

Seed germination, vegetative growth and concentration of some elements in French marigold (*Tageta patula*) as influenced by salinity and ammonium nitrate

Abdolhossein Aboutalebi Jahromi^{1*} and Mehdi Hosseini Farahi²

1. Department of Horticultural Sciences, Jahrom Branch, Islamic Azad University, Jahrom, Iran
2. Young Researchers and Elite Club, Yasooj Branch, Islamic Azad University, Yasooj, Iran

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Abstract

Marigold has special importance and application in landscape designing. Nowadays, various species and cultivars of this plant are grown in many climates, where different environmental stresses such as freezing, salinity and drought can lead to vegetative disorders. This study was carried out to investigate the interactions between salinity and ammonium nitrate on seed germination, vegetative growth and sodium and potassium concentrations in French marigold flowers (*Tageta patula*). The experiment was conducted as a factorial arrangement based on a completely randomized design with two factors: salinity (0, 2, 4, 6 and 8 mmol cm⁻¹) and ammonium nitrate (0, 15 and 30 g l⁻¹) with four replications. Based on the obtained results, different salinity concentrations were negatively influenced all studied parameters except germination rate. The application of ammonium nitrate recovered the studied parameters to the same level as the control plants. Plant height was decreased by increasing salinity concentrations. The tallest plant was observed in the control treatments. Salinity levels negatively influenced shoot fresh weight. Different levels of ammonium nitrate had significant effects on root dry weight and potassium and sodium contents. Ammonium nitrate led to reductions in root dry weight and potassium content, and an enhancement in sodium content in the shoot. Sodium and potassium levels were increased and root dry weight was decreased by increasing salinity. Enhancement of potassium ions in the marigold following salinity stress can improve its tolerance to salinity stress.

Keywords: ammonium nitrate, elements, marigold, salinity, vegetative growth.

Abbreviations: **GR**, Germination rate; **AN**, Ammonium Nitrate; **CRD**, Completely Randomized Design; **RFW**, Root Fresh Weight; **SFW**, Shoot Fresh Weight; **RDW**, Root Dry Weight; **SDW**, Shoot Dry weight; **Na**, Sodium; **K**, Potassium.

Introduction

French marigold (*Tageta patula*) belongs to the Asteraceae family (Hassani, 1998), which is used as a cut or pot flower and marginal planting in landscape. Nowadays, marigold is commercially grown for the

extraction of carotene pigments, especially xanthophylls. The leaves of marigolds contain special aromatic oils. Marigold oil has therapeutic applications, and is insect repulsive. Marigold cultivation is also useful for reduction of nematode populations in the soil. The application of marigold in rotation of cultivation programs can be a replacement

* Corresponding Author, Email: aa84607@gmail.com

for nematicides to reduce populations of scabies-agent nematode (*Meloidogyne* spp.). Nowadays, the existing species of marigolds are cultured in various climates, where problems of environmental stresses such as freezing, salinity and drought are imposed to plants after their establishments. Research on ornamental and non-bearing plants is usually limited due to low economic benefit of these plants. However, the environmental benefits, purification role, aesthetic appeal, soil fixation, erosion prevention, windbreaker role and air modification of these plants highlight the importance of basic studies on these plants.

With regards to the utilization of marigold as a summer flower in green spaces and city landscapes, especially in regions with saline soil, it is important to investigate the response of this plant to salt stresses. Plants use two mechanisms to cope with the stress factor: in resistance mechanism when plants encounter slight stresses they keep their metabolic activities at a high level (similar non-stress conditions), on the other hand when they encounter severe stresses their metabolic activities decrease. In avoidance mechanism, plant reduces its metabolic activities and shows dormancy under extreme stress conditions. In agriculture, scientist paid more attention to stress avoidance mechanism by using plant breeding methods. In nature, both mechanisms are used by different plant species. Salinity and drought have attracted more attention among abiotic stresses (Alizadeh, 2005). Salinity in the agricultural sciences is one of the characteristics of soil or water. It's due to excessive presence of ions in soil or water. Among ions, one valence types such as sodium (Na) and potassium (K), despite their differences, have effective and determinant roles in plant biochemical properties. Saline soils are characterized with $EC > 4$ mmol/cm, exchangeable sodium percentage (ESP) less than 15 and $pH < 8.5$. These soils are known as 'white alkali'—in Russia 'Solonchak'—and, according to the new American

taxonomy procedure, 'Solarthia' (Baybordi and Koohestani, 2004).

Salinity stress leads to reductions in transpiration rate and consequently delays plant growth and reduces crop yield. Generally, salinity stress could be defined as the general effects of salts on plant growth (Hasheminia *et al.*, 1997). Results of previous studies showed that the influence of salinity on plant growth and metabolism results from the reduction of osmotic potential due to salt accumulation (Levitt, 1980) and ion toxicity (Wiggans and Gardner, 1989).

Salinity stress affects various aspects of plant growth and metabolism. Photosynthesis (Staples and Toenniessen, 1984), nitrate reduction (Polard and Wynjones, 1979) and internal unbalancing of plant growth regulators (Shah and Loomis, 1965) are the most important parameters that have been evaluated for plant responses to stress conditions. In saline media, high concentrations of ions in the rhizosphere can affect nutrient uptake by the root (Birendra *et al.*, 1996), which has been mainly attributed to the presence of high concentrations of chloride and sodium (Abo-Kaseam *et al.*, 1995). Decline in the uptake of potassium (Nassery, 1979), calcium, magnesium (Al-Harbi, 1995), ammonium and nitrate (Soliman *et al.*, 1994) are examples of negative effects of salinity stress in relation to plant nutrition. Potassium is involved in stomata conductance, enzyme activity, and transportation within the plant. It has been proved that different strategies are used by different plant species to tolerate salinity stress.

Marigold is a very tolerant plant species to salinity stress based on vegetative growth and flower size, but based on flower number it is a very sensitive plant (Decital and Morris, 1987). Depends on plant species, presence of ions in soil or water can stimulate, inhibit or counteract with germination. Salinity stress generally leads to a delay in germination and a reduction in plantlet growth (Mirmohammadi-Meibodi

and Ghareyazi, 2002). Influence of salinity on plant growth varies at different stages of plant growth; also plant reaction to salinity is also changed at different stages of plant growth. Furthermore, response of different species and even their cultivars are also different in response to salinity stress. For example, rice can tolerate high concentrations of salt at germination stage (more than 30 ds/m) but it is sensitive to salinity at the primary stage of plantlet growth and flowering (Sarraf, 1993). Sugar beet is sensitive at the germination stage but it is tolerant at the other stages (Shannon, 1998); maize is tolerant at the germination stage, its sensitivity to salinity increases at the plantlet stage and decreases at the clustering and seed filling stages (Mass and Hoffman, 1987).

Salinity stress can affect different stages of plant growth, however, the sensitivity of some stages are more than the others. Germination is one of the most critical and sensitive stages of plant growth to salinity stress. Low germination in the saline soils causes poor establishment and weak production of the plant. At the germination stage tolerant plants to salinity finish vegetative stage with more success. Pill and Killan (2000) evaluated effects of osmo-priming on germination percentage and germination rate of parsley in saline stress conditions by different treatments. They showed that in the saline soils, uptake of some elements such as calcium, magnesium and potassium reduces because of competition with sodium (Yeo and Flowers, 1986). High sodium concentrations reduces uptake of other cations and as a result unbalances cations uptake into the plant. For instance, declines in calcium, magnesium and potassium uptake have been reported as a consequence of high sodium concentration in the root medium (Lauchi and Epsetin, 1984). In barley, excessive NaCl concentrations lead to calcium deficiency in plant tissues (Brown and Hayward, 1970). It has been reported that in saline conditions, sodium chloride competes with other cations leading to

growth retardation. Apart from osmotic stress, growth of bean, pea and sweet orange is reduced due to potassium deficiency (Hassan-Shahi *et al.*, 2008). Salinity in the form of NaCl, causes potassium deficiency in the lower parts of squash and sweet clover plants. Therefore, the main reason for growth disorders by NaCl is due to the inhibition of other minerals uptake in competition with sodium. Although high concentrations of sodium can decrease potassium uptake, its low concentrations may increase potassium uptake. High concentrations of sodium can also inhibit calcium uptake (Mirmohammadi-Meibodi and Ghareyazi, 2002).

Nitrogen plays a key role in plant nutrition. In fact, plant life is not possible without this element. Contrary to other elements, nitrogen is not available in the soil materials and rainwater and atmosphere (through biological agents such as nitrogen-fixing bacteria) are the sources of nitrogen for plants. In addition to nitrogen uptake by plants, leaching, erosion, ammonia sublimation and elemental nitrogen are the sources of nitrogen loss from the soil (Ghazan-Shahi, 1999). Usually increasing uptake and accumulation of Cl^- is associated with a reduction in NO_3^- in the plant shoot. Many researchers attribute this subject to the antagonistic effect of Cl^- on NO_3^- uptake (Kafkafi *et al.*, 1982). Negative effects of salinity on root water uptake have been proposed as another reason for NO_3^- deficiency following salinity stress (Lehle *et al.*, 1992). It has been reported that in saline conditions, the uptake of nitrate and ammonium ions reduces, and finally the plant adapts to nitrogen deficiency. This causes a reduction in amino acids and protein synthesis. Furthermore, nitrate reduction in the root is hampered by exposure to salinity stress (Odegaro and Smith, 1969). This study aimed to investigate the interactions between salinity and ammonium nitrate on seed germination, vegetative growth and sodium and potassium concentrations in French marigold flowers (*Tagetes patula*).

Material and Methods

To assess the effect of ammonium nitrate on the reduction of salinity stress in French marigolds (*Tageta patula*), an experiment was conducted as a factorial arrangement based on a completely randomized design (CRD). The first factor was different levels of ammonium nitrate including 0, 15 and 30 g/l and the second factor was different concentrations of NaCl salinity, including solutions with electrical conductivity of 0.49 (tap water as control treatment), 2, 4, 6 and 8 mmos/cm with four replications. The marigold seeds were prepared from a seed production centre in Esfahan, Iran. Benomyl was used to the seeds before culture. Three seeds were sown in 10 l pots containing manure, sand and agricultural soil (1:1:1) and were irrigated daily using the salt solutions. In this study, germination rate, plant height, lateral shoot number, root and shoot fresh weights, root and shoot dry weights, and amount of sodium and potassium were measured. To determine germination rate, the germinated seeds were daily counted, and germination rate was calculated by Equation (1) (Khan *et al.*, 1997), where GR, Ni and Di are germination rate, number of germinated seeds, and number of days from initiation of the experiment, respectively.

$$GR = \sum \frac{N_i}{D_i} \quad (1)$$

In order to measure sodium and potassium levels, the shoot samples were dried in oven 70 °C for 24 h. Then 0.2 g of the powdered sample was placed in 550 °C for 5 h until discoloration of the samples. The plant extracts were then prepared using 2 ml of HCl and boiling water and 50 ml of distilled water. Sodium and potassium concentrations were measured by a flame photometer device (Aboutalebi *et al.*, 2008). The obtained data was analysed by using MSTAT-C software and the mean averages were compared by Duncan's multiple range test (DMRT).

Results and Discussion

The plants irrigated with a solution of 8 mmos/cm were died after the germination stage. Therefore, this salinity level was removed from the analysis.

Germination rate (GR)

Based on the results of analysis of variance, salinity and ammonium nitrate levels had no significant influence on GR. There is no significant interaction between these factors (Table 1). According to the results of the mean comparisons, the highest GR was observed at a salinity of 2 mmos/cm, but it was not significantly different compared to the other concentrations (Table 2). In all growth stages, salinity stress negatively affected the plant growth, but variation in the sensitivity was observed for different stages. Germination is one of the most critical and sensitive stages of plant growth due to exposure to salinity stress (Table 3).

Low germination in the saline soils resulted in poor establishment and production of the plants; at this stage tolerant plants were successfully finished their vegetative stage. The results of this study showed that salinity levels had no significant influence on GR. The highest GR was observed in plants treated with 30 g/l ammonium nitrate but no significant difference was observed among ammonium nitrate levels (Table 2). In evaluation of the interactions between the two factors, the highest GR was observed in 30 g/l ammonium nitrate and control treatments (0.84) and the lowest was observed in 15 g/l ammonium nitrate and control treatments (0.62) (Table 4).

Plant height

Salinity levels had a significant influence ($P < 0.01$) on plant height, but no significant effect was observed for application of different levels of ammonium nitrate. Moreover, significant interaction ($P < 0.05$) was observed between ammonium nitrate and salinity stress (Table 1). Longest plants

Table 1. Analysis of variance for the measured parameters

S.V	D.F	Mean Square (MS)								
		GR	Plant height	Lateral shoot No	RFW	SFW	RDW	SDW	Na	K
AN (A)	2	0.031 ^{ns}	2.4 ^{ns}	0.93 ^{ns}	0.22 ^{ns}	22.5 ^{**}	0.022 ^{ns}	0.014 ^{ns}	0.431 ^{**}	0.896 ^{**}
Salinity(B)	3	0.007 ^{ns}	367.4 ^{**}	74.76 ^{**}	5.47 ^{**}	383.9 ^{**}	0.589 ^{**}	5.727 ^{**}	4.099 ^{**}	0.025 ^{**}
Interaction AB	6	0.011 ^{ns}	1.2 [*]	1.10 ^{ns}	0.35 ^{**}	30.0 ^{**}	0.040 ^{**}	0.54 [*]	0.747 ^{**}	0.080 ^{**}
Error	36	0.016	2.0	1.22	0.07	3.4	0.012	0.098	0.004	0.003
C.V %		17.3	18.3	18.4	18.3	16.3	15.14	16.61	8.05	3.81

^{ns} not significant; ^{*} significant difference at $P < 0.05$ and ^{**} significant difference at $P < 0.01$

(14.6 cm) were observed in the control treatment (Table 3). Negative effects of salinity stress can be due to water deficiency, ion toxicity, ion unbalancing, or combination of them, however, it is hard to exactly conclude which of these problems is the main factor that influenced the plant growth. In the present study plant height was decreased under saline conditions. Growth limitations resulting from salinity stress can be as a result of water stress and ion deficiency (Table 3). Based on the result of previous studies, low water potential in the external plant medium is the more important limiting factor for growth inhibition in several species than ion toxicity under salinity stress conditions (Liu and Staden, 2001). The main effect of sodium acetate is reduction in root volume, leading to decline in water absorption and nutrient uptake (Salisbury and Ross, 1992). Flowers and Yeo (1995) divided the effect of salinity into two short-term and long-term effects: short-term effects can be seen following few days of salinity stress that usually results in shoot growth inhibition due to salt-induced water deficiency. Salinity negatively influences growth of leaf cells. Reduction in leaf growth may occur even if ions desorptions and absorptions are equal, because maintenance of a suitable ion gradient for ion distribution and for the production of different solvents require consumption of energy (Volkmar *et al.*, 1998). Hung and Cox (1988) reported that marigolds showed symptoms of toxicity when EC of the

irrigation water exceed 7.9 ds/m. In such situations, leaves turned to green-yellow and bronze two weeks after imposing of salinity stress, and necrotic symptoms appeared in the margin of its mature leaves.

The Tallest and shortest plants (10.07 and 9.22 cm) were observed in 30 and 15 g/l ammonium nitrate, respectively (Table 2). In evaluating the effect of interactions between ammonium nitrate and salinity, the tallest plants were observed by application of 30 g/l ammonium nitrate and control treatment for the salinity (15.0 cm). In control and salinity 2 mmol/cm, plant height was increased in high concentrations of ammonium nitrate. Salinity decreased plant height. Average of plant height was 14.6 cm in the control treatment, while it decreased to 5.3 cm in plants exposed to 6 mmol/cm salinity (Table 4). There was no significant difference among the levels of ammonium nitrate in each salinity level.

Number of lateral shoots

Salinity levels had a significant influence ($P < 0.01$) on the number of lateral shoots, while there was no significant effect resulting from the application of different levels of ammonium nitrate. Interactions between ammonium nitrate and salinity were not significant (Table 1). Highest number of lateral shoots was obtained in control treatment (6.8 shoots) and the lowest was seen in the plants exposed to 6.0 mmol/cm salinity (Table 3). Number of lateral shoot was decreased by increasing salt concentrations in the irrigation water.

Different plants species and varieties show different levels of resistance to salinity. Salt composition, soil properties, weather, moisture, and plant variety can influence harmful effects of salt on the plants (Mass and Hoffman, 1977). Differences in tolerance to salinity have been observed between plants and even between different species of one plant (Hughes *et al.*, 1975). Number of lateral shoot decreases with increasing levels of ammonium nitrate (Table 2). The highest number of lateral shoot was observed in the control treatment and 15 g/l ammonium nitrate (7.5 shoots). By increasing salinity level, the application of ammonium nitrate had no influence on the number of lateral shoot (Table 4).

Root fresh weight (RFW)

Salinity levels had a significant influence ($P<0.01$) on RFW but there was no significant effect from application of

different levels of ammonium nitrate. Interactions between ammonium nitrate and salinity were significant ($P<0.01$; Table 1). The highest RFW was observed in the control treatment (1.84 g) and the lowest RFW was observed in plants that treated with 6.0 mmos/cm salinity (0.27 g; Table 3). Among ammonium nitrate levels, the highest RFW was obtained from 0 g/l and the lowest RFW was observed in plants treated with 30 g/l ammonium nitrate (Table 2).

Evaluation interaction between ammonium nitrate and salinity levels showed that the highest RFW was observed in control treatment and 0 g/l ammonium nitrate (2.28 g) and the lowest RFW was obtained in plants treated with 6 mmos/cm salinity and 30 g/l ammonium nitrate (0.15 g). Except for 4 mmos/cm, RFW was decreased by increasing ammonium nitrate concentration in all salinity levels (Table 4).

Table 2. Effects of different levels of ammonium nitrate on vegetative parameters and potassium and sodium levels in okra

Ammonium nitrate (AN) Trait	0	15 g/L	30
Germination rate (GR)	†0.74 ^a ± 0.023	0.68 ^a ± 0.037	0.76 ^a ± 0.019
Plant height (cm)	9.6 ^{ab} ± 1.52	9.22 ^b ± 2.03	10.07 ^a ± 2.63
Lateral shoot no.	5.1 ^a ± 0.70	4.9 ^{ab} ± 0.75	4.55 ^b ± 0.96
Root fresh weight (gr)	1.13 ^a ± 0.075	0.88 ^b ± 0.085	0.84 ^b ± 0.048
Shoot fresh weight (gr)	8.75 ^a ± 2.56	7.5 ^b ± 1.96	7.4 ^b ± 0.97
Root dry weight (gr)	0.38 ^a ± 0.063	0.29 ^b ± 0.042	0.25 ^b ± 0.011
Shoot dry weight (gr)	0.92 ^a ± 0.054	0.92 ^a ± 0.062	0.85 ^a ± 0.042
Na (%)	0.49 ^c ± 0.012	0.68 ^b ± 0.014	0.87 ^a ± 0.025
K (%)	0.62 ^a ± 0.042	1.32 ^b ± 0.036	1.38 ^b ± 0.051

†Means in each row with similar letters are not significantly different ($P<0.05$) according to DMRT.

Table 3. Effects of different levels of electrical conductivity on vegetative parameters and potassium and sodium levels in marigold plants

Salinity Trait	0.49 (Control)	2	4	6
		mmos/cm		
Germination rate (GR)	†0.72 ^a ± 0.017	0.76 ^a ± 0.032	0.74 ^a ± 0.028	0.72 ^a ± 0.011
Plant height (cm)	14.6 ^a ± 1.95	10.8 ^b ± 0.97	7.8 ^c ± 1.05	5.3 ^d ± 1.53
Lateral shoot no.	6.8 ^a ± 0.85	4.9 ^b ± 0.52	4.2 ^c ± 0.36	3.5 ^d ± 0.18
Root fresh weight (gr)	1.84 ^a ± 0.091	1.02 ^b ± 0.085	0.67 ^c ± 0.074	0.27 ^d ± 0.015
Shoot fresh weight (gr)	15.53 ^a ± 2.5	8.34 ^b ± 1.6	4.97 ^c ± 0.9	2.74 ^d ± 0.3
Root dry weight (gr)	0.59 ^a ± 0.028	0.35 ^b ± 0.041	0.18 ^c ± 0.029	0.12 ^d ± 0.011
Shoot dry weight (gr)	1.79 ^a ± 0.041	1.05 ^b ± 0.021	0.53 ^c ± 0.032	0.37 ^d ± 0.014
Na (%)	0.04 ^d ± 0.002	0.49 ^c ± 0.006	0.77 ^b ± 0.009	1.4 ^a ± 0.010
K (%)	1.40 ^b ± 0.006	1.41 ^b ± 0.008	1.43 ^{ab} ± 0.007	1.51 ^a ± 0.004

†Means in each row with similar letters are not significantly different ($P<0.05$) according to DMRT.

Table 4. Interactions between ammonium nitrate (AN) and salinity levels in marigold plants

Trait AN (g/L) × salinity (mmos/cm)	germination rate (GR)	Plant height (cm)	Lateral shoot No.	Root fresh weight (gr)	Shoot fresh weight (gr)	Root dry weight (gr)	Shoot dry weight (gr)	Na (%)	K (%)	
0	0.49	0.70 ^{ab} ± 0.06	14.5 ^a ± 3.1	6.2 ^{bc} ± 1.5	2.28 ^a ± 0.5	17.56 ^e ± 2.5	0.74 ^a ± 0.1	1.78 ^a ± 0.3	0.04 ^{gh} ± 0.00	1.66 ^b ± 0.63
	2	0.76 ^{ab} ± 0.07	10.2 ^{bc} ± 2.7	5.4 ^c ± 1.8	1.08 ^{cd} ± 0.3	7.96 ^e ± 0.9	0.34 ^{cd} ± 0.1	1.06 ^b ± 0.5	0.09 ^g ± 0.01	1.46 ^c ± 0.32
	4	0.77 ^{ab} ± 0.07	8.3 ^{cd} ± 1.6	4.4 ^d ± 0.9	0.77 ^{de} ± 0.2	5.51 ^d ± 0.9	0.25 ^{de} ± 0.0	0.62 ^c ± 0.1	0.66 ^{ef} ± 0.03	1.62 ^{bc} ± 0.42
	6	0.71 ^{ab} ± 0.08	5.4 ^{ef} ± 0.6	4.4 ^d ± 1.2	0.38 ^{ef} ± 0.1	4.00 ^d ± 0.5	0.20 ^{de} ± 0.0	0.23 ^c ± 0.0	1.19 ^c ± 0.22	1.75 ^{cd} ± 0.17
15	0.49	0.62 ^b ± 0.06	14.3 ^a ± 2.4	7.5 ^a ± 1.6	1.75 ^b ± 0.6	14.65 ^{de} ± 2.6	0.56 ^b ± 0.2	1.86 ^a ± 0.6	0.04 ^{gh} ± 0.00	1.27 ^b ± 0.32
	2	0.74 ^{ab} ± 0.05	10.1 ^c ± 1.6	5.1 ^{cd} ± 0.8	1.04 ^{cd} ± 0.3	8.88 ^e ± 0.8	0.40 ^b ± 0.1	1.00 ^b ± 0.1	0.57 ^f ± 0.02	1.31 ^c ± 0.23
	4	0.66 ^{ab} ± 0.04	7.4 ^{de} ± 0.6	4.1 ^{de} ± 0.6	0.47 ^c ± 0.0	4.14 ^e ± 0.4	0.15 ^f ± 0.0	0.52 ^{cd} ± 0.1	0.76 ^c ± 0.06	1.29 ^{de} ± 0.21
	6	0.72 ^{ab} ± 0.08	5.1 ^f ± 0.3	2.9 ^f ± 0.3	0.27 ^f ± 0.0	2.38 ^f ± 0.3	0.07 ^g ± 0.0	0.31 ^{de} ± 0.0	1.37 ^c ± 0.41	1.41 ^{cd} ± 0.25
30	0.49	0.84 ^a ± 0.08	15.0 ^a ± 2.8	6.8 ^b ± 0.8	1.48 ^c ± 0.2	14.38 ^{de} ± 2.6	0.49 ^{bc} ± 0.2	1.72 ^a ± 0.9	0.04 ^{gh} ± 0.00	1.28 ^b ± 0.41
	2	0.78 ^{ab} ± 0.06	12.2 ^b ± 2.5	4.9 ^{cd} ± 0.4	0.94 ^d ± 0.1	8.18 ^e ± 0.6	0.31 ^d ± 0.1	1.11 ^b ± 0.4	0.80 ^d ± 0.03	1.46 ^c ± 0.06
	4	0.79 ^{ab} ± 0.09	7.8 ^d ± 0.7	4.2 ^{de} ± 0.3	0.78 ^{de} ± 0.2	5.26 ^d ± 0.5	0.14 ^f ± 0.0	0.46 ^d ± 0.1	0.99 ^d ± 0.05	1.39 ^d ± 0.014
	6	0.72 ^{ab} ± 0.04	5.3 ^{ef} ± 0.2	3.5 ^e ± 0.2	0.15 ^g ± 0.0	1.84 ^f ± 0.4	0.08 ^g ± 0.0	0.13 ^e ± 0.0	1.64 ^d ± 0.41	1.39 ^d ± 0.41

†Means in each column with similar letters are not significantly different ($P < 0.05$) according to DMRT.

Shoot fresh weight (SFW)

Salinity and ammonium nitrate levels had significant influence ($P < 0.01$) on SFW. Interaction between ammonium nitrate and salinity levels was also significant ($P < 0.01$) (Table 1). The highest SFW was observed in control treatment (15.53 g). Salinity led to a reduction in SFW so that the lowest SFW was obtained in a salinity of 6.0 mmos/cm (2.74 g) (Table 3). Different parts of the plant are not equally affected by salinity. In the tuber crops, tubers are more affected by salinity than other parts of the plant (Hoffman and Rawlins, 1971). In cotton, fibres are affected by salinity less than vegetative growth and in wheat, barley and some resistant grasses, salinity affects vegetative growth more than seed production (Ayer *et al.*, 1952). In rice and maize, seed production may be decreased by the influence of salinity without a reduction in straw (Pearson, 1959). Often shoots are more affected by salinity than the root (Meiri and Poljakoff-Mayber, 1970).

Highest and lowest SFW were observed in plants treated with 0 g/l (8.75 g) and 30 g/l (7.4 g) ammonium nitrate, respectively (Table 2). Assessment of interactions between ammonium nitrate and salinity levels showed that the highest SFW was obtained in the control treatment and 0 g/l ammonium nitrate (17.56 g), and the lowest SFW was obtained in plants treated

with 6 mmos/cm salinity and 30 g/l ammonium nitrate (1.84 g; Table 4).

Despite a significant reduction in fresh weight of root and shoot, application of ammonium nitrate inhibited the negative effects of salinity. It has been reported that, in many soils, nitrogen deficiency is one of the main limiting nutrition factors for plant growth. Therefore, apart from soil salinity, nitrogen application can help plant growth and yield improvement. Under saline conditions, reduced nitrogen uptake is one of the determinant factors influencing plant growth (Al-Rawahy *et al.*, 1992). Chloride has an inhibiting effect on the uptake and metabolism of nitrate (Bar *et al.*, 1997). Therefore, under salinity stress, plant need for nitrogen is higher than in non-saline conditions (Grattan and Grieve, 1999).

Root dry weight (RDW)

Based on the results of analysis of variance (Table 1), salinity levels had a significant influence ($P < 0.01$) on RDW, but there was no significant effect for application of different levels of ammonium nitrate. Interaction between ammonium nitrate and salinity levels was also significant ($P < 0.01$). In evaluation of salinity effects, the highest RDW was observed in the control treatment (0.59 g) and the lowest was obtained plants treated with 6.0 mmos/cm salinity (0.12 g; Table 3). RDW in plants treated with 0 g/l

ammonium nitrate was significantly more than RDW in plants treated with 15 or AN 30 g/l. RDW was decreased by increasing ammonium nitrate concentrations (Table 2).

Evaluation of interactions between ammonium nitrate and salinity levels showed that highest RDW was detected in plants treated with 0 g/l ammonium nitrate and the control treatment of salinity (0.74). RDW was decreased by increasing ammonium nitrate levels in each of the salinity levels. In all ammonium nitrate concentrations, RDW was decreased (Table 4) with increasing the salinity levels. The root is an organ that involves in water and nutrient uptake. Most damaging effects of salinity stress occur in the root. The root is the organ that directly confronts saline stress, undertakes osmotic regulation, and serves the avoidance mechanism that reduces salt effects (Carla *et al.*, 2009); therefore it receive a lot of energy from the shoots to cope with the salinity stress. This leads to reductions in root efficiency for providing water and nutrients for other organs. Salinity stress may decrease root dry weight as well (Safarnejad *et al.*, 2005). The results obtained in the present study confirm the above investigations.

Shoot dry weight (SDW)

Salinity levels had a significant influence ($P<0.01$) on SDW but ammonium nitrate had no significant effect. Interactions between ammonium nitrate and salinity levels were significant ($P<0.05$; Table 1). Highest SDW was observed in the control treatment (1.79 g). Salinity caused a significant reduction in SDW (Table 3). Evaluation of the influence of ammonium nitrate on SDW showed that the highest SDW was observed in plants treated with 0 and 15 g/l ammonium nitrate. SDW was significantly decreased with increasing ammonium nitrate from 15 to 30 g/l (Table 2).

Interaction between salinity and ammonium nitrate indicated that the highest SDW was obtained in the control salinity treatment and 15 g/l ammonium nitrate (1.86 g), which had no significant

difference on 0 and 30 g/l ammonium nitrate at the same salinity level. With the application of 15 g/l ammonium nitrate, the reduction in SDW was slower when comparing its values in plants treated with salinity of 2.0 to 6.0 mmos/cm (Table 4). Shanon (1986) reported that salinity in water and soil causes a reduction in shoot and stem growth and affects plant yield. It has been reported that, in many soils, nitrogen deficiency is one of the main limiting nutrition factors for plant growth. Therefore, apart from soil salinity, nitrogen application can lead to improvement of plant growth and yield. Under saline conditions, a reduction in nitrogen uptake is one of the limiting factors influencing plant growth (Al-Rawahy *et al.*, 1992). Chloride has an inhibiting effect on uptake and metabolism of nitrate (Bar *et al.*, 1997). Therefore, compare to non-saline conditions, the plant needs for nitrogen increase under salinity stress conditions (Grattan and Grieve, 1999).

Sodium (Na)

Salinity and ammonium nitrate levels had a significant influence ($P<0.01$) on Na level. Interactions between ammonium nitrate and salinity levels were significant ($P<0.01$; Table 1). The level of Na was 0.04% in the control treatment, while by increasing salinity levels the amount of Na was significantly increased. The highest Na level was observed in a salinity of 6.0 mmos/cm (1.4%; Table 3). Evaluating the effect of ammonium nitrate on Na levels indicated that Na level increased in the shoot by increasing ammonium nitrate concentrations. The highest amount of Na (0.87%) was observed in 30 g/l ammonium nitrate (Table 2).

Investigating the interactions between salinity and ammonium nitrate showed that the highest Na level was observed in 6.0 mmos/cm salinity treatment and 30 g/l ammonium nitrate (1.64 g). Application of ammonium nitrate had no influence on the reduction of Na in the shoot (Table 4).

Based on the obtained results it can be concluded that Na moves easily from root to shoot in French marigold. Furthermore, application of ammonium nitrate caused an increase in Na levels in the shoot.

Potassium (K)

Salinity and ammonium nitrate levels had a significant influence ($P < 0.01$) on K level as well. Interactions between salinity and ammonium nitrate was significant for potassium levels ($P < 0.01$; Table 1). Assessment of the effects of salinity showed that the highest K level was detected in 6.0 mmos/cm salinity treatment (1.51%). The amount of shoot K in the control salinity treatment was statistically same as its level in 4.0 mmos/cm salinity (Table 3). Evaluation of the effect of ammonium nitrate on the level of K showed that the highest K in the shoot was detected in plants treated with 0 g/l ammonium nitrate (1.62%) and the lowest K level was obtained in plants treated with 15 g/l ammonium nitrate (1.32%). K level in the plants treated with 30 g/l ammonium nitrate was more than its level in the plants treated with 15 g/l ammonium nitrate, but both were at a lower level than the plants treated with 0 g/l ammonium nitrate (Table 2).

The interactions between salinity and ammonium nitrate showed that the highest K level was observed in plants treated with 6.0 mmos/cm of salinity and 0 g/l ammonium nitrate (1.75 g). The amount of K in the shoot decreased by increasing ammonium nitrate levels in control salinity and at salinity levels of 4.0 and 6.0 mmos/cm (Table 4). Previous studies in many plant species showed that, potassium ion concentration decreased when salinity increased in the form of Na ions or Na/K ratio (Graifenberg *et al.*, 1995). In contrast, in some plants it was observed that the concentration of potassium in the leaves increased by increasing salinity levels (Cachorro *et al.*, 1993). Our results about reduction in K level through increasing salinity levels is not in agreement with the

findings of Gregorio *et al.* (1998) but is in agreement with the results of Ruiz *et al.* (1997). In fact, ammonium nitrate cannot induce K uptake under high salinity concentrations.

Conclusion

Different levels of ammonium nitrate were somehow affected root and shoot fresh weights, root dry weight and potassium and sodium levels but had no significant influence on germination rate, plant height, number of lateral shoots and shoot dry weight. Increasing ammonium nitrate led to a reduction in root dry weight and potassium, and increased sodium concentration in the shoot. Different salinity concentrations had a significant influence on all of the evaluated traits except germination rate. Increasing salinity caused a significant decrease in studied parameters. Accumulation of potassium ions in the marigold following salinity stress is indicative of resistance against salinity, while in the all salinity levels significant changes in potassium ion were not observed. In saline soils because of high levels of Na and Cl ions and ion unbalancing, and in spite of the presence of microelements in the soil solution- there is no possibility for transferring these elements towards the shoots which results in their deficiency and consequently damage to the plant. Despite a significant reduction in root and shoot fresh weights, the application of ammonium nitrate improved root and shoot fresh weight in each salinity level.

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