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INFLUENCE OF HUMIC AND ASCORBIC ACIDS ON GROWTH PARAMETERS AND ANTHOCYANIN CONTENT OF *ACALYPHA WILKESIANA* IRRIGATED WITH SEAWATER

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Abstract

High salinity plays a serious role in metabolic processes by diminishing the productivity of plants. The current study was carried out to study the influence of humic acid (HA) and ascorbic acid (AA) on vegetative growth and related biochemical attributes as well as mineral nutrition in *Acalypha wilkesiana* plants. In a pot experiment, HA was applied through irrigation at the concentrations of 1000 and 2000 $mgL^{-1}$and AA was applied through foliar spray at the concentrations of 250 and 500 mgL^{-1} under irrigation with diluted seawater at 3.0, 6.0 and 9.0dSm^{-1}. Saline treatments reduced the vegetative growth of pot grown *Acalypha wilkesiana* plants. Also, there is an adversely influence in the total chlorophyll, anthocyanin, proline, total sugar contents, and mineral nutrition i.e., N, P, K and Mg but higher contents of Na^+ and Cl^-. HA application not only alleviated an adversely effects of salinity stress but also improved all vegetative growth attributes. On the other hand, the highest values of vegetative parameters and both of anthocyanin and total chlorophyll contents were obtained with humic acid at 1000 and 2000 mg L^{-1} under the irrigation with diluted seawater at 6.0 dSm^{-1}. An accumulation of carotenoids and total sugar contents were stimulatory enhanced under saline conditions. However, the greatest records of vegetative growth, biochemical features and mineral nutrition with restriction of accumulated $Na^+$and Cl^- ions were recorded under the HA or AA treatments. Stepwise regression appeared that chlorophyll contents, K, $Mg^{+2}$and Na^+ had the most effective on anthocyanin content (R^2 = 0.99).

Keywords: Saline water, Humic acid, Ascorbic acid, *Acalypha wilkesiana*, Anthocyanin.

Introduction

Nowadays, under the climate changes and the lack of some irrigation water sources, the environmental agencies and organizations interested globally with the management and strategies of landscape and garden parks and exploring an alternative water sources for irrigation. (Botequilla Leito & Ahern, 2002). To overcome drought and scarcity of water the use of alternative resources of water should be efficiently occupied. Alternative water sources might be recycled water, treated municipal effluent and brackish groundwater, all of which generally have higher levels of salts compared with potable waters (Niu *et al*., 2007b and Niu & Cabrera, 2010). Treated effluent may also contain nutrients essential for plant growth; if water quality is good (not too saline), treated effluent can improve plant growth and reduce fertilizer requirements (Gori *et al*., 2000); application of industrial and municipal wastewater to land can be an environmentally safe water management strategy (Rodriguez *et al*., 2005; Ruiz *et al*., 2006). The potential physical, chemical or biological problems that are associated with effluent water applied to eatable crops (Kirkam, 1986) are of lesser concern for landscape plant production (Gori *et al*., 2000).

Owing to the expanding of landscape and green parks in the civilized environment, irrigation with saline water is becoming an urgent necessity in landscape where water scarcity leads to the reuse the wastewater for irrigation (Navarro *et al*., 2008; McCammon *et al*., 2009). In coastal gardens and landscapes, salinity is also a reality and where plants are damaged by aerosols originating from the sea (Cassaniti *et al*., 2009a; Ferrante *et al*., 2011). Globally, approximately one third of agricultural land are salt affected, leading a remarkable decrease in crop production (Ravindran *et al*., 2007). However, even though the importance of ornamental plant in Mediterranean areas, studies on salt tolerance of such plants have not been considered to be fully understood (Valdez-Aguilar et al. 2011). An adverse effect of salinity on growth and development of plants by reducing leaf area and stem enhancement subsequent by toxicity from high ionic concentration of Na^+ and Cl^-constituents (Munns, 2002; Azevedo *et al*., 2006; Gama *et al*., 2007; Munns & Tester, 2008; Taffouo *et al*., 2010; Liang *et al*., 2014), in particular, Duranta plants (Naema *et al*., 2017). Due to low osmotic stress of soil solution, salt stress, nutritional imbalances i.e; N, Ca, K, P, Fe, Zn, (Ashraf, 2004; Horie *et al*., 2011)and oxidative stress (Bano and Fatima, 2009), the adverse effects of salinity on plant growth and vegetative growth development at physiological and biochemical stages (Munns and James, 2003), and at the molecular aspect (Tester and Davenport, 2003) and thus limits water uptake from soil. Furthermore, salinity obviously restricts the uptake of phosphorus because phosphate ions precipitate with calcium ions in rhizosphere area (Bano and Fatima, 2009). Excessive accumulation of sodium in cell walls can rapidly lead to osmotic stress and cell death (Munns, 2002 & Zhang *et al*., 2005). When toxic ions such as Na^+ and Cl^- are present in the rhizosphere, they can disturb the uptake of nutrients by interfering with transporters in the root plasma membrane, such as those for K^+ and NO^-_3 (Tester & Davenport, 2003) and excess salt levels in soil result in hyperosmolarity, ion disequilibrium, nutrient imbalance, and production of reactive oxygen species (ROS), leading to plant growth retardation through molecular damage (Nawaz *et al*., 2010).Despite the fact that salt stress causes serious injury in ornamental plants, there are few studies have handled specifically with these plants used in landscapes (Marosz, 2004; Cassaniti *et al*., 2009a).

The efficient application of saline water depends on the convenient dilution and use of suitable plant growth regulators (Ejaz *et al*., 2012). During stress conditions the endogenous levels of growth regulators became low, which can be overcome by their exogenous application of plant growth regulators, fertilizers, and non-enzymatic antioxidants to minimize the adverse effects of salinity on plant growth and yield (Tuna *et al*., 2008; Kaya *et al*., 2010; Kaya *et al*., 2013).

From the most effective growth regulators against abiotic stresses are humic acids (Gulser *et al*., 2010 & Meganid *et al*., 2015) and ascorbic acid (Hossain *et al*., 2017). Ascorbic acid (AA) is considered the most water-soluble antioxidant in plants. Where, it plays an integral role in plants by regulating the redox state and anti-oxidative activity in cell of plants. Also, cell division and plant growth development as Co-enzyme. Recently, AA has been specific functions such as the regulation of the expression of various genes involved in plant growth, hormonal signaling pathways and determine the flowering time of plant, plant abiotic stress systems (Conklin and Barth 2004; Noctor 2006; Barth *et al*., 2010; Gao *et al*., 2011).

Humic acids are the major component increasing cell membrane permeability, respiration, photosynthesis, oxygen and phosphorus uptake, and supplying root cell growth so increasing the plant growth (Arancon *et al*., 2006; Laila *et al*., 2017). The application of humic acid individually or in combination with other materials, caused a marked increase in plant growth and crop yields by improving the hydro physical properties and nutrient availability of soils (Selim and Mosa, 2012). Humic acids enable growing plants to overcome the adverse effects of moderate soil salinity by improving the soil properties such as aggregation, aeration, permeability, water holding capacity, micronutrient uptake and availability, and by the decrease in the uptake of some toxic elements (Tan, 2003; Meganid *et al*., 2015).

Ornamental plants can be considered all the species and/or varieties that provide aesthetic value, improve the environment and the quality of our lives (Savé, 2009). *Acalypha wilkesiana* is a member of the spurge family (*Euphorbiaceae*) belonging to the genus acalypha, and is spreading to most parts of the world, especially the tropics of Africa, America and Asia. It characterized with an evergreen shrub and growing 3 m high and spreading 2 m across. It prefers light well drained soil and is suited to a protected shady position. Also, it can be damaged by both drought and frost (minimum temperature above 10°C). Finally, anthocyanins are especially prominent in the flushing leaf primordia of tropical rainforest species (Richards, 1952) and in the senescing autumn foliage of deciduous trees (Chang *et al*., 1989). These are characterized with water-soluble flavonoids that impart pink to purple colors in leaves and other organs (Harborne, 1988). The objective of this work was planned to appraise the response of acalypha plants to different levels of saline irrigation water under Egyptian conditions. Also, this work aimed to assess the relative efficiency of foliar spraying with ascorbic acid (AA) and soil application with humic acid (HA) under saline irrigation with diluted seawater in concern of growth, biochemical characteristics of acalypha plants under saline conditions.

Materials and Methods

Design of Experiment and Plant Culture

A pot experiment was conducted at Al-Baramoon Agricultural Research Station, Horticulture Research Institute, Mansoura, Dakahlia Governorate, during the two successive seasons of 2015/2016 and 2016/2017. On December 2015 and 2016, one-year old plants of *Acalypha wilkesiana* were obtained from the local commercial nurseries and transplanted into polyethylene containers (45 cm in diameter and 60cm length), filled with a mixture of clay and sand (2:1v: v), then each container had one plant. Before the beginning of treatments, all plants were cut at 30cm in height above the soil surface. The experimental design was split plot design with four replicates. Then main plots were assigned with diluted seawater irrigation levels; 3.0, 6.0 and 9.0 dSm^{-1}. While, the sub-plots consisted of humic acid (HA) and ascorbic acid (AA) concentrations in the following manner;

1. Control (without application).
2. Humic acid 1000mg L^{-1}(soil addition with tap water).
3. Humic acid 2000mg L^{-1}(soil addition with tap water).
4. Ascorbic acid 250mg L^{-1} (foliar-spray addition).
5. Ascorbic acid 250mg L^{-1} (foliar-spray addition).

Ascorbic acid was obtained from AL-Gomhorya Pharmaceuticals Medicinal Plants Production Company, Mansoura, Egypt. Soluble humic acid as potassium humate (80% humic acid, 11-13% K_2O) was produced by the Fertilizers Development Center, El-Delta Fertilizers Plant, Egypt.

Ascorbic acid concentrations were with distilled water which containing 0.02% Tween 20, as a surfactant, (polyoxy ethylenesorbitan monolaurate). Soluble humic acid was dissolved in tap water to make the treatment's solution which added to the soil and plants were sprayed with AA manually by using a spraying bottle. The treatments were started 2 weeks after transplanting and were added in twice times with two weeks between them.

A soil sample was collected from the soil mixture before transplanting and was air-dried, ground and sieved over a 2 mm. Physico-chemical properties was carried out as the following; distribution of particle-size by using the pipette method as described by Dewis and Fertias, (1970), electrical conductivity of saturated soil paste extract; Jackson, (1967), soil pH (saturated soil paste; Richards, 1954), available soil nitrogen was extracted using KCl (2.0 M), available soil phosphorus was extracted and determined using the Olsen method (extracted using $NaHCO_3$ [0.5 M] at pH 8.5 and determined colorimetrically with stannous chloride). Finally, available soil potassium was extracted with ammonium acetate (1.0 M) at pH 7. Some physico-chemical properties of the experimental soil are presented in Table 1.

Table 1 : Some physico-chemical properties of experimental soil.

Particle size distribution (%)				**Texture class**	***Chemical properties***					
Coarse sand	**Fine sand**	**Silt**	**Clay**		**O.M (%)**	**EC (dSm^{-1})**	**pH (1:2.5)**	**Available nutrients ($mg\ kg^{-1}soil$)**		
								N	P	K
1st s season										
7.72	18.18	33.6	40.5	Clay loam	1.33	1.13	8.1	49.78	11.82	298.0
2nd season										
7.61	18.14	33.8	40.45	Clay loam	1.40	1.87	7.9	56.90	12.90	301.98

Irrigation Water

Irrigation water was collected from the Mediterranean Sea, Egypt. Then, the obtained sea water was mixed with fresh tap water that equal 1.0 dSm^{-1} with saline water that measured approximately 50.0 dSm^{-1} to obtain the selected salinity levels i.e., 3.0, 6.0 and 9.0 dSm^{-1}, respectively. Acalypha plants were irrigated with tap water until the initial of saline irrigation treatments. After one week of the first applied to the humic and ascorbic acids, then the irrigation scheduling with diluted sea water was carried out every 8 days in winter and 5 days in summer. Periodically, 500ml of diluted seawater was added to each container.

Vegetative Growth Parameters

At 6 months after transplanting, four plants from each plot were randomly sampled for determination some growth and foliage parameters, i.e. (plant height "cm", number of branches/plants, shoot fresh and dry weight "g/plant). At the end of the experiment, shoot fresh weights were determined by severing the main shoot at the substrate surface and weighted.

Biochemical Analyses

Total Chlorophyll and carotenoids contents (mg/100g fresh weight) were determined according to Lichtenthaler and Wellburn (1983) by using (10ml) methanol alcohol (98%) to extract (0.05g) a fresh sample that was collected from the blade of the 4thupper leaf of the main stem. Extracts were kept overnight in darkness at room temperature and were determined spectrophotometrically at 666, 653 and 470 wavelengths. The pigment amounts were calculated by using the following equations:

Chlorophyll a (mg/ml) = 15.65 A 666-7.34A 653,

Chlorophyll b (mg/ml) = 27.05 A 653-11.21A 666

Total chlorophyll (mg/ml) = Chl a + Chl b

Total carotenoid = 1000A470-2.86 Chl a -85.9 Chl b)/245

Proline contents (µm mg^{-1} dry matter) was determined in dry leaves according to Bates at al., (1973). Total sugar contents (dry matter %) was determined as described by Sadasivam and Manickam, (1996).

Mineral Composition

Shoots were oven dried at 70°C to constant weights and dry weights (DW) were determined. Total nitrogen content (%) was determined using the Kjeldhal apparatus, according to the Association of Official Analytical Chemists (A.0.A.C, 1990). Phosphorus (%) was determined colormetrically as described by Olsen and Sommers (1982). Potassium (K^+%), and sodium (Na^+) contents (in mg kg^{-1} DW) were determined using Flame Photometer (Chapman and Pratt (1982). Finally, magnesium (Mg^{+2}) and chloride (Cl^-) contents (in mg kg^{-1} DW) were determined using EPA methods (U.S.EPA, 1983).

Statistical Analysis

The obtained data were statistically computerized according Duncan's multiple range test for analysis of variance (ANOVA) at confidence levels of 95% by CoStat (Version 6.303, Co Hort, USA, 1998-2004). Finally, to identify the optimum model describing anthocyanin content as affected by soil quality indices, stepwise multiple regression analysis was done using SPSS statistical software 17.0 version.

Results and Discussion

Vegetative Growth Parameters

Statistical analysis revealed that the increased salinity levels of irrigation water significantly ($P < 0.05$) declined plant growth parameters i.e., plant height, number of branches per plant, fresh and dry biomasses (g $plant^{-1}$) of *Acalypha wilkesiana* during the two successive growing seasons of 2016 and 2017 (Table 2).

The highest averages of these parameters were; 70.65 and 68.25cm for plant height, 9.90 and 9.25 branches/plant, 86.70 and 82.86 g $plant^{-1}$ for fresh biomass and 22.92 and 20.23g plant-1 for dry biomass were obtained from the lowest concentration of diluted seawater 3.0 dSm^{-1} during the two seasons respectively comparing with the other concentrations.

Table 2: Effects of humic and ascorbic acids on vegetative growth parameters of *Acalypha wilkesiana irrigated with seawater.*

Treatments		Plant height (cm)		Branches number/plant		Fresh weight (g $plant^{-1}$)		Dry weight (g $plant^{-1}$)	
		1st season	2nd season	1st season	2nd season	1st Season	2nd season	1st season	2nd season
Mean values as affected by seawater irrigation treatments (dSm^{-1})									
3.0		70.65a	68.25a	9.90a	9.25a	86.70a	82.86a	22.92a	20.23a
6.0		61.50b	60.90b	8.45b	8.50b	66.57b	62.74b	13.33b	11.73b
9.0		47.65c	48.75c	5.60c	7.75c	47.07c	45.87c	6.91c	6.22c
Mean values as affected by humic and ascorbic acids treatments (mg L^{-1})									
Without		40.50e	39.00e	6.58e	5.92e	46.74e	42.41e	7.96e	6.70e
HA 1000		73.00b	73.83b	8.67b	7.92b	71.91c	67.49c	15.80b	14.32b
HA 2000		76.92a	78.67a	9.50a	8.83a	81.75a	77.94a	19.66a	16.89a
AA250		47.92d	46.25d	7.00d	7.83d	59.35d	58.40d	12.81d	12.08d
AA500		61.33c	58.75c	8.17c	8.67c	74.14b	72.87a	15.70c	13.66c
Mean values as affected by seawater irrigation combined with humic and ascorbic acid treatments									
3.0 dSm^{-1}	Without	47.75j	45.50	7.75de	7.00def	58.07i	53.00i	11.10f	9.83h
	HA 1000mgL^{-1}	81.00b	82.25b	11.25a	10.00ab	88.70c	85.09bc	24.92b	21.98b
	HA 2000mgL^{-1}	85.75a	86.75a	11.75a	9.0bc	103.73a	99.40a	30.79a	27.11a
	AA250mgL^{-1}	66.25h	63.00h	8.00de	9.25b	84.78d	82.14d	22.87c	20.14c
	AA500mgL^{-1}	72.50f	63.75f	10.75ab	11.00a	98.20b	94.72b	24.93b	22.11b
6.0 dSm^{-1}	Without	40.00l	37.00l	7.00ef	6.50ef	44.94lj	40.95k	7.77h	6.20j
	HA 1000mgL^{-1}	75.00d	74.25d	9.00cd	8.25bcd	75.75g	63.5g	15.73e	12.55f
	HA 2000mgL^{-1}	77.00c	79.25c	9.75bc	10.00ab	78.58f	74.34f	18.08d	15.25d
	AA250mgL^{-1}	42.25k	41.50k	8.00de	8.25bcd	52.14j	55.03i	9.82g	11.07g
	AA500mgL^{-1}	73.25e	72.50e	8.50cde	9.50ab	81.45e	79.87e	15.26e	13.60e
9.0 dSm^{-1}	Without	33.75o	34.50o	5.00g	4.25g	37.20m	33.30m	5.02k	4.06l
	HA 1000mgL^{-1}	63.00i	65.00g	5.75i	5.50fg	51.3k	53.90i	6.76i	8.44i
	HA 2000mgL^{-1}	68.00g	70.00f	7.00ef	7.50cde	62.96h	60.11h	10.12g	8.32i
	AA250mgL^{-1}	35.25n	34.25n	5.00g	6.00ef	41.13n	38.02l	5.73j	5.04k
	AA500mgL^{-1}	38.25m	40.00	5.25g	5.50fg	42.78l	44.01j	6.91i	5.26k

Different letters in the same column indicated to significant differences according to the Duncan Multiple Range Test *($P < 0.05$).*

The reduction percent in fresh biomass was amounted 23.22which then upsurge to 29.30% in the 1st season and from 24.86 to 26.89% in the 2nd season as the salinity increased from 6.0 and 9.0dSm^{-1}, respectively. This reduction may be attributed to minimize the absorption of water, lower transpiration, and prevent water flow by stomata due to minor water potential in the root zone which is corresponded with the plant growth reduction (Demir & Kocacaliskan, 2002; Azevedo *et al.*, 2006; Munns & Tester, 2008; Alvarez & Sanchez-Blanco, 2013; Liang *et al.*, 2014). Under salinity stress, the plants suffer in the different ways; (i) suppress plant growth due to the accumulation of salts within the plant cells (ii) ionic toxicity of Na^+ and/or Cl^- in the plant cells declined root development, reduces photosynthesis products and increases cellular respiration. Then, this leads to low biomass in plants that exhibited to higher concentrations of salts (Akbarimoghaddam *et al.*, 2011).

A marked response for growth parameters were obtained from spraying plants with ascorbic acid or soil application with humic acid at all concentrations as compared to the control plants during the two seasons (Table 2).

Application with HA at 2000 mgL^{-1} were manifested the highest values of all studied growth parameters i.e., 76.92 and 78.67cm plant^{-1} for height, 9.50 and 8.83 plant^{-1} for number of branches, 81.75 and 77.94 g plant^{-1}for fresh biomass and19.66 and 16.89 g plant^{-1}for dry biomass during the both seasons, respectively. Application of humic acid principally associated to the enhancement of nutrients absorption. It enhances protein synthesis, improves photosynthesis products and macro-elements such as K^+, NH_4^+, or Ca^{2+}, and forms aqueous complexes with micronutrients (Nardi *et al.*, 2009; Selim & Mosa, 2012; Hamideh *et al.*, 2013).

The Two-way ANOVA revealed that, there werea significant interactive effect among seawater irrigation supplementary with humic or ascorbic acids treatments on all studied parameters except for number of branches at 9.0dSm^{-1} salinity, there were a slightly effects during both growing seasons (Table 2).

With the low level of salinity treatments, humic acid supply had a positive effective on plant height, fresh and dry biomass weights. Contrary, the reduction was occurred with plants that receiving no humic and ascorbic acid additions. However, it can be noticed that the addition of humic and ascorbic acids mainly alleviated the adverse effects caused by salinity stress and the progress of vegetative growth parameters of *Acalypha wilkesiana* plants. Under 3.0 dSm^{-1} of saline water irrigation treatment, the highest mean values of plant height (85.75 and 86.75 cm plant^{-1}), fresh and dry biomass weights (103.73 and 99.40, 30.79 and 27.11g plant^{-1}) were obtained from addition of 2000 mgL^{-1} humic acid respectively during the both seasons. These were followed by soil application with 1000 mgL^{1}humic acid treatment during the both seasons. On the other hand, the lowest mean values of studied vegetative parameters were 33.75 and 34.50 cm, 37.20 and 33.30, 5.02 and 4.06 g plant^{-1}occurred with irrigation with 9.0 dSm^{-1} of diluted seawater during the two seasons, respectively. The previous studies reported that the addition of humic acid stimulates photosynthesis process (Rady, 2012 and Naema *et al.*, 2017), secondary metabolism under the abiotic stress enabling plant to enhance the gas exchange and gathering light energy in chloroplast (Lotfi *et al.*, 2018) and resistant salinity stress (Pandolfi *et al.*, 2012).Further, ascorbic acid plays as promoter in plant growth aspects such as in cell division, cell wall enlargement and other oxidative pathways (Pignocchi and Foyer, 2003; Hossain *et al.*, 2017).

Biochemical Parameters

In the present study, saline water tended to decrease the chlorophyll, anthocyanin and proline in leaf tissues of plants during the both seasons except for total carotenoids and total sugars as presented in Table 3. Up to an irrigation salinity of 3.0 dSm^{-1}, total carotenoids gradually decreased with the increasing of salinity level, while above 6.0 dSm^{-1} total carotenoids increased with values of 0.22 and 0.19 mg g^{-1}FW, respectively. However, previous studies documented that salinity stress amplified chlorophyll contents in salt-tolerance plants (Higbie *et al.*, 2010&Stefanov *et al.*, 2016) allowing this parameter could be used as a biochemical indicator of salt-tolerance plants. Meanwhile, moderate salinity stress improves the biosynthesis pathways particularly chlorophyll and carotenoids to reserve appropriate performance of the photosynthesis process. In that trend, the obtained results are in agreement with Jiang *et al.* (2017), who found that the irrigation with saline water markedly enhance contents of carotenoids for leaves of tomato plants. Moreover, Pandolfi *et al.* (2012)emphasized that abiotic stress may galvanize some of physiological modification sallowing the plants to withstand salinity.

Table 3: Dataof a two-way ANOVA of biochemical parameters of *Acalypha wilkesiana* by saline irrigation water treatments in combination with different HA and AA applications.

Treatments		Carotenoids (mg g^{-1}FW)		Total Chlorophyll (mg g^{-1}FW)		Anthocyanin (mg100g^{-1} DW)		Proline (µg/g^{-1} DW)		Total Sugars (%DM)	
		1st season	2nd Season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Mean values as affected by saline water irrigation treatments (dSm^{-1})											
3		0.19b	0.18b	2.38a	2.40^{a}	470.89a	477.39a	3.31a	3.37a	3.94c	4.01c
6		0.15c	0.14c	2.12b	2.08^{b}	357.45b	280.91b	2.84b	2.73b	4.68a	4.88a
9		0.22a	0.19a	1.02c	1.03^{c}	175.07c	153.49c	1.23c	1.19c	4.44b	4.72b
Mean values as affected by humic and ascorbic acid treatments (mg L^{-1})											
Without		0.18b	0.18a	1.35d	1.37e	169.79e	141.88e	1.87e	1.71e	3.30d	3.28d
HA 1000		0.20a	0.18a	1.80b	1.79c	245.76d	249.88d	2.26d	2.33c	3.31d	3.50c
HA 2000		0.19b	0.18ab	1.99a	1.96b	451.64a	426.63a	2.64b	2.65b	4.46c	4.94b
AA 250		0.18b	0.16b	1.63c	1.60d	386.46c	323.31c	2.39c	2.30d	4.87b	4.96b
AA 500		0.18b	0.17ab	1.99a	2.00a	418.70b	378.38b	3.17a	3.15a	5.83a	6.02a
Mean values as affected by saline water irrigation combined with humic and ascorbic acid treatments											
3.0 dSm^{-1}	Without	0.25ab	0.25a	1.70e	1.69	266.38h	247.77i	2.60i	2.44i	3.02f	2.67j
	HA 1000mgL^{-1}	0.20 abcd	0.18c	2.32d	2.38cd	370.57g	383.01d	2.91f	2.99e	3.00f	2.75j
	HA 2000mgL^{-1}	0.21 abcd	0.22b	2.57b	2.56b	650.53a	663.36a	3.34c	3.43c	4.24d	4.60f
	AA250mgL^{-1}	0.13cd	0.13d	2.39c	2.46c	550.72b	565.59b	3.15d	3.35d	4.46d	4.82e
	AA500mgL^{-1}	0.14abc	0.12d	2.81a	2.81a	516.26c	527.20c	4.58a	4.65a	4.96c	5.24d
6.0 dSm^{-1}	Without	0.12cd	0.11d	1.42f	1.46g	154.29l	109.81	2.08j	1.81j	3.14f	3.14i
	HA 1000mgL^{-1}	0.15 abcd	0.14d	1.70e	1.65ef	266.64h	253.43g	2.64h	2.74g	3.30f	3.73h
	HA 2000mgL^{-1}	0.13cd	0.12d	2.57b	2.48bc	458.48e	365.83e	3.02e	2.90f	4.30d	4.88e
	AA250mgL^{-1}	0.22abcd	0.22b	1.71e	1.59f	406.74f	310.97f	2.84g	2.71h	5.35b	5.36c
	AA500mgL^{-1}	0.14bcd	0.13d	2.36cd	2.31d	501.10d	364.53e	3.63b	3.49b	7.31a	7.28a
9.0 dSm^{-1}	Without	0.19 abcd	0.17c	0.92g	0.97i	88.72n	68.06n	0.94o	0.89n	3.74e	4.02g
	HA 1000mgL^{-1}	0.24 abcd	0.23ab	1.39f	1.34h	100.08m	111.88k	1.23m	1.25m	3.63e	4.03g
	HA 2000mgL^{-1}	0.21 abcd	0.19c	0.83h	0.85j	245.91i	250.70h	1.55k	1.61k	4.85c	5.34c
	AA250mgL^{-1}	0.19abcd	0.13d	0.77h	0.74k	201.91k	93.39m	1.15n	0.85o	4.78c	4.67f
	AA500mgL^{-1}	0.26a	0.25a	0.80h	0.88j	238.77j	243.41j	1.29l	1.35l	5.22b	5.57b

Different letters in the same column indicated to significant differences according to the Duncan Multiple Range Test ($P < 0.05$).

Anthocyanin followed the same reduction trend in stressed plants, the percent of decrease amounted 24.09 & 51.05% in 1st season and by 41.16% & 45.36% in 2nd season under saline water irrigation treatments from 6.0 up to 9.0 dSm^{-1}, respectively. Data recorded in the current study revealed that the highest mean values of accumulated proline in leaves of *Acalypha wilkesiana* were 3.31and 3.37 µg/g^{-1} (dry basis) occurred with 3.0 dSm^{-1} saline irrigation treatment during the two seasons, respectively. In general, increased salinity level above control significantly amplified the accumulation of total sugars. However, data revealed that there is an increase in total sugars then declined evidently at 9.0 dSm^{-1} of irrigation water treatment. Kerepesi and Galiba, 2000 argued that an accumulation of carbohydrates in sugar form such as glucose, and fructose and starch manufactured belong salt stress and carbohydrates play a marked role in salinity stress by osmo-protection, scavenging of reactive oxygen species (ROS) and carbon storage.

As presented in Table 3, the applied treatments of humic and ascorbic acids were significantly effective increased the biochemical parameters of plants compared with the control. Using the humic acid resulted a significant increase in the carotenoids contents of *Acalypha wilkesiana* plants as compared with the other treatments over both growing seasons. The application of ascorbic acid also markedly amplified chlorophyll, proline and total sugar contents, mainly at the rate of 500 mgL^{-1}, while the maximum content of anthocyanin was produced from the treatment of 2000 mgL^{-1}HA compared with the control during both 2016 and 2017 seasons. These results were supplementary with the function groups of humic compounds such as carboxyls and hydroxyls (Delgado *et al*., 2002) as well asable to regulate the enzymes activity inner glucose metabolism led to enhancement in several physiological and biochemical pathways (Nardi *et al*., 2009).

Concerning the interaction between irrigation with diluted seawater and HA or AA, the two-way ANOVA results revealed that a significantly upsurge in carotenoids content by AA at 500 mg L^{-1} combined with 9.0dSm^{-1} irrigation saline water during the both seasons

(Table 3). Similarly, the highest mean values of total chlorophyll contents (2.81 mg g^{-1}FW) were obtained from foliar spray of 500mg L^{-1}AA combined with 3.0 dSm^{-1}salinity treatment during the both seasons. Further the same treatment gave the highest mean values of proline contents which recorded 4.58 and 4.65 (µg/g^{-1}DW) during the two seasons, respectively. While, the maximum mean values of anthocyanin (650.53 and 663.36 mg100 g^{-1}DW) were occurred with the soil application of 2000mg L^{-1}HA under 3.0 dSm^{-1} salinity treatment respectively during the both seasons. Finally, plants received 500mg L^{-1} of AA with 6.0 dSm^{-1} salinity gave the highest values of total sugar contents during both seasons in comparison with the other treatments.

Mineral Composition

Concerning with the effects of irrigation with saline water, the decreased content of N, P and K nutrients were occurred with particularly salt treated plants at 6.0 and 9.0 dSm^{-1} in comparison to the plants irrigated with saline water at 3.0 dSm^{-1}as presented in Table 4.

Table 4: Effects of saline irrigation water in combination with different HA and AA levels on concentrations of N, P and K (%) of *Acalypha wilkesiana* plants.

Treatments		N		P		K	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Mean values as affected by saline water irrigation treatments (mg L^{-1})							
3.0		1.91a	1.88a	0.28a	0.24a	4.09a	1.07b
6.0		1.27b	1.36b	0.20b	0.28b	2.05b	2.10a
9.0		0.46c	0.55c	0.10c	0.21c	0.73c	0.77c
Mean values as affected by humic and ascorbic acid treatments (mg L^{-1})							
Without		0.65e	0.69e	0.10d	0.12e	1.52c	1.55d
HA 1000		1.19c	1.20c	0.15c	0.24c	2.33b	2.34c
HA 2000		2.11a	2.22a	0.27a	0.29b	2.88a	2.99a
AA250		0.81d	0.93d	0.21b	0.26c	2.24b	2.36c
AA500		1.30b	1.29b	0.21b	0.30a	2.46b	2.45b
Mean values as affected by saline water irrigation combined with humic and ascorbic acids							
3.0 dSm^{-1}	Without	0.92g	0.86	0.13ef	0.12gh	2.56f	2.46f
	HA 1000mgL^{-1}	1.62d	1.43e	0.24d	0.34b	4.11d	3.97d
	HA 2000mgL^{-1}	3.49a	3.62a	0.41a	0.38a	5.14a	5.32a
	AA250mgL^{-1}	1.15f	1.34f	0.30bc	0.13fg	4.27c	4.14c
	AA500mgL^{-1}	2.36b	2.17c	0.31b	0.23d	4.37b	4.46b
6.0 dSm^{-1}	Without	0.76i	0.85h	0.11gh	0.13gh	1.44j	1.57j
	HA 1000mgL^{-1}	1.40e	1.53d	0.15e	0.24d	2.12h	2.26h
	HA 2000mgL^{-1}	2.19c	2.27c	0.28c	0.21e	2.67e	2.78e
	AA250mgL^{-1}	0.82h	0.88h	0.25d	0.40a	1.81i	1.92i
	AA500mgL^{-1}	1.17f	1.26g	0.25d	0.41a	2.22g	2.36g
9.0 dSm^{-1}	Without	0.26n	0.35m	0.05j	0.10h	0.56n	0.64n
	HA 1000mgL^{-1}	0.55k	0.63j	0.07ij	0.15f	0.77l	0.81l
	HA 2000mgL^{-1}	0.67j	0.77i	0.12fg	0.28c	0.84k	0.88k
	AA250mgL^{-1}	0.45l	0.55k	0.08i	0.25d	0.65m	0.68m
	AA500mgL^{-1}	0.36m	0.44l	0.09hi	0.12gh	0.81kl	0.87kl

Different letters in the same column indicated to significant differences according to the Duncan Multiple Range Test ($P < 0.05$).

This result may be attributed to the salt-induced nutritional disorders of plants are very difficult due to the influence of salinity on availability of nutrients, competitive uptake and uptake and accumulation of nutrients into the plants transport or distribution within the plant (Hu and Schmidhalter, 2005 & Akbarimoghaddam *et al*., 2011). Salt induced reducing nitrogen availability may be due to dislike Cl^- between NO_3^- and/or between Na^+ and NH_4^+ ions which eventuallyled to reduce the crop yield or declined the absorption of water by root hairs under salt stress (Lea-Cox and Syvertsen 1993). Also, the reduction in availability of P under salt stress occur due to; (a) the sorption processes that cause strongly fixing for PO_4^{-3} concentrations in soil solution, (b) the reduction in the activity of PO_4^{-3} as a result of ionic strength action and (c) Ca and Pminerals characterized with low solubility. These results are notable for the fact that concentration of phosphate in grown plants declined with amplified salt stress (Qadir and Schubert 2002). Concerning K content in tissue, the reduction in K^+ uptake in plant tissue may be attributed to the competitive uptake of Na^+ and K^+ at rhizosphere media, hence the Na^+ inhibits the K^+ passage through xylem tissues of plant or the reserves the K^+ uptake from soil solution (Khan *et al*., 2001; Hu and Schmidhalter, 2005). Osmotic stress of salt stress affect ion homeostasis, withstands the capability to scavenging reactive oxygen species (ROS) (James *et al*., 2011 & Rahnama *et al*., 2010).

Also, the enhanced ones were registered in treated plants with humic and ascorbic acids and the best results recorded in 2000mg L^{-1}HA.

In the current study, the highest values of N, P and K elements were observed in plants irrigated at 3.0 dSm^{-1} saline concentration after soil addition with humic acid at the concentration of 2000mg L^{-1}, followed by foliar sprayed with 500mg L^{-1}Ascorbic acid. Generally, it was noticed that the plants that received either different levels of soil or foliar addition of HA or AA had greater ionic concentrations in plants compared to those maintained under HA or AA treatments through irrigation.

As shown in Table 5, $Cl^-$and Na^+ concentrations in acalypha plants amplified laterally with the increased salinity level of irrigation water. In the contrary, Mg^{+2}decreased linearly with increasing the salinity concentration. In another study, Hu and Schmidhalter, (2005) stated that Mg^{+2} concentration decreased due to salinity in *T. aestivum* leaves.

Table 5: Effects of saline irrigation water in combination with different HA and AA levels on Cl^-, Na^+ and Mg^{+2} concentrations (mg kg^{-1} DW) of *Acalypha wilkesiana* plants.

Treatments		**Cl^-**		**Na^+**		**Mg^{+2}**	
		1st season	**2nd season**	**1st season**	**2nd season**	**1st season**	**2nd season**
Mean values as affected by saline water irrigation treatments (dSm^{-1})							
3.0		104.01c	112.44c	72.80c	75.26c	42.22a	41.13a
6.0		130.40b	125.42b	80.52b	81.50b	36.34b	37.15b
9.0		160.08a	154.11a	139.84a	136.00a	24.10c	25.45c
Mean values as affected by humic and ascorbic acid treatments (mgL^{-1})							
Without		145.67a	153.35a	117.86a	121.95a	20.50e	19.44e
HA 1000		130.91c	127.20c	97.86b	98.48b	25.18d	25.11d
HA 2000		121.07e	116.25d	86.88d	84.92d	43.08b	44.40b
AA250		132.05b	126.92c	93.49c	90.99c	34.76c	36.95c
AA500		127.79d	129.56b	92.51c	91.60c	47.57a	46.98a
Mean values as affected by saline water irrigation combined with humic and ascorbic acids							
3.0 dSm^{-1}	Without	105.69j	125.66h	90.33f	93.77g	24.99i	22.64k
	HA 1000mgL^{-1}	109.56i	118.87j	73.41i	80.86i	28.15g	25.14j
	HA 2000mgL^{-1}	96.27l	94.60n	62.94m	62.73o	52.98c	55.08b
	AA250mgL^{-1}	105.22j	99.00m	68.30l	66.80n	47.99d	50.79d
	AA500mgL^{-1}	103.31k	124.01i	69.04k	72.16k	57.01a	51.98c
6.0 dSm^{-1}	Without	155.67c	166.43b	98.23e	109.60	20.07l	17.94l
	HA 1000mgL^{-1}	128.43f	118.50j	85.04g	83.79h	25.62h	27.08i
	HA 2000mgL^{-1}	117.82g	107.84k	71.10j	70.10m	48.11d	49.14e
	AA250mgL^{-1}	134.98e	130.64g	75.10h	73.19j	32.92e	35.24f
	AA500mgL^{-1}	115.12h	103.73l	73.16i	70.87l	54.98b	56.35a
9.0 dSm^{-1}	Without	175.66a	167.97a	165.04a	162.47a	16.45m	17.74l
	HA 1000mgL^{-1}	154.74c	144.29f	135.13c	130.78d	21.78k	23.09k
	HA 2000mgL^{-1}	149.12d	146.31e	126.61d	121.97e	28.16g	28.99h
	AA250mgL^{-1}	155.96c	151.08d	137.07b	132.99b	23.37j	24.82j
	AA500mgL^{-1}	164.94b	160.93c	135.33c	131.78c	30.73f	32.61g

Different letters in the same column indicated to significant differences according to the Duncan Multiple Range Test ($P < 0.05$).

Furthermore, the supply of both HA and AA to acalypha plants substantially decreased the concentrations of $Cl^-$and Na^+ uptake (mg kg^{-1}DW), while increased the concentration of Mg^{+2} (mg kg^{-1} DW) during the both seasons. The soil addition of 2000mg L^{-1} HA was the most effective on reducing the concentrations of Cl^- and Na^+ ions as compared to the other treatments. Concerning the Mg^{2+}concentration, ascorbic acid concentration of at 500mg L^{-1} was the most effective as compared to the other treatments. Under salinity conditions, numerous studies argued that Na^+ competes with Ca^{+2} for binding sites and that apoplastic Ca^{+2} directly alleviates symptoms produced by mineral toxicities. The mitigated effect of external Ca^{+2} on plants facing salinity may be associated with the preserve of an optimal K^+/Na^+ ratio and imbalance in the cytosol in relation to an inhibition of Na^+ influx and K^+ efflux or promotion of Na^+ efflux and K^+ influx across the plasma membrane (Chakraborty *et al*., 2016).

Under the present study, the obtained results indicated that salt-induced damages were mitigated due to an amplification of humic and ascorbic acids, particularly the first one. Application of humic and ascorbic acids had slightly salt induced reduction of Cl^- and Na^+ concentrations under all saline concentration treatments during both seasons. While, the highest mean values of Mg^{+2} content were occurred with 500 mg L^{-1}ofascorbic acid combined with 6.0 dSm^{-1} as compared to the others during both seasons, respectively.

Response of anthocyanin in leaves of *Acalypha* plants (µg g^{-1}FW) with studied parameters

In current study, the method of stepwise regression appeared that chlorophyll contents, potassium (K), magnesium (Mg) and sodium (Na)had the most effective on changes of anthocyanin contents with adjusted R^2 = 0.984. On the other hand, branch numbers, fresh and dry weights, total carotenoids, proline contents, total sugars, nitrogen (N), phosphorus (P)and chloride (Cl) didn't have markedlyinfluence on the contents of anthocyanin. Then, anthocyanin content in leaf tissues of acalypha plants can be followed by the linear regression equation:

Anthocyanin= 202.61 + 7.10 Mg + 82.42K- 120.57 Chlorophyll -1.28 Na (R^2= 0.99).

Additionally, the linear regression between anthocyanin content in leaves and the most effective characteristics are shown in figure 1-A, B, C and D, respectively. Figure 1Ashow that the Mg^{+2} contents in leaf tissues had a positive influence on anthocyanin content with the regression coefficient; R^2 = 0.8109. This result may be attributed to Mg^{+2} stimulates and promotes pericarp coloration, while, does not influence on metabolism of sugar in plant (Wang *et al*., 2017), K nutrients and chlorophyll for Mg, 0.81 for K and 0.7140 for chlorophyll). Anthocyanin in red grape cell suspension culture are increased and their catabolism is decreased under Mg^{+2} treatment (Bhaskaran *et al.*, 2011), as is in several ornamental plants by spraying the foliage or by drenching pot plants with Mg^{+2} solution. Aster plants with Mg^{2+} treatment increased pigment concentrations without inducing the activity of key enzymes of the anthocyanin biosynthetic pathway in flower buds (Oren-Shamir *et al*., 2003). Potassium also activates enzymes involved in photosynthesis, where its essential function on CO_2 fixation is clearly demonstrated with isolated intact chloroplasts. External increase of potassium concentration levels to concentration, similar to the intact cell cytosol, stimulates CO_2 fixation three-fold. The counterbalance of the proton pumping into thylakoids during CO_2 fixation is also influenced by potassium concentration. On the contrary, linear regression displayed that the laef-Na had a negative impact on the anthocyanin content in leaf with regression coefficient; R^2= 0.7206) as shown in figure 1-D. Also, the accumulation of soluble salts at soil surface or subsurface layers due to capillarity rise and evaporation of underground water causes a severe reduction in plant growth; inhibits metabolic process; and/or restricts water uptake.

Conclusion

In current study, it is appeared that the destructive effect of irrigation with saline water on vegetative growth and biochemical characteristics of pot-grown *Acalypha swilkesiana* plants were significantly enhanced by HA application. The HA might withstand an adverse effect of salinity by improving antioxidant enzyme sallowing enhanced plant growth. The application of HA or AA generally led to a considerable upsurge in values of almost all the studied growth and biochemical attributes. However, the application of HA through irrigation was more effective than AA application through foliar spray. Under a larger scale, this work requires more study to supplementary estimate the potential using of HA application to improve the growth and provide aesthetic value of *Acalypha wilkesiana* plants under salinity stress, which improve the environment and the quality of our lives.

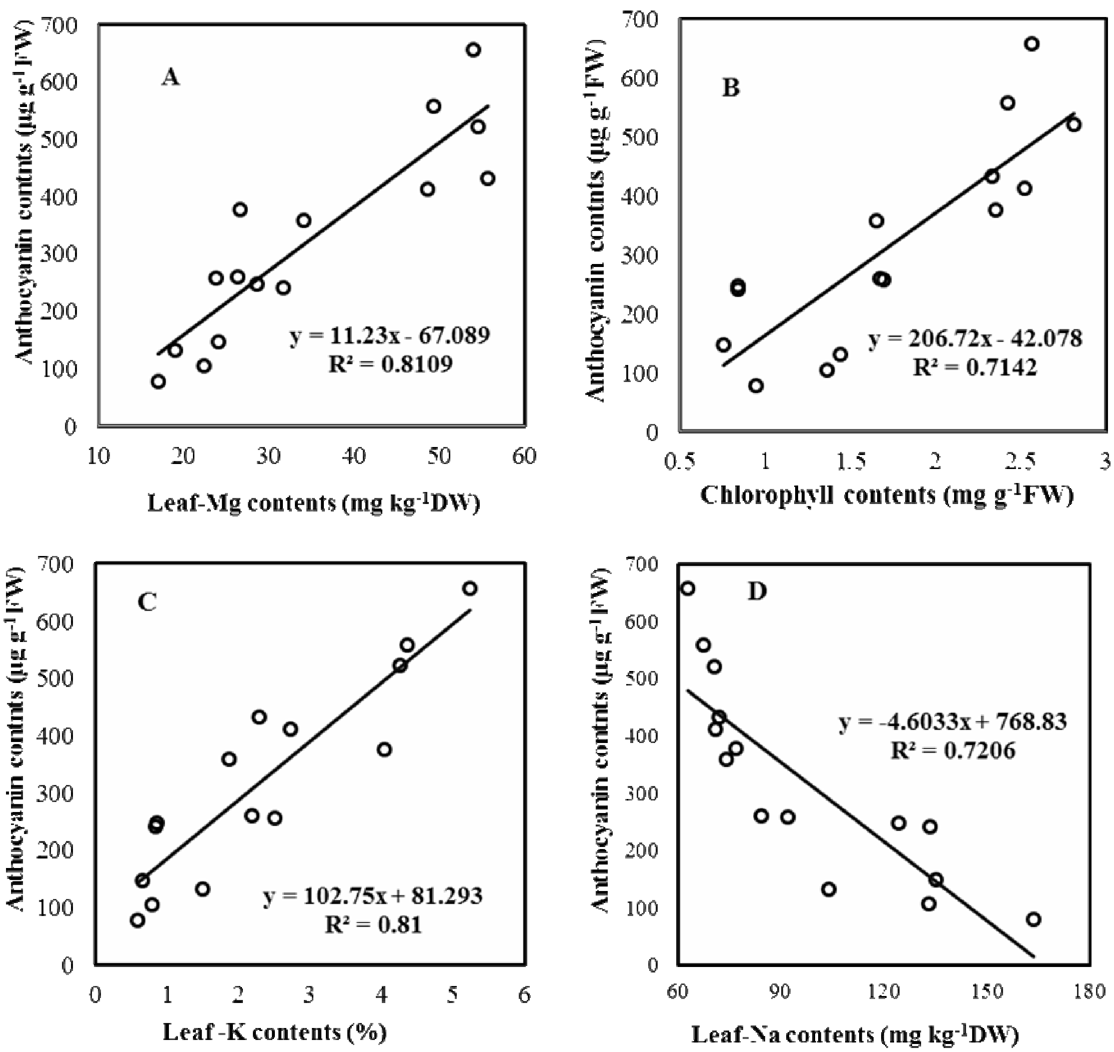

Fig 1 : Linear regression of anthocyanin contents response with: A) Leaf-Mg contents (mg kg^{-1}DW); B) total chlorophyll contents in leaves (mg g^{-1} FW); C) Leaf-K contents (%) and D) Leaf-Na contents (mg kg^{-1}DW) under salinity stress.

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