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Plant growth promoting bacteria effects on growth, photosynthetic pigments and root nutrients uptake of *Avena sativa* L. under drought stress

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Abstract

Drought stress is one of the most important factors affecting plant growth. Plant growth under drought stress may be enhanced by the application of microbial inoculation including plant growth promoting rhizobacteria. This research was conducted as a factorial experiment in a completely randomized design. The first factor included the bio-fertilizer (*A. vinelandii* (A)), *P. agglomerans*+ *P. putida stroin* (P), A + P and control (without bio-fertilizer). The second factor was drought stress at three levels of field capacity (FC), 0.7 FC and 0.4 FC. The results showed that treatment A + P at FC level had the highest effect on increasing photosynthetic pigments ($p<0.01$). While the lowest amount of photosynthetic pigments occurred in the treatment A at FC level. The highest and lowest shoot fresh weight was belonged to the treatments P at 0.7 FC and the treatment A at 0.4 FC, respectively ($p<0.01$). The highest and lowest root fresh weight was respectively was belonged to the control treatment at 0.7 FC and 0.4 FC, respectively ($p<0.01$). The results showed that the use of bio-fertilizers separately had more positive effects on the nutrients uptake of *A. sativa* L. In general, the results of this study suggest that growth promoting bacteria as bio-fertilizers have a greater effect on growth, photosynthesis pigments and nutrient uptake of *A. sativa* L. The use of these bacteria did not actually reduce the effect of drought stress on the plant.

Keywords: *Azotobacter*; *Pseudomona*; Chlorophyll contents; Environmental stress; Plant growth

1. Introduction

The most important problems of arid and semi-arid rangelands are drought and water shortage that affect plants growth and development. Given that the majority of the world's rangelands are located in these areas, the effect of drought stress on these areas' plants is of great importance (Zandi Esfahan and Azarnivand, 2012). Drought stress is one of the main environmental factors limiting the growth and yield of vegetation cover (Nazar *et al*., 2015; Sagdeghi and Rostami, 2016), the most common cause is the increase in temperature and reduced available water to plants (Nazar *et al*., 2015). Water scarcity as a limiting factor of plants' growth prevents seed germination and plants' development and reduces the plant productions around the world (Yan, 2015).

Nowadays, application of microorganisms in the soil as bio-fertilizer is considered as the most natural and desirable solution for maintaining live and active soil system (Zahir *et al*., 2004; Nadeem *et al*., 2014). In addition, the supply of nutrients quite fitting the normal plants' feeding contributing to biodiversity, improving the status and maintaining the health of the environment is one of the most important benefits of bio-fertilizers (Delshadi, 2015).

Bacteria and fungi, especially growth promoting bacteria and materials derived from their activity, are the most important bio-fertilizers. The fertilizers according to growth and development of plants are commonly called yield promoting bacteria (Zahir *et al*., 2004; Nadeem *et al.,* 2014). The mechanism of the plant growth promoting bacteria has not been

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fully understood in order to increase the plant growth, but the bacteria are capable of producing some growth promoting hormones especially a variety of cytokinin, gibberllic acid and auxin, fixing nitrogen, and phosphorus (Vacheron *et al*., 2013). *Azotobacter* spp. and *Pseudomonas* spp. are the most important bacteria that increase soil mineral elements, with production of matters regulating growth and they affect development and yield of plants (Zahir *et al*., 2004; Hayat *et al*., 2010). The use of bio-fertilizers such as nitrogen fixation bacteria of the genus *Azotobacter* and bacteria dissolving phosphate such as *Pseudomonas*, provide nutrients needed by the plant such as nitrogen and phosphorus and thus improves plants' growth and yield in addition to increasing useful soil microorganisms' population (Arancon *et al*., 2004). It should be noted that the effect of growth promoting bacteria depends on the yield of the host plant and soil environment as well as inherent capabilities of bacteria (Nadeem *et al*., 2014). However, growth promoting bacteria play an important role in maintaining soil fertility and improving the plant growth and development, but some concerns have been also reported in some studies (eg. Saharan and Nehra, 2011; Vacheron *et al*., 2013). For example, the production of cyanide is a well-known feature of *Pseudomonas* (Martínez-Viveros *et al*., 2010). Cyanide in fact as an environmental controller can increase the growth and on the other hand has negative impacts on the plant growth (Martínez-Viveros *et al*., 2010). Also the production of auxin by the bacteria at low concentration increased the plant growth and at high concentration reduced the plant growth (Patten and Glick, 2002; Vacheron *et al*., 2013). Although growth promoting bacteria are very effective on the plant growth and development, but specific bacterial species may reduce specific the growth and the negative role may occur under certain conditions. Therefore, it is necessary to choose the appropriate species to obtain the maximum plant production (Nadeem *et al*., 2014).

Avena sativa L. (Gramineae family) is a species of cereal grain grown for its seed. It is suitable for human consumption and livestock feed (Achleitner *et al*., 2008; Nirmalakumari *et al*., 2013). Although, this plant can grow in cold and wet weather and low fertility soil (Ren *et al*., 2007; Buerstmayr *et al*., 2007), but it is sensitive to drought stress (Frey *et al*., 1986). Due to the fact that drought stress is one of the most important factors of reduced plant growth, the present study was conducted to aim: 1. to study the effect of growth promoting bacteria (*Azotobacter vinelandii* and *Pantoea agglomerans* +*P. putida*) under drought stress on growth and photosynthetic pigments of *A. sativa* L., and 2. to find out the effect of plant growth promoting bacteria on the root nutrient uptake in *A. sativa* L. under drought stress.

2. Materials and Methods

2.1. Preparing pots

This study was conducted as a factorial experiment in a completely randomized design, with three replications in the research greenhouse of the University of Zabol (at minimum and maximum temperature of 9±2°C and 35±5°C, respectively). The first factor was the use of bio-fertilizers at four levels, including the control (without bio-fertilizer), bio-fertilizers *A. vinelandii* = A, *P. agglomerans*+ *P. putida*= P and, A+P combination. The second factor was applying drought stress at three levels: Field Capacity (FC), 0.7 FC and 0.4 FC on *A. sativa* L.

The soil samples were air-dried, homogenized and sieved through a 4 mm stainless sieve before analysis. Characteristics of the soil are listed in Table 1. The soil's texture was determined using laser diffractometry (Wang *et al*., 2012); soil pH was determined in a 1:5 soil to distilled water slurry after one hour of agitation using pH-meter (Model 691, Metrohm AG Herisau Switzerland) (Thomas, 1996); Electrical conductivity (ECe) was determined using an EC–meter (DDS–307, Shanghai, China) (Rhoades, 1996); Total soil nitrogen (N_t) was analyzed using Kjeldahl method (Bremner, 1996). Available phosphorus (AP) was determined by the method of Bray and Kurtz (1954). Available potassium (AK) was measured by flame photometry method (Knudsen *et al*., 1982). Organic matter content was determined using the methods described by Lo *et al*. (2011).

Table 1. Some soil physical and chemical characteristics used in the experiments

Soil texture	EC (dS m^{-1})	pH	N t (%)	P (mg kg^{-1})	K (mg kg^{-1})	OM (%)
Loamy sand	0.19	4.9	0.17	16	560	1.71

The seeds were prepared from Isfahan Pakan Bazr Co., Iran. Firstly, empty pots were weighed in order to cultivate the seeds. To prevent the leaching of fertilizers and plant root penetration into the soil, the bases of the pots were covered with a thin cover. Then, 2 kg soil was poured into each pot. The pots were saturated and then irrigated with distilled water. Before planting, the seeds were dipped in a solution of bio-fertilizers (seed treatment). In each pot, 12 seeds were planted in a depth of 1 cm. Until germination, drought stress was applied. The pots were irrigated daily at field capacity (FC), 0.7 FC and 0.4 FC. The plants washed with distilled water to measure plant fresh weight, chlorophyll contents and the amount of nutrient uptake by root of the plant.

2.2. Measuring chlorophyll contents

To measure the "chlorophyll a" and "chlorophyll b" content, total chlorophyll, and carotenoids, 0.1 g of plant fresh tissue was pulverized inside a porcelain mortar with 5 ml of 80% acetone, and then centrifuged. The solution was transferred to centrifuge tubes, and the remnant in the mortar was washed twice with 5 ml of 80%acetone, the solution of which was added to the tubes. Then, the tubes were centrifuged for 10 min at 6000 rpm, the solution of which was transferred to a 250 mm flask, and its volume was adjusted to 25 ml with 80% acetone. Chlorophyll contents were read at wavelengths of 470, 663, 645 nm, using spectrophotometer (WPA–S2000) (Arnon, 1949). The contents of chlorophyll a, b and carotenoids were estimated according to the Eqs.1, 2 and 3. Total chlorophyll was calculated by sum of chlorophyll a and b in terms of milligrams per gram of sample weight (Arnon, 1949).

Chlorophyll a =19.3 × A663-–0.86 ×A645) V/100W (1)

Chlorophyll b = (19.3×A645- 3.6 ×A663)/V (2)

Carotenoides= 100(A470) – 3.27 (mgchl.a)–104 (mgchl.b)/227 (3)

V = volume of filtrated solution (upper solution of centrifuges)
A = absorption of light at wavelengths of 663, 645 and 470 nm
W = wet weight of sample (g)

2.3. Measuring nutrient uptake by the plant

The amount of element uptake was measured in two steps. In the first step, the plant extract was prepared by wet digestion in special tubes using H_2SO_4, Se+H_2O_2 and salicylic acid. To prepare the acid solution, 1.75 g of selenium powder was dissolved in 500 ml of sulfuric acid and heated, on the heater, at a temperature of 150°C for four hours (the solution color changed from black to blue-green and, finally, light yellow, respectively). Then, 100 ml acid solution and 3.6 g salicylic acid was added daily to the solution. The plant shoot (1 g, oven-dried) was poured in digestion tubes and 2.5 ml (for each sample) of the acid solution was added to the samples. After 24 hours, the samples were heated on the heater (150°C). After cooling the tubes three times, at each time 1 ml of hydrogen peroxide was added and this practice continued until obtaining discolor solution. After cooling, the solution reached a volume of 50 ml with distilled water and was then passed through a filter paper for reading elements (Rayan *et al*., 2001). In the second step, phosphorus, N, K, Fe, and Zn were measured. Nitrogen was measured by titration after distillation, using the Kjeldahl method; the amount of phosphorus was measured using colorimetric (yellow molybdate-vanadate) and spectrophotometer, and K was measured by flame photometer (Rayan *et al*., 2001). Iron and Zn were determined using ICP/OES (GBC Avanta, Australia).

2.4. Data analysis

The statistical analyses of the experimental data were performed using the SAS 8.1. All reported results are the means of three replicates and deviations were calculated as the standard error of the mean (SEM). The statistical processing was mainly conducted by multiple analyses of variance. Duncan test post hoc analysis was performed to define which specific mean pairs were significantly different. A probability of 0.05 or lower was considered as significant.

3. Results

3.1. Effect on plant weight

The results of analysis of variance (Table 2) showed that the main effects of bio-fertilizer, drought stress and interaction effects of bio-fertilizer and drought stress on the shoot and root fresh weight ($p<0.01$) were significant. The main effects of bio-fertilizer showed that

treatment P increased shoot fresh weight significantly, and the lowest shoot weight was related to the treatment A + P (Fig. 1). The main effects of drought stress showed that 0.7 FC and 0.4 FC had the highest and lowest shoot fresh weight, respectively (Fig. 1). The interaction effect of bio-fertilizer and drought stress showed that the highest and lowest shoot weight was respectively, related to the treatments P at the 0.7 FC and treatments A at the 0.4 FC (Fig. 1).

The main effects of bio-fertilizers showed that the highest and lowest root fresh weight was respectively, related to the control treatment (without fertilizer) and treatment A + P. The main effect of drought stress showed that the highest and lowest shoot fresh weight was respectively, related to 0.7 FC and 0.4 FC (Fig. 1). The interaction effects of bio-fertilizer and drought stress showed that the highest root fresh weight was related to the control treatment at the 0.7 FC level while the lowest root fresh weight was related to the control treatment at 0.4 FC level (Fig. 1).

Table 2. Analysis of variance of fresh weight of *A. sativa* L. under different drought stress and bio fertilizers

SoV	Df	Mean square		
		Root weight (mg)	Shoot weight (mg)	
Bio fertilizers	3	14.08**	8.27**	** p<0.01
Drought stress	2	22.90**	22.47**	
Bio fertilizers× Drought stress	6	11.59**	8.27**	
Error	24	0.09	0.07	
CV (%)		4.57	3.94	

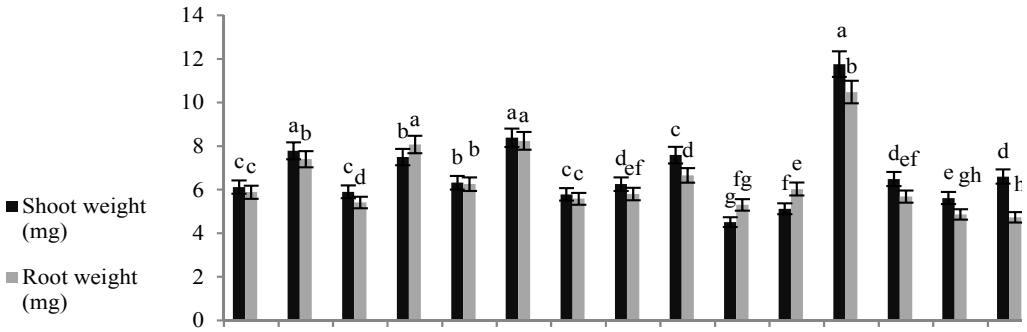

Fig. 1. Effects of bacteria inoculation and drought stress on fresh weight of *A. sativa* L. Error bars represent standard error of the mean. A= *A. vinelandii*, P= *P. agglomerans*+ *P. putida*, A+P= *A. vinelandii* + *P. agglomerans*+ *P. putida.* Different letters above the bars present significant different among the treatments (p<0.05)

3.2. Effect on photosynthetic pigments

The results of data analysis of variance (Table 3) showed that the main effect of bio-fertilizer treatments, drought stress and interaction effects of bio-fertilizer and drought stress on chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were significant (p<0.01). In addition, the effect of drought stress on chlorophyll b and total chlorophyll was significant (p<0.05), but this was not significantly effective on the amount of chlorophyll a and carotenoid (Table 3). The results of a comparison of different treatments of bio-fertilizer showed that the highest increase in chlorophyll a, chlorophyll b and total chlorophyll was related to treatment A + P and the highest amount of carotenoid was associated with the control treatment (without fertilizer). The lowest amount of chlorophyll a, b, total chlorophyll and carotenoids was observed in treatment A (Figs. 2 and 3). The results of a comparison of average levels of drought stress showed that chlorophyll a, b (Fig. 2), total chlorophyll and carotenoids (Fig. 3) had a significant increase at the 0.4 FC level. The lowest amount of chlorophyll a, chlorophyll b (Fig. 2), total chlorophyll and carotenoid (Fig. 3) was observed at 0.7 FC level. Also a comparison of average interaction effects of bio-fertilizers and levels of drought stress showed that treatment A + P in terms of FC had the highest effect on increasing the amount of chlorophyll a, chlorophyll b (Fig. 2), total chlorophyll and carotenoid (Fig. 3). Under the FC level treatment A reduced chlorophyll a, chlorophyll b (Fig. 2), total chlorophyll and carotenoid (Fig. 3).

Table 3. Analysis of variance of photosynthetic pigments of *A. sativa* L. under different drought stress and bio fertilizers

SoV	Df	Mean square				
		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoid	
Bio fertilizers	3	0.026**	0.002**	0.056**	0.0097**	** p<0.01, *p<0.05, n.s p>0.05
Drought stress	2	0.0084$^{n.s}$	0.0012*	0.024*	0.0023$^{n.s}$	
Bio fertilizers× Drought stress	6	0.01**	0.0013**	0.025**	0.0069**	
Error	24	0.02	0.00	0.01	0.02	
CV (%)		13.85	15.80	14.73	9.12	

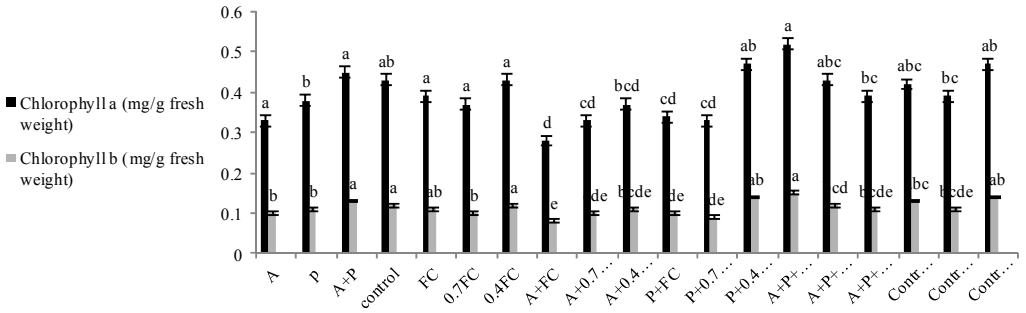

Fig. 2. Effects of bacteria inoculation and drought stress on chlorophyll a and chlorophyll b contents of *A. sativa* L. Error bars represent standard error of the mean. A= *A. vinelandii*, P= *P. agglomerans*+ *P. putida*, A+P= *A. vinelandii* + *P. agglomerans*+ *P. putida.* Different letters above the bars present significant difference among the treatments (p<0.05)

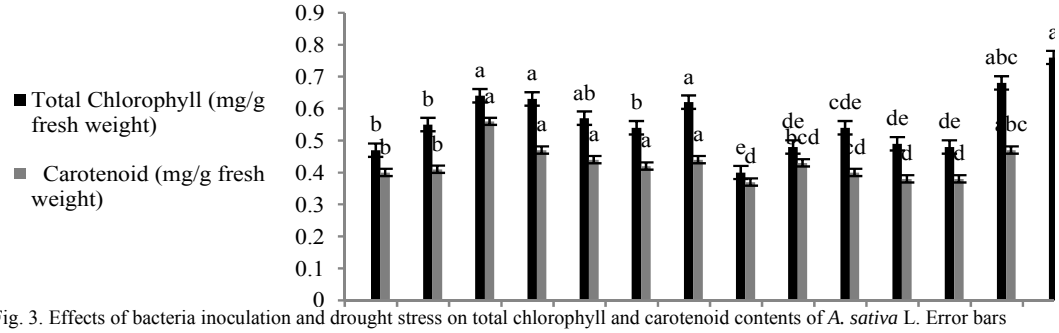

Fig. 3. Effects of bacteria inoculation and drought stress on total chlorophyll and carotenoid contents of *A. sativa* L. Error bars represent standard error of the mean. A= *A. vinelandii*, P= *P. agglomerans*+ *P. putida*, A+P= *A. vinelandii* + *P. agglomerans*+ *P. putida.* Different letters above the bars present significant difference among the treatments (p<0.05)

The results of present study showed that treatment A + P increased the amount of *A. sativa* L. chlorophyll, but treatment A reduced photosynthetic pigments in the plant. Also, in relation to levels of drought stress, 0.4 FC had the highest effect on increasing the level of photosynthetic pigments. The interaction effect of fertilizers and drought showed that *Azotobacter* at FC level reduced photosynthetic pigments in the plant. Also combination of two bacteria at FC level increased photosynthetic pigments.

3.3. Effect on nutrient uptake

The results of analysis of variance (Table 4) showed that the main effects of bio-fertilizer and interaction effects of bio-fertilizer and drought stress on the nutrient uptake were significant in the root of *A. sativa* L. (p<0.01). Drought stress except for Zn had a significant effect on other elements' uptake by the plant root (p<0.01).

The results of mean comparison showed that treatment A increased K and Zn uptake. Treatment P increased N uptake. Treatment A + P had no effect on the absorption of nutrients. Treatment P reduced phosphorus and K concentration and treatment A + P reduced N and Fe concentration. The results showed, the control treatment (without fertilizer) led to increased P and Fe concentration and reduced Zn concentration (Fig. 4).

The comparison of mean of drought stress levels showed that the highest concentration of K, Fe and Zn was measured at 0.7 FC level and the highest concentration of phosphorus and N was measured in terms of FC. Also, at FC level, K and Zn concentration was reduced and, phosphorus and Fe concentration was reduced

in the plant root at 0.4 FC level. The lowest concentration of N was also observed at 0.4 FC level (Fig. 4).

Interaction effect of bio-fertilizer and different levels of drought stress showed that treatment A at FC led to increased phosphorus and N concentration, while increased concentration of N was also observed in the control treatment. The control treatment at 0.7 FC led to reduced concentration of N. Treatment A at 0.7 FC also led to increased concentration of K and Zn. The control treatment at FC also resulted in increased concentration of Fe. Treatment P at 0.4 FC resulted in reduced concentration of phosphorus and K. Fe and Zn concentration was reduced respectively, in treatment A + P at 0.4 FC and the control treatment at FC (Fig. 4).

Table 4. Analysis of variance of root's nutrients uptake of *A. sativa* L. under different drought stress and bio fertilizers

		Mean square					
SoV	Df	N	P	K	Fe	Zn	
Bio fertilizers	3	0.01**	0.09**	0.35**	26514.1**	11263.1**	
Drought stress	2	0.09**	0.29**	0.33**	41475.2**	392.1**	** p<0.01,
Bio fertilizers× Drought stress	6	0.03**	0.10**	0.39**	739.7**	5483.7**	n.s p>0.05
Error	24	0.0014	0.00144	0.00289	196.9	1050.5	
CV (%)		4.51	6.42	8.71	9.49	17.22	

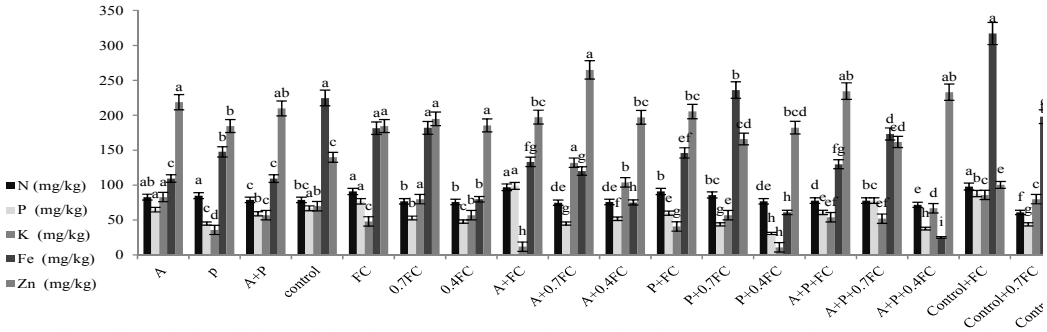

Fig. 4. Effects of bacteria inoculation and drought stress on root's nutrient uptake of *A. sativa* L. Error bars represent standard error of the mean. A= *A. vinelandii*, P= *P. agglomerans*+ *P. putida*, A+P= *A. vinelandii* + *P. agglomerans*+ *P. putida*. Different letters above the bars present significant difference among the treatments (p<0.05)

The results generally showed that the use of bacteria separately had a greater effect on nutrient uptake, compared to using them alone. The interaction effect of bio-fertilizer and drought stress also showed that bio-fertilizer treatments at FC and 0.7 FC increased the concentration of most of nutrients and bacteria use did not practically reduce the negative impact of drought stress on nutrient uptake.

4. Discussion

In general, the results showed, under drought stress, plant growth promoting bacteria increased the plant biomass. The increased biomass can be attributed to better growth and, thus, the necessary nutrients' absorption, such as nitrogen and phosphorus, due to the increase in root development (Goenadi *et al*., 2000). *Pseudomonas* bacteria are able to produce the hormones auxin and gibberellic as well as vitamins. The bacteria, due to an effect on increased nutrients uptake, can increase plant weight and yield. One of the methods of increasing the plant growth and yield by growth-promoting rhizobacteria is the ability to produce siderophore and increase the level of iron in the plant (Bhattacharyya and Jha, 2012). Thus, an increase in the plant weight can be attributed to the ability of the bacteria. Hamidi *et al*. (2010) reported that growth promoting rhizobacteria increased the shoot weight of *Z. mays*. Amiri *et al*. (2012) reported that *Azotobacter* increased the dry weight of *Foeniculum vulgare*. Seyed Sharifi and Khavazi (2012) reported an increased shoot and root weight in *Z. mays*. The results showed that in some cases the root and shoot weight of *A. sativa* L. was reduced in bio-fertilizer treatments compared with the control treatment. The results of the experiments carried out by Khoshbakht *et al*. (2011) on the effect of *P. putida* on *Aloea vera* showed that biomass loss in the treatments was related to the bacteria. Also, Cardinale *et al*. (2015) reported two strains of *Pseudomonas*, which were obtained from the rhizosphere of plants resistant to the salinity (*Hordeum secalinum* and *Plantago winteri*) from the meadows with natural saline soil, reduced the growth of barley (*H. vulgare*). It seems that

producing volatile matters such as cyanide, which is produced by direct contact between growth promoting rhizobacteria and the plant root, can enhance or reduce the plant growth and development. Cyanide that is produced by *Pseudomonas* spp can result in an increase in the plant growth as a biological control agent. On the other hand, these strains deal with pathogens reduced access to iron, resulting in reduced plant growth (Alstrom and Burns, 1989). The results showed that, by increasing drought stress, the root and shoot weight of *A. sativa* L. was reduced. In a natural environment without stress, many of the mechanisms used by growth promoting rhizobacteria for growth increase are common; however, under stressful and difficult conditions, due to the inability to survive and compete, some species cannot survive or react with the host plant and, therefore, the bacteria are not effective on the plant growth and development. As the results of the present study showed, bacteria used at 0.7 FC had a greater effect on the plant growth and yield.

The results of current study showed that under drought stress the amount of *A. sativa* L. chlorophyll decreased. Efeoglu *et al*. (2009) in their study on *Z. mays* showed that under drought stress, chlorophyll a, b and total chlorophyll were reduced. Manivannan *et al*. (2008) showed that under drought stress the amount of chlorophyll and carotenoid was reduced in *Helianthus annus*. Also, Abbaszadeh *et al*. (2008) reported that in a natural environment without stress, the amount of chlorophyll a and total chlorophyll was the highest. It seems that the reason for this decrease is due to increased destruction and/ or production of pigments as well as impaired activity of enzymes responsible for photosynthetic pigments synthesis. Researchers have mentioned reduced cellular proteins under drought stress, increased chlorophyllase and peroxidase enzyme activity as the factors affecting reduced chlorophyll under drought stress. So that reduced chlorophyll contents of the plant under long stress might be somewhat due to reduced flow of nitrogen to the plant tissues and changes in enzymes' activity such as nitrate reductase (Ahmadi and Baker, 2000; Ebrahimi *et al*. 2016). The results generally showed that growth promoting bacteria *Azotobacter* and *Pseudomonas* had no significant effect on reducing negative impacts of drought stress on *A. sativa* L. photosynthetic pigments.

In the present study, *Azotobacter* reduced the amount of chlorophyll in the plant. One of the factors reducing the effect of bacteria such as *Azetobacter* is that *Azetobacter* is of heterotroph bacteria group that for the growth and activity needs simple carbon resources, and is active when an organic matter is present. But the soil used in this experiment was provided from rangeland with poor soil in organic matter. *Azotobacter* is also seen mostly in alkaline to neutral soil, the most appropriate soil pH range for the growth and proliferation of *Azotobacter* is 6.5 to 8, and out the soil pH the population of the bacteria is reduced; while pH of the soil used to cultivate the *A. sativa* L. (Table 1) was 4.9. Studies have shown that the use of growth promoting bacteria with chemical fertilizers, animal manures, vermin compost and other fertilizers leads to increased growth of plants (Delshadi *et al*. 2017). So in the soil used in this study, *Azotobacter* was not able to grow and reproduce and therefore, it had no significant effect on nitrogen fixation and the product growth (Khosravi and Mahmoudi, 2013).

The interaction effect of bio-fertilizer and drought stress also showed that bio-fertilizer treatments at FC and 0.7 FC increased the concentration of most of nutrients and bacteria use did not practically reduce the negative impact of drought stress on nutrient uptake. Khoshbakht *et al*. (2011) showed that *P. putida* increased phosphorus uptake in *A. vera*. Also Esitken *et al*. (2010) reported that the use of growth promoting bacteria of *Pseudomonas* increased the amount of Fe and Mg. Asghari *et al*. (2014) showed that growth promoting bacteria had a positive effect on mineral nutrient uptake in rice. Kizilkaya (2008) showed that *Azotobacter* increased nitrogen uptake of wheat. Similarly Turan *et al*. (2012) reported an increase in absorption of phosphorus and Zn in strawberry. The present study results suggested more efficiency of Pseudomonas in up taking elements such as phosphorus, N in *A. sativa* L. compared with the control treatment. Fallah Nosrat Abad and Shariati (2014) reported that the *P. putida* was the best treatment compared with the control treatment and had the highest effect on increasing concentrations of phosphorus, Fe and Zn. *Pseudomonas* bacteria with the help of changing the acidity of surroundings and also enzymatic processes are able to turn soil dissolved phosphorus to organic phosphorus acids and light phosphorus and increase the element mobility in the soil. The acids reduce soil pH and are effective on dissolved phosphate (Madani *et al*., 2011). Also, another reason of increased nutrient uptake by growth promoting bacteria is increasing the EC in inoculated treatments with bacteria compared

with the control treatment. Rodriguez *et al*. (1999) reported increased phosphorus uptake by plants symbiotic with phosphate solubilizing microorganisms due to the production of carbon dioxide by the microorganisms and the effect on increasing the absorption of phosphorus.

On the interaction of a combination of treatments A and P on increasing the absorption of elements such as Fe, K and Zn, it can be said that the inoculation of growth promoting bacteria a synergic and intensifying relationship is found that can improve microbial biomass and increased activity in order to increase the absorption of minerals from the soil and then through optimized plant growth improve plant growth. It seems that the combination of a variety of growth promoting bacteria can provide the possibility of an intensifying relationship that the result is increased bacteria beneficial effects including increased absorption of water and nutrients from the soil by the plant and as a result the plant increased growth and the plant more products (Delshadi *et al*., 2017). Improved plant growth through seed treatment with bio-fertilizers can be due to the effect of the microorganisms on physiological and metabolic activities of the plant as well as nitrogen fixation and other part of this additive effect is on the improved plant efficiency by hormone cytokinin and auxin stimulating the absorption of water and nutrients (Delshadi, 2015).

Another reason for the increased nutrient uptake is increased electrical conductivity in the treatments inoculated compared with the control treatment. Researchers reported increased phosphorus uptake by plants coexisted with microorganisms' phosphate solubilizing due to carbon dioxide production by microorganisms and the effects on increased absorption of phosphorus (Rodriguez *et al*., 1999). Perhaps the reason of increased uptake elements such as phosphorus in the control treatment is also root development and then increased nutrient absorption in this treatment. Growth promoting bacteria through the production of useful compounds for host plant and facilitate the uptake of nutrients from the soil (Kloepper *et al*., 1987) as well as facilitate the plant growth, by nitrogen fixation of atmosphere, phytohormone and dissolution of minerals such as phosphorus, synthesis and siderophore secretion which may solve and separate iron, increase the availability of nutrients for the plant (Patten and Glick, 2002; and Nadeem *et al*., 2014). Also growth promoting bacteria through the production of amino cyclopropane-1-carboxylate deaminase (ACC) and chitinase enzyme and also the production of materials such as exopolysaccharide that help the plant under stress conditions (Sandhya *et al*., 2009) will increase the plant growth and development.

The bacteria directly (regulate physiology plant through synthesis of plant hormones) and indirectly (increased plant access to the soil nutrients and minerals) increase plant growth and development. More than one mechanism is involved at the same time in improving the plant growth and a mechanism when is evaluated separately will have a less significant effect, and only a combination of mechanisms could be responsible for the accurate measurement of the effect of inoculation with bacteria on plants. Biological nitrogen fixation, synthesis of the plant growth regulators, increasing the mobility, nutrient uptake and solubility of insoluble mineral combinations such as phosphorus, reduce or prevent synthesis of ethylene in plants under stress conditions and increase the plant resistance to environmental stress and biological control of pathogenic microorganisms are effective mechanisms on increasing the yield of plants inoculated with the bacteria (Bashan and Levanony, 1990).

5. Conclusion

The results showed that growth promoting bacteria individually had a greater effect on increasing the plant growth, photosynthesis and nutrient uptake in root of *A. sativa* L., compared with the combined use of growth promoting bacteria. The remarkable note of the results of this study was that growth promoting bacteria under irrigation conditions at 0.7 FC and FC increased studied traits and had no role in mitigating the effects of drought stress. The results of this study showed that growth promoting bacteria can be used in the restoration and improvement of rangelands, but it should be considered the effect of bio-fertilizers depends on the plant species, climate and soil condition. However, the question as to what extent can the rhizobacteria promote the host plant's resistance to drought effects needs further research, so that appropriate strains of each region and plant can be known and used, given that growth-promoting rhizobacteria include a wide range of soil microorganisms. Therefore, a more comprehensive and accurate survey and study in the field is recommended.

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