

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

عصام الشماع\*

### المخلص

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 الجلوكوزيدات 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. إذ لوحظ أن الأوراق المعرضة للملوحة الشديدة على تراكيز أعلى من شوارد البوتاسيوم  $K^+$  وتراكيز أقل من شوارد الكالسيوم  $Ca^{2+}$  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 محتوي 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 التخرر وفقدان الأجزاء الفتية للجذور.

كلما ازداد تركيز الملوحة انخفضت تراكيز شوارد الكالسيوم  $Ca^{2+}$  في حين بقي معدل  $Na^+/K^+$  في الجذور محافظاً على مستوى منخفض، وذلك بسبب التخرر 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.

\* أستاذ مساعد - قسم العقاقير والنباتات الطبية - كلية الصيدلة - جامعة دمشق.

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

Isam Alshammaa \*

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### Abstract

**Background:** Salinity is involved in decline of plant production in arid and semi-arid lands. It is believed that  $\text{Ca}^{2+}$  maintains the structural integrity of the plasmalemma by cross linking the negatively charged head groups of the plasmalemma. 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  $\text{K}^+$  and  $\text{Na}^+$  contents and a lower Ca content than leaves of control plants ,  $\text{K}^+/\text{Na}^+$  ratio of leaves of stressed plants become a lower than 1 when the senescence was started .

$\text{Na}^+/\text{Ca}^{2+}$  ratio became a higher the higher the salinity conditions were and the longer the time of stress. The roots of stressed plants had lower  $\text{K}^+$  content in higher salinity and as longer the time of stress. The  $\text{Na}^+$  content of roots of stressed plants was higher than the  $\text{Na}^+$  content in roots of control plants, but decreased at the end of experiment due to necrosis and loss of young parts of roots.  $\text{Ca}^{2+}$  content in roots was lower as the salinity stress was higher. The  $\text{Na}^+/\text{Ca}^{2+}$  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 cardiotonic glycosides accumulation in the leaves of *Adonis autumnalis* , and in doing so, it leads to increasing the concentrations of the cardinolides in the leaves and roots of *Adonis autumnalis* which have been treated at the salinity of 25mM.

**Keywords:** *Adonis autumnalis*, salinity, cardinoids, Adonitoxinionic disequilibrium

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\* Assistant prof. Dept. of Pharmacognosy – Faculty of Pharmacy –Damascus University.



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  $\text{Ca}^{2+}$  maintains the structural integrity of the plasma membrane by cross linking the negatively charged head groups of the plasma membrane [5]. At high concentration of  $\text{Na}^{+}$  this  $\text{Ca}^{2+}$  is displaced by  $\text{Na}^{+}$  causing an influx of  $\text{Na}^{+}$ . In high external salinity,  $\text{Na}^{+}$  may enter the cytoplasm passively across the plasma membrane because the cytoplasm is electrically negative with respect to the apoplast and the concentration of  $\text{Na}^{+}$  is probably higher outside the cytoplasm. In plants, the  $\text{Na}^{+}$  selective channel has not been identified in the plasma membrane, therefore entry of  $\text{Na}^{+}$  into cytoplasm may be through a  $\text{K}^{+}$  channel with some permeability to  $\text{Na}^{+}$  [6]. In glycophytes, the membrane depolarizes in response to external  $\text{NaCl}$ . Depolarization will open the cation outward rectifier channels and  $\text{Na}^{+}$  could move into the cell at a rate governed by the electrochemical gradient for  $\text{Na}^{+}$  and the effective permeability of the channels to  $\text{Na}^{+}$  [7]. Summarising, salt stress reduced plant growth through water deficits, ion toxicity, ionic imbalance or a combination of any of these factors, and plays an important role on turgor, permeability, enzymatic activity and hormonal balance [8]. All these effects cause a primary metabolism fall, thus intermediary products could be 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 Arnou and stood solution and used as a control, the second was rinsed with the same solution, but 25 mM  $\text{NaCl}$  was added (25mM  $\text{NaCl}$  plants), the third with

50 mM  $\text{NaCl}$  added to it (50mM  $\text{NaCl}$  plants) and the fourth with 100 mM  $\text{NaCl}$  (100mM  $\text{NaCl}$  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),  $\text{Na}^{+}$ ,  $\text{K}^{+}$ ,  $\text{Ca}^{2+}$ , and total cardenolide contents.

Figure (2) Adonis autumnalis  
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#### Dry weight

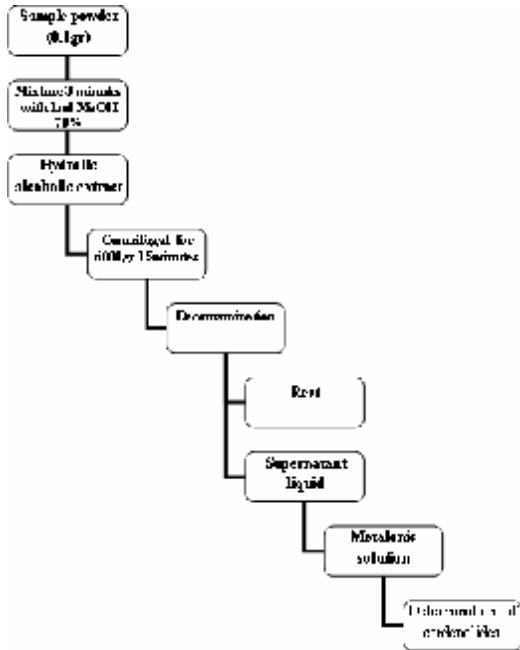
Dry weight determinations were made by heating leaves and roots separately at  $60^{\circ}\text{C}$  for 48 hours and then to constant weight.

#### Ion determination

$\text{Na}^{+}$ ,  $\text{K}^{+}$  and  $\text{Ca}^{2+}$  determination were made by emission spectroscopy for plasma icp in a Mixer 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 cardenolides

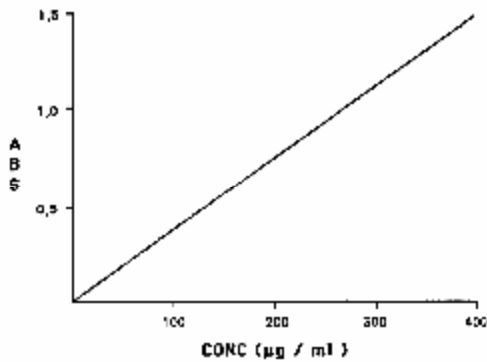


Figure (3)  
Curve patron of cardenolides  
ABS: Absorption, CONC: constriction



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

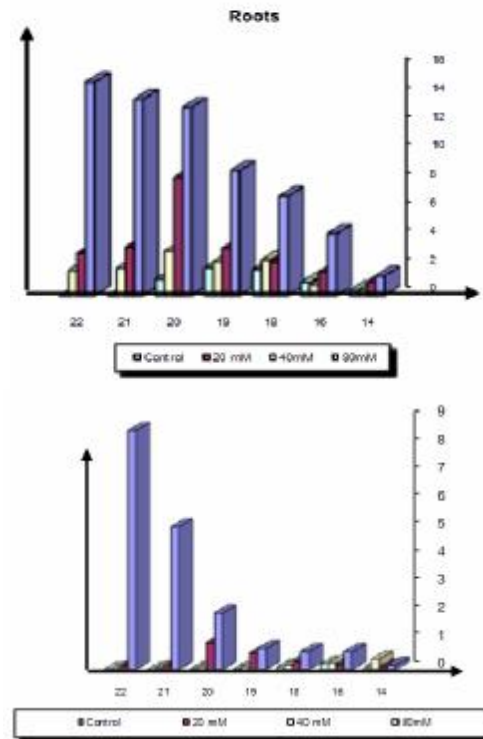
The content of  $K^+$  in roots and leaves of control plants remained relatively constant and in the interval considered as normal during the first 17 weeks of growth and sink down at the end of the experiment (tab. 1and2) , probably due to inert substances accumulation in plants . Roots and leaves of 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 non-halophytic plants [13]. Tot M contents in roots of

stressed plants were similar to content of  $\text{Na}^+$ . 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  $\text{K}^+$  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  $\text{K}^+$  and  $\text{Na}^+$  in leaves of salt sensitive plants. Contents of  $\text{Na}^+$  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  $\text{Na}^+$  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  $\text{K}^+/\text{Na}^+$  ratio is lower than 1 (Table 3). Our results showed that  $\text{K}^+/\text{Na}^+$  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 weeks respectively. We infer that 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**

$\text{K}^+$ ,  $\text{Na}^+$  and Tot M ( $\text{Na}^+$ ,  $\text{K}^+$ ) 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
<b>Na<sup>+</sup></b>				
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<sup>+</sup>**

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<sup>+</sup> + K<sup>+</sup>**

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	-

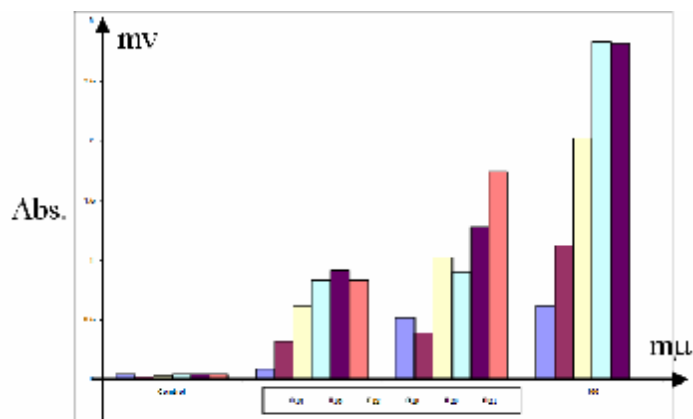


Figure (8)

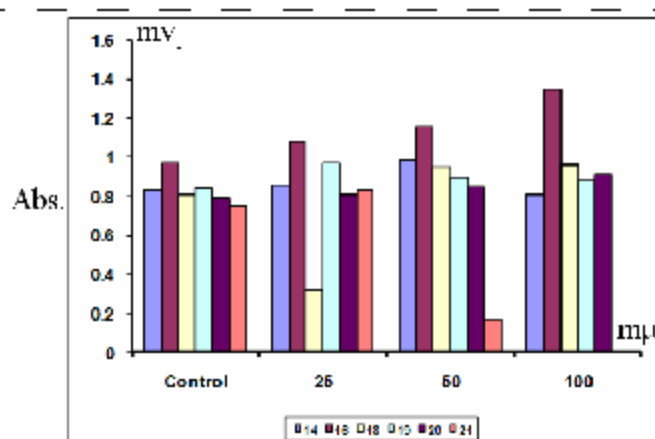


Figure 2

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

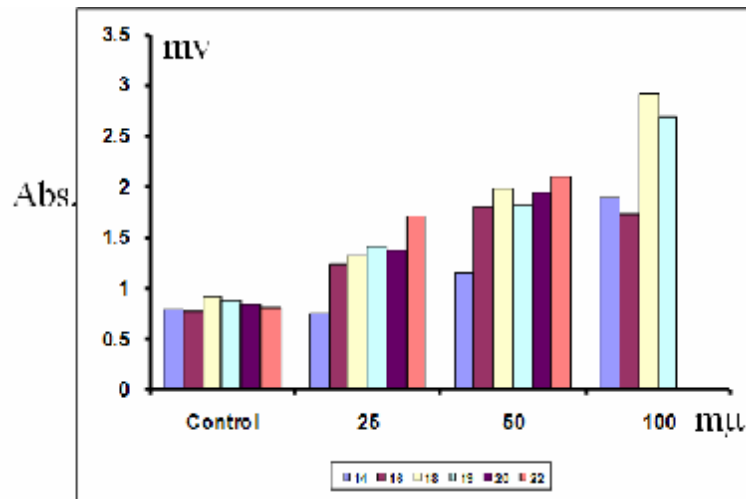


Figure 3

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

The Ca<sup>2+</sup> 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 Ca<sup>2+</sup> 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<sup>+</sup>/Ca<sup>2+</sup> 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<sup>+</sup>/Ca<sup>2+</sup> ratios damage semi permeability and permit Na<sup>+</sup> and K<sup>+</sup> uptake but lower

Na<sup>+</sup>/Ca<sup>2+</sup> ratio maintain semi permeability. We must not consider the decrease of Na<sup>+</sup>/Ca<sup>2+</sup> ratio in the stressed roots as a protective mechanism, due to necrosis and loss of young parts of roots, because Ca<sup>2+</sup> linked to the older part of plants. The levels of Ca<sup>2+</sup> 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 Ca<sup>2+</sup> for 18 weeks and then decreased due loss of old leaves rich in Ca<sup>2+</sup>. The content of Ca<sup>2+</sup> in leaves of 80 mM plants increased during the experiment because their leaves were always in senescence. The Na<sup>+</sup>/Ca<sup>2+</sup> 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<sup>+</sup>, 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<sup>+</sup>/Ca<sup>2+</sup> 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.

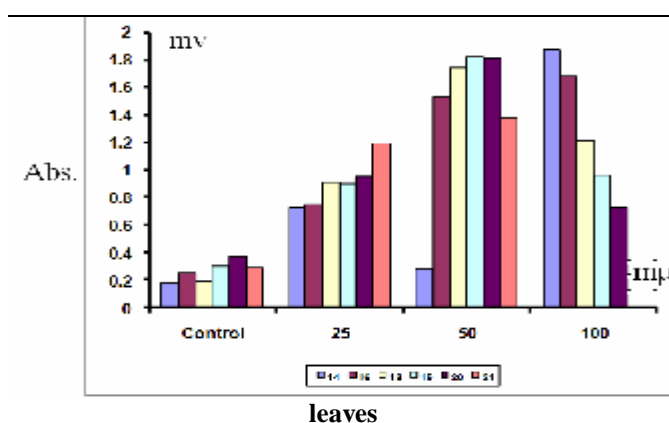
The slight ionic imbalance, which reaches weak levels of salinity, leads to the activation of the cardiotoxic glycosides accumulation in the leaves of *Adonis*, and in doing so, it leads to increasing the concentrations of the cardenolides in the leaves and roots of *Adonis* which have been treated at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



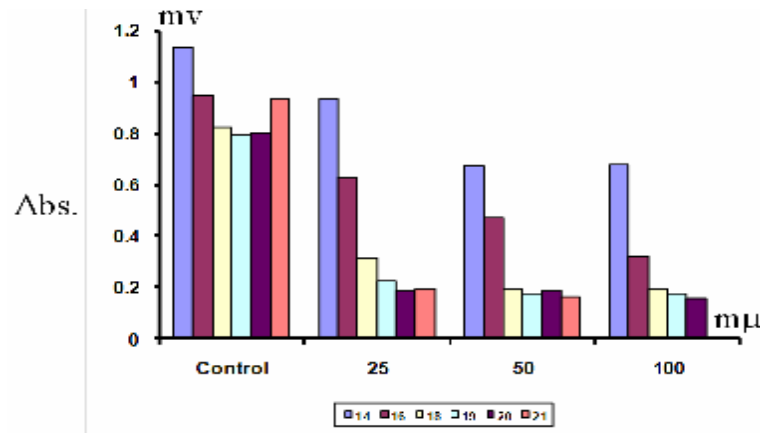
of 25mM. Generally speaking , it can be concluded concentrations of the cardinolides in the leaves and that the concentration of the cardinolides can be roots can be decreased by the salinity of 50 and increased by the slight salinity of 25mM, while the 100mM.

**Table 2**  
**K<sup>+</sup>,Na<sup>+</sup> and Tot M (Na<sup>+</sup>,K<sup>+</sup>) 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 <sup>+</sup>			
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 <sup>+</sup>				
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 <sup>+</sup> + K <sup>+</sup>				
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	-







### Roots

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

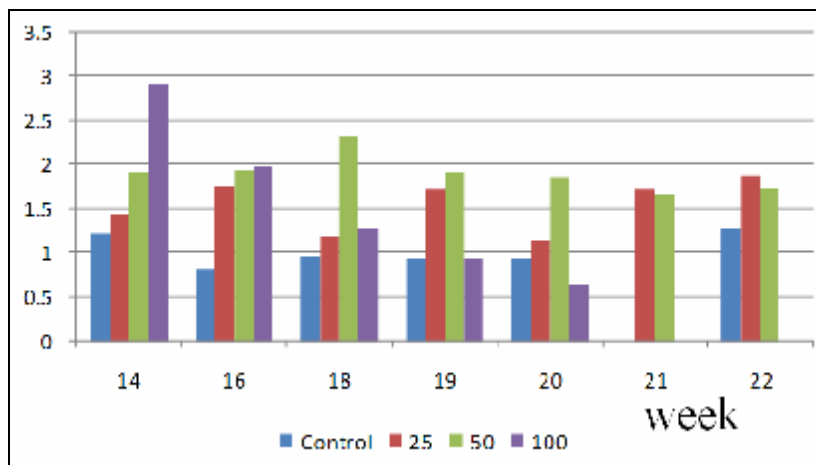


Figure 4. Tot M (Na<sup>+</sup> and K<sup>+</sup>) content in leaves of treated and control plants, expressed in meq/g DW. Data are the average of six analyses. Weeks : weeks of treatment

Table 3  
K<sup>+</sup>/Na<sup>+</sup> 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
<b>Roots</b>				

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

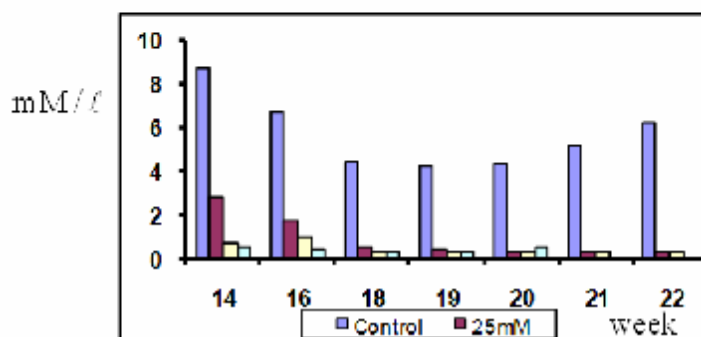
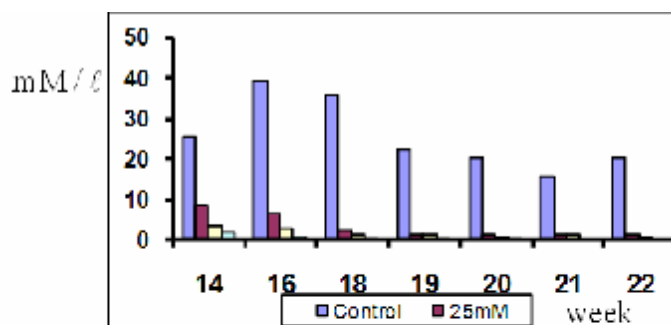


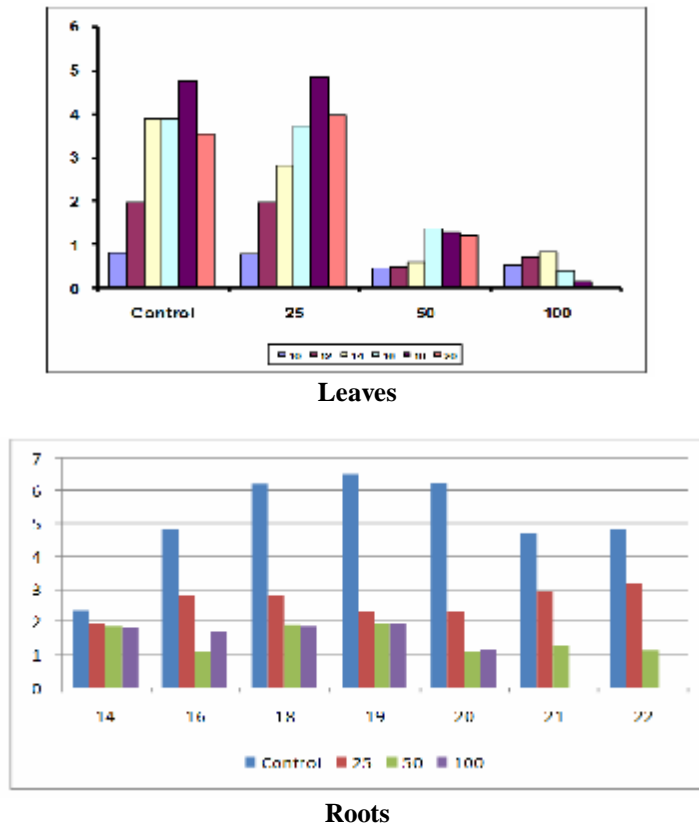
Figure 5

K<sup>+</sup>/Na<sup>+</sup> contents ratio in leaves and roots

Table 4

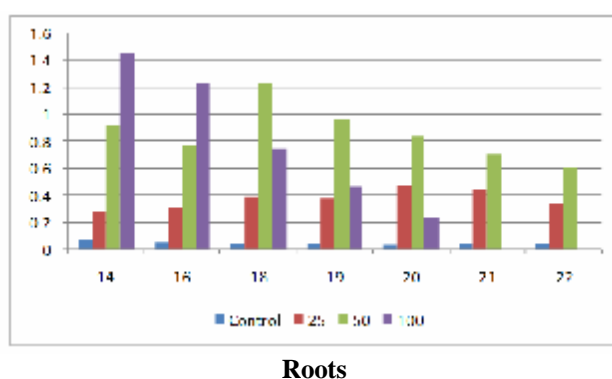
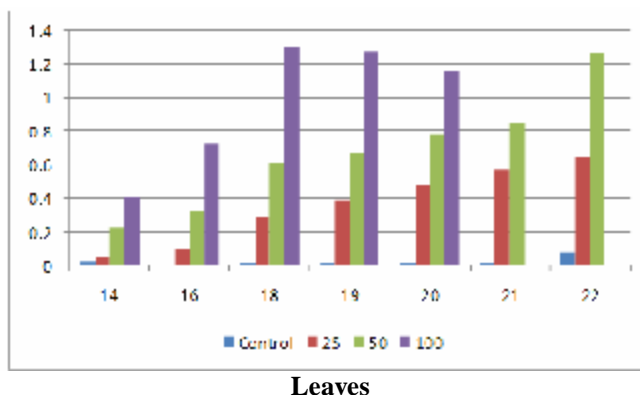
Ca<sup>2+</sup> values in leaves and roots expressed in meq/g DW. Data are the average of six analysis. Weeks: weeks of treatment .

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	
Weeks	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	-

Figure 6. Ca<sup>2+</sup> values in leaves and roots expressed in meq/g DW.

**Table 5**  
Na<sup>+</sup>/ Ca<sup>2+</sup> contents ratio in leaves and roots

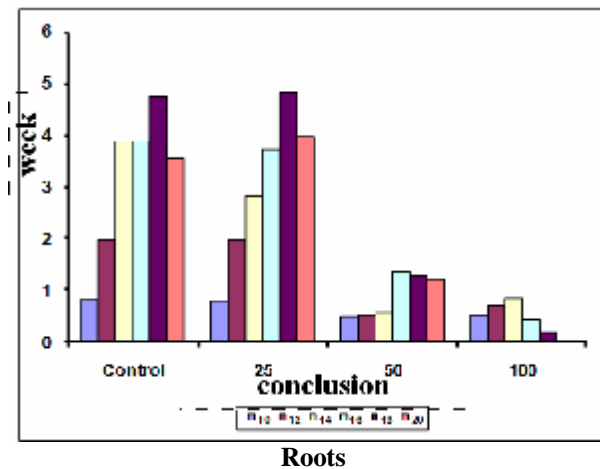
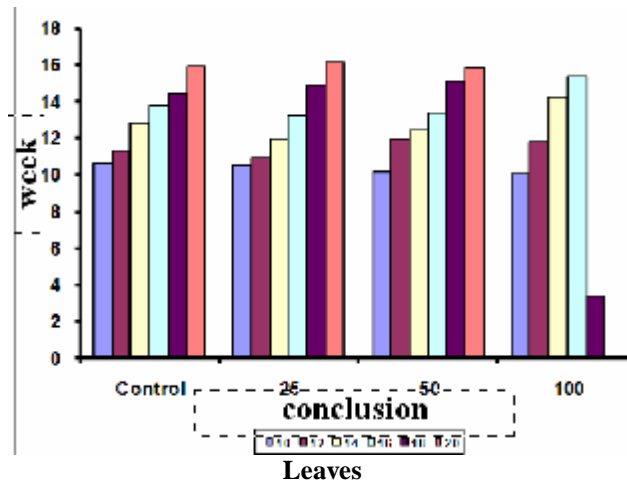
Weeks	Leaves			
	Control	25mM	50 mM	100mM
14	0.027	0.061	0.233	0.412
16	0.009	0.097	0.327	0.731
18	0.017	0.291	0.618	1.313
19	0.018	0.395	0.673	1.274
20	0.016	0.481	0.783	1.164
21	0.017	0.572	0.855	0
22	0.079	0.644	1.267	0
Roots				
14	0.071	0.291	0.914	1.461
16	0.052	0.315	0.781	1.236
18	0.048	0.392	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



**Figure 5**  
K<sup>+</sup>/Na<sup>+</sup> contents ratio in leaves and roots

**Table 6**  
cardenolides contents in leaves and roots expressed in mg/DW

Weeks	Control	20mM	40mM	80mM
<b>leaves</b>				
14	10.61±0.04	10.51±0.06	10.10±0.11	10.01±0.03
16	11.24±0.03	10.92±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	-
<b>Roots</b>				
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



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