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Research Article

Bio-fertilizers Efficiency on Physiological Growth and Yield of *Aloe vera* L. in arid lands of Iran

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Abstract: This research was carried out to assess bio-fertilizers (*Azospirillum brasilense* and *Pseudomonas fluorescens*) for replacing chemical fertilizers to produce organic crop with improvement of *Aloe vera* root for increasing absorption efficiency micro and macro elements to prevent of ecological effects in the glassy greenhouse in Hassan Abad, Semnan. *Aloe vera* was planted an offshoot in the pots on 20/6/2009. This research was designed as Randomized Complete Blocks Design (RCBD) in three repetitions with four treatments as follows: (first treatment: control, second treatment: *Azospirillum brasilense* bacteria, third treatment: *Pseudomonas fluorescens* bacteria and fourth treatment: integration of *A.brasilense* and *P.fluorescens*. Physiological growth parameters were; total fresh weight, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, length of the biggest leaf, length of the biggest root, leaf number, offshoot number, area of the biggest leaf, plant total area, measuring of Nitrogen, Phosphorus and Potassium elements of the plant in two continuous years. Data were analyzed by using MSTAT-C software and comparison of means by Duncan's multi-range test (LSD). The results showed that *P.fluorescens* treatment vs. control and second treatment (*A.brasilense*) and fourth treatment (combination of two bacteria) was statistically higher in fresh and dry weight of shoot, number, length, and area of the leaf, plant total area and amount of Nitrogen, phosphorous and potassium elements. *A.brasilense* treatment vs. control and third treatment (*P.fluorescens*) and fourth treatment (combination of two bacteria) was statistically higher in total fresh weight and offshoot number. The fourth treatment (integration of two bacteria) vs. control and second treatment (*A.brasilense*) and third treatment (*P.fluorescens*) was statistically higher in fresh and dry weight of root and length of the biggest root. The results declare that use of *P.fluorescens* bio-fertilizer as compared with other treatments increases the yield of *Aloe vera*.

Keywords: *Aloe vera*, *Azospirillum brasilense*, Bio-fertilizers, Growth, Iran, *Pseudomonas fluorescens*

INTRODUCTION

Aleo vera L. is the most important ornamental and medicinal plant that has been used to treat various diseases for many years. The plant can evaluate for the mass cultivation, according to the conditions of the arid and semi-arid of the country. This results in better economy condition, people employment, self-sufficiency and higher productivity of the poor agricultural lands and the optimum use of irrigation water. This plant uses in the medicinal industrials, cosmetic, sanitary and food as a multipurpose plant. So, it must be free from any chemical residues and it also is better to replace the bio-fertilizers instead of chemical fertilizers to prevent environmental pollutions. Sustainable and organic products with high quality in the international markets is essential according to the welcome of farmers and greenhouse owners to produce and provide proper cultivation plan and optimize culture medium. Application of biological fertilizers with improved root status can increase the absorption of nutrients. Therefore, this research will study the best and most suitable biological fertilizer and culture medium in order to raise the quality of the product and increase the yield per unit area. According to the International Aloe Science Council (IASC), the value of the world trade of raw crop is about 125 million dollars and the value of the final crops is 110 billion dollars (Rodriguez, 2004).

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Aloe vera is perennial brush with succulent leaves. Its height is about 60 to 80 cm and it has spear-shaped leaves. This plant is xerophyte, always green, vigorous, branched tuberous roots, short and thick stems and almost branched, yellow and bright red flowers, self-incompatible (i.e. anthers arrive earlier than pistil and flag filament is longer than the length of pistil), as well as pollination, is performed by bee. The leaves without a petiole, succulent, spear-shaped, rosette with thorny margins, leaf cuticle is covered with a wax layer. Fruits are a capsule and flourish that ripe in maturity (Satyabrata, 2002, Iranian Herbal Pharmacopoeia, 2002).

Aloe vera approximately consists of 95% of water. The amount of its minerals is low, approximately 1-2%. Dry and firm leachate is obtained by squeezing or simple flow of leachate from *Aloe vera* leaves, at least 15% Anthracene hydraulic derivatives (Iranian Herbal Pharmacopoeia, 2002). This yellow fluid located in the peripheral cells and attached to the parenchyma of leaves immediately exits after cutting leaves. The fluid is dried in front of sunshine or the fire. This leachate contains varying levels of aluminum, aloe-emodin, chrysophanic acid, volatile oils and resin (Ziaei *et al.*, 2005).

A study was carried out on the effect of *P. fluorescens* rhizobacteria on *Origanum*. *majorana* L. and was observed that parameters including essential fats, plant length, shoot weight, leaf number, nodule number, and root dry weight showed a significant difference as compared with control (Banchio *et al.*, 2008). In other studies, the effect of growth stimulus rhizobacteria (*Azotobacteria*, *Mesorhizobium*, *Azospirillium*, *and Pseudomonas*) on increasing dry matter and Chickpea (*Cicer arietinum* L.) yield was studied and results showed that the combination of this bacteria increased the number of nodules, yield, dry matter and protein function as compared with control (Rokhzadi *et al.*, 2008). In this study, the effect of the use of valuable strains of *A.brasilense* and *P.fluorescens* and the integrating of two bacteria was investigated for organic production of *Aloe vera*.

MATERIALS AND METHODS

Study Area

This study was performed in Hassan Abad village, Semnan province in June 2009. The region has a coordinate of 17° 22" longitude and 39° 35" latitude, with a height of 1060 meters above sea level.

Aloe vera offshoot was prepared from a greenhouse Hassan Abad, Semnan. to measure morphological parameters devices such as meter, a normal digital scale, bucket autoclave machine, digital incubator, microbiological hood class II and oven for drying samples were used. The site of the experiment was in the village of Hassan Abad, Semnan, an area of 10,000 m^2. Physical and chemical properties of the soil and the chemical properties of irrigation water are presented in Tables 1 and 2.

Table 1. Physical and chemical properties of the soil

pH	EC ds/m	T.N.V %	O.C. %	Total N %	P(AV.) p.p.m	K(AV.) p.p.m	Clay %	Silt %	Sand
7.41	1.886	12	3.4	0.3	>28	1200	30	20	50

EC= Electrical conductivity, T.N.V= Total neutral materials, O.C= Organic carbon, N= Nitrogen, P(AV.)= Available phosphorus, K(AV.)= Available potassium.

Table 2. Chemical properties of irrigation water

EC ($Ec*10^6$)	pH	(Co^3)$^{-2}$ (Meq/L)	Cl^- (Meq/L)	So^4 (Meq/L)	Sum Anions (Meq/L)	Ca^{2+} + Mg^{2+} (Meq/L)	Na^{2+} (Meq/L)	total Cations (Meq/L)	S.A.R
1880	6.8	5.9	10	3	18.9	10.68	8	18.68	3.4

Randomized complete block design with three replications and four treatments was applied. First treatment: control, second treatment: *A.brasilense* bio-fertilizer, third treatment: *P.fluorescens* bio-fertilizer and fourth treatment: integrated *P.fluorescens* and *A.brasilense* fertilizer. The comparison of the means was carried out by Duncan's multi range test (LSD) using the MSTATC software and graphs were drawn with Excel.

Preparation of bed and planting

After preparing the *Aloe vera* offshoots, 72 plastic pots with a height of 22 cm and a 16 cm diameter were prepared and were filled with 20% rotten peat, 20% manure, 10% garden soil and 50% washed sand.

Figure 1. Treatment properties registration and *Aloe vera* planting

Preparation of *A. brasilense* and *P. fluorescens* bacteria

Strains were prepared from the Natural Resources and Animal Affairs Research Center of Semnan province. Samples first placed at a bucket autoclave for disinfection and sterilization, and then put at the microbiological hood class II with NB medium (nutrient agar) for 24 hours. Then, they placed in an incubator at 37°C and after growing, they were concentrated by spectrophotometer with a MacFarland-half scale to be used for inoculation of the roots.

Watering Systems

In this experiment, pressured system in greenhouse and furrow in the field was used. The first irrigation was conducted on 20/6/2009, every 2 days until the end of August, due to warming weather, every 4 days from September with cooling weather, every 5 days from October to March and every two days from April to June 2010 due to increasing temperature (figure 2).

Figure 2. pressured system in greenhouse

Measurements of Morphological Parameters

To measure the largest leaf length, two pots from each plot (that has six replicates) were randomly selected and the largest leaf of each plant was measured from the base to the end of the leaf and the mean of two observations was recorded. To measure the length of the roots, after removing selected plants from the pots, the soil around the roots cleaned and cut and the number of them was counted and the length of the largest root was measured by the ruler and the mean of two observations was recorded. Also, the number of leaves of each observation was recorded during the experiment and the mean of two observations was recorded. To measure the fresh weight of the shoot, it was weighed up with a large digital scale. To measure the root fresh weight, the cut-off roots of each observation were weighed on a scale with calibrated scale (figure 3).

Figure 3. some instrument for testing

To measure the dry weight of the shoot, the shoots of each observation were placed inside the paper envelopes, and the observations, treatments and repeats were recorded on it and dried the inside of the oven for 48 hours at 80°C, and then, it was measured by balance and recorded. The dry weight of the roots of each observation was measured same as the dry weight of the shoot.

To measure the nutrient content of the leaves, two observations were randomly taken from each plot and three leaves from each observation. The leaves were sent to the water and soil laboratory of the research center. The methods of them were as follows: measurement of the nitrogen by Kjeldahl method, measurement of phosphorus by digestion method in acid and colorimeter and measuring by spectrophotometer. The potassium initially was digested in acid and then measured by flame photometer method. The measuring the area of the largest leaf was done using a Leaf area meter (made in England) (table 3). All experiments were being done at the Natural Resources and Animal Affairs Research Center of Semnan Province (figure 3).

Table 3. Results of nutrient levels of leaves

Treatment	Available N (%)	Available P (%)	Available K (%)
Control	0.99	0.18	4.36
A.brasilense	1.38	0.26	6.82
P.fluorescens	1.50	0.33	9.56
Integrated bacteria	1.02	0.24	5.86

RESULTS

Total fresh weight

As shown in table 4, the effect of bio-fertilizers on the total weight of *Aloe vera* was significant (P≤0.05). The comparison of means showed that total fresh weight was higher in bio-fertilizer treatments than the control (table 5). The highest was belonged to the "*A.brasilense"* and then, "*P.fluorescens*" and "integration of two bacteria", respectively. All showed an increase than to the control and statistically were on the highest level (Figure 4a).

Shoot fresh weight

According to the analysis of variance (Table 4), the effect of bio-fertilizers on the total weight of *Aloe vera* was significant (P≤0.05). Shoot fresh weight was higher in bio-fertilizer treatment than control (Table 5). The highest increase was related to "*A. brasilense"* against the control, and "*P. fluorescens*" and "integration of two bacteria", respectively, and finally, all treatments statistically were higher against the control (Figure 4b).

Shoot dry weight

The effects of bio-fertilizers on shoot dry weight of *Aloe vera* wasn't significant (p>0.05) in table 4, but the comparison of means (Table 5) showed that the highest increase in shoot dry weight was related to "*P. fluorescens"* and subsequently, the "integration of two bacteria" and "*A. brasilense*", respectively, as compared with the control. All showed a significant increase as compared with the control (Figure 4c).

Table 4. Analysis of variance (MS) of bio-fertilizer effects on vegetative and morphological traits of *Aloe vera*

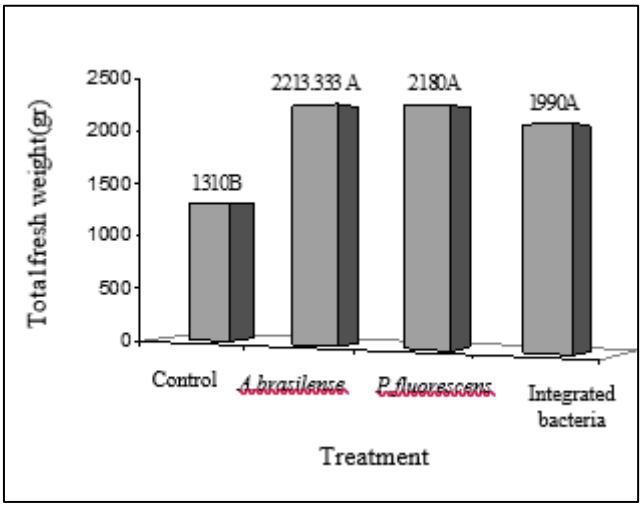
Resources of variations	treatment	repetition	error
Degree of freedom	3	2	6
Total fresh weight	530600*	19033.333	89500
Shoot fresh weight	506397.556 *	2711.583	69305.372
Shoot dry weight	2498.011^{ns}	272.053	674.116
Root fresh weight	5421.482*	128.43	797.121
Root dry weight	1288.738^{ns}	49.688	425.222
Length of the largest leaf	144.833**	2.146	6.729
Length of the largest root	463.222*	80.583	88.139
Number of leaves	6.306^{ns}	0.583	2.139
Number of offshoots	0.307^{ns}	5.687	1.187
The largest leaf area	4276.778**	671.396	145.674
Total plant area	605129.003*	73118.11	88437.957
N absorbed by the plant	0.247^{ns}	0.198	0.102
P absorbed by the plant	0.042**	0.002	0.002
K absorbed by the plant	14.338*	6.085	2.96

* significant on level 5%, ** significant on level 1% and ns is insignificant.

Table 5. Comparison of means of the effect of treatments on growth parameters of *Aloe vera*

treatments / **traits**	**Control**	***A.brasilense***	***P.fluorescens***	**Integration of *A.brasilense* and *P.fluorescens***
Total fresh weight	1310 B	2213.333 A	2180 A	1190 A
Shoot fresh weight	1191 B	2058.333 A	2066.667 A	1766.667 A
Shoot dry weight	43.767	81.813	114.207	83.893
Root fresh weight	67.090 A	117.210 AB	143.053 A	166.133 A
Root dry weight	16.980	42.123	38.103	67.5
Length of the largest leaf	34.5 B	48 A	49.5 A	47.333 A
Length of the largest root	18.333 B	34.667 AB	40 A	47.667 A
Number of leaves	14.667 B	16.333 AB	18 A	15.333 AB
Number of offshoots	5.5	6.133	5.890	5.470
The largest leaf area	114 C	174.667 B	202 A	180.667 A
Total plant area	1379.420 B	2244.717 A	2324.023 A	2256.04 A
N absorbed by the plant	0.897	1.383	1.497	1.017
P absorbed by the plant	0.120 C	0.260 B	0.410 A	0.240 B
K absorbed by the plant	4.360 B	6.823 AB	9.557 A	5.866 B

* significant on level 5%, ** significant on level 1% and ns is insignificant.



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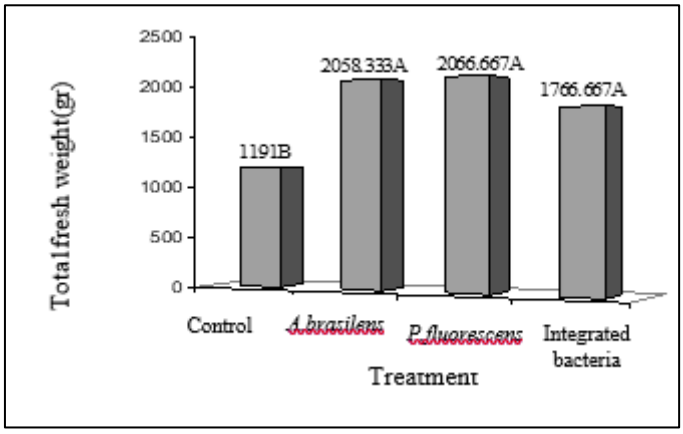

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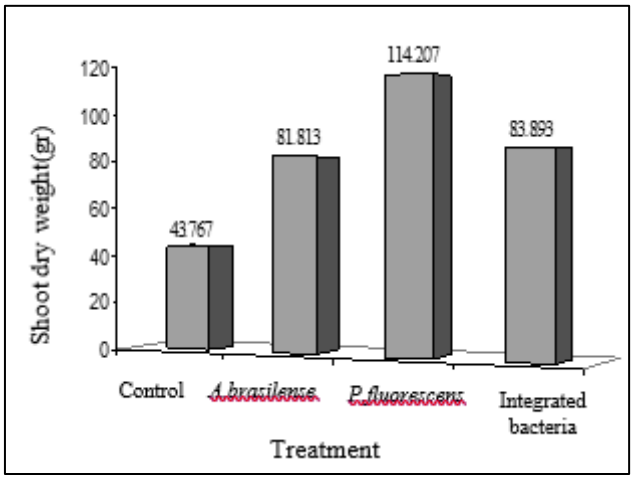

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Figure 4. a. Effects of bio-fertilizers (AZ.>Sod.>AZ.+Sod.> Control) on total fresh weight, b. effects of bio-fertilizers (Sod. > AZ. >AZ.+Sod.> Control) on shoot fresh weight and c. effect of bio-fertilizer (Sod.>AZ.+Sod.>AZ.>Control) on shoot dry weight

Root fresh weight

According to the analysis of variance (Table 4), the effect of bio- fertilizers on the root fresh weight of *Aloe vera* was significant (P≤0.05). The comparison of means showed that the fresh weight of the root of the plant in the bio-fertilizer treatment was higher than the control (Table 5). The highest of the root fresh weight was related to the "integration of two bacteria" and then, "*P. fluorescens*" and " *A. brasilense*" as compared with the control, respectively. All statistically were at the highest level (Figure 5a).

Root dry weight

According to the analysis of variance (Table 4), the effect of bio-fertilizers on root dry weight of *Aloe Vera* wasn't significant (p>0.05), according to the results of Table 5, the highest of the root dry weight was related to "integraion of the two bacteria" as compared with the control and then, "*A.brasilense"* and "*P.fluorescens"* as compared with the control, respectively. All showed a significant increase as compared with the control (Figure 5b).

Length of the largest leaf

According to the analysis of variance (Table 4), the effect of bio-fertilizers on the length of the largest leaf of *Aloe vera* was significant (P≤0.02). The comparison of the means (Table 5) showed that the length of the largest leaf was higher in the bio-fertilizer treatment than the control. The highest leaf length belonged to the "*P. fluorescens*" and "*A. brasilense*" and "integration of two bacteria", respectively and all showed a significant increase as compared with the control and they statistically were on the highest level (Figure 5c).

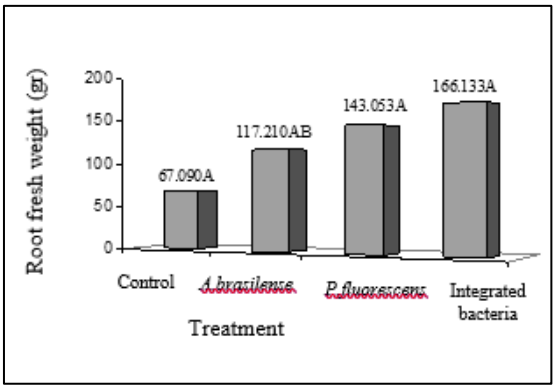

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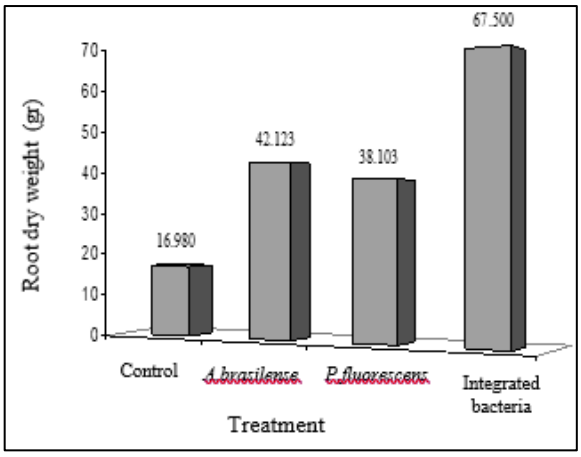
Fig 5. a. Effect of bio-fertilizer (AZ.+Sod.> Sod.>AZ. >Control) on root fresh weight



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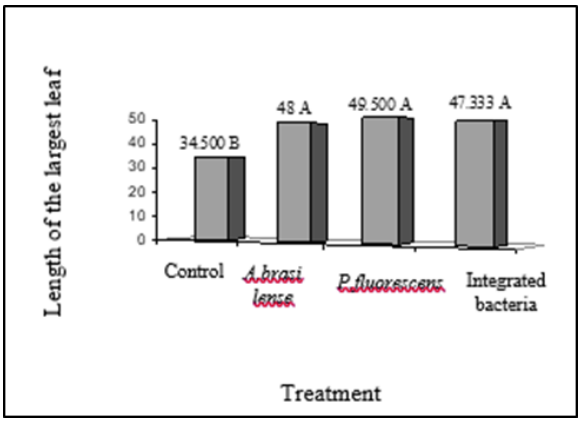

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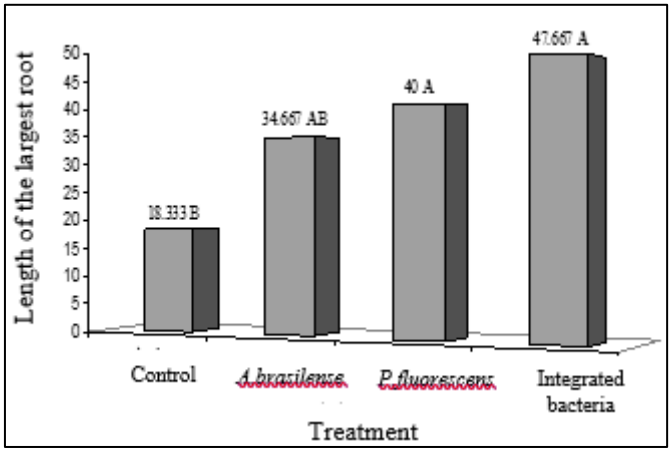
Fig 5. b. effect of bio-fertilizer (AZ.+Sod.>AZ.> Sod. >Control) on root dry weight and c. effect of bio-fertilizers (Sod.>AZ.>AZ.+Sod.>Control) on the length of the largest leaf

Length of the largest root

As shown in table 5, the effect of bio-fertilizers on the length of the largest root of *Aloe vera* was statistically significant (P≤0.05) and based on the comparison of the means (Table 5), the length of the largest root of the plant in the treatment with biological fertilizers was higher than the control. The highest increase in root length was related to the "integration of two bacteria", "*P.fluorescens*" and "*A.brasilense*", respectively, as compared to the control and all showed a significant increase than the control (Figure 6a).

Number of leaves

According to the analysis of variance (Table 4), the effect of biological fertilizers on the number of leaves of *Aloe vera* wasn't significant (p>0.05), but the comparison of the means (Table 5) showed that the highest increase in the number of leaves was belonged to "*P.fluorescens*", "*A.brasilense"* and "integration of two bacteria" as compared with the control, respectively, and all showed a significant increase compared to the control (Figure 6b).



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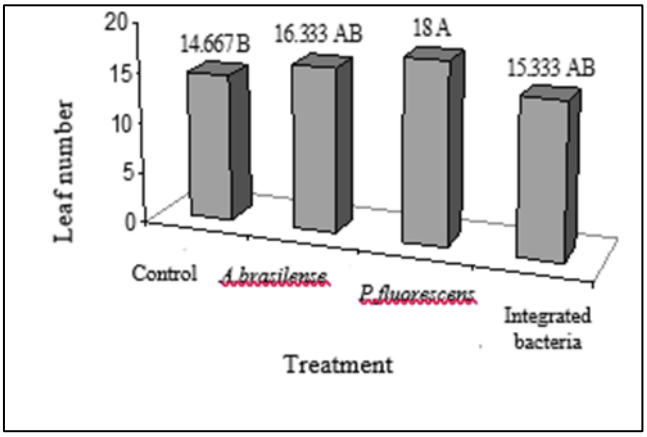


b

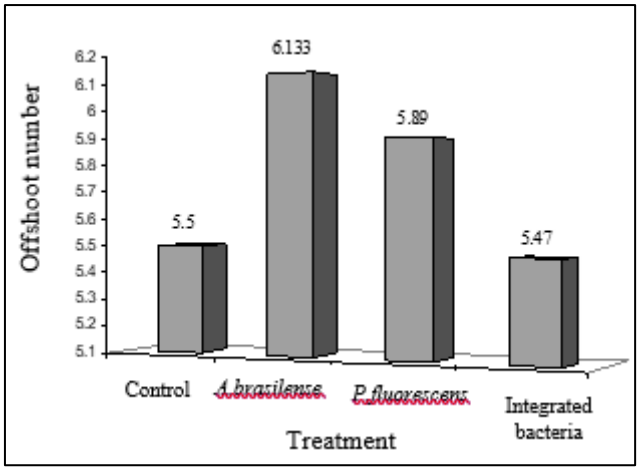
Fig 6. a. Effect of bio-fertilizers (AZ.+Sod.> Sod.>AZ. >Control) on the length of the largest root and b. effect of bio-fertilizers (AZ.+Sod.> Sod.>AZ. >Control) on number of leaves

Number of offshoots

According to the analysis of variance (Table 4), the effect of bio-fertilizers on the number of offshoot of *Aloe vera* wasn't significant (p>0.05), but the comparison of the means (Table 5) showed that the highest increase in the number of offshoots was related to "*A.brasilense"* and "*P.fluorescens"*, respectively, as compared with the control, and the number of *Aloe vera* offshoots increased by biological fertilizers as compared with the control (Figure 7a).

The largest leaf area

As shown in Table 4, the effect of bio-fertilizers on the largest leaf area of *Aloe vera* significantly was significant (P≤0.01). Based on Table 5, the highest increase of leaf area was related to "*P. fluorescens*" treatment, "integration of two bacteria" and "*A. brasilense*", respectively, as compared with the control (Figure 7b).



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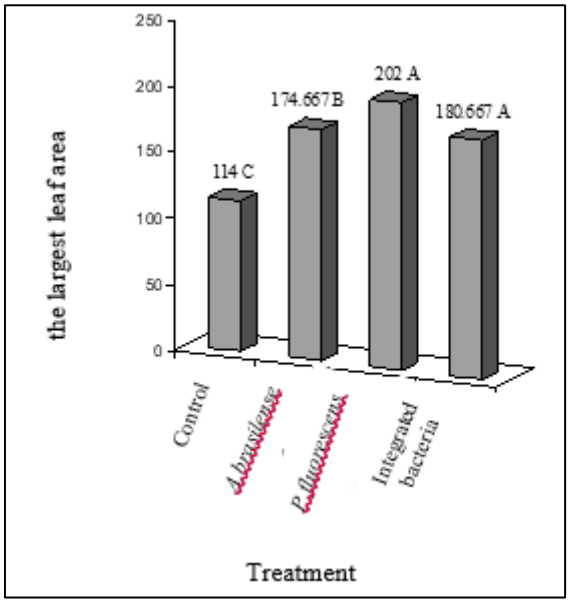


b

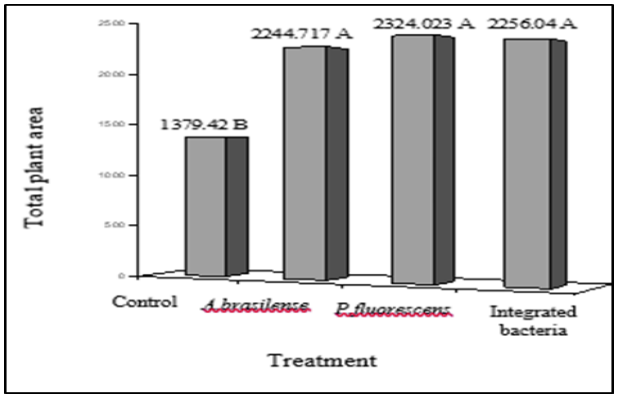
Fig 7. A. effect of bio-fertilizers (Sod.>AZ.+Sod.<Control>AZ.) on offshoot number and b. effect of bio-fertilizer (Sod.>AZ.<AZ.+Sod.>Control) on the largest leaf area

Total plant area

The effect of bio-fertilizers on the total area of *Aloe vera* (Table 4) was significant on the level ($P \leq 0.01$). The comparison of the means (Table 5) showed that the highest increase of area was related to "*P.fluorescens*" and "integration of two bacteria" and "*A.brasilense*" respectively, as compared with the control. Bio-fertilizer treatments showed an increase than the control (Figure 8a).

The amount of nitrogen absorbed by the plant

According to the table of variance analysis (Table 4), the effect of bio- fertilizers on the amount of nitrogen adsorbed by *Aloe vera* was not significant ($p>0.05$). Considering the comparison of means (Table 5), the highest increase of nitrogen absorbed was related to the "*P. fluorescens*, "*A. brasilense*" and "integration of two bacteria", respectively, as compared with the control (Figure 8b).



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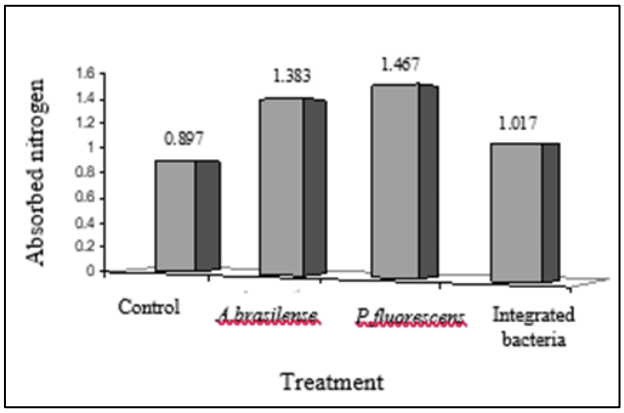

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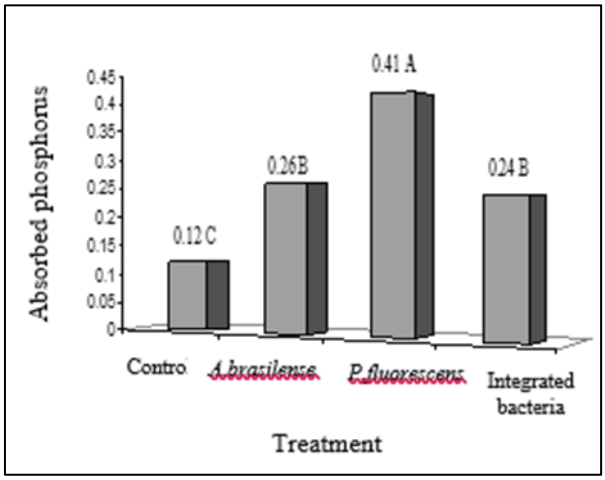
Fig 8. a. effect of bio-fertilizer (Sod.>AZ.<AZ.+Sod.>Control) on the total plant area and b. effect of bio-fertilizers (Sod.>AZ.>AZ.+Sod.>Control) on nitrogen absorbed by the plant

The amount of phosphorus absorbed by the plant

According to the analysis of variance (Table 4), the effect of biological fertilizers on the amount of phosphorus absorbed by the *Aloe vera* was significant ($P \leq 0.01$). Considering the comparison of the means (Table 5), the highest increase of phosphorus adsorbed was related to "*P. fluorescens*", "*A. brasilense*" and "integration of two bacteria", respectively, as compared with the control, and all significantly increased as compared with the control (Figure 9a).

The amount of potassium absorbed by the plant

The analysis of variance (Table 4) showed that the effect of bio-fertilizers on the amount of potassium absorbed by *Aloe vera* statistically was significant ($P \leq 0.05$). According to the results of comparison of means (Table 5), the highest increase of absorbed potassium belonged to the "*P. fluorescens"*, "*A. brasilense"* and "integration of two bacteria", respectively, as compared with the control and all showed a significant increase as compared with the control (Figure 9b).



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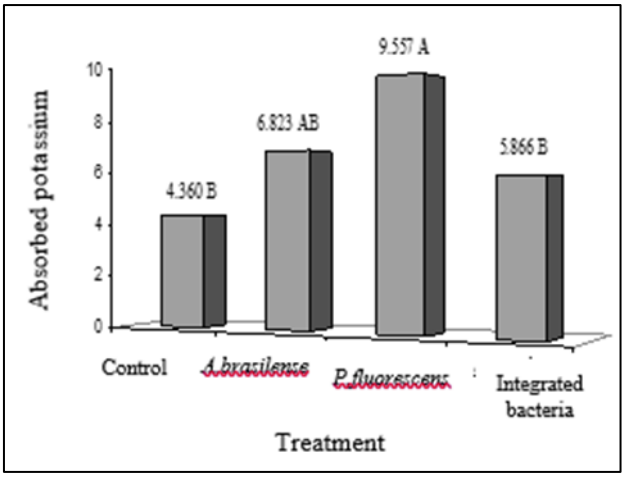

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Fig 9. a. effect of bio-fertilizers (Sod.>AZ.>AZ.+Sod.>Control) on phosphorus absorbed by the plant and b. effect of bio-fertilizers (Sod.>AZ.>AZ.+Sod.>Control) on potassium absorbed by the plant

DISCUSSION

Evaluation of the use of biological fertilizers (*A. brasilense* and *P. fluorescens*) on cultivation and production of *Aloe vera*

This project was done for first and newest project in Iran for evaluating the best bio-fertilizers on growth parameters of *Aloe vera* including fresh weight, shoot fresh weight, shoot dry weight, root dry weight, number of leaves, number of offshoots, the length of the largest leaf and root, the absorption of macro elements such as nitrogen, potassium and phosphorus (NPK) by plant, the largest leaf area and total plant area, and, finally, the yield of *Aloe vera* for organic production in Iran. So far, the use of these bacteria has been studied on many crops, but no experiment has been conducted on *Aloe vera*.

Many kinds of research have been carried out on two bacteria *A.brasilense* and *P.fluorescens* in various crops including wheat (Ardakani *et al.,* 2001), wheat (Ashrafi Soltani, 2007), sunflower (Behbodi *et al.,* 2005), *Organum majorana* L. (Banchio *et al.,* 2008), forest plants (Teimori *et al.,* 2004), maize cultivars 700 and 704 (Hamidi et al, 2009), wheat (Diaz-Zortia *et al.,* 2008), pea (Rokhzadi *et al.,* 2008), maize 700 and 704 cultivars and a promising hybrid (Ghalavand *et al.,* 2006), which increased the yield and weight of the aerial organs and the production of more auxin.

The fresh weight of the plant by using "*A. brasilense"* bacteria showed an acceptable yield against the control, and then there was a significant difference between the "*P. fluorescens*" and "integration of two bacteria" against the control, respectively, whereas, the use of *P. fluorescens*, more than *A. brasilense* showed a significant difference in fresh weight of aerial organs against the control.

Root fresh weight, root hairs, root number and root length affected by the bio-fertilizers of *A. brasilense* and *P. fluorescens* were investigated. The results obtained were similar to those obtained in various crops such as wheat (Cv. Mahdavi) (Ardakani *et al.,* 2001), wheat (Cv. Ghods, Roshan and Omid) (Amoaghaei *et al.,* 2002), peach (Roza *et al.,* 2008), wheat cultivars cultivated in the area (Askari *et al.,* 2009), and wheat (Cv. Ghods, Roshan and Omid) (Mostajeran *et al.,* 2002), that the use of these bacteria increased root fresh weight, root hairs system, root length, IBA and IAA hormones and the resistance of the root to fungal diseases and rotten of the root.

Root fresh weight and length showed the highest increase with "integration of two bacteria" bio-fertilizer against the control, and then, the bacteria of "*P. fluorescens*" and "*A. brasilense*" showed the highest increase, respectively.

The dry weight of aerial organs and root

Shoot and root dry weight in this study did not show a significant difference with bio-fertilizers. However, the use of *A. brasilense* and *P. fluorescens* bacteria in different crops such as wheat (Alamri and Mostafa, 2009), thymus (Banchio *et al.,* 2008), forest plants (Teimouri *et al.,* 2003), pea (Rokhzadi *et al.,* 2008), wheat (Cv. Ghods, Roshan and Omid) (Amoaghaei *et al.,* 1382), wheat (Diaz-Zortia *et al.,* 2008) and wheat (Cv. Ghods, Omid and Roshan) (Mostajeran *et al.,* 2005) increased the dry weight of the aerial organs and root and even grains.

Considering the fact that the fresh weight of aerial organs and root was significantly increased, but the dry weight of these parts did not show a significant difference as compared with the control. This can be explained by the presence of gel and high water content of *Aloe vera* (>95%). They could not show a significant difference in dry weight when these plants dried up in the oven and losed the gel and water.

The length of the leaves in this study showed a significant difference with the bio-fertilizers as

compared with the control. The results were similar to those obtained by Hamidi et al. (2007) on *Zea mays* L. (Cv. 700 and 704), *Matricaria chamomilla* (Azizi *et al.*, 2008) and *Medicago sative* (Okan *et al.*, 2002). As observed, the *P. fluorescens* fertilizer increased the length of leaves as compared with control and two other fertilizers. It can be concluded that " *P. fluorescens* " fertilizer is better than two other fertilizers, and considering the sale of *Aloe vera* through the leaf, it can have high revenue for producers.

The absorption of nutrients such as phosphorus and potassium in this study showed an increase against the control. The obtained result is similar to the results obtained by using biological fertilizers on crops such as wheat (Alamri and Mostafa, 2009), which decreased sodium uptake and increased the adsorption of calcium, phosphorus, potassium, and nitrogen concentrations in a plant. In a study by Reihani Tabar et al. (2002) caused to absorb iron and potassium elements, and in a study conducted by Amoaghaei et al. (2003) on wheat (Cv. Ghods, Roshan and Omid), nitrogen was more stabilized. In a study by Askari et al. (2009), it caused to increase NPK levels in wheat. Also, in a study by García *et al.*, 2003, led to the stabilization of nitrogen, or in a study (Mostajeran *et al.*, 2005) on wheat (Cv. Ghods, Omid and Roshan), increased nitrogen, or in a study (Okan et al. 2002), it increased the absorption of iron, water, and nutrients and stabilizing nitrogen. In contrast to the other researches, the use of "*A. brasilense*" and "*P. fluorescens*" bacteria didn't show a significant difference in nitrogen uptake, due to the fact that there was no nitrogen deficiency in the tested plants, therefore, a change in the amount of nitrogen absorbed by the plant was not observed.

In this study, the number of leaves didn't significantly different, however, the highest increase in leaf number was belonged to the *P. fluorescens* fertilizer with the maximum mean, and then, the *A. brasilense* fertilizer, and the "integrated fertilizer", respectively, as compared with the control. It can be concluded that the maximum growth of plants led an insignificant difference.

In this study, there was a significant difference by using the *A. brasilense* and *P. fluorescens* against the control and the leaf area was increased. The increase can be attributed to the increase of adsorption of phosphorus and potassium macro-elements in the plant, which increased LAI photosynthesis and, finally, increased plant leaf area. As the plant area increases, the yield of the crop is also increased.

CONCLUSION

The results of this study indicate that the use of bio-fertilizer "*P. fluorescens*" increased the number, length and area of leaf, which led to an increase in dry and fresh weight of the aerial organs, as well as the absorption of macro nutrients (N, P and K) by plant showed the most significant difference as compared with the control, so that, it had the highest level as compared with biological fertilizers "*A. brasilense*" and "integration of two fertilizers". Bio-fertilizer "*A. brasilense*" was at the highest level in the fresh weight of the plant and number of offshoots as compared with the control and bio-fertilizers of "*P. fluorescens"* and "integration of two fertilizers". The integration *A. brasilense* and *P. fluorescens* in some morphological parameters such as the fresh and dry weight of the root and root length were higher as compared with the control and showed the most significant difference between two *A. brasilense* and *P. fluorescens* fertilizers. As mentioned, the useable part of *Aloe vera* is leaves, therefore, by increasing aerial organs weight, the amount of gel will dramatically increase which use in the food, pharmaceutical, and sanitary industries. In addition, by increasing the total area, the yield per area unit will increase. The use of bio-fertilizer "*P. fluorescens*" increases the absorption of macro elements (N, P, and K), which will no longer require the use of chemical fertilizers in *Aloe vera*. The most important point in this research is the use of new methods to reduce the environmental damages, which can lead to increase the yield of the crop, thereby, more job creation, self-sufficiency, and the avoidance of currency departure from the country.

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