تأثير الملوحة في اختلال توازن الشوارد وفي اصطناع الغلوكوزيدات المقوية للقلب في نبات حشيشة الصياد

عصام الشماع*

الملخص

خلفية البحث وهدفه: تؤثر الملوحة في إنتاج الأرض، وذلك من خلال التراكيز الموجودة فيها، ويعتقد أن الكالسيوم يؤثر في مستوى الشوارد المعدنية +Na بطريقة تنافسية. ويظهر هذا البحث تأثير الملوحة في اصطناع الغلوكوزيدات المقوية للقلب في نبات حشيشة الصياد.

مواد البحث وطرائقه: زرع نبات حشيشة الصياد Adonis autumnalis الموجود في مدينة حلب (جبل الحص- كفركار)، في ثلاثة تراكيز مختلفة من الملوحة 25,50,100M بإضافة محلول كلوريد الصوديوم NaCl ورقبت خلال 22 أسبوعاً. إذْ لوحظ أن الأوراق المعرضة للملوحة الشديدة على تراكيز أعلى من شوارد البوتاسيوم +K وتراكيز أقل من شوارد الكالسيوم +Ca2 مقارنةً بأوراق مجموعة الشاهد control .

النتائج: لوحظ انخفاض النسبة الشاردية +K+/Na لأوراق النباتات المعرضة للملوحة المركزة إلى أقل من الواحد عندما بدأت أعراض الشيخوخة بالظهور. كما أصبح معدل النسبة الشاردية +Na+/K أعلى بارتفاع نسبة الملوحة، وكلما طال زمن التركيز الشاردي المرتفع في التربة انخفض تركيز جذور النباتات المعرضة لظروف الإجهاد الملحي محتوى أقل من +K وازدادت الملوحة وطال زمن التعرض لظروف الإجهاد الملحي. وقد كان تركيز شاردة +Na لجذور النباتات المعرضة للملوحة المركزة أعلى من تراكيز شوارد الصوديوم +Na في جذور النباتات في الشاهد، ولكنها تناقصت في نهاية التجربة بسبب التنخر وفقدان الأجزاء الفتية للجذور.

كلما ازداد تركيز الملوحة انخفضت تراكيز شوارد الكالسيوم +Ca2 في حين بقي معدل +Na+/K في الجذور محافظاً على مستوى منخفض، وذلك بسبب التنخر والتموت النسيجي وضياع الأجزاء الفتية للجذور.

الاستنتاجات:

لم تتداخل الملوحة وحالة عدم التوازن الشاردي بشكل ملحوظ مع الاستقلاب الحيوي.

زادت نسبة تركيز القلويدات المقوية للقلب في النباتات الخاضعة للمعالجة في الملوحة 25mM .

الكلمات المفتاحية: حشيشة الصياد، الملوحة، Adonitoxin، اختلال توازن الشوارد.

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Effect of Salinity on Ionic Dise Quilibrium and Cardinolids Synthesis in Adonis Autumnalis

Isam Alshammaa *

Abstract

Background: Salinity is involved in decline of plant production in arid and semi-arid lands. It is believed that Ca^{2+} maintains the structural integrity of the plasmo membrane by cross linking the negatively charged head groups of the plasmo membrane. In this paper we study the effect of salinity on cardinolid synthesis in Adonis autumnalis plants.

Methods: Plants of Adonis autumnalis (Aleppo) were grown in three salinity conditions: 25, 50 and 100 mM NaCl, during 22 weeks. Results : Leaves of stressed plants had a higher K^+ and Na^+ contents and a lower Ca content than leaves of control plants , K^+/Na^+ ratio of leaves of stressed plants become a lower than 1 when the senescence was started .

Na^+/Ca^{2+} ratio became a higher the higher the salinity conditions were and the longer the time of stress. The roots of stressed plants had lower K^+ content in higher salinity and as longer the time of stress. The Na^+ content of roots of stressed plants was higher than the Na^+ content in roots of control plants, but decreased at the end of experiment due to necrosis and loss of young parts of roots. Ca^{2+} content in roots was lower as the salinity stress was higher. The Na^+/Ca^{2+} ratio in roots was maintained low, due to necrosis and loss of young parts of roots. Salinity and ionic disequilibrium did not interfered significantly on cardinolids metabolism.

Conclusion: The content of cardinolids did not changed significantly in salinity conditions until the death of the plants.

The slight ionic imbalance, which reaches weak levels of salinity, leads to the activation of the cadiotonic glycosides accumulation in the leaves of Adonis autumnalis , and in doing so, it leads to increasing the concentrations of the cardionolides in the leaves and roots of Adonis autumnalis which have been treated at at the salinity of 25mM.

Keywords: Adonis autumnalis, salinity, cardinoids, Adonitoxinionic disequilibrium

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Figure(1) Adonis autumnalis

Introduction

Salinity is involved in decline of plant production in arid and semi-arid lands [1,2] . The activity of many enzymes is affected by addition of salts during the experiment [3] . Some enzymes show marked inhibition by inorganic ion concentration above 100 mM [4] . It is believed that Ca2+ maintains the structural integrity of the plasma membrane by cross linking the negatively charged head groups of the plasme membrane [5]. At high concentration of Na+ this Ca2+ is displaced by Na+ causing an influx of Na+ . In high external salinity , Na+ may enter the cytoplasme passively across the plasma membrane because the cytoplasm is electrically negative with respect to the apoplast and the concentration of Na+ is probably higher outside the cytoplasm . In plants , the Na+ selective chanel has not been identified in the plasme membrane , therefore entry of Na+ into cytoplasm may be through a K+ channel with some permeability to Na+ [6] .In glycophytes, the membrane depolarizes in response to external NaCl . Despolarization will open the cation outward rectifier channels and Na+ could move into the cell at a rate governed by the electrochemical gradient for Na+ and the effective permeability of the chanels to Na+ [7].

Summarising , salt stress reduced plant growth through water deficits , ion toxicity , ionic inbalance or a combination of any of these factors , and plays an important role on turgor,permeability, enzimatic activity and hormonal balance [8]. All these effects cause a primary metabolism fall , thus intermediary products could by used in secondary metabolite synthesis. In a previous work [9] we reported the effect of salinity in the nicotine production in Nicotina rustica plants . In this paper we study the effect of salinity on cardenolide synthesis in Adonis autumnalis plants .

Materials and methods

Culture conditions

Seeds of Adonis autumnalis (Ranunculaceae)were sown in equal since flower pots containing a mixture of sand and vermiculite . Pots were moistened with the same amount of deionised water three times a week , when the germination began , the plantules were divided into four groups : the first was rinsed with Arnon and stood solution and used as a control , the second was rinsed with the same solution , but 25 mM NaCl was added (25mM NaCl plants), the third with 50 mM NaCl added to it (50mM NaCl plants) and the fourth with 100 mM NaCl (100mM NaCl plants). The plants were harvested after 14, 16, 18, 19, 20 , 21, 22, weeks of the treatments. In each sample three different plants were harvested and each plant was analyzed twice . Thus , the total number of analysis for each sample was six. Leaves and roots were sampled separately. The following measurements and analysis were carried out : measurement of growth (dry weight) , Na+ , K+ , Ca2+ , and total cardenolide contens.

Figure (2) Adonis autumnalis حلب - جبل الحص- كفركار 2010/4/2

Dry weight

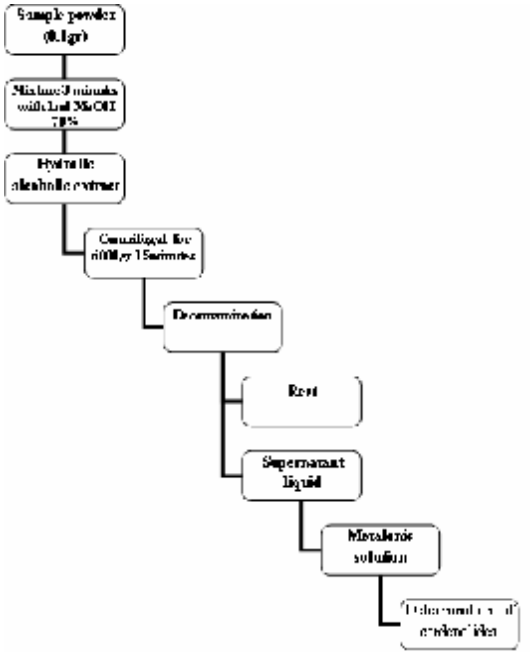
Dry weight determinations were made by heating leaves and roots separately at 60°C for 48 hours and then to constant weight .

Ion determination

Na+ , K+ and Ca2+ determination were made by emission spectroscopy for plasma icp in a Mixter spectrometer multichannel sequenical pf plasma icpjy70 plus [10]

Cardenoloide determination

The extraction and purification of cardenolids glycoside were carried out as described by Lught and Oordhock-Ananias (1974) [11]. Determination of cardenoloide was made by means of colorimetric method based on a Baljet colour reaction, modified by Khafegy and Girgis 1974 [12]. Total cardenolide contents measured as adinotoxine



Scheme (1) : Extraction of cardinolides

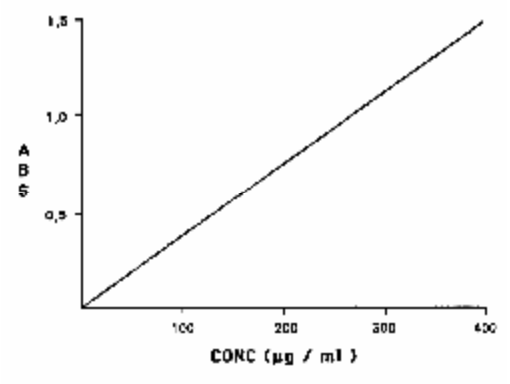


Figure (3)
Curve patron of cardinolides
ABS: Absorption, CONC: constriction

Figure (4)

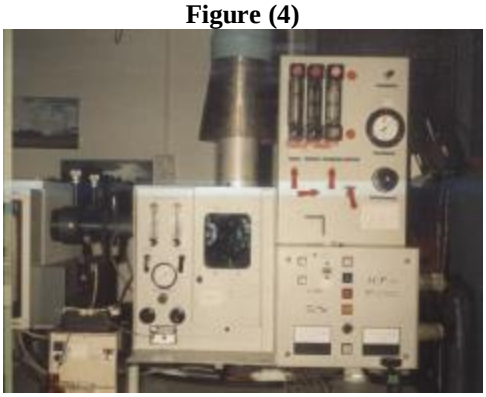


Figure (5)

Results

Dry weight

Plants grown under salinity conditions had lower dry weight (DW) values than control plants . This effect was more pronounced in plants grown on higher NaCl concentration , and was observed in both leaves and roots (Fig. 6) .

Univalent ions content

The content of K+ in roots and leaves of control plants remained relatively constant and in the interval considered as normal during the first 17 weeks of growth and sink down at the end of the experiment (tab. 1and2) , probably due to inert substances accumulation in plants . Roots and leaves of control plants had a very low content of Na+ and total M (Na+ + K+) contents were very similar to K+ (tab. 1and2) .The roots of stressed plant had K+ contents lower with higher salinity conditions of growth and longer time of stress . Na+ contents of 25 mM plants roots increased during the experiment but 50 mM plants roots Na+ contents increased only until the 18th week of growth and then decreased , until the end of the experiement; and finally Na+ contents of 100 mM plants roots reached very high levels during the first 14 weeks of growth decreasing thereafter until the end of the experiment .

Discussion

A high content of Na+ inhibited the root growth and led to necrosis and loss of young roots tissues in no-halophytic plants [13]. Tot M contents in roots of

stressed plants were similar to content of Na+ .The results showed that in our experimental conditions , the limit for normal life of root of Adonis autumnalis had a content of total M 2 times higher than the roots of control plants . Roots of 50 mM and 100 mM plants reached this limit after 18 weeks and 14 weeks of growth respectively , then they underwent necrosis and death . The content of K+ in leaves of stressed plants were slightly higher than in leaves of control plants , specially in those of 40 mM plants . These results agree with [14] , who reported an increase in contents of K+ and Na+ in leaves of salt sensitive plants . Contents of Na+ and Tot M ions were notably higher in the leaves of plants grown in salinity conditions than in the leaves of control plants and were higher the greater the salinity conditions than in the leaves of control plants and were higher the greater the salinity conditions and time of stress . Our results showed that , generally , when the Na+ contents in leaves reached 0.7 meq/g DW , their content of univalent ions was two times higher than in control plant leaves , this high content is the limit of viability on Adonis autumnalis leave-cells.

On the other hand [15] showed that senescence is started when the K+/Na+ ratio is lower than 1(Table 3) . Our results showed that K+/Na+ ratio in leaves of 25 mM , 50 mM and 100 mM plants were lower than 1 at 19,18, 19 and 16 weeks respectively , and the roots of these plants at 18 and 16 respectively . We infer than these plants suffered stronger degrading processes the higher the salinity conditions were.

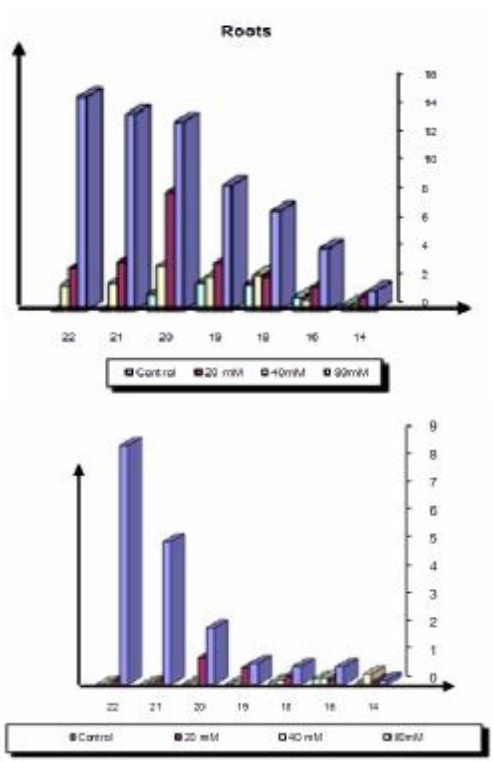


Figure 6
Dry weight (DW) expressed in g. Data are the average of six analyses.
Weeks : weeks of treatment

Table1
K+,Na+ and Tot M (Na+,K+) content in roots of treated and control plants, expressed in meq/g DW . Data are the average of six analyses. Weeks : weeks of treatment.

Weeks	Control	25mM	50mM	100mM
		Na+		
14	0.042±0.002	0.885±0.003	0.512±0.002	0.613±0.021
16	0.018±0.001	0.313±0.030	0.391±0.018	1.121±0.091
18	0.029±0.009	0.612±0.041	1.021±0.081	2.018±0.289
19	0.041±0.021	0.831±0.071	0.892±0.092	2.831±0.189
20	0.01±0.040	0.911±0.091	1.273±0.122	2.812±0.218
21	0.042±0.125	0.831±0.123	1.735±0.873	-
22	0.031±0.041	0.913±0.821	1.843±0.075	-
		K+		
14	0.832±0.065	0.854±0.074	0.983±0.082	0.812±0.153
16	0.972±0.0751	1.081±0.0912	1.152±0.092	1.352±0.175
18	0.812±0.076	0.321±0.083	0.953±.0.054	0.962±0.097
19	0.841±0.081	0.971±0.086	0.894±0.068	0.883±0.073
20	0.792±0.074	0.811±0.051	0.851±0.061	0.913±0.089
21	0.751±0.047	0.832±0.052	0.172±0.065	-
22	0.687±0.042	0.786±0.062	0.837±0.045	-
		Na+ + K+		

14	0.781±0.063	0.852±0.081	1.132±0.060	1.875±0.878
16	0.762±0.047	1.751±0.036	1.803±0.084	1.721±0.092
18	0.911±0.078	1.471±0.101	1.965±.0.100	2.921±0.076
19	0.871±0.063	1.523±0.0971	1.818±0.149	2.675±0.117
20	0.831±0.076	1.661±0.089	1.935±0.164	-
22	0.791±0.042	1.7114±0.121	2.102±0.128	-

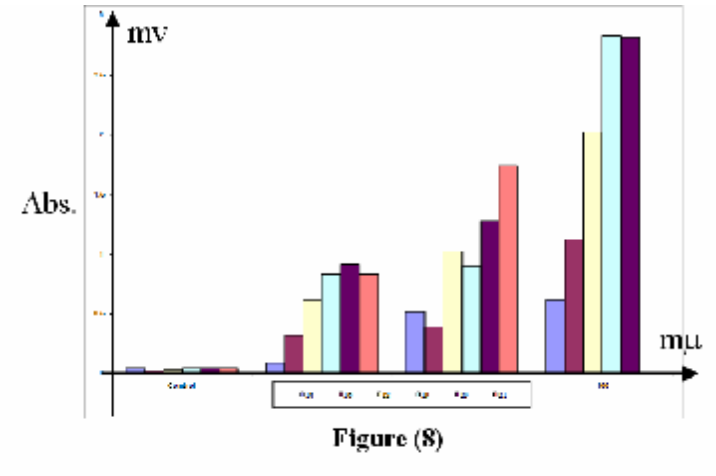


Figure (8)

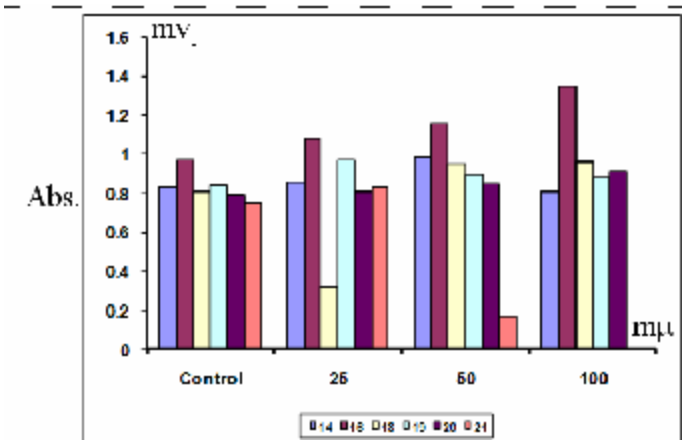


Figure 2

Na+, K+ content in roots of treated and control plants, expressed in meq/g DW. Data are the average of six analyses. Weeks : weeks of treatment.

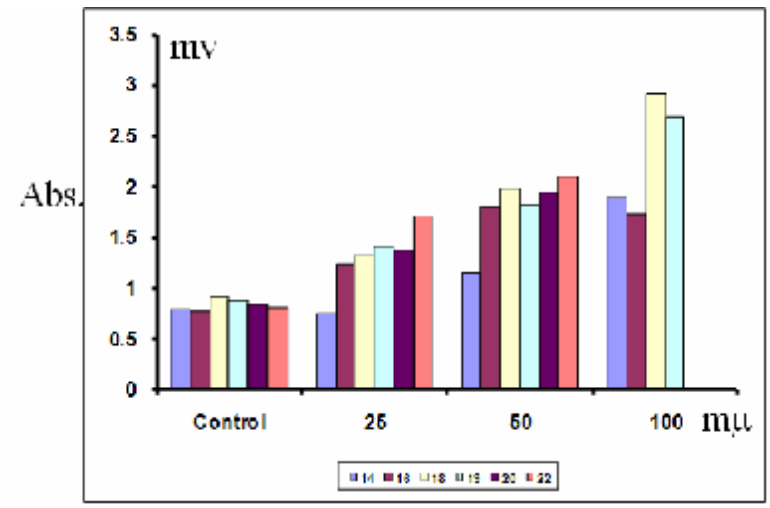


Figure 3

Tot M (Na+ and K+) content in roots of treated and control plants, expressed in meq/g DW. Data are the average of six analyses. Weeks : weeks of treatment Ca2+ content

The Ca2+ content in roots of plants control increased until the 20th week and then decreased until the end of experiment , probably due to necrosis and loss of the young parts of roots (Table 4) .The levels of Ca2+ in the roots of stressed plants of Adonis autumnalis increased during the course of the experiment , but they were lower than the levels in roots of control plants and decrease the consent the salinity conditions of grown , and the longer the time of stress . The Na+/ Ca2+ ratio (Table 5) was low and relatively constant in the roots of control plants during the course of the experiment , but this ratio increased and then decreased in the roots of 20mM and 40 mM plants and decreased continuously in the roots of 80 mM plants [16] considered that high Na+/ Ca2+ ratios damage semi permeability and permit Na+ and K+ uptake but lower

Na+/Ca2+ ratio maintain semi permeability . We must not consider the decrease of Na+/ Ca2+ ratio in the stressed roots as a protective mechanism , due to necrosis and loss of young parts of roots , because Ca2+ linked to the older part of plants . The levels of Ca2+ in the leaves of Adonis autumnalis control plants were sufficient and increased slightly during the course of the experiment due to the increase in the number , of old leaves with respect to young ones . On the contrary , leaves of 20 mM and 40 mM plants increased their contents in Ca2+ for 18 weeks and then decreased due loss of old leaves rich in Ca2+ . The content of Ca2+ in leaves of 80 mM plants increased during the experiment because their leaves were always in senescence . The Na+/Ca2+ ratio was low in the leaves of control plants , but very high in leaves of stressed plants , showing the damage in the latter .

Cardenolide content

The content of cardenolides in leaves and roots of control and stressed plants first increased and then decreased during the course of experiment (Fig.8). The salinity did not interfere in the cardenolide accumulation since that cardenolide in stressed plants was only slightly lower than in the control plants , and proportionally stressed plants had higher number of adult leaves . These facts suggest that ionic disequilibrium does not significantly interfere in the enzymatic systems of cardenolide synthesis or degradation**.**

Conclusions

The plants of Adonis autumnalis stressed by salinity , did not have a suitable mechanism not to uptake Na+ , generally , had a high content in Tot M mainly in leaves . In roots the levels of total M , decreased in stressed plants in the course of the experiment due to necrosis and loss of young roots .The Na+/ Ca2+ ratio was lower in leaves of control plants than in leaves of stressed plants , but during the course of the experiment this ratio decreased in leaves of stressed plants. This is due to loss of old leaves, but not to a protective mechanism .

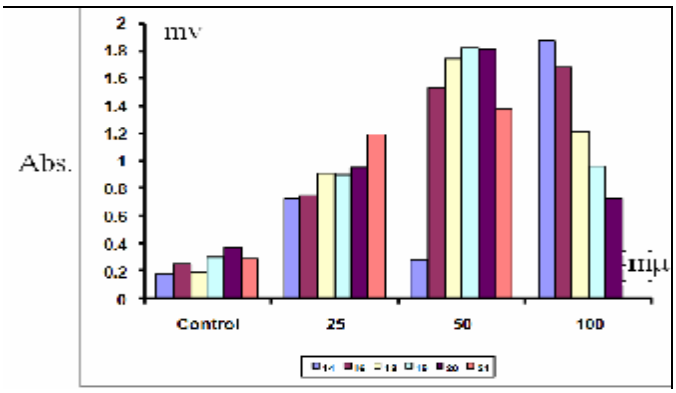
The content of cardenolide did not change significantly in salinity conditions until the death of the plants . We must infer that there was no clear effect of ionic disequilibrium on enzymatic systems either of cardenolide synthesis or degradation .

The slight ionic imbalance, which reaches weak levels of salinity, leads to the activation of the cardiotonic glycosides accumulation in the leaves of Adonis, and in doing so, it leads to increasing the concentrations of the cardinolides in the leaves and roots of Adonis which have been treated at the salinity

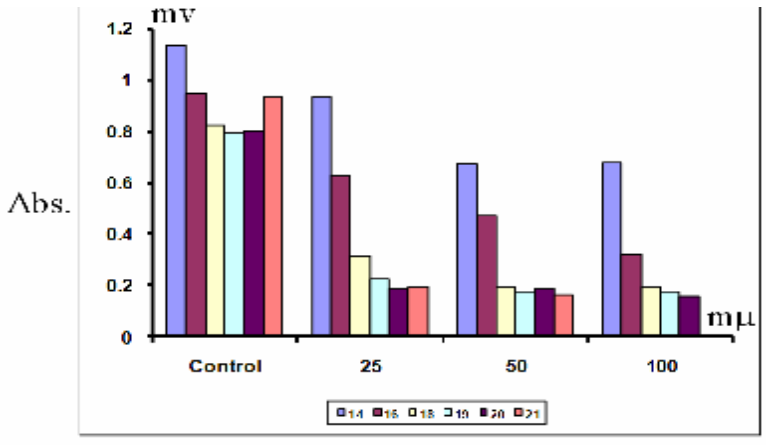
of 25mM. Generally speaking , it can be concluded that the concentration of the cardinolides can be increased by the slight salinity of 25mM, while the concentrations of the cardinolides in the leaves and roots can be decreased by the salinity of 50 and 100mM.

Table 2
K+,Na+ and Tot M (Na+,K+) content in leaves of treated and control plants, expressed in meq/g DW . Data are the average of six analyses. Weeks : weeks of treatment.

Weeks	Control	25mM	50mM	100mM
	Na+			
14	0.182±0.019	0.725±0.0723	0.284±0.0112	1.872±0.181
16	0.252±0.019	0.752±0.0812	1.521±0.124	1.681±0.167
18	0.189±0.018	0.914±0.069	1.742±0.192	1.213±0.211
19	0.301±0.051	0.897±0.037	1.817±0.097	0.961±0.072
20	0.371±0.041	0.948±0.073	1.811±0.131	0.731±0.046
21	0.295±0.031	1.193±0.087	1.376±0.163	-
22	0.378±0.038	1.937±0.121	1.471±0.131	-
	K+			
14	1.135±0.081	0.934±0.091	0.672±0.071	0.681±0.079
16	0.946±0.061	0.631±0.053	0.475±0.091	0.321±0.081
18	0.827±0.052	0.315±0.031	0.195±.0.041	0.192±0.021
19	0.791±0.071	0.224±0.022	0.178±0.073	0.1751±0.009
20	0.798±0.079	0.189±0.037	0.187±0.051	0.159±0.091
21	0.931±0.089	0.195±0.043	0.163±0.061	-
22	0.952±0.091	0.174±0.018	0.152±0.061	-
	Na+ + K+			
14	1.218±0.176	1.427±0.148	1.916±0.197	2.936±0.284
16	0.819±0.123	1.751±0.186	1.937±0.198	1.984±0.183
18	0.968±0.073	1.182±0.261	2.309±.0.217	1.298±0.175
19	0.937±0.081	1.715±0.139	1.918±0.894	0.943±0.128
20	0.941±0.09	1.153±0.097	1.853±0.197	0.654±0.071
21	0.986±0.083	1.727±0.198	1.659±0.187	-
22	1.288±0.171	1.873±0.191	1.741±0.182	-



leaves



Roots

Figure 3Na+, K+ content in leaves of treated and control plants, expressed in meq/g DW. Data are the average of six analyses. Weeks : weeks of treatment

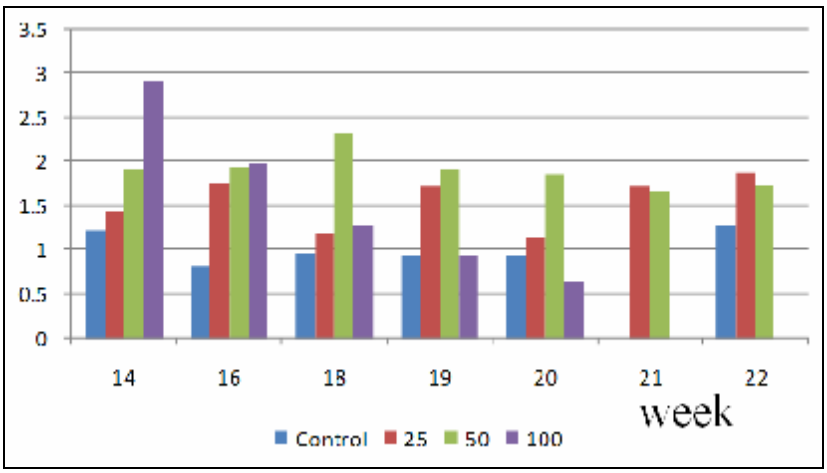


Figure 4. Tot M (Na+ and K+) content in leaves of treated and control plants, expressed in meq/g DW. Data are the average of six analyses. Weeks : weeks of treatment

Table 3
K+/Na+ contents ratio in leaves and roots

Weeks	Leaves			
	Control	**25mM**	**50mM**	**100mM**
14	25.47	8.125	2.89	1.79
16	39.14	5.98	2.78	0.99
18	35.71	2.17	0.95	0.49
19	22.18	1.27	0.91	0.51
20	19.91	0.93	0.73	0.49
21	15.31	0.85	0.89	0
22	20.36	0.78	0.65	0
Roots				

14	8.62	2.76	0.69	0.43
16	6.71	1.74	0.91	0.37
18	4.41	0.52	0.28	0.29
19	4.15	0.42	0.27	0.28
20	4.25	0.29	0.28	0.45
21	5.16	0.29	0.27	0
22	6.21	0.28	0.26	0

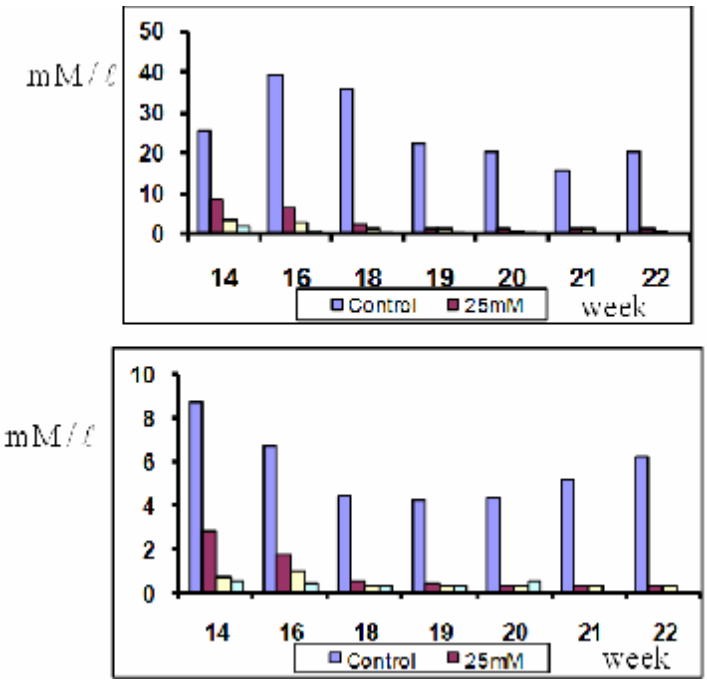
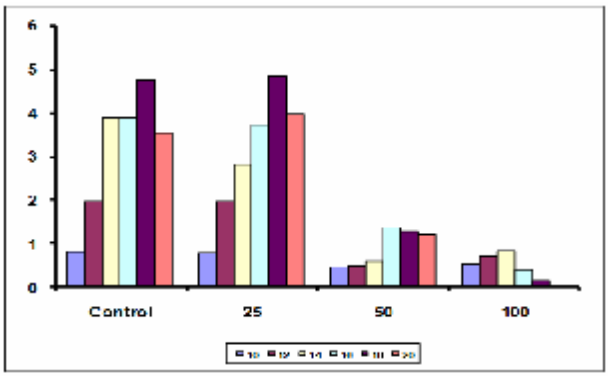


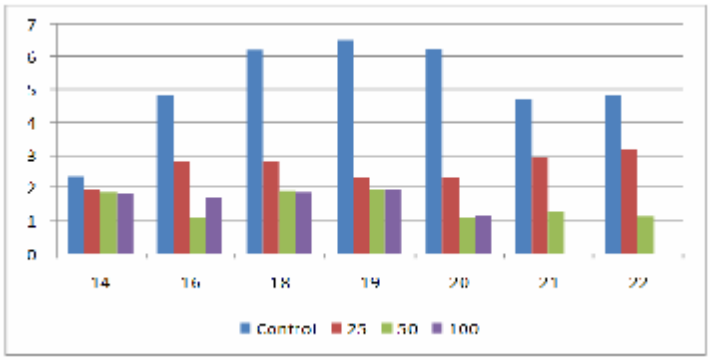
Figure 5
K+/Na+ contents ratio in leaves and roots

Table 4
Ca2+ values in leaves and roots expressed in meq/g DW. Data are the average of six analysis. Weeks: weeks of treatment .

Weeks	Leaves			
	Control	**25mM**	**50mM**	**100mM**
14	1.968±0.151	1.954±0.182	1.871±0.173	1.574±0.213
16	1.931±0.234	1.981±0.194	1.837±0.175	1.973±0.177
18	1.991±0.196	1.951±0.175	1.758±0.211	1.871±0.189
19	2.635±0.212	1.951±0.181	1.692±0.157	1.951±0.179
20	2.841±0.234	1.882±0.191	1.825±0.178	1.912±0.214
21	2.916±0.251	1.753±0.162	1.879±0.138	0
22	2.973±0.282	1.869±0.177	1.564±0.0	0
Roots				
14	2.371±0.28	1.971±0.0981	1.879±0.177	1.841±0.175
16	4.825±0.382	2.831±0.195	1.121±0.191	1.725±0.187
18	6.223±0.691	2.842±0.193	1.937±0.188	1.872±0.183
19	6.514±0.415	2.314±0.133	1.961±0.137	1.945±0.189
20	6.271±0.392	2.353±0.246	1.111±0.128	1.176±0.136
21	4.711±0.318	2.931±0.283	1.251±0.178	-
22	4.831±0.291	3.212±0.235	1.134±0.184	-



Leaves

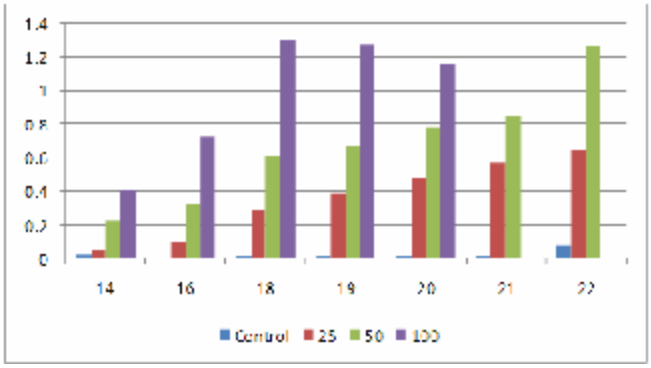


Roots

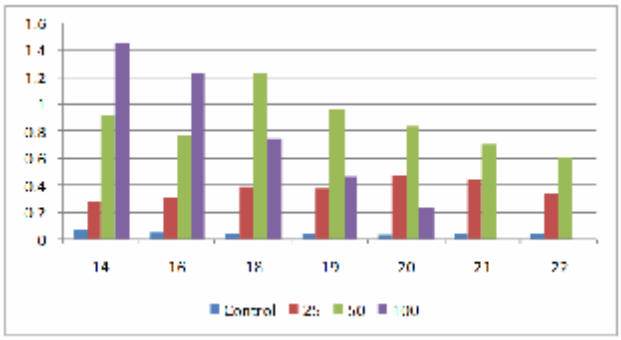
Figure 6. Ca2+ values in leaves and roots expressed in meq/g DW.

Table 5
Na+/ Ca2+ contents ratio in leaves and roots

Weeks	Leaves			
	Control	**25mM**	**50 mM**	**100mM**
14	0.027	0.061	0.233	0.412
16	0.009	0.097	0.327	0.731
18	0.017	0.291	0.618	1.313
19	0.018	0.395	0.673	1.274
20	0.016	0.481	0.783	1.164
21	0.017	0.572	0.855	0
22	0.079	0.644	1.267	0
Roots				
14	0.071	0.291	0.914	1.461
16	0.052	0.315	0.781	1.236
18	0.048	0.392	1.234	0.752
19	0.046	0.381	0.976	0.467
20	0.041	0.474	0.841	0.242
21	0.045	0.453	0.711	0
22	0.045	0.349	0.613	0



Leaves

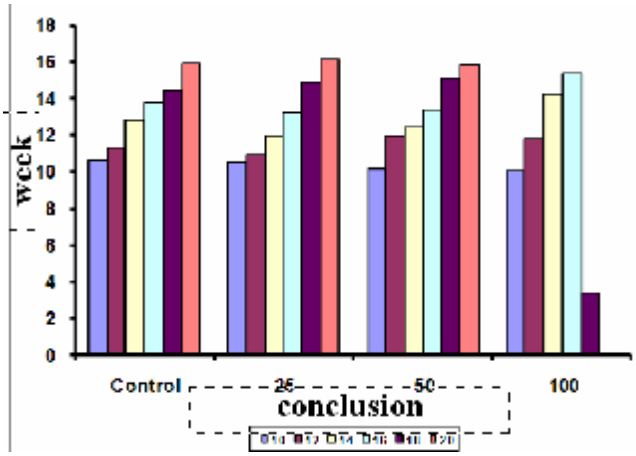


Roots

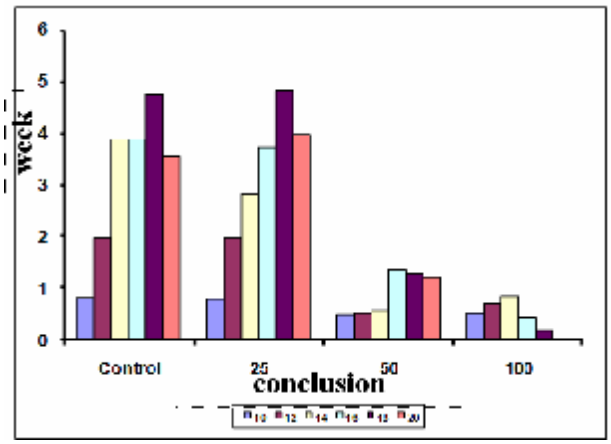
Figure 5
K+/Na+ contents ratio in leaves and roots

Table 6
cardenoloides contents in leaves and roots expressed in mg/DW

Weeks	Control	20mM	40mM	80mM
leaves				
14	10.61+0.04	10.51+0.06	10.10+0.11	10.01+0.03
16	11.24+0.03	1092+0.05	11.95+0.01	11.73+0.01
18	12.81+0.12	11.95+0.07	12.42+0.06	14.23+0.07
19	13.72+0.05	13.15+0.01	13.32+0.07	15.39+0.04
20	14.43+0.01	14.87+0.03	15.11+0.03	3.27+0.02
21	15.92+0.03	16.12+0.08	15.81+0.02	0
22	16.01+0.02	17.21+0.03	15.21+0.05	-
Roots				
14	0.81±0.005	0.78±0.011	0.48±0.002	0.53±0.003
16	1.98±0.020	1.98±0.003	0.51±0.009	0.71±0.001
18	3.89±0.020	2.81±0.005	0.59±0.011	0.84±0.005
19	3.87±0.008	3.72±0.20	1.36±0.005	0.42±0.002
20	4.75±0.002	4.83±0.004	1.27±0.002	0.17±0.009
21	3.54±0.010	3.96±0.003	1.21±0.010	0
22	2.36±0.005	3.11±0.002	0.92±0.003	0



Leaves



Roots

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