Journal of Plant Physiology and Breeding

2017, 7(2): 81-90 ISSN: 2008-5168

Physico-Chemical Evaluation of Some Wormwood (*Artemisia absinthium* L.) Ecotypes Under Salt Stress Condition

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Received: October 29, 2016 Accepted: May 30, 2017
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Abstract

The physiological and biochemical responses of three ecotypes of *Artemisia absinthium* L. from Iran were evaluated under salinity conditions. Salinity treatments were made by NaCl with EC of 0, 5, 10 and 15 dS/m. Physiological and biochemical attributes were measured 30 days after the salt treatments imposed. Highest reduction in the shoot and root fresh weights was observed in the ecotypes of East Azarbaijan and Guilan at 10 and 15 dS/m salinity levels. The ecotype of Semnan was affected lesser by the same salinity levels than the control. Total chlorophyll showed a significant decrease at all salinity levels in all ecotypes. But the ecotype of East Azarbaijan showed a higher reduction at 10 and 15 dS/m salinity. Increasing the concentration of exterior salt led to increase the Na^+ and Cl^- concentrations in leaves, stems and roots. The highest Na accumulation in stems was found in the ecotype of East Azarbaijan at 10 and 15 dS/m. The highest proline was detected in the ecotype of Semnan. The results indicated that there was different responses to salinity among the studied *Artemisia absinthium* ecotypes. The ecotypes of Semnan and Guilan were more salt tolerant than the East Azarbaijan ecotype. The relative tolerance of the ecotype of Semnan was due to the high amount of output capacity of Na^+, Ca^{2+} concentration, as well as its proline content.

Keywords: *Artemisia absinthium*; Ion content; Proline; Salinity

Introduction

One of the inhibiting factors in agriculture is the salinity in semi-arid and arid regions (Flowers *et al*. 1977). More than 800 million hectares of land throughout the world are salt affected (FAO 2008).

Artemisia absinthium L. (wormwood) belongs to the Asteraceae family and is distributed in Europe and Asia. In past, this plant was grown as an ornamental plant. It is a yellow-flowering perennial herbal plant. The aerial parts of this plant has a wide range of pharmacological and biological activities (Tan *et al*. 1998). *Artemisia absinthium* L. is a drought tolerant plant. However, there is little information about its salinity tolerance. In a study, the essential oil and its constituents in *Artemisia absinthium* was enhanced by increasing salinity levels. (Abd-El Nabi and Hussein 1996). The vegetative yield of *Artemisia annua* increased significantly with increasing salinity to 6 dS/m, but further salinity led to decrease in its yield (Prasad *et al*. 1998). On the other hand the chlorophyll content decreased in *Artemisia scoparia* and *A. vulgaris* cv. “variegate” under the salinity stress, with a higher reduction in *A. scoparia*. Also, the contents of proline, MDA, soluble carbohydrate, and Na^+ increased under salt stress in both species, but *A. vulgaris* “variegate” had a higher level of proline and soluble carbohydrates and lower level of MDA and Na^+ than other species (Guan *et al*. 2013). Chamomile (*Matricaria recutita* L.) under the salinity stress showed a reduction of fresh flower yield by increasing salinity. However, the saline irrigation water had no significant influence on the oil quantity and quality (chemical composition) (Baghalian *et al*. 2008). Salinity did not change the root or shoot biomass of the three *Echinacea* species studied (Sabra *et al*. 2012). NaCl concentrations of below 100 mM did not change the growth of safflower (*Carthamus tinctorius* L.) seedlings in terms of plant height, root length and plant dry weight, however, it increased the medicinal flavonoid content of the leaves in this plant (Gengmao *et al*. 2015). Irrigation with saline water increased the essential oil content and its main components in *Calendula officinalis* but decreased fresh weight and dry weight of flower heads and pigment contents (Khalid and da Silva 2010). The Na^+ concentration was higher in *Atriplex halimus* than in *Atriplex nummularia* and its accumulation in the roots of the *A. halimus* MOR2 clone was far greater than in the leaves (Belkheiri and Mulas 2013). Regarding to pharmaceutical importance of *Artemisia* ssp. and needs to use saline lands for growing minor crops including medicinal plants, this study was conducted to evaluate *Artemisia absinthium* response to salinity using physiological and biochemical characteristics.

Materials and Methods

The experiment was carried out in Department of Horticulture, Islamic Azad University, Karaj Branch, Iran. Seeds of *Artemisia absinthium* L. (ecotypes of Semnan, East Azarbaijan, Guilan) obtained from IBRC (Iranian Biological Research Center). The seeds were sown in trays containing a mixture of peat: perlite (1:1, v: v). After 30 days, seedlings were transferred to pots (25 cm diameter and 25 cm deep) containing a mixture of field soil (Table 1): perlite: peat: sand (1:1:1:1, v:v:v:v). The plants were grown under the following greenhouse conditions: 16/8 photoperiod, average day/night temperature of 35/15 ºC, 70% RH and when the light intensity was lower than 13 Klux, natural light was supplemented with artificial lights of PHILIPS Tempo 3 series, model RVP351 SON-T 400 W S.K. with sodium vapor tubular lamps (Philips SON-T 400 W). The plants were watered every 3-4 days and fertilized every 2 weeks with the nutrient solution of Phusamko™ 4 (Kalvin Co., Iran). The established plants (60 days old, 30 cm height) were subjected to different saline irrigation water including 0 (control), 5, 10 and 15 dsm^{-1}. The solutions were made by dissolving appropriate amounts of NaCl in distilled water and the EC of the solutions was adjusted by a portable EC meter instrument (EUTECH Instruments, Ecoscan, Con5, Singapore). To avoid salinity shock, NaCl was daily added to the irrigation water by 2 dsm^{-1} increment until reaching the required concentration. The plants were subjected to saline irrigation water corresponded to field water capacity of the soil. Four weeks after applying salt treatments, physiological attributes and essential oil were measured. The physiological characters were as follows: relative water content (RWC) (Barrs and Weatherley 1962), cell membrane stability (CMS) (Blum and Ebercon 1981), free proline (Bates *et al*. 1973), soluble carbohydrates (Morris 1948) and photosynthetic pigments. The pigments were assayed by two procedures: by SPAD instrument (Konica Minolta, SPAD-502 Plus) and by the spectrophotometer (Arnon 1949). The concentrations of Na^+, K^+ and Ca^{2+} ions were

Table1. Chemical and physical characteristics of the soil

Sand (%)	Silt (%)	Clay (%)	O.C (%)	ECe (dS/m)	pH	Na^+ (mg/L)	Cl^- (mg/L)	Mg^{2+} (mg/L)	Ca^{2+} (mg/L)
18	36	46	0.76	1.34	8.08	119.97	92.3	34.32	110

determined based on Temminghoff and Houba (2004). To extract essential oil, 50 g of dried shoots from each replicate of all treatments was exposed to hydro distillation for 3 h by using a Clevenger-type apparatus (Clevenger 1928). The salinity of water (0, 5, 10, 15 dS/m) and ecotypes (Semnan, East Azarbaijan, Guilan) were considered as two factors for analyzing the data. The experiment was carried out as factorial based on a completely randomized design with six replications. Mean comparisons were made by the method of Duncan's multiple range test at 5% probability level. All statistical analyses were done by SAS v. 9.1 software (SAS Institute 2004).

Results

RWC decreased with increasing the salinity levels (Figure 1A). The ecotype of Semnan maintained the highest RWC at the control and all salinity levels. A significant reduction in the salinity of 15 dsm^{-1} was found in the ecotype of Guilan. There was a high cell membrane stability in the ecotype of Semnan as compared to the ecotypes of Guilan and East Azarbaijan (Figure 1B). Chlorophyll a (Chl a) was not affected in the ecotypes at all salinity levels (Figure 2A). Chlorophyll b (Chl b) was not affected significantly by 5 dS/m as compared to the control in all ecotypes (Figure 2B). Total chlorophyll decreased significantly at all salinity levels and all ecotypes. The ecotype of East Azarbaijan showed higher reduction than the control as compared to other ecotypes at 10 and 15 dS/m salinity (Figure 2C). Carotenoids were not affected by all salinity levels in the ecotype of Semnan while they were increased by increasing salinity levels in the ecotypes of East Azarbaijan and Guilan (Figure 2D). In response to EC of 15 dS/m, the amount of proline was obviously increased in the ecotype of Semnan (Figure 3A). On the other hand, soluble carbohydrates increased in the Semnan ecotype with increasing salinity levels. Although salinity increased soluble carbohydrates in the ecotypes of East Azarbaijan and Guilan, there were no significant differences among salinity levels (Figure 3B). The amount of sodium concentration elevated in the leaves, stems of all ecotypes by increasing salinity concentration (Figure 4A). The Na^+ concentration in the leaves of the Semnan ecotype was significantly increased until 15 dS/m salinity while in the ecotypes of East Azarbaijan and Guilan it was not affected by 10 and 15 dS/m salinity levels (Figure 4A). The highest sodium concentration of stems and roots were found in the East Azarbaijan ecotype at 10 and 15 dS/m salinity (Figures 5A and 6A). The chloride concentration of leaves, stems and roots in all ecotypes were elevated by increasing salinity (Figures 4B, 5B and 6B). The potassium concentration of leaves in the Semnan ecotype didn't not change at all salinity conditions while

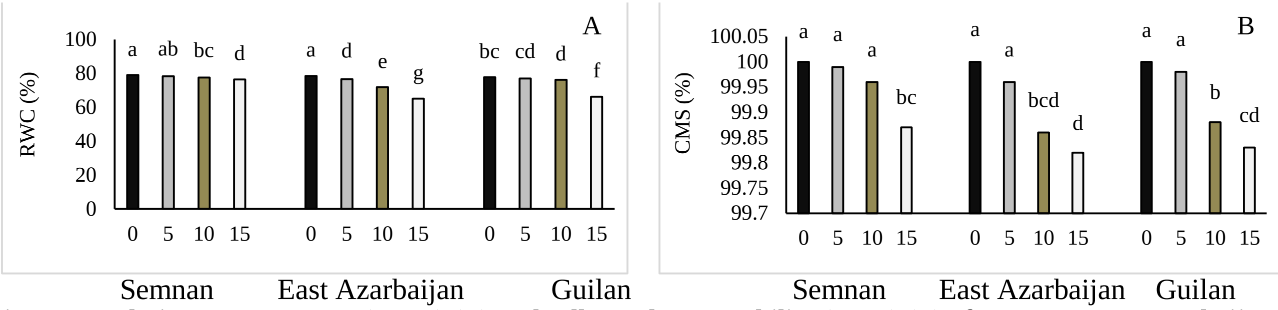

Figure 1. Relative water content (RWC) (A) and cell membrane stability (CMS) (B) of Semnan, East Azarbaijan and Guilan ecotypes of *Artemisia absinthium* L. exposed to 0, 5, 10 and 15 dS/m NaCl for four weeks. Values are the mean of 6 replicates ± S.E. Means are considered different at p≤0.05 when followed by different letters within the character measured.

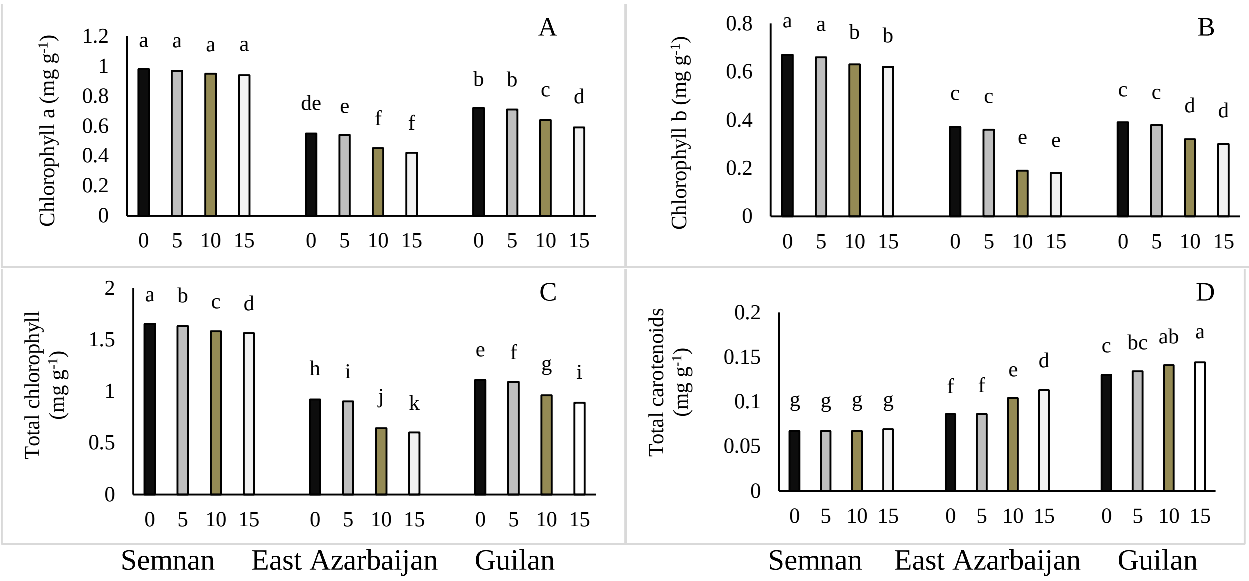

Figure 2. The photosynthetic pigments (chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and total carotenoids (D)) of Semnan, East Azarbaijan and Guilan ecotypes of *Artemisia absinthium* L. exposed to 0, 5, 10 and 15 dS/m NaCl for four weeks. Values are the mean of 6 replicates ± S.E. Means are considered different at p≤0.05 when followed by different letters within the character measured.

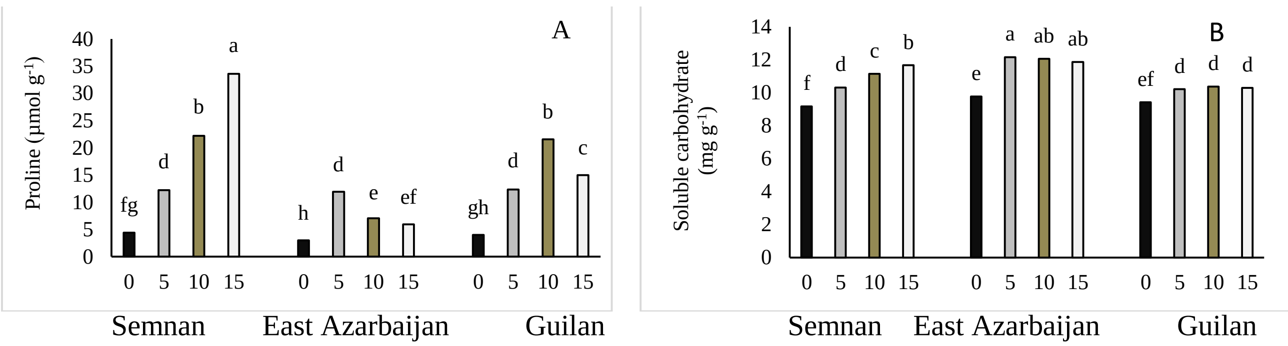

Figure 3. The proline (A) and soluble carbohydrate (B) concentrations of Semnan, East Azarbaijan and Guilan ecotypes of *Artemisia absinthium* L. exposed to 0, 5, 10 and 15 dS/m NaCl for four weeks. Values are the mean of 6 replicates ± S.E. Means are considered different at p≤0.05 when followed by different letters within the character measured.

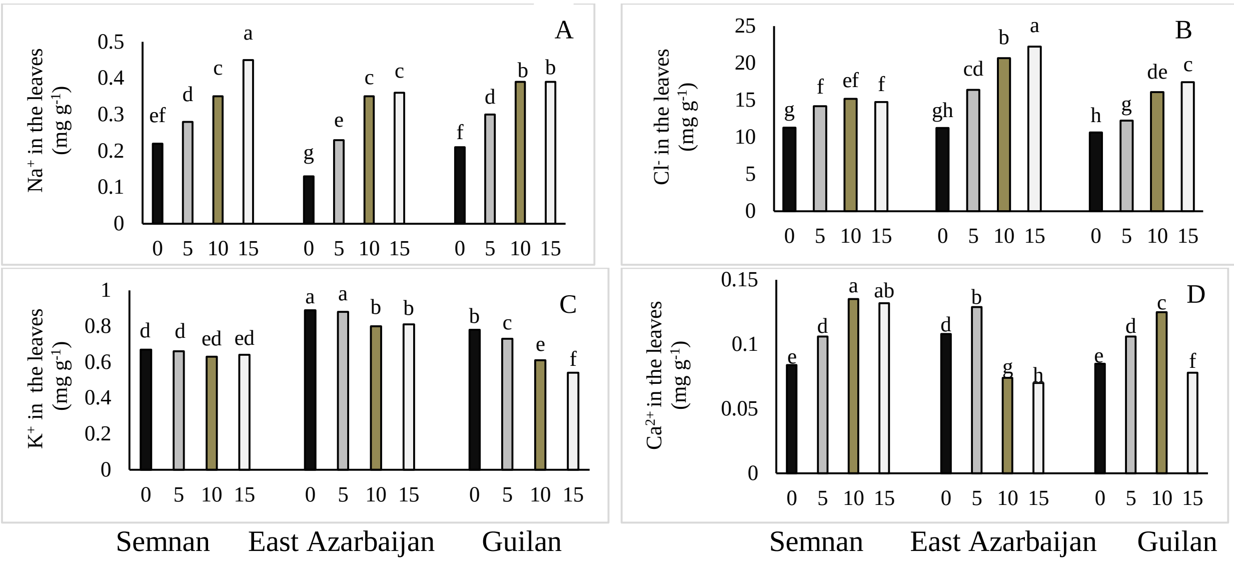

Figure 4. The sodium (Na^+) (A), chloride (Cl^-) (B), potassium (K^+) (C) and calcium (Ca^{2+}) (D) accumulations in the leaves of *Artemisia absinthium* ecotypes exposed to 0, 5, 10 and 15 dS/m NaCl for four weeks. Values are the mean of 6 replicates ± S.E. Means are considered different at p≤0.05 when followed by different letters within the character measured.

while it decreased in the ecotypes of East Azarbaijan and Guilan by increasing salt concentration (Figure 4C). The trend of the potassium concentration in stems and roots displayed different responses of the ecotypes, since the ecotype of Semnan was different than other ecotypes. In this ecotype, potassium concentration decreased by elevation of salinity (Figures 5C and 6C). High concentrations of calcium in the leaves were found in the ecotype of Semnan at higher salinity levels while the calcium concentration in other ecotypes decreased at 10 dS/m salinity (Figure 4D). Calcium concentration in stems had a decreasing trend in all ecotypes (Figure 5D). The K^+/Na^+ ratio was significantly reduced under salinity conditions in leaves, stems and roots, but it was drastically decreased in leaves and stems of East Azarbaijan Ecotype. The Ca^{2+}/Na^+ ratio had similar trend to K^+/Na^+ ratio, however, the ecotype of Semnan changed slightly in relation to the control as compared to other ecotypes. The highest essential oil content was found in the ecotype of Semnan at 10 and 15 dS/m salinity levels. Essential oil increased in the ecotypes of East Azarbaijan and Guilan until 5 and 10 dS/m salinity, respectively (Figure 7).

Discussion

The different response of plants to the salinity levels depended on the concentrations and compositions of ions in the solution as well as the genotypes under study (Greenway and Munns 1980). The decrease in RWC and CMS under the salinity stress in all ecotypes was in confirmation with other reports about Kharachia 65 and KRL 19 genotypes in wheat (Sairam *et al.* 2002). However, RWC and CMS of the ecotype of Semnan decreased less than others. The results showed the same trend in both RWC and CMS data in *Artemisia absinthium*. The total chlorophyll decreased by increasing salinity levels, however, the extent of reduction was lower in Semnan than the other ecotypes. In other studies, the total chlorophyll of *Artemisia annua* (Qureshi *et al.* 2005; Guan *et al.* 2013) and *Artemisia scoparia* (Guan *et al.* 2013) decreased slightly by increasing salinity levels. The carotenoids of the ecotype of Semnan showed no significant change under salinity levels as compared to the control, however, the carotenoids of East Azarbaijan and Guilan ecotypes elevated by increasing salinity. Other researchers also showed the lack of significant difference of 5 and 10 dS/m salinity with the control treatment in *Echinacea pallida* (Sabra *et al.* 2012) and *Carthamus tinctorius* (Gengmao *et al.* 2015) in terms of carotenoids content. The carotenoids are often known as a phyto-pigments that are involved in plant responses to environmental stresses and act as an antioxidant to overcome reactive oxygen species (ROS) problems. The decrease in plant growth and yield under the salinity maybe affected by changing the transportation of the photosynthetic assimilates to the roots. The reduction of shoot growth, particularly leaves, by salinity could be the consequence of stomatal closure, low efficiency of the photosynthetic system and the change of the ion homeostasis (Heidari Sharifabad 2001). Salinity tolerance may be assessed by the amount of compatible solutes such as ammonium compounds (i.e. proline) or carbohydrates in halophytic plants. The amount of accumulation of these solutes has been attributed to the osmotic stress of the other effects of salinity (Munns 2002). The highest proline concentration was found in the ecotype of Semnan at 15 dS/m salinity, however, it also increased in the ecotypes of East Azarbaijan and Guilan until 5 and 10 dS/m salinity levels, respectively. Other researchers also showed the increase in proline concentration due to the increase of salinity levels in *Cynodon dactylon* (Hameed and Ashraf 2008), *Artemisia vulgaris* (Guan *et al*. 2013) and *Atriplex halimus* (Belkheiri and Mulas 2013). Similar to proline, the soluble carbohydrates have a key role in the osmotic regulation. The soluble carbohydrates of *Artemisia absinthium* ecotypes changed in salt treatments. The highest sodium concentration of leaves was found in the ecotype of Semnan at 15 dS/m salinity, while the highest sodium concentrations of stems and roots were observed in the ecotype of East Azarbaijan at 10 and 15 dS/m. In other researches, the sodium concentration levels in the plant organs of *Helianthus annuus* L. (Flagella *et al.* 2004) and *Carthamus tinctorius* (Gengmao *et al*. 2015) were increased also by increasing salinity level. The chloride (Cl^-) was increased by increasing salinity levels in all organs of the ecotypes under study. Falagella *et al*. (2004) in *Helianthus annuus* and Koyro (2006) in *Plantago coronopus* reported similar results about the concentration of Cl^- in leaves due to salinity. There were no significant differences among the salt doses in the ecotype of Semnan for potassium concentration in leaves. According to Gengmao *et al*. (2015), the potassium concentration of leaves and stems in *Carthamus tinctorius* at 5 and 10 dS/m salinity levels were not significantly different from the control. The K^+/Na^+ ratio of leaves and stems decreased in all of the ecotypes under study. The high amounts of Na^+ concentration in the soil would cause to decrease available amounts of Ca^{2+}, K^+ and Mg^2

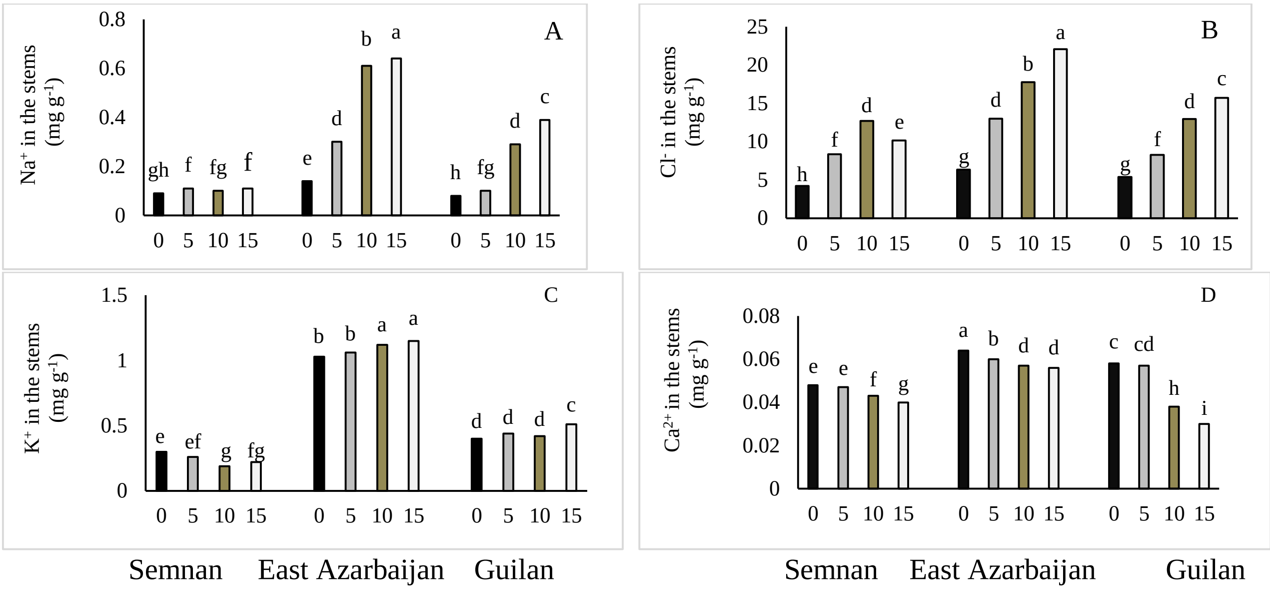

Figure 5. The sodium (Na^+) (A), chloride (Cl^-) (B), potassium (K^+) (C) and calcium (Ca^{2+}) (D) accumulations in the stems of *Artemisia absinthium* ecotypes exposed to 0, 5, 10 and 15 dS/m NaCl for four weeks. Values are the mean of 6 replicates ± S.E. Means are considered different at p≤0.05 when followed by different letters within the character measured.

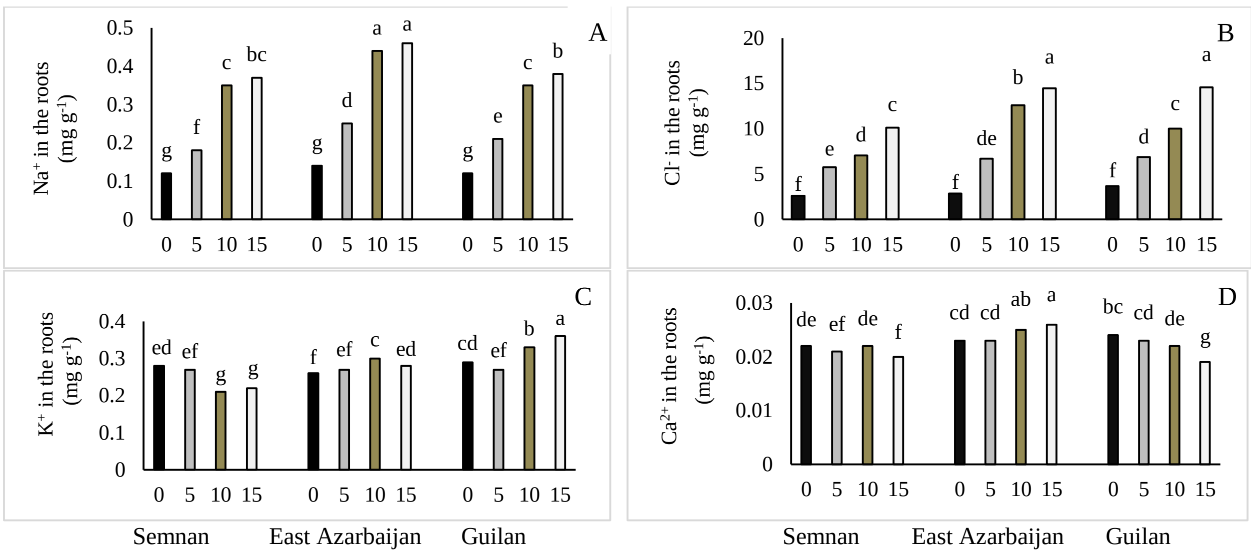

Figure 6. The sodium (Na^+) (A), chloride (Cl^-) (B), potassium (K^+) (C) and calcium(Ca^{2+}) (D) accumulations in the roots (C) of *Artemisia absinthium* ecotypes exposed to 0, 5, 10 and 15 dS/m NaCl for four weeks. Values are the mean of 6 replicates ± S.E. Means are considered different at p≤0.05 when followed by different letters within the character measured.

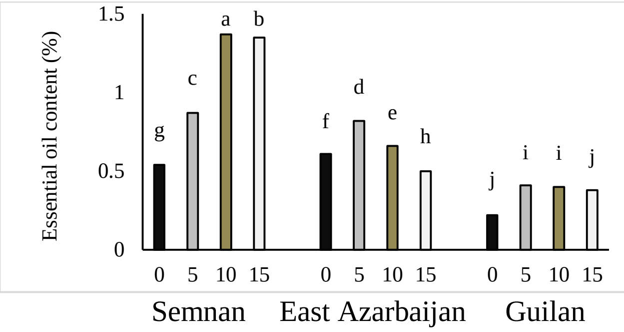

Figure 7. The essential oil content in the shoots of *Artemisia absinthium* ecotypes exposed to 0, 5, 10 and 15 dS/m NaCl for four weeks. Values are the mean of 6 replicates ± S.E. Means are considered different at p≤0.05 when followed by different letters within the character measured.

(Cramer *et al*. 1985). Moreover when Na^+ as a cofactor creates interference with the potassium function, it exerts a direct toxic effect (Khan *et al*. 2000). Na^+ is able to substitute K^+ in a special osmotic functions in the vacuole. Therefore, because of the lack of K^+, the extra amount of Na^+ can upgrade the plant growth. The extent to which Na^+ can replace K^+ varies among plant species, different cultivars of the same species and even in different leaves of the same plant, with younger leaves relying on more K^+ than older leaves (Lindhauer *et al*. 1990).

The calcium concentration of leaves and the amount of proline had similar trend in response to salinity. The Ca^{2+}/Na^+ ratio of leaves decreased by increasing salinity levels. Ca^{2+} regulates and also alleviates the negative influence of salinity stress on plant growth (Ahmad and Prasad 2011; Sarwat *et al*. 2013).

The essential oil content was increased in the Semnan ecotype at 10 and 15 dS/m salinity. Abd-El Nabi and Huseein (1996) also reported an increase in the essential oil content of *Artemisia absinthium* under salt stress. The stimulation of more essential oil production may be due to higher density of trichomes that contain essential oils under salinity conditions. The salt stress may indirectly affect the accumulation of essential oil by uptake and distribution of the nutrients in the growth and development processes (Charles *et al*. 1990). The decrease in essential oil production attributes to the decrease of plant anabolism, however, the increase of essential oil under the salinity is due to the decrease of primary metabolites which are used in the synthesis of secondary metabolites (Morales *et al*. 1993). The effect of salinity on essential oil and its components has been mainly attributed to the effect of enzyme activity and metabolism (Burbott and Loomis 1969).

Conclusion

Salinity induced several physiological and biochemical changes in *Artemisia absinthium* ecotypes. However, the changes were different among ecotypes. The ecotype of Semnan is a salinity tolerant ecotype because it had higher proline and soluble carbohydrates under saline condition as compared to the control because these compounds led to maintain high RWC and CMS. Thus not only these compounds led to maintain high RWC and CMS but also could be helpful to lessen the effect of salinity on chlorophyll a and chlorophyll b. Also potassium concentration of the Semnan ecotype did not change from normal to salinity treatments in contrast to other ecotypes. Furthermore, calcium concentration of Semnan leaves increased until the 15 dS/m salinity, but decreased in other two ecotypes at this salinity level. In addition, the essential oil concentration of Semnan ecotype had the highest increase at 10 and 15 dS/m salinity levels relative to the control. The ecotype of Guilan indured salt until 10 dS/m salinity. The ecotype of East Azarbaijan was the most salt sensitive ecotype based on the measured traits. In general, the increase in Ca^{2+}, proline and soluble carbohydrates in leaves and also essential oil content can be regarded as the salinity tolerant mechanisms in the studied *Artemisia absinthium* ecotypes under this experimental condition.

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