# The effects of methanol and ethanol foliar application under salinity stress on some physiological characteristics of Pelargonium graveolens L. 

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#### Abstract

To study the effects of methanol and ethanol foliar application ( 0,10 and $20 \% \mathrm{v} / \mathrm{v}$ ) and NaCl salinity stress ( 0,75 and 150 mM ) on yield and some physiological traits of geranium (Pelargonium graveolens L.), a factorial experiment was conducted based on completely randomized design with three replications. Salinity showed significant effect on all characteristics, except root dry weight, chlorophyll b and Fe content. Effect of foliar application of methanol and ethanol was significant on proline, protein, chlorophyll a, essential oil, Fe and K content, root dry weight and $\mathrm{IC}_{50}$. The results also revealed the significant interaction of salinity by foliar application of alcohol in relation to the chlorophyll a and protein content. The greatest protein and chlorophyll a contents were recorded by the $\mathrm{NaCl}_{0}+$ methanol $_{20 \%}$ treatment, which was significantly different from the corresponding control. Dry weight of aerial parts, K/Na ratio, essential oil, K, P, Fe and Zn contents were negatively affected by the salinity stress. With increasing salinity stress the amounts of malondialdehyde and $\mathrm{H}_{2} \mathrm{O}_{2}$ were elevated. Among alcohol treatments, methanol foliar application was more effective than ethanol. Methanol had better effect on $\mathrm{IC}_{50}$, root dry weight, $\mathrm{Fe}, \mathrm{K}$ and essential oil content, while ethanol ${ }_{20 \%}$ increased the proline content significantly as compared to the methanol and control treatments. Overall, the results indicated that foliar application of methanol ameliorated the negative effects of salinity in geranium, when averaged over the salinity levels under study.


Keywords: Chlorophyll; Essential oil; Malondialdehyde; NaCl; Pelargonium graveolens L.

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## Introduction

Geranium (Pelargonium graveolens L.) is an important evergreen medicinal and aromatic ornamental plant. This plant is valued for its rose scented oil (Becker and Brawner 1996). Geranium oil has also antibacterial (Ghannadi et al. 2012) and anti-inflammatory (Elmann et al. 2010; Boukhatem et al. 2013) activities. Moreover, the oil of geranium is used as relaxant and anti-depressant in aroma therapy (Rashidi Fakari et al. 2015; Abouhosseini Tabari et al. 2018). It is used to decrease the diastolic blood pressure (Rashidi Fakari et al. 2015).

The most important components identified in the geranium oil are citronellol, geraniol and citronellyl formate (Boukhatem et al. 2013).

Abiotic stresses, particularly salinity stress, is one of the major limitations of plant productivity and causes considerable yield and economic losses for the producers (Shrivastava and Kumar 2015). High salinity imposes damages on plants including growth inhibition, necrosis (Sivritepe and Eris 1999), nutrients imbalance, osmotic stress (Leidi et al. 1992) and impaired metabolism (Larcher 2003). Plants exposed to salinity stress produce reactive
oxygen species (ROS) that are toxic to proteins, lipids and carbohydrates. Prolonged stressful condition commonly damage cell membrane and eventually lead to cell death (Gill and Tuteja 2010). Therefore, it is important to use cheap and available methods to enhance salinity stress tolerance in plants.

Nowadays, with increasing population, researchers tend to use growth enhancers to improve crop production. Photosynthesis is the essential process for the production of organic matter in plants. Using methanol and ethanol have been confirmed as a nontoxic and easy way to encourage plant growth. Foliar spray of alcohol directly affects the metabolic pathways related to plant growth and development (Gout et al. 2000), and pathways related to plant defense mechanisms (Dorokhov et al. 2018). According to Gout et al. (2000), application of methanol on sycamore (Acer pseudoplatanus L.) was incorporated into the methyl groups of molecules, such as serine, methionine and phosphatidylcholine. Methanol spray increased crop $\mathrm{CO}_{2}$ fixation in tomato and sugar beet (Zbiec et al. 2003). Methanol in plants is the by-product of pectin metabolism during cell wall synthesis (Fall and Benson 1996). The methylotrophic bacteria, which stimulate the production of auxins and cytokinins, volatilize or consume the internal methanol at the leaf surfaces (Lee et al. 2006). Nadali et al. (2010) reported that methanol spraying increased sugar yield of the sugar beet crop. In the study conducted by Vojodi et al. (2017) on Calendula officinalis, it was found that the flower and leaf dry weight as well as RWC
and total soluble solids were affected by the methanol spray and the highest value for the flower dry weight was recorded at $30 \%$ methanol foliar spray.

Nowadays, due to the high demands for the geranium oil on the international market, improving the oil quantity and quality of this plant is important. In recent years, due to the drying of Urmia lake (a saline lake in Iran), salinity level in the neighboring areas has begun to increase rapidly. To cope with the soil salinity, it will be useful to study the effects of different economic and easily available plant growth promoters on plants. Therefore, we investigated the effects of methanol and ethanol foliar application on some growth and physiological characteristics of geranium under salinity stress.

## Material and Methods

This work was conducted in a greenhouse of Azarbaijan Shahid Madani University, Tabriz, Iran, during the growing season of 2015-2016. The growing conditions were as follows: light intensity (fluorescent lamp) of about $450 \mu \mathrm{~mol} \mathrm{~m} \mathrm{~m}^{-2} \mathrm{~s}^{-1}, 16: 8$ hours (day/night) of photoperiod and $25: 20^{\circ} \mathrm{C}$ day/ night temperature regime. The root cuttings ( 20 to 30 cm long) of Pelargonium graveolens were planted in plastic pots ( 5 L ), filled with the mediumsized perlite. The pots were fed with the $1 / 2$ Hoagland's nutrient solution for the first three weeks for better establishment. Then, the NaCl salinity stress at three levels ( 0,75 and 150 mM ) were applied. To prevent salts accumulation in the medium, the pots were washed with tap-water once
a week. To avoid the sudden shock from the salinity stress, salinity treatment was begun from 25 mM , reaching to the defined levels by adding up 25 mM every seven days. Forty days after planting, the plants were treated by methanol and ethanol with three concentrations ( 0,10 and $20 \% \mathrm{v} / \mathrm{v}$ ). All solutions were freshly prepared before spraying. Plants were treated with ethanol and methanol 10 days after the completion of the salinity stress. The second treatment was applied 20 days after the first treatment. Forty days after applying the second treatment, the plants were harvested and plant dry weight, antioxidant activity, chlorophyll content, content of several elements ( $\mathrm{N}, \mathrm{P}, \mathrm{K}, \mathrm{Na}, \mathrm{Fe}, \mathrm{Zn}$ ), malondialdehyde, $\mathrm{H}_{2} \mathrm{O}_{2}$, protein, essential oil and proline were determined. The experiment was arranged as factorial based on completely randomized design with three replications. The data were subjected to the standard analysis of variance. To compare the means, LSD values were calculated at the $5 \%$ level of significance.

## Dry weight of roots and aerial parts

Dry weight of roots and aerial parts was determined after drying in a drying machine at $25^{\circ} \mathrm{C}$ for one week.

## Chlorophyll a and b

The chlorophyll pigments were extracted and determined according to Prochazkova et al. (2001). The absorbance was read by a spectrophotometer at 663 and 645 nm .

## Antioxidant Activity

Antioxidant activity was measured through the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH). First, 0.5 mM of the control solution was prepared in methanol. Second, 1 M of the control solution was added to 3 mL of different concentrations of each sample. The samples were put in dark at room temperature for 30 minutes. Then, the absorbance was read at 517 nm . The percent scavenging was calculated by the following formula (Zhang and Hamauzu 2004):

$$
\text { Percent scavenging }=\left(\mathrm{A}_{0}-\mathrm{A}_{1} / \mathrm{A}_{0}\right) \times 100
$$ where $\mathrm{A}_{0}$ and $\mathrm{A}_{1}$ are the absorbance of the control and test samples, respectively.

Antioxidant compounds were measured at different concentration of samples to obtain the amount of $\mathrm{IC}_{50}$. $\mathrm{IC}_{50}$ is defined as the concentration of antioxidant necessary to decrease the initial DPPH concentration by $50 \%$. IC $_{50}$ was derived from the regression of \% scavenging activity on antioxidant concentration and is expressed as $\mathrm{mg} / \mathrm{ml}$.

## Elements

Na and K content of the ground dried leaves were determined by the flame photometric method (Corning, 410, England). N and P were determined by the Kjeldahl and vanadate-molybdate methods, respectively. Zn and Fe content were measured by the atomic absorption apparatus (Shimadzu, AA6300, Japan) according to the methods described by AOAC (1990).

## $\mathrm{H}_{2} \mathrm{O}_{2}$ and MDA content

$\mathrm{H}_{2} \mathrm{O}_{2}$ content of the leaf samples was determined as the method described by Gondim et al. (2013). Leaf samples were homogenized with 0.5 (W/V) trichloroacetic acid and centrifuged at 12000 g for 15 min at $4{ }^{\circ} \mathrm{C}$. Then, 0.5 ml of supernatant was mixed with 1 ml of fresh potassium iodide and 0.5 ml of 100 mM potassium phosphate buffer $(\mathrm{pH}=7)$. After allowing the reaction to develop for one hour in dark, absorbance was read at 390 nm . Lipid peroxidation (MDA) was quantified according to the method of Hodges et al. (1999).

## Essential oil

Essential oil was extracted by Clevenger type apparatus. 30 g of dry plant material was extracted by water distillation for 3 h . The extracted essential oils were dried by anhydrous sodium sulfate (Vojodi Mehrabani et al. 2017).

## Protein content

Protein content was quantified by the spectrophotometer at 595 nm ( $\mathrm{T} 80^{+}$made in china), according to the method of Bradford (1976).

## Proline content

Proline content was determined by the method of Bates et al. (1973). Toluene was employed as the reference standard reagent.

## Results and Discussion

## Analysis of variance

Results of analyses of variance for the traits under
investigation were presented on Table 1. Effect of salinity was significant on all characteristics, except root dry weight, chlorophyll b and Fe content. Foliar application of methanol and ethanol was also significant on proline, protein, chlorophyll a, essential oil, Fe and K content, root dry weight and $\mathrm{IC}_{50}$. Interaction of salinity $\times$ alcohol foliar application was only significant for protein and chlorophyll a content. Thus, for protein and chlorophyll a content, the differences between alcohols were not similar at all salinity levels.

## Dry weight of aerial parts

Salinity stress had adverse effect on the dry weight of aerial parts and the highest amount ( 4.8 g ) was observed in the control plants. High salinity stress $(150 \mathrm{mM})$ decreased the plant dry weight up to 39.5 \% as compared to the control (Table 2). Akca and Samsunlu (2012) in walnut and Baatour et al. (2010) in Origanum majorana reported the significant decrease in plant dry weight by increasing the salinity stress. The reduced growth potential under salinity stress can be attributed to ions competition and imbalance at the rhizosphere area (Baatour et al. 2010; Alipour 2018) that influence the photosynthesis rate, stomatal conductance (Alipour 2018) and finally reduce the plant yield. On the other hand, plant response to the stressors are different depending on the intensity of stress (Valifard et al. 2017), duration of stress (Gupta and Huang 2014), and growth and developmental stages (Flowers and Yeo 1995).

## Root dry weight

According to Table 3, root dry weight was affected by the effects of foliar applications of 10 and $20 \%$ methanol. These treatments increased root dry weight ( 1.86 and 1.74 g , respectively) as compared to the control. The results of this investigation are in harmony with the findings of Vojodi et al. (2017). However, Valizadeh-Kamran et al. (2019) noted that methanol application under salinity condition didn't influence root dry weight of Lavandula, but the foliar methanol treatment improved the flowers dry weight. Possibly, methanol foliar application improves chlorophyll content, leaf area and photosynthesis (Ramadan and Omran 2005) and correspondingly allocates more assimilates to the root growth and development.

## Chlorophyll

Chlorophyll a content for the combinations of salinity levels with alcohols' foliar application are shown in Table 4. The highest chlorophyll a content was determined in $\mathrm{NaCl}_{0}+$ methanol ${ }_{20 \%}, \mathrm{NaCl}_{0}+$ ethanol ${ }_{10 \%}$ and $\mathrm{NaCl}_{75}+$ methanol ${ }_{20 \%}$. The lowest amount of chlorophyll a ( $0.8 \mathrm{mg} \mathrm{g} \mathrm{g}^{-1} \mathrm{FWt}$ ) was recorded with 150 mM NaCl with no foliar application. According to Nguyen et al. (2017), the chlorophyll content of the ethanol-treated plants was higher than the control plants under high salinity. Methanol foliar application positively influenced the chlorophyll content in Caliendula officinalis (Vojodi et al. 2017).

Although there was a salinity $\times$ alcohol interaction for the chlorophyll a content, but salinity at 150 mM reduced the value of this trait in geranium, with or without alcohol application. This reduction can be attributed to the destruction of chloroplasts under salinity stress (Alipour 2018).

## Essential oil

Positive and significant effect of $20 \%$ methanol foliar application on essential oil content of geranium was observed in this study; however, it was not significantly different from the methanol concentration of $10 \%$ (Table 3). We did not observe any significant difference between the control, $10 \%$ ethanol and $20 \%$ ethanol in terms of essential oil content (Table 3). Bagheri et al. (2014) also reported the positive effect of methanol spray on lavender plants. It seems that foliar application of methanol influences the metabolic (Gout et al. 2000) and stress-defensive (Nguyen et al. 2017) pathways, which possibly favors the essential oils biosynthesis and accumulation.

Although both salinity levels (75 and 150 mM ) didn't significantly affect the essential oil content, but the salinity of 150 mM reduced the essential oil content significantly as compared to 75 mM (Table 2). Valizadeh Kamran et al. (2019) also reported the significant reduction in essential oil content of Lavandula stoechas L. at higher salinity level ( 100 mM ) compared with the mild salinity ( 50 $\mathrm{mM})$ and control treatments.

Table 1. Analysis of variance for the effects of foliar application of methanol and ethanol on some physiological traits, elements, MDA and $\mathrm{H}_{2} \mathrm{O}_{2}$ contents of Pelargonium graveolens under salinity conditions.

| Source of <br> variation | df | Mean squares |  |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | Proline | $\mathrm{IC}_{50}$ | Protein | Essential <br> oil | Chl b | Chl a | RDW | ADW

Table 1 continued

| Source of variation | df | Mean squares |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Fe | Zn | K/Na | K | Na | P | N | MDA | $\mathrm{H}_{2} \mathrm{O}_{2}$ |
| Salinity (S) | 2 | 47899** | 230** | $33.2 * *$ | $117.2^{* *}$ | 440** | 1835947** | $1.6{ }^{\text {ns }}$ | 490** | 396** |
| Foliar application (FA) | 4 | 23313* | 20.9 | 0.91 | $130^{* *}$ | 13.5 | 463183 | 1.2 | 20 | 26.6 |
| $\mathrm{S} \times \mathrm{FA}$ | 8 | 12469 | 19.6 | 0.27 | 4.7 | 29 | 376731 | 0.36 | 26.6 | 24.7 |
| Error | 30 | 8646 | 8.7 | 4.4 | 14.7 | 48 | 222452 | 0.76 | 29.9 | 21.7 |
| CV |  | 17 | 18.4 | 14.3 | 15.3 | 11.9 | 13.9 | 15.9 | 17.1 | 10.3 |

* and ${ }^{* *}$ significant at $\mathrm{p} \leq 0.05$ and $\mathrm{p} \leq 0.01$, respectively; Chl: chlorophyll; RDW: root dry weight; ADW: dry weight of aerial parts; MDA: malondialdehyde.

Table 2. Means for essential oil content, dry weight of aerial parts, proline, $\mathrm{H}_{2} \mathrm{O}_{2}$, $\mathrm{MDA}, \mathrm{IC}_{50}$ and elements' content of Pelargonium graveolens under different salinity levels.

| Salinity level <br> $(\mathrm{mM})$ | Proline <br> $\left(\mathrm{mg} \mathrm{g}^{-1} \mathrm{FWt}\right)$ | Dry weight of <br> aerial parts $(\mathrm{g})$ | IC 50 <br> $(\mathrm{mg} / \mathrm{ml})$ | MDA <br> $(\mathrm{nmol} \mathrm{g}-1 \mathrm{FW})$ | H 2 O 2 <br> $(\mu \mathrm{~mol} / \mathrm{g} \mathrm{FWt)}$ | Essential <br> oil <br> $\left(\right.$ Lha $\left.^{-1}\right)$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0 | $1.8^{\mathrm{b}}$ | $4.8^{\mathrm{a}}$ | $2.6^{\mathrm{ab}^{\mathrm{b}}}$ | $12^{\mathrm{b}}$ | $10.6^{\mathrm{b}}$ | $3.5^{\mathrm{bb}}$ |
| 75 | $1.9^{\mathrm{b}}$ | $4.0^{\mathrm{ab}}$ | $2.2^{\mathrm{b}}$ | $15^{\mathrm{b}}$ | $14.5^{\mathrm{b}}$ | $3.9^{\mathrm{a}}$ |
| 150 | $2.8^{\mathrm{a}}$ | $2.9^{\mathrm{b}}$ | $2.9^{\mathrm{a}}$ | $23^{\mathrm{a}}$ | $20.8^{\mathrm{a}}$ | $3.02^{\mathrm{b}}$ |

Table 2 continued

| Salinity level (mM) | $\begin{gathered} \mathrm{Na} \\ \left(\mathrm{mg} \mathrm{Kg}^{-1} \mathrm{Dwt}\right) \end{gathered}$ | $\begin{gathered} \mathrm{K} \\ \left(\mathrm{mg} \mathrm{Kg}^{-1} \mathrm{Dwt}\right) \end{gathered}$ | $\begin{gathered} \mathrm{P} \\ \left(\mathrm{mg} \mathrm{Kg}^{-1} \mathrm{Dwt}\right) \end{gathered}$ | K/Na | $\begin{gathered} \mathrm{Fe} \\ (\mathrm{mg} \mathrm{Kg} \\ \text { - } \mathrm{Dwt}) \end{gathered}$ | $\begin{gathered} \mathrm{Zn} \\ \left(\mathrm{mg} \mathrm{Kg}^{-1} \mathrm{Dwt}\right) \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0 | $9^{\text {b }}$ | $21^{\text {a }}$ | $2291{ }^{\text {a }}$ | $3.8{ }^{\text {a }}$ | $1123{ }^{\text {a }}$ | $19.5{ }^{\text {a }}$ |
| 75 | $11.5{ }^{\text {b }}$ | $16^{\text {b }}$ | $2025{ }^{\text {ab }}$ | $1.7{ }^{\text {ab }}$ | $1053{ }^{\text {ab }}$ | $15.8{ }^{\text {b }}$ |
| 150 | $19.6{ }^{\text {a }}$ | $16^{\text {b }}$ | $1598{ }^{\text {b }}$ | $0.9{ }^{\text {b }}$ | 785 ${ }^{\text {b }}$ | $11.7^{\text {c }}$ |

Means with similar letters in each column are not significantly different based on LSD test at $\mathrm{p} \leq 0.05$; MDA: malondialdehyde.

Table 3. Means for proline, $\mathrm{Fe}, \mathrm{K}$, root dry weight, essential oil and $\mathrm{IC}_{50}$ of Pelargonium graveolens at different foliar application levels of methanol and ethanol.

| Alcohol foliar application levels (\%) | $\begin{gathered} \text { Proline } \\ \left(\mathrm{mg} \mathrm{~g}^{-1} \mathrm{FWt}\right) \end{gathered}$ | $\begin{gathered} \mathrm{Fe} \\ \left(\mathrm{mg} \mathrm{Kg}^{-1} \mathrm{Dwt}\right) \end{gathered}$ | $\begin{gathered} \mathrm{K} \\ (\mathrm{mg} \mathrm{Kg} \end{gathered}$ | Root dry weight (g) | $\begin{aligned} & \text { Essential oil } \\ & \left(\text { Lha }^{-1}\right) \end{aligned}$ | $\begin{gathered} \mathrm{IC}_{50} \\ (\mathrm{mg} / \mathrm{ml}) \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0 | $1.9{ }^{\text {b }}$ | $700^{\text {c }}$ | $17.4{ }^{\text {b }}$ | $1.1{ }^{\text {bc }}$ | $2.9{ }^{\text {b }}$ | $2.5{ }^{\text {ab }}$ |
| Methanol (10\%) | $1.7{ }^{\text {b }}$ | $1105^{\text {a }}$ | $21.2^{\text {a }}$ | $1.86{ }^{\text {a }}$ | $3.8{ }^{\text {ab }}$ | $2.09{ }^{\text {b }}$ |
| Methanol (20\%) | $1.79{ }^{\text {b }}$ | $1113{ }^{\text {a }}$ | $20.1{ }^{\text {a }}$ | $1.74{ }^{\text {ab }}$ | $4.4{ }^{\text {a }}$ | $1.9^{\text {b }}$ |
| Ethanol (10\%) | $2.6{ }^{\text {ab }}$ | $891{ }^{\text {b }}$ | $14.2^{\text {c }}$ | $1.03{ }^{\text {c }}$ | $3.1{ }^{\text {b }}$ | $2.7{ }^{\text {ab }}$ |
| Ethanol (20\%) | $2.9{ }^{\text {a }}$ | $900^{\text {b }}$ | $13.2{ }^{\text {c }}$ | $1.11^{\text {bc }}$ | $3.06{ }^{\text {b }}$ | $3.2{ }^{\text {a }}$ |

Means with similar letters in each column are not significantly different based on LSD test at $\mathrm{p} \leq 0.05$.

Table 4. Means of treatment combinations of salinity and foliar application levels of methanol and ethanol in relation to chlorophyll a and protein contents of Pelargonium graveolens.

| Salinity (mM) | Alcohols (\%) | Chlorophyll a ( $\mathrm{mg} \mathrm{g}^{-1} \mathrm{FWt}$ ) | $\begin{gathered} \text { Protein } \\ \left(\mathrm{mg} \mathrm{~g}^{-1} \mathrm{FWt}\right) \end{gathered}$ |
| :---: | :---: | :---: | :---: |
| 0 | 0 | $1.3{ }^{\text {cdef }}$ | $181{ }^{\text {bc }}$ |
| 0 | Methanol (10\%) | $2.4{ }^{\text {ab }}$ | $236{ }^{\text {ab }}$ |
| 0 | Methanol (20\%) | $2.8{ }^{\text {a }}$ | $260^{\text {a }}$ |
| 0 | Ethanol (10\%) | $2.6{ }^{\text {a }}$ | $192^{\text {bcd }}$ |
| 0 | Ethanol (20\%) | $2.1{ }^{\text {ab }}$ | $110^{\text {f }}$ |
| 75 | 0 | $1.4{ }^{\text {cdef }}$ | $179{ }^{\text {bcd }}$ |
| 75 | Methanol (10\%) | $2.4{ }^{\text {ab }}$ | $199{ }^{\text {abcd }}$ |
| 75 | Methanol (20\%) | $2.6{ }^{\text {a }}$ | $203{ }^{\text {abc }}$ |
| 75 | Ethanol (10\%) | $1.5{ }^{\text {bcde }}$ | $195{ }^{\text {bcd }}$ |
| 75 | Ethanol (20\%) | $2.2{ }^{\text {abc }}$ | 197abcd |
| 150 | 0 | $0.8{ }^{\text {f }}$ | $102{ }^{\text {f }}$ |
| 150 | Methanol (10\%) | $1.5{ }^{\text {bcdef }}$ | $125{ }^{\text {ef }}$ |
| 150 | Methanol (20\%) | $1.4{ }^{\text {cdef }}$ | $138{ }^{\text {def }}$ |
| 150 | Ethanol (10\%) | $1.6{ }^{\text {bcde }}$ | $160^{\text {cdef }}$ |
| 150 | Ethanol (20\%) | $1.6{ }^{\text {bcde }}$ | $178{ }^{\text {bcde }}$ |

## Proline

The result revealed the significant effects of salinity stress (Table 2) and alcohol foliar application (Table 3 ) on the proline content. Foliar application of ethanol at the concentration of 10 and $20 \%$, increased the proline content about 37 and $53 \%$, respectively, compared to the control plants; however, only the value for ethanol ${ }_{20 \%}$ was significantly higher than the control (Table 3). Also the proline content at 150 mM salinity was about $56 \%$ higher than the control plants. Proline is produced by plants under environmental stresses, and protect them by accomplishing several functions such as scavenging of ROS and maintenance of osmotic balance (Kalsoom et al.
2016). Nanjo et al. (1999) stated that plants manufacture proline under salinity stress to protect themselves and to control their physiological status. Akca and Samsunlu (2012) noted the enhanced
proline content with increasing salinity level. However, according to Ayala-Astorga and AlcarazMelendez (2010), proline content in Paulownia imperialis significantly increased at 20 and 40 mM of sodium chloride and decreased at higher sodium chloride concentrations. On the other hand, in $P$. fortunei, the proline content significantly decreased at all salt concentrations as compared to the control plants. They stated that $P$. imperialis was more tolerant to salt stress at the salinity conditions tested. cell membrane maintenance and regulating the cytosol activity under stressful conditions.

## IC ${ }_{50}$

No consistent results were obtained about the effect of salinity and alcohol foliar application on $\mathrm{IC}_{50}$. Although the highest IC50 values were obtained at 75 mM NaCl , and at $20 \%$ methanol, but they were not significantly different from the corresponding controls (Table 2). However, Valifard et al. (2017)
reported that with increasing salinity stress antioxidant activity was increased. Also, Bagheri et al. (2014) reported that methanol foliar application had positive effects on the total phenolics content and consequently increased antioxidant activity. In the study conducted by Nguyen et al. (2017), ethanol enhanced salinity stress tolerance by detoxifying ROS. They also showed that the expression of ROS signaling-related genes was linked with salinity tolerance and the genes were upregulated by ethanol under salt stress condition. These scientists reported that ethanol treatment in Arabidopsis thaliana reduced the accumulation of $\mathrm{H}_{2} \mathrm{O}_{2}$.

## Protein

The highest protein content was obtained at $\mathrm{NaCl}_{0}+$ methanol ${ }_{20 \%}$; however, it was not significantly different from the following treatments: $\mathrm{NaCl}_{0}+$ methanol ${ }_{10 \%}, \mathrm{NaCl}_{75}+$ methanol $_{20 \%}, \mathrm{NaCl}_{75}+$ methanol ${ }_{20 \%}$ and $\mathrm{NaCl}_{75}+$ ethanol ${ }_{20 \%}$ (Table 4). Although the interaction of salinity $\times$ alcohol foliar application was significant, but high salinity stress ( 150 mM NaCl ) decreased the protein content compared to no-saline treatment. The nutrients imbalances caused by salinity, affect the involvement of minerals in protein bio-synthesis and photosynthesis (Helal and Mengel 1979). In a study conducted by Hernandez et al. (2000), methanol foliar application increased protein content in peanut.

## $\mathrm{H}_{2} \mathrm{O}_{2}$ and MDA

$\mathrm{H}_{2} \mathrm{O}_{2}$ and MDA content were influenced by the salinity stress (Table 2). At the salinity of 150 mM , the amounts of MDA ( $23 \mathrm{nmol} \mathrm{g}{ }^{-1} \mathrm{FW}$ ) and $\mathrm{H}_{2} \mathrm{O}_{2}$ ( $20.8 \mu_{\mathrm{molg}^{-1}} \mathrm{FWt}$ ) were significantly greater than the control (Table 6). In our experiment there was no significant change in the MDA and $\mathrm{H}_{2} \mathrm{O}_{2}$ contents in the plants treated with alcohol. However, in the study conducted by Nguyan et al. (2017) the application of ethanol increased the tolerance of rice plants to the salinity stress through the detoxification of $\mathrm{H}_{2} \mathrm{O}_{2}$.
$\mathrm{Na}^{+}$accumulation under salinity triggers $\mathrm{H}_{2} \mathrm{O}_{2}$ accumulation and MDA over-expression, which results in the cell membrane instability and decrease in plant growth and productivity (Sairam et al. 2002). Other researchers have also indicated the increase in MDA content under the salinity stress (Sreenivasulu et al. 2000; Bandeoglu et al. 2004; Gunes et al. 2007). The accelerated MDA production in the sensitive plants under saline conditions may be because of the hastened ROS production or the low efficiency of the antioxidant system in scavenging the oxidative radicles. Thus, tolerance of plants to salinity depends upon the ROS scavenging potential of the plants under stress conditions.

## $\mathbf{N a}^{+}$and $\mathrm{K}^{+}$

$\mathrm{K}^{+}$content was influenced by the independent effects of salinity and methanol foliar applications
(Tables 2 and 3) and the highest values were recorded at both concentrations of methanol (Table 3). Furthermore, the highest $\mathrm{K}^{+}$content was recorded in the control plants and with increasing the salinity stress, the amount of $\mathrm{K}+$ decreased significantly (Table 2). $\mathrm{Na}^{+}$content was influenced by the salinity stress, and with increasing salinity stress to 150 mM , the amounts of Na increased significantly as compared to the control plants (Table 2). Sharifi et al. (2007), Baatour et al. (2010), Boyrahmadi et al. (2011) and Akca and Samsunlu (2012) reported that salinity stress increased Na content in plants. Under salinity stress, $\mathrm{Na}^{+}$is absorbed and accumulated in the cytoplasm which is toxic to the plants and could induce the cytosolic $\mathrm{K}^{+}$efflux, which consequently results in nutrient deficiency and retarded growth (Assaha et al. 2017).

## $\mathrm{K}^{+} / \mathbf{N a}^{+}$ratio

Salinity stress influenced $\mathrm{K}^{+} / \mathrm{Na}^{+}$ratio and the highest data was recorded at normal condition (Table 2). The salinity level of 150 mM had negative effect on $\mathrm{K}^{+} / \mathrm{Na}^{+}$ratio and was significantly different from the control (Table 2). The similar results were reported by Akca and Samsunlu (2012) and Boyrahmadi et al. (2011). K ${ }^{+}$ accumulation in the roots helps in the regulation of osmotic potential of roots and the translocation of $\mathrm{K}^{+}$in to xylems (Pardo and Rubio 2011). High $\mathrm{K}^{+}$ content under salinity conditions could be used as a sign of salinity tolerance in plants (Tester and Davenport 2003; Chen et al. 2007).

## P

Control plants had the highest P content and the lowest amount was recorded at 150 mM salinity stress (Table 2). In a research done on Triticum aestivum by Boyrahmadi et al. (2011) they reported that salinity stress had negative effect on the P content. In saline soils, salt stress suppresses the absorption of phosphorus, which leads to physiological nutritional deficiency (Tian and Wang 2016) and finally impaired plant growth and development.

## Fe and Zn

Salinity decreased the Fe and Zn content significantly and the lowest amount belonged to the 150 mM salinity stress (Table 2). On the other hand, methanol foliar application at both concentrations (10 and 20\%) increased Fe content in geranium significantly as compared to ethanol and control treatments (Table 3). Zn may contribute to protein biosynthesis, cell membranes integrity, cell elongation (Cakmak 2008) and the tolerance of plants to the environmental stresses (Aravind and Majeti 2003; Cakmak 2000). Iron is also an essential micronutrient for plants because it plays vital role in DNA synthesis, respiration and photosynthesis, and regulates many metabolic pathways (Rout and Sahoo 2005).

## Conclusions

Methanol and ethanol are inexpensive compounds and may protect plants from the salinity stress
conditions. Our results showed that alcohol spray improved root dry weight, chlorophyll, essential oil, protein, K and Fe content in Pelargonium graveolens, on the average of salinity levels. Salinity had negative effect on the dry weight of aerial parts, K/Na ratio, essential oil, K, P, Fe and

Zn content. The beneficial effects of alcohols, especially methanol, on several characteristics of the geranium plants suggests their possible usefulness under saline stress conditions. However, commercial application of these compounds needs more detailed studies.

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# تاثير محلول پاشى با اتانول و متانول بر برخی ويزگَىهاى فيزيولوزيكى شعمدانى عطرى تحت تنش شورى 

لميا وجودى مهربانى

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## جكيده









 شمعدانى عطرى، در ميانگَين سطوح شورى، شد.
واثههاى كليدى: اسانس؛ شعمدانى عطرى؛ كلروفيل؛ مالون دى آلدئيد؛ NaCl

