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Ameliorative Effect of Ammonium Sulfate on Salt Tolerance and Ion Homeostasis in Lemon (C. limon) Seedlings

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Abstract
To investigate interaction effects of salinity and nitrogen on growth, mineral composition and salinity tolerance of lemon seedlings, a greenhouse experiment with four sodium chloride concentrations (0, 250, 500 and 1000 mg kg^{-1} soil) and four nitrogen levels (0, 50, 100, and 200 mg kg^{-1} soil ammonium sulfate) was carried out. Experiment was conducted in a completely randomized design with three replications. Sodium and chloride ions in plant tissues were increased to toxic levels with increase in salinity which resulted in a significant reduction of plant dry weight. Nitrogen consumption up to 100 mg kg^{-1} soil increased plant dry weight. As the concentration of sodium chloride was increased, the improvement effect of nitrogen on plant growth was decreased. Although nitrogen had no considerable effect on sodium concentration in root and shoot, it made a decrease in chloride concentration in shoot and an increase in root concentration of this element. Salinity decreased essential nutrients concentration in plant shoot. Therefore, it can be concluded that adverse effect of salinity is to some extent due to reduction of required elements to suboptimal ranges in plant tissues. Nitrogen improved the adverse effects of salinity on plant nutrients by increasing their concentration in plant tissues. Therefore it can be inferred that part of the ameliorative effects of nitrogen on salinity adverse effects is related to the maintenance of essential nutrient concentrations in plant tissues. The results of the present study indicate that nitrogen can be applied in amounts higher than optimal level to reduce the harmful effects of salinity.

Keywords: Dry Weight, Ion Accumulation, Salinity Stress.

Abbreviations: Na, Sodium; **Cl,** Chloride; **N,** Nitrogen; **NaCl,** Sodium chloride; **K,** Potassium; **P,** Phosphorus; **Fe,** Iron; **Cu,** Copper; **Zn,** Zinc; **Mn,** Manganese.

Introduction

Progressive salinization of agricultural lands throughout the world results in significant reduction of crops production (Munns, 2002). Therefore finding efficient ways to overcome this problem, especially in arid and semi arid regions of the world is receiving lots of attention. It is estimated that approximately a third of the world's irrigated lands and half of the lands in semiarid and costal regions are affected by salinity (Al-Yassin, 2004). Soil and water salinity are critical problems because of their adverse effects on the growth and yield of many

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crops. Generally four reasons are suggested for reduction of plant growth under salt stressed conditions: (i) Osmotic stress caused by the reduction of soil water availability, (ii) Specific ion toxicity effects disturbing the metabolic processes in plant cell, (iii) Nutritional imbalances caused by the complex condition of growth medium and (iv) A combination of the abovementioned factors (Niu et al., 1995; Hajlaoui et al., 2010). Citrus is considered as a salt-sensitive crop (Storey and Walker, 1999). Similar to the most other plants, salinity reduces citrus trees' growth and causes physiological disorders (Al-Yassin, 2005). These effects are associated with the accumulation of excessive concentration of sodium (Na) and/or chloride (Cl) in the leaves (Camara-Zapata et al, 2004; Al-Yassin, 2005; Garcia-Sanchez and Syvertsen, 2009). Citrus response to salinity depends on several factors such as rootstock and scion combinations, irrigation system, soil type and climate, fertilization and some more (Cedra et al., 1990; Levy and Syvertsen, 2004; Sadeghi Lotfabadi et al., 2010). Changing one or more of these factors, with the same salinity level, can produce entirely different results. Salinity may cause nutrient deficiencies or imbalances, due to the competition of Na^+ and Cl^- with nutrients such as K^+, Ca^{2+}, Mg^{2+} and NO_3^- (Romero-Aranda et al., 1998). It has been reported that an adequate fertilization for instance application of additional nitrogen (N) under salinity stress condition can enhance plant growth due to reduction of Cl^- and Na^+ toxicities (Camara-Zapata et al., 2004) and maintenance of nutrient balances (Hu and Schmidhalter, 2005). On the other point of view, a number of laboratory and greenhouse studies have shown that salinity can reduce N accumulation in plants (Pessarakli, 1991; Al-Rawahy et al., 1992). Therefore, under salt stress conditions, the N requirement of plant is higher than non-saline conditions. Recently, horticultural researchers have tried to test the hypothesis that N consumption can reduce the harmful effects of salinity in field studies. Irshad et al. (2002) reported that, under salinity stress, application of N more than optimum level to the growth medium reduces the concentration of Cl in avocados and citrus trees. In a study conducted by Tabatabaei (2006), the relation of N and salinity on olive trees was studied. The results showed that increase of N up to 200 mg/l in hydroponics conditions led to a decrease in salinity adverse effects on plant growth. Gimeno et al. (2009) reported that additional soil or foliar KNO_3 decreases leaf Cl^{-1} concentration and increases leaf K^+ concentration in salt stressed lemon trees grafted on Citrus macrophylla or Sour orange rootstocks. Sousa et al. (2016) concluded that the greater N supply was effective in decreasing the negative effects of high salinity to transpiration, stomatal conductance and CO_2 assimilation rate in three scion-rootstock combinations of citrus.

Since citrus is a salt-sensitive plant and its growth and development is significantly affected by high salt concentrations, this study was conducted to investigate the harmful effects of salinity on the growth and nutrients absorption capacity of lemon seedlings and also to investigate the ameliorative effects of ammonium sulfate on the salt tolerance and ion homeostasis in lemon plants.

Materials and Methods

This study was carried out at the Islamic Azad University of Jahrom branch in Iran (28º 30′ N, 53º 31′ E and 1,050 m elevation from sea level). A bulk sample was collected from surface horizon (0-20 cm) of a fine, mixed (calcareous), mesic Typic Calcixerpts soil that was homogenized by plowing. Prior to analysis and potting, the soil was air dried, crushed and passed through a 2-mm sieve. Some physical and chemical characteristics of the soil sample are shown in Table 1. The experiment was performed in a greenhouse under natural photoperiods during the spring and summer. One-year-old lemon seedlings with equal shape and size were planted in 4 kg pots filled with the analyzed soil. The experiment was factorial based on a completely randomized design with three replications. Factors consisted of four sodium chloride (NaCl) levels (0, 250, 500 and 1000 mg kg^{-1} soil which after balancing with soil, caused salinity amounts of 1.13, 2.33, 3.65 and 5.95 dS m^{-1} respectively) and four concentrations of N (0, 50, 100 and 200 mg kg^{-1} soil as ammonium sulfate).

Before planting the uniform amounts of 2, 5, 5, 5 and 10 mg kg^{-1} soil of copper (Cu), zinc (Zn) and manganese (Mn) in the form of their sulfate salt, iron (Fe) as EDDHA chelate and phosphorus (P) in the form of mono-potassium phosphate were added to all pots to prevent the limiting effect of the above mentioned elements on plant growth. One month after seedlings establishment, salinity and N treatments were applied. In order to avoid shocks caused by salinity, NaCl total amount for each treatment was applied in three equal splits every 15 days. The levels of N were divided into two equal parts and consumed at the beginning and two months after seedling establishment. From the beginning of the treatments, the experiment lasted for five months. During the course of the experiment, pots were irrigated with distilled water to keep soil moisture content near the field capacity moisture. At the end of the experiment, plants were harvested from the soil, dried at 65^{o}C for 24 hours and weighed. N was measured in ground plant samples by Kjeldahl method. Also, one gram of dried plant samples were placed in a furnace for half an hour at 250° C and then for 5 hours at 550° C. Samples were dissolved in hydrochloric acid 2N and after passing the filter paper, the extract was filtered in 50 ml volume. In the extract, the concentration of Na, Cl, P, potassium (K), Zn, Cu, Mn and Fe were measured by standard methods. In addition to the shoot, the roots were removed from the soil and Na and Cl concentrations were measured in a similar way as for shoot samples. Finally, the data were subjected to statistical analysis of variance (ANOVA) and the Duncan's multiple range tests were also performed to identify the homogenous sets of data. Correlation analysis between determined traits was conducted using Pearson’s method.

Results

Shoot dry weight

The effects of NaCl and N levels on shoot dry weight were significant at 1%probability level (Table 2).

Table 1. Some physical and chemical properties of the soil

Soil property	Quantity
Sand (%)	24
Silt (%)	42
Clay (%)	34
OM (%)	0.80
CCE (%)	43.7
EC (dSm^{-1})	1.13
CEC ($cmol_c$ kg^{-1})	10.8
pH (saturated paste)	7.8
NH4-OAc extractable- K (mg kg^{-1})	189
$NaHCO_3$ extractable- P (mg kg^{-1})	6.2
DTPA extractable- Fe (mg kg^{-1})	7.6
DTPA extractable- Mn (mg kg^{-1})	4.7
DTPA extractable- Zn (mg kg^{-1})	0.56
DTPA extractable- Cu (mg kg^{-1})	0.78

(OM: organic matter, CCE: calcium carbonate equivalent, EC: electrical conductivity, CEC: cation exchange capacity, NH4-OAc: ammonium acetate, DTPA: di ethylene tri amine penta acetic acid)

Table 2. Analysis of variance (mean square) and F test for plant studied traits

Source of variance	DF	Dry wieght	Shoot Na	Shoot Cl	Root Na	Root Cl	Shoot N
N	3	0.453^{**}	0.001^{ns}	0.105^{**}	0.002^{ns}	0.168^{**}	2.65^{**}
NaCl	3	5.984^{**}	0.190^{**}	2.44^{**}	0.919^{**}	7.911^{**}	3.88^{**}
NaCl ×N	9	0.071^{*}	0.001^{ns}	0.046^{ns}	0.002^{ns}	0.062^{**}	0.173^{**}
Cv	-	19.35	12.75	33.36	16.38	17.52	12.73

(*, ** and ns are significant at 5 and 1 percent probability level and non-significant respectively)

As it was expected increase in salinity levels from 250 to 1000 mg kg^{-1} soil caused a significant decrease in shoot dry matter weight (Table 3). At all levels of salinity, increase in N levels up to 100 mg kg^{-1} soil increased the average shoot dry weight of lemon while higher consumption of N (200 mg kg^{-1} soil) caused a significant reduction in shoot dry weight of lemon. Interactive effects of salinity and N on shoot dry weight were significant (Table 2). It indicated that the ameliorative effect of N on plant growth was dependent on the amount of applied NaCl. As the level of applied NaCl was increased the ameliorative effect of N on plant growth was decreased. For example at 250 mg NaCl kg^{-1} soil increasing N from zero to 100 mg kg^{-1} soil increased shoot dry weight by 20% while at 1000 mg NaCl kg^{-1} soil this increase was reduced to 16%.

Na and Cl concentrations in plant root and shoot

While salinity stress significantly affected the Na content of lemon aerial parts, N levels did not cause any significant influence on this plant response (Table 2). Increase in NaCl application made a significant increase in the Na concentration of lemon shoot (Table 4). However, the effect of N on the shoot Na concentration was negligible (Table 4). Although increasing N levels made a slight reduction in Na concentration in the lemon shoot, the effect of N on total Na uptake was not significant (The total uptake data of the elements is not shown but they can be calculated from the multiplication of dry matter weight with the elements concentration). Therefore this slight reduction in Na concentration can be attributed to dilution effect resulted from the enhancement of plant growth by N application. In fact enhancement of plant growth, without any change in Na absorption rate, leads to the dilution of Na in the plant's tissue and the reduction of its concentration.

The Cl concentration of the lemon shoot is presented in Table 4. Increase in salinity significantly increased the Cl concentration in lemon aerial parts. So that in salinity level up to 1000 mg NaCl kg^{-1} soil, the Cl concentration of lemon aerial parts was 17 times more than control. The data presented in Table 4 indicates that at each level of salinity, increasing N levels from zero to 100 mg kg^{-1} soil caused a significant reduction in

Table 3. Effect of salinity and N application on plant dry matter

N levels (mg kg^{-1} soil)	NaCl levels (mg kg^{-1} soil)				
	0	250	500	1000	Main effect
	Shoot dry weight (g pot-1)				
0	2.60 c	2.47 cd	2.07 e	1.23 f	2.09 B
50	2.53 cd	2.57 cd	2.10 e	1.30 f	2.13 B
100	3.37 a	2.97 b	2.30 de	1.43 f	2.52 A
200	2.97 b	2.63 c	2.03 e	1.17 f	2.20 A
Main effect	2.87 A	2.66 B	2.12 C	1.28 C	

For main effects the means with the same capital letters and for interactions, the means with similar small letters have no significant difference on the basis of Duncan's multiple range tests at 95% probability level

Table 4. Effect of salinity and N applications on Na and Cl concentrations in lemon shoot and root

N levels (mg kg^{-1} soil)	NaCl levels (mg kg^{-1} soil)				
	0	250	500	1000	Main effect
			Shoot Na (%)		
0	0.033 gh	0.073 e	0.140 c	0.327 a	0.143 A
50	0.030 gh	0.077 e	0.110 d	0.300 b	0.129 B
100	0.023 h	0.053 f	0.137 c	0.307 b	0.130 B
200	0.020 h	0.047 fg	0.133 c	0.307 b	0.127 B
Main effect	0.027 D	0.062 C	0.130 B	0.310 A	
			Shoot Cl (%)		
0	0.067 f	0.303 def	0.530 cd	1.37 a	0.567 A
50	0.067 f	0.278 edf	0.373 de	1.10 b	0.457 AB
100	0.053 f	0.230 ef	0.343 def	0.730 c	0.339 B
200	0.053 f	0.233 ef	0.413 de	1.20 ab	0.475 A
Main effect	0.060 C	0.263 C	0.415 B	1.10 A	
			Root Na (%)		
0	0.045 e	0.110 d	0.271 c	0.701 a	0.282 A
50	0.043 e	0.116 d	0.301 c	0.684 b	0.286 A
100	0.044 e	0.114 d	0.282 c	0.592 b	0.258 A
200	0.038 e	0.110 d	0.290 c	0.668 a	0.276 A
Main effect	0.042 D	0.113 C	0.286 B	0.661 A	
			Root Cl (%)		
0	0.090 i	0.518 h	0.866 de	1.77 c	0.811 C
50	0.082 i	0.528 h	0.814 ef	1.84 bc	0.816 C
100	0.086 i	0.611 gh	0.943 de	1.93 b	0.892 B
200	0.094 i	0.695 fg	0.997 d	2.48 a	1.07 A
Main effect	0.088 D	0.588 C	0.905 B	2.00 A	

For main effects the means with the same capital letters and for interactions, the means with similar small letters have no significant difference on the basis of Duncan's multiple range tests at 95% probability level

lemon shoot concentration and total uptake of Cl. However, the reduction of Cl concentration was more evident at 1000 mg NaCl kg^{-1} soil than other salinity treatments. In other words, the effect of N on the reduction of Cl concentration was improved by increase in salinity levels. So that at the level of 250 mg NaCl kg^{-1} soil the increase in N levels from zero to 100 mg kg^{-1} resulted in 24% reduction in Cl concentration but this reduction for the level of 1000 mg NaCl kg^{-1} soil was 47%. At 200 mg N kg^{-1} soil the concentration of Cl in lemon shoot was significantly higher than other N levels. Since this level of N did not show any significant difference than other N levels in respect to total Cl uptake, this increase can be related to the reduction of plant growth and the consequent increase of Cl concentration in plant tissues. The effects of N and NaCl levels on the Na and Cl concentrations of lemon root are presented in Tables 2 and 4. The results showed that increasing salinity levels caused significant increase in Na and Cl concentrations in lemon root. Assessment of Na and Cl concentrations obtained from the plant shoot and root indicated that higher concentrations of Na and Cl were accumulated in root when compared to their concentrations in the shoot (Table 4). The effect of N levels on Na concentration of lemon root was not significant. In contrast the data presented in Table 4 showed that increase in N consumption lead to a significant accumulation of Cl in the lemon root which of course was more evident in higher levels of salinity.

Shoot N, P and K concentrations

The effect of N, NaCl and their interactions on N concentration in lemon shoot was significant at 1% probability level (Table 2). An increase in N consumption in each level of NaCl increased the concentration and total uptake of N in lemon shoot (Table 5). In contrast, increase in NaCl levels in each level of N caused a significant decrease in N concentration and its total uptake. It is worth noting that as the level of applied NaCl is increased the effect of N consumption on the provision of plant N requirement is decreased. For example at the rate of 250 mg NaCl kg^{-1} soil, increasing the amount of N from zero to 200 mg kg^{-1} soil increased the N concentration by 143% while the similar number for 1000 mg NaCl kg^{-1} soil was only 65%. The optimum N content in citrus leaves is about 2.2 - 2.4% on dry weight basis. In the present experiment, in the absence of salinity stress, the optimum N concentration for lemon was achieved with the application of 50 mg N kg^{-1} soil while at 250 and 500 mg NaCl kg^{-1}soil it was attained by consumption of 200 mg N kg^{-1} soil. However in 1000 mg NaCl kg^{-1} soil none of the N rates were able to bring N concentration to the optimum level (Table 5).

Table 5. Effect of salinity and N applications on N, P and K concentrations in lemon shoot

N levels (mg kg^{-1} soil)	NaCl levels (mg kg^{-1} soil) 0	250	500	1000	Main effect
			Shoot N (%)		
0	1.76 f	1.10 ij	1.10 ij	0.767 l	1.18 D
50	2.18 de	1.57 g	1.33 h	0.831 kl	1.48 C
100	2.45 c	2.03 e	2.32 d	0.967 jk	1.92 B
200	2.94 a	2.67 d	2.10 de	1.27 hi	2.24 A
Main effect	2.33 A	1.84 B	1.69 C	0.958 D	
			Shoot P (%)		
0	0.21 abc	0.17 d	0.13 ef	0.12 fg	0.16 C
50	0.22 ab	0.20 bc	0.10 g	0.14 e	0.17 B
100	0.23 a	0.21 abc	0.19 c	0.16 d	0.20 A
200	0.20 c	0.21 abc	0.12 fg	0.17 d	0.17 B
Main effect	0.21 A	0.20 B	0.14 D	0.15 C	
			Shoot K (%)		
0	1.83 ab	1.03 fg	0.98 g	0.93 g	1.16 B
50	1.87 a	1.17 d-g	1.13 efg	1.10 g	1.17 B
100	1.87 a	1.63 a-e	1.53 a-f	1.23 c-g	1.57 A
200	1.67 a-d	1.73 abc	1.07 fg	1.33 b-g	1.45 A
Main effect	1.67 C	1.39 B	1.18 BC	1.15 C	

For main effects the means with the same capital letters and for interactions, the means with similar small letters have no significant difference on the basis of Duncan's multiple range tests at 95% probability level.

The effect of N and NaCl levels on the concentration of P in lemon shoot is presented in Table 6. In all levels of applied salinity, N consumption up to 100 mg kg^{-1} soil significantly increased the concentration and total uptake of P in lemon shoot (Table 5). At the same time consumption of 200 mg N kg^{-1} soil reduced P concentrations. In each level of applied N increase in soil salinity reduced the concentration and total uptake of P in lemon shoot (Table 5). Although N consumption up to 100 mg kg^{-1} soil significantly increased concentration of P in the lemon shoot, this increase was correlated with the amount of NaCl added to the soil so that no such increase was observed at zero level of salinity. The optimum concentration of P in citrus leaves is about 0.2% of dry matter. Based on the results presented in Table 5, irrespective of the amount of consumed N, in salinity treatments of 500 and 1000 mg NaCl kg^{-1} soil the concentration of P was lower than the optimum level in lemon shoot. The optimum concentrations of P were only observed in salinity control treatment and at 250 mg NaCl kg^{-1} soil. The effects of N and NaCl levels on the concentration of K in lemon shoot are presented in Tables 5 and 6. The results of Table 5 indicate that increasing N consumption up to 100 mg kg^{-1} soil significantly increased the K concentration and its total uptake. In contrast, in each level of applied N, salinity caused decrease in the concentration and total uptake of K in lemon shoot (Table 5).

The optimum K content in citrus is about 1.7% on leaf dry weight basis. Based on the results presented in Table 5 the concentration of K in the lemon shoot is less than its optimum level in most salinity treatments and the optimum K concentrations were only observed in the absence of salinity.

Table 6. Analysis of variance (mean square) and F test for plant studied traits

Source of variance	DF	Shoot nutrients concentration					
		P	K	Fe	Mn	Zn	Cu
N	3	0.004**	0.447**	266**	147**	21.7**	3.30*
NaCl	3	0.017**	0.684**	7064**	84.8**	556**	7.58**
NaCl ×N	9	0.001**	0.127 ns	118*	40.6**	27.0**	2.47*
Cv	-	14.51	21.74	6.98	5.54	7.84	11.05

(*, ** and ns are significant at 5 and 1 percent probability level and non-significant respectively)

Table 7. Effect of salinity and N applications on Fe, Mn, Zn and Cu concentrations in lemon shoot

N levels (mg kg^{-1} soil)	NaCl levels (mg kg^{-1} soil)				
	0	250	500	1000	Main effect
			Shoot Fe (mg kg^{-1})		
0	132 a	111 d	92.7 e	73.6 f	102 C
50	130 ab	111 d	108 d	73.1 f	105 BC
100	134 a	119 bcd	125 abc	73.0 f	113 A
200	135 a	117 cd	112 d	79.0 f	111 AB
Main effect	133 A	115 B	109 C	74.7 D	
			Shoot Mn (mg kg^{-1})		
0	78.1 bc	75.0 cd	74.2 cd	69.7 de	74.2 B
50	78.1 bc	74.2 cd	74.4 cd	68.1 e	73.3 B
100	76.3 bc	80.6 bc	89.0 a	77.2 bc	80.6 A
200	81.0 b	81.2 b	77.1 bc	76.3 bc	78.7 A
Main effect	78.2 A	77.4 A	78.4 A	72.7 A	
			Shoot Zn (mg kg^{-1})		
0	41.3 abc	36.3 de	33.0 f	26.7 h	34.3 B
50	41.7 ab	36.0 e	40.7 abc	29.3 g	36.9 A
100	41.0 abc	35.7 e	39.0 bc	27.3 gh	35.7 A
200	43. a	38.7 cd	35.0 ef	19.3 i	34.0 B
Main effect	41.7 A	36.7 B	36.9 B	25.7 C	
			Shoot Cu (mg kg^{-1})		
0	10.3 b-e	10.7 bcd	8.70 ef	8.33 f	9.50 B
50	10.7 bcd	10.0 b-f	12.7 a	9.23 c-f	10.7 A
100	11.3 ab	10.3 b-e	11.0 abc	9.07 def	10.4 A
200	10.7 bcd	11.3 ab	10.3 b-e	9.73 b-f	10.5 A
Main effect	10.7 A	10.6 A	10.7 A	9.08 B	

For main effects the means with the same capital letters and for interactions, the means with similar small letters have no significant difference on the basis of Duncan's multiple range tests at 95% probability level

Shoot Fe, Mn, Zn and Cu concentrations

The effects of experimental treatments on the concentrations of Fe, Mn, Zn and Cu in lemon shoot are presented in Tables 6 and 7. Regardless of the applied salinity levels, N consumption up to 100 mg kg^{-1} soil increased the concentration and total uptake of Fe, Mn and Zn in lemon shoot but consumption of 200 mg N kg^{-1} soil resulted in a significant reduction in the above mentioned responses (Table 7). In contrast to N, salinity reduced the concentration and total uptake of Fe, Mn, Zn and Cu in the lemon shoot. The optimum concentration of Fe in citrus is about 100 mg kg^{-1} dry matter. According to the data presented in Table 7 the concentration of Fe was not under optimum level except at the highest salinity levels. In contrast to Fe, the concentration of Mn and Zn was almost optimum (25-100 mg kg^{-1} dry matter) in all salinity treatments (Table 7). Although the increase in N consumption had no significant effect on the copper concentration (Table 7), N significantly increased the total uptake of this element in lemon shoot. Finally, in most treatments the copper concentrations were higher than the optimum level for this element (6-16 mg kg^{-1} dry matter).

Discussion

Shoot dry weight

Salinity stress caused a significant reduction in plant growth. Soil salinity has been reported to interfere with the plant physiological processes which lead to the reduction of plant growth and yield. The reduction of plant growth in citrus due to salinity stress has been reported by Al-Yassin (2004), Gimeno et al. (2009), Wei et al. (2013) and Sousa et al. (2016). Under salinity stress, the improvement of plant growth due to the application of N was predictable. Razavi Nasab et al. (2014) showed that in low and moderate salinity stresses, N-fertilizer had a positive effect on plant mechanisms in pistachio seedlings and sodium toxicity was alleviated by N treatments. Although there is no clear explanation, the reduction of plant growth at the level of 200 mg N kg^{-1} soil may be attributed to the toxicity of ammonia volatilization in excess amounts of N consumption. Ammonia volatilization occurs when large amounts of ammonium fertilizers are added to the environments with alkaline pH which suffer from the lack of nitrifying bacteria. Among soil attributes, pH plays an important role in the ammonia volatilization; in the pH higher than 7.5, ammonia volatilization can be significantly increased (Mattos et al., 2003; Wang et al., 2004). Since in the present study the tested soil was alkaline in nature and the ammonium sulfate fertilizer can also produce significant amounts of ammonium, the toxicity of ammonia volatilization was not unexpected.

Na and Cl concentrations in plant tissues

Enhancement in both Na and Cl concentrations in plant tissues is another opposing effect of salinity. Pearson correlation analysis showed that the plant dry weigh had negative and highly significant correlation with the concentration of Na and Cl in plant shoot and root (Table 8). Garcia-Sanchez et al. (2005) studied the effect of different salinity levels (0, 10, 20, 40 and 80 mM NaCl) on three citrus rootstocks including Sour orange, Citranje and Macrophyla. Their results showed that Cl and Na concentrations were significantly increased by salinity levels which in turn made a noticeable reduction in plant growth for all studied rootstocks. Similar results were also reported by Wei et al. (2013) in four citrus cultivars grafted on Trifoliate orange. In the case of Na the critical concentration for the toxicity of this element in citrus was reported to be 0.1 to 0.25% on leaf dry matter weight basis (Syvertsen et al., 1988). The results obtained from current study showed that toxic Na concentrations in plant shoot were occurred at 500 and 1000 mg of NaCl kg^{-1} soil (Table 4) which was in correspondence with soil salinities of 3.65 and 5.95 dS m^{-1}. Furthermore, it has been reported that symptoms of Cl toxicity appear when the concentration of this ion reaches about 1% of leaf dry weight while yield loss can occur at concentrations higher than 0.2% (Syvertsen et al., 1988). The results presented in Table 4 indicate that, regardless of the amount of N consumed, in all salinity treatments, Cl concentration in lemon shoot is more than 0.2%. Therefore the Cl-induced toxicity and plant yield reduction are not unexpected. Significant reduction in Cl concentration and its total uptake by N consumption are obviously observed from the obtained results. Some researchers have stated that increase of nitrate (NO_3^-) in growth medium reduces the uptake and accumulation of Cl^- in a large number of garden plants. This is attributed to anionic competition and the inhibitory effect of NO_3^- on Cl uptake (Grattan and Grieve, 1999). Bar et al. (1997) indicated that addition of NO_3^- to a non saline growth medium at concentrations higher than optimum level causes a reduction in Cl^- concentration of leaves and improvement of avocado (Persea spp.) and lemon growth. These researchers also reported that addition of NO_3^- higher than optimum level reduces the concentration of Cl in avocado and citrus trees under salinity stress conditions. Similar results have also been reported by Tabatabaei (2006) and Irshad et al. (2002) in olive and wheat plants respectively. A clear negative correlation between N and Cl concentrations in plant shoot was indicative of the inhibitory effect of N on Cl uptake and accumulation (Table 8). In contrast to plant shoot, Cl concentration in plant root was increased by enhancement in N application. In fact, the nitrate absorbed by the plant blocks transferring of Cl to aerial parts and causes Cl accumulation in the root. As it was already stated the anionic competition between Cl and nitrate has been also reported by other researchers (Grattan and Grieve, 1999; Bar et al., 1997; Irshad et al., 2002; Tabatabaei, 2006). Results indicated that higher concentrations of Na and Cl are accumulated in root rather than in shoot (Table 4). In this direction, similar results were also reported by Garcia-Sanchez et al. (2005) in three citrus rootstock seedlings.

Accumulation of nutrients in plant shoot

Salinity decreased N, P, K, Fe, Mn, Zn and Cu concentrations in plant shoot. It was reported by Grattan and Grieve (1999), Munns and Tester (2008) and Wang et al. (2012) that in plants under salt stress, nutritional imbalances appear in different ways. The loss of balance may be due to the effect of salinity on the availability of nutrients, competition in absorption, disturbances in the transmission or distribution of the nutrients in plant or may be due to the inactivation of a nutrient which in turn increases the plant's internal demand for that special element. In the present study, we found that the shoot and root Cl and Na contents generally showed negative and significant correlation coefficients with the above mentioned macro and micro-nutrient concentrations (Table 8).

Table 8. Pearson correlation coefficients between determined traits.

Parameter	shoot dw	shoot Na	shoot Cl	root Na	root Cl	shoot N	shoot P	shoot K	shoot Fe	shoot Mn	shoot Zn	shoot Cu
Shoot dw	1.00**	-0.825**	-0.764**	-0.811**	-0.814**	0.663**	0.621**	0.581**	0.780**	0.357*	0.711**	0.360*
Shoot Na	-0.825**	1.00**	0.912**	0.977**	0.946**	-0.730**	-0.563**	-0.461**	-0.904**	-0.381**	-0.884**	-0.499**
Shoot Cl	-0.764**	0.912**	1.00**	0.927**	0.890**	-0.709**	-0.541**	-0.475**	-0.860**	-0.494**	-0.857**	-0.503**
Root Na	-0.811**	0.977**	0.927**	1.00**	0.939**	-0.705**	-0.606**	-0.454**	-0.903**	-0.401**	-0.858**	-0.460**
Root Cl	-0.814**	0.946**	0.890**	0.939**	1.00**	-0.645**	-0.514**	-0.394**	-0.883**	-0.311*	-0.921**	-0.443**
Shoot N	0.662**	-0.730**	-0.709**	-0.705**	-0.645**	1.00**	0.590**	0.627**	0.814**	0.610**	0.679**	0.471**
Shoot P	0.620**	-0.564**	-0.541**	-0.607**	-0.514**	0.590**	1.00**	0.640**	0.601**	0.373**	0.409**	0.181^{ns}
Shoot K	0.581**	-0.461**	-0.475**	-0.454**	-0.393**	0.627**	0.640**	1.00**	0.553**	0.456**	0.370**	0.308*
Shoot Fe	0.780**	-0.904**	-0.860**	-0.902**	-0.883**	0.814**	0.601**	0.553**	1.00**	0.563**	0.874**	0.542**
Shoot Mn	0.357*	-0.381**	-0.494**	-0.401**	-0.311*	0.609**	0.373**	0.456**	0.563**	1.00**	0.381**	0.351*
Shoot Zn	0.711**	-0.884**	-0.857**	-0.858**	-0.921**	0.679**	0.409**	0.370**	0.874**	0.381**	1.00**	0.578**
Shoot Cu	0.360*	-0.499**	-0.503**	-0.460**	-0.443**	0.471**	0.182^{ns}	0.309*	0.542**	0.351*	0.578*	1.00**

(*, ** and ns are significant at 5 and 1 percent probability level and non-significant respectively)

It indicates that salinity causes decrease in the concentration of necessary nutrients below the optimum levels. Under salt stress conditions the reduction in plant N concentration and uptake can be attributed to the inhibitory effect of Cl on NO_3^- absorption and metabolism (Bar et al., 1997). Usually increase in the absorption and accumulation of Cl^- is associated with a decrease in NO_3^- concentration in the plant's body (Francisco et al., 2002; Anjum, 2008). While some researchers attributed this event to the inhibitory effect of Cl^- on NO_3^- adsorption (Bar et al., 1997), others ascribe this response to the effect of salinity on reducing water availability and absorption which in turn lessens the inactive uptake of nitrate (Lea-Cox and Syvertsen, 1993). To investigate the scion effects on salt tolerance of citrus, four sweet orange cultivars were grafted on Trifoliate orange and exposed to NaCl salinity by Wei et al. (2013). Their results showed that not only the concentration of N but also the concentrations of P, K, Ca and Mg were reduced by salinity in leaf and root of all scion-rootstock combinations. In the case of P it is probable that the reduction of P availability in saline soil may be due to changes in ionic balance and the control of phosphate concentration in soil solution by low Ca-P dissolution. Some studies indicated that salinity stress may increase plant need for P. For example Awad et al. (1990) found that by increasing the concentration of NaCl in a nutrient solution from 10 to 50 and 100 mM, the concentration of P needed in the youngest mature tomato leaves to produce 50% of plant production increased from 58 to 77 and 97 mM respectively. These results were confirmed by the appearance of P deficiency symptoms on the plants grown under high salinity and their absence in low salinity while plant P concentrations in both conditions were the same. Reduction in K uptake by salinity has been reported by other researchers as well (Wei et al. 2013). In fact as the salinity and Na concentrations of the soil increases, the K uptake is inhibited by the competitive effect of Na in K absorption. Under soil salinity and Na accumulation, high levels of Na^+ not only hinder K^+ absorption by the root but also disrupt the root membrane selectivity for ions absorption (Perez-Alfocea et al., 1996). Usually under salinity stress conditions the solubility of micronutrients is particularly low and plants grown in these soils often suffer from micronutrients deficiencies (Page et al., 1990; Zayed et al., 2011). The adverse effect of salinity on Fe, Mn, Zn and Cu availabilities and absorptions has also been reported by Al-Harbi, (1995) and Rahman et al. (1993). Also reduction in copper uptake with increasing salinity was already reported by Eskandari et al. (2014) in pistachio seedlings. In the present study N had positive effect on macro and micro-nutrients concentrations and uptakes. In fact, nitrogen caused an ion homeostasis and retained the concentration of nutrients in lemon seedlings. The positive and significant correlation between N and other measured nutrients clearly demonstrate these relationships (Table 8). As it was stated by Ohtani et al. (2007) the effect of N on P uptake and accumulation can be attributed to the reduction of salinity harmful effects, improvement of plant growth, decrease in local soil pH due to the oxidation of ammonium to nitrate in the soil, the absorption of NO_3^- by plant and the release of hydrogen ion from the root, higher root growth and its better contact with the soil and finally the secretion of the acid-forming compounds from the root. Similar results have been also reported by Singh and Swarup (1982) and Sheila and Gregory (1989) concerning the effect of N on the improvement of Cu and Mn availabilities. In our study such beneficial effects were also observed in respect to the determined nutrients.

Conclusion

Increasing NaCl levels caused increase in the concentration of Na and Cl ions in the lemon shoot to the toxic levels. As a result, significant reductions in plant dry weight were observed. Also the concentrations of N, P, K, Fe, Mn, Zn and Cu in lemon shoot were noticeably reduced by salinity. Therefore it can be concluded that the destructive effect of salinity on plant growth is partly related to the reduction of the essential nutrient elements lower than optimum level in plant tissues. N application improved the plant growth. Although N had no significant effect on the concentration of Na in lemon shoot and root, Cl concentration in shoot and root showed a significant reduction and then induction by high N levels. In fact it can be concluded that the effect of N on the improvement of plant growth is partly due to its effect on reducing Cl absorption and preventing the transition of this element from root to shoot. Furthermore, N alleviated the harmful effect of salinity on reduction of plant essential nutrients by increasing their concentration in shoot tissue. Therefore it can be stated that the effect of N on the alleviation of salinity adverse effects is to some extent linked with ion homeostasis and maintaining of essential nutrients concentration in plant under saline conditions. Finally the results of the present study indicated that under salinity stress condition N can be applied in amounts higher than optimum levels to reduce the harmful effects of salinity.

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