*Caspian J. Env. Sci. 2010, Vol. 8 No.1 pp. 79~87*


CJES
Caspian Journal of Environmental Sciences

**[Research]**

# Effect of salinity on growth, ion content and water status of glasswort (*Salicornia herbacea L.)*

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## ABSTRACT
Salinity is one of the major environmental stresses that limit plant growth and productivity. Glasswort (*Salicornia herbacea L.)* is one of the native halophytic plants of Iran that widely spread in salt areas. The purpose of this study was to determine the physiological and growth responses of *S. herbacea* to salinity stress. Plastic pods (15 cm diameter, 20 cm height) with the Silica sand bed were used for the experiment. The solution used for the study consisted of 0 (control), 100, 200, 300, 400, 500 mM of NaCl and $Na_2SO_4$. *S .herbacea* seeds cultivated at five pots for each treatment in green house condition. Plants were irrigated with half strength Hoagland's nutrient solution for 6 months. Salt treatments were applied for 45 days. Shoot and root dry weights, proline, glucose, ion concentration, Osmotic Potential (OP), Relative Water Capacity (RWC), Water Use Efficiency (WUE)، Net Assimilation Rate (NAR), Specific Leaf Area (SLA) and Leaf Area Ratio (LAR) were measured. Data analysis showed that $Mg^{2+}$, $Ca^{+2}$ and $K^+$ decreased when salinity increased but $Na^+$ increased. $CL^-$ increased when NaCl increased but significantly inhibited at higher $Na_2SO_4$. Dry weight, WUE, SLA, NAR and LAR increased in up to 100 to 300 mM NaCl and $Na_2SO_4$ but decreased with a further increase in salinity. *S. herbacea* uptakes more ions in chloride soil than that of sulfate soil. WUE, NAR and dry weight are more at sulfate soil. It is also assumed that salt tolerance mechanism of *S. herbacea* changes at different salts. Measurement of osmotic potential showed that it did not significantly increased when salinity increased. In addition, glucose did not promote up to 400 mM. Therefore, *S. herbacea L.* is a high tolerant halophyte which grows well up to 500 mM of NaCl and $Na_2SO_4$ salt. Salinity enhances the growth of *S. herbacea* and its optimum growth occurs at 100 – 300 mM. Proline, glucose and osmotic potential remain unchanged at moderate salt concentrations. Also, it tolerates salinity via uptake of ions at NaCl and also ions repulsing and increasing WUE at $Na_2SO_4$. *S. herbacea* grows at sulfate better than chloride.

**Keywords**: *Growth, Ion Content, Salicornia herbacea L., Salinity, Water Statue*

## INTRODUCTION
Salinity is one of the most important challenges for human life in recent decades. Soils can become saline due to geo-historical processes or man-made activities. Salinity affects at least 20% of world's arable land and more than 40% of irrigated land to various degrees (Rhoades & Loveday, 1990). Iran is a classical country with saline soils and Kavirs. Saline and alkaline soils are expanding in arid and semi arid areas of Iran and cover 12.5 % (204800 km$^2$) of the total area of the country (Akhani & Ghorbanli, 1993). Most of the salt stresses in plant are due to the abundance of NaCl and $Na_2SO_4$ in soil (Martin et al. 1993; Flowers et al. 1977; Jafari, 1994). In these regions, halophytes can tolerate high environmental stresses. Successful utilization of these areas depends on the advances in the knowledge of many factors involved in salt tolerance of plant species. Scientists have focused on salt tolerance mechanisms of halophytes. Because, the use of native halophytic plants to reclaim the saline areas would not only be economically beneficial but would also be ecologically relevant (Khan et al. 2000). According to the

literatures, halophytes tolerate salinity through the uptake or repulsion of ions, increasing of organic solutes, change of stomata, water content and other physiological changes (Khan et al. 2001; Aghaleh et al. 1994; Yoshie & Hideo, 1994; Parks, 1987; Wang & Zhao, 2004). There are little detailed information concerning halophytic plants in Iran and it is necessary to recognize halophytic plants and their salinity tolerance mechanisms in the country(Jaafari, 1994; Akhani & Ghorbanli, 1993). Glasswort (*Salicornia herbacea L.)* is one of the native halophytic plants in Iran, distributed in saltpan of Orumieh province, Hozsultan of Qom province, Kashafrood of Khorasan province, Persian Gulf and Oman seashore, and other salt playas of Iran. It is annual succulent species of chenopodiaceae family with 5–35 cm length and leafless. It is used as human food, oil and cattle fodder. Its ashes are used for glassmaking and soap making (Moghimi, 2005). *Salicornia sp*. is included among the group of halophytes where they grow larger and benefits from NaCl concentrations above the minimal requirements as micronutrients in plants (Khan et al. 2001).

The main objectives of this study were to determine organic solutes, ion balance and physiological responses of Glasswort to salinity under greenhouse condition.

## MATERIALS AND METHODS

Seeds of Glasswort (*S. herbacea)* were collected from the Coasts of Orumieh Lake, during October 2008. Seeds were surface sterilized with % 70 alcohol for 15 seconds and washed with distilled water three times. Plastic pods (15 cm diameter, 20 cm height) with the Silica sand bed were used for the experiment. The solution used for the study consisted of 0 (control), 100, 200, 300, 400, 500 mM of NaCl and $Na_2SO_4$. Seeds cultivated at five pots for each treatment in greenhouse conditions.

Potted plants were irrigated with half strength Hoagland's nutrient for 6 months (Hoagland & Arnon, 1950). Then, salt treatments were initiated by adding NaCl and $Na_2So_4$ per liter of the culture solution according to table 1. 10 litter Barrels were used for each treatment and salinity stresses were applied for 45 days in each potted plants.

**Table 1.** Amount of salt at each salinity treatment.

| $Na_2SO_4$(gr $L^{-1}$) | NaCl(gr $L^{-1}$) | Salt concentration(mM) |
|---|---|---|
| - | - | 0(control) |
| 14.2 | 5.85 | 100 |
| 28.4 | 11.7 | 200 |
| 42.6 | 17.55 | 300 |
| 56.8 | 23.4 | 400 |
| 71 | 29.25 | 500 |

Root and shoot Dry weight, Water Use Efficiency( WUE), Net Assimilation Rate (NAR), Leaf Area Ratio (LAR), Specific Leaf Area(SLA), Osmotic Potential (OP), Relative Water Content (RWC), proline, glucose and ion concentration of $Mg^{2+}$,$Ca^{+2}$, $Na^+$, $K^+$, $CL^-$ were measured according to the standard methods described in Rasouli (2008). (SLA, NAR and LAR with Beadle (1993) method, RWC with Weatherley (1950) method, OP with Richardson & Mckell (1980) method, WUE with Claussen (2002) method, Proline with Bates(1973) method, Glucose with Kochert (1978) method, Ion concentration with Wilson (1983) method and $Cl^-$ with Mohr(1969) method). Leaf area determined with the leaf area meter in Windias package.

A one way ANOVA and Duncan Means test were carried out to determine the differences among treatments. Parried T-Test sample was used to determine differences among NaCl and $Na_2So_4$.

**RESULTS**

Results showed that salinity significantly affected most of the studied factors in both salts. But, proline at NaCl, glucose and shoot dry weight at $Na_2SO_4$ and osmotic potential in both salts did not vary statistically (table2).

Results of Duncan test indicated that $Mg^{2+}$, $Ca^{2+}$ and $K^+$ contents decreased when salinity increased and all mentioned parameters had the highest amount in control treatment (figure 1-B, 1-C, 1-D). $Na^+$ content did not vary in up to 200 mM NaCl and 300 mM $Na_2SO_4$ But it increased with a further increase in salinity (figure 1-A). $Cl^-$ content enhanced when NaCl increased but significantly inhibited at higher $Na_2SO_4$ (figure 1-E).

In terms of osmotic potential, despite the different higher values of Osmotic Potential at salinity treatments, it did not decrease significantly at different both salt concentrations and control treatment (figure 2-A). LAR, NAR, WUE, SLA, RWC, root and shoot dry weight increased with salinity increasing in up to 300 mM but eclined with more increases in salinity, with two exceptions of shoot dry weight at $Na_2SO_4$ which remain unchanged and RWC which decreased at moderate $Na_2SO_4$ and promoted at higher salinity. Also, there were not significant differences between low (100 mM) and moderate (300mM) NaCl treatments (figures 2-B, 2-C, 2-D, 2-E, 2-F, 2-G).

Also, results showed that glucose and proline did not vary at $Na_2SO_4$ and NaCl salts, respectively. But, proline increased at low $Na_2SO_4$ concentrations and glucose was significantly higher at 500 mM of NaCl, (figures 3-A, 3-B).

**Table 2.** Results of One-Way Analysis of Variance of Characteristics by Salinity

| Dependent variable | $Na_2SO_4$ | NaCl | Dependent variable | $Na_2SO_4$ | NaCl |
|---|---|---|---|---|---|
| $Ca^{2+}$ | ***16.01 | ***17.12 | Root dry weight | **10.13 | *3.68 |
| $Mg^{2+}$ | ***15.537 | ***25.11 | Shoot dry weight | ns 2.09 | **8.79 |
| $K^{2+}$ | ***88.67 | ***33.88 | WUE | **6.20 | **9.67 |
| $Na^{2+}$ | ***89.61 | ***16.00 | NAR | **5.23 | **6.78 |
| $Cl^-$ | ***6.22 | ***33.35 | LAR | *4.89 | **6.00 |
| OP | ns 0.56 | 1.58 ns | SLA | 2.81* | *3.37 |
| Proline | ***21.94 | ns 2.88 | RWC | *3.23 | **6.62 |
| Glucose | ns 2.34 | *4.47 | | | |

Note: Numbers represent F values, *P< 0.05, **P< 0.01, ***P< 0.001. ns. Non-significant.

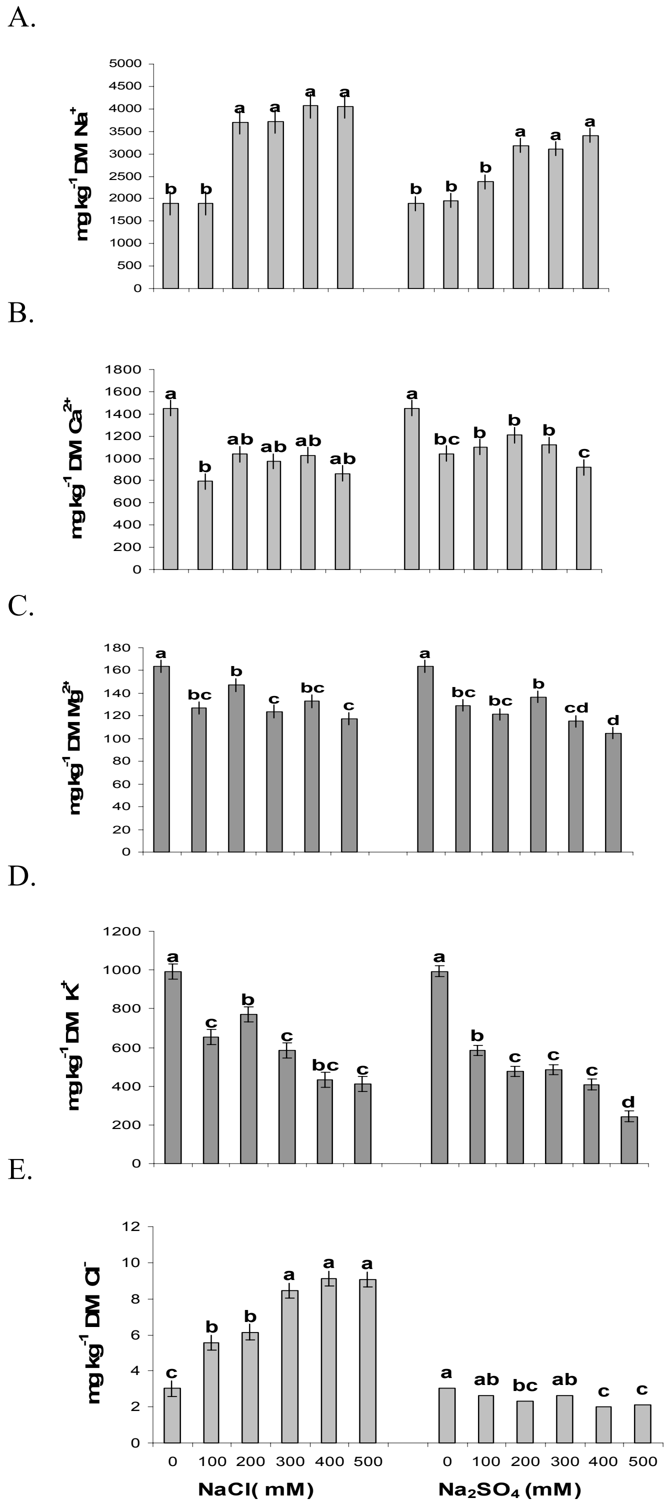



**Fig. 1.** Duncan test of $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$ and $Cl^-$ at different concentrations of NaCl and $Na_2SO_4$ - Bars represent mean ± standard error. Different letters represent a significant difference P < 0:05 between treatments.

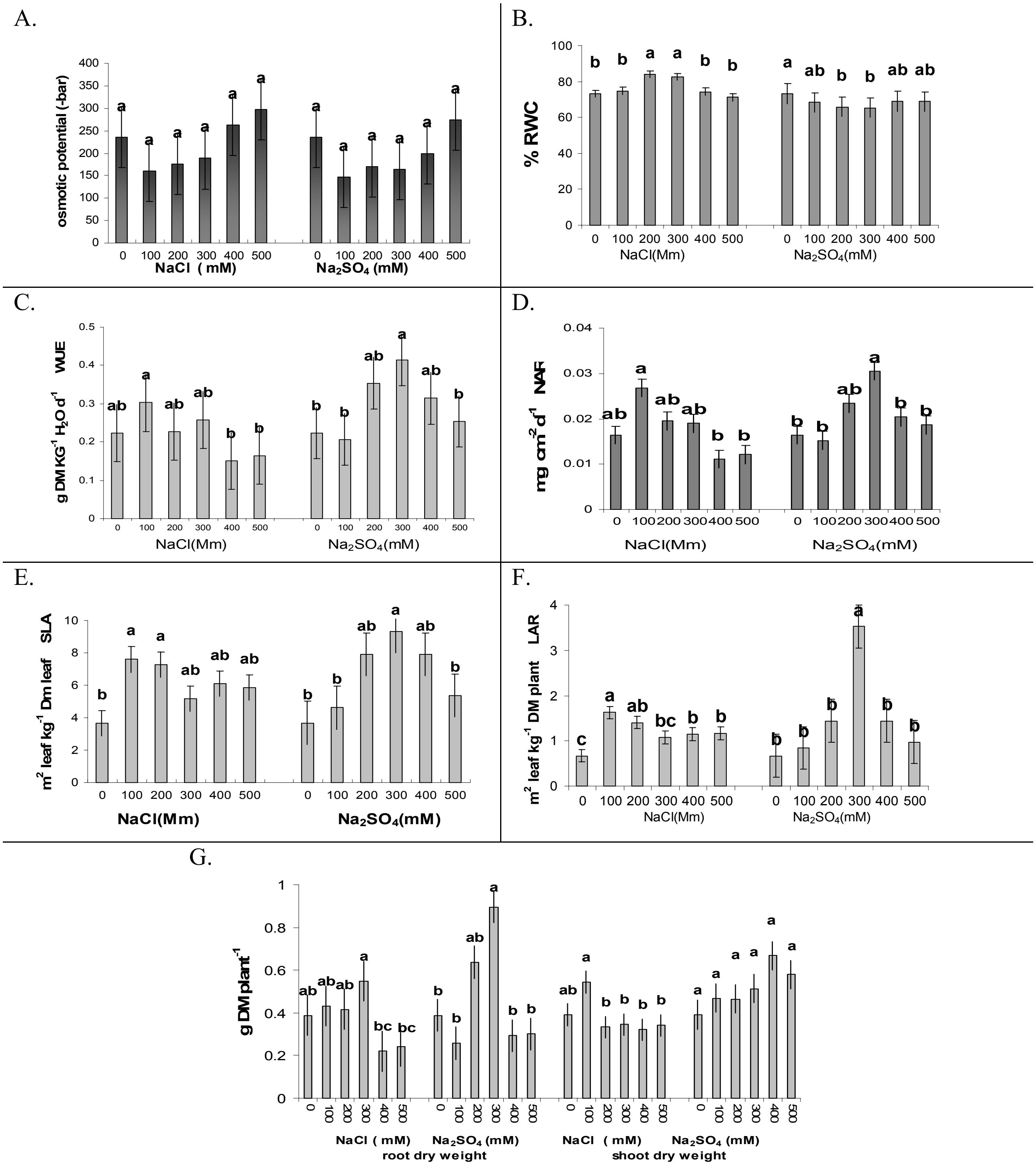
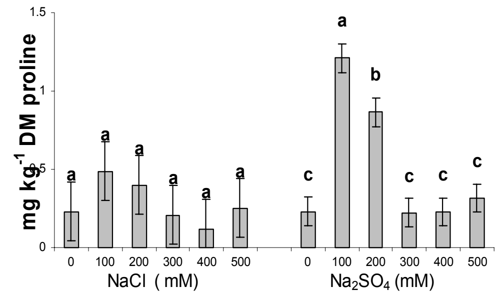



**Fig. 2.** Duncan test of osmotic potential (A), RWC (B), WUE (C), NAR (D), SLA (E), LAR (F), root and shoot dry weight (G) at different concentrations of NaCl and $Na_2SO_4$ - Bars represent mean -± standard error. Different letters above bars represent a significant difference P < 0:05 between treatments.




A.

B.

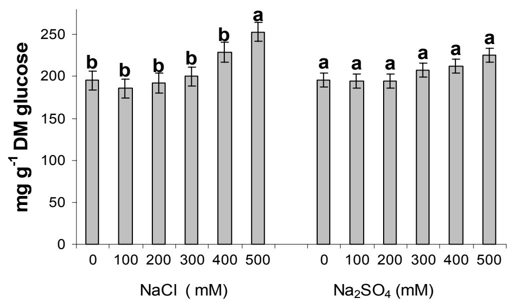



**Fig.3**. Duncan test of Proline (A) and glucose (B) at different concentrations of NaCl and $Na_2SO_4$ - Bars represent mean -± standard error. Different letters represent a significant difference P < 0:05 between treatments.

Results of T-Test between NaCl and $Na_2SO_4$ showed that ion concentration, Osmotic potential and RWC at NaCl are more than those at $Na_2SO_4$. . While, NAR, WUE, root and shoot dry weight at $Na_2SO_4$ is more. SLA, LAR, $Ca^{2+}$, proline and glucose did not vary statistically (table2).

**Table 2.** Results of T-Test between NaCl and $Na_2SO_4$ in *S. herbacea*

| variable | salt | means | SE | t | variable | salt | means | SE | t |
|---|---|---|---|---|---|---|---|---|---|
| SLA | $Na_2SO_4$ | 7.018 | 0.997 | 0.484 ns | NAR | $Na_2SO_4$ | 0.0208 | 0.0023 | 3.37** |
| | NaCl | 6.53 | | | | NaCl | 0.0129 | | |
| proline | $Na_2SO_4$ | 0.563 | 0.0871 | 2.009 ns | LAR | $Na_2SO_4$ | 1.645 | 0.394 | 1.171 ns |
| | NaCl | 0.393 | | | | NaCl | 1.184 | | |
| glucose | $Na_2SO_4$ | 207.002 | 5.772 | -0.267 ns | $Ca^{2+}$ | $Na_2SO_4$ | 363.75 | 17.08 | -0.771 ns |
| | NaCl | 208.54 | | | | NaCl | 376.59 | | |
| RWC | $Na_2SO_4$ | 67.52 | 2.09 | **-4.734 | $Mg^{2+}$ | $Na_2SO_4$ | 112.9 | 6.52 | **-4.039 |
| | NaCl | 77.42 | | | | NaCl | 139.28 | | |
| Osmotic potential | $Na_2SO_4$ | -152.46 | 18.61 | **3.067 | $K^+$ | $Na_2SO_4$ | 565.54 | 38.38 | **-4.641 |
| | NaCl | -209.55 | | | | NaCl | 743.68 | | |
| Root dry weight | $Na_2SO_4$ | 0.437 | 0.0647 | 2.078* | $Na^+$ | $Na_2SO_4$ | 4140.7 | 347.24 | **-3.97 |
| | NaCl | 0.297 | | | | NaCl | 5519.35 | | |
| Shoot dry weight | $Na_2SO_4$ | 0.539 | 0.0589 | 3.63** | $Cl^-$ | $Na_2SO_4$ | 4.67 | 0.949 | **-11.22 |
| | NaCl | 0.325 | | | | NaCl | 15.33 | | |
| WUE | $Na_2SO_4$ | 0.283 | 0.0317 | 3.37** | | | | | |
| | NaCl | 0.176 | | | | | | | |

Note: * p<0.01, ** p< 0.001, ns; non significant

**Discussion**

This study shows that decreasing of $Ca^{2+}$, $Mg^{2+}$ and $K^+$ and increasing of $Na^+$ when salinity increases is an active mechanism of salt tolerance in Glasswort (*S. herbacea L.*) that has been reported by Khan et al. (2001); Khan et al. (2000); Tikhomirova et al. (2005); Karimi 2004; Yarnia (2001) and Moghaieb et al. (2004). It seems that there are mechanisms for $Na^+$ transfer against other ions but uptake of $Cl^-$ depends on soil salt. Although, NaCl is the major salt in Iran's soil, $Cl^-$ increases with salinity increasing. In contrast, $Cl^-$ decreases in *S. herbacea* at $Na_2SO_4$ salt. It may be suggested that uptake of $Cl^-$ depends on its abundance and existence of other anions in soil.

Exposure to salinity concentration increases succulence of plant so that ions accumulate in vacuoles; but ions accumulation decreased with more increases in salinity (Khan et al. 2001; Gul et al. 2000; Reimann & Breckle, 1995). Our results showed the optimal NAR, LAR, WUE, SLA, RWC and dry weight of Glasswort (*S. herbacea* L.) at 100 to 300 mM NaCl and $Na_2SO_4$. Ungar (1978), Austenfeld (1974), Webber (1997) and Khan et al. (2000) showed that the optimum growth of *Salicornia sp* is from 170 to 340 mM NaCl. The growth response at moderate salinity may be considered as consequence of $Na^+$ use as an essential nutrient element (Pessarakli, 2001), higher WUE and higher photosynthesis via increasing leaf area; but at high salinity, growth reduction can be consequence of high uptake of $Na^+$ and $Cl^-$ , nutrient deficiencies and excessive demand on the energy requirement of compatible soluble production (Reimann & Breckle, 1995; Raschke, 1977). It seems that reduction of RWC at $Na_2SO_4$ is related to the high affinity of $Na^+$ content and RWC (Yoshie, 1994). Na+ content did not vary in up to 300 mM of $Na_2SO_4$ , While cations and $Cl^-$ decreased at this salinity concentration. Therefore, RWC decreased in up to 300 mM.

Growth and survival of halophytes depend on the high level of ion accumulation for the maintenance of turgor and osmotic adjustment (Ungar, 1987). Water relations and the ability to adjust osmotic pressure have been found as important determinants of the growth response (Gul et al. 2000; khan et al. 2000; Flowers et al. 1977; Parks et al. 2002). Measurement of osmotic potential showed that it did not decline significantly with salinity increasing. Also, glucose promotion in up to 400 mM and proline at high salinity were not reported. It may be suggested that uptake of $Na^+$ and $Cl^-$ together with repulse of $K^+$, $Ca^{2+}$ and $Mg^{2+}$ do not relate to osmotic adjustment in up to 500 mM salinity and these ions replacements are due to ionic characteristics of $Na^+$ and $Cl^-$.

Also, Glasswort (*S. herbacea L.)* uptakes more ions in chloride soil than that of sulfate soil but WUE, NAR and dry weight are more at sulfate soil. It is also assumed that salt tolerance mechanism of *S. herbacea* changes at different salts. More ion absorption was observed through increasing of osmotic potential at NaCl. But *S. herbacea* repulses ion at $Na_2SO_4$. Dry weight and WUE stimulation by $Na_2SO_4$ indicate that the growth of *S. herbacea* is higher in sulfate soil than that of chloride soil. These results are also similar in line with Rezaee et al. (2004) and Indulkar & More (1984). But Rasouli (2008), Olga & Babourin (2000) and Merit et al. (2008) represent that sulfate sodium is more toxicant than chloride sodium.

In summary, Glasswort (*S. herbacea L.)* is a high tolerant halophyte which grows well up to 500 mM of NaCl and $Na_2SO_4$ salt. Salinity enhances the growth of *S. herbacea* and its optimum growth occurs at 100 – 300 mM. Proline, glucose and osmotic potential remain unchanged at moderate salt concentrations. Also, it tolerates salinity via uptake of ions at NaCl and also ions repulsing and increasing WUE at $Na_2SO_4$. *S. herbacea* grows at sulfate better than chloride.

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(Received, 31 Jan. 2010, Accepted, 25, May, 2010)