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Effect of saline water irrigation and amino acid treatments on growth, productivity and alkaloids productivity of *Catharanthus roseus* L. G. Don under Sinai conditions

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ABSTRACT

Cultivation of *Catharanthus roseus* L. under saline water irrigation as a simulation of water salinity in Sinai, conditions were investigated in response to amino acid treatments. Two pot experiments were conducted at the Desert Research Center, Mataria, Cairo Egypt, and its experimental farm at El-Maghara Experimental Station in North Sinai Governorate, Egypt during the two successive seasons of 2014 & 2015.Established seedlings were irrigated with freshly prepared saline water using NaCl at 2000, 4000 and 8000 ppm plus tap water as a control treatment. All treatments received amino acid treatments as a foliar spray at 0, 0.5 and 1 g/liter using Amino zeid 42/85 a commercial product containing 85% total amino acids and 42.3 % highly concentrated free amino acids.Results indicated that damage of salinity was severed in a higher concentration of saline water irrigation treatment to reach the death of plants irrigated with 8000 ppm after the first cut. Alkaloids production in different plant parts was increased within saline water concentration increase levels. Meanwhile, most of the growth traits were enhanced using amino acid treatments under other saline water irrigation conditions. Combining irrigation with amino acids treatments at 1 g/l resulted in enhancing of growth traits under 2000 and 4000 ppm treatment. Although amino acids enhanced growth under moderate salinity condition, results indicate that *Catharanthus roseus* plants still need susceptible to salinity damage. From our observation the use of amino acids for enhancing *C. roseus* salinity tolerance still more research using higher concentration or other methods of amino acids application
Keywords: *Catharanthus roseus*, saline water, salinity stress, amino acids, yield, alkaloid

INTRODUCTION

Catharanthus roseus (Fam. Apocynaceae), is an endemic to Southeastern and Eastern Madagascar, widely cultivated and is naturalized in subtropical and tropical regions in the different parts of the world, known with different English names occasionally used include the Madagascar periwinkle, vinca, Cape periwinkle, rose periwinkle, and rosy periwinkle. Based on flower colors, *C. roses* was named as var 'Rosea' that is the pink flowered and 'Alba' for the white flowered. Jaleel *et al*., (2007a)

periwinkle had been used for long time in folk medicine for treating different cases, using different plant parts for treating i.e. viz. diabetes, hypertension, and cancer. Aslam et al., (2010). *C. roseus* represents a commonly choice of herbal cancer therapy in Egypt, China, India, and Greece.

Advanced research on Catharanthus medicinal properties reveal the discover of 130 indole group alkaloids mainly formed in the root bark with various ranges from 0.15 to 1.34% and even more in some strains, thus it is considered very toxic and well known of cancer treatment Singh and Jagdev, (1996).

A purified form of two kinds if these alkaloids (vincristine, and vinblastine) proved successfully treating common types of leukemia and lymphoma. Purified vincristine and vinblastine derived from vinca leaves are considered the main source of anti-cancer treatments for leukemia (non-Hodgkin's lymphoma) which represents 11 % of cancer cases in Egypt Ibrahim *et al*., (2014). Since the discovery and use of vincristine, the survival rates of childhood leukemia increased with an average of 80 % Andrade *et al*., (2013).

Alkaloids production in such important plants are mainly affected by abiotic stress conditions such as salinity. Plants tend to increase the production of certain secondary products i.e. alkaloids to defend its survival against such hard conditions. Although plants could survive stress condition ,through the production of alkaloids, but plant growth and biological mass production are still severely reduced .Selmar, (2008)

Cultivation of medicinal plants for drug production is mainly located in isolated farms in the desert to avoid drug contamination. Such sandy soils usually suffer from high salinity could be a limiting factor in many areas. Salinity could severely affect the production of different medicinal crops. Damage could reach in some cases more than 50% of the crop production in major fields, along with other abiotic stresses Mahajan and Tuteja, (2005).

Under Sinai condition salinity of irrigation water from the underground reservoir which represents the main source of irrigation water which varie in salinity from 1408 to 10240 ppm depending on location and raining season Magdy *et al*., (2013). The most known damage of salinity mainly referred to salinity water content of Na^+ and Cl^- ions for their role in soil structure deterioration and their toxicity to plants Hasegawa *et al*., (2000). In order to enhance plant growth under salinity stress condition, different approaches were used over the last decade i.e. but not limited to the amino acids application.

Plants tend to increase amino acids accumulation, function as osmotically active solutes as a physiological response to salinity stress (Serraj and Sinclair, 2002 and Osman *et al*., 2007). Amino acids enhance plant growth under salinity through their anti-senescence and anti-stress effects due to their acid neutralizing and antioxidant properties as well as for their membrane and cell wall stabilizing abilities Zhao and Yang, (2008). Exogenous application of amino acids has been used as alternative routes of polyamines synthesis in plants for direct or indirect enhancement of physiological activities under different plantation Boras and zidan,(2011).

The successful plantation of medical plants for commercial uses against collection from wild sources is a hard equation involve trials to improve active ingredients production and maintaining the appropriate vegetative growth at the same time. Therefore, the aim of this research is to enhance both vegetative growths as well as alkaloids production from *C. roseus* with the application of amino acid treatments under the condition of irrigation with high salinity water used for irrigation in Sinai, as a promising area of medicinal plants production.

MATERIAL AND METHODS

Location and growing media: This study was conducted to at Desert Research Center, Mataria, Cairo Egypt, and its experimental farm at El-Maghara Experimental Station in North Sinai Governorate, Egypt, during the two successive seasons of 2013/14 and 2014/15 to investigate the effect of amino acids treatments on salinity tolerance and alkaloid production on *C. roseus* (L.) G.Don.

Two pot experiments were conducted using a soil mixture of three parts of sand, one part compost (v:v) mixed together and composted for one month before use. Polyethylene bags were filled with the soil mix and irrigated once before seedling transplanting.

Plant material: Seed pods of *C.roseus* were collected from mature plants growing at Floriculture Nursery of Horticulture Department, Faculty of Agriculture, Tanta University. Seeds were sown in seedling, slightly covered and located in the plastic greenhouse until germination. After germination, seedling trays were moved under saran greenhouse (63% shading) until reaching the appropriate size for transplanting, approximately 50 days after germination .Seedling ≈ 7 cm height containing 2-3 pairs of true leaves were transplanted into pots 25 cm diameter filled with the experimental soil mixture and arranged under full sun condition. Immediately after transplanting, the seedlings were irrigated with fresh water and irrigation with fresh water lasted for two weeks before the beginning of the experiment.

Salinity treatments: In a simulation of Sinai salinity water, seedlings were irrigated with freshly prepared saline water saline water using tap water and sodium chloride from El Nasar Pharmaceutical Chemicals Company with purity of 99%. The used concentrations used were 2000, 4000 and 8000 ppm plus tap water as a control treatment. Plants received 200 ml of saline water at two days intervals. To avoid salinity accumulation, pots were irrigated with fresh water after two times of saline water irrigation treatments.

Amino acids application: Amino zeid 42/85 a commercial product from United for Agriculture Development Co., Cairo, Egypt containing 85% total amino acids 42.3 % highly concentrated free amino acids was used as a source for the amino acid application. Plants were sprayed with amino acids at 0.5 and 1g/liter plus the control at two weeks interval. The components of amino zeid 42/85 are presented in Table (A)

Table (A): The amino zeid 42/85 components.

Amino acids	% w/w	Amino acids	% w/w	Amino acids	% w/w
Aspartic	3.29 %	Isoleucine	1.11 %	Phenyl alanine	0.99 %
Tyrosine	0.52 %	Leucine	2.03 %	Serine	4.99 %
Glutamic	8.18 %	Lysine	1.75 %	Threonine	3.57 %
Glycine	2.03 %	Arginine	4.64 %	Organic nitrogen	10 %
Alanine	2.26 %	Histidine	0.56 %	Potassium oxide	2.5%
Valine	2.51 %	Proline	3.96 %		

Experiment layout and statistical analysis

Treatments were arranged as a factorial in a randomized complete block design. Salinity treatments were arranged in the main plot and three treatments of the amino acid foliar application as sub plot. Each treatment contained three replicates of 7 plants each. The experiments were conducted over a period of four months from June to September of each season. Collected data for both seasons were pooled and the obtained results were analyzed using MSTATC Program.Bricker,(1991) Means were compared using LSD test at 0.05 level according to Snedecor and Cochran,(1968).

Growth analysis: Data on *C.roseus* growth was collected two times per seasons at two months interval. Data recorded for the first cut were conducted on 1 July followed by the second cut on 1 August for both seasons. Data on root growth were only recorded in the second cut. Harvest was done early as possible, plant height (cm) and shoot number/plant were recorded before harvest. After harvest, plants were removed for sampling and fresh herb measurements i.e. root length (cm), shoots fresh weight (g), shoots fresh yield per feddan (kg), roots fresh weight (g) and roots fresh yield per feddan (kg). Samples were air dried under shade to estimate shoots dry weight (g), shoots dry yield per feddan (kg), relative dry matter, root dry weight (g) and roots dry yield per feddan (kg).

Photosynthetic pigments and carbohydrates determination: Chlorophyll a, b, total chlorophyll, and carotenoids were determined following Saric *et al*., (1967).Total carbohydrates were determined following procedures described by Herbert *et al*., (1971).

Nutrient element%: Nitrogen was determined by modified micro-Kjeldahl method as described by A.O.A.C., (1970) . Phosphorus was determined using the ammonium molybdate method according to Murphy and Riley, (1962).

Potassium and sodium % were estimated using flame photometric method according to Cottenie *et al*., (1982).

Proline content: Estimation of proline content in fresh samples was done by colorimetric method described by Bates *et al*., (1973). Proline concentration in plant samples was determined by referring to standard curve and calculated on a fresh weight basis as following:

$$\mu\text{mole proline/g of fresh sample} = \frac{(\mu g\ \text{proline/ml})\ x\ \text{ml toluene}\ x\ 5}{(115.5\ \mu g/\mu\text{mole})\ x\ g\ \text{sample}}$$

Total alkaloids: Total alkaloids were measured quantitatively according to Harbone, (1973) using extraction solution of glacial acetic acid and 70 % ethanol.

RESULTS AND DISCUSSION

Data presented in Table (1) clearly indicate that irrigation with saline water, amino acid application, and their interaction significantly affected vegetative growth parameters of *Catharanthus roseus*. Results show a reduction in vegetative growth parameters within increasing saline water concentration that caused the death of all plants irrigated with 8000 ppm saline water after the first cut. Meanwhile, all amino acids application recorded significant increase compared to untreated plants. Tap water irrigation treatment significantly recorded the highest values for plant height in both cuts 37.06 and

30.91cm respectively. Meantime, the interaction effect of amino acid application with saline water irrigation shows that the possibility of enhancing growth under high salinity. Amino acid treatment with1 g/l recorded the same close significant values 36.97 and 35.56 cm for plants irrigated with 2000 and 4000 ppm salinity water, respectively and came in the second place compared to 36.77 cm for plants irrigated with tap water and 1g/l amino acids in the first cut. In the second cut, a similar effect was recorded only when irrigation with 2000 ppm saline water was combined with 1g/l. The similar effect was not clear as that case of No. branches, amino acids at 0.5 g/l recorded the second place when combined with 2000 ppm treatment only compared to other combinations, while treating plants with 4000 ppm saline water and 1 g/l amino acids in the second cut recorded the second best value (12.83) compared to plant irrigated with tap water. The fresh, as well as dry weight of shoots, were severely affected by saline water irrigation, all treatments with amino acids recorded the second place for fresh weight when plants received 2000 ppm saline water treatment only in the first cut and the similar effect was recorded for dry weight in the second one. As subsequent results, the same pattern was recorded in all cases. Meanwhile, the relative dry matter didn't show any significant improvement in the first cut after amino acids treatment, while in the second cut, most of the plants irrigated with high salinity recorded the same significant values compared to irrigation with tap water. Results indicated that *C. roseus* growth damage was caused by irrigation with saline water severely in some parameters. The plant height and No. branches were reduced with increasing salinity concentration as salinity defect number of physiological processes in plant especially metabolic processes, leading to growth inhibition in most cases Lambers, (1985). Reduction in metabolic processes directly affects dry weight accumulation leading to decrease both fresh, as well as dry weights. Meantime, relative dry matter reflects the plant responses to increasing osmotic potential of living cells to enhance water absorbance from the growing medium and as an indicator of plant physiological and productive status. Plants irrigated with saline water combined with amino acids tend to increase the relative dry matter due to the role of amino acids in the production of new cell biomass and final dry weight and relative dry matter increases Bush, (1993). *C. roseus* root growth was suppressed under saline after irrigation, the root length was shortened under higher levels of saline water irrigation while amino acids treatments increased the root length Table (2). Regarding the treatments combination, the only plants irrigated with tap water recorded the highest values compared to all other combination. The similar pattern was observed regarding root fresh as well as dry weights, except that, 2000 ppm saline water treatment combined with 1 g/l amino acids treatment recorded the same close significant values compared to tap water treatment combined with same treatment of amino acids. Increased fresh and dry weights of roots after amino acid combination with saline water irrigation treatments, the overall yield of roots fresh as well as dry yields were increased in the same trend.

Saline water irrigation retarded root growth of *C. roseus* as salinity causes depletion of protein in all leaf pairs and the root as mentioned by Misra *et al*., (2014). Roots are the first to sense salinity, and therefore the first to suffer the specific toxicity of NaCl stress which can cause severe ion toxicity depending on the plant status and species Dogan *et al*., (2010). The combination of both treatments also showed an intermediate increase in parameters due to the role of amino acids against saline including the role of exogenous application of proline as free radical scavenger, electron sink, the stabilizer of macromolecules and a cell wall component Matysik *et al*., (2002). Beside the role of other amino acids on in the production of new biomass production Bush, (1993).

Table (1): Effect of water salinity and amino acid application and their interaction on vegetative growth of *Catharanthus roseus* L.G.Don

Salinity (ppm)	Amino acids (g/L)	Plant height (cm)		No. Branches/plant		Shoots fresh weight (g)		Shoots dry weight (g)		Shoots fresh yield (kg/feddan)		Shoots dry yield (kg/ feddan)		Relative dry matter	
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd	1 st cut	2 nd cut
Tap water		37.06	30.91	12.04	13.67	25.46	24.45	6.34	5.75	242.5	232.8	60.35	54.76	27.69	23.78
2000		34.67	30.00	9.20	12.56	24.18	19.14	5.74	4.86	230.2	182.3	54.66	46.31	24.72	25.55
4000		31.81	24.39	6.5٩	11.94	18.53	12.78	5.24	3.25	176.5	121.7	49.87	30.97	22.90	25.17
8000		21.81		5.95		13.54		2.41		128.9		22.92		17.32	
LSD at 0.05 %		1.00	1.02	0.75	1.98	2.64	1.12	0.34	0.40	25.14	10.71	3.28	3.81	1.05	2.66
	0	28.37	26.32	8.86	12.09	20.44	18.21	4.30	4.42	194.6	173.4	40.89	42.13	21.13	24.88
	0.5	31.58	28.20	9.32	14.43	20.36	18.34	4.94	4.66	193.8	174.7	47.04	44.39	22.94	25.37
	1	34.07	30.78	7.15	11.65	20.49	19.81	5.56	4.78	195.1	188.7	52.91	45.52	25.40	24.26
LSD at 0.05 %		0.66	0.90	0.55	0.98	1.04	1.21	0.22	0.25	9.92	11.55	2.03	2.41	0.68	1.94
	0	35.23	27.94	13.94	12.89	27.41	24.40	5.8٥	5.06	261.0	232.4	55.65	48.20	25.45	20.90
Tap water	0.5	36.77	31.00	13.89	16.11	25.00	23.16	6.38	6.14	238.0	220.6	60.79	58.46	27.76	26.82
	1	39.18	33.78	8.28	12.00	23.98	25.78	6.78	6.05	228.4	245.5	64.60	57.61	29.88	23.63
	0	32.65	27.72	8.72	13.06	24.17	17.82	5.0٥	5.01	230.2	169.7	48.08	47.67	23.25	28.05
2000	0.5	34.37	29.06	11.00	14.50	24.67	19.13	5.89	4.63	234.9	182.2	56.12	44.07	24.32	24.37
	1	36.97	33.22	7.89	10.11	23.69	20.47	6.2٨	4.96	225.6	195.0	59.77	47.19	26.58	24.23
	0	27.65	23.28	7.22	10.33	18.31	12.40	4.47	3.21	174.4	118.1	42.59	30.53	20.83	25.69
4000	0.5	32.23	24.56	5.72	12.67	18.20	12.74	5.1٤	3.22	173.3	121.4	48.92	30.63	22.50	24.91
	1	35.56	25.33	6.88	12.83	19.08	13.18	6.10	3.33	181.7	125.5	58.09	31.74	25.37	24.91
	0	17.94		5.57		11.85		1.81		112.9		17.25		14.98	
8000	0.5	22.94		6.68		13.56		2.3٥		129.1		22.35		17.19	
	1	24.55		5.61		15.19		3.06		144.7		29.16		19.78	
LSD at 0.05 %		1.21	1.55	1.10	1.70	2.08	1.71	0.430	0.438	19.84	20.00	4.07	4.18	1.36	3.36

Means in the same column for each trait are the average of two independent experiments (n = 6). Values significantly differ for each other according to LSD test at P=0.05.

Table(2):Effect of water salinity and amino acid nutrition application and their interaction on root parameters of *Catharanthus roseus* L.

Salinity (ppm)	Amino acids (g/pot)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Roots fresh yield (kg/fed)	Roots dry yield (kg/fed)
Tap water		37.96	11.19	1.82	106.60	17.36
2000		27.24	10.44	1.69	99.41	16.09
4000		23.11	6.415	1.35	61.09	12.88
LSD at 0.05 %		1.14	0.10	0.05	0.92	0.45
	0	26.48	8.21	1.43	78.14	13.60
	0.5	29.76	9.66	1.59	91.95	15.15
	1	32.07	10.18	1.85	96.98	17.58
LSD at 0.05 %		0.73	0.18	0.06	1.71	0.58
	0	34.11	10.43	1.71	99.31	16.33
Tap water	0.5	38.39	11.37	1.84	108.20	17.54
	1	41.39	11.78	1.91	112.20	18.20
	0	24.33	8.43	1.47	80.31	14.03
2000	0.5	27.44	11.21	1.53	106.8	14.55
	1	29.94	11.67	2.07	111.20	19.68
	0	21.00	5.75	1.09	54.81	10.42
4000	0.5	23.44	6.39	1.40	60.84	13.36
	1	24.89	7.10	1.56	67.61	14.87
LSD at 0.05 %		1.26	0.31	0.11	2.60	1.01

Means in the same column for each trait are the average of two independent experiments (n = 6). Values significantly differ from each other according to LSD test at P=0.05.

Generally, the accumulation of chlorophyll a,b and total chlorophyll, as well as carotenoids, was decreased compared to the control with increasing water salinity level as data presented in Table (3). The pigments content was degraded to reach their lowest values when plants were irrigated with 8000 ppm saline water in the first cut and 4000 ppm in the second one. Meanwhile, amino acid treatments showed the different trends as plants treated with amino acids showed a significant increase in both chlorophyll and carotenoids for both cuts. Meantime, the interaction effect of amino acids application with saline water irrigation showed that amino acids treatment combination with salinity didn't improve pigments content, especially under high-salinity treatments.

Salinity harm the plant tissues in different means causing oxidative stress and significant decrease to photosynthetic systems including pigments damage photosystems (Koyro, 2006 and Sicher, 1999). The reduction of chlorophyll content in *C. roseus* under salinity condition considers a presentable result even under the condition of amino acid treatments. Sever damage for chlorophyll content in leaf tissues could be referred either to the reduction of pigments biosynthesis or due to the increasing activity of chlorophyll degradation enzymes. Xu *et al*., (2008).

Table (3): Effect of water salinity and amino acid nutrition application and their interaction on the photosynthetic pigment of *Catharanthus roseus* L.

Salinity (ppm)	Amino acids (g/L)	Chl. a		Chl. b		Total chl.		Carotenoids	
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
Tap water		0.616	0.567	0.171	0.164	0.787	0.730	0.258	0.232
2000		0.417	0.324	0.112	0.089	0.529	0.413	0.200	0.165
4000		0.218	0.181	0.062	0.053	0.280	0.233	0.159	0.101
8000		0.078		0.027		0.105		0.068	
LSD at 0.05 %		0.0007	0.0002	0.0007	0.0002	0.0007	0.0002	0.0007	0.0002
	0	0.320	0.337	0.092	0.097	0.412	0.435	0.162	0.165
	0.5	0.338	0.363	0.092	0.102	0.431	0.465	0.175	0.164
	1	0.339	0.369	0.095	0.107	0.432	0.476	0.178	0.169
LSD at 0.05 %		0.0006	0.0002	0.0006	0.0002	0.0006	0.0002	0.0006	0.0002
	0	0.605	0.559	0.168	0.159	0.773	0.718	0.234	0.246
Tap water	0.5	0.620	0.568	0.172	0.163	0.791	0.731	0.269	0.221
	1	0.625	0.570	0.172	0.171	0.797	0.741	0.272	0.228
	0	0.399	0.283	0.110	0.085	0.510	0.368	0.194	0.149
2000	0.5	0.426	0.343	0.112	0.089	0.538	0.433	0.202	0.170
	1	0.426	0.344	0.113	0.092	0.540	0.436	0.203	0.176
	0	0.209	0.169	0.063	0.048	0.272	0.218	0.154	0.098
4000	0.5	0.219	0.179	0.063	0.053	0.283	0.232	0.1603	0.102
	1	0.224	0.193	0.061	0.056	0.285	0.250	0.163	0.104
	0	0.067		0.026		0.093		0.064	
8000	0.5	0.085		0.032		0.117		0.068	
	1	0.081		0.02183		0.103		0.073	
LSD at 0.05 %		0.0012	0.0004	0.0012	0.0004	0.0012	0.0004	0.0012	0.0004

Means in the same column for each trait are the average of two independent experiments (n = 6). Values significantly differ from each other according to LSD test at P=0.05.

Reduction in carotenoids content may be due to their protective role against reactive oxygen species. Carotenoids can protect the photosynthetic system against reactive oxygen species generated under salt stress (Parida and Das, 2005_and Parviz *et al*, 2008). Salinity stress causes a reduction in photosynthetic pigments and degree of reduction depends on the salt tolerance of plant species Dogan and Tipirdamaz, (2010).Similarly, salinity level above 90 mM NaCl decreased total carotenoids in *Pisum sativum* Hernandez *et al*., (1999).

Nutritional elements and sodium%, as well as chlorine % in the leaf tissues of *C. roseus*, was significantly affected by both saline water and amino acids treatments as indicated in Table (4). Results show that nitrogen and phosphorus were decreased within increasing saline water concentration to reach the lowest values at 8000 and 4000 ppm treatments for both cuts, respectively. Meanwhile, both sodium and chlorine were increased to reach their highest values at the same levels of saline water treatments. However, the highest level of potassium content was recorded at 2000 ppm saline water treatments. A totally opposite trend was observed in the case of amino acid treatments. The highest levels of nutrient elements were recorded for the amino acid at 1g/l in most cases during both cuts while the highest levels of sodium and chlorine were recorded for untreated plants. Meantime, the interaction effect of amino acid application with saline water irrigation showed the possibility of enhancing mineral uptake and reducing toxic ions (sodium and chlorine) under high salinity . Combining amino acids at 1 g/l with saline water irrigation in most cases recorded the same close significant values of 2000 and 4000 ppm treatments compared to tap water treatment for both cuts Meanwhile, toxic ions % in leaf tissues recorded the highest values in all saline water treatments in the absence of amino acids, while the presence of amino acids at any concentration recorded lower values

Table (4): Effect of water salinity and amino acid nutrition application and their interaction on mineral % of *Catharanthus roseus* L.G.Don

Salinity (ppm)	Amino acids (g/l)	N %		P%		K%		Na %		Cl %	
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
Tap water		2.19	1.60	0.967	0.236	3.50	3.02	0.57	0.61	3.32	4.10
2000		2.06	1.55	0.970	0.154	3.70	2.97	3.37	4.06	7.35	7.29
4000		1.38	1.50	0.403	0.177	3.36	3.34	3.54	5.97	9.73	8.27
8000		1.14		0.294		2.42		4.33		11.41	
LSD at 0.05 %		0.022	0.033	0.022	0.024	0.119	0.129	0.34	0.47	0.53	1.14
	0	1.57	1.41	0.597	0.205	2.67	2.90	3.66	4.13	8.72	8.10
	0.5	1.70	1.56	0.701	0.146	3.35	3.32	2.68	2.95	8.14	5.93
	1	1.81	1.77	0.679	0.217	3.73	3.10	2.52	3.55	7.00	5.64
LSD at 0.05 %		0.032	0.037	0.018	0.022	0.052	0.068	0.17	0.40	0.26	0.62
	0	2.07	1.45	1.060	0.313	2.67	2.66	0.35	0.77	2.92	5.13
Tap water	0.5	2.19	1.50	0.972	0.083	3.35	3.09	0.73	0.50	4.02	4.16
	1	2.31	1.76	0.870	0.312	3.72	3.30	0.63	0.56	3.02	3.02
	0	1.97	1.45	0.690	0.205	0.02	2.46	3.87	4.73	8.40	9.363
2000	0.5	2.07	1.59	0.977	0.138	3.70	3.15	3.17	3.42	6.75	5.61
	1	2.15	1.75	1.243	0.120	4.19	3.29	3.08	4.03	6.89	6.91
	0	1.26	1.31	0.495	0.097	3.20	3.58	4.36	6.90	11.21	9.81
4000	0.5	1.37	1.60	0.640	0.217	3.68	3.73	3.27	4.94	10.44	8.03
	1	1.50	1.80	0.073	0.218	4.22	2.71	3.01	6.06	7.53	6.98
	0	0.99		0.143		3.15		6.06		12.34	
8000	0.5	1.17		0.210		3.73		3.56		11.36	
	1	1.27		0.530		3.20		3.38		10.55	
LSD at 0.05 %		0.064	0.064	0.037	0.037	0.104	0.118	0.34	0.69	0.53	1.07

Means in the same column for each trait are the average of two independent experiments (n = 6). Values significantly differ from each other according to LSD test at P=0.05.

Meanwhile, toxic ions % in leaf tissues recorded the highest values in all saline water treatments in the absence of amino acids, while the presence of amino acids at any concentration recorded lower values.

The reduction in nutrient minerals % after saline water irrigation treatment using NaCl-stressed plant application may be due to the competition of Na^+ and Cl^- with other mineral ions in the uptake Khan, (2001) . It also seems logical that this kind of stress leads to the increase of toxic aion (sodium and chlorine) as they are the main constituents of the salt. Also Rother *et al*., (1983) demonstrated that Cl^- sharply decreased the NO3 uptake by the roots, incorporation of nitrogen into organic compounds and translocation of nitrogen to the leaves. A decrease in potassium % may be due to either the toxic effect of NaCl on plant growth or competition by other ions, which in turn exercised a regulatory control on potassium uptake. Potassium % reduction in NaCl-stressed plants was also reported in the finding of (Warwick and Halloran, 1991 and Gadallah, 1999). The ability of the plant to phytoremediator this kind of stress mostly depend on the plant nutrient status and the ability to adsorb minerals and other nutrient from the soil under salinity conditions. Amino acids had been extensively used to enhance plant ability to survive under salinity conditions as naturally plants tend to increase the cells osmotic solutes to increase the osmotic pressure in order to enhance water and nutrients absorbing. Saeed *et al*., (2005)

Data presented in Table (5) summarize the physiological responses of *C. roseus* after saline water irrigation, amino acid application, and their interaction. Data indicated different physiological responses related to saline water irrigation while amino acids application didn't show any significant differences in some cases.

Total carbohydrates: Results showed that Carbohydrates % was gradually decreased with increasing salinity level during the tow cuts of treatment compared to control samples (Table5). The highest concentrations of 8000 and 4000 ppm treatment recorded the lowest values for both cuts, respectively. Meantime, there were no much differences recorded in carbohydrates % as untreated plants recorded the same significant values compared to 0.5 g/l with maximum carbohydrates % after 1 g/l application. Regarding the interaction effect, data indicated that amino acids application couldn't help to enhance carbohydrates contents under saline water irrigation except for in the case of tap water irrigation.

Total carbohydrates in *C. roseus* was severely reduced which can be explained by the effect of salinity on chlorophyll damage as our results indicated. Degradation of chlorophyll directly affects photosynthesis process Pasternak, (1987) and overall the accumulation of carbohydrates in plant tissues. Suppressing metabolism due to salinity could be attributed to the nutritional imbalance Liu *et al*., (1998), specific toxic effects of salinity Niu *et al*., (1995) and hyperosmotic stress Greenway and Munns, (1980)

Proline content: Proline accumulation in *C. roseus* tissues proves to increase within saline water concentration increase, while amino acids treatment didn't record any significant difference in both cuts in this concern. Meantime, the interaction effect of amino acid application with saline water irrigation reveals that plants irrigated with saline water still show responses of stress even when amino acids were applied, as plants irrigation with high saline water concentrations still accumulates proline in significant values compared to other interaction treatments. Plants responses to salinity via proline accumulation had been documented in many cases (Osman *et al*., 2007 and Zhonghua *et al*., 2011) . Proline levels were linked to salinity and other stress conditions as it acts as free radical scavenger, electron sink, stabilizer of macromolecules and a cell wall component Matysik *et al*., (2002), proline functions as a component of signal transduction pathways that regulate stress-responsive genes in Arabidopsis Kiyosue *et al*., (1996) and *Pancratium maritimum* Khedr *et al*., (2003). Besides, intermediates of proline biosynthesis, an increase in the expression of several osmotically regulated genes were found Iyer and Caplan, (1998).

Total alkaloids % : Alkaloids accumulation significantly varied in the response of saline water irrigation. Treatments of amino acids recorded the same significant values among treatments. In the first cut, shoots total alkaloids increased within saline water concentration decreases to record the highest values at 8000 ppm treatment even when combined with amino acids application, all other combinations between irrigation treatments and amino acids application recorded the same close significant values and ranked in the second place. As plant didn't survive the second cut under 8000 ppm treatment, plant irrigated with 4000 ppm treatment combined with amino acids treatment recorded the highest values in this concern. Roots total alkaloids % recorded different results, as the accumulation of alkaloids in roots was estimated after the double time of growth compared to shoots. Involving amino acids and its effect on enhancing growth for longer period resulted in different results. Amino acids treatment at the highest concentration recorded the highest total alkaloids % in roots 1.325 % The highest total alkaloids % on roots was recorded for 2000 ppm treatment and with amino acids, treatment in the interaction both 2000 and 4000 ppm saline water treatment combined with 1 g/l amino acids application recorded the highest root total alkaloids 1.621 and1.557 % respectively. Salinity is manifested as osmotic stress, resulting denaturation of functional and structural proteins Smirnoff, (1998). Thereby, they can activate similar cell signaling pathways Zhu, (2001) resulting in the production of stress proteins and accumulation of compatible solutes Cushman and Bohnert, (2000) mainly alkaloids in the case of *C. roseus*. The increase in roots and leaves alkaloids was linked to salinity stress through arginine accumulation which induces nitric oxide biosynthesis which acts as chemical elicitor for indole alkaloid production of *C. roseus* (Hu *et al*., 2003 and Xu *et al*., 2005) .

Table (5): Physiological responses parameters of *Catharanthus roseus L.G.Don* after saline water, amino acid treatments and their interaction

Salinity (ppm)	Amino acids (g/l)	Carbohydrates (%)		Proline (mmol/g fresh weight)		Shoot Total alkaloid (%)		Root total alkaloid (%)
		1 st cut	2 nd	1 st cut	2 nd cut	1 st cut	2 nd cut	
Tap water		15.47	8.10	0.54	0.44	0.91	1.40	0.76
2000		11.55	5.93	1.08	1.90	1.19	1.48	1.39
4000		7.50	5.64	2.77	2.71	1.34	2.91	1.28
8000		4.11		3.91		3.17		
LSD at 0.05 %		0.136	0.276	0.170	0.280	0.257	0.047	0.033
	0	9.46	9.35	2.45	1.71	1.63	2.02	0.96
	0.5	9.50	9.41	2.64	1.64	1.70	1.87	1.14
	1	10.02	9.80	2.42	1.71	1.63	1.91	1.33
LSD at 0.05 %		0.178	0.273	0.149	0.143	0.210	0.048	0.022
	0	14.99	13.54	0.53	0.06	0.86	1.39	0.72
Tap water	0.5	15.15	14.69	0.60	0.46	0.86	1.41	0.77
	1	16.28	15.45	0.50	0.80	0.99	1.41	0.80
	0	11.15	9.94	0.975	1.66	1.13	1.85	1.04
2000	0.5	11.66	10.32	1.17	1.86	1.20	1.29	1.51
	1	11.83	11.13	1.10	2.18	1.24	1.31	1.62
	0	8.04	4.56	2.33	3.40	1.43	2.81	1.13
4000	0.5	6.99	3.22	3.23	2.60	1.50	2.90	1.14
	1	7.48	2.81	2.76	2.14	1.09	3.01	1.56
	0	3.65		5.95		3.11		
8000	0.5	4.20		5.55		3.21		
	1	4.49		5.33		3.20		
LSD at 0.05 %		0.356	0.473	0.298	0.247	0.421	0.083	0.037

Means in the same column for each trait are the average of two independent experiments (n = 6). Values significantly differ from each other according to LSD test at P=0.05.

Meanwhile, the increase of roots and leaves alkaloids within treatment combination could also be attributed to the exogenous application of arginine. Alkaloids are considered an adaptation for many conditions i.e. salinity, in the case of NaCl salinity, studies indicated that it exerts a remarkable influence on individual alkaloids of plant parts Misra and Gupta, (2006). Previous studies proved that the maximum % of alkaloids in *C. roseus* found in the root park particularly during flowering Jaleel *et al*., (2007b) and seems to increase under NaCl stress when compared to unstressed plants Jaleel *et al*.,(2008) which agree with our findings.

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تأثير الرى بالمياة مرتفعة الملوحة والاحماض الأمينية على النمو، الانتاجية وانتاج القلويدات لنبات الونكا تحت ظروف سيناء

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أجرى هذا البحث بغرض دراسة إنتاجية نبات الونكا كمحاكاة لظروف الرى بمياه مرتفعة فى مستوى الملوحة بمنطقة شمال سيناء. تم رى النباتات النامية فى أصص بلاستيكية بثلاث مستويات من الملوحة تم تحضيرها باستخدام ملح كلوريد الصوديوم بتركيز ٢٠٠٠، ٤٠٠٠ و ٨٠٠٠ جزء فى المليون بالاضافة للرى بماء الصنبور كمعاملة كنترول. ولتحسين نموالنبات تحت ظروف الملوحة فقد تم رش النباتات بالأحماض الأمينية باستخدام مركب تجارى (amino zeid42/85). أظهرت النتائج حساسية نبات الونكا للملوحة خاصة فى المستويات المرتفعة حيث ادى الرى بمياه تركيز ملوحتها ٨٠٠٠ جزء فى المليون الى موت جميع النباتات مباشرة بعد الحشة الأولى. وبينما صاحب إرتفاع مستوى ملوحة ماء الرى زيادة ملحوظة فى إنتاج القلويدات فى أجزاء النبات المختلفة وقد لوحظ أيضا تحسن فى صفات النمو للنباتات المعاملة بالاحماض الامينية بمعدل ١جم/لتر تحت المستويات المعتدلة للملوحة. وبصفه عامة تشير النتائج إلى حساسية نبات الونكا للملوحة بشكل كبير مما يفتح المجال لمزيد من الدراسة حول إستخدام الأحماض الأمينية لزيادة مقاومة النبات للملوحة من خلال إستخدام طرق أخرى للتطبيق أو إستخدام تركيزات أعلى.