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Influence of plant-growth-promoting bacteria on germination, growth and nutrients' uptake of *Onobrychis sativa* L. under drought stress

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ABSTRACT

The study was conducted to investigate the effect of growth-promoting bacteria (*Azotobacter vinelandii* = A, *Pantoea agglomerans* + *P. putida* = P, combined A + P, control) on the germination, growth and nutrient uptake of *Onobrychis sativa* L. under drought stress (field capacity (FC), 0.7 FC, 0.4 FC). The results showed that the highest and lowest germination rate (GR) was related to the P and control treatments in FC level, respectively. The maximum root and shoot length at the FC level was related to the treatment A. In the A + P treatment, the maximum shoot dry weight was measured in 0.7 FC level. The lowest root dry weight was also related to the A + P treatment in the FC level. Bio-fertilizers had the maximum impact in increasing the nutrients uptake in 0.7 FC and FC levels. The fertilizers in 0.7 FC and FC levels increased the plant traits and played no significant role in mitigating the effects of drought stress.

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KEYWORDS
Azotobacter; *pseudomonas*; seed germination; plant growth; *onobrychis sativa* L

Introduction

Due to the crisis of environmental pollution, especially pollution of soil and water resources that has constantly extended to human food sources and has threatened the human health, extensive efforts have been started to find proper strategies for improving soil quality and agricultural products with the removal of pollutants (Delshadi 2015). Chemical fertilizers are kinds of the pollutants that, in addition to high-energy consumption, are expensive and indiscriminate use of them in addition to economic disadvantage will cause irreparable damage to the environment (Amin Deldar et al. 2012).

With the growing population, the problem of nutrient shortages in developing countries is of utmost importance. On the other hand, in most countries that are confronting with nutrient shortages, quality and quantity of protein is the power key issue of nutrition (Afshari et al. 2014). In Iran, livestock industry development requires the serious attention to the supply of forage and animal feed in way that meet the needs of the growing population in protein products (Safikhani et al. 2014). Rangelands are one of the most important and valuable natural resources that proper utilization coupled with the resuscitation and modification operations of them can play a vital role in maintaining soil and water supply people's needs in the field of protein products (Jangali et al. 2012). In this regard, due to the reduction in forage production of rangelands by natural and human factors, it is necessary to increase the feed production in various ways. One of these ways is the seeding operations of important and palatable plant species in rangelands that, if successful, can increase the production of fodder in rangelands (Ghaderi et al. 2013). The most important problems of arid and semi-arid rangelands are drought and water shortage that affect the growth and development of plants (Zandi Esfahan & Azarnivand 2012). Drought stress is one of the main environmental factors limiting plant growth; the most common cause of that is increase in temperature and reduction in available water to plants (Nazar et al. 2015). Water shortage as a factor limiting in the germination stage inhibits growth and establishment of the plant and reduces the crop production (Yan 2015).

The first step that a plant may be confronted with drought is germination. Since the germination begins with water uptake, water shortage at this stage, in terms of duration and intensity of stress, causes to non-germination or reduce in percentage and rate of germination and delays plant establishment finally (Smithson et al. 1985). The drought resistance is changed in plant life cycle and most of the plants in the germination stage are more susceptible to drought. On the other hand, a significant number of existing bacteria and fungi in the soil have functional relations with plants and have beneficial effects on their development (Kaya et al. 2006). Nowadays, utilization of beneficial soil organisms as bio-fertilizers are raised as the most natural and most desirable solution for keeping active and alive of vital soil system (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). Some of these bacteria species are widely used in agriculture so as to increase seeds' production as well as yield, and in disease control. The bacteria directly (by regulating the physiology of plants through synthesis of plant hormones) and indirectly (increased plant access to the soil's nutrients and minerals) increase plant growth and development. *Azotobacter* spp. and *Pseudomonas* spp. are the most important bacteria that, in addition to increasing soil mineral elements, with the production of matters regulating growth, affect the development and yield of plants (Zahir et al. 2004; Hayat et al. 2010).

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According to most of the studies conducted, the effect of growth-promoting bacteria on plant growth and development has been positive and can be a good alternative to chemical fertilizers. Also, increased plant resistance and reduced negative effects of environmental stress, including drought stress and reduced environmental risks to other fertilizers, are among the advantages of this type of fertilizer (Delshadi 2015).

Major research studies conducted on the effects of growth-promoting bacteria on plants are in the field of agriculture and crops, while a few studies have been conducted on the use of this type of fertilizer for rangeland plants for rangeland restoration. Due to the fact that the majority of the world's rangelands are in arid and semi-arid areas (Zandi Esfahan and Azarnivand 2012; Delshadi 2015), and drought stress is one of the most important factors in reduced plant growth, the present study was conducted with the hypothesis that growth-promoting rhizobacteria *Azotobacter vinelandii* and *Pantoea agglomerans* + *P. putida*, under drought stress, increase germination, plant growth and the absorption of nitrogen, phosphorus, potassium, iron, zinc and manganese in the shoots of *Onobrychis sativa* L. The objectives of this study included: (1) The study of the effect of growth-promoting rhizobacteria under drought stress on the germination and growth of *O. sativa* L.; and (2) Assessing the effect of plant-growth-promoting rhizobacteria on the nutrient uptake in *O. sativa* L. shoots under drought stress conditions.

Materials and methods

Plant species

O. sativa L. belongs to the *Leguminosae* family. It has been cultivated over the centuries in different parts of the world such as Iran and farmers have used it for feeding their livestock. The plant is important not only due to high production and palatability, but can also be used for rangelands' restoration and conversion of abandoned dry farming lands. The plant has a high tolerance to abiotic stresses, particularly drought stress and hence can be used to feed production in dry areas. *O. sativa* L., due to the having capabilities such as high adaptability to different weather conditions, high forage production in low irrigation condition, high nutritional value, insects absorption due to the sweet nectar to produce honey and due to having deep roots to prevent soil erosion and improve cultivating soil structure and quality, is one of the most important forage crops (Berard et al. 2011; Boschma et al. 2011).

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 9.1°C and 35°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 were considered. The second factor was applying drought stress at three levels: FC, 0.7 FC and 0.4 FC.

The soil samples were air-dried, homogenized and sieved through a 4 mm stainless sieve before analysis. The soil's texture (loamy sand) was determined using laser diffractometry (Wang et al. 2012); soil pH (4.9) was determined in a 1:5 soil to distilled water slurry after one hour of agitation using pH-meter (Thomas 1996); electrical conductivity (EC) (0.19 dS m^{-1}) was determined using an EC–meter (Rhoades 1996); Total soil nitrogen (0.17%) was analyzed using Kjeldahl method (Bremner 1996). Available phosphorus (16 mg kg^{-1}) was determined by the method of Bray and Kurtz (1954). Available potassium (560 mg kg^{-1}) was measured by flame photometry method (Knudsen et al. 1982). Organic matter content (1.71%) was determined using the methods described by Lo et al. (2011).

The seeds were prepared from Isfahan Pakan Bazr Co., Iran. Firstly, in order to cultivate the seeds, empty pots were weighed. 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, two 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. For this purpose, the pots were irrigated daily at FC, 0.7 FC and 0.4 FC. To measure the GR and percentage, the number of seedlings was recorded daily.

Determine seed germination

Germination percentage (GP) and GR, respectively were calculated using the following formula (Bajji et al. 2002):

$$GP = (Ni/S) \times 100$$

GP is germination percentage, Ni is the number of seeds germinated per in day *i* and, *S* is the total number of seeds planted.

$$GR = \sum Ni/Di$$

GR is germination rate (in terms of the number of germinated seeds per day), and Ni is the number of germinated seeds in daily, and Di is the number of days from the initial sowing.

After 10 days of planting, the plants were thinned. In each pot, seven plants remained. After 90 days, the plants were harvested. The plants were washed with distilled water to measure root and shoot lengths, plant dry weight, chlorophyll contents and the amount of nutrients' uptake.

Determination of nutrients' uptake

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 h, the samples were heated on the heater (150°C). After cooling the tubes three times, at each time one ml of hydrogen peroxide was added and this practice continued

until obtaining a 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, Zn and Mn 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, Zn and Mn were determined using ICP/OES (GBC Avanta, Australia).

Statistical analysis

All of the data were analyzed using the SPSS 18.0. The data (three replicates) were analyzed by analysis of variance (ANOVA). Distribution was tested for normality by Kolmogorov–Smirnov. Equality of variance among treatments was tested using the Levene's test for homogeneity of variance. Post hoc Duncan test was performed to determine the significant differences among treatments. When the *p*-value was lower than .05, it was considered significantly difference.

Results

Seed germination

The results of data variance analysis (Table 1) showed that the main effect of the bio-fertilizer treatments, drought stress and interaction effects of bio-fertilizer and drought stress on the GR were significant ($p < a.01$), while the treatments had not significant effects on the GP.

Table 1. Analysis variance of seed germination of *O. sativa* L. under different drought stress and bio-fertilizers.

		Mean square	
SOV	Df	Germination rate	Germination percentage
Bio-fertilizers	3	114.39**	202.77n.s
Drought stress	2	60.25**	144.44n.s
Bio-fertilizers ×Drought stress	6	48.90**	177.77n.s
Error	24	0.28	252.77
CV (%)		2.02	20.81

Note: SOV, source of variations; CV, coefficient variation.
**$p < .01$.
n.s, $p > .05$.

The results of the main effects of the bio-fertilizers showed that the highest GR was related to the treatment P and the lowest GR was measured in the control treatment (Figure 1). The results of the main effects of drought stress showed that the highest and lowest GRs were related to the levels of 0.4 FC and 0.7 FC, respectively (Figure 1).

The results of comparison mean of interaction effect of bio-fertilizers and drought stress showed that the highest and lowest GRs were related to the treatments P and control at FC level (Figure 1).

Plant growth

The results of data variance analysis (Table 2) showed that the main effect of the use of bio-fertilizers, different levels of drought stress and the interaction effects of bio-fertilizer and drought stress compared with the control treatment had not significant effects on the root length of *O. sativa* L. The main effect of bio-fertilizers had no significant effect on the shoot length, but different levels of drought stress ($p < .01$) and interaction effects of bio-fertilizer and drought stress had significant effect on the shoot length ($p < .05$).

The results of comparison mean showed that treatment A significantly increased the root length. Meanwhile, reduction

Table 2. Analysis variance of root and shoot length, root and shoot dry weight of *O. sativa* L. under different drought stress and bio-fertilizers.

		Mean square			
SOV	Df	Root dry weight	Shoot dry weight	Shoot length	Root length
Bio-fertilizers	3	0.35**	6.44**	0.65n.s	0.72n.s
Drought stress	2	0.27**	1.12**	10.53**	3.24n.s
Bio-fertilizers × Drought stress	6	0.17**	1.31**	3.05*	3.05n.s
Error	24	0.00	0.03	2.12	1.46
CV (%)		3.25	19.84	10.92	9.67

Note: SOV, source of variations; CV, coefficient variation.
**$p < .01$.
*$p < .05$.
n.s, $p > .05$.

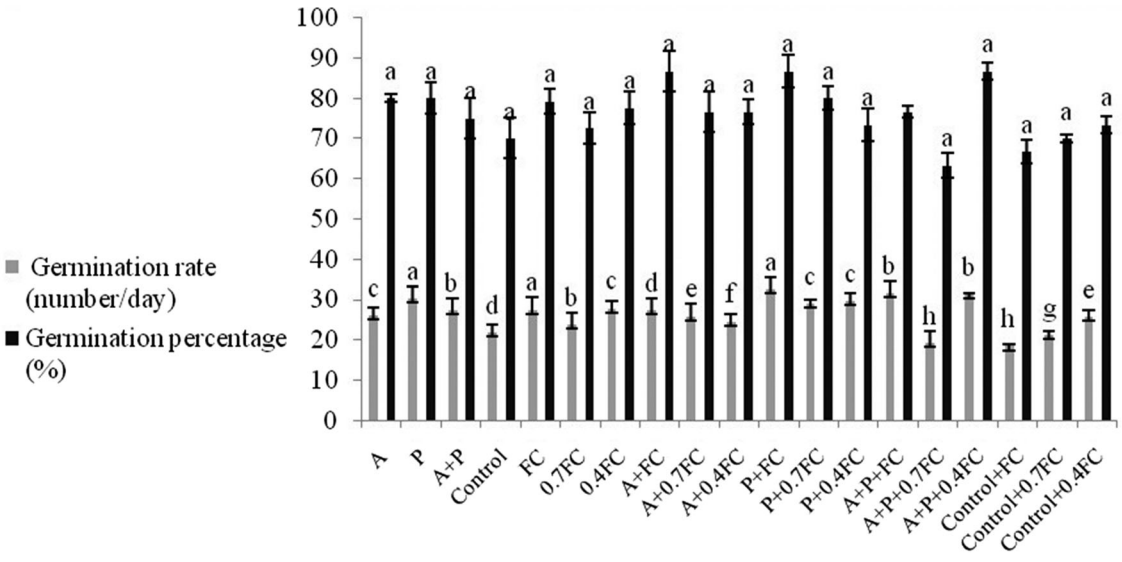

Figure 1. Effect of bacteria inoculation and drought stress on GR and GP of *O. sativa* L. seeds. Error bars represent standard error of the mean. A = *A. vinelandii*, P = *P. agglomerans* + *P. putida*, A + P = *A. vinelandii* + *P. agglomerans* + *P. putida*.

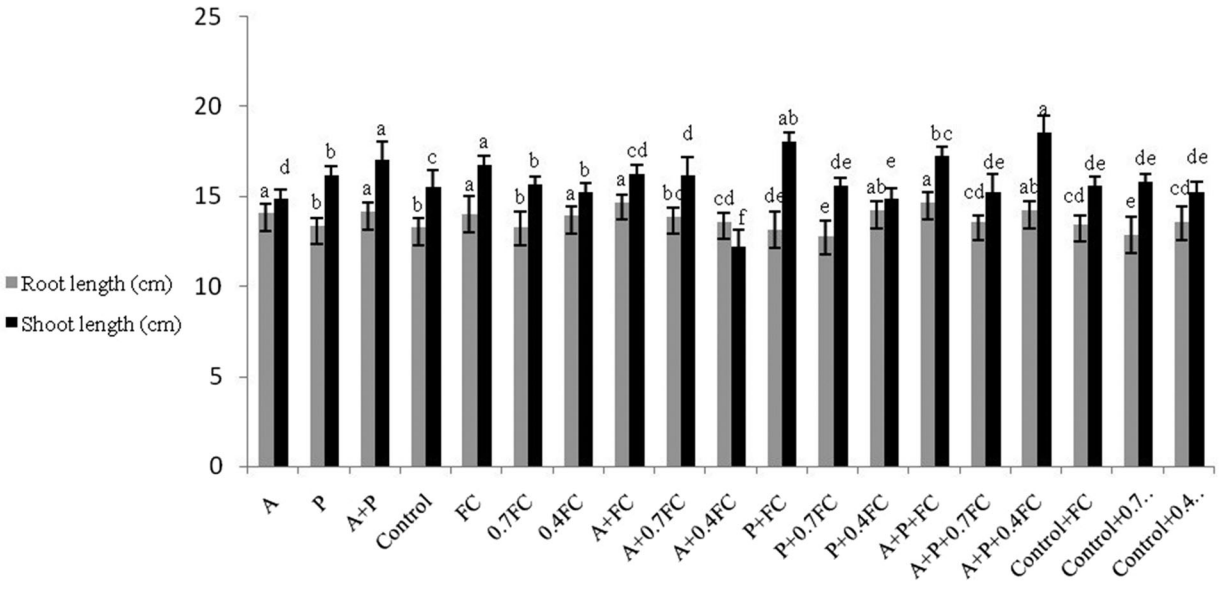

Figure 2. Effects of bacteria inoculation and drought stress on root and shoot length of *O. sativa* L. Error bars represent standard error of the mean. A = *A. vinelandii*, P = *P. agglomerans* + *P. putida*, A + P = *A. vinelandii* + *P. agglomerans* + *P. putida*.

in root length was observed in the treatment P. The maximum and minimum plant shoot length was related to the A + P and P treatments, respectively (Figure 2). The results of main effects of drought stress showed that the maximum root and shoot length was related to FC level (without stress), and the minimum root and shoot length was measured in treatment A at the level of 0.4 FC (Figure 2).

The results of the interaction effects of bio-fertilizers and drought stress showed that the maximum length of root and shoot was related to the treatment A at the FC level, while the minimum length of root and shoot was related to the treatment A and at 0.4 FC level and the control treatment at 0.7 FC level (Figure 2).

The results of analysis of variance (Table 2) showed that the effects of the bio-fertilizer, different levels of drought stress and the interaction effects of the bio-fertilizer and drought stress had a significant effect on the root and shoot dry weight ($p < .01$).

The results of the main effects of the bio-fertilizers showed that the bio-fertilizers increased root and shoot dry weight of the plant, compared with the control treatment. The highest root and shoot dry weights were observed in the treatments A + P. The lowest root and shoot dry weights were observed in the treatments P (Figure 3). The results of the main effects of drought stress showed that the highest and lowest shoot and root dry weights were observed at 0.7 FC and FC level, respectively (Figure 3).

The results of the interaction effect of bio-fertilizers and drought stress showed that, in the A + P treatment under 0.7 FC level, shoot dry weight was increased; however, in the treatment P, shoot dry weight was reduced under FC level. The highest and lowest root dry weights were related to the treatment A + P at FC levels (Figure 3).

Nutrients' uptake

The results of data variance analysis (Table 3) showed that the main effects of bio-fertilizer and different levels of drought stress were significant on the uptake of K, Fe, Zn and Mn in the shoot of plant ($p < .01$). Also, bio-fertilizers had a significant effect on the amount of N ($p < .01$). Different levels of drought stress had no significant effect

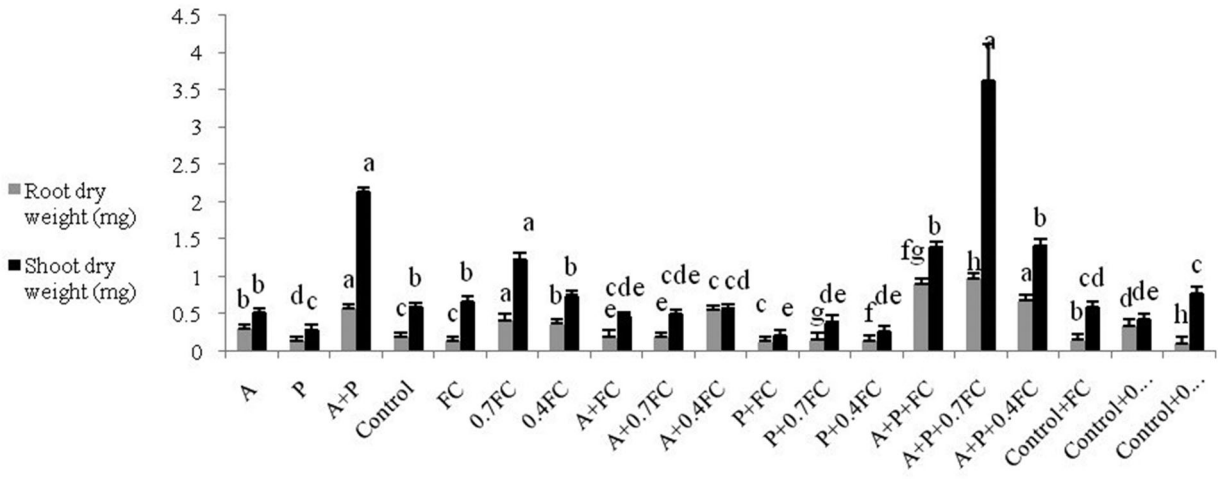

Figure 3. Effects of bacteria inoculation and drought stress on root and shoot dry weight of *O. sativa* L. Error bars represent standard error of the mean. A = *A. vinelandii*, P = *P. agglomerans* + *P. putida*, A + P = *A. vinelandii* + *P. agglomerans* + *P. putida*.

Table 3. Analysis variance of nutrients uptake of *O. sativa* L. under different drought stress and bio-fertilizers.

		Mean square					
SOV	Df	N	P	K	Fe	Zn	Mn
Bio-fertilizers	3	0.16**	1.04**	0.10**	3419.05**	120.91**	146.04**
Drought stress	2	0.01n.s	0.90**	0.25**	961.31**	83.81**	583.74**
Bio-fertilizers × Drought stress	6	0.03**	0.76**	0.06**	1081.46**	48.64**	371.66**
Error	24	0.07	0.02	0.00	2.21	0.54	0.24
CV (%)		9.55	18.35	5.07	2.09	3.57	2.33

Note: SOV, Source of variations; CV, coefficient variation.
**$p < .01$.
n.s, $p > .05$.

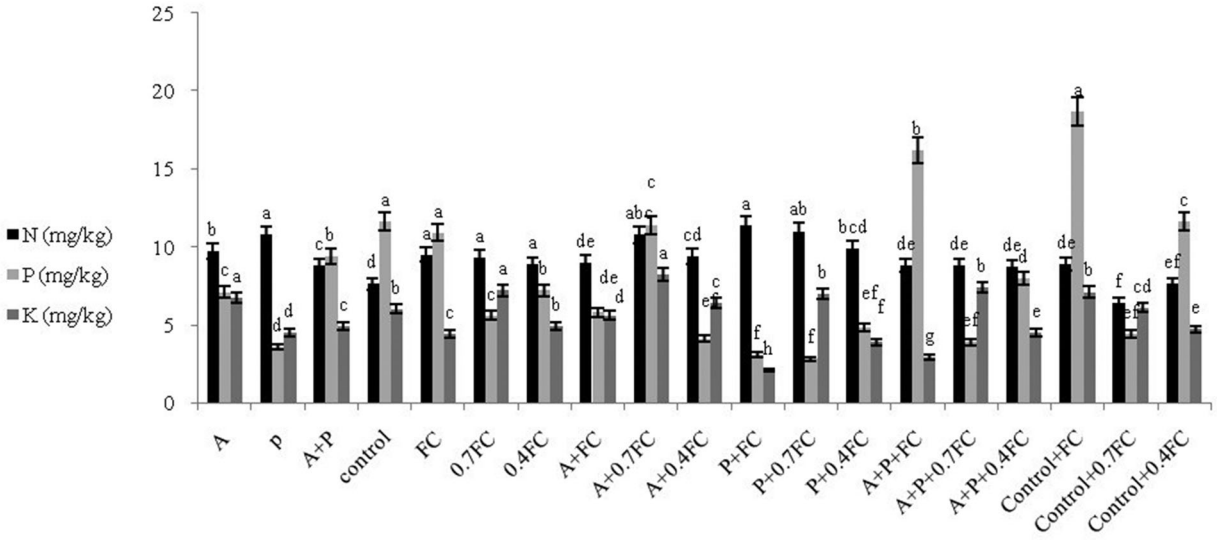

Figure 4. Effects of bacteria inoculation and drought stress on concentration of N/P/K in shoot of *O. sativa* L. Error bars represent standard error of the mean. A = *A. vinelandii*, P = *P. agglomerans* + *P. putida*, A + P = *A. vinelandii* + *P. agglomerans* + *P. putida*.

on the amount of N in the shoot of plant. The results showed that the interaction effects of bio-fertilizers and drought stress had significant effect on the amount of N ($p < .01$), while these factors had no significant effect on uptake of Mn.

Results of the comparison mean of bio-fertilizers showed that maximum and minimum of concentration of N was observed in the treatment P and control, respectively. The control treatment showed a significant increase in the phosphorus uptake and the P treatment reduced the uptake of phosphorus in comparison with other treatments. The results showed that treatment A led to increase in K (Figure 4), Zn and Mn uptake (Figure 5), while the minimum concentrations of K (Figure 4) and Zn (Figure 5) were measured in the treatment P and the minimum concentration of Mn was measured in the control treatment. The maximum and minimum concentrations of Fe were measured in the P and A treatments. The maximum and minimum concentration of Mn was observed in the control and A + P treatments, respectively (Figure 5).

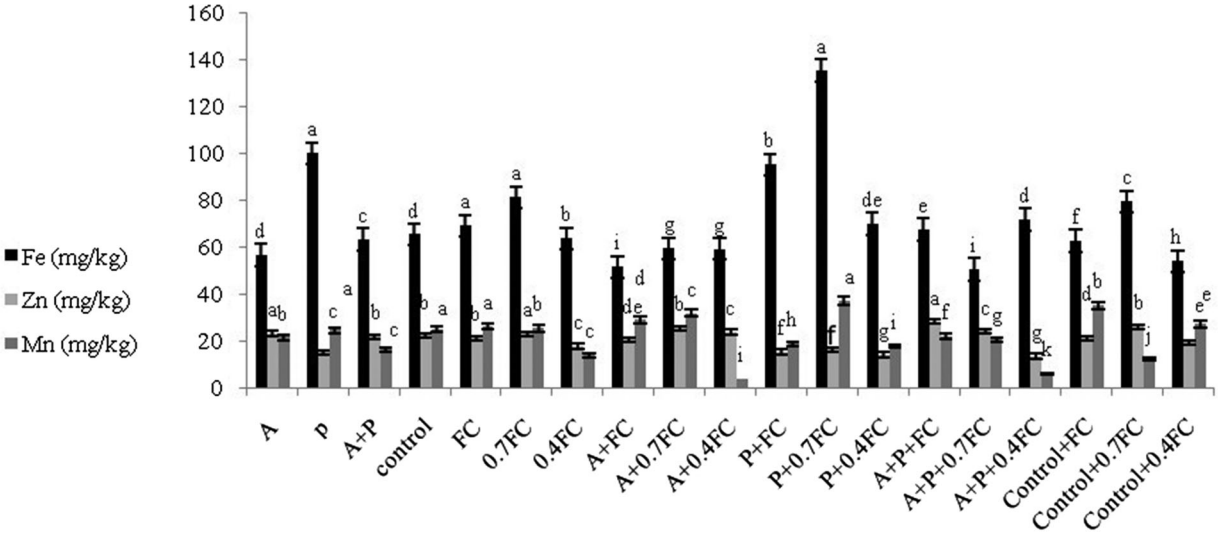

Figure 5. Effects of bacteria inoculation and drought stress on concentration of Fe/ Zn/ Mn in shoot of *O. sativa* L. Error bars represent standard error of the mean. A = *A. vinelandii*, P = *P. agglomerans* + *P. putida*, A + P = *A. vinelandii* + *P. agglomerans* + *P. putida*.

Results of the comparison mean of different levels of drought stress showed the maximum concentrations of P, N (Figure 4) and Mn (Figure 5) and the minimum amount of K were measured in the level of FC (Figure 4). The maximum concentrations of K (Figure 4), Fe, Zn and Mn (Figure 5) and the minimum amount of phosphorous were measured in the level of 0.7 FC (Figure 4). The minimum concentrations of N (Figure 4), Fe, Zn and Mn (Figure 5) were related to level of 0.4 FC (Figure 4). Results of interactions effects of bio-fertilizers and drought stress showed that the maximum concentration of N was related to the P treatment in the FC level and the minimum N uptake was measured in the control treatment at the level of 0.7 FC. The maximum and minimum concentration of phosphorus was observed in the control treatment in level of FC and treatment P at the level of 0.7 FC. The maximum and minimum concentration of K was related to the treatment A at level of 0.7 FC and treatment P at FC level, respectively (Figure 4). Also, the maximum and minimum concentration of Fe was obtained in the P and A + P treatments, at the level of 0.7 FC, respectively (Figure 5). Treatment A + P in the FC level, caused to maximum increasing of concentration of Zn and, the minimum uptake of Zn was related to treatment A + P at level of 0.4 FC (Figure 5). The maximum and minimum concentration of Mn was measured at the level of 0.7 FC and treatment A at the level of 0.4 FC (Figure 5). The maximum and minimum concentration of Mn was observed in the treatment A at the level of 0.7 FC and the treatment P at level 0.4 FC, respectively (Figure 5).

Discussion

In general, the results showed, under drought stress, treatment P at FC level increased the seed GR of *O. sativa* L., while it had no significant effect on the GP. In this context, Shaukat et al. (2006) reported *Azotobacter* spp. and *Pseudomonas* spp. had a significant effect on germination and seedling growth of *Zea mays* L. Similar results were also reported by Gholami et al. (2009) on improved seedling germination of *Z. mays* inoculated with growth-promoting rhizobacteria. Also, Nuncio-Orta et al. (2015) showed that *Azotobacter* increased *Capsicum annuum* L. germination. In explaining this, it can be stated that some enzymes (Hidrolitil enzyme) are involved in the process of seed germination, and in the presence of growth-promoting rhizobacteria, activities were faster than observed with the control treatment (without fertilizer) and therefore the seed GR and percentage of this treatment is compared more with the control treatment (Delshadi 2015).

The results showed that treatment A significantly increased the root and shoot lengths of the plant at FC level, while the treatment A at level of 0.4 FC reduced the root and shoot lengths and had no effect in reduction of drought stress on the plant growth. The effects of growth-promoting rhizobacteria, including the production of the plant growth regulators, such as gibberllic acid, cytokinin and auxin, can directly and indirectly provide favorable conditions for the plant growth (Nadeem et al. 2014). Microbial inoculation changed the hormone levels within a gene, that later led to changes in the growth and development of plants and plants'-inoculated elevation gain (Glick et al. 2007). The bacteria, by increasing the volume and root development, increased the plant's access to nutrients and water, thereby attracting the plant nutrients. Finally, the plant nutrients' uptake increased the plant shoot growth (Davoodifard et al. 2012).

Another mechanism of growth-promoting rhizobacteria to stimulate plant growth is to reduce ethylene levels. Amino Cyclopropane-1-Carboxylate deaminase (ACC) is an ethylene precursor that, by ACC-occidaz enzyme activity, is converted to ethylene. ACC-deaminase enzyme bacteria are able to use ACC as the only source of nitrogen and, by reducing ethylene concentration and preventing the accumulation of this material under drought stress, can control the negative effects on root growth and, therefore, protect it (Glick et al. 2007). Cotton and wheat seeds inoculated with growth promoters increased shoot and root height (Narula et al. 2005; Kumar et al. 2007). An increased height of *Z. mays*. seedling inoculated with *Azotobacter* and *Pseudomonas* has also been reported (Zahir et al. 2004). Walker et al. (2012) also reported that plant height was increased in the presence of *Pseudomonas* bacteria. Sarcheshmehpour et al. (2013) reported a positive effect of plant-growth-promoting rhizobacteria under drought stress on the growth and nutrition of pistachio seedlings. Similar results have also been reported by Rana et al. (2015) on the positive effects of growth-promoting rhizobacteria on rice and wheat.

In the present study, it was observed that the treatment A reduced the shoot length of the plant under drought stress. Khosravi and Mahmoudi (2013) reported a similar reduction in wheat growth indices in the presence of *Azotobacter*. One of the factors that can prevent plant height and the transfer of auxin is ethylene acetic acid (Vacheron et al. 2013). Under normal conditions, drought stress increases ethylene concentration of the plant. Also, the reduced plant height, as a result of applying drought stress, can be attributed to impaired photosynthesis due to the dehydration and reduced production of materials for submission to the plant growing parts and, finally, lack of access to the genetic potential in terms of the plant height (Jamshidi et al. 2012).

The results of the present study showed that the treatment A + P increased the shoot dry weight of the plant. It seems that the reason for this increase is due to extra absorption of the macro and micro nutrients and increased hormone production. The increased dry matter production 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 acid as well as vitamins. The bacteria, due to an effect on increased nutrient uptake, can increase plant dry 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 & Jha 2012). Thus, an increase in the plant dry weight can be attributed to the ability of the bacteria. Hamidi et al. (2010) reported that growth-promoting rhizobacteria increased the shoot dry 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 dry weight in *Z. mays*.

The results showed that the shoot dry weight of the plant was reduced in bio-fertilizer treatments under FC level compared with the control treatment. Reduced root dry weight was observed in the A + P treatment in FC level. The results of the experiments carried out by Khoshbakht et al. (2011)

on the effect of *P. putida* on *Aloea vera* showed that shoot dry weight loss in the treatments was related to the bacteria. Similar results were also reported on reduced lettuce and bean growth, which was affected by the bacteria *Pseudomonas* (Alstrom & Burns 1989). Also, Cardinale et al. (2015) reported that 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 bacteria used at 0.7 FC had a greater effect on the plant growth and yield.

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.

The results of the present study showed that the use of bio-fertilizers separately had more effects on nutrients' uptake in comparison with combination application and the control treatment. The interaction effect of bio-fertilizer and drought stress also showed that bio-fertilizers at FC and/or 0.7 FC increased the nutrients' uptake, while using bacteria practically did not reduce the negative impact of drought on the uptake of nutrients. The results of a study by 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 rhizobacteria of *Pseudomonas* increased the amount of elements such as Fe and Mg. Asghari et al. (2014) showed that growth-promoting rhizobacteria had a positive effect on mineral nutrient uptake in rice.

The results of the present study showed a higher yield of the treatment P in absorbing elements, such as N and phosphorus in *O.sativa* L., compared to 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, as well as being able to change the acidity of their surroundings and also enzymatic processes, are able to turn soil-dissolved phosphorus into organic phosphorus acids and light phosphorus, and increase the element mobility in the soil. The acids reduce soil pH and are effective in dissolving phosphates (Madani et al. 2011). Also, another reason for the increased nutrients' uptake by growth-promoting bacteria is an increase in the EC in inoculated treatments with bacteria compared with the control treatment. Rodrı´guez and Fraga (1999) reported an increased phosphorus uptake by plants inoculated with phosphate-solubilizing microorganisms due to the production of carbon dioxide by the microorganisms, as well as the effect on increasing the absorption of phosphorus.

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

Conclusion

The results of the present study showed that the use of bio-fertilizers, separately or in combination, increased the germination of *O. sativa* L., Also, according to the results, it seems that these fertilizers at 0.7 FC and FC levels were effective in increasing the plant growth. They had little impact on reducing the negative impacts of drought stress. According to the results of this study, it can be stated that the use of bio-fertilizers containing rhizobacteria can be effective on germination and nutrients' uptake.

Another aspect that is important when using these types of microorganisms is the selection of the appropriate strains of each climate, the type of plant and environmental conditions, as the bacteria can have the highest effect on the growth of a plant species. In general, the results of this study showed that the effects of bio-fertilizers on *O. sativa* L. are positive in terms of them being used in the restoration and improvement of rangelands. 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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Disclosure statement

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