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Efficacy of biofertilizer (*Azospirillum brasilense*) and Growmore foliar liquid on growth performance of pomegranate seedlings (*Punica granatum* L.)

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Abstract

This study was conducted to evaluate the effect of the biofertilizer *Azospirillum brasilense* and the chemical foliar fertilizer Growmore, individually and in combination, on improving some vegetative growth characteristics of pomegranate (*Punica granatum* L.) Wonderful seedlings 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 seedling height, number of leaves, stem diameter, number of branches, leaf area, and leaf content of macronutrients NPK. 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 seedling^{-1} bacterial fertilizer and 300 ml L^{-1} Growmore, which achieved an increase over the control in seedling height by 120% and stem diameter by 180%. The the leaf area and leaf carbohydrates 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 seedlings.

Keywords: *Azospirillum*, fruit trees, organo-fertilization, sustainable agriculture

Introduction

The pomegranate (*Punica granatum* L.) is a deciduous fruit tree of significant economic and nutritional importance with an adaptability to diverse environmental conditions in semi-arid regions and its high market value (Chandra et al., 2010). The Wonderful variety occupies a prominent position among globally and locally cultivated pomegranate varieties due to its abundant yield, high-quality fruit, and high content of active compounds (Tinebra et al., 2021). This makes it a focus of modern horticultural research, particularly in the seedling production stage and the improvement of vegetative growth. The success of pomegranate cultivation and achieving high productivity depends largely on the efficiency of plant nutrition programs, especially during the early stages of growth (El-Gioushy, 2016). This stage is fundamental for developing a strong vegetative system capable of supporting fruit production in later stages (Maity et al., 2019).

In this context, biofertilizers have emerged as a sustainable alternative or supplement to chemical fertilizers, playing a vital role in improving soil fertility, increasing nutrient availability, and stimulating plant growth through the secretion of natural growth regulators and nitrogen fixation (Ranjan et al., 2020; Bhardwaj et al., 2014). Among these biofertilizers, the bacterium *Azospirillum brasilense* is of particular interest due to its high efficiency in promoting plant growth through multiple mechanisms, including nitrogen fixation, root stimulation, increased nutrient uptake, and the secretion of auxins, gibberellins, and cytokinins (Suhameena et al., 2020; Singh & Sadawarti, 2020; Bashan et al., 2004).

On the other hand, foliar conventional fertilizers, especially those containing macro- and micronutrients (Al-Waeli et al.,

2023), remain an important tool for providing the plants with nutritional needs and improving photosynthetic efficiency and vegetative growth (Hussein et al., 2024). Therefore, this research aims to study the response of pomegranate seedlings of the Wonderful variety to different levels of the biofertilizer *Azospirillum brasilense* and the foliar fertilizer Growmore, both individually and in combination, across several vegetative growth indicators. This research aims to study the complementary effect between different levels of the *A. brasilense* bio-inotrope and balanced foliar fertilizer in improving vegetative growth indicators and chemical content of pomegranate seedlings of the 'Wonderful' variety under the conditions of Najaf Governorate.

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. The experiment included two factors at four application levels, the first is the conventional foliar fertilizer Growmore which constitutes Major and Micronutrients including: 20% Total Nitrogen, 5.9% Nitrate, 3.9% Ammonium, 10.2% Urea, 20% Available Phosphate ($P_2 O_5$), 20% Soluble Potassium (K_2 O) Chelated Iron Fe 0.15%, Chelated Manganese (Mn) 0.05%, Chelated Zinc (Zn) 0.10%, Chelated Copper (Cu) 0.05%, Boron (B) 0.02%, and Molybdenum (Mo) 0.0005% applied at 0, 100, 200, and 300 mg L^{-1}. While the second one is the biofertilizer *Azospirillum brasilense* at 0, 4, 8, and 12ml $seedling^{-1}$ (10^6 CFU ml^{-1}).

The foliar spray with Growmore was applied one a month for 5 times starting at April 1 to October 1 except was not applied for July and August. As for the biofertilizer, the first application was on April 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 (Sparks et al., 2020).

Table1. Some of physical and chemical properties of the potting soil used in the experiment

Properties	Units	Value
pH	---	7.03
E.C.	Ds m^2	3.1
N	mg Kg^{-1}	12.4
P		12.9
K		14.3
Soil texture	Sand-loam	
Clay	%	24
Silt	%	16
Sand	%	60

The experiment treatments were distributed in a two-factor experiment using a randomized complete block design (RCBD). The first factor included four levels of biofertilizer (0, 4, 8 and 12ml $seedling^{-1}$), and the second included four levels of chemical fertilizer Growmore (0, 100, 200, and 300 mg L^{-1}). The growth traits were measured including seedling height (cm), stem diameter (mm), leaf area (cm^2 $seedling^{-1}$). While, nutritional indicators were estimated based on leaf content of nitrogen %, phosphorus % and potassium% in five randomly selected fully developed leaves from each experimental unit (Duboies et al., 1956).

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 (Payne et al., 2009). Differences between means were compared using the least significant difference (LSD) test at a probability level of 0.05. (Hoshmand, 2018).

Results and Discussion

Seedling height

The results showed a significant effect of biofertilizer treatment, conventional fertilizer application, and their interaction on plant height (Table 2). The treatment 12 ml $seedling^{-1}$ 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 seedling^{-1} and 300 mg L^{-1} of chemical fertilizer resulted in the highest average seedling height of 86.03 cm, compared to lower values in all other treatments.

Table2. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on plant height increase of pomegranate 'Wonderful' seedlings

Biofertilizer *Azospirillum brasilense* ml seedling^{-1}	**Conventional foliar Growmore mg L^{-1}**				**Average**
	Cntrol	**100**	**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**				

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 seedling^{-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 seedling^{-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

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

Biofertilizer	**Conventional foliar Growmore mg L^{-1}**	**Average**

A. brasilense ml seedling^{-1}	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**				

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 4.356 cm^2 respectively (Table4). 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 seedling^{-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.

Table4. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on seedling's leaf area (cm^2) in pomegranate 'Wonderful' variety

Biofertilizer *Azospirillum brasilense* ml seedling^{-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**				

Biochemical indicators

Plants content of nitrogen

Regarding the effect of the treatments on seedling content of nitrogen, it was observed that the leaf nitrogen increased with increasing biofertilizer concentration (Table5). As the *A. brasilense* at 12 ml seedling^{-1} significantly outperformed the control treatment, recording nitrogen level of 3.15% compared to the control with only 2.04%. Similarly, the Growmore spray treatment at a concentration of 300 mg L^{-1} recorded 3.15% with a significant difference from the control of only 2.38% leaf nitrogen. The interaction between biofertilizer and conventional fertilizer had a significant effect on leaf nitrogen, especially since the highest average of 3.95% in seedlings treated with 12 ml seedling^{-1} Biofertilizer + 300 mg L^{-1} Growmore spray.

Table5. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on seedling's content of nitrogen in pomegranate 'Wonderful' variety

Biofertilizer *Azospirillum brasilense* ml seedling^{-1}	**Conventional foliar Growmore mg L^{-1}**				**Average**
	Control	**100**	**200**	**300**	
Control	1.62	1.99	2.17	2.38	2.04
4	2.51	2.52	2.55	2.55	2.53
8	2.65	2.65	2.68	3.73	2.92
12	2.87	2.88	2.91	3.95	3.15
Average	2.41	2.51	2.57	3.15	
L.S.D 0.05	**Azo. / Gro.= 0.35 Interaction= 0.76**				

Plants content of phosphorus

Phosphorus content in seedlings also increased in the biofertilizer treatments, as 12 ml seedling^{-1} treatment resulted in leaf content of phosphorus of 0.89% which was significantly higher than that of the control (0.31%) treatment (Table6). The same table also shows that the 300 mg L^{-1} Growmore spray treatment achieved much higher average of leaf phosphorus recoding 1.01% compared to only 0.29% from control treatment. Overall, the interaction treatment of *A. brasilense* 12 ml seedling^{-1} and Growmore 300 mg L^{-1} achieved the highest leaf phosphorus of 1.95% achieving significant difference from most of the individual and interaction treatments.

Table6. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on seedling's content of phosphorus in pomegranate 'Wonderful' variety

Biofertilizer *Azospirillum brasilense* ml seedling^{-1}	Conventional foliar Growmore mg L^{-1}				Average
	Control	100	200	300	
control	0.20	0.27	0.39	0.40	0.31
4	0.26	0.42	0.50	0.80	0.49
8	0.33	0.45	0.69	0.90	0.59
12	0.38	0.36	0.88	1.09	0.89
Average	0.29	0.37	0.61	1.01	
L.S.D 0.05	Azo. / Gro.= 0.044 Interaction= 0.098				

Plants content of potassium

The findings (Table7) also showed significant increase leaf content of potassium recording an average of 1.99% and 1.81 in the seedlings treated with *A. brasilense* at 12 ml seedling^{-1} and 300 mg L^{-1} Growmore, respectively compared to much lower levels in the control treatments which ranged from 1.14 to 1.42% respectively. It was also observed in the same table (7) that the interaction of the both factors at the highest concentrations resulted in the highest seedling content of potassium (2%) with a significant difference from most individual and interaction treatments.

Table7. Effect of biofertilizer *A. brasilense* and conventional foliar Growmore on seedling's content of potassium in pomegranate 'Wonderful' variety

Biofertilizer *Azospirillum brasilense* ml seedling^{-1}	Conventional foliar Growmore mg L^{-1}				Average
	Control	100	200	300	
Control	0.39	1.45	1.25	1.48	1.14
4	1.54	1.72	1.59	1.81	1.66
8	1.80	1.84	1.87	1.95	1.86
12	1.97	2.11	1.90	2.00	1.99
Average	1.42	1.78	1.65	1.81	
L.S.D 0.05	Azo. / Gro.= 0.083 Interaction= 0.133				

The results showed that the seedlings growth was positively affected by the biofertilizer. This effect is attributed to the ability of *Azospirillum* bacteria to fix atmospheric nitrogen and convert it into a form available to the plant. The bacteria secrete plant growth regulators such as auxins, gibberellins, and cytokinins, which stimulate cell division and cell elongation, leading to increased vegetative growth (Fukami et al., 2018). Soil-assisted biofertilizer bacteria have a high capacity to produce growth regulators such as auxins, gibberellins, and cytokinins, which are responsible for cell elongation and division, thus increasing the vegetative growth of seedlings (Sumbul et al., 2020). The cytokinins produced by these microorganisms promote bud formation and vegetative growth, increasing the number of developing leaves. The bacteria also fix essential nitrogen in the synthesis of chlorophyll, amino acids, proteins, and nucleic acids (RNA and DNA). This is reflected in increased plant height, number of leaves, and leaf area in treated seedlings (Suhameena et al., 2020).

The levels of nutrients macro-elements NPK in seedlings were observed to be higher due to both experimental biofertilizer and the commercial foliar liquid. The increase reflects higher levels of chlorophyll and higher efficiency of photosynthesis activities in plant growth indicated by cell division, elongation, and volume increase, thus leading to increased plant vegetative growth indicators (Al-Dulaimy, 2022; Ibrahim and Abd Elkawy, 2018; Taiz and Zeiger (2010). This is attributed to the fertilizer's content of the macronutrient NPK, which is directly involved in plant tissue building and the regulation of metabolic processes, in addition to micronutrients such as iron, zinc, and manganese, which play a pivotal role in chlorophyll formation and the activation of several enzymes involved in photosynthesis and respiration. Mengel and Kirkby (2012) and Broadley et al. (2012) indicated that the availability of these elements in sufficient quantities leads to improved photosynthetic efficiency and carbohydrate production, which positively impacts vegetative growth.

The clear interaction between biofertilizer and foliar fertilizer confirmed the complementary role of biofertilizer in improving nutrient availability and increasing their absorption efficiency from the soil, and the role of foliar fertilizer in providing nutrients directly and rapidly through the leaves. Several studies have confirmed that combining biofertilizers and chemical fertilizers leads to a significant

improvement in the growth and productivity of fruit trees compared to using either one alone (Stino et al., 2009; Pandey et al., 2020).

The chemical fertilizer Growmore contains balanced amounts of NPK and micronutrients that play an important role in cell elongation and division processes, and activate several important enzymes in auxin production. Phosphorus plays a role in the formation of a strong root system that helps in the absorption of minerals that promote growth (Hopkins and Huner, 2004). Potassium plays a vital role in many physiological processes such as photosynthesis and respiration. It also plays a role in metabolism, activating many enzymes, promoting cell division, and the growth of plant tissues (Pandey et al., 2020). It also plays a role in the osmotic regulation of stomatal opening and closing, as well as involved in the synthesis of ATP by activating phosphorus uptake (Broadley et al., 2012). It is an essential element for accelerating the transport and filling of sieve tubes with photosynthetic byproducts, such as high molecular weight compounds like proteins and carbohydrates. This increases the cell diameter, maintains osmotic pressure, and causes cell swelling, leading to the opening and closing of stomata (Xu, 2020). Kandile et al. (2010) stated that nitrogen and potassium contribute to cell division and cell proliferation, resulting in increased vegetative growth. All these factors enhance plant vitality and activity by stimulating enzyme systems and the formation of DNA and RNA, which in turn boost vegetative activity (Citak & Sahriye, 2010). The increased nitrogen concentration in the seedlings treated with biofertilizer is attributed to the activity of bacteria that effective in fixing nitrogen in the form of ammonium readily available for uptake by roots (Suhameena et al., 2020), the activity of bacteria affected the physiological metabolic processes of seedlings, leading to increased vegetative and root growth indicators. This may positively impact the availability of bioavailable nitrogen for growing seedlings and the uptake of phosphorus and potassium. Furthermore, some bacterial species secrete hormones such as auxins, cytokinins, and gibberellins, which positively affect nutrient uptake (Fukami et al., 2018). The bacteria used in this research may have the ability to improve vegetative growth indicators by secreting siderophores, chelating compounds of elements such as phosphorus, iron, manganese, and others. This leads to increased availability of these elements in

the soil, which in turn increases the content of these nutrients in the plant. This aligns with the findings of Hayat et al. (2010).

Conclusion

The results of the study demonstrated the possibility of increasing pomegranate seedlings 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 seedlings growth and content of biochemicals which were 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.

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