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Effect of Natural Substances on Carnation Plants (*Dianthus caryophyllus* L.) Growing under Saline Irrigation

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Abstract: This investigation was carried out during 2015 and 2016 seasons on *Dianthus caryophyllus* L. grown in 30 cm diameter plastic pots at a private nursery in Itay El- Baroud, El-Beheira Governorate, Egypt. The aim of this work was to study the effect of different levels of saline water of irrigation (tap water, 1000, 2000 and 3000 ppm) and three anti - salt stress materials namely, potassium humate at (8gm / plant) as a soil drench, spraying with proline at 50 ppm or spraying with ascorbic acid at 500 ppm and spraying with distilled water as a control and their interactions on vegetative growth, flower quality, vase life period and the chemical composition of carnation plants. From the obtained results it was concluded that increasing the salinity concentration to 2000 or 3000 ppm led to significant decrease in plant height, fresh and dry weights, number of leaves, flower diameter, fresh and dry weights of the flowers , vase life and photosynthetic pigments content (chlorophyll a, b and total carotenoids) of carnation plants, but increased proline content. Treating carnation plant with potassium humate, proline and ascorbic acid significantly increased plant height, fresh and dry weights of the plant, vase life and reduce the adverse effect of salinity on carnation plants. None of the three anti-stress agents affect the number of leaves on the plant. Potassium humate achieved the highest fresh weight of the flowers followed by the proline and ascorbic acid. Treatment with potassium humate and proline significantly increased chlorophyll a, b, total carotenoids and proline content.

Key words: Carnation (*Dianthus caryophyllus* L.) · Proline · Potassium humate · Ascorbic acid · Salinity · Vegetative growth · Quality of flowers · Chemical composition

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is one of the most important commercial cut flower in the global florist trade. It belongs to family *Caryophyllaceae*. Carnations are excellent for cut flowers, bedding, pots, borders, edges, indoors and rock gardens. They give a unique softness to the rock gardens. Though cut carnations are traded in the world market year around, they are in particular demand for the Valentine's Day, Easter, Mother's Day and Christmas. Miniature carnations are now gaining popularity for their potential use in floral arrangement [1].

Salinity, a severe environmental factor, has negative influences on growth and productivity of plants. Salinity problems have become increasingly more prevalent in over the last 10 years. Emphasis on water conversation strategies that use non – potable, alternative irrigation sources has been a primary contributor [2]. Salinity is one of the most serious problems limiting plant growth and productivity. Under such conditions it becomes very important to select plant species and varieties that are capable of tolerating high salinity levels and to determine the maximum salt concentration that can be used for irrigation without a significant effect on plant growth, quality and appearance.

Proline is an amino acid that preserves the plant cell under conditions of drought and salinity because it prevents and reduces the breakdown of protein in the cell and maintains the vitality of the plant. In addition to its role in protein synthesis and the plant cells response to environmental stresses, circumstantial evidence suggest that proline may also play a role in flowering and development both as a metabolite and as a signal molecule. Although there is a growing consensus that

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proline is of special importance throughout the reproductive phase (from flower transition to seed development) a general agreement on the molecular and genetic mechanisms proline is involved in, is yet to be established [3].

Humic substances have shown the beneficial effects on soil aggregation, structure, fertility, moisture holding capacity, microbial activity and cation exchange capacity [4,5]. Also, application of humic substances has been shown to increase membrane permeability, oxygen uptake, respiration and photosynthesis, phosphate uptake, nitrogen use efficiency and root elongation [6]. Moreover, application of humic substances has stimulatory effects on cytokinin and auxin or gibberellin-like substance along with indirect effect on plant metabolism [7]. The most exciting effects of humic acid appear in sandy and saline soil, where humic acid has improved the capacity of elements in sandy soil. Through its association with sodium, the plant helps to tolerate its high concentrations and protect against toxicity and osmosis problems associated with these high concentrations.

Ascorbic acid is a small molecular soluble in water and insoluble in other solvents as chloroform, benzene and fats. Ascorbic acid has antioxidant properties and acts as a primary substrate in the cyclic pathway for enzymatic detoxification of number of reactive oxygen species such as H_2O_2 and many other harmful to normal function of plant metabolism. Ascorbic acid participates in a variety of processes including photosynthesis, cell wall growth and cell expansion, resistance to environmental stresses and synthesis of ethylene, gibberellins, anthocyanins and hydroxyl proline [8]. Exogenous application of ascorbic acid to nutrient solution of bean plants grown under salt stress conditions increased antioxidant enzyme activity, resistance to salt stress, chlorophyll content and prevents abscisic acid accumulation as well as stimulated the vegetative growth and yield of different vegetable crops [9].

The aim of this work was to study the effect of different concentrations of saline of irrigation water and the treatment of proline, potassium humate and ascorbic acid on the growth and quality of flowers and the chemical content of carnation plant (*Dianthus caryophyllus* L.).

MATERIALS AND METHODS

The present study was carried out in the two successive seasons 2015 and 2016 at a private nursery in Itay El- Baroud Town, El-Beheira Governorate, Egypt. The rooted cuttings with length about 5 to 8 cm and having about 4 to 6 leaves of *Dianthus caryophyllus* L. "Crimson Tempo" dark red from "FLORAMIX" company for flowers and ornamental pot plants, Egypt, as a product of Hilverda Kooij Holland were planted in 10 cm diameter pots. One rooted cutting was transplanted to 30 cm diameter plastic pots filed with a sandy clay soil on March, 27st, 2015 and the plants were pinched to form five regular branches on the plant to produce only five flowers per plant. These processes were repeated at the same date in the second season (2016). The analysis of the soil medium presented in Table (1) was carried out in the soil testing laboratory, Faculty of Agriculture, Fayoum University according to Wild *et al.* [10] and Page *et al*. [11]. There are 16 treatments which are all the possible combinations of the four concentrations of saline irrigation water (tap water, 1000, 2000 and 3000 ppm) using pure NaCl and four various treatments with anti-stress substances, namely: proline at 50 ppm as foliar spray, Potassium humate at(8g/ plant) as a soil application, ascorbic acid at 500 ppm as a foliar application and distilled water as foliar spray (control) and their interactions on morphological characters as well as chemical constituents of carnation (*Dianthus caryophyllus*). These concentrations were sprayed early in the morning three times (at 30, 40 and 50 days after transplanting) on the plant foliage until the run off point while Potassium humate applied as a soil application twice, during planting and with the first portion of fertilization. All experimental unites received fertilization dose (1.5 g N ammonium nitrate (33% N) + 2.25 g P_2O_5 calcium superphosphate (15.5% P_2O_5) + 2.25 g K_2O potassium sulphate (48 % K_2O) / plant) as recommended by Ibrahim [12].Calcium superphosphate fertilizer was broadcasted during soil preparation while ammonium nitrate and potassium sulphate fertilizers were side banded at two equal portions; 3 and 6 weeks after transplanting. The treatments of irrigation had started after two weeks of transplanting. The plants were irrigated daily by 200 ml of the treated water for 6 day a week, in the seventh, all plants irrigated by tap water. All other agro-management practices such as pests control, pinching, disbudding, fertilization *etc*. were performed whenever it was necessary and as recommended in the commercial production of carnation. Flowers were cut at full flowering stage and stems were trimmed to 35 cm, stem ends were cut off inclined and put into bottles filled with distilled water. Vase life was recorded as the number of days from harvesting to senescence, which was detected in-rolling of petal margin and wilting of whole petals as well as a color change.

Table 1: The mechanical and chemical properties of the planting soil medium in 2015 and 2016 seasons

2015 Season	2016 Season	2015 Season	2016 Season	2015 Season	2016 Season	2015 Season	2016 Season	2015 Season	2016 Season
The mechanical properties									
Clay (%)				Silt (%)				Sand (%)				Soil texture	
41	44			24	22			35	34			Sandy clay	
The chemical properties									
pH				EC (ds/m)				HCO_3 mm/100g				SO_4 mm/100g				K mm/100g	
8	8.5			1.6	1.5			0.62	0.61			8.46	8.44			0.66	0.68
Ca mm/100g				Mg mm/100g				Na mm/100g				Cl mm/100g				---	---
10.96	10.92			4.94	4.82			3.55	3.64			2.57	2.43			---	---

The following data were recorded; plant height(cm), number of pairs of leaves per plant, plant fresh weight (g), plant dry weight (g), flower diameter (cm), flower fresh weight (g), flower dry weight (g), vase life (days), proline content according to Bates *et al*. [13], chlorophyll a and b contents and total carotenoids contents according to Moran and Porath [14]. The experimental design was factorial experiment in complete randomized block design with three replicates; sixteen treatments were replicated three times with 15 pots for each replicate. Five pots from each replicate unit specialized for cutting flowers while ten pots specialized for measuring of vegetative characters and determination of chemical constituents.

Data were subjected to analysis of variance (ANOVA) using the SAS program [15] and the mean values were compared using ref tukey's test at L.S.D at 0.05 level [16].

RESULTS AND DISCUSSION

Vegetative Growth Characters

Plant Height: Data in Table (2) shows that in the first season, plant height was not affected by increasing the salinity of irrigation water to 1000 ppm. Increasing salinity of the irrigation water to 2000 or 3000 ppm, led to significant decrease in plant height compared to irrigation by tap water. Irrigation with higher salinity concentration (3000 ppm) reduced plant height by 14.9% in first season and 20.7% in the second one than tap water.

Regarding the anti - stress materials, results showed that, in the first season both plants treated by potassium humate or sprayed with ascorbic acid achieved the tallest plants as compared to plants treated by proline or sprayed with distilled water. At the same time, spraying plants by proline significantly increased plant height than plants sprayed with distilled water.

In the second season, both of proline and Potassium humate caused a significant decrease in plant height as compared to control, while the control was on bar with treatment with ascorbic acid. Regarding the interaction effect, when the plants irrigated with 2000 ppm or 3000 ppm saline water, all plants which treated with the various tested anti-stress materials were better than those treated with distilled water, but Potassium humate and ascorbic acid were the most effective in the first season. In the second season, spraying with ascorbic acid or addition of Potassium humate to plants irrigated with 2000 or 3000 ppm were the best to reduce the harmful effect of salinity on plant height in the first season, while in the second one, ascorbic acid was the best.

Number of Pairs of Leaves/ Plant: The results in Table (2) shows that, firstly, with regard to the effect of irrigation with saline water, results of statistical analysis displayed that, the number of pairs of leaves formed on the plant gradually decreased morally with increasing salinity of water irrigation in an inverse relationship in both seasons of the study. The results showed that the treatment with the three tested anti-stress materials had no significant effect on the number of leaves formed on the plant in the two seasons of the study.

Regarding the effect of saline water irrigation combined with anti-stress materials, the results showed that the highest number of pairs of leaves was formed on plants irrigated with tap water and sprayed with distilled water in both seasons. Whereas the lowest values were produced by plants irrigated with saline water at 3000ppm combined with proline in both seasons. Potassium humate application and ascorbic acid insignificantly reduced the adverse effect of salinity at 2000 and 3000 ppm on the number of pairs of leaves / plant in both seasons.

Plant Fresh and Dry Weights: The results arranged in Table (2) show that, in the first season, irrigation with saline water at low concentrations (1000 ppm) did not reflect significant change in plant fresh and dry weights. But, increasing salinity concentration to 2000 or 3000 ppm, the fresh and dry weights of the plant was significantly lower than that of the tap water. Irrigation with saline water at the highest concentration (3000ppm) produced

Table (2): Means of plant height (cm), number of pairs of leaves per plant, plant fresh weight (g) and plant dry weight (g) of *Dianthus caryophyllus* L. as influenced by the different levels of Saline irrigation water (S), anti-stress substances (A) and their interaction (S×A) in the two seasons of 2015 and 2016

	Saline irrigation water (ppm) S					Saline irrigation water (ppm) S				
	Tap water	1000	2000	3000	Mean	Tap water	1000	2000	3000	Mean
	2015 Season					2016 Season				
Anti-stress substances (A)	Plant height (cm)					Plant height (cm)				
Control	49.2eg	49.6df	48.6Eg	42.9 h	47.5 C	44. 0a	40.6 ad	37.3 de	33.3 fg	38.8 A
Proline	53.8 bc	50.4 cf	49.7 Df	46.0 gh	50.0 B	42.1 a-c	37.3 de	37.3 de	30.3 g	36.6 B
Humic	57.5 a	56.6 ab	52.9 Cd	47.5 fg	53.6 A	43.0 ab	38.6 d	35.0 ef	31.6 g	37.01B
Ascorbic acid	56.5 ab	53.7 bc	52.1 Ce	48.4 fg	52.6 A	40.3 bd	39.6bd	38.0 de	39.0 cd	39.2 A
Mean	54.2 A	52.6 AB	50.8 B	46.1 C	---	42.3 A	39.1 B	36.9 B	33.5 C	---
	Number of pairs of leaves per plant					Number of pairs of leaves per plant				
Control	17.3 a	15.3 ad	15.0a d	13,0d	15.2 A	16.0 a	14.6ac	15.0 ab	12.0 de	12.4 A
Proline	16.0 ab	14.6 bd	15.0Ad	10.3e	14.0 A	15.0ab	13.6ad	14.6ac	11.0e	13.5 A
Humic	15.6ac	14.0 bd	13.3Cd	14.0b-d	14.3 A	15.0ab	14.0ad	12.0de	13.0 be	13.5 A
Ascorbic acid	15.0ad	15.0 ad	14.3Bd	13.3cd	14.42 A	15.0ab	14.0ad	13.6ad	12.3ce	13.7 A
Mean	16.0A	14.8 AB	14.4 B	12.6 C	---	15.3 A	14.1 AB	13.8 B	12.1 C	---
	Plant fresh weight (g)					Plant fresh weight (g)				
Control	76.6 bd	63.8 ef	64.6 Ef	55.3 f	65.1 B	60.1 a	53.2 bc	50.9be	42.2 f	51.6 A
Proline	85.6 ab	82.8 ac	76.15 bd	67.5 de	78 A	52.2be	46.7ce	52.2be	42.4 f	48.4 A
Humic	84.3 ab	89.5 a	63.6 Ef	66 df	75.8 A	56.4ab	52.0be	48.7 cf	46.2 ef	50.8 A
Ascorbic acid	90.8 a	82.0 ac	74.6 be	71.8 ce	79.8 A	54.4ac	52.7be	47.2 df	48.1de	50.6 A
Mean	84.3 A	79.5 A	69.7 B	65.1 B	---	55.8 A	51.1AB	49.7 B	44.7 C	---
	Plant dry weight (g)					Plant dry weight (g)				
Control	17.5 be	18.0 df	16.5 F	16.8 ef	17.2 C	10.2 d	13.8 ad	12 cd	10.5 cd	11.6 B
Proline	23.5 a	21.8 ac	22.1 ab	18.3 df	21.4 B	17.2 ab	13.1 bd	11.8 cd	9.6 d	12.2 AB
Humic	22.3 ab	23.5 a	19.8 be	20.8 ad	21.6 A	17.8 a	14.9 ac	12.9 bd	11.5 cd	14.3 A
Ascorbic acid	22.6 ab	21.8 ac	19.0 Cf	20.0 bd	20.8 B	14.7 ac	14.1 ad	11.2 cd	11.4 cd	12.9 AB
Mean	21.5 A	21.3 AB	19.3 B	19.01 C	---	14.9 A	13.96 A	11.98 B	10.7 B	---

*Values marked with the same letter (s) within the main and interaction effects are statistically similar using LSD test at P = 0.05. Uppercase letter (s) indicate differences between main effects and lowercase letter (s) indicate differences within interactions of each character.
Saline irrigation water (S), Anti-stress substances (A)

the lowest plants fresh and dry weights, the fresh weight was reduced by about 22.8 % and 19.9% in the first and second season, respectively compared to the plants irrigated with tap water.

In terms of anti-stress materials, the results showed that treatment with the three tested substances significantly increased fresh and dry weights of the plant compared to plants sprayed with distilled water (control) without significant differences between them in the first season. While in the second season, all tested materials did not show any differences than control, except humic in the second season which increased dry weight significantly compared to control.

Regarding the combined effect of saline water irrigation and anti-stress materials, the results indicated that when saline irrigation water was used at 2000 ppm in the first season, combined with both of proline and ascorbic acid achieved the highest values of the fresh and dry weight of the plant . In the second season, the effect of all the tested materials was equal to that of distilled water combined with irrigation at a concentration of 2000 ppm. When water irrigation salinity concentration increased to 3000 ppm in the first season combined with proline, ascorbic acid and Potassium humate the fresh and dry weights of the plant significantly increased compared to the plants sprayed with distilled water. The increment was significant for proline and ascorbic acid but was on bar with those treated by Potassium humate. In the second season, only ascorbic acid combined with 3000 ppm increased plant fresh weight significantly by about 13.98% than plant sprayed with distilled water combined with 3000 ppm. Proline treatment combined with 3000 ppm saline water irrigation significantly increased the dry weight / plant compared with plants irrigated with tap water (control).

Flower Characters

Flower Diameter, Fresh and Dry Weight: The results in Table (3) reflected that there is a gradual decrease in the mean of diameter, fresh and dry weights of the flowers by increasing the concentration of the salinity irrigation water. The decrease in flower diameter or flower dry weight were insignificant in the first season between tap water and low concentration (1000 ppm), while the decrease in flower fresh weight was significant. In the second season, increase salinity concentration to 1000 ppm caused a significant decrease in both flower diameter and flower fresh weight, while the decrease in flower dry weight was insignificant. When the salinity concentration of water of irrigation rose to 2000 or 3000 ppm, all the above mentioned characters decreased significantly compared to irrigation by tap water.

Regarding the influence of anti-stress materials, the results showed that treatment of potassium humate in the first season recorded the largest mean diameter, the highest fresh and dry weights of the flowers by a significant differences as compared to the other treatments, except dry weight was on bar with ascorbic acid treatment. While the effect of spraying with distilled water (control) was equal to that of spraying by proline to recorded least values of flowers diameter and flower fresh weight. In the second season, the results showed no significant differences between the plants sprayed with distilled water or sprayed with potassium humate or proline on flower diameter. While spraying with ascorbic acid caused a significant decrease as compared to control and was on bar with the spraying by proline. Only, potassium humate treatment significantly increased flower fresh weight compared to the other treatments, while both of humic and ascorbic acids increased flower dry weight.

For the combined effect, the combined treatments of irrigation with saline water at concentrations of 1000, 2000 ppm and (3000 ppm in the second season) treated with potassium humate were recorded the largest diameter of carnation flowers. While irrigation with a concentration of 3000 ppm, the largest diameter of the flowers was recorded when combined with ascorbic acid in the first season.

In the case of irrigation with saline water at 1000 or 3000 ppm, the highest fresh weight of flowers was obtained in the first season when the plants were treated with potassium humate or sprayed with ascorbic acid weight without significant differences between them in the second season. When saline water is used at a concentration of 2000 ppm, in both seasons, all tested anti-stress materials are equal.

With regard to flower dry weight, in the first season, the combined treatments irrigation with tap water or saline water at 1000 ppm concentration combined with the treatment of potassium humate, resulted in the heaviest dry weight of flowers at all. When the plants were irrigated with saline water at concentration of 2000 ppm combined with treatment by humic or ascorbic acid resulting in highest flower dry weight. Increasing the salinity concentration of irrigation water to 3000 ppm combined with spraying with proline or ascorbic acid were the best, while the combined with potassium humate had no effect compared with distilled water.

Vase Life: The results in Table (3) reflected an inverse relationship between the concentration of irrigation water salinity and the period of survival of flowers in the vase *i.e*. the plants irrigated with tap water recorded the longest vase life period with a significant differences compared with irrigation by saline water at 1000 or 2000 and 3000 ppm. The results of the second season confirmed the results of the first one.

About the impact of anti-stress substances, the data presented in the same Table (3) indicate that, spraying carnation plants with proline was the best to record the longest period of flower keeping quality compared with the effect of the treatment with humic followed by ascorbic with a significant differences than proline. All the tested substances led to a significant increase in the vase life of the flowers as compared to spraying with distilled water (control).

The tabulated data showed that the combination of irrigation with tap water and spraying with proline or humic recorded the longest vase life for carnation flowers during the two seasons of study. When the plants were irrigated with saline water at concentration of 1000 ppm combined with spraying with proline was the best. When the plants irrigated with saline water at 2000 ppm combined with potassium humate, the longest period of vase life was recorded. On the other hand, the shortest period was recorded in plants sprayed with distilled water in the two seasons.

The irrigation of carnation plants with saline water at concentration of 3000 ppm combined with spraying with proline, achieves the longest period of vase life during the two seasons of study. The results clearly showed that plants irrigated by saline water at 3000 ppm combined with spraying with proline reflected an increase in vase life by 46.9 and 77% compared to plant irrigated with the same concentration of saline water combined with spraying with distilled water.

Table 3: Means of flower diameter (cm), fresh and dry weight (g) and vase life (days) of *Dianthus caryophyllus* L. as influenced by the different levels of saline irrigation water (S), anti-stress substances (A) and their interaction (S×A) in the two seasons of 2015 and 2016

	Saline irrigation water (ppm) S					Saline irrigation water (ppm) S				
	Tap water	1000	2000	3000	Mean	Tap water	1000	2000	3000	Mean
	2015 Season					2016 Season				
Anti-stress substances (A)	Flower diameter (cm)					Flower diameter (cm)				
Control	6.8 a	6.4 ad	5.5 Gh	5.1 h	5.9 BC	6.9 a	6.4 bd	5.9 df	5.0 g	6.1 A
Proline	5.6 fg	6.1 cf	5.6 fh	5.8 eg	5.7 C	6.8 ab	6.2 ce	6.1 cf	4.6 g	5.9 AB
Humic	6.5 ab	6.5 ac	6.8 A	5.9 eg	6.5 A	7.0a	6.4 bd	5.8 ef	5.5 f	6.2 A
Ascorbic acid	6.5 ab	6.3 be	5.5 Gh	6.1 df	6.1 B	6.5 ac	5.9 df	5.6 f	4.7 g	5.6 B
Mean	6.4 A	6.3 A	5.8 B	5.7 B	---	6.8 A	6.2 B	5.8 B	4.9 C	---
	Flower fresh weight (g)					Flower fresh weight (g)				
Control	4.6 ef	4.5 eg	4.4 Fg	3.5 ij	4.28 B	5.6 bc	4.1 g	5.4 bc	4.6 ef	4.9 B
Proline	4.9 d	4.2 gh	4.4 Fg	3.3 j	4.21 B	5.8 b	4.7 ef	4.8 de	4.4 fg	4.9 B
Humic	5.7 a	5.1 cd	4.7 Df	4.1 gh	4.93 A	6.4 a	5.4 bc	4.4 eg	4.5 eg	5.2 A
Ascorbic acid	5.6 ab	5.1 cd	4.4 Fg	4.1 gh	4.79 A	5.2 cd	5.6 bc	4.5 eg	4.6 ef	5.0 AB
Mean	5.2 A	4.7 B	4.4 C	3.7 D	---	5.7 A	4.9 B	4.8 BC	4.5 C	---
	Flower dry weight (g)					Flower dry weight (g)				
Control	0.53 de	0.57 ce	0.10 F	0.60 ce	0.45 C	0.43 ad	0.39 c-e	0.46 ad	0.34 de	0.40 B
Proline	0.73 c	0.67 cd	0.70 C	0.67 cd	0.69 B	0.55 ad	0.47 a-d	0.44 ad	0.26 e	0.43 B
Humic	1.03 a	1.00 a	0.93 B	0.50 e	0.84 A	0.56 a	0.54 ab	0.49 ac	0.37 ce	0.48 A
Ascorbic acid	0.67 cd	0.53 de	0.90 B	0.67 cd	0.69 B	0.51 ac	0.50 ac	0.42 bd	0.42 bd	0.46 A
Mean	0.74 A	0.69 AB	0.66 AB	0.61 B	---	0.51 A	0.47 A	0.45 AB	0.35 B	---
	Vase life (days)					Vase life (days)				
Control	9.33 cf	10.00 ce	8.33 Df	5.67 g	8.33 C	8.33 df	9.00 ce	7.00 ef	4.33 g	7.17 C
Proline	15.33 a	11.33 bc	10 Ce	7.67 eg	11.08 A	14.33 a	10.33 cd	9.00 ce	8.33 df	10.50 A
Humic	14.67 ab	10.00 ce	11.33 bc	7.00 fg	10.75 AB	13.33 ab	9.00 ce	10.67 c	6.00 fg	9.75 AB
Ascorbic acid	10.00ce	11.00 c	10.33 cd	7.00 fg	9.58 B	11.33 bc	9.33 df	9.33 df	6.30 fg	9.07 B
Mean	12.33 A	10.58 B	10.00 B	6.84 C	---	11.83 A	9.42 B	9.00 B	6.24 C	---

*Values marked with the same letter (s) within the main and interaction effects are statistically similar using LSD test at P = 0.05. Uppercase letter (s) indicate differences between main effects and lowercase letter (s) indicate differences within interactions of each character.
Saline irrigation water (S), Anti-stress substances (A)

The pronounced negative effect of salinity of irrigation water on morphological characters can be explained as follows:

Initially soil salinity is known to represses plant growth in the form of osmotic stress which is then followed by ion toxicity [17]. During the initial phases of salinity stress, water absorption capacity of root systems decreases and water loss from leaves is accelerated due to osmotic stress of high salt accumulation in soil and plants and therefore salinity stress is also considered as hyperosmotic stress [18]. Osmotic stress in the initial stage of salinity stress causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes and decreased photosynthetic activity and decrease in stomatal aperture [19]. Salinity stress is also considered as a hyperionic stress. One of the most detrimental effects of salinity stress is the accumulation of Na^+ and Cl^- ions in tissues of plants exposed to soils with high NaCl concentrations. Entry of both Na^+ and Cl^- into the cells causes severe ion imbalance and excess uptake might cause significant physiological disorder. High Na^+ concentration inhibits uptake of K^+ ions which is an essential element for growth and development that results into lower productivity and may even lead to death [20].

In response to salinity stress, the production of ROS, such as single oxygen, superoxide, hydroxyl radical hydrogen peroxide, is enhanced [21]. Salinity-induced ROS formation can lead to oxidative damages in various cellular components such as proteins, lipids DNA, interrupting vital cellular functions of plants. Our results are in agreement with those obtained by Bass *et al*. [22] on gerbera concluded that, salinity decreased plant height, fresh and dry weights, number of flowers per plant and fresh and dry weights of the flowers and peduncle length. Haouala and Jaziri [23] used 3 concentrations of NaCl (50, 100 and 150mM) in irrigation water to irrigate

carnation (*Dianthus caryophyllus* L). The results indicated that, rooting rate and the length of the main root were clearly reduced in presence of NaCl, this results are supported by the results of Eid *et al*. [24] on *Anthurium andreanum* plant.

The stimulating impact of proline on the morphological characters can be discussed on the basis that, proline is an amino acid, plays a highly beneficial role in plants exposed to various stress conditions. Besides acting as an excellent osmolyte. Proline plays three major roles during stress, *i.e.*, as a metal chelator, an antioxidative defense molecule and a signaling molecule. Review of the literature indicates that a stressful environment results in an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance, stabilizing membranes thereby preventing electrolyte leakage bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative burst in plants. The amino-acid (proline) may play a role in protecting membranes and proteins against adverse effects of higher concentrations of inorganic ions and temperature extremes. The mechanisms utilized by the plants to overcome the adverse effects of salt stress might be via accumulation of free amino acids especially proline during salt, drought and water [25, 26]. Results of this study are supported by the results of Abd El-Samad *et al*., [27] which concluded that, when maize and broad bean plants sprayed with proline or phenylalanine results showed that, dry mass, water content, leaf area were increased. Kahlaoui *et al*. [28] stated that, as a result of the proline applied to tomato particularly with low concentration of proline (10mg.L^{-1}) led to significant increase of leaf area, growth length and fruit yield, Faraz *et al*. [29] mentioned that , proline as foliar spray to two varieties of *Brassica juncea* significantly increased all growth parameters.

While the enhancing effect of humic acid on morophological characters of carnation can be explained on the physiological fact that the mechanism by which humic acid stimulated plant growth similar to that of plant growth regulators. Humic substances contained cytokinin and auxins and thus affect plant growth in a positive mannar [30]. Humic substances were reported to stimulate plant growth through regulating the carbon cycle, increased cell division and optimized uptake and assimilation of major and minor elements, enzyme activation and / or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production [31].

Our results are in agreement with the findings of Eissa *et al*. [32] on anna apple seedling he found that spraying the plants with humic acid at either 1000 or 2000 ppm, significantly, produced taller plant height and more number of leaves and branches $plant^{-1}$ compared to control. Mohammadipour *et al*. [33] on *calendula officinalis* indicated that application of potassium humate significantly increased plant height, number of branches and flowers $plant^{-1}$, flowers fresh and dry weights and flower diameter than untreated control.

The stimulatory influence of spraying ascorbic acid on morphological characters of carnation can be explained on basis that, ascorbic acid is rapidly oxidized to dehydroascorbic acid, which in turn can be reduced again by copper-containing enzymes. The ascorbic acid oxidase enzyme is present in the plant. Because of its ability to reverse oxidation and reduction of ascorbic acid, its role is that it is an adjunct to phosphoric processes in photogravure, an important regulator of oxidation and reduction of protoplasm, as an oxidative effect and activity of some important enzymes within the plant [34]. Under salinity stress conditions Reactive Oxygen Species (ROS) such as superoxide (O_2) radical, hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) produced which are responsible for the damage of membranes and other essential macromolecules such as photosynthetic pigments, proteins, DNA and lipids [35].

Our results are consistent with the results obtained by Rady [9] on pea plants; he mentioned that ascorbic acid as a foliar spraying caused an increase in all morphological characters in the above-mentioned plants.

Chemical Constituents

Photosynthetic Pigments Content: Results in Table (4) indicated that there were opposite relation between photosynthetic pigments content in carnation plant (chlorophyll a, b and total carotenoids) and the concentrations of saline water during the two seasons of the study *i.e.* the highest content of chlorophyll a, b and carotenoids was recorded in plants irrigated with tap water while the lowest magnitudes were obtained from plants irrigated with saline irrigated water at 3000 ppm.

Concerning the effect of anti - stress substances, spray the carnation with proline increased plant content of chlorophyll a and b significantly compared to all other tested materials followed by humic and ascorbic acid which were on bar and differed significantly than control (distilled water). This was true during the two seasons of study. Foliar application of ascorbic acid or addition of

Table 4: Chlorophyll a, b and Total carotenoids (mg/g fresh weight) and proline (mlg/g fresh weight) of *Dianthus caryophyllus L.* as influenced by the different levels of Saline irrigation water (S), Anti-stress substances (A) and their interaction (S×A) in the two seasons of 2015 and 2016

	Saline irrigation water (ppm) S					Saline irrigation water (ppm) S				
	Tap water	1000	2000	3000	Mean	Tap water	1000	2000	3000	Mean
	2015 Season					2016 Season				
Anti-stress substances (A)	Chlorophyll a (mlg/g fresh weight)					Chlorophyll a (mlg/g fresh weight)				
Control	0.635 b	0.470 de	0.398 fg	0.277 i	0.445 C	0.637 b	0.473 de	0.397 fg	0.275 i	0.445 C
Proline	0.783 a	0.673 b	0.458 def	0.422 ef	0.584 A	0.781 a	0.675 b	0.455 def	0.420 ef	0.583 A
Humic	0.625 b	0.514 cd	0.429 ef	0.351 gh	0.480 B	0.627 b	0.516 cd	0.426 ef	0.350 gh	0.479 B
Ascorbic acid	0.636 b	0.541 c	0.422 ef	0.299 hi	0.475 BC	0.638 b	0.543 c	0.422 ef	0.297 hi	0.475 BC
Mean	0.670 A	0.550 B	0.427 C	0.337 D	---	0.671 A	0.552 B	0.425 C	0.335 D	---
	Chlorophyll b (mlg/g fresh weight)					Chlorophyll b (mlg/g fresh weight)				
Control	0.418 abc	0.318 es	0.194 hi	0.185 i	0.279 B	0.415 abc	0.321 es	0.196 hi	0.183 i	0.279 B
Proline	0.372 cde	0.351 de	0.253 gh	0.235 ghi	0.303 AB	0.376 cde	0.350 de	0.251 gh	0.238 ghi	0.304 AB
Humic	0.459 a	0.389 bcd	0.250 gh	0.223 ghi	0.330 A	0.456 a	0.392 bcd	0.252 gh	0.221 ghi	0.330 A
Ascorbic acid	0.436 ab	0.344 de	0.260 fg	0.258 fg	0.325 A	0.433 ab	0.346 de	0.262 fg	0.258 fg	0.325 A
Mean	0.421 A	0.351 B	0.239 C	0.225 C	---	0.420 A	0.352 B	0.240 C	0.225 C	---
	Total carotenoids (mlg/g fresh weight)					Total carotenoids (mlg/g fresh weight)				
Control	0.164 a	0.108 def	0.076 gh	0.066 h	0.103 B	0.169 a	0.102 def	0.073 gh	0.071 h	0.104 B
Proline	0.121 cde	0.106 def	0.097 efg	0.080 gh	0.101 B	0.120 cde	0.111 def	0.097 efg	0.083 gh	0.103 B
Humic	0.149 ab	0.128 bcd	0.110 def	0.095 fg	0.120 A	0.145 ab	0.123 bcd	0.116 def	0.092 fg	0.119 A
Ascorbic acid	0.153 a	0.142 abc	0.107 def	0.096 fg	0.124 A	0.157 a	0.140 abc	0.109 def	0.092 fg	0.124 A
Mean	0.147 A	0.121 B	0.098 C	0.084 C	---	0.148 A	0.119 B	0.099 C	0.084 C	---
	Proline (mlg/g)					Proline (mlg/g)				
Control	0.01 h	0.03 gh	0.10 c	0.03 gh	0.04 C	0.05 l	0.28 fg	0.28 fg	0.24 gh	0.21 C
Proline	0.002 i	0.08 cd	0.06 d-f	0.52 a	0.17 A	0.11 jk	0.55 c	0.61 b	0.46 D	0.43 A
Humic	0.004 i	0.04 fg	0.07 de	0.39 b	0.13 B	0.15 j	0.21 hi	0.16 ij	0.78 A	0.33 B
Ascorbic acid	0.03 gh	0.08 cd	0.01 h	0.05 eg	0.04 C	0.008 kl	0.30 ef	0.57 bc	0.35 E	0.32 B
Mean	0.01 C	0.06 B	0.06 B	0.25 A	---	0.10 D	0.33 C	0.41 B	0.46 A	---

*Values marked with the same letter (s) within the main and interaction effects are statistically similar using LSD test at P = 0.05. Uppercase letter (s) indicate differences between main effects lowercase letter (s) indicate differences within interactions of each character

Saline irrigation water (S), Anti-stress substances (A)

potassium humate significantly increased the content of total carotenoids compared with the other tested substances.

Regarding the interaction effect, generally, foliar application of proline combined with any of tested saline concentrations of irrigation water caused in the highest content of chlorophyll a, b as compared to the other tested substances during the two seasons of the study. The highest values of carotenoids contents were obtained from plants irrigated with the tap water and treated with Ascorbic acid or potassium humate in the two seasons of study while the lowest magnitudes were recorded in plants irrigated with saline water at 3000ppm and sprayed with distilled water.

Free Proline: The results in Table (4) clearly showed that plant content of free proline was significantly increased by increasing the salinity concentration of irrigation water. All the tasted concentrations resulted in a significant increase of proline content as compared to control in both seasons of study. The results indicated that, the plants treated with proline achieved the highest content of free proline in the plant during the two seasons of study followed by plants that were treated with potassium humate. In the second season, potassium humate was equal to the effect of foliar application of ascorbic acid on the content of free proline and the two differed significantly than treatment with distilled water.

Concerning the interaction effect, the highest plant content of free proline caused by plants that irrigated with a concentration of 3000 ppm combined by spraying with proline in the first season or those treated with potassium humate in the second one.

The stimulating impact of ascorbic acid on the content of free proline can be discussed on the basis that proline is an amino acid plays a major role in osmotic adjustment *i.e.* osmoprotectants and its accumulation is widely observed in various organisms under salt stress

[26]. The amino-acid (proline) may play a role in protecting membranes and proteins against adverse effects of higher concentrations of inorganic ions and temperature extremes.

The mechanisms utilized by the plants to overcome the adverse effects of salt stress might be via accumulation of free amino acids especially proline during salt, drought and water stress.

The Inhibition of effect of salinity of irrigation water on accumulation photosynthetic pigments contents in the leaves of carnation plants can be explained as follows: Osmotic stress in the initial stage of salinity stress causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes and decreased photosynthetic activity decrease in stomatal aperture [19]. Similar results were obtained by many investigators such as Aroiee *et al*. [36] mentioned that salinity stress increased the amount of free proline in the leaves of pumpkin compared to the control, Navarro *et al*. [37] studied the effect of different electrical conductivity (EC) value on carnation and mentioned that, contents of leaves from chlorophyll a, b and carotenoids decreased as EC of irrigation water increased. On the other hand, proline accumulation increased. Soliman and Shanan [38] found that, total chlorophylls, carotenoid contents, total carbohydrates in *Lagerstroemia indica* grown at various sea salt concentrations significantly decreased. On the contrary, proline content recorded a significant increase.

Our results are in agreement with the results of Dawood *et al.* [39] stated that, exogenous application of proline to *Vicia faba* under seawater stress. The results showed that, the concentration of 25 mM increased photosynthetic pigments and total carbohydrates. Alam *et al.* [40] suggestd that proline improves salt tolerance of maize by increasing the K+ / Na+ ratio and nutrient uptake, particularly P uptake. Our results are parallel with the findings of Azzaz *et al*. [41] stated that addition of potassium humate (HA) at 6 kg fed^{-1} to *Calendula officinalis* increased the value of chlorophyll a by 11.67 % than the untreated plants, also potassium humate treatments led to a significant increase in chlorophyll b and the best result of carotenoids content compared to control plants in both seasons. Prakash *et al*. [42] reported that, potassium humate to stevia plant, resulted in highest chlorophylls content in plant Tadayyon *et al*. [43] on niger plant, showed that foliar application of potassium humate resulted in a significant increase in plant content of chlorophylls and carotene.

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