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ORIGINAL ARTICLE

# Growth, biochemical components and ion content of Chamomile (*Matricaria chamomilla* L.) under salinity stress and iron deficiency

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**Abstract** The effect of salinity and iron deficiency on growth, proline, carbohydrate and ion content in Chamomile (*Matricaria chamomilla* L.) was evaluated in controlled environment. Salinity treatment was 0, 50, 100 and 150 mM NaCl in nutrient solution and iron (Fe) treatment was $F_0$ = the same nutrient solution without Fe and $F_1$ = standard nutrient solution containing 100 μM Fe (Fe-sufficient medium). Results indicated that increasing salinity from 0 to 150 mM, decreased fresh weight of shoot (76.3%) and increased of root fresh weight (53.8%). However, application of Fe to the nutrient solution significantly increased fresh weight of root and shoot. A two-way ANOVA indicated a significant main effect of salinity and iron on the proline and soluble carbohydrate contents in plant leaves. Salinity significantly increased proline and soluble carbohydrate in leaves. Maximum proline and carbohydrate content in leaves of chamomile plants were obtained at salinity and iron deficiency treatments. Salinity treatment significantly increased $Na^+$ concentration of plants, whereas potassium concentration of plants in shoot (37.6%) and root (46.1%) decreased. Salinity also decreased Fe content in root and shoot of chamomile plants. By application Fe into nutrient solution, $Na^+$ concentration in shoot and root decreased but $K^+$ and Fe content in root and shoot increased.



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## 1. Introduction

Iron (Fe) is a vital element for plant growth and development, since it is essential for the proper functioning of multiple metabolic and enzymatic processes such as those related to oxygen and electron transport, nitrogen fixation, DNA and chlorophyll biosynthesis and photosynthesis (Jeong and Guerinot, 2009). Despite the ubiquitous presence of this element in the earth's crust, the low solubility of Fe compounds in many soils especially under high pH, aerobic conditions limits the bioavailability of Fe and induces the occurrence of Fe deficiency symptoms in plants, which are primarily observed in young

leaves (Jeong and Connolly, 2009). Iron chlorosis is chiefly associated with plant growth on high pH, calcareous soils, and to the presence of high bicarbonate concentrations which can inhibit Fe uptake mechanisms (Lucena, 2006).

There is numerous data in literature about the separate effects of salinity and inadequate Fe supply on plant growth and nutrient uptake, concentration and distribution. There is also some evidence that root or foliar Fe supply might favoursunflower and maize, grown in saline medium (Salama et al., 1996). On the other hand, high NaCl might affect iron absorption, and might aggravate Fe deficiency or Fe toxicity (Rabhi et al., 2007).

Salt stress creates both ionic as well as osmotic stress on plants. Also, ionic toxicity generated from salt contaminated soil has negative effects on plant growth and development (Munns et al., 2006). However, there are many defense mechanisms in plants which are tolerant to water-deficit and salt stresses, such as osmoregulation, ion homeostasis, antioxidant and hormonal systems (Mahajan and Tuteja, 2005), helping plants to survive and grow under severe environmental conditions prior to their reproductive stages. In contrast, the defense mechanisms in sensitive plant species are weaker, leading to growth retardation and yield reduction.

Plant biochemicals [ascorbate peroxidase (AOX), glutamine synthetase (GS), proline, glycine betaine, photosynthetic pigments, soluble proteins and mineral elements] and physiological changes in plants growing under salt or water-deficit conditions have been investigated in many plant species such as rice (Chaum et al., 2007) and cabbages (Maggio et al., 2005). Biochemical and physiological parameters in higher plants cultivated in salt or water-deficit conditions have been developed as effective indices for tolerant screening in plant breeding programs (Ashraf and Foolad, 2007).

Chamomile (*Chamomilla chamomilla* L.) is one of the important herbal medicine plant, it is used for the treatment of many diseases. Chamomile plant has been used medicinally for thousands of years and is widely used in Europe. It is a popular treatment for numerous ailments, including sleep disorders, anxiety, digestion/intestinal conditions, skin infections/inflammation (including eczema), wound healing, infantile colic, teething pains, and diaper rash. In the United States, chamomile is best known as an ingredient in herbal tea preparations advertised for mild sedating effects. German chamomile (*Matricaria chamomilla*) and Roman chamomile (*Chamaemelum nobile*) are the two major types of chamomile used for health conditions (Simpson, 2001).

Therefore, the objective of this investigation was to identify the interaction between salinity and Fe on the growth, biochemical changes such as proline and carbohydrate content and nutrient uptake of German Chamomile (*Matricaria chamomilla* L.).

## 2. Materials and methods

### 2.1. Plant growth

This study was conducted in a greenhouse at the University of Zabol, Iran during April–June 2010. The experiment was laidout in a complete randomized factorial design with three replicates. Surface-sterilized seeds representing German Chamomile were germinated in the dark on sand moistened with distilled water and the resulting seedlings were transferred to containers containing the following continuously aerated standard nutrient solution: 0.7 mM $K_2SO_4$, 0.1 mM KCl, 2 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.1 mM $KH_2PO_4$, 10 μM $H_3BO_3$, 0.5 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.2 μM Cu $SO_4$ and 0.01 μM $(NH_4)_6MO_7O_{24}$ (Mori and Nishizawa, 1987).

When the seedlings were seven days old they were canvassed for uniform size, these transferred to plastic containers and placed under hydroponic culture with 2 L of nutrient solution. The plants were grown under greenhouse conditions with a 12 h photoperiod of natural daylight, maximum and minimum temperatures of 26 °C and 18 °C, respectively and relative humidity of 70% on average. Four salinity treatments ($S_0$ = 0 (control), $S_1$ = 50, $S_2$ = 100 and $S_3$ = 150 mM NaCl) were imposed by to the nutrient solution after the plants were ten days old. Iron (Fe) treatment was $F_0$ = the same nutrient solution without 100 μM Fe on nutrient solution and $F_1$ = standard nutrient solution containing 100 μM Fe (Fe-sufficient medium).

The culture solution was weekly renewed and its pH was initially adjusted to 6.5. Twenty days after salt treatment, the plants were harvested. Just before harvest, leaves were cut from plants growing under each treatment and prepared for assays of soluble carbohydrates and proline. Thirty days after growth in the 2.5L pots, the test plants were harvested and dried separating shoots from roots. Shoot and root biomass samples were taken after drying the plants in an electric oven at 100 °C for ten minutes, and then at 70 °C until sacrificed samples indicated that a constant weight had been reached.

### 2.2. Determination of proline and soluble carbohydrate

The extracts of the mature leaf material were used to determine soluble carbohydrates (Irigoyen et al., 1992). Free proline was estimated according to Bates et al. (1973), in leaf samples which were homogenized in 5 ml of sulphosalycylic acid (3%) using mortar and pestle. With about 2 ml of extract in a test tube, 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added. The mixture was boiled in a water bath at 100 °C for 30 min. and allowed to cool. When the reaction mixture was cool, 6 ml of toluene was added and the combination transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and the absorbance read at 520 nm in a spectrophotometer against a toluene blank.

### 2.3. Plant ion analysis

After the plants were harvested and separated into roots and shoots. Roots were carefully washed with 1% (v/v) HCl in order to get rid all adhering particles then rinsed several times with distilled water. After drying at 50 °C during 72 h, samples were weighed for biomass determination then ground with an agate grinder for nutrients extraction. The contents of $Na^+$, $K^+$ and Fe were determined by using a Jemway PFP7 Flam photometer and atomic absorbtion (variance, 220 FS).

### 2.4. Statistical analyses

All data were analyzed using the SAS Institute Inc. Version 6.12 Software. Initially, the data were analyzed in an analysis-of-variance (ANOVA) test to determine significance

($P \leqslant 0.05$) of the treatment effects. Significant differences between individual means were determined using Fisher's protected least significant difference test.

## 3. Results

### 3.1. Shoot and root growth

The effects of increasing amounts of salinity and Fe treatment on growth of chamomile plants shoot and the roots are shown in Table 1. Seedling growth was recorded in terms of shoot and root fresh weight at different levels of NaCl salinity and Fe treatment (Figs. 1 and 2). The increase in NaCl concentrations decreased the shoot and increased of root fresh weight of chamomile plants. The reduction in shoot growth was greater than root growth. Increasing salt concentration from 0 to 150 mM NaCl, strongly reducted shoot (76.3%) and increased root(53.8%) fresh weight (Figs. 1 and 2).

Shoot and root growth of chamomile plants were strongly restricted by iron-deficient treatment ($F_0$ = −Fe). Table 1 shows the effects of Fe on growth plants. In chamomile plants to which Fe had been applied to the nutrient solution, root and shoot fresh weight were significantly increased.

The interaction between saline and Fe treatment showed, however shoot and root growth reduction caused by salinity, but by application Fe on the nutrient solution fresh weight in root and shoot significantly increased (Figs. 1 and 2).

### 3.2. Soluble carbohydrate and proline

The soluble carbohydrate and proline measured in the leaves of chamomile plants varied significantly with salinity and Fe treatment (Table 1).

Osmotic adjustment by the chamomile plants with the accumulation of organic solutes might have occurred. A two-way ANOVA indicated a significant main effect of salinity ($P < 0.001$) with the proline and soluble carbohydrate contents in plant leaves (Table 1). This tended to occur regardless of Fe treatment. The salinity treatment caused a significant

**Table 1** Results of two-way analysis of variance (ANOVA) of salinity (S) and iron (I) and their interaction (S × F) for the variables listed.

| Dependent variable | Independent variable | | |
|---|---|---|---|
| | S | I | S × I |
| Shoot FW | 0.00109*** | 0.0085*** | 0.00017** |
| Root FW | 0.000167** | 0.0057** | 0.0011ns |
| Proline | 113.19** | 7.92* | 11.36** |
| Soluble carbohydrate | 0.407*** | 1.137** | 0.120** |
| Shoot $Na^+$ | 0.235*** | 0.0319*** | 0.0133*** |
| Root $Na^+$ | 0.126*** | 0.0685*** | 0.0305*** |
| Shoot $K^+$ | 17.5*** | 8.255*** | 1.788*** |
| Root $K^+$ | 7.503*** | 10.424*** | 0.271*** |
| Shoot Fe | 0.00046** | 0.0000025ns | 0.00089** |
| Root Fe | 0.00000021ns | 0.00000136* | 0.00000031ns |

Numbers represent *F* values at 5% level; ns, not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

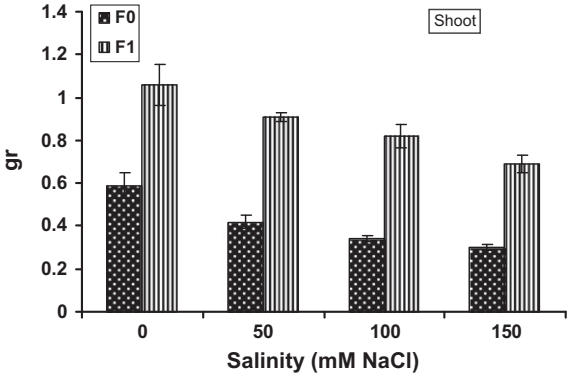


**Figure 1** Effects of NaCl salinity and Fe treatment on shoot FW.

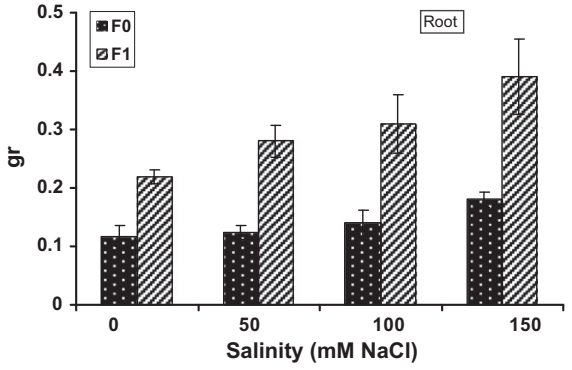


**Figure 2** Effects of NaCl salinity and Fe treatment on root FW.

increase in the concentrations of proline and soluble carbohydrate in the leaves of plants (Figs. 3 and 4). The greatest accumulations of proline and carbohydrate were observed in 150 mM NaCl.

Interaction between NaCl and Fe treatment were found to be significant for proline and soluble carbohydrate (Table 1). This can be related to the evidence that the iron supplied to plants interacts with the salinity tolerance of the plants. In this study, the interaction of salinity and Fe treatment significantly affected proline and soluble carbohydrate accumulations (Table 1). Whereas, the proline of the Fe deficiency treatment ($F_0$) plants increased significantly with increasing NaCl, along with higher soluble carbohydrate in Fe deficiency ($F_0$) plants subjected to 150 mM NaCl (Figs. 3 and 4).

### 3.3. Sodium, potassium and iron

Based on the analysis of variance, the overall effect of salinity was highly significant ($P < 0.01$) on the concentrations of $Na^+$ and $K^+$ in the root and shoot tissues of chamomile plants (Table 1). Salt treatments increased significantly $Na^+$ concentration of plants, whereas potassium concentration of plants in shoot (37.6%) and root (46.1%) decreased (Figs. 5–8). A two-way ANOVA indicated salt treatment only had significantly effect on Fe content in shoot and had not significantly effect on Fe in root (Table 1). Salt treatments decreased significantly

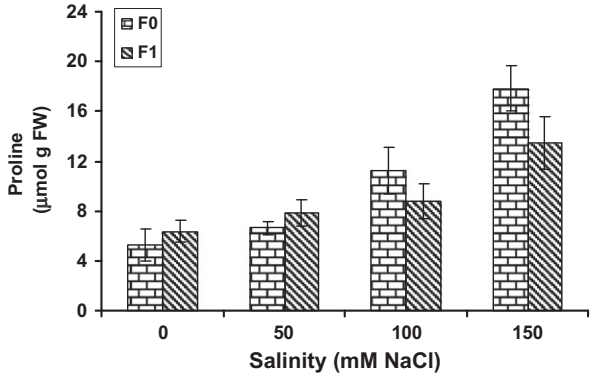


**Figure 3** Effects of NaCl salinity and Fe treatment on proline content.

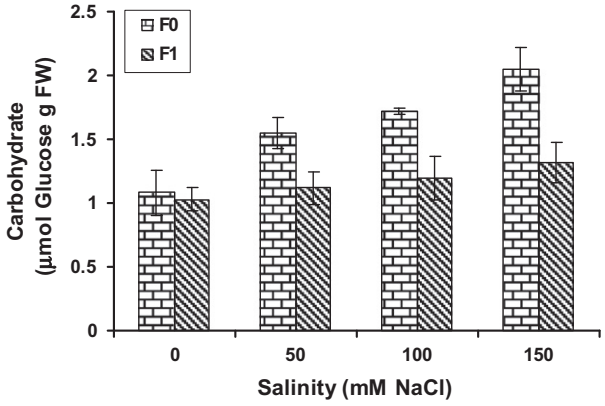


**Figure 4** Effects of NaCl salinity and Fe treatment on carbohydrate content in shoot.

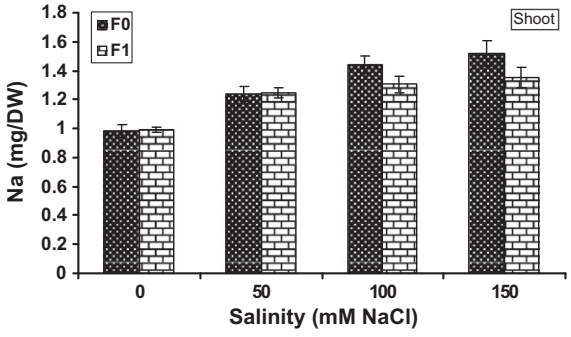


**Figure 5** Effects of NaCl salinity and Fe treatment on $Na^+$ content in shoot.

Fe concentration of shoot plants especially at $F_0$ treatment but by application iron treatment, Fe content increased until 100 mM NaCl and then decreased (Fig. 9).

Fe application significantly ($P < 0.01$) affected the shoot potassium, sodium and Fe concentration in chamomile plants (Table 1). Figs. 5–10 showed that with application of 100 µM Fe into nutrient solution, potassium (in root) and Fe content in plants shoot under salinity treatments significantly increased and sodium (in shoot) content decreased.

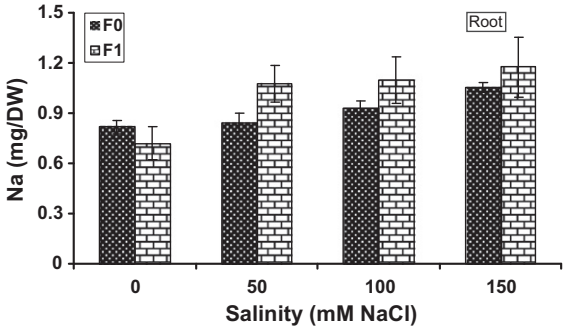


**Figure 6** Effects of NaCl salinity and Fe treatment on $Na^+$ content in root.

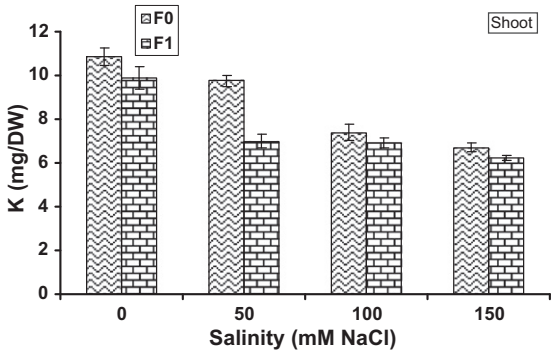


**Figure 7** Effects of NaCl salinity and Fe treatment on $K^+$ content in shoot.

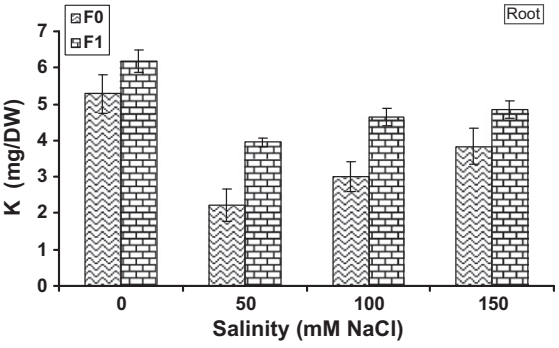


**Figure 8** Effects of NaCl salinity and Fe treatment on $K^+$ content in root.

## 4. Discussion

Salinity affects on the growth of plants by reduction of shoot hight and biomass. The reason for the decrease in plant growth may be explained by the in osmotic pressure due to increasing salt level, which lessens the available water to plant (Huang et al., 2006). In this study, by increasing salinity from 0 to 150 mM, shoot fresh weight (76.3%) decreased and root (53.8%) increased (Figs. 1 and 2).

Under iron deficiency, shoot growth was greatly affected than root growth. By application Fe in the nutrient solution, root and shoot fresh weight were significantly increased.

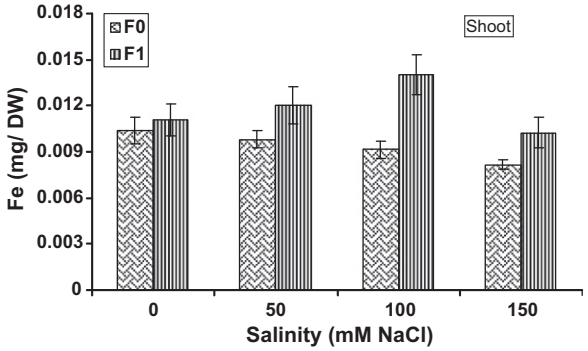


**Figure 9** Effects of NaCl salinity and Fe treatment on Fe content in shoot.

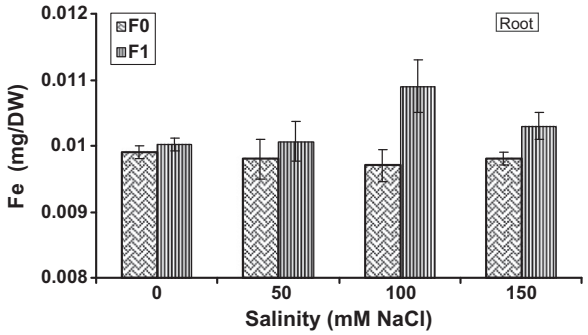


**Figure 10** Effects of NaCl salinity and Fe treatment on Fe content in root.

Marschner (1995) indicated that iron is involved in the formation of chlorophyll, N assimilation, nitrate reduction, and protein synthesis.

Proline, sucrose, and other organic sugars in quinoa contribute to osmotic adjustment during stress and protect the structure of macromolecules and membranes during extreme dehydration (Prado et al., 2000). Melonid et al. (2001) suggest that proline also serves: as an important source of nitrogen in plant metabolism, as a readily available source of energy, and as a reducing agent. Osmotic adjustment by the chamomile plants with the accumulation of organic solutes might have occurred. Salinity stress had significantly increased proline and soluble carbohydrate contents in plant shoot (Figs. 3 and 4).

However by application of Fe into the nutrient solution, proline and carbohydrate content in shoot increased but data in this study showed that the highest proline and carbohydrate obtained under iron deficiency treatment ($F_0$). There is numerous data in literature about the separate effects of salinity and inadequate Fe supply on plant growth and nutrient uptake, concentration and distribution. There is also some evidence that root or foliar Fe supply might favour sunflower and maize, grown in saline medium (Delgado and Sanchez-Raya, 1998). On the other hand, high NaCl might affect iron absorption, and might aggravate Fe deficiency or Fe toxicity (Yousfi et al., 2007). Often a combination of two abiotic stresses is more harmful to crops, since its deleterious effect exceeds the effect of the individual stresses. According to Mittler (2006), the stress combination should be regarded as a new state of abiotic stress, as the response of plants to it is unique and cannot be directly extrapolated from their response to each different stress.

Salinity stress disturbs the uptake and accumulation of essential nutrients (Shalan et al., 2006). Generally, $Ca^{2+}$ and $K^+$ are decreased in plants under saline conditions. These decreases could be due to the antagonism of $Na^+$ and $K^+$ at uptake sites in the roots, the effect of $Na^+$ on $K^+$ transport into the xylem or the inhibition of uptake processes (Al-Harbi, 1995).

Our results indicated $Na^+$, $K^+$ and Fe content in shoot and root in plants were strongly affected by both iron-deficient and salinity treatments (Table 1). Salinity and iron application significantly ($P < 0.01$) affected the $K^+$, Fe concentration in the shoot and sodium concentration in root and shoot of chamomile plants (Table 1). Figs. 5–8 showed sodium content in leaves increased but potassium and Fe content in shoot and root decreased with increasing salinity levels from 0 to 150 mM NaCl.

The decrease in $K^+$ content can be attributed to $Na^+$ competition with $K^+$ for binding sites on the plasma membrane which suppressed the influx of $Na^+$ from the external solution (Al-Harbi, 1995).

The depressive effect of salt on iron uptake was confirmed by the strong reduction of the iron uptake efficiency in the presence of salt. With respect to this parameter, neo-formed roots should distinguished from the pre-existing ones for a more accurate determination of salt impact on iron uptake efficiency (Yousfi et al., 2007).

## 5. Conclusions

NaCl concentration caused reduction in the growth of chamomile plants. Results obtained indicated that the difference in growth at the salinity treatments can be attribute differences in ion transfer rates to the leaves, proline and carbohydrate accumulation. It can be inferred that, under the studied conditions, the proline and carbohydrate concentration was promoted by salinity but iron application compared to Fe deficiency treatment had no effect on increase them at the highest salinity treatment. Different salinity treatment increased $Na^+$ content in root and shoot of chamomile plants and decreased $K^+$ and Fe content in root and shoot. Fe application in this study had positive effect on decreasing of $Na^+$ and increasing of $K^+$ and Fe content in shoot and root of chamomile plants.

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