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Is the Performance of Snapdragon Plants (*Antirrhinum majus* L.) Influenced by Some Bio-Stimulators under Salinity Stress?

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Abstract: This study was carried out during the two seasons of 2014/2015 and 2015/2016, at the nursery of the Ornamental Hort. Dept., Fac. Agric., Cairo Univ., Egypt. The aim of this research was to determine the influence of foliar application of three anti-salinity agents: chitosan, setter-2 and citric acid on growth, flowering and chemical composition of *Antirrhinum majus* L. plants exposed to salinity stress. The plants were irrigated with saline water containing NaCl at the concentrations of 20, 40, 60 and 80 mM, while the control plants irrigated with tap water. Plants irrigated with different salinity levels were foliar sprayed every two weeks with chitosan, setter-2 or citric acid at the concentration of 2000 ppm. The results revealed that raising salt concentration to 40 mM or more decreased plant height, leaf area, shoot fresh and dry weights, inflorescences length and inflorescences fresh weight. All salt concentrations increased number of leaves/plant and root length, while had no significant effect on stem diameter and inflorescences dry weight. Increasing salt concentrations from 20 to 80 mM decreased total chlorophylls, total carbohydrates and N, P and K %, while increased proline content, Na and Cl% in leaves. Foliar application of chitosan or setter-2 significantly increased the most growth and flowering parameters, total carbohydrates and N, P and K % and reduced the accumulation of proline content, Na and Cl% and appeared to be more effective than citric acid in this respect. Under the different concentrations of salinity, in most case, treating plants with chitosan or setter-2 improved most of the studied growth and flowering traits, increased total carbohydrates, N, P and K % while reduced the accumulation of proline content, Na and Cl % in leaves at the highest salt concentration compared to control (plants only irrigated with salt concentrations). From these results it can be conducted that the adverse effects of salinity on *A. majus* could be alleviated by foliar application of chitosan or setter-2 at the concentration of 2000 ppm.

Key words: *Antirrhinum majus* · Saline water · Chitosan · Setter-2 · Citric acid

INTRODUCTION

Snapdragon (*Antirrhinum magus*) belongs to Scrophalariaceae family, is one of the important winter flowering plants, native to the Mediterranean region that usually cultivated as an annual in Egypt. The plants are desirable for use as cut flowers for their wide range of petal colors and fragrance, they also used in gardens landscape in cool weather in flowering beds or borders as bedding and container plants. The leaves are simple, entire, lanceolate or oblong-lanceolate, each to three inches long, soft and tender. The flowers are showy, tubular, on elongated, terminal spikes, each to one-and-a-half inches long, with different flower colors except blue. The upright shoots covered with buds that open from bottom to top, providing shining color for an extended time period through spring to early summer. The plants ranged in size from 4 inches to 2 to 3 feet tall, depending on the cultivar [1-2]. Snapdragon cultivars are classified by height into three categories; dwarf, medium and tall. The dwarf plants, 6 to 12 inches, are bushy and used for border edging or in mixed containers. The medium-sized plants, 12 to 24 inches, often are used as border fillers or as cut flowers. Tall cultivars, 24 to 48 inches, have a dominant, single flower shoot and mostly are used as cut flowers or in the back of a border. In addition, there are trailing types that are used in containers and hanging baskets [3].

Salinity as one of abiotic stress has become a serious problem affects the growth and productivity of many plants especially in arid and semi-arid regions due to the shortage in fresh water resources. Salinity in irrigation

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water and in soils causes change in plants metabolic activities such as alteration in hormones balance, photosynthesis and respiration, uptake of minerals and inhibition of enzymatic activates [4]. The injurious impact of salinity is attributed to its effects osmotic stress, nutritional disorders and ions toxicity. Accumulation of Na^+ and Cl^- ions in the soil affects the soil porosity and also decreases the soil water potential that results in reduction in water and nutrients absorption. Also, excess the amount of ions in plant cells cause enzyme inhibition and metabolic dysfunction such as degradation of photosynthetic pigments and cause an imbalance of the cellular ions resulting in ion toxicity [5-7]. Furthermore, high salinity causes production of reactive oxygen species (ROS) which are responsible for various stresses by inducing damage to macromolecules and cellular structure that may lead to plant death [8].

Plant bio- stimulators are any substance applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or plant quality parameters, regardless of its nutrients content [9]. Among of such bio-stimulators are chitosan, Setter-2 or citric acid.

Chitosan is a biopolymer natural, cheap safe on the environment, produced from chitin deacetylation, it is similar to cellulose differing only by the presence or absence of nitrogen. Chitosan has received much interest in agriculture due to its excellent biocompatibility, biodegradability and bioactivity. In addition to its stimulation effect on plant growth, chitosan has been proven to induce the tolerance of various horticultural plants to abiotic and biotic stress. Chitosan affects different physiological process such as plant immunity, defense mechanisms involving various enzymes and antioxidant enzymatic activities viz., superoxide dismutase, catalase and peroxide against adverse conditions, also chitosan could interact with chromatin and directly affect gene expression [10-11]. Chitosan application was carried out on some ornamental plants and showed its positive effect on enhancing growth, flowering, photosynthesis, content of chlorophylls and mineral nutrient uptake [12-16]. It has been showed that chitosan able to induce some ornamental plants against abiotic stress like salinity because of its antioxidant activities [17]. Additionally, recent studies have been reported that salinity stress could be avoided by chitosan which scavenging reactive oxygen species induced by salt stress [18].

Setter-2 is a commercial product of bio-stimulators containing ascorbic acid (vitamin c), citric acid, micro and macro-elements. Ascorbic acid plays a vital role in plant growth as a cofactor of enzymes involved in regulating; photosynthesis, cell elongation, cell division, hormone biosynthesis, cell wall expansion as well as inducing the tolerance to ROS [19-21]. It is regarded as one of the most effective antioxidants abundantly against abiotic stresses [22-23]. Previous studies reported that ascorbic acid mitigate the drastic effects of salinity stress on different plants growth characteristics [24-28]. Citric acid is one of the organic acids that serve as the source of carbon skeleton and cellular energy, which are utilized in the respiratory cycle and other biochemical pathways [29]. It has been reported that citric acid was closely related with improved growth and flowering and increase vase life and chlorophyll content [30-32]. Citric acid has also been confirmed to alleviate salinity stress [33]. Additionally, earlier study found that foliar application of Setter-2 mitigate the harmful effect of salinity stressed plants and was effective in reducing the accumulation of Na and Cl ions in plant tissues [34].

The present study aimed to evaluate the influence of three anti-salinity bio-stimulators compounds; chitosan, setter-2 and citric acid on improving vegetative growth and flower quality of *Antirrhinum majus* L. plant irrigated with NaCl-saline water.

MATERIALS AND METHODS

A pot experiments were carried out at the Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during seasons of 2014/2015 and 2015/2016. This study aimed to investigate the response of *Antirrhinum majus* plants irrigated with different concentrations of saline water to foliar application of chitosan, Setter-2 and citric acid as plant growth bio-stimulators.

On 1st of October in the first season and 7th of October in the second one, seeds of *Antirrhinum majus* cv.‘ Common Pink were sown in plastic trays. After 6 weeks, in both seasons, when the seedlings reached about 10 cm in length and had 6-8 leaves they were transplanted individually in 25-cm plastic pots filled with a mixture of clay and sand (1:1, v/v). After two weeks from transplanting, the plants were irrigated with saline water containing NaCl at the concentrations of 20, 40, 60 and 80 mM, in addition to the control plants which irrigated with tap water. The irrigation was applied every five days at 500 ml/pot. In both seasons, plants irrigated with different salinity levels were foliar sprayed every two weeks with three different plant bio-stimulators including, chitosan (poly-(1.4-B-D-glucopyranosamine);2-Amino-2-deoxy-(1-

>4)-B-D glucopyranan), setter-2 (commercial compound consists of 0.5% ascorbic acid, 0.5% citric acid, 5% total N, 0.1% Mn, 0.1% Cu, 9% chelated Ca^{++} and 1.5% chelated boron) or citric acid at the concentration of 2000 ppm for each one of them. In addition, the control plants continued to be sprayed only with tap water. The different bio-stimulators compounds were obtained from United Agric. Development Co., Egypt. Wetting agent (Bio-new film at 1 ml /L) was added to the solutions of bio-stimulators. The plants foliage was sprayed until run off point. The common cultural practices were done including hand picking of weeds and monthly plants fertilization with comercial kristalon (NPK 19-19-19) at the rate of 2.5 g/pot.

The experiment was arranged in randomized complete block design with 20 treatments [5 salt concentrations (including the control) X4 plant bio-stimulators treatments (including the control)], with 3 blocks (replicates), each block consisting of 80 plants (4 plants/treatment).

After 90 days from transplanting the experiment was terminated and the vegetative and flowering parameters were recorded including plant height (cm), number of leaves/plant, stem diameter (mm, at 5 cm above soil surface), leaf area (cm^2), root length (cm), leaf area ($cm^{2,}$ using digital leaf area meter), shoot fresh and dry weights (g/plant), number of inflorescences /plant, inflorescence length as well as fresh and dry weights of inflorescences (g/ plant). In addition the following chemical constituents were determined including total chlorophylls in fresh leaf samples using chlorophyll meter Model SPAD 502 [35], total carbohydrates % in dried leaves [36], proline content (μ moles /g fresh matter of leaves) in fresh leaves [37]. N, P, K Na, Ca and Cl% were also determined [38-39].

The data recoded on the two seasons for vegetative growth, flowering and chemical constituents were subjected to an analysis of variance (ANOVA) and the Least Significant Difference (L.S.D.) test at the 5% level was calculated to compare the mean values of different parameters [40].

RESULTS AND DISCUSSION

Vegetative Growth and Flowering Parameters

Effect of Salt Concentrations: The data presented in Tables 1 and 2 showed that in both seasons, plant height, leaf area, shoot fresh and dry weights, inflorescences length and inflorescences fresh weight of *Antirrhinum majus* plants were significantly decreased as a result of irrigation with using saline water at 40, 60 and 80 mM, while the lowest salt concentration (20 mM) had no significant effect on these parameters as compared to the control. These reductions in the studied vegetative growth and flowering traits as a result of increasing salt stress are similar to finding of several researches [41-54]. Such reductions caused by increasing salinity levels may be due to the unfavorable effects of salts absorbed and accumulated in plant tissues and affecting on photosynthetic rate, enzyme activity and growth hormones. Also, accumulation of salts in soil decreases water and minerals uptake by plants which lead to decrement growth and flowering biomass [4, 55].

Number of leaves/plant and root length (in both seasons) as well as number of inflorescences (in second season) showed different trend in response to irrigation water salinity. In most cases, increasing salt concentrations from 20 to 80 mM significantly increased these parameters compared to the control. These results confirmed the reports of other researches [35, 54, 56] which found increases in root length and number of leaves as the salinity concentrations increased. In addition, stem diameter, number of inflorescences (in the first season) and inflorescences dry weight were not significantly affected by any concentration of saline water compared to the control.

Effect of Bio-Stimulators Treatments: Data in Tables 1 and 2 indicated that vegetative growth and flowering parameters of *Antirrhinum majus* plants were considerably affected by foliar application of the three bio- stimulators treatments. In both seasons, in most cases treating the plants with chitosan or setter-2 significantly increased most of growth and flowering parameters compared to the control plants sprayed with tap water. The few exceptions to this prevalent trend were detected in both seasons with plants foliar sprayed with chitosan or setter-2 that had insignificantly higher stem diameter than the control, also inflorescences dry weight in the second season showed insignificant increase in response to foliar application of setter-2 compared to the control.

However, foliar application of citric acid had no significant effect on increasing most of vegetative growth and flowering parameters including, plant height, number of leaves /plant (in the first season), stem diameter, root length and inflorescences dry weight (in both seasons), shoot dry weight, inflorescences length and inflorescences fresh weight (in the second season). In addition this treatment caused significant reduction in leaf area in both seasons compared to the control plants.

Table 1: Effect of salt concentrations and foliar application of bio-stimulators treatments on plant height, number of leaves, stem diameter, root length and leaf area of *Antirrhinum majus* during the 2014/2015 and 2015/2016 seasons

	1st season					2nd season				
	Bio-stimulators treatments (B)					Bio-stimulators treatments (B)				
Salt concentration (S), mM	Control	Chitosan	Setter-2	Citric acid	Mean (S)	Control	Chitosan	Setter-2	Citric acid	Mean (S)
					Plant height (cm)					
Control	40.90	66.93	64.00	56.40	57.06	47.03	70.20	60.06	53.70	57.75
20	49.30	65.30	60.86	51.05	56.63	50.26	63.60	60.66	54.13	57.16
40	48.50	60.93	52.86	44.87	51.79	46.33	53.80	52.46	47.26	49.96
60	44.50	55.56	53.93	41.77	48.94	42.93	48.06	43.56	42.03	44.15
80	40.27	50.43	48.07	42.23	45.25	34.90	47.23	43.73	40.10	41.49
Mean (B)	44.69	59.83	55.94	47.26	----	44.29	56.58	52.09	47.44	----
L.S.D. (0.05)										
S	1.87					4.02				
B	3.36					2.52				
S X B	7.52					5.65				
					Number of leaves /plant					
Control	119.86	167.03	109.96	111.10	126.99	110.83	140.60	136.90	110.30	124.66
20	150.26	208.16	188.16	130.53	169.28	105.67	176.87	167.83	108.44	139.70
40	110.60	163.26	148.43	132.76	138.76	111.57	152.56	158.07	142.97	141.29
60	119.63	141.90	132.16	136.13	132.46	123.63	145.90	146.53	135.20	137.82
80	104.30	148.80	128.46	131.16	128.18	102.47	139.50	126.70	105.43	118.53
Mean (B)	120.93	165.83	141.43	128.34	----	110.83	151.09	147.21	120.47	----
L.S.D. (0.05)										
S	15.74					12.89				
B	11.74					8.628				
S X B	25.56					19.29				
					Stem diameter (mm)					
Control	0.07	0.11	0.09	0.09	0.09	0.08	0.11	0.08	0.08	0.09
20	0.09	0.15	0.14	0.07	0.12	0.08	0.18	0.17	0.07	0.13
40	0.09	0.12	0.12	0.09	0.11	0.08	0.17	0.14	0.19	0.14
60	0.05	0.11	0.09	0.08	0.08	0.07	0.11	0.09	0.08	0.09
80	0.06	0.08	0.07	0.06	0.07	0.07	0.10	0.10	0.08	0.09
Mean (B)	0.07	0.12	0.10	0.08	----	0.08	0.13	0.12	0.10	----
L.S.D. (0.05)										
S	0.09					0.10				
B	0.07					0.82				
S X B	0.16					0.17				
					Root length (cm)					
Control	9.54	15.03	10.76	10.03	11.34	9.51	16.11	10.02	9.89	11.38
20	9.03	18.22	18.59	10.04	13.97	10.94	31.32	15.96	13.42	17.91
40	13.99	26.51	19.31	13.59	18.35	12.58	36.79	15.97	14.14	19.87
60	8.57	32.48	22.76	13.86	19.42	12.64	36.79	17.64	13.90	20.24
80	10.19	32.15	18.49	12.24	18.27	12.53	35.59	32.92	14.97	24.00
Mean (B)	10.26	24.88	17.98	11.95	----	11.64	31.32	18.50	13.26	----
L.S.D. (0.05)										
S	2.55					4.16				
B	2.59					1.73				
S X B	5.78					3.87				
					Leaf area (cm^2)					
Control	8.84	11.16	7.62	2.70	7.58	8.86	12.25	8.35	5.41	8.72
20	6.61	9.33	9.09	5.94	7.74	7.94	9.93	10.41	7.54	8.96
40	4.21	9.70	8.14	5.38	6.86	7.69	10.17	8.54	6.56	8.24
60	5.21	8.95	7.94	4.61	6.68	6.55	10.29	8.34	5.24	7.61
80	3.56	8.92	6.64	3.65	5.69	3.25	10.30	6.93	3.46	5.99
Mean (B)	5.69	9.61	7.89	4.46	----	6.86	10.59	8.51	5.64	----
L.S.D. (0.05)										
S	0.60					0.38				
B	0.40					0.40				
S X B	0.89					0.88				

Table 2: Effect of salt concentrations and foliar application of bio-stimulators treatments on shoots fresh and dry weights, number of inflorescences /plant, inflorescence length as well as inflorescences fresh and dry weights of *Antirrhinum majus* during the 2014/2015 and 2015/2016 seasons

	1st season					2nd season				
	Bio-stimulators treatments (B)					Bio-stimulators treatments (B)				
Salt concentration (S), mM	Control	Chitosan	Setter-2	Citric acid	Mean (S)	Control	Chitosan	Setter-2	Citric acid	Mean (S)
					Shoots fresh weight (g/plant)					
Control	11.82	21.59	16.50	11.94	15.46	10.80	19.32	17.29	12.68	15.02
20	15.85	20.76	16.32	15.09	17.01	13.14	19.19	17.18	11.88	15.35
40	11.02	12.10	14.71	14.49	13.08	11.31	15.78	15.55	14.58	14.31
60	12.46	12.71	12.84	17.31	13.83	11.78	14.24	14.15	14.05	13.56
80	10.82	13.80	13.85	13.67	13.04	10.45	14.79	12.38	11.62	12.31
Mean (B)	12.39	16.19	14.84	14.50	----	11.50	16.66	15.31	12.96	----
L.S.D. (0.05)										
S	1.69					0.64				
B	1.38					1.04				
S X B	3.10					2.32				
					Shoots dry weight (g/plant)					
Control	2.84	4.77	6.13	4.97	4.68	2.42	5.98	4.45	4.05	4.23
20	4.44	7.17	4.75	4.28	5.16	3.34	5.57	6.46	3.61	4.75
40	2.35	5.94	4.06	3.30	3.91	2.61	4.63	4.06	3.14	3.61
60	2.74	3.27	2.58	4.35	3.24	3.90	4.63	2.02	3.21	3.44
80	1.96	4.18	4.02	3.94	3.53	2.42	3.92	3.40	3.84	3.40
Mean (B)	2.87	5.07	4.31	4.17	----	2.94	4.95	4.08	3.57	----
L.S.D. (0.05)										
S	0.76					0.62				
B	0.80					0.75				
S X B	1.79					1.69				
					Number of inflorescences /plant					
Control	4.56	14.57	14.27	10.43	10.96	6.20	17.07	13.23	10.90	11.85
20	7.03	20.20	14.26	7.21	12.18	9.27	23.27	19.26	11.63	15.86
40	7.96	15.27	16.77	8.63	12.16	10.73	17.90	14.27	11.60	13.63
60	4.23	17.56	15.27	5.60	10.67	7.63	19.13	16.43	11.60	13.70
80	4.30	17.30	14.60	6.86	10.77	7.37	20.26	15.00	11.80	13.61
Mean (B)	5.62	16.98	15.03	7.75	----	8.24	19.53	15.64	11.51	----
L.S.D. (0.05)										
S	1.83					1.36				
B	1.18					1.78				
S X B	3.09					2.63				
					Inflorescence length (cm)					
Control	8.33	13.50	12.00	8.33	10.54	9.67	12.69	10.67	10.00	10.76
20	7.33	12.55	11.97	8.33	10.05	8.33	11.45	11.67	8.67	10.03
40	7.67	12.57	11.00	6.67	9.48	7.67	10.25	7.67	7.33	8.23
60	4.67	8.22	7.33	4.67	6.22	4.67	8.02	6.33	5.33	6.09
80	2.67	6.00	6.33	4.33	4.83	4.33	5.26	4.67	4.33	4.65
Mean (B)	6.13	10.57	9.73	6.47	----	6.93	9.53	8.20	7.13	----
L.S.D. (0.05)										
S	0.51					0.83				
B	0.26					0.38				
S X B	0.96					1.13				
					Inflorescences fresh weight (g/plant)					
Control	13.41	17.88	16.61	13.69	15.40	13.10	16.38	15.47	14.98	14.98
20	12.96	16.54	14.96	13.87	14.58	12.74	15.35	13.92	12.94	13.74
40	12.71	15.78	14.59	14.32	14.35	12.96	15.00	13.49	13.11	13.64
60	12.24	15.28	14.53	12.27	13.58	11.77	15.42	12.72	12.12	13.01
80	11.71	15.02	14.05	12.09	13.22	11.45	14.00	12.82	11.31	12.40
Mean (B)	12.61	16.10	14.95	13.25	----	12.40	15.23	13.68	12.89	----
L.S.D. (0.05)										
S	0.83					1.30				
B	0.43					0.73				
S X B	1.36					2.09				
					Inflorescences dry weight (g/plant)					
Control	2.51	2.70	2.25	2.09	2.39	2.10	2.67	2.48	2.19	2.36
20	2.23	2.48	2.42	2.15	2.32	2.04	2.48	2.23	2.02	2.19
40	1.97	2.56	2.48	2.29	2.33	2.07	2.47	2.16	2.10	2.20
60	1.81	2.42	2.32	1.87	2.11	1.88	2.03	1.84	1.94	1.92
80	1.56	2.31	2.25	1.79	1.98	1.80	2.22	2.05	1.81	1.97
Mean (B)	2.02	2.49	2.34	2.04	----	1.98	2.37	2.15	2.01	----
L.S.D. (0.05)										
S	0.42					0.77				
B	0.17					0.28				
S X B	0.69					1.39				

The data also in Tables 1and 2 cleared that when the three tested bio- stimulators were applied at the same concentration, chitosan was the most effective treatment for increasing the mean values of most studied parameters followed by setter-2, whereas citric acid treatment was the least effective one. These results are is in harmony with findings of previous researches which reported increases in vegetative growth and flowering parameters as a result of bio-stimulators chitosan treatments [13, 15, 57- 61] or application of bio-stimulators such as setter-2 treatments [62- 64].

The favorable effects of these bio-stimultatos may be due to its role in the different physiological processes; chitosan induces synthesize of endogenous plant hormones [65] or induces stomatal closure which reduces transpiration [66]. It might have a potential as a free radical scavenger or DNA-protective properties and chitosan scavenging mechanism may be attributed to its structure which features large numbers of hydroxyl and amino groups available to react with reactive oxygen species [13]. Setter-2 including organic acids of ascorbic acid and citric acid that improves water absorption, uptake of essential nutrients and plant growth rate. Also, it induces plant hormones, protein synthesis, delaying senescence and protect plant cells from the oxidative stress [33, 67]. In addition, Mn, B (existing in Setter-2) plays important roles in photosynthesis, metabolism of both nitrogen and carbohydrate, sugar translocation, cell division, differentiation, growth and respiration [68].

The Interaction Effect Between Salt Concentrations and Bio-Stimulators Treatments: the data in Tables 1 and 2 revealed that in most cases, the lowest values for most vegetative growth and flowering characters were obtained from plants irrigated with the highest salt concentration (80 mM) and sprayed with tap water, whereas the highest values for plant height, leaf area, shoots fresh weight, inflorescences length and inflorescences fresh and dry weights were resulted from plants irrigated with tap water and foliar sprayed with chitosan. The highest values for number of leaves, stem diameter, shoots dry weight and number of inflorescences were produced from plants irrigated with saline water at the concentration of 20 mM and sprayed with chitosan. The tallest root length were obtained from plants irrigated with saline water at the concentration of 60 mM and sprayed with chitosan. Under the different levels of salinity, foliar application of chitosan or setter-2 was the most effective treatments than citric acid since produced significantly higher values, in most case, for most of studied parameters as compared to the plants irrigated and sprayed with tap water (control). In this regard some researchers reported increases in vegetative growth and floral characters of salt stressed plants as a result of chitosan treatments [69-70] or application of setter-2 [34].

From the above results it is worth to mention that, treating *Antirrhinum majus* plants grown under salinity stress up to 80 mM NaCl with chitosan or setter-2 resulted in great effects on ameliorating the deleterious effect of salinity on vegetative growth and flowering traits and the favorable effect of such bio- stimulators may be due to their important roles on the different physiological processes.

Chemical Constituents

Total Chlorophylls, Total Carbohydrates N, P and K%: The results found in Table 3 showed that accumulation of total chlorophylls and total carbohydrates contents as well as N, P and K% were decreased significantly, in most cases, as the salt concentration in I rrigation water was increased from 20 to 80 mM compared to the control. The only two exceptions to this general trend were detected with the lowest salt concentration (20 mM) which caused insignificant reduction in total chlorophylls content and P% in the first season compared to the control. These results are in agreement with finding of many researches which reported decrement in total chlorophylls [71-72], total carbohydrates [52], N, P and K % [73- 75] as a result of increasing salinity stress.

The reduction in total chlorophylls could be due to enzymatic chlorophyll degradation [76] which decreased photosynthetic activity and consequently reduced carbohydrates accumulation. The reduction in accumulation N, P and K% with increasing salt concentration may be due to the adverse effect of accumulated salt in soil which decreased the absorption of nutrients as a result of decreased soil water potential [77]. The ions of Na^+ and Cl^- in soil may be causes nutritional imbalance as Na^+ competing with K^+ ions and inhibits its uptake, while Cl^- competing with NO_3^- and $H_2PO_4^-$ that the main sources of N and P in agricultural soil and inhibits their uptake by plants [78-80], consequently the reductions in these parameters reflecting the reductions in growth and flowering parameters observed in the present study.

Table 3: Effect of salt concentrations and foliar application of bio-stimulators treatments on total chlorophylls, total carbohydrates as well as N, P and K (% of dry matter) in leaves of *Antirrhinum majus* during the 2014/2015 and 2015/2016 seasons

	1st season					2nd season				
	Bio-stimulators treatments (B)					Bio-stimulators treatments (B)				
Salt concentration (S), mM	Control	Chitosan	Setter-2	Citric acid	Mean (S)	Control	Chitosan	Setter-2	Citric acid	Mean (S)
					Total chlorophylls content (SPAD)					
Control	48.67	54.50	58.27	52.03	53.37	52.22	57.25	54.60	54.50	54.64
20	47.93	57.20	56.60	50.67	53.10	51.27	56.30	55.07	53.57	54.05
40	45.67	55.21	53.87	49.73	51.12	48.80	54.10	53.87	52.00	52.19
60	44.97	53.27	52.47	45.60	49.08	47.50	53.10	52.50	50.43	50.88
80	40.28	53.90	53.40	45.00	48.15	47.00	53.50	53.78	52.88	51.79
Mean (B)	45.50	54.82	54.92	48.61	----	49.36	54.85	53.96	52.68	----
L.S.D. (0.05)										
S	0.34					0.54				
B	0.10					0.25				
S X B	1.06					1.10				
					Total carbohydrates (% of dry matter)					
Control	11.91	18.54	18.11	14.76	15.83	13.08	19.68	16.80	14.17	15.93
20	10.89	18.20	17.48	13.14	14.93	11.60	18.49	16.06	12.80	14.74
40	10.50	17.22	16.16	11.91	13.95	12.12	14.40	13.81	11.28	12.90
60	9.84	13.14	12.72	10.51	11.55	11.52	11.30	11.70	10.69	11.30
80	9.40	12.47	10.24	9.56	10.42	10.08	10.68	10.80	10.17	10.43
Mean (B)	10.51	15.91	14.94	11.98	----	11.68	14.91	13.83	11.82	----
L.S.D. (0.05)										
S	0.77					0.44				
B	0.82					0.78				
S X B	1.83					1.75				
					N (% dry matter)					
Control	1.73	2.17	2.60	2.17	2.17	1.97	3.62	3.00	2.90	2.87
20	1.56	1.96	1.87	1.80	1.80	1.83	3.19	2.60	2.13	2.44
40	1.42	1.97	1.67	1.64	1.68	1.47	2.63	1.90	1.63	1.91
60	1.17	1.88	1.47	1.37	1.47	1.33	2.60	1.77	1.70	1.85
80	1.37	1.93	1.55	1.52	1.59	1.43	2.36	1.62	1.89	1.83
Mean (B)	1.45	1.98	1.83	1.70	----	1.61	2.88	2.18	2.05	----
L.S.D. (0.05)										
S	0.08					0.10				
B	0.12					0.11				
S X B	0.26					0.23				
					P (% dry matter)					
Control	0.24	0.28	0.24	0.22	0.25	0.28	0.35	0.33	0.29	0.31
20	0.21	0.30	0.25	0.20	0.24	0.27	0.38	0.30	0.28	0.31
40	0.18	0.24	0.23	0.18	0.21	0.27	0.32	0.29	0.26	0.29
60	0.18	0.23	0.19	0.20	0.20	0.19	0.29	0.23	0.21	0.23
80	0.17	0.20	0.21	0.19	0.19	0.15	0.25	0.26	0.21	0.22
Mean (B)	0.20	0.25	0.22	0.20	----	0.23	0.32	0.28	0.25	----
L.S.D. (0.05)										
S	0.03					0.03				
B	0.02					0.03				
S X B	0.05					0.05				
					K (% dry matter)					
Control	1.43	1.58	1.52	1.56	1.52	1.27	1.44	1.35	1.28	1.34
20	1.36	1.55	1.51	1.41	1.46	1.24	1.34	1.44	1.24	1.32
40	1.22	1.39	1.40	1.35	1.34	1.20	1.33	1.30	1.21	1.26
60	1.18	1.35	1.33	1.17	1.26	1.22	1.19	1.26	1.11	1.20
80	1.16	1.28	1.32	1.24	1.25	1.14	1.18	1.25	0.88	1.11
Mean (B)	1.27	1.43	1.42	1.35	----	1.21	1.30	1.32	1.14	----
L.S.D. (0.05)										
S	0.05					0.03				
B	0.04					0.02				
S X B	0.12					0.05				

Regarding the effect of bio-stimulators treatments the data in Table 3 revealed that treating the plants with any type of the three tested bio-stimulators had a favorable impact on enhancing the accumulation of total chlorophylls and total carbohydrates contents as well as N, P and K% in leaves of *Antirrhinum majus* plants. In most cases, plants sprayed with chitosan, setter-2 or citric acid resulted in significantly increases in such parameters compared to the control. The exceptions of this trend were recorded in second season with plants foliar sprayed with citric acid which caused insignificant increase in total carbohydrates content and P% as well as decreased K% significantly compared to the control. Among the different bio- stimulators applied at the same concentration, chitosan was the most effective treatment flowed by setter-2 for increasing the studied components than citric acid. Similar results have been obtained by earlier researches that reported that chitosan treatments increased total chlorophylls [13, 15, 81-83], total carbohydrates contents [83-84] or N, P and K% [83-88]. Also, the increase in previous mentioned chemical constituents traits have been obtained as a result of setter-2 bio- stimulator treatments [64].

Regarding the interaction effect between salt concentration and bio-stimulators treatments the data in Table 3 revealed that, in most cases, within each bio-stimulator treatment total chlorophylls and total carbohydrates N, P and K% were reduced as salt concentration was increased. On contrast, in most cases within each salt concentration treatment the values of these traits were increased as the three bio-stimulators were applied compared to the control (plants only treated with salt concentrations). In most cases, spraying plants irrigated with tap water with chitosan resulted in the highest values. This result was detected with setter-2 in few cases. On contrary, the lowest values obtained from plants irrigated with saline water at the highest concentration (80 mM) and sprayed with tap water. In this concern previous researches stated that setter-2 increased the content of chlorophylls, carbohydrates N, P and K% in plants irrigated with saline water [34], also previous studies reported that seed-pretreatment with chitosan increased chlorophylls content under salinity stress [70].

Proline Content, Na and Cl %: It is clear from data in Table 4 that, in most cases, raising the salt concentration from 20 to 80 mM resulted in significant increase in proline content, Na and Cl % compared to the control. The only exception to this general trend was detected in the first season with plants irrigated with the lowest salt concentration (20 mM) which had insignificantly higher Cl% in their leaves than those of control plants. These results are in agreement with those obtained by many researches which reported increment in proline content [43, 52, 72], Na and Cl % [71-73, 89-92] due to raising salinity stress.

The accumulation of proline considered as one of the adaptive mechanisms to decrease the harmful effects of salinity [93]. The functions attributed to the proline accumulation are increase in the enzyme activities, stabilization of proteins, protein complexes and membranes, maintenance of cell redox homeostasis, reserve of carbon and nitrogen, cytosolic pH control and removal of free radicals [94].

As for the effect of bio- stimulators treatments, the data in Table 4 revealed that in most cases, foliar application of studied bio- stimulators treatments (chitosan, setter-2 or citric acid) caused significant decrease in proline content, Na and Cl % in both seasons compared to the control. The only one exception to this dominant trend was found in the second season with plants foliar sprayed with citric acid which had significantly higher Na% in their leaves than those recorded with the control.

Concerning the interaction effect between the two studied factors the data in Table 4 indicated that, in most cases, within each bio-stimulator treatment increasing salt concentration increased the values of proline content, Na and Cl % compared to the control. However, within the highest salt concentration (80 mM) the accumulation of these components in plants sprayed with chitosan, setter-2 or citric acid was significantly lesser than that in the control (plants only irrigated with highest salt concentration). In both seasons, the highest values of Na% was recoded with plants irrigated with the highest salt concentration (80 mM) and sprayed with tap water, while the lowest values were obtained from plants irrigated with tap water and sprayed with chitosan followed by setter-2. In this concern previous researches [34] stated that setter-2 increased the content of proline, decreased Na and Cl% in plants irrigated with saline water.

Table 4: Effect of salt concentrations and foliar application of bio-stimulators treatments on proline contents as well as Na and Cl (% of dry matter) in leaves of *Antirrhinum majus* during the 2014/2015 and 2015/2016 seasons

	1st season					2nd season				
	Bio-stimulators treatments (B)					Bio-stimulators treatments (B)				
Salt concentration (S), mM	Control	Chitosan	Setter-2	Citric acid	Mean (S)	Control	Chitosan	Setter-2	Citric acid	Mean (S)
	Proline content (μ moles/g fresh matter))									
Control	0.91	0.86	0.83	0.85	0.86	0.89	0.85	0.82	0.85	0.85
20	1.03	0.95	0.85	0.96	0.95	0.92	0.88	0.87	0.87	0.89
40	1.12	1.01	0.94	1.05	1.03	0.96	0.93	0.92	0.91	0.93
60	1.18	1.06	1.03	1.21	1.12	1.16	1.10	1.08	0.96	1.08
80	1.17	1.03	0.96	0.98	1.04	0.97	0.90	0.91	0.80	0.90
Mean (B)	1.08	0.98	0.92	1.01	----	0.98	0.93	0.92	0.88	----
L.S.D. (0.05)										
S	0.06					0.04				
B	0.03					0.02				
S X B	0.09					0.07				
	Na (% dry matter)									
Control	1.52	1.56	1.50	1.67	1.56	1.61	1.67	1.51	1.88	1.67
20	1.67	1.65	1.56	1.73	1.65	1.92	2.01	1.97	2.05	1.99
40	1.83	1.77	1.67	1.85	1.78	2.31	2.29	2.19	2.26	2.26
60	2.00	1.89	1.98	2.11	2.00	2.33	2.25	2.23	2.32	2.28
80	2.12	1.88	1.70	1.57	1.82	2.32	1.99	2.15	2.19	2.16
Mean (B)	1.83	1.75	1.68	1.79	----	2.10	2.04	2.01	2.14	----
L.S.D. (0.05)										
S	0.09					0.05				
B	0.04					0.03				
S X B	0.09					0.08				
	Cl (% dry matter)									
Control	0.43	0.44	0.44	0.47	0.45	0.42	0.44	0.44	0.46	0.44
20	0.41	0.49	0.48	0.54	0.48	0.40	0.45	0.46	0.49	0.45
40	0.55	0.58	0.57	0.58	0.57	0.48	0.61	0.50	0.60	0.55
60	0.61	0.60	0.62	0.66	0.62	0.54	0.58	0.52	0.58	0.56
80	0.97	0.55	0.59	0.56	0.67	0.90	0.55	0.50	0.53	0.62
Mean (B)	0.59	0.53	0.54	0.56	----	0.55	0.53	0.48	0.53	----
L.S.D. (0.05)										
S	0.03					0.05				
B	0.02					0.02				
S X B	0.05					0.05				

CONCLUSION

From the above results, it can be conducted that the adverse effects of salinity up to 80 mM Nacl on growth, flowering and chemical composition of *Antirrhinum majus* L. plants could be alleviated by foliar application of chitosan or setter-2 at the concentration of 2000 ppm.

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