text{min} \times \text{kg}$ (plant tissue)											
	First cut						First cut					
0.0	0.35	0.37	0.48	0.42	0.50	0.42	0.19	0.20	0.21	0.19	0.22	0.20
2000	0.34	0.38	0.49	0.42	0.54	0.43	0.19	0.19	0.21	0.20	0.22	0.20
4000	0.34	0.36	0.49	0.42	0.49	0.42	0.19	0.19	0.21	0.20	0.21	0.20
6000	0.33	0.37	0.48	0.42	0.48	0.41	0.19	0.19	0.20	0.20	0.21	0.20
Means	0.34	0.37	0.49	0.42	0.50		0.19	0.19	0.21	0.20	0.21	
LSD at 5%	S = 0.02 ^{ns} F = 0.03 ^{***} (S) * (F) = 0.07 ^{ns}						S = 0.01 ^{ns} F = 0.01 ^{***} (S) * (F) = 0.02 ^{ns}					
	Second cut						Second cut					
0.0	0.24	0.25	0.33	0.27	0.34	0.28	0.29	0.41	0.45	0.41	0.49	0.41
2000	0.21	0.26	0.30	0.27	0.34	0.27	0.32	0.40	0.45	0.42	0.48	0.41
4000	0.23	0.26	0.35	0.26	0.32	0.28	0.31	0.40	0.48	0.42	0.50	0.42
Means	0.22	0.26	0.32	0.26	0.33		0.31	0.41	0.46	0.42	0.49	
LSD at 5%	S = 0.08 ^{ns} F = 0.04 ^{***} (S) * (F) = 0.08 ^{ns}						S = 0.13 ^{ns} F = 0.07 ^{**} (S) * (F) = 0.12 ^{ns}					
Aniline 4-hydroxylase activity $\mu\text{mol}/\text{min} / \text{mg}$ protein												
	First cut						First cut					
0.0	0.31	0.34	0.40	0.35	0.41	0.36	0.29	0.34	0.37	0.32	0.39	0.34
2000	0.31	0.34	0.39	0.33	0.41	0.36	0.30	0.33	0.38	0.33	0.39	0.35
4000	0.31	0.35	0.40	0.35	0.41	0.36	0.30	0.32	0.37	0.34	0.38	0.34
6000	0.29	0.34	0.38	0.34	0.34	0.34	0.31	0.32	0.35	0.33	0.34	0.33
Means	0.30	0.34	0.39	0.34	0.39		0.30	0.33	0.37	0.33	0.37	
LSD at 5%	S = 0.01 [*] F = 0.011 ^{***} (S) * (F) = 0.02 ^{**}						S = 0.05 ^{ns} F = 0.02 ^{***} (S) * (F) = 0.05 ^{ns}					
	Second cut						Second cut					
0.0	0.25	0.32	0.39	0.34	0.39	0.34	0.25	0.32	0.36	0.31	0.38	0.32
2000	0.26	0.33	0.39	0.36	0.40	0.35	0.26	0.33	0.38	0.34	0.38	0.34
4000	0.35	0.33	0.38	0.36	0.39	0.36	0.32	0.31	0.34	0.35	0.37	0.34
Means	0.29	0.33	0.38	0.35	0.39		0.27	0.32	0.36	0.33	0.37	
LSD at 5%	S = 0.04 ^{ns} F = 0.04 ^{***} (S) * (F) = 0.08 ^{ns}						S = 0.08 ^{ns} F = 0.03 ^{***} (S) * (F) = 0.05 ^{ns}					
Values in the same column not followed by the same letter are significantly different at the 5% level of probability.												

DNA breakage and Plant survival %

As shown in Table (11) the highest level of seawater (6000 ppm) slightly increased DNA breakages % in both cutting of the first season. On the other hand, all concentrations of ascorbic acid and proline decreased the percentage of DNA breakages in both seasons and cuttings. Proline at 80 ppm and ascorbic acid at 200 ppm were the best.

Salinity irrigation at 4000 and 6000 ppm decreased significantly plant survival (%) in both cuttings and seasons. The plant survival decreased by 66.7 and 75.0% in the first and second seasons respectively when plant irrigated by seawater at 6000 ppm without spraying with proline or ascorbic acid. While, spraying plants with all concentrations of proline and ascorbic acid increased significantly plant survival (%). Proline treatments at 80 ppm increased survival % for plant that irrigated by seawater at 6000 ppm by 75 and 133% in the first cut of the first and second seasons respectively. The increased reached to 200.0% for plants irrigated by seawater at 4000 ppm in the second cut of the both seasons

Table 11

Effects of ascorbic acid and proline and their interaction treatments on DNA breakages (%) and plant survival (%) 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)
	DNA breakages (%)											
	First cut						First cut					
0.0	9.40	5.37	5.68	5.33	7.77	6.71	9.31	7.24	5.84	5.57	7.80	7.15
2000	8.30	8.07	4.28	4.57	6.86	6.41	8.93	8.48	4.30	4.72	6.68	6.62
4000	8.41	6.02	5.78	7.04	3.23	6.09	7.82	5.08	5.90	6.97	3.34	5.82
6000	8.86	8.45	8.29	7.31	6.47	7.87	8.27	8.37	8.29	7.36	6.50	7.76
Means	8.74	6.98	6.01	6.06	6.08		8.58	7.29	6.08	6.15	6.08	
LSD at 5%	S = 2.90 ^{ns} F = 1.81* (S) * (F) = 3.62 ^{ns}						S = 2.53 ^{ns} F = 1.96 ^{ns} (S) * (F) = 3.93 ^{ns}					
	Second cut						Second cut					
0.0	9.77	7.02	4.42	6.35	8.88	7.29	9.79	7.11	4.66	6.37	8.92	7.37
2000	8.05	7.67	5.601	9.52	4.19	7.01	8.20	7.72	5.78	9.54	4.48	7.14
4000	7.97	6.06	5.99	6.82	6.23	6.61	7.99	6.45	6.06	6.82	6.27	6.72
Means	8.59	6.92	5.34	7.56	6.43		8.66	7.09	5.50	7.57	6.56	
LSD at 5%	S = 4.10 ^{ns} F = 2.69 ^{ns} (S) * (F) = 4.66 ^{ns}						S = 4.13 ^{ns} F = 2.70 ^{ns} (S) * (F) = 4.68 ^{ns}					
	Plant survival (%)											
	First cut						First cut					
0.0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2000	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
4000	66.67	83.33	100.00	91.67	100.00	88.33	58.33	83.33	100.00	91.67	100.00	86.67
6000	33.33	33.33	50.00	50.00	58.33	45.00	25.00	33.33	50.00	33.33	58.33	40.00
Means	75.00	79.17	87.50	85.42	89.58		70.83	79.17	87.50	81.25	89.58	
LSD at 5%	S = 13.21 ^{***} F = 5.53 ^{***} (S) * (F) = 11.07 ^{**}						S = 9.56 ^{***} F = 5.53 ^{***} (S) * (F) = 11.07 ^{***}					
	Second cut						Second cut					
0.0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2000	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
4000	33.33	66.67	100.00	83.33	100.00	76.67	33.33	83.33	100.00	66.67	100.00	76.67
Means	77.78	88.88	100.00	94.44	100.00		77.78	94.44	100.00	88.89	100.00	
LSD at 5%	S = 19.99* F = 10.26 ^{***} (S) * (F) = 17.76 ^{***}						S = 7.56 ^{**} F = 13.57* (S) * (F) = 23.50 ^{**}					
Values in the same column not followed by the same letter are significantly different at the 5% level of probability.												

Discussion

The application of proline to the leaves is a shotgun strategy to reducing the negative impacts of salt. Ascorbic acid and proline enhanced plant growth, they increased plant height and leaves dry weight of *Duranta* under salinity stress. In addition, Siddique et al. (2015) found that foliar proline administration resulted in a considerable boost in rice plant growth metrics. Butt et al. (2016) discovered that application of proline under salinity stress conditions increased plant height of chilli plants. Rady et al. (2016) established that application of proline of levels (3, 6 or 9 mM) to lupine types resulted in a significant boost in growth characteristics as compared to the control. Maia et al. (2017) and Soltaniet al. (2014) reported that using some foliar applications such as ascorbic acid and proline gave better various photosynthetic and enhanced plant growth and productivity under saline irrigation water treatments. Moreover, the decrease in photosynthesis and growth is caused by the increase of free radicals (ROS) in chloroplasts and the breakdown of chlorophyll molecules under salinity stress (Sadaket al. 2020). This increase in chlorophyll content could be owing to larger levels of amino acids being available to the treated plants, as amino acids aid to boost chlorophyll content, which could lead to an increase in certain growth parameters (Awadet al. 2007). In similar studies Alotaibiet al. (2019) showed

the enhanced chlorophyll content in leaves on *Simmondsia chinensis* (Link) Schneider, **Abd-El-Hady et al. (2019)** on *Acalypha Wilkesiana*, **Malkiewicz and Smolik. (2019)** on *Petunia×atkinsiana* any further reduction in stomatal conductance and photosynthetic rate inhibit plant growth and reduce productivity (**Tester and Davenport 2003**). **Bosabalidis and Kofidis (2002)** discovered plants anatomical changes, particularly in their leaves, which are the plant's major organs for water loss. Changes in cuticle thickness, stomata density, non-glandular scales, epidermal and mesophyll cells are examples of such adaptations to preserve water under stress conditions. The stomatal conductance is linked with a water use efficiency of the plants, closing the stomata during most of the day, avoiding water loss by transpiration. As a result of the current investigation, it can be concluded that exogenous proline administration increased stomatal conductance by maintaining sufficient cellular turgor (**Kamran et al., 2009**), allowing for greater sub-stomatal accumulation and CO₂ absorption. **Ramadan et al. (2019)** stated that Salt stress alters the availability, competitive absorption, and translocation of nutrients to plant parts. Plant nutrition is unbalanced as a result of other components interfering with excessive concentrations of Na and Cl ions in the soil. The increase in Na⁺ absorption caused by salinity was accompanied by a decrease in K⁺ concentration, indicating an apparent antagonism relationship between K⁺ and Na⁺ (**Cuinet al. 2009**). Spraying with Proline reduces sodium absorption and enhances potassium absorption (**Abd El-Samad 2010**) on maize. On the other hand, **Ahmed (1996)** concluded that, application of ascorbic acid enhancement the amount of organic acids excreted into the soil by the roots, hence increasing the solubility of most nutrients that dissolve slowly into the rhizosphere zone. These study agreement with **Wuet al. (2017)** and **Ibrahim et al. (2019)** who discussed the application of proline to the leaves is strategy to reducing the negative impacts of salt. From the previous results, it is clear that application of exogenous proline reduces sodium absorption and the toxicity caused by salt, and this is consistent with **Alotaib et al. (2019)**, **Hussein et al. (2017)**. Water stress enhanced TBARS accumulation, which was partially prevented when plants were given exogenous ascorbic acid and proline as was the case with salt stress. The level of osmotic stress was too low to allow a parallel of the effects on plant survival. Several data suggested that the salt-induced transition to permanent wilting was linked to increased cellular activity of potentially harmful active oxygen species (AOS). Ascorbic acid scavenged reactive oxygen species and inhibited protein oxidation and degradation, according to **Dolatbadian et al. (2010)** Non-enzymatic antioxidant mechanisms such as GSH responded differently to the adverse effect treatment of NaCl (**Jaleel et al. 2007**). Endogenous ascorbate at a high level is required to adequately sustain the antioxidant system that protects plants from oxidative damage caused by abiotic stress. GSH is a nonenzymatic antioxidant that protects proteins containing sulfhydryl groups, participates in amino acid membrane transport, and increases plant stress tolerance (**Zhao et al. 2019**). Proline contributes to the maintenance of the glutathione pool in the reduced state, which is used by dehydroascorbate reductase and other GSH-dependent enzymes involved in the antioxidant defense and glyoxalase systems (**Hoque et al. 2008**). Proline also has a regulatory role in the activity and function of CAT enzymes in plant cells, as well as their engagement in metabolic response development in response to environmental stimuli, according to **Abdallah and El-Bassiouny (2016)**. The antioxidant enzyme system (the first line of defence) protects stressed plant cells from oxidative damage by scavenging active oxygen species (O[•], OH[•], and H₂O₂) within the cells. Foliar application of ascorbic acid in general, significantly increased the all enzyme activities as compared to the corresponding salinity levels. Ascorbic acid serves as a main substrate in the cyclic pathway for enzyme detoxification of hydrogen peroxide (**Shalata and Neumann 2001**). SOD reduced ROS production, thus reducing cell oxidation, these results are agreement with **Soliman et al. (2015)**.

In this study, treated plants with ascorbic acid and proline increased cytochrome b5 in leaves. The enzyme is a component of an ER-associated redox chain that delivers electrons to cytochrome b5, an intermediate electron donor in a variety of lipid modification processes including as NADH-dependent fatty acid desaturation, sterol precursor desaturation, and fatty acid hydroxylation (**Rahier et al. 1997**). Spraying plants with proline and ascorbic acid increased CYP450. CYP450 is a plant enzyme that is involved in the manufacture of secondary metabolites such as phenylpropanoids, terpenoids, alkaloids, and lignins, as well as signalling molecules, fatty acids, lipids, defense-related chemicals, and plant hormones as reported by **Goujon et al. (2003)**. However, pretreatment with proline and ascorbic acid reduced the effect of free radicals and helped maintain levels of the enzymes such as amidopyrine N-demethylase in plants. Amidopyrine N-demethylase is an enzyme of the cytochrome P450 that helps in the metabolism of cells as reported by **Marabini et al. (1994)**.

In this study treated *Duranta erecta* L. Var. *Variegata* plants with proline and ascorbic acid increased aniline 4-hydroxylase. Because the activity of aniline 4-hydroxylase is induced by an H₂O₂ signal generated by various environmental stresses. Aniline 4-hydroxylase are important for the AOS-scavenging system, the investigation on aniline 4-hydroxylase has seldom been reported (**Sawada et al. 2006**).

Finally, proline and ascorbic acid increased antioxidant enzyme systems to protect plant cells from oxidative enzyme and decreased the toxic ions, DNA breakage % and increased photosynthesis, growth and survivability (%).

Conclusions

Using proline at a rate of 80 ppm or ascorbic acid at a rate of 200 ppm for four months was recommended to get the best growth of *Duranta erecta* L. var. *variegata* plants and increased antioxidant enzyme system, which irrigated with seawater concentrations. Plants irrigated with 6000 ppm seawater need to continuously spraying with proline at 80 ppm to keep alive.

Declarations

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Competing Interests

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Author Contributions

All authors contributed to the study conception, design, and Material preparation. Data collection was performed by [Magda Ibrahim El-Desouky Hamoud] and [Sabah El banna]. Data analysis, the first draft of the manuscript were performed by [Magda Ibrahim El-Desouky Hamoud] and [Gehan Gaber Mostafa]. All authors reviewed and commented on previous versions of the manuscript, read and approved the final manuscript

Data Availability

The datasets estimated during and/or analysed during the current study are available from the corresponding author on reasonable request

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