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The effects of growth stimulant bacteria on qualitative and quantitative yield of *Aloe vera*

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ABSTRACT

In this study the effects of Azispirilium brasilense, Pseudomonas florescens and Azotobacter chroococcum bacteria on Aloe vera planted in greenhouse conditions were studied. The study was conducted based on completely randomized blocks design with three replication and eight treatments. The treatments included: 1-Bacteria inoculation using Azispirilium brasilense bacterium 2- Bacteria inoculation using Pseudomonas florescens bacterium 3- Bacteria inoculation using Azotobacter chroococcum bacterium 4,5,6- Bacteria inoculation using a mixture of two bacteria 7- Bacteria inoculation using a mixture of all three bacteria and 8- a control. The statistical analysis suggested that plant growth stimulant bacteria tested in this research had significant effect on the increase of growth traits specially the gel weight of fully grown leaf, the bulk and mass of fully grown leaf, the average width of the leaves on a bush, and vitamin C, but they did not have any effect on the weight of the root and the length of the bush. The most significant effect was reported to be from Aztobacter bacterium. The findings of the research show the high potential of applying growth stimulant bacteria for improving both quantitative and qualitative aspects of Aloe vera production.

Key words: *Aloe vera* , vitamin C , gel , growth stimulant bacteria

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INTRODUCTION

Aloe vera is a xerophyte plant belonging to *Aloeaceae* plant sub-family and *Liliales* plant family. Almost 40 different species are explained in the *Aloe Barbadensis Miller* type of Aloe (10). *Aloe vera* is a durable species and its biomass is usually displayed via leaves. Its growth happens in the form of rosettes around a small area of the stem which can barely be as big as 5 centimeters (3).

The use of bio-fertilizers in sustainable agricultural systems is of great importance in increasing the yield and saving and retaining the sustainable fertility of soil (12). Bio-fertilizers are rhizosphere colonies including plant root growth promoting bacteria. These bacteria help the plants via supplying nutrients, biological controlling, producing pseudo-hormone substances of the plant, decreasing the ethylene surface of the plant, and making the plant resistant against different kinds of stress including water and nutrients deficiency and decreasing the contamination effect of plant's heavy elements(4). Using the *Azotobacter chroococcum*, *Azispirilium brasilense*, and *Pseudomonas florescens* bacteria and mycorrhizal fungi as bio-fertilizers cause an increase in nitrogen and phosphor uptake and consequently the promotion of the roots of several farm plants (4, 6,9) .These bacteria may accumulate either in the rhizosphere or even in root or internal cellular space of the plants (13). Bio-fertilizers enhance the efficiency of both organic and chemical fertilizers on agricultural crop yield by increasing the activities of plant growth-promoting bacteria.

The effects of biologic fertilizers on two herbs of wild chamomile and marigold were studied in Cuba. While they caused an increase in flower yield, they did not have any effect on the quality (7, 11). In a greenhouse experiment conducted on marjoram plant, it was found that bio-fertilizers including Azotobacter, Azospirillium, and phosphate soluble bacteria had significant effect on growth indices and the amount of its essence (8). Nanda et al. (1995) found that bacteria inoculation of maize seedlings with Azotobacter and Azispirilium led to an increase in green crop yield in treatments enjoying different amounts of nitrogen fertilizers (15). In this research, the effect of *Azispirilium brasilense*, *Pseudomonas florescens* and *Azotobacter chroococcum* bacteria and their combination on *Aloe vera* plant's growth traits are investigated and studied.

MATERIALS AND METHODS

The research was done in mid-summer 2011 as random blocks design with 8 treatments and 3 repetitions in the Avicena Herb Garden located in the south of Hamedan city. Before greenhouse experiments were done, in order to find the physical and chemical characteristics of the soil, a sampling of the soil used in research was carried out. The physical and chemical characteristics of the soil used are presented in table 1.

Table 1: The physical and chemical characteristics of the soil

Silt %	Sand %	Clay %	Soil Texture	EC (ds/m)	pH	TDN %	Potassium (ppm)	Phosphate (ppm)	Nitrogen %	Organic Carbon %
16	36.69	14.64	SL	1.44	7.3	5	320	68.21	%546	5.46

The experimental treatments included the following:
A- Bacteria inoculation using *Azotobacter chroococcum* of Strain 12
B- Bacteria inoculation using *Pseudomonas florescens* of Strain 41
C- Bacteria inoculation using *Azispirilium brasilense*
D- Bacteria inoculation using a combination of the bacteria A+B
E- Bacteria inoculation using a combination of bacteria A+C
F- Bacteria inoculation using a combination of bacteria B+C
G- Bacteria inoculation using a combination of all three bacteria of *A.chroococcum*, *A.brasilense*, and *P. florescens*
H- Control treatment (no use of bio-fertilizers)

First, eight sterilized containers were provided having 1384cc distilled water and 116cc bacteria (for single-bacterium treatments), while 58 and 38 cc of each bacterium were added as the combination of two and three bacteria treatments. Therefore a consistent layer of these inoculating liquids would form on the baby plants. Then the control treatment was conducted on a plant using 1500cc distilled water. For the purpose of inoculation, the roots were immersed in bio-fertilizers for 15 hours. The soil used in the experiment was a 2 – 1 – 1 combination of soil, animal manure, and sand consecutively. Sampling started 6 months after plantation. Sampling was carried out as the bushes were taken out of soil along with their roots and they were cut from shoots and the following traits were studied: the increase in bush length, the fully-grown leaf gel weight, root weight, the biomass of fully-grown leaf, the average width of bush leaves, and the vitamin c of gel. Vitamin C was measured using a 2, 6-Dichlorophenol powdering method. The variance analysis of the data using SAS software and also comparing the average effects of treatments using Duncan test at 5% were carried out.

RESULTS AND DISCUSSION

In figure 1, Comparison of average effect of bacteria inoculation on increasing the length of the plant is displayed. The mono-bacterial treatments have not made any significant difference compared with the control treatment, while they have been remarkably significant when compared with combined bacterial treatments. Different researchers have reported an increase in the height of maize plants via applying Azospirillium bacterium (16) and Azotobacter (2). Though the height of the *Aloe vera* plant did not react properly to bacteria inoculation.

In figure 2 the mean comparative effects of bacterial inoculation on the average width of the leaves of a plant are presented. The Azotobacter inoculation treatment enjoying on average a 5 cm increase had the largest width of the leaves in the plant and had a significant increase compared with the control which amounted to almost 42% On the other hand, the other inoculation treatments were statistically at almost the same level as the control.

In figure 3, comparison of average effects of bacterial inoculation on the biomass of the fully-grown leaves is displayed. The inoculation treatment using Azotobacter with an average of 583 cm^3 caused the largest biomass of the leaves. Its effect was quite significant compared with the control and equaled to almost 94% in increasing the biomass. The second most effective treatment was the combined inoculation (coinoculation) of two bacteria of

Azispirilium brasilense, and *Pseudomonas florescens* that resulted in the largest biomass of the leaves and was very significant compared with the control. And the least effective treatment was by the inoculation of *Azispirilium brasilense* , and coinoculation of *Azispiriliumbrasilense* and *Azotobacter*, *Pseudomonas florescens* and Azotobacter and the coinoculation of all three bacteria, which concerning the results, were at the same level as the control.

Figure 1:The effect of the different levels of bio-fertilizers on the length of the plant

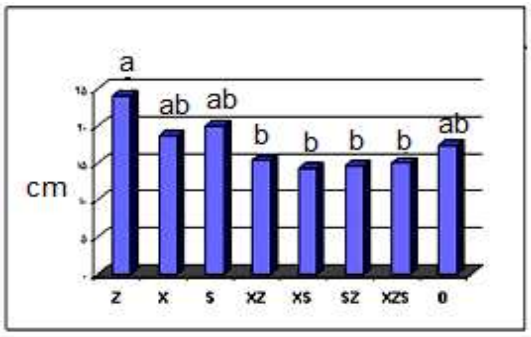
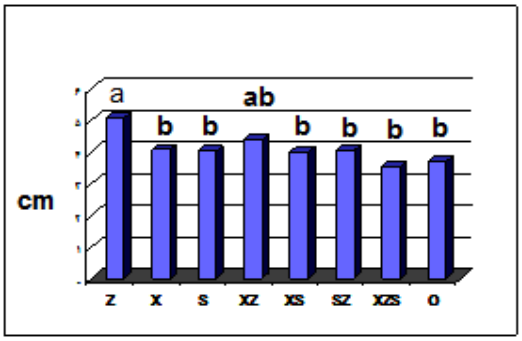


Figure 2: The effect of the different levels of bio-fertilizers on the average width of the leaves of a plant



O: Control, Z: Azotobacter chroococcum strain 12, X: Azospirillium brasilense strain of, S: Pseudomonas florescent strain 41.

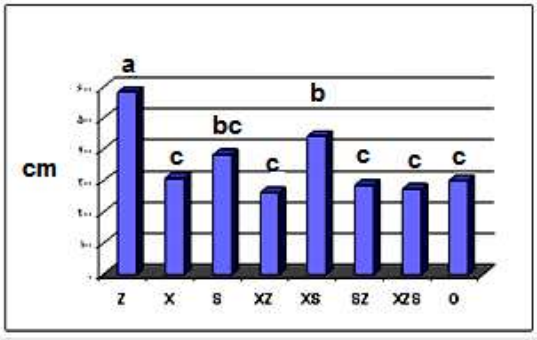


Figure 3: The effects of different levels of bio-fertilizers on the fully-grown leaves' biomass

In figure 4, comparisons of average effects of bacterial inoculation on the gel weight of the leaves are seen. The largest weight of the gel is achieved in *Azotobacter chroococcum* and *Pseudomonas florescens* treatments compared with that of the control. The weight in fact increased by 38% and 32 % consecutively and the least amount of the weight increase was caused by the coinoculation of two bacteria of *Azotobacter chroococcum* and *Pseudomonas florescens* with an average of 70 gr and the other treatments have not led to any better situation compared with the control.

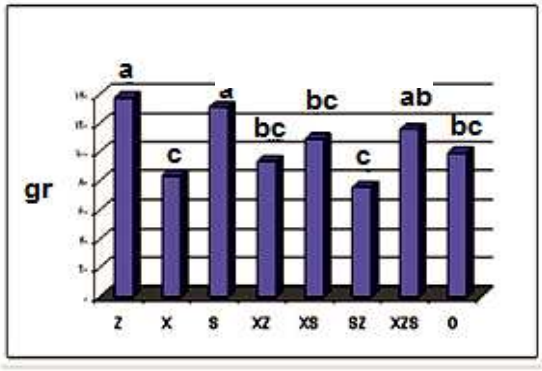


Figure 4: The effect of the different levels of bio-fertilizers on the average gel weight of the fully-grown leaves

In table 5, comparison of average effects of bacterial inoculation on the root weight is displayed. *Azotobacter chroococcum* inoculation and the combination of three bacteria had a similar effect on the root while other treatments were at a lower level compared with control. In fact the least amount of effect was caused by the coinoculation of Azispirillium and Azotobacter with an average of 44 gr. Applying these bacteria led to an increase in wet root weight, the root hairs system and the length of the root, and it caused to increase of internal hormones of IBA and IAA as a result of stimulating the bacteria (1). But the results of this study suggest that the symbiosis of some bacteria with the plant root has had no effect compared with control and even has had negative effect in some treatments.

The cooperation between the maize root and the *Azispirilium brasilense* strain of bacterium through the inoculation of two varieties of maize seedlings suggested that the dry aerial mass and the root increased in milk stage of the corns and the nitrate reductase activity in the leaves of the inoculated seedlings' plants increased (5).

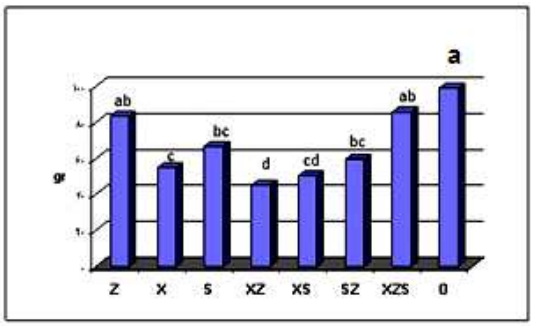


Figure 5: The effect of different levels of bio-fertilizers on the root weight

In table 6, the comparisons of average effects of bacterial inoculation on the amount of vitamin C are seen. The inoculation treatments combining both strain of bacteria (Azotobacter and Azispirillium), (*Pseudomonas florescens* and *Azispirilium brasilense*) and a combination of all three of the bacteria have led to the highest amount of vitamin C and caused a significant difference in comparison with control. Also the control has produced the least amount of vitamin C. Consequently, all of the inoculation treatments have caused an increase in *Aloe vera* gel quality compared with control.

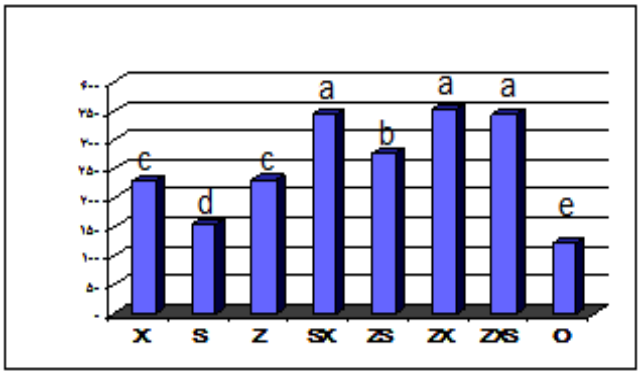


Figure 6:The effect of different levels of bio-fertilizers on vitamin C

CONCLUSION

Treatments inoculated with *Azotobacter chroococcum* of strain 12 have been more effective on the quantitative traits of *Aloe vera* in comparison with other fertilizers. All bacteria have an increasing effect on vitamin C. But the highest level of effect in inoculated treatments using a combination of three and two bacteria (*Azotobacter chroococcum* of strain 12 and *Azispiriliumbrasilense* strain of) , (*Pseudomonas florescens* of strain 41 and a *Azospirillium brasilense*). Therefore, one can use the growth stimulant bacteria as bio-fertilizers for improving the growth and crop production of *Aloe vera.*

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