JFST No. 174, Vol. 23, August 2026

Journal of Food Science and Technology (Iran)

Homepage: www.fsct.modares.ir

Scientific Research

Impact of Bacterial Fertilization and Foliar Growmore Application on the Growth Parameters of Pomegranate 'Wonderful' Saplings

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ARTICLE INFO

Article History:

Received: 2026/01/31
Review: 2026/04/05
Accepted: 2026/05/11

Keywords:

Azospirillum,

fruit trees,

organo-fertilization,

sustainable agriculture

DOI: **10.48311/fsct.2026.119057.83038**

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ABSTRACT

This study was conducted to evaluate the effect of the biofertilizer *Azospirillum brasilense* and the chemical foliar fertilizer Grow More, individually and in combination, on improving some vegetative growth characteristics of pomegranate (*Punica granatum* L.) Wonderful saplings under the Najaf Governorate environmental conditions. The experiment was RCBD factorial included 3 levels of biofertilizer and the commercial balanced Growmore foliar fertilizer, to study the effect of each and their interaction on a number of vegetative growth indicators. The findings showed that the treatments differently affected studied growth parameters namely the increase in sapling height, number of leaves, stem diameter, number of branches, leaf area, and total leaf chlorophyll content. The soil applied biofertilizer *A. brasilense* resulted in significantly higher values in most traits especially at 12 ml seedling^{-1} compared to the control. Similar but lower effectiveness was detected in the individual treatments with foliar balanced Growmore where the best results were obtained at 300 mg L^{-1}. Overall findings indicate that the highest percentage increase was in the interaction treatment of 12 ml sapling^{-1} bacterial fertilizer and 300 ml L^{-1} Growmore, which achieved an increase over the control in sapling height by 120%, in the number of leaves by 135%, and in stem diameter by 180%. The number of branches also increased from less than 7 to 35 branches/seedling, and the leaf area and leaf chlorophyll content recorded an increase over the control by 189% and 200% respectively. This study is confirming that biofertilization with *A. brasilense* and/or commercial foliar balanced NPK were effective for producing vigor fast growing pomegranate saplings.

1-Introduction

The pomegranate (*Punica granatum* L.) is a deciduous fruit tree of considerable economic and nutritional importance due to its adaptability to various environmental conditions in semi-arid regions and high market value [1]. Wonderful is one of the most important and widely grown varieties, both globally and locally, because it produces high-quality fruits with relatively large amounts of active compounds [2]. Therefore, it has attracted much attention in modern horticultural research with emphasis on technology for seedling production and techniques for enhancing vegetative growth. Like most other fruit trees, successful pomegranate cultivation to realize the full potential of high yields depends primarily on nutrition quality and nutrient utilization efficiency, particularly during the early establishment of rapid vegetative growth [3,4].

In the light of the above, biofertilizers have emerged as a sustainable option with or without chemical fertilizers to enhance soil fertility, nutrient availability, and better growth of plants by excreting organic growth regulators and nitrogen fixation [5,6]. *Azospirillum brasilense* bacteria is one of the attractive biofertilizers as it efficiently promotes plant growth by fixing nitrogen and also rooting stimulation that later leads to better nutrient uptake; besides, it secretes auxins, gibberellins, and cytokinins [7,8].

Furthermore, foliar traditional fertilizers, particularly those that contain macro- and micronutrients, are still considered a primary source to satisfy the dietary needs of plants [9]. They also improve the efficiency of photosynthesis and promote vegetative growth [10]. Therefore, the present study was carried out to investigate the response of Wonderful pomegranate seedlings to different levels of biofertilizer *Azospirillum brasilense* and foliar fertilizer Growmore both individually and in combination on some parameters of vegetative growth. This, in turn, will assist in establishing an environmentally sound and integrated fertilization program for pomegranate seedlings in Najaf Governorate.

2-Materials and Methods

The experiment was conducted during the 2025 growing season at the research station of the College of Agriculture, University of Kufa, in Najaf Governorate, from April 1, 2025, to October 1, 2025, on 1-year-old American pomegranate seedlings of the wonderful variety. The plants were obtained from a government nursery affiliated with the Horticulture Department of the Iraqi Ministry of Agriculture on March 20. The seedlings were transferred to 10 kg pots after the experimental site was prepared before planting, including leveling the land and removing weeds. A mixture of sandy soil and peat moss was placed in the pot in equal quantities, and the seedlings were planted. All necessary maintenance operations, such as hoeing and weed control, were carried out uniformly throughout the experiment. The pots were placed inside a wooden shade structure. Initial measurements of the treatments were taken, and foliar spraying of the plants began on April 1, at a rate of 5 sprays per month, except for August. As for the biofertilizer, the first application was on October 4th and the second on June 25th, i.e., after 75 days. Random samples were taken from the soil in which the seedlings were growing and then mixed homogeneously. The soil was then analyzed to determine some of its chemical and physical properties [11].

The experiment was conducted as a factorial experiment using a randomized complete block design (RCBD) with two factors. The first factor included four levels of biofertilizer (0, 4, 8, and 12 ml sapling), and the second factor included four levels of chemical fertilizer (0, 100, 200, and 300 mg L^{-1}). The growth traits were measured including sapling height (cm), stem diameter (mm), number of branches (branch $sapling^{-1}$), number of leaves (leaf $sapling^{-1}$), leaf area (cm^2 $sapling^{-1}$), as well as leaf content of Chlorophyll (mg.$100g^{-1}$ FW) was estimated in five randomly selected fully developed leaves from each experimental unit [12].

Statistical analysis

Data were collected at the end of the experiment on October 1, 2025, and were statistically analyzed using ANOVA and factor analysis with interactions using the GenStat 12.1 VSN International statistical software [13]. Differences between means were compared using the least significant difference (LSD) test at a probability level of 0.05 [14].

3-Results and Discussion

Sapling height

The results showed a significant effect of biofertilizer treatment, conventional fertilizer application, and their interaction on plant height (Table 1). The treatment 12 ml sapling^{-1} biofertilizer resulted in a significantly higher average plant height of 68.43 cm, compared to 47.35 cm in the control treatment. The treatment 300 mg L^{-1} conventional foliar fertilizer achieved the highest average height of 66.12 cm, compared to its control which did not exceed 50.47 cm. Table 1 also indicates that the interaction of *A. brasilense* at 12 ml sapling^{-1} and 300 mg L^{-1} of chemical fertilizer resulted in the highest average sapling height of 86.03 cm, compared to lower values in all other treatments.

Table1. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on plant height increase of pomegranate 'Wonderful' saplings

Biofertilizer *Azospirillum brasilense* ml sapling^{-1}	**Conventional foliar Growmore mg L^{-1}**				**Average**
	Cntrol	**1001**	**200**	**300**	
control	39.10	44.57	50.37	51.37	47.35
4	50.46	52.47	54.03	56.53	53.57
8	54.73	55.43	62.63	66.53	60.85
12	57.63	61.03	69.03	86.03	68.43
Average	50.47	54.58	59.17	66.12	
L.S.D 0.05	**Azo. / Gro.= 2.02 Interaction= 4.226**				

Number of leaves

Regarding the effect of the treatments on the number of leaves, it was observed that the number of leaves increased with increasing biofertilizer concentration. As the *A. brasilense* at 12 ml sapling^{-1} significantly outperformed the control treatment, recording the highest average of 178.43 leaf palnt^{-1} compared to only 102 leaf palnt^{-1}. Similarly, the Growmore spray treatment at a concentration of 300 mg L^{-1} recorded 151.84 leaf palnt^{-1}, compared to 126.19 leaf palnt^{-1} in the control. The interaction between biofertilizer and conventional fertilizer had a significant effect on the rate of increase in the number of leaves, especially since the highest average of 201.58 leaf palnt^{-1} was recorded in saplings treated with 12 ml sapling^{-1} Biofertilizer + 300 mg L^{-1} chemical fertilizer spray. The highest rate of increase in the number of leaves was recorded in saplings treated with 12 ml sapling-1 *A. brasilense* and 300 mg L^{-1} foliar Growmore, compared to the lowest increase of 85.77 leaves. sapling^{-1} in the control treatment.

Table2. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on sapling's number of leaves (leaf sapling^{-1}) increase in pomegranate 'Wonderful' variety

Biofertilizer *Azospirillum brasilense* ml sapling^{-1}	**Conventional foliar Growmore mg L^{-1}**				**Average**
	Cntrol	**1001**	**200**	**300**	
control	86.77	97.13	111.10	117.87	102.97
4	122.67	125.14	127.61	133.09	127.13
8	137.27	143.40	148.70	154.82	146.05
12	159.05	167.15	185.93	201.58	178.43
Average	126.19	133.21	143.33	151.84	
L.S.D 0.05	**Azo. / Gro.= 11.023 Interaction= 16.206**				

Stem diameter

The results in Table (3) show that stem diameter was significantly affected by the levels of biofertilizer, chemical fertilizer, and their interaction. Among other concentrations, the *A. brasilense* at 12 ml sapling^{-1} also resulted in a significantly higher stem diameter 3.30 mm compared to 1.49 in the control treatment. In similar manner, the 300 mg L^{-1} Growmore recorded much higher stem diameter of 2.64 mm sapling^{-1}, compared to the control treatment with only 2.21 mm. It was also observed (Table 3) that the interaction of the highest concentrations of both factors resulted in the highest average stem diameter of 3.46 mm with a significant differences from most individual and interaction treatments.

Table3. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on sapling's stem dimeter (mm) increase in pomegranate 'Wonderful' variety

Biofertilizer *A. brasilense* ml sapling^{-1}	**Conventional foliar Growmore mg L^{-1}**				**Average**
	Cntrol	**1001**	**200**	**300**	
control	1.2633	1.4400	1.5867	1.6967	1.4967
4	1.8733	2.0400	2.2133	2.3967	2.1308
8	2.5833	2.7333	2.8567	3.0200	2.7983
12	3.1367	3.2433	3.3800	3.4600	3.3050
Average	2.2142	2.3642	2.5092	2.6433	
L.S.D 0.05	**Azo. / Gro.= 0.133 Interaction= 0.284**				

Number of Branches

As for the treatments effects on the number of branches, findings (Table 4) showed a significant increase in number of branches due to biofertilizer application. The 12 ml sapling^{-1} *A. brasilense* significantly recorded much higher number of branches (31.74 branch sapling^{-1}) than the control treatment (9.89 branch sapling^{-1}). Foliar spray with Growmore at 300 mg L^{-1} also resulted in number of branches as higher as 23.39 compared to 18 branch sapling^{-1} in the control which did not differ from Growmore at 200 mg L^{-1} that of 18.90 branch sapling^{-1}. The interaction of *A. brasilense* and the conventional fertilizer had a significant increase in number of branches where the highest average of 35.17 branch sapling^{-1} in the plants treated with 12 ml sapling^{-1} Biofertilizer + 300 mg L^{-1} chemical fertilizer spray.

Table4. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on sapling's number of branches in pomegranate 'Wonderful' variety

Biofertilizer *Azospirillum brasilense* ml sapling^{-1}	**Conventional foliar Growmore mg L^{-1}**				**Average**
	Control	**100**	**200**	**300**	
control	6.29	7.86	9.24	16.16	9.89
4	19.62	16.24	14.92	16.23	16.75
8	17.95	20.86	22.72	25.99	21.88
12	28.13	30.64	33.02	35.17	31.74
Average	18.00	18.90	19.97	23.39	
L.S.D 0.05	**Azo. / Gro.= 1.233 Interaction= 2.682**				

Leaf area

The results for the treatments effects on leaf area indicated a significant effect of the biofertilizer treatments, with the 12 ml Seedling-1 treatment significantly outperforming the control treatment, achieving the highest average leaf area of 9.597 cm^2, compared to an average of 4.356 cm^2. The same table also shows that the 300 mg L^{-1} Growmore spray treatment achieved the highest average leaf area of 7.193 cm^2 compared to the lower concentration and the control treatment with an average as low as 5.698 cm^2. Overall, the interaction treatment using 12 ml $sapling^{-1}$ biofertilizer and 300 mg L^{-1} Growmore achieved the highest leaf area of 11.118, and significantly outperforming most of the individual and interaction treatments.

Table5. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on sapling's leaf area (cm^2) in pomegranate 'Wonderful' variety

Biofertilizer *Azospirillum brasilense* ml $sapling^{-1}$	**Conventional foliar Growmore mg L^{-1}**				**Average**
	Control	**100**	**200**	**300**	
Control	3.826	4.227	4.598	4.772	4.356
4	5.066	5.378	5.503	5.730	5.419
8	6.170	6.348	6.760	7.153	6.608
12	7.729	8.981	10.560	11.118	9.597
Average	5.698	6.233	6.855	7.193	
L.S.D 0.05	**Azo. / Gro.= 0.48 Interaction= 0.96**				

Plant content of total chlorophyll

In case of treatments effects on leaf content of total chlorophyll, findings showed that biofertilization with *Azospirillum brasilense* had much higher effect on chlorophyll level than the that recorded by the commercial foliar Growmore (Table6). As the treatment of 12 ml $sapling^{-1}$ biofertilizer resulted in sapling content of chlorophyll of 19.764 mg $100g^{-1}$ FW while Growmore at the highest concentration 300mg L^{-1} resulted in chlorophyll level of 16.535 mg $100g^{-1}$ FW compared to significantly lower value in the control that range from 10.25 to 13.201 mg $100g^{-1}$ FW. Table 6 also indicates that the interaction of *A. brasilense* at 12 ml $sapling^{-1}$ and 300 mg L^{-1} of foliar Growmore resulted in the highest average sapling content of chlorophyll of 23.468 mg $100g^{-1}$ FW which significantly differed from all the other interaction and individual treatments.

Table6. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on sapling's content of chlorophyll (mg $100g^{-1}$ FW) in pomegranate 'Wonderful' variety

Biofertilizer *Azospirillum brasilense* ml $sapling^{-1}$	**Conventional foliar Growmore mg L^{-1}**				**Average**
	Control	**100**	**200**	**300**	
control	7.730	10.549	11.212	11.545	10.259
4	12.816	13.135	13.575	14.467	13.498
8	14.811	15.159	15.967	16.661	15.649
12	17.447	18.255	19.884	23.468	19.764
Average	13.201	14.275	15.159	16.535	
L.S.D 0.05	**Azo. / Gro.= 0.42 Interaction= 0.86**				

Biofertilizer *Azospirillum brasilense* significantly enhanced all studied vegetative growth characteristics of "wonderful" pomegranate saplings. All levels of biofertilizer resulted in significant increases in sapling height, stem diameter, number of branches and leaves, leaf area, and total chlorophyll content. These results are due to nitrogen-fixing bacteria, *Azospirillum*, which can fix nitrogen in the air and then change it to a form that can be used by the plant. Another reason is that these bacteria produce plant growth hormones, including auxins, gibberellins, and cytokinins, which raise cell division and cell elongation, hence increasing vegetative growth [15]. The findings are consistent with those of Bashan et al. (2004) and Bhardwaj et al. (2014) who demonstrated the role of growth-promoting bacteria in improving the vegetative growth parameters of fruit crops [6,16].

The level of 300 mg/L^{-1} proved to be superior in most of the traits under study as compared to the control treatment in assessing the effect of Grow More foliar fertilizer. This is because of the NPK nutrient content of the fertilizer. NPK is a macronutrient that directly participates in building up plant tissues and in the regulation of metabolic processes; the other components are micronutrients like iron, zinc, and manganese. They play a very important role in the formation of chlorophyll and in the activation of a number of enzymes taking part in photosynthesis and respiration. According to Mengel and Kirkby (2012) and Broadley et al. (2012), when these elements are available in adequate amounts, the efficiency of photosynthesis is improved hence more carbohydrate is realized and this has a positive effect on vegetative growth [17,18].

The chlorophyll content in leaves indicates the nutrition level of the plant and its capacity for photosynthesis. The results of this study indicated a significant increase in total chlorophyll content with increasing levels of both the biofertilizer and the foliar fertilizer. This rise in chlorophyll indicates higher photosynthesis efficiency and carbohydrate metabolism in plant growth as reflected by cell division, elongation, and volume increment; hence, it leads to increased indicators of vegetative growth of the plant [19,20]. Iron and manganese also play a role in this process due to their participation in chlorophyll formation and the electron transport chain in photosynthesis, while nitrogen is involved in the formation of proteins and enzymes that catalyze the process of photosynthesis. These findings are consistent with those of Taiz and Zeiger (2010) and Ibrahim and Abd Elkawy and (2018) in their studies on fruit trees [21,22].
These results could have been attributed to the positive interaction between the biofertilizer and foliar fertilizer. It could be assumed that the biofertilizer made the nutrients more available and the absorption efficiency from the soil while the foliar fertilizer supplied the nutrients directly and quickly through the leaves. Similar results were reported by Stino et al. (2009) and Pandey et al. (2020) who stated that biofertilizers in combination with chemical fertilizers significantly improved growth and productivity in fruit trees compared to single applications of each nutrient source [23,24]. The findings of this study generally prove that integrated fertilization programs involving applications of bio-fertilizer *Azospirillum brasilense* and foliar fertilizer Grow More contribute to the production of strong pomegranate seedlings with balanced vegetative growth and higher nutrient use efficiency. The trend is a positive one for sustainable agriculture, as it reduces the reliance on traditional chemical fertilizers.

4-Conclusion

The results of the study demonstrated the possibility of increasing pomegranate saplings growth and vigor with aid of soil-applied biofertilizer *Azospirillum brasilense* especially when used at 8 or 12 ml saplin^{-1}. Spraying with foliar nutrient Growmore was also effective in increasing saplings growth and content of chlorophyll which was increased as the concentration increases to 300 mg L^{-1}. Findings indicated that all the growth parameters under study showed a gradual increase with increasing concentrations used within the limits of this study. Best results were obtained in the combined interaction of both factors especially when used at the highest application levels.

Conflict of interest

The authors have no conflicts of interest to disclose that are compatible with the subject matter of this article.

Consent to participate

All authors have expressed their authorization to engage in this publication.

Funding

This research did not receive any specific funding.

Ethical Statement

Except for sensory evaluation, we have not conducted any human or animal investigations during this study. Regarding the sensory case.

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