

Increased Resistance to Salt Stress of *Duranta plumieri* Irrigated with Seawater by Using Thiamin, Humic Acid and Salicylic acid.

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ABSTRACT

Global climatic changes limited the fresh water supply, which led to increasing the need for irrigation water. For this reason, the main objective of this study was to investigate the role of humic acid (HA), salicylic acid (SA) and thiamin (Vitamin B1) in increasing the *Duranta plumieri* resistance to irrigate with diluted seawater. The experiment was conducted on *Duranta plumieri*, (synonym to *Duranta erecta L.*) during the two successive seasons of 2014 and 2015 to estimate the effects of soil application with humic acid at 1000 and 2000ppm concentrations and foliar spraying plants with salicylic acid or thiamin at 250 and 500ppm combined with irrigation with diluted seawater at three concentrations: 2000, 4000 and 6000ppm on vegetative growth and associated with physiological parameters. The results revealed that the plant vegetative growth parameters were considerably affected by salinity stress. Plant height, the number of branches/plant and shoot fresh and dry weight were linearly decreased with the increasing in saline water concentration. Also, salt stress was negatively affected the total carbohydrate contents, photosynthetic pigments, reducing and non-reducing sugars and mineral uptake (N,P,K and Mg) as well as higher contents of Na⁺ and Cl⁻ elements. In contrast to this, SA, B1 or humic acid were induced a stimulatory effect on the all vegetative growth parameters in plants which irrigated with all diluted seawater concentrations. The highest values of vegetative parameters and total chlorophylls were obtained with humic acid at 1000 and 2000ppm concentrations under all salinity concentrations. SA enhanced the physiological processes in *Duranta* plants under saline conditions through increasing significantly the total carbohydrate and carotenoids contents, and reducing and non-reducing sugars. The exogenous application of thiamin significantly increased proline content in salinity stressed plants. The greatest contents of N,P,K and Mg in plants under salinity stress were obtained with soil application of humic acid followed them with the treatment of spraying with SA and B1. Also, these treatments led to decrease the contents of Na⁺ and Cl⁻. It could be recommended that soil application with humic acid at 2000ppm can ameliorate the negative effects of irrigation with diluted seawater up to 6000ppm salinity concentration and spraying *Duranta* plants with SA or B1 at 500ppm increased the growth and physiological processes under 4000ppm salinity.

Keywords: *Duranta plumieri*, salt stress, Diluted seawater, Salicylic acid, Thiamin, Humic acid.

INTRODUCTION

Ornamental plants have dramatically positive effects on the global environment by several ways such as: reducing the CO₂ and thermal emissions as well as cleaning the air pollutants (whether dust or chemicals). Economically, the ornamental plants are the most important using in the tourist cities for many purposes like as: opening gardens, golf spaces, interior designs, etc. Because of most ornamental plants needs a lot of irrigation water, the tendency to use saline irrigation water is the greatest trend. Saline water for irrigation ornamental plants reduces plant size and growth resulting in smaller leaves, shorter stem and sometimes fewer green leaves which reducing aesthetic value. The previous parameters necessary are often assessed based on growth reduction for salt tolerance of landscape plants but should be based primarily on aesthetic value.

To increase the sustainable landscape as much as the use of saline water, there are two aspects have to be considered: choice the plants that tolerance to salinity and treating the plants to enhance their cope to the salinity (Cassaniti *et al.* 2012). At the latest two decades, the global climate relatively had changed which already affected on the environment in many ways. The most impacts of global climate change are climate drought and a shortage of irrigation water, especially in arid and semiarid regions. Prospective, Egypt will be suffering the plight of water shortage in next decades. Therefore, the search for other sources of irrigation water should be a priority. One of another source of water irrigation is the use of diluted seawater to compensate for the shortage of irrigation water.

The high concentration of ionic elements in seawater is the main restricting factor in the utilization of seawater for irrigation (Xiao-Hua *et al.*, 2009). There are several disadvantages when irrigation with saline water induced abiotic stress and toxic effects on plants which lead to gradually declined in photosynthesis and respiration rates and deterioration in, proteins and nucleic acids (Manai *et al.*, 2014). Salinity stress push plants to accumulate the reactive oxygen species which combined to the reduction of antioxidant defense reflects on a damage of the membranes, photosynthetic pigments, proteins, DNA and lipids and inhibits the photochemical activities (Sairam and Tyagi, 2004). Salinity stress induced decreasing the root and shoot biomass, chlorophyll, nitrate and NPK contents of the plant (Aydin *et al.* 2012).

There are many reports suggested that exogenous application of non-enzymatic antioxidants; salicylic acid (SA), thiamin (vitaminB1) and humic acid (HA) could mitigate injurious effects of salinity on plants under saline conditions. Humic acid (HA) is a commercial product contains many elements, which improve the soil fertility and increase the availability of nutrients and consequently increased plant growth. Humic acid increased the tolerance and growth of vegetable grown under saline conditions (Cimrin *et al.* 2010 and Aydin *et al.* 2012). Salicylic acid (SA) is an endogenous growth regulator of phenolic nature and acting an important role in abiotic stress tolerance by increasing the activities of antioxidant enzymes and scavenging the excess reactive oxygen species (ROS) which resulted in ameliorating the physiological processing and enhancing plant growth (He and Zhu, 2008). Application of SA

enhanced the photosynthetic rate, maintained the stability of membranes and lowered the electrolyte leakage in salt-stressed plants resulted in decreasing uptake of Na^+ and Cl^- (El-Tayeb, 2005). Thiamin (Vitamin B1) has been reported to mitigate the severity effects of several environmental stresses in plants. Foliar applied non-enzymatic antioxidative compounds (such as thiamin) relieved the harmful effects of salinity and increased the plant tolerance to salinity by an organization the antioxidative defense system (Tuna et al., 2013). Thiamine acts as a prospective coenzyme in many key metabolic pathways (Friedrich, 1987).

Duranta plumier L. family Verbenaceae, is an evergreen flowering shrub and it considers outdoor ornamental shrub. It is widely using in landscape design as accent shrub, fence plant or container plant. It can be trimmed and pruned lightly to make an evergreen compact hedge. For these reasons, the keeping aesthetic value and quality of *Duranta plumier* and increasing its resistance to saline irrigation with diluted seawater is the main goal of this investigation. Therefore, soil application with humic acid and foliar application with salicylic acid and thiamin (V.B1) were the major trends for realization the main goal in this research.

MATERIALS AND METHODS

This study was carried out at the nursery of AL-Baramoon Agricultural Research Station, Mansoura, Dakahlia Governorate, during the two successive seasons of 2014/2015 and 2015/2016 to estimate the impact of foliar spraying with thiamin (V.B1) and salicylic acid (SA) and soil application with humic acid (HA) under saline irrigation with diluted seawater on *Duranta plumier* growth and some related physiological characteristics.

Table 1. Physico-chemical properties of experimental soil (average two seasons).

Particle Size Distribution				pH	T.S.S		Cations				Anions			
Sand	Silt	Clay	Texture	1:2.5	%	K^+	Na^+	Mg^{++}	Ca^{++}	HCO_3^-	CO_3^-	Cl^-	So_4^-	
56.39	18.41	25.31	Sandy Clay Loom	7.37	0.14	0.3	2.33	0.85	1.09	0.4	-	1.66	2.70	

Chemicals and saline irrigation water treatments:

Thiamin (B1) and salicylic acid (SA) were obtained from AL-Gomhorya Pharmaceuticals Medicinal Plants Production Company, Mansoura, Egypt and dissolved in methyl alcohol before making the spraying solutions by distilled water which containing 0.02% Tween 20, (polyoxyethylenesorbitan monolaurate), as a surfactant. Plants were sprayed with (SA) and (B1) solutions manually by using a spraying bottle until it flooded on the plants.

Soluble humic acid (HA) as potassium humate (80% humic acid, 11-13% K_2O) was produced by the Fertilizers Development Center, El-Delta Fertilizers Plant, Egypt and dissolved in tap water to make the treatment's solutions and added to the soil at the rate of 200mL pot⁻¹. The treatments were started 2 weeks after transplanting and were added in twice times with two weeks interval. Seawater was obtained from the Mediterranean Sea in Gamas City, Dakahlia, Egypt. Saline irrigation water was prepared by mixing seawater

Plant materials and experimental design:

One year old plants of *Duranta* were obtained from the local commercial nurseries in 1st December 2014 and 2015 and transplanted into polyethylene containers (45 cm in diameter and 60cm length), filled with a mixture of clay and sand (1:2 V:V). They remained until the end of the experiment. Each container had one plant. Before transplantation, 4.5g pot⁻¹ of super phosphate (15.5% P_2O_5) was mixed with the soil. Ammonium sulphate (20.5% N) and potassium sulphate (48% K_2O) were added every two months throughout the experiment in doses of 3g pot⁻¹. All plants were cut at 30cm in height above the soil surface before the beginning of treatments. The experiment was designed in factorial randomized complete block with four replicates for each treatment. Each replicate was included 21 treatments, four pots/treatment, which were the combination of three salinity levels 2000, 4000 and 6000 ppm and seven chemical treatments as follows: control, humic acid (1000 and 2000ppm), thiamin (250 and 500ppm) and salicylic acid (250 and 500ppm). At 6 months after transplanting, four plants from each replicate were randomly sampled for determination some vegetative growth parameters.

A random soil sample was collected from the soil mixture before transplanting and was air-dried, ground and sieved over a 2mm. Physico-chemical properties were carried out as the following: distribution of particle size by using the pipette method as described by Dewis and Fertias (1970), electrical conductivity and the soil pH of saturated soil paste extract according to Jackson (1967) and Richards (1954). Data of soil analysis were presented in Table (1).

with fresh tap water to obtain the selected concentrations. Plants were irrigated with tap water until the beginning of saline irrigation treatments. After one week of the first applied to the chemicals, plants were irrigated with diluted seawater (500mL/container in every irrigation time) every 8 days in winter and 5 days in summer.

Vegetative growth measurements:

Vegetative growth parameters: plant height "cm", number of the branches/plant, shoot fresh and dry weights "g/plant". At the end of the experiment, shoot fresh weights were determined by severing the main shoot at the substrate surface and weight. Shoots were oven-dried at 70°C to constant weights and dry weights were determined supplementary with a number of physiological parameters.

Determination of chemical analysis:

The numbers of physiological parameters were determined as follows:

Total Chlorophyll and carotenoids contents (mg/100g fresh weight) in fresh sample were estimated

according to Lichtenthaler and Wellburn (1983). Total sugar content (%Dry Matter) in dry sample was determined as described by Sadasivam and Manickam, (1996). Reducing and non-reducing sugars (%Dry Matter) were determined according to James (1995). Nitrogen (N) content (%) was determined according to the Association of Official Analytical Chemists (A.O.A.C, 1990). Phosphorus (P) (%) was determined as described by Olsen and Sommers (1982). Potassium (K) (%) was determined by as described by Jackson (1967). Proline contents ($\mu\text{m}/\text{mg}$ dry matter) determined in dry leaves according to Bates et al. (1973). Magnesium (Mg^{++}) (ppm) was determined spectrophotometrically according to Chapman and Pratt (1982). Chloride element (Cl^-) (ppm) was determined by EPA method 300.0 (U.S.EPA, 1983). Sodium element (Na^+) (ppm) was determined by EPA method 200.7 (U.S.EPA, 1983).

Statistical analysis was done by using the analysis of variance technique by means of CoStat Computer Software. Data of treatment means were compared by using Duncan's test method as mentioned by Gomez and Gomez (1984) at 5 % significance level.

RESULTS

1. Vegetative growth parameters:

The vegetative growth parameters (plant height, number of branches, shoot fresh and dry weight) were significantly linearly decreased with increasing the salinity concentrations during the both seasons (Tables 2 & 3). However, the highest mean values were occurred with 2000ppm saline water in comparing with the other salinity concentrations (84.25 and 72.68cm for plant height, 20.14 and 20.04 branches/plant for the number of branches, 124.07 and 123.67g/plant for shoot fresh weight and 43.44 and 42.97g/plant for shoot dry weight during the two seasons respectively). The obtained results revealed that *Duranta* plants received humic acid at 2000ppm significantly were the highest values in plant height (90.83 and 84.50cm) and number of branches (29.75 and 31.0 branches/plant) for the both seasons respectively (Table 2). Meanwhile, plants sprayed with thiamin at 500 ppm significantly increased in shoot fresh weight in the two seasons (107.35 and 111.75g/plant respectively).

Table 2. Effect of irrigation with diluted seawater, soil application with humic acid and foliar spray with salicylic acid and thiamin on plant height (cm/plant) and the number of branches/plant of *Duranta plumieri* at 2014 and 2015 seasons.

Treatments	diluted seawater concentrations (ppm)							
	1 st season				2 nd season			
	2000	4000	6000	Means	2000	4000	6000	means
Control	58.75 ^{hi}	51.00 ^k	45.75 ^l	51.83 ^l	55.50 ^{hi}	53.00 ^j	48.75 ^k	52.42 ^g
Humic acid 1000ppm	95.25 ^b	79.75 ^d	56.75 ^{ij}	77.25 ^b	85.25 ^b	77.25 ^c	57.25 ^h	73.25 ^b
Humic acid 2000ppm	108.25 ^a	85.25 ^c	79.00 ^d	90.83 ^a	92.25 ^a	86.50 ^b	74.75 ^d	84.50 ^a
Thiamin 250ppm	79.50 ^d	57.00 ^{ij}	53.25 ^{jk}	63.25 ^e	71.25 ^e	54.25 ^{ij}	50.25 ^k	58.58 ^f
Thiamin 500ppm	85.75 ^c	69.75 ^e	64.50 ^{fg}	73.33 ^c	71.25 ^e	70.25 ^e	60.25 ^g	67.25 ^d
Salicylic acid 250ppm	89.75 ^c	63.00 ^{gh}	53.00 ^{jk}	68.58 ^d	71.25 ^e	64.50 ^f	55.50 ^{hi}	63.75 ^e
Salicylic acid 500ppm	72.50 ^e	72.50 ^e	68.25 ^{ef}	71.08 ^{cd}	62.00 ^g	75.25 ^{cd}	70.75 ^e	69.33 ^c
means	84.25 ^a	68.32 ^b	60.07 ^c		72.68 ^a	68.71 ^b	59.64 ^c	
Treatments	number of branches/plant							
	1 st season				2 nd season			
	2000	4000	6000	Means	2000	4000	6000	means
Control	9.50 ^l	8.00 ^m	7.50 ^m	8.33 ^g	10.25 ^{kl}	7.50 ^m	8.50 ^m	8.75 ^g
Humic acid 1000ppm	28.50 ^c	25.25 ^d	20.00 ^f	24.58 ^b	29.50 ^c	23.00 ^e	21.25 ^{fg}	24.58 ^b
Humic acid 2000ppm	33.50 ^a	30.50 ^b	25.25 ^d	29.75 ^a	34.50 ^a	31.50 ^b	27.00 ^d	31.00 ^a
Thiamin 250ppm	18.25 ^g	17.00 ^{gh}	14.50 ⁱ	16.58 ^d	17.00 ^h	18.00 ^h	10.75 ^k	15.25 ^d
Thiamin 500ppm	22.5 ^e	21.50 ^e	16.50 ^h	20.17 ^c	21.00 ^g	22.75 ^{ef}	14.25 ⁱ	19.33 ^c
Salicylic acid 250ppm	12.50 ^j	11.00 ^k	9.75 ^{kl}	11.08 ^f	13.25 ^{ij}	9.00 ^{lm}	7.50 ^m	9.92 ^f
Salicylic acid 500ppm	16.25 ^h	16.00 ^h	14.00 ⁱ	15.42 ^e	14.75 ⁱ	14.25 ⁱ	11.75 ^{jk}	13.58 ^c
means	20.14 ^a	18.46 ^b	15.36 ^c		20.04 ^a	18.00 ^b	14.43 ^c	

Mean values followed by the same letters are not significantly different at the $P < 0.05$ according to Duncan's test.

Regarding the effect of interactions between saline irrigation water and either humic acid, SA or B1 on vegetative growth parameters, data presented in Table (2) indicated that humic acid at 2000ppm supplementary with saline irrigation at 2000ppm led to increasing significantly the plant height and number of branches (108.25 and 92.25cm in height and 33.50 and 34.50 branches/plant respectively for the two seasons). Also, the same treatment gave the highest values in the same characters under 4000 and 6000ppm saline water

compared with the (SA) and (B1) treatments. While for the dry weight parameter, the humic acid at both concentrations gave the highest values under 2000ppm salinity (Table 3). Spraying plants with thiamin at 500ppm under 2000 or 4000ppm saline water were increased significantly the fresh weight (148.28 and 149.21 g/plant, 103.92 and 113.32g/plant) respectively during the two seasons.

2. Physiological parameters

Photosynthetic Pigments content:

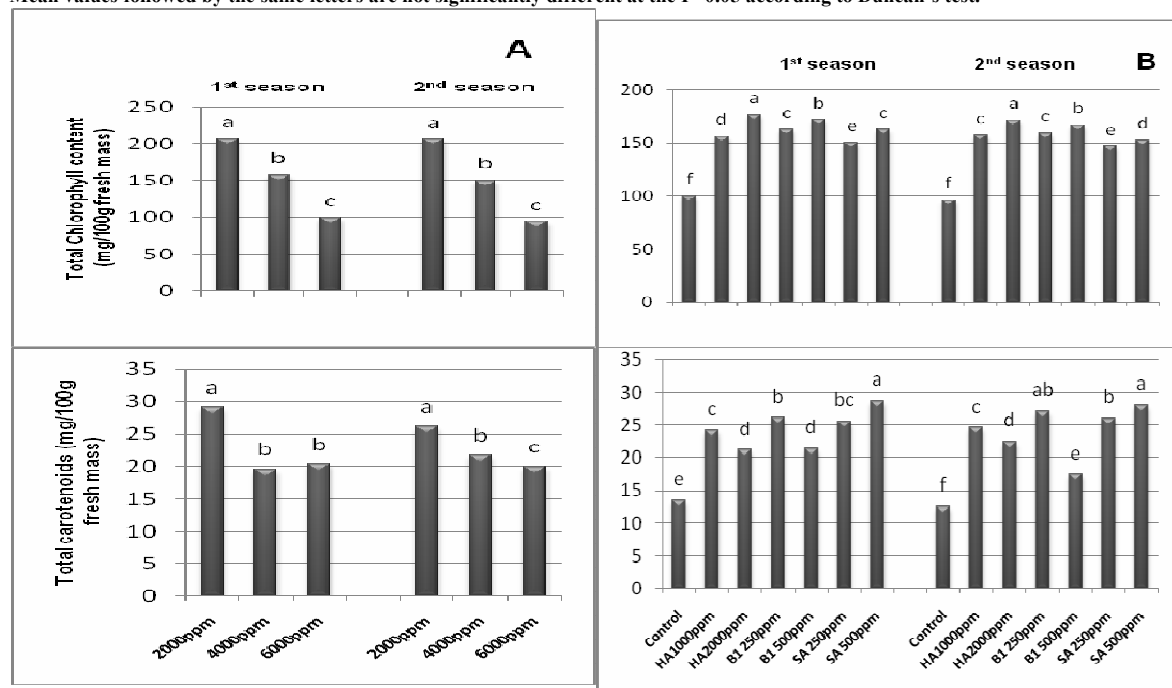
Plants irrigated with saline water at all concentrations decreased sharply in total chlorophyll contents and a steep increase in carotenoids especially under 6000ppm salinity (Fig.1). On the other hand,

treated plants with SA, B1 or humic acid resulted in increasing the total chlorophyll and carotenoids contents during the two seasons in comparing with the control (Fig.1).

Table 3. Effect of irrigation with diluted seawater, soil application with humic acid and foliar spray with salicylic acid and thiamin on fresh and dry weights (g/plant) of *Duranta plumieri* at 2014 and 2015 seasons.

Treatments	diluted seawater concentrations (ppm)							
	fresh weight (g/Plant)				dry weight (g/plant)			
	1 st season				2 nd season			
	2000	4000	6000	means	2000	4000	6000	means
Control	94.80 ^f	63.47 ^{kl}	46.53 ⁿ	68.26 ^f	98.06 ^g	69.03 ^k	53.13 ⁿ	73.41 ^l
Humic acid 1000ppm	136.39 ^c	66.84 ^{jk}	84.56 ^h	95.93 ^c	138.42 ^b	68.96 ^k	91.03 ^h	99.47 ^b
Humic acid 2000ppm	140.54 ^b	74.19 ⁱ	87.44 ^{gh}	100.72 ^b	132.64 ^d	68.95 ^k	78.28 ⁱ	93.29 ^d
Thiamin 250ppm	136.78 ^c	89.67 ^g	64.56 ^{kl}	97.00 ^c	135.68 ^c	91.53 ^h	56.79 ^m	94.66 ^c
Thiamin 500ppm	148.28 ^a	103.92 ^e	69.86 ^j	107.35 ^a	149.21 ^a	113.32 ^e	72.73 ^j	111.75 ^a
Salicylic acid 250ppm	103.90 ^e	70.30 ^j	63.18 ^l	79.12 ^d	106.84 ^f	67.19 ^k	59.70 ^l	77.91 ^e
Salicylic acid 500ppm	107.80 ^d	48.65 ⁿ	59.75 ^m	72.06 ^e	104.86 ^f	48.08 ^o	54.74 ^{mn}	69.23 ^g
means	124.07 ^a	73.86 ^b	67.98 ^c		123.67 ^a	75.29 ^b	66.63 ^c	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's test.

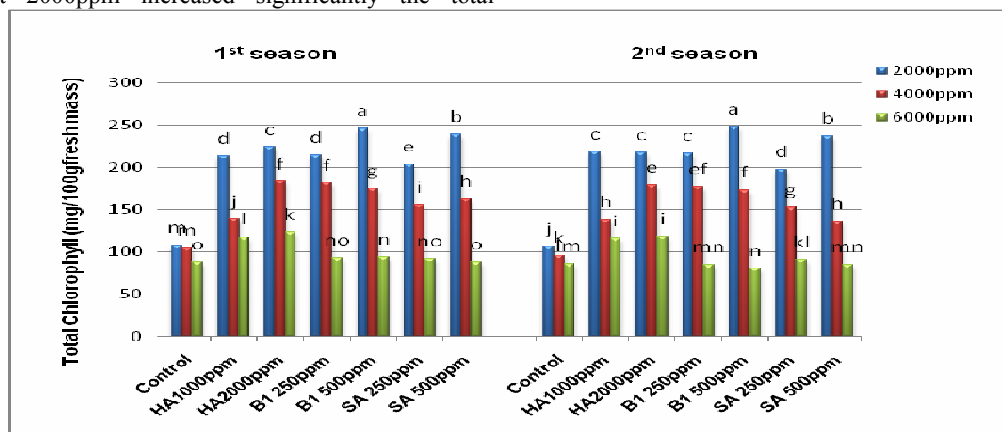


Columns labeled by the same letters are not significantly different at the P<0.05 according to Duncan's test.

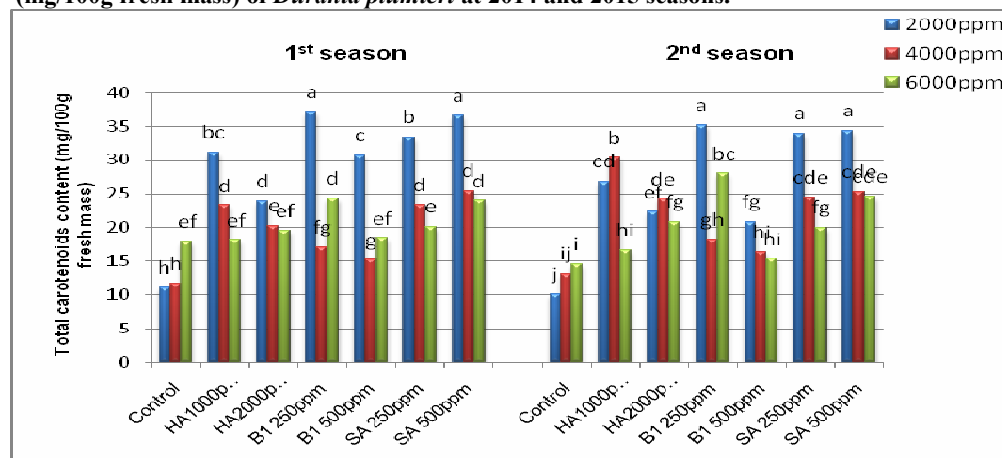
Fig. 1. Effect of irrigation with diluted seawater (A) and soil application with humic acid (HA) and foliar spray with thiamin (B1) and salicylic acid (SA) (B) on total chlorophyll and carotenoids contents (mg/100g fresh mass) of *Duranta plumieri* at 2014 and 2015 seasons.

Plants sprayed with B1 or SA at 500ppm gave the highest values in total chlorophyll contents under 2000ppm salinity (Fig.2). While, the same treatments were increased the carotenoids content in shoot plants under 6000ppm salinity (Fig.3). Furthermore, humic acid at 2000ppm increased significantly the total

chlorophyll content combined with 4000 and 6000ppm saline irrigation water. Plants sprayed with SA at both concentrations and irrigated with diluted seawater at 2000 or 4000ppm, displayed significant increasing in carotenoids content (Fig.3).



Columns labeled by the same letters are not significantly different at the $P < 0.05$ according to Duncan's test. **Fig. 2.** Effect of interaction between the irrigation with diluted seawater, soil application with humic acid (HA) and foliar spray with salicylic acid (SA) and thiamin (B1) on total chlorophyll contents (mg/100g fresh mass) of *Duranta plumieri* at 2014 and 2015 seasons.



Columns labeled by the same letters are not significantly different at the $P < 0.05$ according to Duncan's test. **Fig. 3.** Effect of interaction between the irrigation with diluted seawater, soil application with humic acid (HA) and foliar spray with salicylic acid (SA) and thiamin (B1) on total carotenoids content (mg/100g fresh mass) of *Duranta plumieri* at 2014 and 2015 seasons.

Total sugar contents and reducing and non-reducing sugars:

A negative response from irrigation with diluted seawater was recorded for total sugar contents and reduced and non-reduced sugar contents during the two seasons (Tables 4&5). In contrast, spraying plants with SA at both concentrations gave the highest values in total sugar contents under all salinity concentrations and increased the reducing and non-reducing sugar contents under 6000ppm salinity during the two seasons. Furthermore, humic acid at 2000ppm gave the same results. Plants grown under salinity stress at 4000ppm and sprayed with B1 at 500ppm showed significantly higher non-reducing sugar compared with

the other treatments under the same stress salinity in both seasons (Table 5).

Proline:

Proline content in shoots was steep decreased with increasing in saline irrigation water as presented in Table (6). Contrary, plants gave the highest response to accumulating proline in the shoots when treated with either non-enzymatic antioxidants or humic acid. Data showed that plants sprayed with B1 at both concentrations gave the highest values in proline content comparing with the other treatments under all salinity concentrations. Additionally, under 6000ppm salinity, all treatments resulted in increasing the proline content comparing with the control plants.

Table 4. Effect of irrigation with diluted seawater, soil application with humic acid and foliar spray with salicylic acid and thiamin on total sugar content (% dry matter) of *Duranta plumieri* at 2014 and 2015 seasons.

Treatments	diluted seawater concentrations (ppm)							
	1 st season				2 nd season			
	2000	4000	6000	means	2000	4000	6000	means
Control	8.32 ^g	7.10 ⁱ	5.97 ^k	7.13 ^c	9.68 ^t	7.96 ^l	6.90 ^m	8.18 ^c
Humic acid 1000ppm	7.26 ⁱ	6.17 ^k	7.09 ^j	6.84 ^f	8.34 ^{hi}	6.39 ⁿ	8.16 ^{jl}	7.63 ^f
Humic acid 2000ppm	9.00 ^f	9.04 ^f	9.77 ^e	9.27 ^c	9.86 ^{ef}	9.80 ^{ef}	11.13 ^d	10.26 ^b
Thiamin 250ppm	5.55 ^l	6.17 ^k	7.98 ^h	6.57 ^g	6.68 ^m	9.80 ^m	9.22 ^g	7.57 ^f
Thiamin 500ppm	8.87 ^f	6.52 ^j	8.23 ^{gh}	7.87 ^d	9.95 ^e	7.15 ^l	9.64 ^f	8.91 ^d
Salicylic acid 250ppm	12.39 ^a	8.23 ^{gh}	10.99 ^c	10.54 ^a	13.49 ^a	8.45 ^h	12.08 ^b	11.34 ^a
Salicylic acid 500ppm	11.36 ^b	7.00 ⁱ	10.11 ^d	9.49 ^b	10.92 ^d	7.63 ^k	11.62 ^c	10.06 ^c
means	8.96 ^a	7.17 ^c	8.59 ^b		9.84 ^a	7.74 ^b	9.82 ^a	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's test.

Table 5. Effect of irrigation with diluted seawater, soil application with humic acid and foliar spray with salicylic acid and thiamin on reduced and non-reduced sugar contents (% dry matter) of *Duranta plumieri* at 2014 and 2015 seasons.

Treatments	reduced sugar(% dry matter)							
	1 st season				2 nd season			
	2000	4000	6000	means	2000	4000	6000	means
Control	6.80 ^h	6.23 ⁱ	5.14 ^l	6.05 ^e	7.64 ^f	7.11 ^h	5.80 ^l	6.85 ^e
Humic acid 1000ppm	6.18 ⁱ	5.25 ^l	6.28 ⁱ	5.90 ^f	7.37 ^g	5.68 ^l	7.10 ^h	6.72 ^f
Humic acid 2000ppm	6.98 ^g	8.20 ^d	8.20 ^d	7.79 ^b	7.90 ^e	8.85 ^d	9.03 ^c	8.59 ^b
Thiamin 250ppm	3.68 ^o	4.78 ^m	5.90 ^j	4.79 ^g	4.84 ⁿ	5.20 ^m	6.19 ^j	5.41 ^g
Thiamin 500ppm	8.02 ^e	3.89 ⁿ	6.89 ^{gh}	6.27 ^d	9.09 ^c	4.17 ^o	7.59 ^f	6.95 ^d
Salicylic acid 250ppm	8.67 ^b	6.25 ⁱ	9.00 ^a	7.97 ^a	9.81 ^a	7.41 ^g	9.87 ^a	9.03 ^a
Salicylic acid 500ppm	7.34 ^f	5.60 ^k	8.50 ^c	7.14 ^c	6.94 ⁱ	5.96 ^k	9.44 ^b	7.45 ^c
means	6.81 ^b	5.74 ^c	7.13 ^a		7.66 ^b	6.34 ^c	7.86 ^a	

non-reduced sugar(% dry matter)

Treatments	non-reduced sugar(% dry matter)							
	1 st season				2 nd season			
	2000	4000	6000	means	2000	4000	6000	means
Control	1.52 ^f	0.87 ^h	0.84 ^h	1.08 ^e	2.04 ^{de}	0.84 ^{kl}	1.11 ⁱ	1.33 ^f
Humic acid 1000ppm	1.08 ^{gh}	0.91 ^h	0.81 ^h	0.93 ^e	0.97 ^{ijk}	0.72 ^l	1.06 ^{ij}	0.92 ^g
Humic acid 2000ppm	2.03 ^d	0.84 ^h	1.57 ^f	1.48 ^d	1.96 ^{ef}	0.95 ^{ijk}	2.10 ^{de}	1.67 ^e
Thiamin 250ppm	1.87 ^{de}	1.39 ^f	2.08 ^d	1.7 ^c	1.84 ^{fg}	1.60 ^h	3.03 ^c	2.17 ^c
Thiamin 500ppm	0.85 ^h	2.63 ^c	1.34 ^{fg}	1.61 ^d	0.86 ^{kl}	2.98 ^c	2.05 ^{de}	1.96 ^d
Salicylic acid 250ppm	3.72 ^b	1.98 ^d	1.99 ^d	2.57 ^a	3.67 ^b	1.04 ^{ijk}	2.21 ^d	2.31 ^b
Salicylic acid 500ppm	4.01 ^a	1.41 ^f	1.62 ^{ef}	2.35 ^b	3.98 ^a	1.67 ^{gh}	2.18 ^d	2.61 ^a
means	2.15 ^a	1.43 ^b	1.46 ^b		2.19 ^a	1.40 ^c	1.96 ^b	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's test.

Table 6. Effect of irrigation with diluted seawater, soil application with humic acid and foliar spray with salicylic acid and thiamin on proline (µg/mg dry matter) of *Duranta plumieri* at 2014 and 2015 seasons.

Treatments	diluted seawater concentrations(ppm)							
	1 st season				2 nd season			
	2000	4000	6000	means	2000	4000	6000	means
Control	2.48 ^h	1.60 ^k	1.10 ^l	1.73 ^g	2.50 ^h	1.59 ^j	1.85 ^k	1.98 ^g
Humic acid 1000ppm	2.21 ⁱ	2.34 ⁱ	2.70 ^f	2.42 ^f	2.23 ^j	2.25 ^j	3.11 ^f	2.53 ^f
Humic acid 2000ppm	2.34 ⁱ	2.77 ^{ef}	3.10 ^d	2.74 ^e	2.36 ⁱ	2.76 ^g	3.92 ^c	3.02 ^e
Thiamin 250ppm	3.29 ^b	3.19 ^e	3.29 ^b	3.26 ^b	3.32 ^d	4.06 ^{ab}	4.11 ^a	3.83 ^a
Thiamin 500ppm	3.20 ^c	3.58 ^a	3.60 ^a	3.46 ^a	3.18 ^{ef}	3.08 ^f	4.11 ^a	3.46 ^b
Salicylic acid 250ppm	2.60 ^g	2.79 ^e	3.61 ^a	3.00 ^c	2.59 ^h	3.26 ^{de}	4.03 ^{abc}	3.29 ^c
Salicylic acid 500ppm	2.51 ^h	2.62 ^g	3.34 ^b	2.83 ^d	2.51 ^h	3.08 ^f	3.99 ^{bc}	3.19 ^d
means	2.66 ^b	2.70 ^b	2.96 ^a		2.67 ^c	2.87 ^b	3.59 ^a	

Mean values followed by the same letter are not significantly different at the 5% probability level according to Duncan's test.

Mineral element contents:

It was noticeable that there was a gradual decreased in plant contents of N, P, K and Mg⁺⁺ with the increasing in saline water concentration (Table7) combined with a gradually increasing in Na⁺ and Cl⁻ contents in shoots during the two seasons. On the contrary, these elements were increased with the applying of SA, B1 and humic acid and decreased the shoot contents of Na⁺ and Cl⁻ (Fig.4). SA and humic acid at both concentrations significantly increased the shoot contents of N, P and K elements under all salinity concentrations compared with the other treatments

during the two seasons (Table7). Under 2000ppm saline water, the highest values of N content were obtained when applied humic at 2000ppm (2.88 & 199%) and sprayed with SA at 500ppm (2.15 & 2.42%) respectively during the two seasons. Also, the same treatments gave the highest values of P and K elements under 2000ppm salinity.

Spraying with thiamin or salicylic acid at 500ppm significantly increased the shoot contents of Mg⁺⁺ under 2000ppm salinity during the two seasons (Fig.5). Soil application with humic acid increased the Mg⁺⁺ element content under all diluted seawater

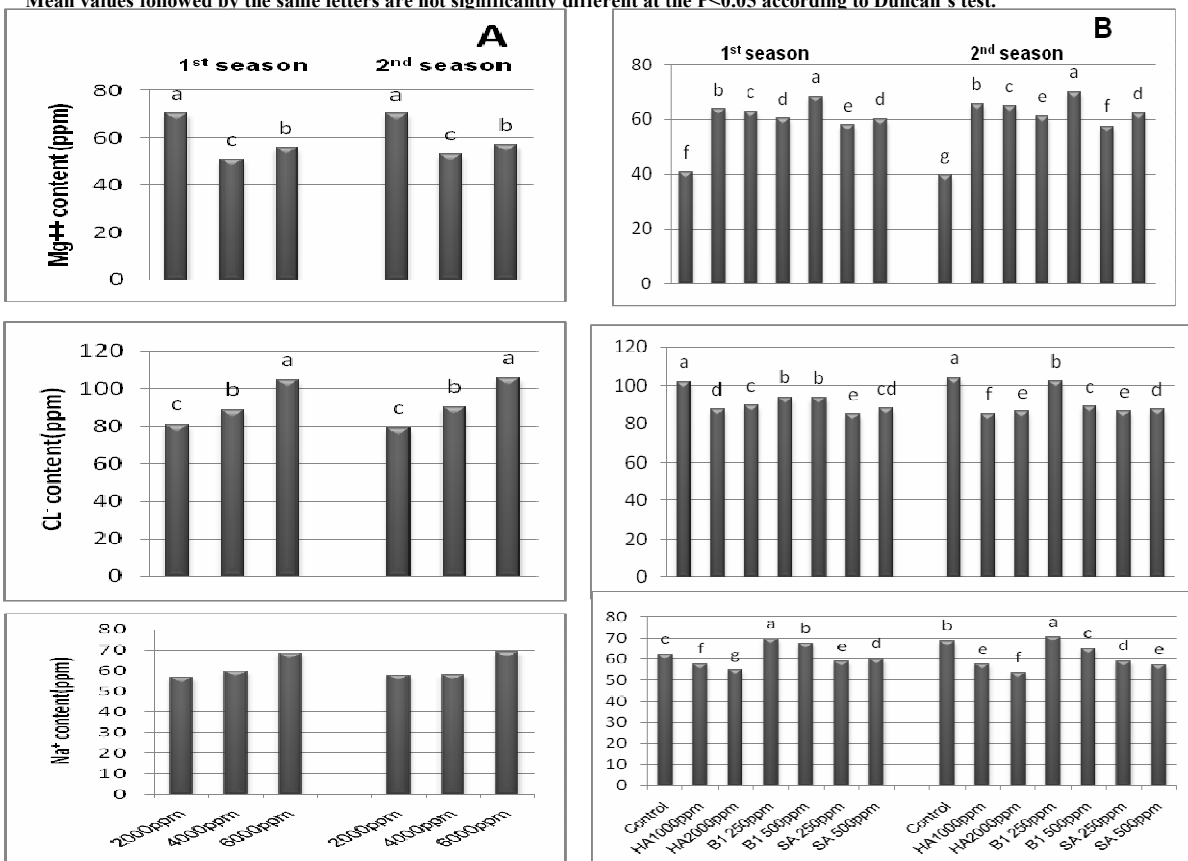
concentrations during the two investigation seasons. Treatments of humic acid and SA at both concentrations significantly decreased the plant contents of Na⁺ and Cl⁻ under all saline irrigation water as illustrated in Figs.

(6&7). Whereas, thiamin treatments at both concentrations were increased the Na⁺ contents in shoot plants under all concentrations of salinity.

Table 7. Effect of irrigation with diluted seawater, soil application with humic acid and foliar spray with salicylic acid and thiamin on Nitrogen, Phosphor and Potassium contents (% dry matter) of *Duranta plumieri* at 2014 and 2015 seasons.

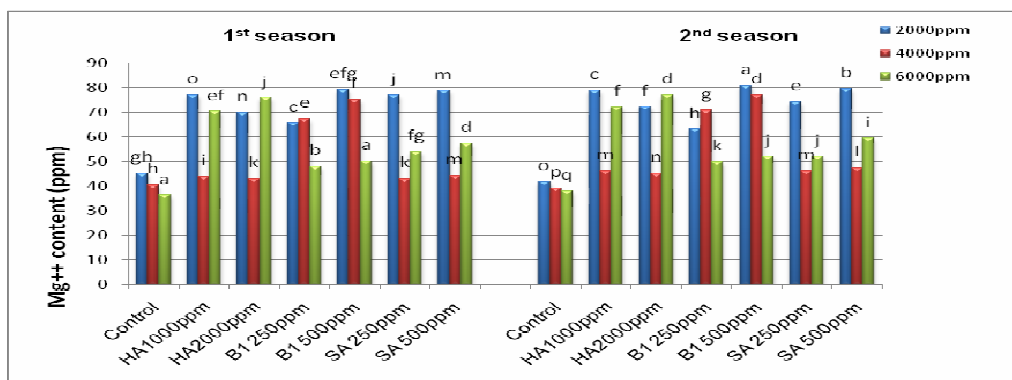
Treatments	diluted seawater concentrations (ppm)															
	1 st season				2 nd season				1 st season				2 nd season			
	N%				P%				K%				K%			
	2000	4000	6000	means	2000	4000	6000	means	2000	4000	6000	means	2000	4000	6000	means
Control	1.37 ^h	0.67 ^k	0.28 ^l	0.77 ^g	0.12 ^b	0.14 ^{lm}	0.13 ⁿ	0.13 ^g	2.05 ^l	1.90 ^j	1.67 ^l	1.87 ^c	2.05 ^l	1.90 ^j	1.67 ^l	1.87 ^c
Humic acid 1000ppm	1.98 ^d	1.77 ^{ef}	1.25 ^{hi}	1.66 ^c	0.34 ^b	0.26 ^d	0.15 ^{jk}	0.25 ^b	2.66 ^{gh}	2.50 ^{gh}	2.13 ⁱ	2.43 ^d	2.66 ^{gh}	2.50 ^{gh}	2.13 ⁱ	2.43 ^d
Humic acid 2000ppm	2.88 ^a	2.61 ^b	1.75 ^{ef}	2.41 ^a	0.38 ^a	0.32 ^c	0.13 ^{mn}	0.28 ^a	3.99 ^a	3.74 ^{bc}	2.93 ^{ef}	3.55 ^a	3.99 ^a	3.74 ^{bc}	2.93 ^{ef}	3.55 ^a
Thiamin 250ppm	1.56 ^g	0.89 ^j	0.76 ^{jk}	1.07 ^f	0.13 ^o	0.15 ^{jk}	0.15 ^{ij}	0.14 ^f	2.98 ^e	2.92 ^{ef}	2.69 ^{fg}	2.86 ^c	2.98 ^e	2.92 ^{ef}	2.69 ^{fg}	2.86 ^c
Thiamin 500ppm	1.74 ^{efg}	1.19 ⁱ	1.15 ⁱ	1.36 ^e	0.23 ^c	0.20 ^f	0.16 ⁱ	0.20 ^c	2.91 ^{ef}	3.24 ^d	2.41 ^h	2.85 ^c	2.91 ^{ef}	3.24 ^d	2.41 ^h	2.85 ^c
Salicylic acid 250ppm	1.85 ^{de}	1.66 ^{fg}	1.13 ⁱ	1.55 ^d	0.32 ^c	0.17 ^{gh}	0.17 ^h	0.22 ^c	3.54 ^c	3.14 ^{de}	3.14 ^{de}	3.27 ^b	3.54 ^c	3.14 ^{de}	3.14 ^{de}	3.27 ^b
Salicylic acid 500ppm	2.15 ^c	1.80 ^{ef}	1.83 ^{def}	1.93 ^b	0.32 ^c	0.14 ^{kl}	0.17 ^g	0.21 ^d	3.85 ^{ab}	2.46 ^{gh}	3.23 ^d	3.18 ^b	3.85 ^{ab}	2.46 ^{gh}	3.23 ^d	3.18 ^b
means	1.93 ^a	1.51 ^b	1.16 ^c		0.26 ^a	0.20 ^b	0.15 ^c		3.14 ^a	2.84 ^b	2.60 ^c		3.14 ^a	2.84 ^b	2.60 ^c	
Treatments	2000				4000				6000				means			
Control	1.05 ^h	0.51 ^k	0.33 ^l	0.63 ^g	0.12 ^m	0.12 ^{lm}	0.12 ^{klm}	0.12 ^f	1.92 ⁱ	1.62 ^j	1.60 ^j	1.72 ^f	1.92 ⁱ	1.62 ^j	1.60 ^j	1.72 ^f
Humic acid 1000ppm	1.76 ^{de}	1.72 ^{ef}	1.63 ^{fg}	1.70 ^c	0.32 ^b	0.22 ^{ef}	0.14 ^{ijk}	0.25 ^b	2.49 ^{fg}	2.68 ^e	2.26 ^h	2.48 ^e	2.49 ^{fg}	2.68 ^e	2.26 ^h	2.48 ^e
Humic acid 2000ppm	1.99 ^b	1.85 ^{cd}	1.92 ^{bc}	1.92 ^b	0.33 ^{ab}	0.34 ^a	0.15 ^{hi}	0.28 ^a	3.79 ^a	3.95 ^a	3.05 ^d	3.60 ^a	3.79 ^a	3.95 ^a	3.05 ^d	3.60 ^a
Thiamin 250ppm	1.53 ^g	1.15 ^h	0.65 ^j	1.11 ^f	0.11 ^m	0.16 ^h	0.13 ^{kl}	0.14 ^c	2.70 ^e	2.69 ^e	3.29 ^c	2.89 ^d	2.70 ^e	2.69 ^e	3.29 ^c	2.89 ^d
Thiamin 500ppm	1.73 ^{ef}	1.10 ^h	1.84 ^{cd}	1.56 ^d	0.20 ^f	0.22 ^c	0.16 ^h	0.20 ^d	2.71 ^e	3.40 ^{bc}	2.84 ^{gh}	2.82 ^d	2.71 ^e	3.40 ^{bc}	2.84 ^{gh}	2.82 ^d
Salicylic acid 250ppm	1.54 ^g	1.94 ^{bc}	0.87 ⁱ	1.45 ^e	0.29 ^c	0.19 ^g	0.16 ^h	0.21 ^c	3.38 ^{bc}	2.96 ^d	2.99 ^d	3.11 ^c	3.38 ^{bc}	2.96 ^d	2.99 ^d	3.11 ^c
Salicylic acid 500ppm	2.42 ^a	1.98 ^b	1.90 ^{bc}	2.10 ^a	0.26 ^d	0.15 ^{hij}	0.16 ^h	0.19 ^d	3.53 ^b	2.64 ^{ef}	3.49 ^b	3.22 ^b	3.53 ^b	2.64 ^{ef}	3.49 ^b	3.22 ^b
means	1.72 ^a	1.46 ^b	1.31 ^c		0.23 ^a	0.20 ^b	0.15 ^c		2.93 ^a	2.85 ^b	2.72 ^c		2.93 ^a	2.85 ^b	2.72 ^c	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's test.

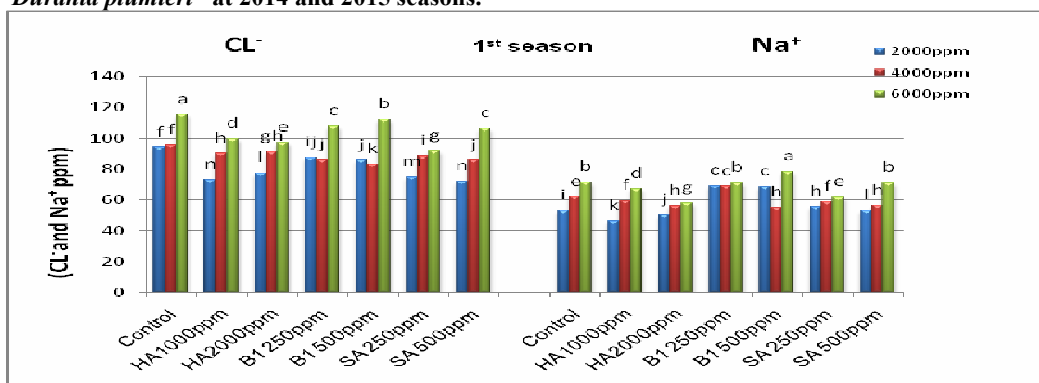


Columns labeled by the same letters are not significantly different at the P<0.05 according to Duncan's test.

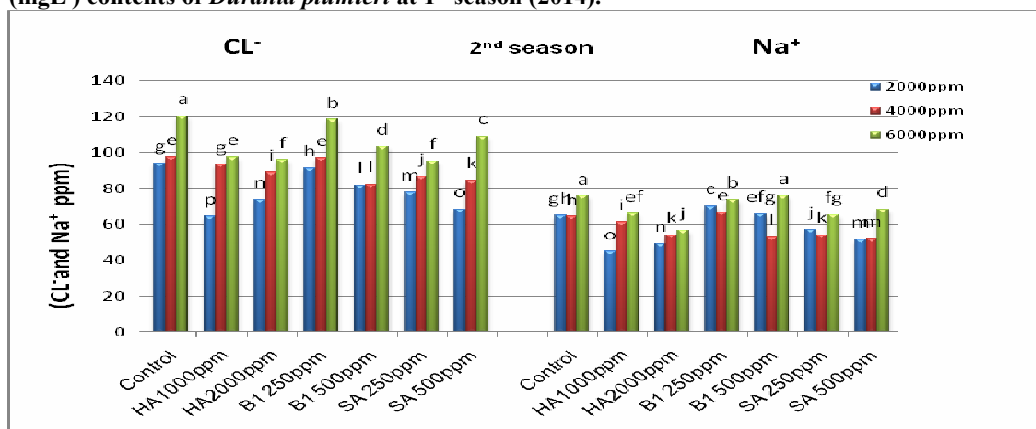
Fig. 4. Effect of irrigation with diluted seawater (A) and soil application with humic acid (HA) and foliar spray with thiamin (BI) and salicylic acid (SA) (B) on Magnesium (Mg⁺⁺) (mgL⁻¹), Chloride (Cl⁻) and Sodium (Na⁺) (mgL⁻¹) contents of *Duranta plumieri* at 2014 and 2015 seasons.



Columns labeled by the same letters are not significantly different at the $P < 0.05$ according to Duncan's test.
Fig.5. Effect of interaction between the irrigation with diluted seawater, soil application with humic acid (HA) and foliar spray with salicylic acid (SA) and thiamin (B1) on Magnesium (Mg^{++}) (mgL^{-1}) content of *Duranta plumieri* at 2014 and 2015 seasons.



Columns labeled by the same letters are not significantly different at the $P < 0.05$ according to Duncan's test.
Fig.6. Effect of interaction between the irrigation with diluted seawater, soil application with humic acid (HA) and foliar spray with salicylic acid (SA) and thiamin (B1) on Chlorid (Cl^{-}) and Sodium (Na^{+}) (mgL^{-1}) contents of *Duranta plumieri* at 1st season (2014).



Columns labeled by the same letters are not significantly different at the $P < 0.05$ according to Duncan's test.
Fig.7. Effect of interaction between the irrigation with diluted seawater, soil application with humic acid (HA) and foliar spray with salicylic acid (SA) and thiamin (B1) on Chlorid (Cl^{-}) and Sodium (Na^{+}) (mgL^{-1}) contents of *Duranta plumieri* at 2nd season (2015).

DISSCUTION

Response of plants to salinity stress were reflected by decline in different morphological, physiological and biochemical processes (Munns and Tester, 2008). So, the salinity stress considered as the major factor limiting plant growth and productivity (Farouk 2005). In this investigation, *Duranta* plants

irrigated with diluted seawater linearly decreased in vegetative growth parameters: plant height, the number of branches, and shoot fresh and dry weight with the increasing in saline water concentration. These results are consistent with Niu *et al.*, (2012), Hafez *et al.* (2011), Cassaniti *et al.* (2009b) and Munns & Tester (2008). They attributed these negatively responses to the reduction in root parameters, shortage in cell elongation

and division in leaves reflects on their final size, leading to a decrease in shoot dry weight. Rodriguez *et al.* (2005) treated *Asteriscus maritimus* plants with saline water (at 7 and 14 dS m⁻¹) and found a reduction of relative growth rate, net assimilation rate and stem and leaf dry weight. Saline water induced a high osmotic potential around the roots resulted in decreasing in water supply to the leaf cells (Blum, 1986). In addition, there was a decrease in photosynthetic pigments (total chlorophyll and carotenoids), minerals contents (N, P, K and Mg⁺⁺), carbohydrate contents, reducing and non-reducing sugar, and proline and increasing in contents of Na⁺ and Cl⁻ elements. These are combined with Shanan (2015) and Mazher *et al.* (2006). Salinity stress may be damaged the photosynthesis of plants at various stages, such as in plastids, gas exchange in stomata, structure and function of the thylakoid membrane, the electron transport system and directly effect on stomatal closure and consequently a reduction in the relative growth rate pointing to the photosynthesis might be the growth-limiting factor (Sudhira and Murthy (2004) and Sanchez-Blanco *et al.*, 2002). In contrast to the effects of saline irrigation water, data obtained from this work indicated to spraying *Duranta* plants with SA and B1 or application soil with humic acid increased all vegetative growth parameters, carbohydrate and sugar contents and decreased Na⁺ and Cl⁻ contents under salinity. Some previous studies reported that there was a positively relationship between plant growth and the addition of either SA, B1 or humic acid under saline irrigation water (Rady, 2012; Horvath *et al.*, 2007 and Szepesi *et al.*, 2005). Recently, the use of humic acid has been increasing for both improving plant resistance and soil quality under salinity. Rady (2012) confirmed that humic acid have directly and indirectly effects on the physiological processes of plant growth. The humic acid directly affects on plants through the influences on photosynthesis, respiration, nucleic acid synthesis and ion uptake (Nardi *et al.*, 2002). Humic substances ,as indirectly affects, can change the physical and chemical properties of soil through the impact on the soil water holding capacity, the release of plant nutrients from soil minerals, the increased availability of trace minerals, consequently, improving salt-affected soil fertility (Ouni *et al.*, 2014). Humic substances supply a high concentration of Ca, Mg and K elements resulted in decreased soil Na, Ec and the pH. These mineral elements protect the cation-exchange sites on soil particles, minimizing adsorption of Na⁺, consequently promoting Na leaching losses during drainage (Lakhdar *et al.*, 2009). Aydin *et al.* (2012) demonstrated that when added humic acid to saline soil significantly increased plant nitrate, nitrogen and phosphorus, proline and enhanced root and shoot dry weight , and they were attributed these positively effects to the enhancement of humic acid to release nutrients and water to the plant as needed. Our results indicated that spraying *Duranta* plants with SA reduced the Na⁺ and Cl⁻ uptake ,supplementary with increasing the total carbohydrate, reducing and non-reducing sugar contents, photosynthetic pigments and proline. These results are

consistent with those of El-Sayed and Abdel-Rahman (2015) and Gunes *et al.* (2007). SA acts as endogenous signal molecule responsible for enhancing the abiotic stress tolerance in plants especially salt stress. Several previous studies attributed increasing salt tolerance in many crops to the ameliorative effects of SA (Azooz, 2009, Kang *et al.*, 2012 and Gunes *et al.*, 2007). They reported that SA highly inhibited Na⁺ and Cl⁻ accumulation, whereas stimulated N, K and Mg concentrations of salt stressed plants. One of the main parts of the salinity tolerance mechanism is the maintenance solidity of the cellular membranes (Stevens *et al.*, 2006). Exogenous application of SA maintained the stability of membranes (El-Tayeb, 2005) and thereby lowered the electrolyte leakage in salt stressed plants resulted in decreasing uptake of Na⁺ and Cl⁻ and increasing the N, P, K and Mg concentrations in dry shoot under all saline water concentrations. Szepesi *et al.*, (2005) conclude that the salt tolerance induced by SA was associated with the previous activation of the oxidative defense mechanisms, the accumulation of osmolytes, increasing of CO₂ assimilation and photosynthetic rate and with increasing of mineral uptake. Irrigation with saline water led to oxidative stress through the excess production of reactive oxygen species (ROS) which distraction the photosynthetic pigments, activate lipid peroxidation and confusion in mineral nutrient metabolisms (Turan and Tripathy, 2013). Spraying with SA led to increasing the activities of antioxidant enzymes and scavenging the excess ROS which resulted in ameliorating the physiological processing and enhancing plant growth (He and Zhu, 2008). Previous studies showed that salt stress induced accumulation of thiamine in the plants resulted in enhanced tolerance to oxidative stress (Rapala-Kozik *et al.* 2008, Tunc-Ozdemir *et al.* 2009). In the present study, sprayed *Duranta* plants with thiamine improved the vegetative growth parameters under salinity. These results are similar to that observed in sunflower by Sayed and Gadallah (2002) who showed that root or shoot applied thiamine enhanced the sunflower growth, which was found to conjugated with thiamine induced reduction in membrane injury and increased leaf relative water content, chlorophyll content and soluble sugars. Nazar *et al.*, (2011) reported that both enzymatic and non-enzymatic antioxidant defense systems protect plants against oxidative stress, and they found a relation between increasing of activity and/or content of antioxidants and the improvement of salt tolerance. Rapala-Kozik *et al.*, (2008) suggested that total thiamine content in maize seedling leaves increased and was associated with changes in the distribution of free thiamine, thiamine monophosphate (TMP) and thiamine diphosphate (TDP) suggesting the important function of thiamine metabolism in the plant's response to salinity. In another study, Rapala-Kozik *et al.* (2012) have proved the participation of biosynthesis of thiamine under salinity and osmotic stress indicator and adaptation processes in *Arabidopsis thaliana*. Results obtained in this investigation indicated to an improvement in photosynthetic pigments and high biomass obtained due

to exogenous application of thiamine. Carotenoids act as potential antioxidants in plants and scavenge singlet oxygen species (Taiz and Zeiger,2010) resulted in increasing the plant tolerance to salinity stress. According to results obtained in this investigation, thiamine enables to reduce the negative effects of salinity stress by affecting as a growth factor to increase vegetative growth under saline irrigation water.

CONCLUSION

The results presented in this investigation confirmed that humic acid ,amongst other treatments, significantly improved the plant height, number of branches, fresh and dry weight, chlorophyll contents and mineral uptake and decreased Na^+ and Cl^- contents under all of diluted seawater concentrations. Exogenous application with SA increased the endogenous levels of total carbohydrate, reducing and non-reducing sugars and total carotenoids under salinity stress. Foliar application with thiamin increased the proline content and also improved the all vegetative growth and biochemical parameters comparing with plants just irrigated with saline water. It can be concluding that soil application with humic acid at 2000ppm was the highest treatment to increase the resistance to salinity stress with diluted seawater up to 6000ppm and spraying with salicylic acid or thiamin at 500ppm increased the resistance to salinity up to 4000ppm of *Duranta plumeri* plants.

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زيادة مقاومة الإجهاد الملحي في نبات الدورانتا الناجم عن الري بماء البحر باستخدام الثيامين و حمض الهيوميك وحمض السالسليك.

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التغيرات المناخية أدت الى الحد من مصادر المياه العذبة مما أدى الى زيادة الحاجة لمياه الري. ولهذا السبب كان الهدف الرئيسي لهذه الدراسة هو دراسة دور حمض الهيوميك و حمض السالسليك و الثيامين في زيادة مقاومة نبات الدورانتا للري بماء البحر المخفف. أجريت التجربة على الدورانتا خلال موسم ٢٠١٤ و ٢٠١٥ لدراسة تأثير الاضافة الأرضية ب حمض الهيوميك بتركيز ١٠٠٠ و ٢٠٠٠ مللي/لتر و الرش الورقي ب حمض السالسليك أو الثيامين بتركيز ٢٥٠ و ٥٠٠ مللي/لتر مع الري بماء البحر المخفف لثلاث تركيزات (٢٠٠٠، ٤٠٠٠ و ٦٠٠٠ مللي/لتر) على القياسات الخضرية و الفسيولوجية و المحتوى المعدني لنباتات الدورانتا. وأظهرت النتائج أن القياسات الخضرية تأثرت بشكل كبير نتيجة تأثير الإجهاد الملحي. ارتفاع النبات و عدد الأفرع/نبات و الوزن الطازج و الجفاف انخفضت مع زيادة التركيز الملحي للمياه. أيضا الإجهاد الملحي كان له تأثير سلبي على الكربوهيدرات، صبغات التمثيل الضوئي، السكريات المختزلة و الغير مختزلة و امتصاص المعادن (نيتروجين، فوسفور، بوتاسيوم و مغنيسيوم) بالاضافة الى زيادة المحتوى من الصوديوم و الكلوريد. وعلى النقيض من ذلك، حمض السالسليك و الثيامين و حمض الهيوميك كان لهم تأثير تحفيزي على جميع الصفات الخضرية في النباتات المروية بماء البحر المخفف بجميع التركيزات. تم الحصول على أعلى القيم للقياسات الخضرية و الكلوروفيل الكلي مع حمض الهيوميك عند تركيز ١٠٠٠ و ٢٠٠٠ مللي/لتر تحت جميع تركيزات الملوحة. حمض السالسليك نشط العمليات الفسيولوجية في نبات الدورانتا تحت الظروف الملحية من خلال الزيادة المعنوية في الكربوهيدرات الكلية و الكاروتين و السكريات المختزلة و الغير مختزلة. الأضافة الخارجية للثيامين أدت الى زيادة معنوية في البرولين في النباتات تحت تأثير الإجهاد الملحي. تم الحصول على أعلى امتصاص من عناصر النيتروجين، الفوسفور، البوتاسيوم و المغنيسيوم في النباتات تحت تأثير الإجهاد الملحي مع الاضافة الأرضية من حمض الهيوميك يليها معاملة الرش ب حمض السالسليك و الثيامين. أيضا أدت هذه المعاملات الى نقص المحتوى من الصوديوم و الكلور. وبناء على هذا البحث، يمكن أن يوصى بأن الاضافة الأرضية ب حمض الهيوميك بتركيز ٢٠٠٠ مللي/لتر يمكن أن يحسن من الآثار السلبية للري بماء البحر المخفف حتى تركيز ٦٠٠٠ مللي/لتر وكذلك رش نباتات الدورانتا ب حمض السالسليك أو الثيامين بتركيز ٥٠٠ مللي/لتر أدى لزيادة النمو و العمليات الفسيولوجية حتى تركيز ٦٠٠٠ مللي/لتر ماء ملح.