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*Original Article*

# The Responses of *Salsola orientalis* to Salt Stress

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ABSTRACT

**Objective:** Salt stress is a world-wide problem and soil salinity is common in arid and semi-arid regions. This study was undertaken to investigate salt tolerance in *Salsola orientalis* in laboratory and natural conditions and recognize the mechanisms that allow it to tolerate these conditions. **Methods:** This study had two parts of greenhouse and natural habitats. The treatment solutions for salinity tests were different concentrations of NaCl and $Na_2SO_4$ (0, 100, 200, 300, 400, and 500 mM) with three replicates and growth parameters and proline and soluble sugar were determined in vegetative growth stage in greenhouse. Soil (two depths of 0-10 cm and 10-45 cm) and plant (root and shoot) samples have been harvested from three 200 meters transects in three provinces of Esfahan, Semnan and Markazi. Plant proline and soluble sugar and soil texture and EC were measured in laboratory. Collected data were analyzed using a factorial experiment and means were compared by DMRT method by SPSS software. **Results:** Results indicated that proline and soluble sugar were significantly affected by salinity levels and increased with salinity increase. The rate of growth parameters increased with an increase in salinity up to 300 mM while salinity levels more than 300 mM NaCl caused all growth characteristics decline. Data obtained from the laboratory experiment confirmed the findings noted during the field study. It has to be mentioned that nature is unpredictable and observing unexpected trends under specific conditions is not impossible.

## 1.INTRODUCTION

Soil salinity is one of the major problems having influence on species productivity in arid and semi-arid regions. Throughout the world, 100 million hectares or 5% of the arable land is adversely affected by high salt concentration, which reduces crop growth and yield (Ghassemi *et al*., 1995). Almost 50% of the irrigated land is affected by high salinity (Zhu, 2001), often resulting in secondary salinization due to inappropriate use of saline irrigation water. In warm and dry areas, salt

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concentrations increase in the upper soil layer due to evapotranspiration exceeding precipitation (Abrol *et al*., 1988). Understanding the responses of plants to salinity has great importance in screening of plant species for the afforestation of saline deserts, and also understanding the mechanisms plants use in the avoidance and/or tolerance of salt stress.

Many investigators have reported retardation in growth of seedlings at high salinity (Bernstein, 1962; Garg and Gupta, 1997; Ramoliya and Pandey, 2003). However, plant species differ in their sensitivity or tolerance to salts (Brady and Weil, 1996). There are many different types of salts and almost an equally diverse set of mechanisms of avoidance or tolerance. In addition, organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Munns, 1993; Ashraf, 1994). It is reported that soil salinity suppresses shoot growth more than the root growth (Ramoliya and Pandey, 2003; Maas and Hoffman, 1977). However, fewer studies on the effect of soil salinity on root growth have been conducted (Garg and Gupta, 1997). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. In saline soil, salt induced water deficit is one of the major constraints for plant growth. As soils dry down soil salinity is exacerbated. Frequent droughts are a regular phenomenon in saline deserts. In addition, many nutrient interactions in salt-stressed plants can occur which may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied (e.g. Maas and Grieve, 1987; Cramer *et al*., 1989), but the relationship between micro-nutrient concentrations and soil salinity is rather complex and remains poorly understood (Tozlu *et al*., 2000). Zandi (2010) studying *Suaeda vermiculata* and *Atriplex leucoclada* pointed to the direct relationship between salinity and proline. Panahi *et al*. (2012) studied *Salsola* responses to salt stress and concluded that proline and soluble sugars were significantly affected by salinity levels and increased with salinity increase and the rate of growth parameters increased with an increase in moderate salinity while in high salinity levels caused all growth characteristics decline. Daoud *et al*. (2013) studied the salt response of halophytes (like *Sporobolus spicatus*, *Spartina alterniflora*, *Batis maritime* and etc.) and stated that maximum growth occurred in low and moderate salinities (25 and 50% seawater). Brakez *et al*. (2013) studied the performance of *Chenopodium quinoa* under salt stress and reported that maximum biomass was registered in 20% seawater treatment (low salinity level). Tawfik *et al*. (2013) studied saline land improvement through testing *Leptochloa fusca* and *Sporobolus virginicus* and concluded that increasing soil salinity significantly increased soluble carbohydrates and proline concentration in the plant tissues.

The aim of this study is to investigate the salinity tolerance level and the mechanisms that allow *Salsola orientalis* to endure increases in salinity under exposure to salinity variations in laboratory and natural conditions.

## 2. MATERIALS AND METHOD

This study had conducted in two stages: laboratory (greenhouse) and natural habitats.

### 2.1. Study Area and Plant Species Choice

As best result of salt tolerance screening can be expected from the species that grow naturally in saline environments (Panahi *et al*., 2012a), the native and palatable species of *Salsola orientalis* has been chosen in its natural habitats. The Irano-Turanian species of Chenopodiaceae family which is mostly characteristic for arid to semiarid and/or saline habitats has great importance in livestock grazing and also in salty and dry range improvement (Panahi *et al*., 2012a). It forms an important component of the flora and vegetation of desert environments. Chenopodiaceae are, however, taxonomically not well investigated due to the limitation of practical taxonomical characters, the fleshy nature of many species, late flowering and fruiting time and the fact that they are not attractive for most collectors and the botanists (Hedge *et al*., 1997).

### 2.2. Data Collection

One part of experiments was conducted under greenhouse conditions at University of Tehran, Iran. Seeds were sown in pots filled with sand in growth chambers (alternating light periods of 8-h dark and 16-h light and 25°C temperature). After two weeks, the pots were transferred to greenhouse and irrigated with Hoagland's nutrient solution. Seeds were raised from April to October under non-saline conditions. The treatment solutions for salinity tests were different concentrations of NaCl and $Na_2SO_4$ (0, 100, 200, 300, 400, and 500 mM) which were added to Hoagland's nutrient solution. Three replicates were used for each treatment. Two months after the start treatments, seedlings were harvested and transferred to laboratory and the following parameters were measured at the harvest time. Growth parameters (Root and shoot fresh and dry weight, length and diameter) were determined. Proline and soluble sugar were also considered in vegetative growth stage.

Soil and plant samples have been harvested from three 200 meters transects in three provinces of Esfahan, Semnan and Markazi. Plant samples were collected from root and aerial organs and proline and soluble sugars were measured. Soil sampling was done in two depths (0-10 cm and 10-45 cm). Soil texture and EC were measured in soil laboratory. All sampled materials were oven-dried (70°C for 48-h) to obtain the dry weight. Proline was determined by the ninhydrin method described by Bates *et al*. (1973). In this method, proline was extracted from 0.5 g of fresh leaf tissue into 10 ml of 3% sulfosalicylic acid and filtered through Whatman No.

42 filter papers and determined in Shimadzu UV- 1201 model spectrophotometer. In order to measure the content of soluble sugars, 0.5 g of dry leaves was homogenized with 5ml of 95% ethanol. One-tenth ml of alcoholic extract preserved in refrigerator mixed with 3ml anthrone (150 mg anthrone, 100 ml of 72% sulphuric acid, W/W). The samples placed in boiling water bath for 10 minutes. The light absorption of the samples was estimated at 625 nm using a PD-303 model spectrophotometer. Contents of soluble sugar were determined using glucose standard (Irigoyen *et al*., 1992). EC meter and hydrometer method were used to determine Electrical Conductivity and soil texture, respectively. Collected data were analyzed using a factorial experiment and means were compared by DMRT method by SPSS software.

## 3. RESULTS AND DISCUSSION

### 3.1. Greenhouse

### 3.1.1.Growth parameters

Results indicated that aerial organ fresh and dry weight significantly ($p$<0.05) increased with salinity increase up to 300mM NaCl and $Na_2SO_4$. Increasing salinity more than the specified value caused fresh and dry weight significant ($p$<0.05) decrease. Root fresh ($p$<0.05) and dry ($p$<0.01) weight significantly increased when salinity increased up to 300mM (Tables 1-2, Figures 1-2). Plant's root have importance in survival of plants in presence of salts due to the ability of the root system to entry control of ions to shoots (Hajibagheri *et al*., 1989), and the lack of water uptake by plants due to high salinity causes reduced shoot and root growth (Werner and Finkelstein, 1995). Morphologic adaptation of halophytes to salinity is a part of their evolution and natural selection in saline environments (Kuz Mina and Treshkin, 2006). Salinity also causes specific structural changes such as fewer and smaller leaves, fewer stomata per leaf area, leaves thicker cuticles and waxy surfaces, differentiation and development of vascular tissue and premature root lignification. These changes vary depending on the species and the type of salt (Poljakoff-Mayber, 1975).

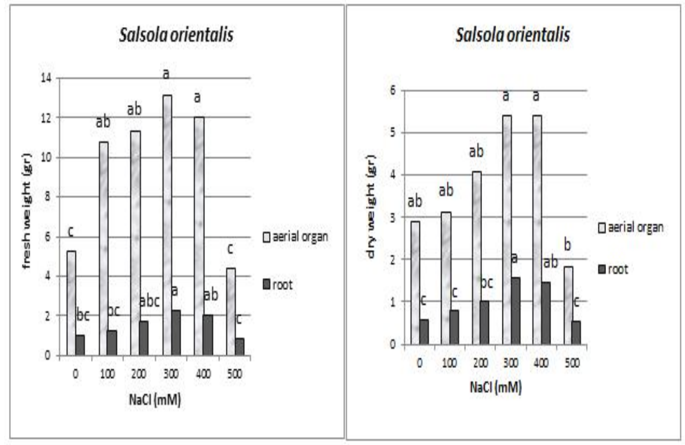


**Figure 1.** The effect of NaCl salinity on fresh and dry weights in S. orientalis.

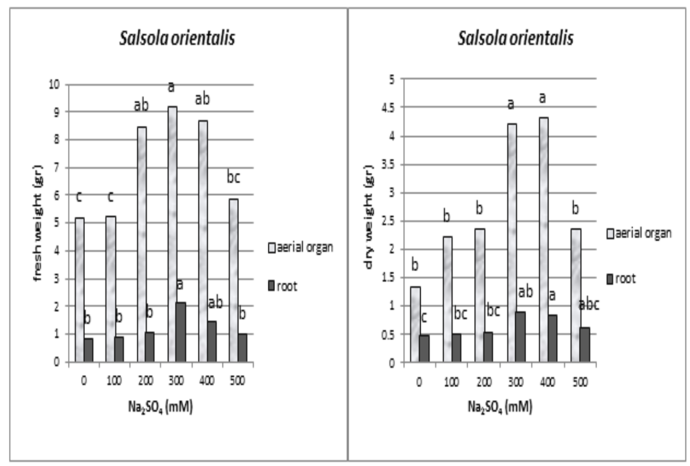


**Figure 2.** The effect of $Na_2SO_4$ salinity on fresh and dry weights in *S. orientalis*.

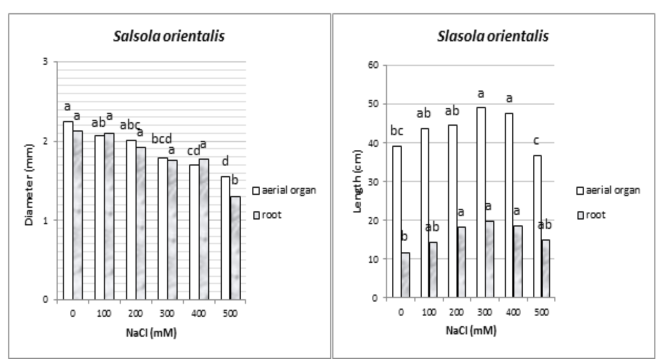


**Figure 3.** The effect of NaCl salinity on length (cm) and diameter (mm) in *S. orientalis*.

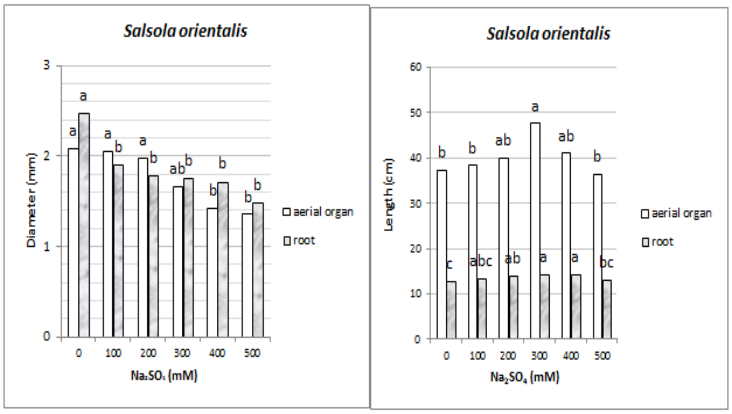


**Figure 4.** The effect of $Na_2SO_4$ salinity on length (cm) and diameter (mm) in *S. orientalis*.

Shoot and root length are the most important parameters of salt stress, because root has direct contact with soil and absorbs water from soil and shoot spreads it to the other parts of the plant. So shoot and root length are main indicators of plant responses to salt stress (Jamil and Rha, 2004). Increasing salt concentration up to 300 mM caused a significant increase in plant height ($p$<0.01) and root length ($p$<0.05), although higher salinity levels significantly affected and reduced plant height (Tables 1-2). Salinity increase also resulted significantly ($p$<0.01) in stem diameter decrease, root diameter didn't show any significant different but up to 400 mM and with increasing salinity up to 500 mM, root diameter reduced significantly ($p$<0.05) (Tables 1-2, Figures 3,4).

Our results suggest that growth parameters decrease with increasing salinity levels more than the specified levels. Garg and Gupta (1997) reported that salinity causes reduction in leaf area as well as in the rate of photosynthesis which together result in reduced crop growth and yield. Jamil *et al.* (2006) studying sugar beet, cabbage and amaranth reported that salinity limits plant growth (shoot and root length and fresh weight). In this study, results showed a reduction in growth parameters of *S. orientalis* with increasing salinity more than 300mM concentration. These results coincide with those obtained by Daoud *et al.* (2013) in the investigation of salt response of halophytes and Brakez *et al.* (2013) studying *Chenopodium quinoa*. They expressed that the salt-tolerant species survived at all salinity treatments, and maximum growth occurred in low and moderate salinity.

**Table 1.** Results of analysis of variance for the growth parameters under NaCl salinity.

| source | df | M.S. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Shoot fresh weight | Root fresh weight | Shoot dry weight | Root dry weight | Plant height | Root length | Stem diameter | Root diameter |
| Salinity | 5 | 41.232* | 0.963* | 6.174* | 0.584** | 68.39** | 28.01* | 0.205** | 0.273* |
| error | 12 | 11.155 | 0.278 | 1.772 | 0.079 | 12.084 | 7.708 | 0.039 | 0.057 |
| error | 12 | 2.097 | 6.722 | 0.529 | 2.611 | 3.536 | 3.536 | 0.126 | 0.356 |

**Table 2.** Results of analysis of variance for the growth parameters under $Na_2SO_4$ salinity.

| source | df | M.S. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Shoot fresh weight | Root fresh weight | Shoot dry weight | Root dry weight | Plant height | Root length | Stem diameter | Root diamete r |
| Salinity | 5 | 10.51* | 0.683* | 4.306** | 0.149* | 64.664* | 1.281* | 0.317** | 0.332* |
| error | 12 | 2.472 | 0.155 | 0.496 | 0.047 | 19.848 | 0.27 | 0.051 | 0.088 |

*significantly different at $p$<0.05; **significantly different at $p$<0.01; ns no significant difference

In the presence of NaCl and $Na_2SO_4$, with increasing salinity up to 300 mM, all studied growth parameters significantly increased compared with control, and decreased with increasing salinity over 300 mM (Figures 1-2).Ravindran *et al*. (2007) reported that low NaCl concentrations simulate growth of some halophytic species. Such stimulatory effect of moderate salinity on growth of some halophytic plants may be attributed to improved shoot osmotic status as a result of increased ion uptake metabolism. On the other hand, the reduction in growth as a result of high soil salinity levels may be attributed mainly to the osmotic inhibition of water absorption, the excessive accumulation of ions such as $Na^+$ or $Cl^-$ in plant cells, and inadequate uptake of essential nutrients (Munns and Tormaat, 1986).

### 3.2. Proline

Proline concentration in salt-tolerant plants is more than salt-sensitive plants (Javed Khan, 2007). Increasing salinity levels significantly increased the rate of proline in plant shoots and roots (Tables 3-4, Figures 5-6). In this study, increasing salinity levels of both NaCl and $Na_2SO_4$, a significant increase ($p<0.01$ in aerial organs and $p<0.05$ in root) of proline levels could be observed. Studying *Suaeda salsa*, Song *et al*. (2006) stated that proline is one of the organic solutions having influence on salt stress decrease.

**Table 3.** Results of analysis of variance for the effect of NaCl salinity on soluble sugar and proline in *S. orientalis*.

| Source | df | M.S. | | | |
|---|---|---|---|---|---|
| | | Root soluble sugar | Aerial organs soluble sugar | Root proline | Aerial organs proline |
| salinity | 5 | 916040.876** | 720516.296** | 2.741* | 3.445** |
| error | 12 | 27121.373 | 27062.716 | 0.731 | 0.281 |

*significantly different at $p<0.05$; **significantly different at $p<0.01$; $^{ns}$ no significant difference

**Table 4.** Results of analysis of variance for the impact of $Na_2SO_4$ salinity on soluble sugar and proline in *S. orientalis*.

| Source | df | M.S. | | | |
|---|---|---|---|---|---|
| | | Root soluble sugar | Aerial organs soluble sugar | Root proline | Aerial organs proline |
| salinity | 5 | 1523288.837** | 1494002.526* | 1.56** | 7.321** |
| error | 12 | 97090.552 | 386820.109 | 0.207 | 0.946 |

*significantly different at $p<0.05$; **significantly different at $p<0.01$; $^{ns}$ no significant difference

The results of this research confirm a direct link between salinity increase and proline content increase which is in conformity with the results obtained by Joshi and Lyengar (1987) studying *Suaeda nudiflora*, Bajji *et al*. (1998) studying *Amaranthus tricolor,* Zandi (2010) studying *Suaeda vermiculata* and *Atriplex leucoclada, and* Tawfik *et al*. (2013) studying *Leptochloa fusca* and *Sporobolus virginicus* who also pointed to the direct relationship between salinity and proline.

The observed accumulation of proline in the plants growing under saline conditions may be attributed to the enhancement of hydrolytic effect of salinity on protein which led to the accumulation of the intermediary substances containing nitrogen such as ammonia, amino acids, and urea (Munns *et al*., 2002). Youssef (2009) suggested that proline functions as a source of solute for intracellular osmotic adjustments under saline conditions.

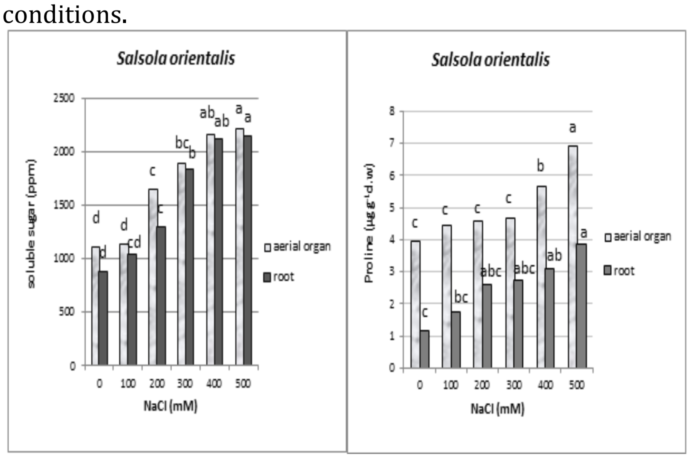


**Figure 5.** The effect of NaCl salinity on soluble sugar and proline in *S. orientalis*

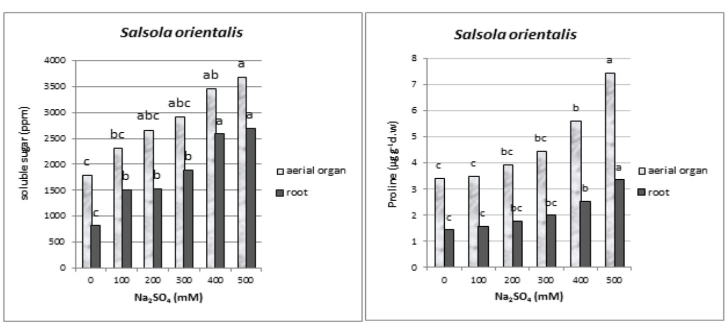


**Figure 6.** The effect of $Na_2SO_4$ salinity on soluble sugar and proline in *S. orientalis***.**

### 3.3. Soluble sugars

In this study, the rate of soluble sugars in S. *orientalis* was also increased significantly ($p$<0.01) in shoots and roots with increasing salinity levels (Tables 3-4, Figures 5-6). Soluble sugars increase in shoots is the important response of plant against water shortage and probably the result of starch hydrolysis in stress conditions is low water content and loss of soil water potential (Jones and Qualset, 1984). In saline environments, accumulation of soluble sugars in plant may be observed in response to osmotic pressure that their concentrations depending on species and in various plant tissues are different (Javed Khan, 2007). In S. *orientalis*, the accumulation of soluble sugars in shoots was more than roots (Figures 5-6).

The results of this study confirm a direct correlation between increased salinity and increased proline and soluble sugars in *S. orientalis*. These results coincide with those obtained by Tawfik *et al*. (2013) studying *Leptochloa fusca* and *Sporobolus virginicus*. The observed increase in soluble carbohydrates at high levels of soil salinity was considered as a protective mechanism against protein denaturation or dehydration (Turnner *et al*., 1978). Chevan and Karadge (1986) attributed the increase in soluble carbohydrates by increasing salinity to the incomplete utilization of sugars in polysaccharide synthesis. Moreover, Weimberg (1987) stated that the increase of soluble sugars under salinity stress may play a role in osmotic adjustment.

**Table 5.** Results of analysis of variance for four soil properties in two depth and 3 locations in *S. orientalis*.

| Source | M.S. | | | |
|---|---|---|---|---|
| | EC | sand | silt | clay |
| Location | $70.21^{**}$ | $118.167^{ns}$ | $55.389^{ns}$ | $55.722^{ns}$ |
| Depth | $5.12^{**}$ | $112.5^{ns}$ | $37.556^{ns}$ | $280.056^{ns}$ |
| Location*depth | $6.392^{**}$ | $215.167^{ns}$ | $54.056^{ns}$ | $70.722^{ns}$ |
| error | 0.106 | 99.111 | 20.778 | 69.167 |

$^{*}$significantly different at $p$<0.05; $^{**}$significantly different at $p$<0.01; $^{ns}$ no significant difference.

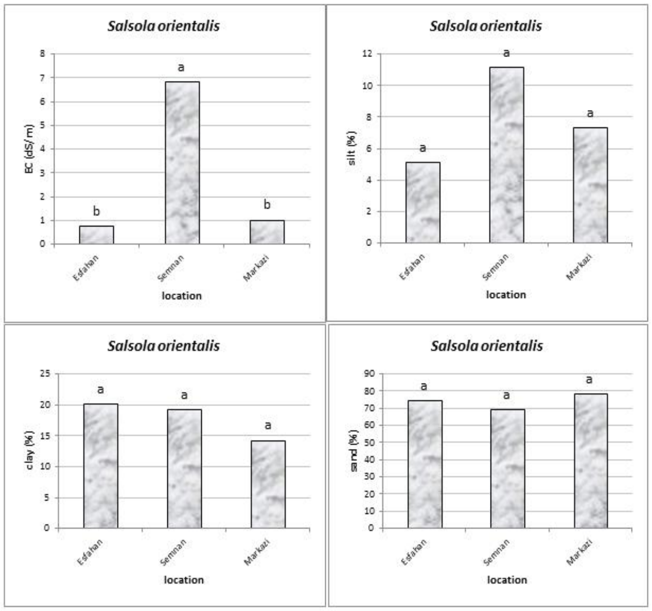


**Figure 7.** Mean differences of four soil properties in three provinces of Iran in *S. orientalis* habitats.

## Field

Statistical analyses showed a significant difference ($p$<0.01) between the rate of EC in three habitats of *S. orientalis* (Table 5). The maximum and minimum rate of EC and sand was observed in Semnan and Esfahan locations, respectively (Figure 7). Results also indicated a significant difference ($p$<0.05) between the rates of soluble sugar of three locations (Table 6) and no significant difference ($p$>0.05) between the rates of proline in three locations and different organs (Table 6, Figures 8-9). It has to be mentioned that the maximum rate of soluble sugar and proline was observed in Markazi province (Figure 9).

**Table 6.** Results of analysis of variance for proline and soluble sugar in aerial organ and root in three locations.

| Source | M.S. | |
|---|---|---|
| | Soluble sugar | proline |
| Location | 165381.5$^{*}$ | 0.04$^{ns}$ |
| Organ | 51084.33$^{ns}$ | 0.218$^{ns}$ |
| Location*Organ | 20311.09$^{ns}$ | 0.424$^{ns}$ |
| Error | 39465.44 | 0.449 |

$^{*}$significantly different at $p$<0.05; $^{**}$significantly different at $p$<0.01; $^{ns}$ no significant difference.

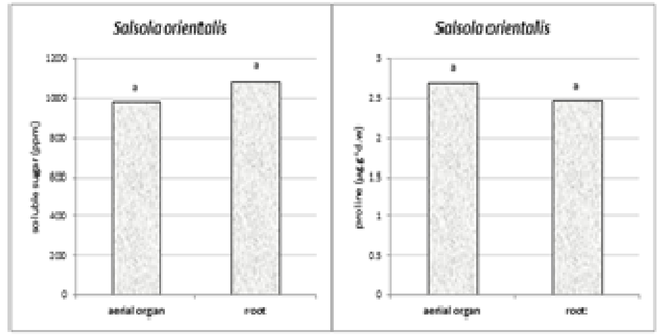


**Figure 8.** Mean differences of soluble sugar and proline in aerial organ and root in *S. orientalis* habitats.

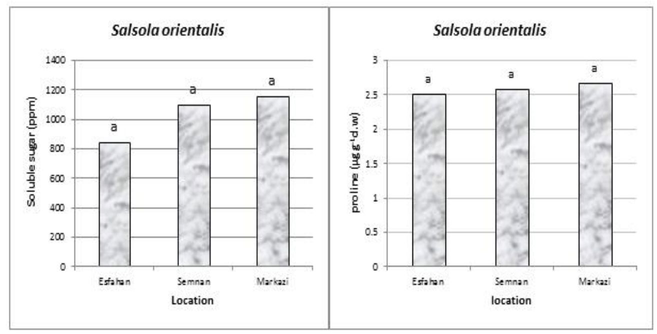


**Figure 9.** Mean differences of soluble sugar and proline in three provinces of Iran in *S. orientalis* habitats.

The high rate of proline and soluble sugar in all three habitats can be a result of climatic conditions such as high temperature, rainfall distribution and low annual precipitation. Nature is unpredictable and observing unexpected trends under specific conditions is not impossible and there is the probability of observing various reactions of species in different climates (Panahi *et al*., 2013). These results coincide with those obtained by Panahi *et al*. (2013) studying *Salsola arbuscula.* The results obtained from greenhouse confirmed the results obtained from field and there is a correlation between the results of laboratory, greenhouse and field.

## CONCLUSION

Best performance of *Salsola orientalis* was obtained in medium salinity (300 mM). However, increasing salinity more than the optimal rate, growth efficiency of plant decreased. According to the results of greenhouse and field, it can be concluded that different salinity levels have significant influence on growth parameters, proline and soluble sugars accumulation in *S. orientalis* and according to the obtained results, using this species can be suggested in salt affected areas. It has to be mentioned that reaching the best results in this field, various studies on the species of this family is needed.

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