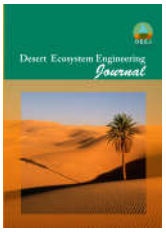
DEEJ (2019) 8 (4) 13-22

University of Kashan

Desert Ecosystem Engineering Journal

Journal homepage: http://deej.kashanu.ac.ir



Providing a Strategy to Confronting the Salinity Stress by Using the PGPR in a Desert Species (*Calligonum comosum* L`Her) in Greenhouse Conditions

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Received: 12/01/2019 Accepted: 13/11/2019

Abstract

The purpose of this study was to evaluating the philological effects of a plant growth regulator in the reduction of salinity stress in the *Calligonum comosum* L. In this regard, a factorial randomized complete block design (RCBD) in the greenhouse condition was implemented. The salinity levels included 0 in control, 2.5, 5, 10 ds/m (mild, moderate and severe stress) respectively. Also the PGPR used as a plant growth regulator with concentrations 0 as control, 10^{-10}, 10^{-8} and 10^{-6} mmol. After applying the desired treatments for 3 months, total chlorophyll, the yield and antioxidant activity of essential oil in the *Calligonum comosum* L. Were measured with international standard methods. The essential oil, extracted by a Clevenger apparatus, and antioxidant activity examined by DPPH method. The results showed, the highest amount of total chlorophyll was observed was observed in the interaction of control treatment of salinity (0) and 10^{-8} mmol PGPR with values of 0.85mlg/g. The results of essential oil yield showed although the increase in salinity level reduced the essential oil yield, by using the PGPR could improve the essential oil yield to a satisfactory level. The highest amount of essential oil yield obtained in the mild salinity stress (2.5ds/m) and 10^{-8} mmol PGPR concentration with values of 0.39g/cm. The results of antioxidant activity showed by using of PGPR the antioxidant activity improved in all of salinity stress level. Briefly, the results of this study showed that the use of PGPR is a suitable way to cope with the negative effects of salinity stress and increase the physiological performance of *Calligonum comosum* L.

Keywords: PGPR, Salinity, Essential oil yield, *Calligonum comosum* L., Chlorophyll.

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DOI: 10.22052/jdee.2020.167444.1043

1. Introduction

Salinity stress is one of the main environmental stresses that limit the growth and yield of plants by decreasing the osmotic potential and disrupting the absorption of some nutrients (Demir Kaya et al., 2006). To reduce the salinity effects, in addition to farming practices methods (management of irrigation, seeding at appropriate places, optimal use of chemical fertilizers and biological methods) and breeding methods (using salinity resistant cultivars), recently the use of biological supplementary methods has become common. Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular mycorrhizal fungus are two of these most important biological substances (Fallahi et al, 2009; Abd El-Hadi Nadia et al., 2009). Plant Growth Promoting Rhizobacteria has increased the tolerance of plants in a wide range of environmental stresses such as drought, salinity, and cold stresses (Clouse et al., 1992; Tabrizi et al., 2008). In addition, using the PGPR increase plant resistance to various environmental stresses such as water shortages, nutrients, and toxicity of heavy metals (Wu et al., 2005; Vessey, 2003). In the study to investigate the effect of PGPR in order to reduce the damages by saline water, a pot experiment was conducted on rice, in this study the root of the plants was planted in the pot after inoculation with the bacteria and then irrigated with salt water, the results showed that with increasing salinity, grain yield, the weight of one thousand seeds, the number of spikes and plant height decreased, But in some strains of bacteria, the above indicators were improved (Rajabi et al., 2009; Faker Baher et al, 2001; Mahfouz & Sharaf-Eldin, 2007).

Also, the growth and production of essential oils in the medicinal plants can be influenced by the use of plant growth regulators. Swamy et al (2008) showed that plant growth and essential oil content significantly increased with the use of stimulants, and by applying a few these substances in the amount of a microgram.,. In other studies, Tarraf & Ibrahi (1999) found that the essential oils of *Lavandula stoechas* have increased significantly with the use of plant growth regulators.

In a study that conducted by Koochaki et al. (2008), the effect of different fertilizers, including fertilizers containing nitrogen stabilizing bacteria, such as nitroxin, super nitroplas, also fluorescent pseudomonas PGPR, were investigated on the *Hyssopus officinalis,* the results indicated that during the 2 years, the application of biological fertilizers in addition to increasing plant height and diameter, and the fresh and dry weight of the plant, increased the essential oil yield in comparison with the control (planting in the manure bed). Also, among the treatments, nitroplas fertilizer and then microorganism Pseudomonas fluorescent treatments had the most effect on increasing the studied traits. Therefore, the performance of a medicinal plant is economically feasible when the amount of primary and secondary metabolites reached to the desired level. So, by managing environmental factors, the maximum product can be achieved (Jafarnia et al., 2016; Upadhyay et al, 2011; Mohsen &Ismail, 2016). Demir Kaya et al., (2006) showed that salinity reduces oil yield in Polygonaceae family, and on the other hand, drought stress can increase the percentage of essential oils in the most medicinal plants, since more metabolites are produced in these conditions and these substances prevent oxidation in the cell. The reducing of essential oil yield in the Polygonaceae family, probably related to the limited supply of cytokinin from the roots to the branches resulting in a change in the ratio between the cytokinin Abscisic acid in the leaf (Dow et al., 1981).

Calligonum comosum L`Her is from Polygonaceae family. This plant species adapted to desert conditions in sandy lakes as a species that is resistant to drought and dehydration. This plant has a special place in different conditions of dry and semi-arid climate, especially sandy hills and its dissemination range is very wide, and it is present in most of Iran's central sandy beaches such as Kerman, Khour and bibanbak, Nain, Dasht-e-Kavir, Damghan, Kashan, and Aran and Bidgol (Figure 1).

C. comosum L. is frequently used as sources of medicine by rural people of south Tunisia. Indeed, anti-inflammatory, anti-ulcer and anti-cancer activities of *C. comosum* L. have been

reported in rat and shrimp animal models (Badria et al., 2017)

The aerial parts of the plant showed protective activity against haloperidol-induced oxidative stress, antiosteoporotic, anti-ulcer and anti-inflammatory, hypoglycaemic, cytotoxicity and antioxidant activities. The fruits of the plant showed antihelmentic activity (Sabry et al. 2016). Antimicrobial activity for different plant parts was reported. Previous phytochemical studies on C. polygonoides reported the isolation of kaempferol, quercetin, (+)-catechin, dehydrodicatechin A, kaempferol-3-O-rhamnopyranoside, quercitrin, isoquercitrin, kaempferol-3-Oglucuronide, quercetin-3-O-glucuronide, pro-cyanidines, b-sitosterol-3-O-glucoside, violaxanthin and neoxanthin carotenoids (AbdelSattar et al. 2018).

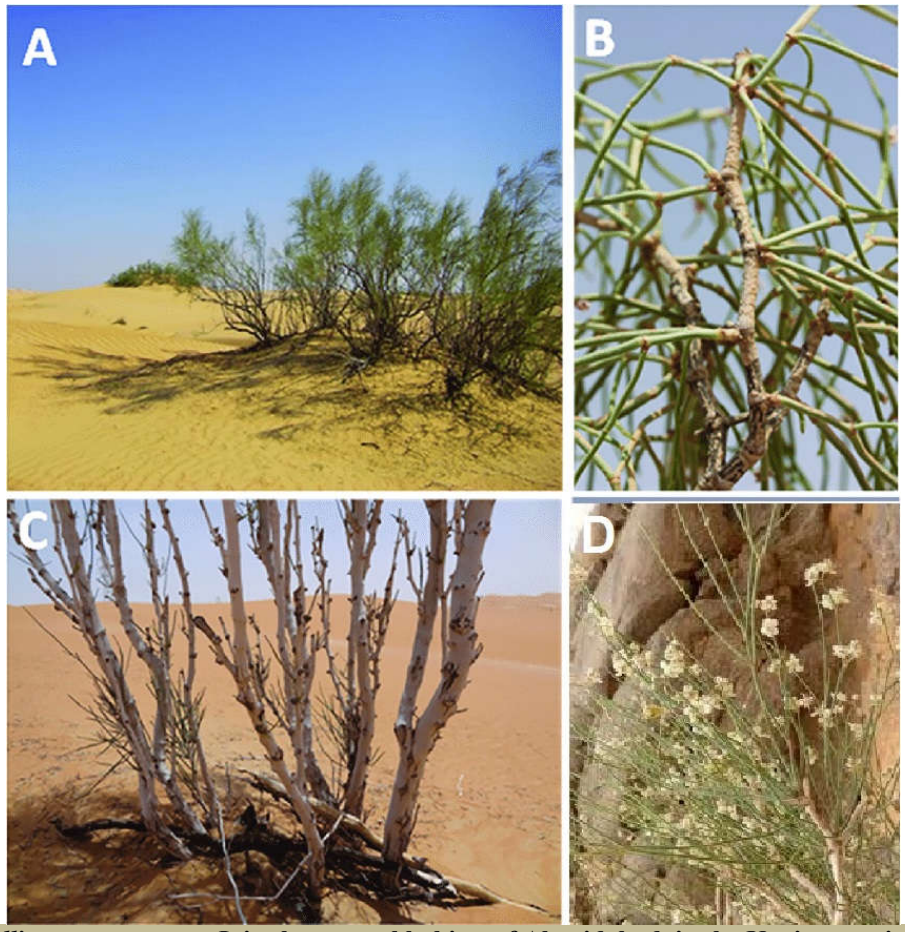

Figure (1): *Calligonum comosum* L in the natural habitat of Abuzidabad, in the Kavirat section of Aran and Bidgol city/November 2017; (A & B), Fresh stems and flowers (C&D)

Growth period of this plant in the studied area begins from mild of December and the emergence of leafy leaves extends to mid-April, its tiny and white flowers begin to form in late March and by the end of May and give a very sophisticated landscape to the faces of the sandstones. Fruits appear in June gradually, groovy fruits are white, pink, red that cover all the plant limb. The height of the plant varies between 1 to 3 meters (Bahmani et al., 2009).

Since environmental stresses, especially salinity stress, are one of the main obstacles in reducing the production of medicinal plants in many parts of the world, including Iran, so doing related experiments and the use of effective substances to reduce the effects of adverse stresses to achieve the economic thresholds of the crop and medicinal plant performance are important. In this regard, the use of PGPR to improve the effects of salt stress *Calligonum comosum* has been examined in this study.

2. Materials and Methods

This experiment was conducted in the greenhouse (with nylon cover, with natural light and temperature of 2° celsius) of Plants Production and Duplication Center of Parks and Green Landscape of Kashan Facilities Municipality using randomized complete block design (RCBD) with four replications. For conducting the experiment, the seeds of *Calligonum comosum* were planted in pots containing of (6 kg of sandy soil of its natural habitat) in November of 2017 and irrigated to the extent of field capacity (FC) for three months to insure roots were formed. When the height of the seedlings reached to 35 cm, the treatments were performed. The treatments were salinity levels included 0 in control and 2.5, 5, 10 dS/m (mild, moderate and severe stress) respectively. Also the other treatment was plant growth regulator (PGPR) with concentrations 0 in control, 10^{-10}, 10^{-8}, 10^{-6} mmol. The plant growth regulator used in this experiment was *Pseudomonas fluorescent*, from the CHAO strain (*Pseudomonas fluorescence* CHAO) that was obtained from the soil in the soil in the department of soil science, faculty of Agriculture university of Tehran, Karaj, Iran. The duration of the stress on the plants lasted 3 months. During this time, each pot was irrigated with 1.5 liters extent of field capacity (FC). Then the plants were cut from the surface of soil separately and dried in a standard method (Omidbeygi, 2009).

2.1. Essential oil Extraction

Aerial parts of *C. comosum* include (leaves, flowers and fruits) were crushed separately in a grinder. Essential oil was obtained by hydrodistillation for 3.5 h using an all-glass Clevenger-type apparatus as recommended by European Pharmacopoeia (Amakura et al., 2002). The oils were dried over anhydrous sodium sulfate and stored in the dark at low temperature (4 ºC) until analysis. Finally, the essential oil yield calculated by equation 1.

Equation 1:

$$\text{Essential oil yield} = \frac{\text{weight of Essential oils}}{\text{weight of dry matter}} * 100$$

2.2. Total chlorophyll

In order to determine the total chlorophyll content, plants in two leaf stage, the 0.5 g of seedling fresh tissue crushed and with 5 mL of acetone 80% centrifuged with13000 r/m and were kept at 4 ° C for 15 minutes. Then the extracts were placed in the spectrophotometer at 663 and 645 nm wavelengths. The following equation 2, used for calculation of total chlorophyll. In these equations, V(mlgr) is the volume of extract and W(g) is the sample weight (Jahan et al., 2015).

Equation 2:

$$Ch_T = 20.2\ (A645) + 8.02\ (A663) \times V/1000W$$

2.3. Antioxididant activity

The antiradical activity of the essential oil measured using the stable radicals of DPPH- according to the Oliveira Method (Oliveira et al., 2010). In this method, 0.2 ml of essential oil at various concentrations was added to 4 ml of methanol solution 6×10^{-5} molar of DPPH free radical and kept for 60 minutes at ambient temperature. Then, the absorbance of the solution at 517 nm wavelength was read using the metering spectrum (Arnao et al., 2001; Zahir et al, 2004).

A sample containing 0.2 ml of methanol with 4 ml of DPPH solution was used as a control sample and methanol solution used to calibrate the device. The experiment was performed in three replications. The rate of radical absorption of the essential oil was determined by the following equation 3.

Equation 3:

$$\%\ \text{RSA} = 1 - \frac{\text{A Control} - \text{A sample}}{\text{A Control}} * 100$$

In this equation:

A_{sample} = Sample Absorption Rate

$A_{control}$ = Contorol Absorption Rate

RSA= Radical Scavenging Activity

The data were analyzed using MSTAT-C statistical software and the mean of evaluating traits was compared using Duncan multiple range test at the 5 % level.

3. Results and Discussion

Based on the results, the total chlorophyll content of *Calligonum comosum* L. with increasing the salinity level showed a decrease at 1% level significantly, the effect of salinity

levels on the total chlorophyll content was a decreasing effect. There was significant difference between the different levels of PGPR and total chlorophyll content and in the 10^{-8} concentration, most significant differences were observed (0.8575mlg/g in the 0 treatment of salinity and 0.3425mg/g in the 10 treatment of salinity). Although the increase in salinity levels had a significant reduction in the total chlorophyll content of this plant, but as shown in (Figure 2), the use of PGPR increased the total chlorophyll content compared the control. According to the results, environmental stresses such as salinity stress, decrease the total chlorophyll content in the most of the medicinal plants, because in the stress conditions, more metabolites are produced and these substances prevent the oxidation process in the cell. The interaction between PGPR and salinity stress was not significant.

Due to decreasing effects of the salinity level on the total chlorophyll content, the results showed that the interaction of salinity stress and PGPR on the total chlorophyll content was in the different statistical groups. (Table 2).

The results showed that the salinity stress had a significant effect on essential oil yield. In sum, the effect of salinity levels on essential oil yield was an increasing effect. Based on the results, the different levels of salinity stress on essential oil yield was significant at the 5 % level. In the PGPR (0) with moderate (5ds/m) and severe (10ds/m) salinity stress treatments the essential oil yield obtained (0.1836) and (0.0993) that showed a decrease of 58% and 85% respectively compared with the control treatment while, using the 10^{-8} mmol of PGPR in mild, moderate and severe stress increased the essential oil yields to 0.3920, 0.3024 and 0.2784 (g/cm^2) that shoved 55%, 33% and 20% respectively compared to the control plants. So the results showed that the use of this PGPR strain increased the essential oil yield in this plant species and it is a suitable solution for dealing with salinity stress conditions (Table 1 and Figure 3).

Table 1: The changes of some philological indices of *Calligonum comosum* L. at different levels of salinity and PGPR concentration

PGPR Concentrations (mmolar)	(ds/m) Salinity levels	Total Chlorophyll (mlg/g)	(g/cm^2) Essential oil Yield	% RSA (µg/mL)
0	0	0.7130 a	0.2014 a	1.8±0.3 ab
	2.5	0.4322 b	0.2563 ab	2.3±0.6b
	5	0.35424 c	0.1836 b	2.2±0.4 bc
	10	0.2630 d	0.0933 c	1.4±0.9 d
10^{-10}	0	0.7566 ab	0.3125 bc	2.6±0.2 bc
	2.5	0.4935 bc	0.3425 bc	3.5±0.2 c
	5	0.38731 c	0.3325 c	3.1±0.8 bc
	10	0.3556 cd	0.3021d	2.4±0.6 bc
10^{-8}	0	0.8575 c	0.2754 dc	4.1±0.6 e
	2.5	0.5070 cb	0.3920 d	5.3±0.3 e
	5	0.3935 c	0.3024 cd	4.9±0.4 g
	10	0.3425 cd	0.2784	4.1±0.8 h
10^{-6}	0	0.7805 d	0.2540 d	4.9±0.6 gh
	2.5	0.3941 dc	0.3698 cd	6.2±0.2 g
	5	0.3850 dc	0.3025 d	5.7±0.4 f
	10	0.3521d	0.3698 d	5.1±0.7 e

Table (2): The mean squares of the interaction of salinity and PGPR

SOV	Df	Total Chlorophyll(mlg/g)	Essential oil Yield (g/cm^2)	DPPH test (µg phenolic/mL)	F	Sig
Salinity factor	2	0.503 **	1.021 *	2.045 *	0.238	0.03
PGPR factor	3	0.011 ns	3.241 **	5.101 **	0.125	0.003
PGPR× Salinity	6	0.003 ns	0.439 *	1.530 *	0.114	0.005
Error	33	0.002	0.204	0.308	-	
CV(%)	-	2.17	2.80	2.68	-	

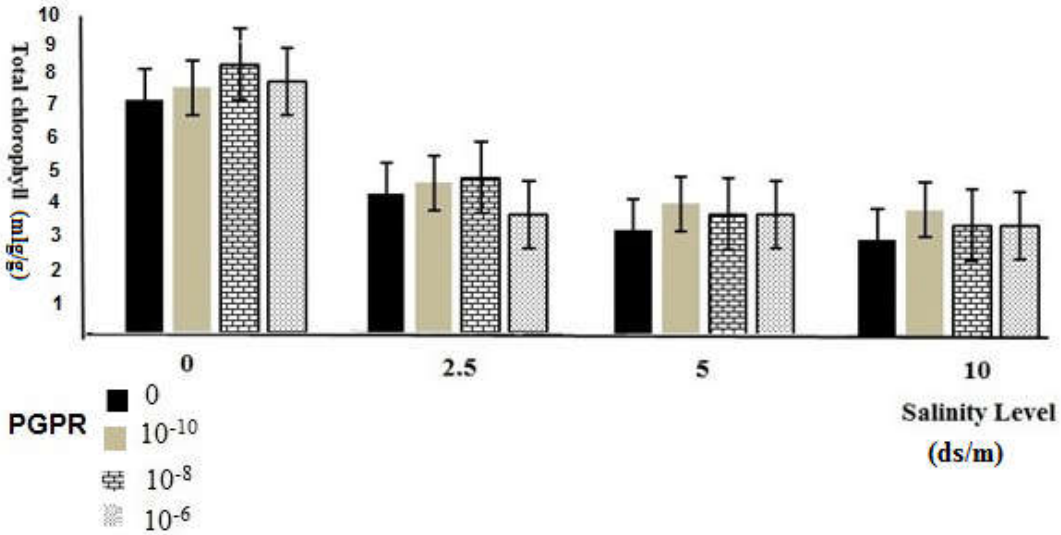

Figure (2): Changes of total chlorophyll content of *Calligonum comosum* L. at different concentrations of PGPR in salinity stress

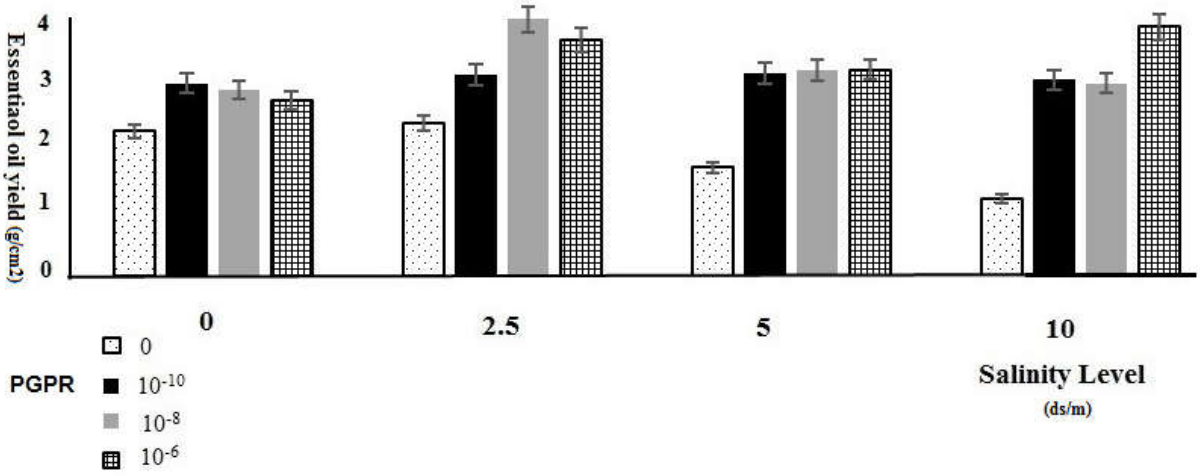

Figure (3): Changes of essential oil yield of *Calligonum comosum* L. at different concentrations of PGPR in salinity stress

The results showed, the effect of different levels of salinity stress on the antioxidant activity was significant at the 5 % level. The effect of salinity levels on this parameter was an increasing effect (Tables 2, and Figure 4). The results showed that the use of PGPR increased the antioxidant properties of essential oil significantly at the 1 % level. The most antioxidant activity of the essential oil was related to the treatment of the PGPR 10^{-6} M and with a mean of 6.2 ± 0.2 which showed an increase compared to control treatment.

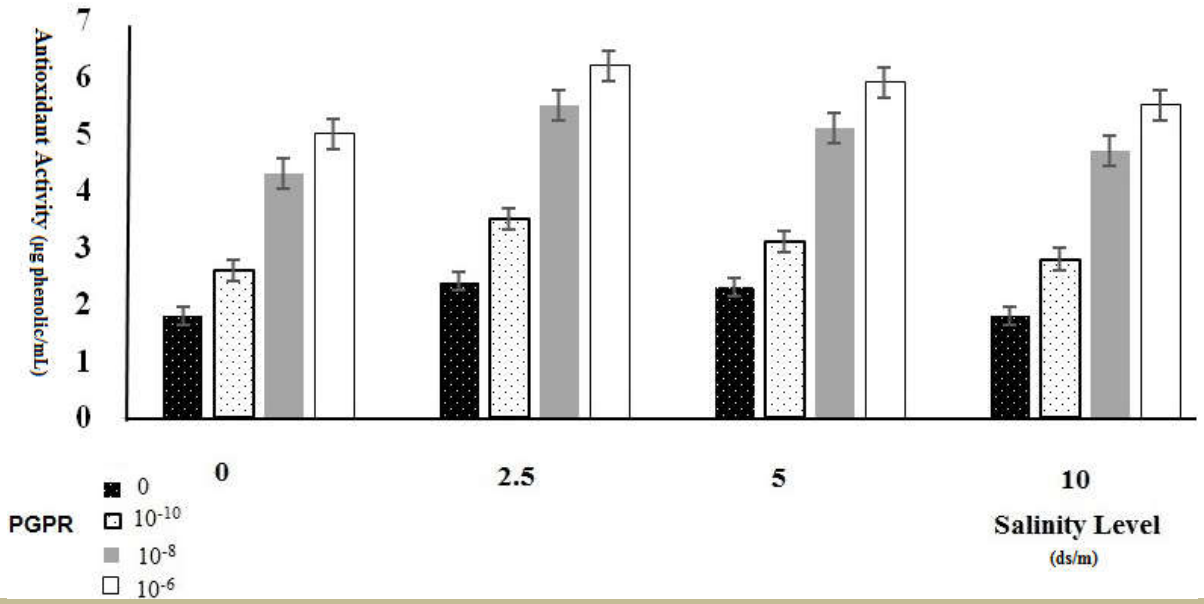

Figure (4): Changes of antioxidant activity of *Calligonum comosum* L. at different concentrations of PGPR in salinity stress

The results of Table 2 and Figure 3 showed that use of PGPR increased the essential oil yield at 1% level. The highest essential oil yield was related to the treatment of 10^{-8} mmol PGPR with a mean of 39g/m^2, which was 67% more than the control treatment, this result confirmed in results of (Tarraf et al, 1999, Lucy et al, 2004) that studied the physiological response of the *Calligonum comosum* L. and found the amount of essential oils has increased significantly with the use of growth regulators. Also, (Ingram and Bartels, 1996; Gharib et al., 2008) in their research showed that using growth regulators, provide a good protection for plants against a number of non-living stresses.

The interaction effects of salinity levels and different concentrations of PGPR had significant effects on the essential oil yield at the 5 % (Table 2). The comparison of the means showed that the interaction of salinity stress and PGPR on essential oil yield were in different statistical groups. As it is seen in the Figure 2, under mild stress conditions (2.5 ds/m), the use of 10^{-8} and 10^{-10} mmol PGPR concentrations increased the essential oil yield significantly. Regarding the effect of growth regulators, we can say that these materials, by improving the growth parameters in the normal and stress conditions, increase the performance of the aerial part of plants and increase the essential oil yield in the plants. Rahmani et al. (2008) ; Daneshmandi & Azizi (2009) showed that drought and salinity stress had a significant effect on the yield and content of essential oil of *Calendula officinalis*. The interaction effects of salinity levels and different concentrations of PGPR on the antioxidant activity of essential oil were significant at the 5 % level. Also, the comparison of the means showed that the effects of salinity stress and PGPR on antioxidant properties were in the different statistical groups (Table 2). This result confirmed results of (AliabadiFarahani & Karima, 2004).

The results of this study showed that increased salinity stress from mild to severe level caused a decrease in the total chlorophyll content (Table 2, Figure 2),. Also, the interaction effect of salinity stress and PGPR on the essential oil yield of *Calligonum comosum* L., the results indicated that moderate and severe stress reduced the essential oil yield. Briefly, the results of this research indicated that the use of 10^{-8} M concentrations of PGPR in mild stress (2.5 ds/m) with stimulation of growth parameters increases the essential oil yield, as this increase is significant in comparison to the control treatment. So the use of PGPR justified economically.

4. Conclusion

According these results we can found that the use of plant growth regulator such as PGPR is essential and justifiable for maintaining the economic performance of plants under stress conditions, on the other hand, to find the point of collision between two treatments (applying salinity stress and the use of PGPR) is important for determining the economic performance of *Calligonum comosum* L. essential oil. It seems that plant growth regulators increase the plant's resistance to these stresses by inhibiting the stresses inflicted on the plant. There have been many studies on the interaction between growth regulators and a variety of stresses that confirm this theory (Ali et al, 2007; Ben Taarit et al, 2009). But more studies are needed to determine the effect of these substances in the production of essential oils in the plants. Also, under moderate and severe stress conditions, the use of PGPR caused a significant increase in essential oil yield compared to control treatment, which confirms the positive role of this material in reducing the negative effects of salt stress. Since the research was conducted under greenhouse conditions, it is recommended that these experiments be carried out in the field conditions to obtain better and more precise results.

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