



RESEARCH ARTICLE

Alleviation of salt stress by stimulative compounds in *Matthiola incana* L. plants

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Abstract

The aim of this study is to determine the influence of two stimulative compounds; amino-suam and setter-2 on improving vegetative growth and flower quality of *Matthiola incana* L. plant irrigated with saline water. To achieve this aim, a pot experiment was carried out at the nursery of the Ornamental Horticultural Department, Agricultural Faculty, Cairo University, Egypt, in two seasons; 2012/2013 and 2013/2014. The results revealed that, starting from 3200 ppm salinity concentration, the values of vegetative growth and floral characters were reduced, except for number of leaves/plant. Salt stress increased Na and Cl% and proline content. It reduced total carbohydrates and chlorophylls as well as N, P and K contents. Setter-2 significantly increased growth, floral characters, total carbohydrates, chlorophyll content as well as N, P and K and proline contents more than amino-suam either in saline or non-saline condition. At the same time both stimulative compounds significantly reduced the Na and Cl%.

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INTRODUCTION

Matthiola is a [genus](#) of family [Brassicaceae](#). The common name "stock" usually refers to the species *M. incana* L. and it may be applied to the whole genus. It is a common garden flower, available in a variety of colors. The beauty of the flower and pleasant sweet smell makes the stock an odd member of its family. *M. incana* is a short-lived perennial along the Mediterranean. Seeds are rich in oils and up to 65% of the oil consists of Omega-3-linolenic acid; one of the fatty acids essential to good health ([Heuer et al., 2005](#) and [Abd El Aziz et al., 2011](#)).

Recently Egypt is one of many countries in Africa was suffering for a long time from the shortage in fresh water resources available for agriculture. Saline irrigation water up to 4.5 dS/m is one of many reasons that cause secondary soil salinization in Egypt ([El-Hendawy, 2004](#)). Saline water may provide a valuable water source for the irrigation of selected floriculture crops as water quality and quantity becomes limited and as demand for quality water increases. There are two major approaches to improving and sustaining productivity in a saline environment; modifying the environment to suit the plant and modifying the plant to suit the environment. Both these approaches have been used, either singly or in combination ([Tyagi and Sharma, 2000](#)), but the first has been used more extensively because it enables the plants to respond better not only to water but also to other production inputs.

In the arid and semi-arid agricultural soils, like that in Egypt, the evaporative process increased which increased the soil salinity and in turn caused a limitation on flower production due to microclimate differences associated with this soil. Therefore, it was necessary to search for another alternative methods or substances to make a balance with irrigation demand. Out of these methods is planting the salt resistance species which is the most useful approach ([Rasmussen et al., 2009](#)), or using some substances, *i.e.* stimulative compounds as amino acids.

Amino acids have traditionally been considered as precursors and constituents of proteins. Plants subjected to stress show accumulation of proline and other amino acids. Accumulation of amino acids in plants play different roles; for example regulating and signaling molecules, regulation of ion transport and detoxification of heavy metals. Amino acids also affect synthesis and activity of some enzymes and gene expression ([Rai, 2002](#)). Moreover, amino

acids act as buffers which help to maintain favorable pH value within the plant cell, since they contain both acid and basic group, they remove the ammonia from the cell. The main effect of amino acids had been studied by several authors, *i.e.* **Abdel Aziz et al. (2009)** on *Antirrhinum majus* and **Talaat et al. (2005)** on *Catharanthus roseus* L.

Amino-suam, as natural extract of amino acids, is characterized by its double effect where it link with magnesium in plant cells which directly influence the plant efficiency and synthesis. This will reflected on forming storage nutrition and a mass of energy. Consequently, enhance the plants to forming a resistance compounds and overcome the different faces of stress; *i.e.* temperature, water shortage, salinity or diseases infection.

Setter-2 has physiological mode of action on stimulate and balance the internal natural growth hormones on buds and flowers. This will enhance the vitality and fertility of pollen grains and ovule and reduce the flowers abscission. These will help the plants to tolerant the stress conditions; *i.e.* high temperature, salinity or forest. Ascorbic acid (vitamin C) is one of the components of setter-2, plays an important role in decreasing the effects of salt and drought stresses (**Rafique et al., 2011, Yazdanpanah et al., 2011**). It involved in the regulation of many critical biological processes; such as photo-inhibition, cell elongation and cell cycle (**Smirnoff, 2000**). It acts as cofactor for enzymes and regulating photosynthesis, hormone biosynthesis, regenerating other antioxidants, cell division and growth (**Gallie, 2012**) Moreover, ascorbic acid appears to link flowering time, developmental senescence, programmed cell death and responses to pathogens (**Barth et al., 2004 and Pavet et al., 2005**).

The goal of this study was to investigate the influence of foliar application by stimulative compounds; amino-suam and setter-2 on the vegetative growth and flower quality of *M. incana* L. plant grown as pot plant irrigated with different concentrations of saline water.

MATERIAL AND METHODS

This study was carried out at the nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during two seasons; 2012/2013 and 2013/2014. Seeds of *M. incana* L. were sown in plug trays on 21st of October in both seasons. On the 2nd of Dec., the seedlings reached approximately 10 cm in length and had 4 leaves. Seedlings were transplanted to pots, 20 cm inner diameter and containing a mixture of clay and sand (1:1, v/v). Each pot had one seedling.

The plants were sprayed three times every two weeks, starting from 16th of Dec., with two stimulative compounds (each at the rate of 1.5 ml/liter); amino-suam (extraction solution of natural amino acids) and setter-2 (commercial name with active ingredients contain; ascorbic acid, citric acid, total N, Cu, chelated Ca⁺⁺ and boron). The plant irrigated with saline water at different concentrations; 1600, 3200 and 4800 ppm, in addition to tap water as control treatment. To prepare the stock of saline water for irrigation; sodium chloride (NaCl), calcium chloride (Ca Cl₂) and magnesium chloride (Mg Cl₂) salts were used. After 30 days from transplanting, the irrigation saline water at a rate of 400 ml/pot was applied every five days. The plants were fertilized by the commercial fertilizer; Life Green (20N:20P₂O₅:20K₂O) at the rate of 1g/pot every two weeks.

This experiment was designed as a completely randomized block with 2 factors including 12 treatments and 3 replicates, each replicate contain 4 plants (12 plants/treatment). Data were subjected to statistical analysis of variance and the means were compared using the "Least Significant Difference (L.S.D.)" test at 5% level, as described by **Little and Hills (1978)**.

Data recorded:

Growth and Floral parameters

Plant height (cm), number of leaves/plant, leaf area (cm²), fresh and dry weights of shoot (g/plant) and inflorescence; number, length (cm) and weight (g/plant).

Chemical Analysis

Total chlorophylls (SPAD) determined by using fresh leaf samples as described by **Netto et al. (2005)**, total carbohydrates (%) determined by using dried leaves as described by **Dubois et al. (1956)**, N (%) determined by using extract of dried leaves with the modified micro-Kjeldahl method, P (%) determined as described by **Jackson (1967)**, K and Na (%) determined by using a flame spectrophotometer (**Jameel and Kahayri, 2002**) and proline content (µm/mg fresh weight) determined in fresh leaves according to **Bates et al. (1973)**.

RESULTS AND DISCUSSION

Growth parameters

Salinity stress at 3200 and 4800 ppm, significantly reduced the plant height, leaf area and total fresh and dry weights of shoot compared to 1600 ppm and control treatment, this may be due to direct effects of ion toxicity or indirect effects of saline ions that cause soil/plant osmotic imbalance (**Abdel Latef and Chaoxing, 2011**). Death of plants and/or decreases the productivity could be occurred due to the detrimental effects of high salinity. Moreover,

the decrease in shoot dry weight and leaf area were the first visible effects of salinity both in sensitive and tolerant species such as *Cotoneaster lacteus* and *Eugenia myrtifolia* (Cassaniti et al., 2009). This decrement in fresh and dry weights caused by salinity may be due to the reduction in water and minerals absorption and/or the reduction in upper ground growth (Mazher et al., 2006). While the leaf area reduction could be caused by decreasing the leaves turgor, as a consequence of changes in cell wall properties or the reduction of photosynthetic rate. Other wards, the reduction in cell elongation and division in leaves reduces their final size, resulting in a decrease in leaf area (Munns and Tester, 2008). These results were confirmed Abd EL-Aziz et al. (2011) on *M. incana*. Saline conditions reduced the ability of plants to absorb water causing rapid reductions in growth rate, and induced many metabolic changes. Therefore, the reduction in growth was explained by lower osmotic potential in the soil which leads to decreased water uptake and reduced transpiration and closure of stomata which is associated with growth reduction (Sánchez-Blanco et al., 2002)

Applying stimulative compounds declined the reduction occurs in the values of the abovementioned characters comparing to the untreated plants under the same saline stress. The values of plant height and leaf area were exceeded with highly significant difference in the plant treated with setter-2 at salinity concentrations 3200 and 4800 ppm the plants treated with amino-suam or tap water, in both seasons. The heaviest fresh and dry weights of shoot were in plants treated with setter-2 at salinity concentrations 1600 ppm, comparing to the other treatments. This is may be due to that the stimulative compounds enable the cells to maintain higher osmotic potential for increasing their osmotic concentration, which lead to the maintenance of plant growth and improve the ability of the plant to tolerate salt stress by protecting the cellular protein contents against the damage caused by salt injury. Other wards, stimulative compounds significantly improved the growth parameters in the salt-stressed plants compared to control plants. This effect may be attributed to the production of secondary metabolites (antibiotic and plant hormones), which improve the physiological processes (water absorption capacity) by increasing root hydraulic conductivity and uptake of essential macro and micro-nutrients, consequently improves the plants growth (de Varennes and Goss, 2007 and Kaschuk et al., 2009).

The value of number of leaves/plant had different behavior, where it increased as the salinity concentrations increased. The greatest values, with highly significant differences, were in plants treated with amino-suam and irrigated with 4800 ppm saline water comparing to those treated with setter-2 under the other salinity concentrations, in both seasons (Tables 1, 2). As a result of using appropriate salinity concentrations and stimulative compounds, *Matthiola* plants become compact. This means that, with proper management for salinity, the internode length and/or number could be reduce. Under salt stress, the stem tall and rangy plants are shortened to a more attractive length and no need for support as in *Chrysanthemum* (Lee and Van Iersel, 2008).

Table 1. Effect of foliar application by amino-suam and setter-2 on some vegetative characters of *Matthiola* plants under salinity concentrations during seasons 2013-2014.

Salinity concen. (ppm)	1 st season												
	Plant height (cm)			Mean	Number of leaves			Mean	Leaf area (cm ²)			Mean	
	Treat.		Control		Treat.		Control		Treat.		Control		
	Control	Amino-suam		Setter-2	Control	Amino-suam		Setter-2	Amino-suam	Setter-2			
0.0	32.67	48.33	47.33	42.78	25.67	28.33	27.33	27.11	10.82	17.66	18.10	15.53	
1600	31.67	41.67	44.33	39.22	28.33	30.00	29.00	29.11	8.50	13.82	15.99	12.77	
3200	30.00	41.33	40.33	37.22	31.33	39.67	36.00	35.67	7.22	11.23	12.88	10.44	
4800	29.00	37.00	38.67	34.89	34.33	40.33	39.67	38.11	5.81	8.71	9.22	7.76	
Mean	30.84	42.08	42.67		29.92	34.58	33.00		8.09	12.86	14.05		
L.S.D. 5%													
S		2.01				2.26				0.13			
T		1.17				3.00				1.23			
SXT		0.83				1.79				1.05			
Salinity concen. (ppm)	2 nd season												
	Control	Amino-suam	Setter-2	Mean	Control	Amino-suam	Setter-2	Mean	Control	Amino-suam	Setter-2	Mean	
	0.0	33.33	48.67	45.00	42.33	25.67	28.00	27.33	27.00	9.02	12.75	12.45	11.41
	1600	29.67	48.33	44.67	40.89	27.67	31.00	29.00	29.22	8.99	9.78	10.77	9.85
3200	28.33	35.33	37.67	33.78	32.33	36.00	35.00	34.44	7.63	9.15	10.28	9.02	
4800	28.33	27.33	31.67	29.11	36.67	40.67	41.00	39.45	7.06	8.02	8.57	7.88	
Mean	29.92	39.92	39.75		30.59	33.92	33.08		8.18	9.93	10.52		
L.S.D. 5%													
S		0.88				2.32				0.73			
T		1.35				3.78				1.18			
SXT		1.01				1.29				0.32			

Table 2. Effect of foliar spray by amino-suam and setter-2 on some vegetative characters of *Matthiola* plants under salinity concentrations during seasons 2013-2014.

Salinity concn. (ppm)	1 st season								
	Total fresh weight (g/plant)				Mean	Total dry weight (g/plant)			Mean
	Treat.			Control		Treat.		Setter-2	
	Control	Amino-suam	Setter-2		Amino-suam				
0.0	50.26	64.17	61.01	58.48	8.04	10.27	9.76	9.36	
1600	41.91	54.08	52.36	49.45	7.71	8.65	8.38	8.25	
3200	33.69	42.18	47.93	41.27	5.57	6.75	7.67	6.66	
4800	28.54	38.39	36.53	34.49	5.57	6.14	5.85	5.85	
Mean	38.60	49.71	49.46		6.72	7.95	7.92		
L.S.D. 5%									
S		4.16				0.86			
T		3.94				1.40			
SxT		2.13				0.71			
Salinity concn. (ppm)	2 nd season								
	Control	Amino-suam	Setter-2	Mean	Control	Amino-suam	Setter-2	Mean	
	0.0	51.79	65.41	62.88	60.03	8.29	10.47	10.07	9.61
1600	45.98	49.17	50.93	48.69	7.36	7.87	8.15	7.79	
3200	36.50	41.62	44.64	40.92	5.84	6.66	7.14	6.55	
4800	28.64	35.39	38.74	34.26	4.58	5.66	6.20	5.48	
Mean	40.73	47.90	49.30		7.66	7.67	7.89		
L.S.D. 5%									
S		3.88				0.52			
T		2.41				0.93			
SxT		1.36				0.13			

Floral characters

The maximum mean values of inflorescence number, length and weight of *Matthiola* plants, in both seasons, were in plants grown under zero salinity. These values were gradually decreased as the salinity concentrations increased. **Abdel Aziz et al. (2011)** reported that on *Amaranthus tricolor* increasing the levels of salinity from 2000 to 3000 ppm significantly decreased the inflorescences; number, stalk length, fresh and dry weights compared with control plant. This reduction may be due to the inhibition of the photosynthesis of plants via the changes of chlorophyll contents and components and damage of photosynthetic apparatus. It also inhibits the photochemical activities and decreases the activities of enzymes in the Calvin cycle (**Sairam and Tyagi, 2004**). While **Kucukahmetler (2002)** revealed that, salinity caused a reduction of flower quality; color, size, thickness, length and yield of ornamental plants.

In both seasons, the plants treated by setter-2, at any salinity level, have significantly greatest values for these characters, followed by those plants treated with amino-suam compared to the untreated ones. The latter plants had the lowest values of these characters under any salinity levels (Table 3). Foliar and root application of commercial amino acids led to better development of the inflorescences characters (Cerdán et al., 2006). El-Quesni (2009) foliar application of ascorbic acid as one of setter-2 component, significantly increased number and fresh and dry weights of flowers of *Hibiscus rosa sinenses* L. plants compared with untreated plants.

Table 3. Effect of foliar application by amino-suam and setter-2 on some floral characters of *Matthiola* plants under salinity concentrations during seasons 2013-2014.

Salinity concen. (ppm)	1 st season											
	Inflorescence Number/plant			Mean	Inflorescence Length (cm)			Mean	Inflorescence Weight (g/plant)			Mean
	Treat.				Treat.				Treat.			
	Control	Amino-suam	Setter-2	Control	Amino-suam	Setter-2	Control	Amino-suam	Setter-2			
0.0	30.00	34.33	34.67	33.00	18.00	25.33	24.67	22.67	1.64	2.63	4.93	3.07
1600	27.67	33.00	36.33	32.33	11.67	18.33	21.00	17.00	0.95	2.11	4.07	2.38
3200	25.33	29.33	36.00	30.22	10.67	14.33	15.67	13.56	0.93	2.06	3.14	2.04
4800	16.33	25.67	30.67	24.22	8.67	13.33	13.00	11.67	0.87	2.02	2.83	1.91
Mean	24.83	30.58	34.42		12.25	17.83	18.59		1.10	2.21	3.74	
L.S.D. 5%												
S		2.43				1.22				0.07		
T		2.20				2.07				0.29		
SXT		1.05				1.79				0.04		
Salinity concen. (ppm)	2 nd season											
	Control	Amino-suam	Setter-2	Mean	Control	Amino-suam	Setter-2	Mean	Control	Amino-suam	Setter-2	Mean
	Control	Amino-suam	Setter-2		Control	Amino-suam	Setter-2		Control	Amino-suam	Setter-2	
	0.0	31.00	42.33	43.33	38.89	12.33	28.33	22.00	20.89	1.89	3.69	4.17
1600	25.00	34.00	41.67	33.56	10.67	20.00	19.33	16.67	0.95	2.52	3.94	2.47
3200	20.67	33.00	34.00	29.22	9.33	16.33	16.00	13.89	0.94	2.01	2.61	1.85
4800	15.00	24.67	32.67	24.11	8.67	13.33	14.00	12.00	0.81	1.08	1.90	1.26
Mean	22.92	33.50	37.92		10.25	19.50	17.83		1.15	2.33	3.16	
L.S.D. 5%												
S		2.55				1.38				0.02		
T		2.19				1.11				0.25		
SXT		1.01				0.88				0.04		

Chemical characters

Increasing salinity concentration causes reduction in total chlorophylls and carbohydrates content. Treated the plants with amino-suam presented significantly an increase in values of these characters, followed those treated with setter-2 both compared to the untreated ones at any salinity concentrations (Fig.1). Effect of salinity could be due to the antagonistic effect of Na Cl on N absorption, which is a main component of the structure of chlorophyll molecule. As amino-suam and setter-2 enhancing the growth parameters, as mentioned earlier, consequently enable the plants to maintaining photosynthesis process and osmotic balance in addition to reducing the toxic effects of Na ions by reducing its uptake which indirectly help in maintaining the chlorophyll and carbohydrates contents of the plant (Swaefy et al., 2007 and Kaya et al., 2009). Moreover, the stimulants may also contribute to maintaining cell-cycle progression, synthesis of many hormones and pigments (Pignocchi and Foyer, 2003 and Gest et al., 2013).

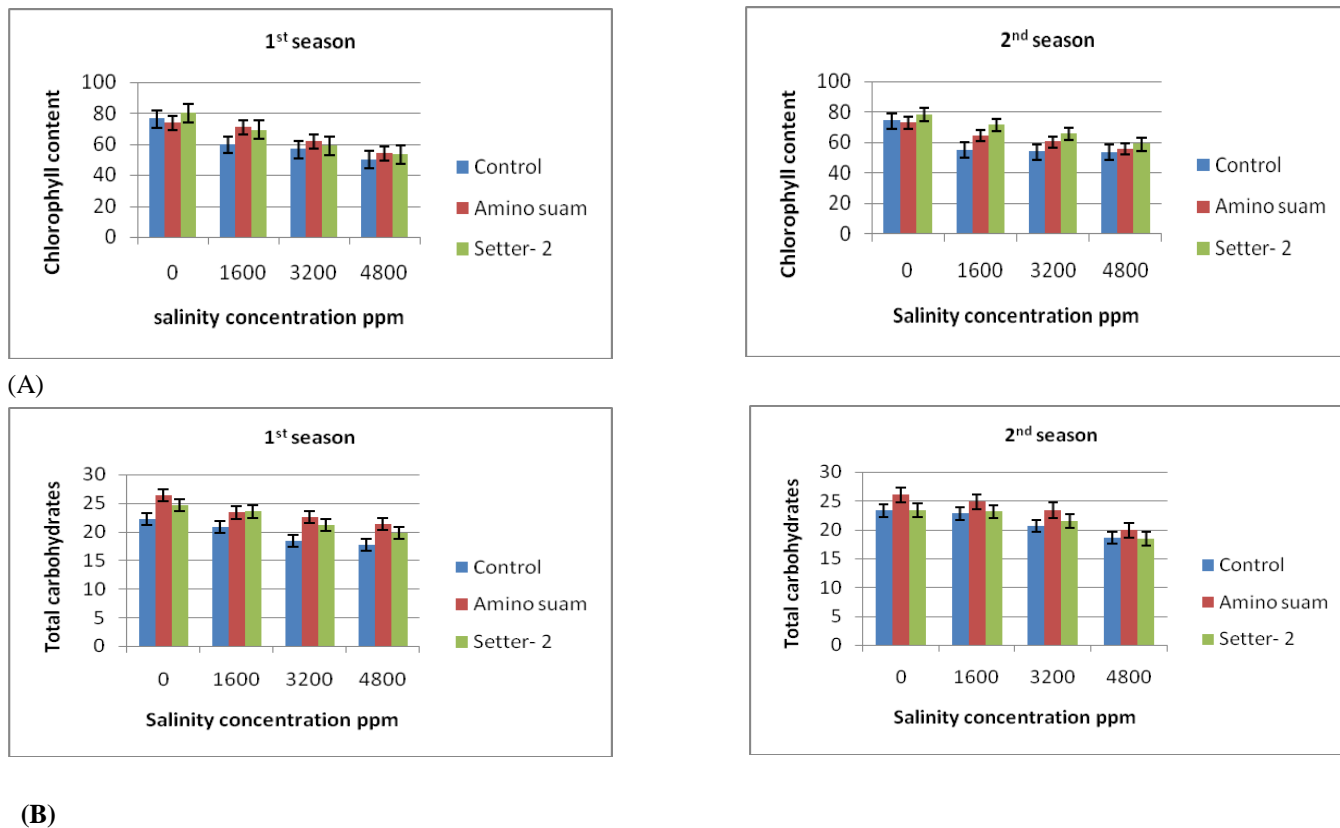


Fig. 1. Effect of amino-suam and setter-2 on total chlorophyll (A) and total carbohydrates (B) under different salinity levels during two seasons.

N, P and K% were decreased in the plants as the salinity concentrations increased. While applying growth stimulants enhance these values. The highest N and K% were in plants treated with amino-suam then setter-2 as compared to the untreated plants, at any saline levels, in both seasons. Contrary in case of P%, where this percentage was superior in plants treated with setter-2 followed by amino-suam than that of the untreated plants at all salinity levels in the 1st season (Fig. 2).

Decreasing in N, P and K% associated with an increasing in proline content which might indicate disturbance in protein metabolism. The diminishing values of nutrients uptake by plants irrigated with saline water were mainly due to the depressive effect of high level of salinity on plant dry weight. In this respect, the decrease in N% might be attributed to the inhibition of cell division and elongation which reflecting on plant height as stated earlier. The reduction in P uptake of different plant organs at 2000 ppm salinity could be referred to decreasing the solubility and available P in soil irrigated with saline water. While in case of K% and uptake, the reduction occurred may be due to the composition of saline irrigation water used which tended to encourage the accumulation of Na and in turn depressed concentration of K in Schefflera plant (El-Quesni et al., 2010).

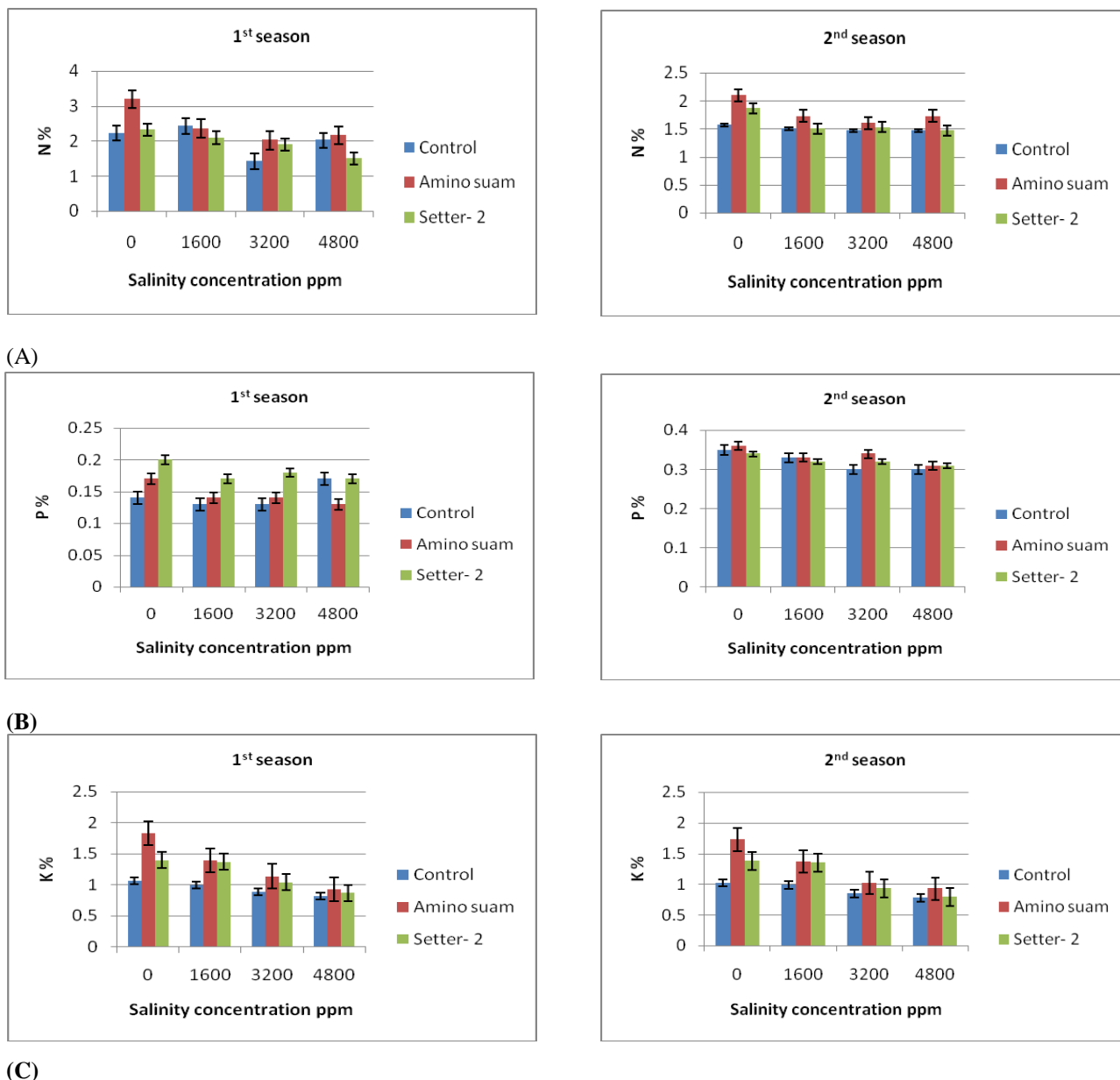


Fig. 2. Effect of amino-suam and setter-2 on N% (A), P% (B) and K % (C) under different salinity levels during two seasons.

It was noticed that the Na and Cl% were increased as the salinity concentration increased. The accumulation of these elements in leaves was associated with a significant reduction in the main minerals; N, P and K %. This is due to that the plants under salt stress tend to uptake more Na resulting in decreased K uptake. Na ions compete with K for binding sites essential for various cellular functions. While, P ions precipitate with Ca, Mg and Zn ions in salt stressed soils and become unavailable to plants. Also, salinity interferes with nitrogen (N) acquisition and utilization by influencing different stages of N metabolism, i.e. NO_3 uptake and reduction the protein synthesis (**Abdel Latif and Chaouing, 2011 and Soliman et al., 2012**). Moreover, the presence of toxic ions such as Na^+ and Cl^- in the root rhizosphere can disrupt the uptake of nutrients (such as K^+ and NO_3) by interfering with transporters in the root plasma membrane (**Tester and Davenport, 2003**).

In the plant treated by setter-2 or amino-suam, Na and Cl% in leaves were decreased than that of the control plants under all saline irrigation water. This result explained the role of stimulants in enable the plant to be adapted with salt stress which positively affect the growth and floral parameters (Fig. 3).

Proline content in leaves of *Matthiola* plants reached its maximum in the plants irrigated by 4800 ppm salinity to make plants more adapted to this unsuitable condition. The differences in proline content were not significant between the untreated plants or those treated with setter-2 or amino-suam starting from 1600 ppm salinity, in both seasons. Moreover, there was no significantly difference in proline content between plants treated with setter-2 or amino-suam in the 1st season, while this difference was quite high in plants treated with amino-suam in the 2nd season (Fig.3). The high level of proline content enables the plants to maintain osmotic balance when growing under salinity and acts as a major reservoir of energy and nitrogen for utilization by plants subjected to salinity stress (**Rabie and Almadini, 2005**). Increasing proline in plant considered as indicator for some mechanism to resist the salinity stress (**Jampeetong and Brix, 2009**). Contrary to the present result, applying amino acids on *Matricaria recutita* plants significantly increased proline content (**Omer et al., 2013**).

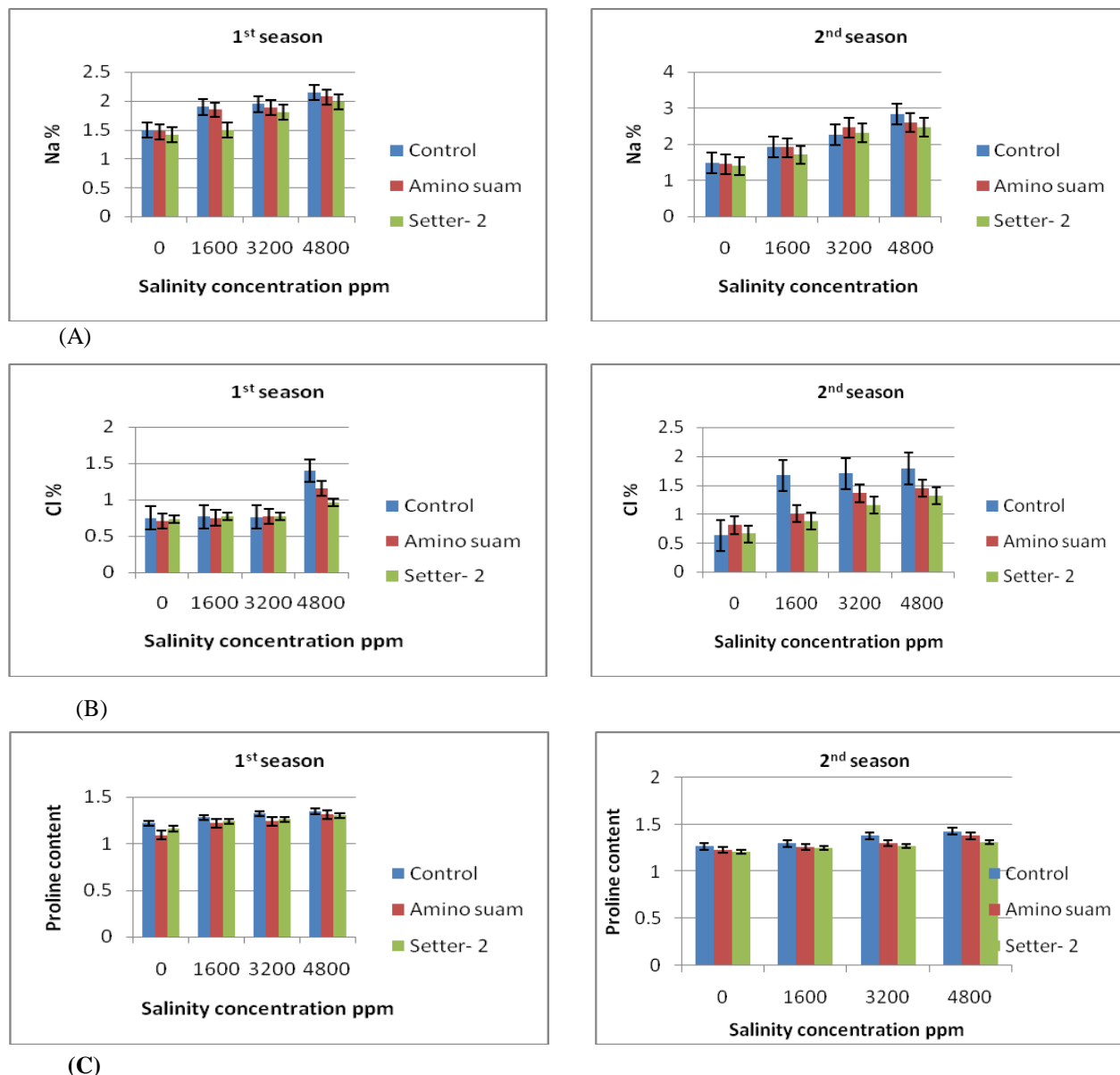


Fig. 3. Effect of amino-suam and setter-2 on Na% (A) , Cl % (B) and proline content (C) under different salinity levels during two seasons.

CONCLUSION

The results of this study provided the evidence that the positive effect of stimulative compounds in protecting *Matthiola incana* plants against the salt injury through enhancing salt-resistance mechanisms of plants due to increasing the total carbohydrates and proline accumulation and decreasing Na and Cl %, all associated with a significant increasing in the main minerals; N, P and K % which reflecting on morphological characteristics.

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