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Effect of PGPB on leaf nutrient status and growth of *Begonia semperflorens*

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Abstract

Bacteria are increasingly used in agriculture as eco-friendly alternatives to promote plant growth through mechanisms such as nitrogen fixation, phosphate solubilization, hormone production, and improved nutrient uptake. This study aims to examine the effects of plant growth-promoting bacteria on the growth, quality, and nutrient content of *Begonia semperflorens*, a commercially valuable ornamental plant known for its long flowering period and vibrant blooms. In this study, one-month-old *Begonia semperflorens* seedlings were dipped into bacterial suspensions (10^8 CFU ml^{-1}) of *Pseudomonas chlororaphis* MF-1, *Bacillus megaterium* M-3, and *Agrobacterium radiobacter* A-16 for 20 minutes prior to planting. After planting, the seedlings were irrigated with bacterial suspensions as watering solution at twice at 15-day intervals. At the end of one month, plant growth and quality parameters (plant height, number of leaves, leaf area, plant fresh weight, plant dry weight, stem diameter, flower stem, plant crown width, number of blooming flowers). were evaluated, along with macro- and microelement contents (total N, P, K, Ca, Mn, Mg, and Fe) in both plant leaves and the growing medium. All bacterial treatments significantly improved plant growth and quality compared to the control group, with M-3 exhibiting the most pronounced effects, increasing plant height by 40%, leaf number by 133%, leaf area by 348%, and flower number by 61%. A-16 enhanced crown width (45%) and flower stem length (26%), while MF-1 improved plant height (43%) and flower production (51%). Additionally, M-3 increased total nitrogen in leaves by 43%, while bacterial treatments enhanced various elements in plant leaves. The results show that these bacterial isolates improve begonia plant characteristics, providing high-quality plants and offering a sustainable alternative as microbial fertilizers in ornamental plant cultivation.

Keywords: *Pseudomonas chlororaphis*, *Bacillus megaterium*, *Agrobacterium radiobacter*, *Begonia semperflorens*, Microbial fertilizer

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INTRODUCTION

Efforts to find solutions that contribute to reducing the use of chemical fertilizers in agricultural production have gained momentum within the framework of sustainable agriculture. As a sustainable substitute for heavy chemical use, biofertilizers support plant growth by releasing a variety of growth-promoting hormones and enhancing the availability of essential inorganic macro- and micronutrients (such as phosphorus solubilization, potassium solubilization, biological nitrogen fixation, zinc solubilization, and the production of hydrolytic enzymes like mannanase, phytase, etc.) (Sarıbuga et al., 2014; Karagöz et al., 2021). Therefore, studies focused on the use of microorganisms and their products to promote plant growth will contribute to the reduction of chemical fertilizer use in agricultural production and environmental pollution, thereby supporting the development of sustainable agriculture.

Scientific research has shown that a variety of microorganisms have been successfully used in organic agriculture (Aksoy and Yılmaz, 2008). Among these, bacteria are undoubtedly the most commonly used microorganisms in agriculture. Within the realm of bacteria, those found in the plant root rhizosphere play a crucial role in controlling plant diseases and pests, promoting plant growth, enhancing plant systemic resistance to stress

conditions, and facilitating the breakdown of organic and inorganic substances in the soil for easier plant uptake. (Karakurt et al., 2011; Tozlu et al., 2012; Şenol et al., 2014; Dikbas et al., 2023). Plant growth promoting bacteria (PGPB) mostly belong to the genera *Acinetobacter, Aereobacter,Alcaligenes,Agrobacterium, Artrobacter, Azospirillum, Azotobacter, Beijerinckia, Burkholderia, Bacillus, Erwinia, Enterobacter, Micrococcus, Pseudomonas, Flavobacterium, Paenibacillus Rhizobium, Rhodosprilum, Rhodobacter, Serratia* and *Xanthomonas* (Dandurand et al., 1994; Daza et al., 2000; Dadasoğlu et al., 2013). The most studied PGPB species belong to the genera *Paenibacillus, Bacillus, Pseudomonas* and *Azospirillum* (Çakmakçı et al., 2006; Sezen, 2012). Successful results have been observed in terms of plant development and yield with the use of PGPBs. (Liu et al., 1995; Altın, 2004; Wang et al., 2005; Altın and Bora, 2015; Karagoz et al., 2018; Tozlu et al., 2019; Şenol Kotan, 2020).

PGPB is widespread in agricultural and natural environments and is isolated from plants, flowers, seeds, water, soil, animals, and humans (Wang et al., 2022). It exists as both an epiphyte and an endophyte in plants (Pantoea agglomerans, 2024). PGPB exhibits beneficial antibacterial activity against phytopathogens, making it a preferred biocontrol agent and plant growth promoter (Guevarra et al., 2021). It provides many benefits to plant symbionts through direct and indirect mechanisms. It facilitates nitrogen fixation in plant roots by utilizing atmospheric nitrogen (Dobbelaere et al., 2003; Çakmakçı et al., 2005; Shridhar, 2012; Mohammadı, 2018). It also solubilizes potassium, ammonia, and phosphorus in symbiont plants, effectively serving as a natural fertilizer. Additionally, it uses bacterial siderophores to degrade heavy metals such as iron, offering bioremediation applications for soil pollution caused by heavy metals (Khan 2005; McCauley et al., 2009; Anonymous, 2024).

The ornamental plant industry is continually advancing with new technologies and cultivation methods to deliver high-quality plant material. However, challenges like rising production costs, pests, disease spread, and climate change persist (Cardoso et al., 2022). As a result, it's crucial to develop next-generation yields and establish standards for more efficient and sustainable production. This study was conducted due to the limited utilization of *Begonia semperflorens* in bacterial applications, despite its frequent use in various studies on ornamental plants. In this study, the effects of *Pseudomonas chlororaphis* MF-1, *Bacillus megaterium* M-3 and *Agrobacterium radiobacter* A-16 isolates on the growth parameters, quality traits and macro and micro element content of Begonia semperflorens plant were evaluated for the first time. The study aims to contribute to sustainable applications in ornamental plant cultivation and to reveal new bacterial isolates that will be evaluated as biofertilizer candidates especially for begonia.

MATERIALS AND METHODS

Bioagent bacteria and plant material used in the study

Pseudomonas chlororaphis MF-1*, Bacillus megaterium* M-3*, Agrobacterium radiobacter* A-16 bacteria were chosen for its biological controls and plant growth-promoting properties (Kotan et al. 2004; Karakurt et al., 2011; Tozlu et al., 2012; Senol et al. 2014; Şenol Kotan et al., 2024). The bacteria were obtained from the culture collection unit at the Plant Protection Department of Atatürk University's Faculty of Agriculture in Turkey. Begonia semperflorens, an evergreen perennial species renowned for its extended blooming period and vibrant flowers, was selected for this study due to its substantial commercial value in landscape and floral design, as well as to examine its response to plant growth-promoting bacterial isolates; the seedlings used in the experiments were sourced from a retailer in Turkey.

Calculation of viable bacterial counts

For bacterial enumeration, 10 ml serial dilutions (10^{-1}-10^{-9}) were made by transferring a 1 ml gr^{-1} sample into 9 ml of sterile peptone. Each dilution was mixed and 100 µl was spread on NA-containing petri dishes. The dilutions were inoculated into three Petri dishes each, incubated at 28°C for 24-48 hours, and bacterial counts were determined from three replicates, with the total count (CFU) calculated by multiplying the dilution factor (Anonymous, 2005).

Greenhouse studies

One-month-old seedlings were dipped into the prepared bacteria and planted in pots after waiting for 20 minutes (Figure 1). Bacteria isolates were used for bacterial treatment, and begonia seedlings were irrigated twice at 15-day intervals with bacteria-containing media. Sterile nutrient broth was used in the control application. 25 ml of bacteria solution (10^8 CFU ml^{-1}) was given to each pot depending on the application. Experimental soil peat, characterized by a particle size of 0–40 mm, a pH ($CaCl_2$) of 5.2–6.0, porosity of 95–99%, dry bulk density of <55–90 g/L, and organic matter content of 95–99%, was used as the growth medium. The seedlings were planted in pots with a volume of 0.5 L. The plants were irrigated with equal water every 2-3 days according to their water needs. The experiment was conducted from July 1 to August 1 in a greenhouse in Erzurum, Türkiye, under natural photoperiod conditions (14–15 h daylight). Average daytime and nighttime temperatures were 24–28 °C and 16–20 °C, respectively. Relative humidity was maintained at 50–70%. After a 30-day period, data regarding both plant growth and the growing medium were collected.

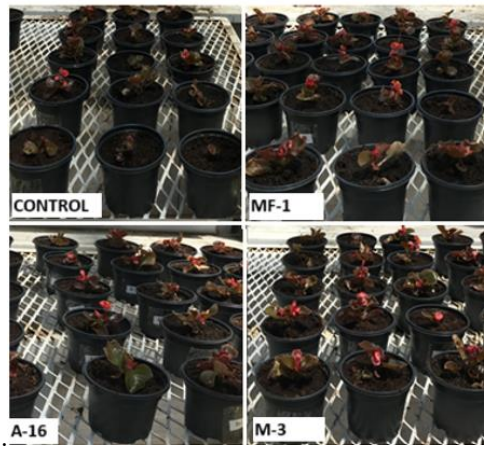

Figure 1. Potted image of one month old begonia seedlings after applications

Determination of plant growth parameters

The study evaluated several growth parameters (plant height (mm), number of leaves (leaves.plant^{-1}), leaf area (cm^2), plant fresh weight (g), plant dry weight (g), stem diameter (mm), flower stem (mm), plant crown width (mm), number of blooming flowers (number.plant^{-1})). For dry weight measurement, the leaves of the plants were placed in paper bags and dried in an oven at 80°C for 48 hours. After drying completely, their dry weights were measured using a precision balance with an accuracy of 0.001 grams (Karagöz et al., 2021).

Plant and growing medium nutrient analysis

Total nitrogen content in plant samples was measured using the micro Kjeldahl method (Bremner 1996). The determination of nutrients (P, K, Ca, Mg, Fe) in plants was carried out using an ICP OES spectrophotometer (Inductively Coupled Plasma spectrophotometer) (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) as described by Karagöz et al. (2021). For the growing medium, extractable P, K, Ca, and Mg contents were determined using the Mehlich-III extraction method, as outlined in the Soil and Plant Analysis Council (2000). Elemental concentrations in the extracts were also measured using ICP-OES.

Experimental Design and Statistical Analysis

The pots used in the greenhouse studies were distributed according to the randomized block design, each treatment consisted of 5 replications, and in each replication, 3 pots were used. In total, 15 pots were used for each treatment. Data analysis was performed using analysis of variance (one way-ANOVA) in SPSS version 20.0 (SPSS Inc.), with mean differences assessed through Tukey's HSD multiple range test. Statistical significance was set at a p-value of less than 0.05. All experiments were conducted in five times, and the results are presented as the mean ± standard deviation with a 95% confidence interval (Karagöz et al., 2021).

RESULTS AND DISCUSSION

Bacterial isolates were applied to *Begonia semperflorens*, a species widely used in the ornamental plant industry, at 15-day intervals. Their effects on plant growth and quality were evaluated at the end of the experiment (Table 1). According to ANOVA results, bacterial treatments had a statistically significant effect ($p < 0.05$) on several growth and quality parameters, including plant height, number of leaves, leaf area, fresh and dry weight, stem diameter, flower stem length, crown width, and number of blooming flowers. Among the tested isolates, *B. megaterium* (M-3) showed the most notable effect, particularly on vegetative growth characteristics.

Table 1. Vegetative growth of begonia plant in response to bacteria suspension treatments

Plant Parameters	Applications			
	Control	MF-1	A-16	M-3
Plant height (mm)	89.0±20 ^{b}	127.7±8 ^{a}	117.8±14 ^{a}	125.2±16 ^{a}
Number of leaves (leaves·plant^{-1})	19±2.4 ^{c}	33±4.5 ^{b}	31±2.4 ^{b}	44.3±4.2 ^{a}
Leaf area (cm^2)	160.9±12.3 ^{c}	449.2±28.0 ^{b}	642.1±36.1^{a}	721.3±35.4 ^{a}
Plant fresh weight (g)	53.92±6.2 ^{d}	108.3±1.9 ^{c}	110.8±5.5 ^{b}	118.2±14.2 ^{a}
Plant dry weight (g)	2.1±0.4 ^{c}	3.89±0.1 ^{b}	3.73±0.2 ^{b}	3.97±0.4 ^{a}
Stem diameter (mm)	7.76±0.9 ^{b}	7.83±0.8 ^{b}	9.14±1.4 ^{a}	9.55±1.0 ^{a}
Flower stem (mm)	32.9±4.1 ^{b}	35.81±4.9 ^{a}	41.68±6.8 ^{a}	37.27±3.8 ^{a}
Plant crown width (mm)	121.6±1^{b}	159.6±18 ^{a}	177.0±14 ^{a}	161.7±15 ^{a}
Number of blooming flowers (number.plant^{-1})	4.7±2.3 ^{b}	7.1±1.6 ^{a}	7.4±1.9 ^{a}	7.6±1.8 ^{a}

*Mean values in the same column by the same letter are not significantly different from the HSD Tukey's multiple comparison test. ($P < 0.05$). (MF-1: *P. chlororaphis*, A-16: *A. radiobacter,* M-3: *B. megaterium*)

The effects of bacteria on begonia plant growth parameters were studied of this study. In begonia plants, bacterial treatments led to significant improvements in various growth parameters compared to the control. Plant height increased by 43% with the MF-1 *P. chlororaphis* isolate, 40% with the M-3 *B. megaterium* isolate, and 32% with the A-16 *A. radiobacter* isolate. The number of leaves increased by 133% with the M-3 isolate, while leaf area increased by 348% with the M-3 isolate and by 299% with the A-16 isolate. Furthermore, the leaf fresh weight increased by 119% and leaf dry weight by 89% with the M-3 isolate. Stem diameter increased by 23% with the M-3 isolate and by 17% with the A-16 isolate. Flower stem length increased by 26% with the A-16 isolate. Plant crown width increased by 45% with the A-16 isolate, 32% with the M-3 isolate, and 31% with the MF-1 isolate. The number of flowers increased by 61% with the M-3 isolate, 57% with the A-16 isolate, and 51% with the MF-1 isolate compared to the control. Bacterial applications showed significantly higher values than the control in all plant parameters considered statistically significant

In a previous study; It was determined that many bacterial formulations caused increases in both plant development and plant chlorophyll amount (Aktaş 2014). It has been noted that the bacterial strain *Pseudomonas fluorescens* positively impacts plant characteristics, including plant height, leaf surface area, and the number of flowers per plant in *chrysanthemums* and *dahlias*. (Göre & Altın, 2006), *Azospirillum* and *Bacillus* strains on *Petunia* (Hoda & Mona, 2014), *Paenibacillus polymixa*, *Achromobacter xylosoxidans*, *Pseudomonas putida*, *Pantoea agglomerans*, *Brevibacillus brevis*, *Bacillus megaterium*, *Bacillus megaterium* strains on *Cyclamen persicum* (Girgin 2019) showed an increase in flowering date, number of flowers/branches and flowering duration. In the study conducted by Şekerci & Ünlü (2023), it was stated that two different bacterial formulations using *B. subtilis* and *B. cereus* provided a two-fold increase in seedling height and number of leaves and a four-fold increase in seedling fresh weight in kalanchoe plants. In addition, PGPB applications; It has been reported that it has positive increases on plant growth parameters in species such as geranium (Mishra et al., 2010), tulip (Parlakova, 2014), jasmine (Jayamma et al., 2014), calendula (Arab et al., 2015) (Dalda Şekerci & Ünlü 2023). It was determined that all bacterial isolates used in the study also provided significant increases in plant growth parameters in begonia plants. Begonia plants at the end of the study are shown in Figure 1.

Figure 1. The effect of control and bacteria applications on plants (MF-1: *P. chlororaphis*, A-16: *A. radiobacter,* M-3: *B. megaterium*)

As a result of the application of bacteria to the plant, the nutrient element content in the plant leaf was examined and the results are given in Table 2. According to the plant nutrient element analysis results performed on the leaves of the plants, it was determined that there were statistically significant differences in Total N, P, Ca, K and Mg elements. It was observed that there was no statistically significant difference in Fe elements.

Table 2. Results of nutrient analysis in begonia leaves

Nutrition Elements	Applications			
	Control	MF-1	A-16	M-3
Total N (%)	2.75±0,02 ^{d}	3.06±0,03 ^{c}	3.20±0,04 ^{b}	3.95±0,06 ^{a}
P (%)	0.78±0.01 ^{d}	0.90±0.04 ^{a}	0.79±0.08 ^{c}	0.86±0.01 ^{b}
K (%)	3.86±0.06 ^{d}	4.51±0.05 ^{a}	4.16±0.02 ^{b}	4.06±0.06 ^{c}
Ca (%)	0.64±0.01 ^{b}	0.65±0.01 ^{b}	0.66±0.01 ^{a}	0.59±0.01 ^{c}
Mg (%)	0.79±0.01 ^{b}	0.80±0.01 ^{a}	0.74±0.01 ^{c}	0.68±0.01 ^{d}
Fe (ppm)	18.77±1.05^{ns}	20.07±0.39 ^{ns}	18.60±0.48 ^{ns}	18.61±0.20 ^{ns}

*Mean values in the same column by the same letter are not significantly different from the HSD Tukey's multiple comparison test. ($P < 0.05$), NS: Statistically insignificant result. (MF-1: *P. chlororaphis*, A-16: *A. radiobacter,* M-3: *B. megaterium*)

According to the nutrient element analysis carried out on plant leaves, the highest value for total N element was reached with M-3 isolate, the highest value for P, K and Mg elements was reached with MF-1 isolate, and the highest value for Ca element was reached with A-16 isolate. For Fe element, where no statistically significant difference was found, the highest value was again reached with MF-1 isolate. Nitrogen, which is one of the most important nutrients that plants must definitely take from their growing environment, is an important factor limiting plant growth after water (Vitousek & Howarth 1991). Total nitrogen in plant leaves was found to be 43% higher with the application of M-3 isolate and 16% higher with the application of A-16 isolate compared to the control application. In general, the sufficiency level of total nitrogen in the leaf was reported by Pritss and Handley (1998) as 2.0-2.8%, and by İbrikçi et al. (1994) as 2.50-3.20%. According to the studies, it was found that the total nitrogen amount in the leaf was sufficient and bacterial applications increased the total nitrogen amount compared to the control application. It has been shown that nitrogen-fixing bacteria provide resistance to pathogens, reduce growth period, stimulate plant development, and increase flowering, yield and/or quality (Bashan & de-Bashan 2002; Shridhar 2012; Mohammadı 2018).

Phosphorus is an important element required by plants for the formation of Adenosine Triphosphate (ATP), sugars and nucleic acids. The P element in plant leaves was found to be 15% higher in the MF-1 isolate and 10% higher in the M-3 isolate compared to the control application. Potassium increases the resistance of plants to diseases, provides seed maturation and development of the root system of the plant (Kacar & Katkat 2010). The amount of potassium in plant leaves was found to be 17% higher for the MF-1 isolate application and 8% higher for the A-16 isolate application compared to the control application. Çakmakçı et al. (2005) reported that P-solubilizing *Bacillus* RC07*, Bacillus* M-13, and *Rhodobacter* RC04, *Paenibacillus* RC05 bacteria increased N and P uptake. In addition, *Bacillus* RC07 and *Bacillus* M-13 were reported to be able to solubilize calcium phosphates. In another study, it was reported that PGPB applications increased the uptake of micronutrients as well as essential nutrients such as N, P and K by the plant (Dobbelaere et al. 2003; Çakmakçı et al. 2005; Karlıdağ et al. 2007). According to the results of the study, it can be said that the bacterial isolates used stimulate plant systemic resistance and support plant root development by increasing the uptake of potassium element into the plant. Magnesium helps the uptake of other elements, especially phosphorus, and plays a role in the activation of many enzymes (Plaster, 1992; Gardiner & Miller, 2008). The Mg element rate in plant leaves was found to be 3% higher for the MF-1 isolate application than the control application.

Iron element plays a very important role in plant respiration and photosynthesis reactions. In its deficiency, the amount of chlorophyll decreases and plant growth slows down (McCauley et al., 2009). The amount of Fe element in plant leaves was found to be 7% higher for the MF-1 isolate application compared to the control application. Iron plays an important role in the absorption of calcium and magnesium. Gardiner and Miller (2008). It was determined that PGPRs caused an increase in plant height, stem diameter, plant fresh weight, plant dry weight, root length, root dry weight, yield, fruit size and fruit number at varying rates. Khan (2005) revealed that bacteria such as *Acinetobacter* and *Pseudomonas* increased in Fe, Mg, Zn, K, Ca and P contents in the plant. The increase in Fe uptake by MF-1 may have stimulated chlorophyll synthesis and the plant growth parameters.

The nutrient element results obtained from the growing medium of the plants as a result of the application of bacterial isolates to the plant are given in Table 3.

Table 3. Results of nutrient analysis in growth medium

Nutrition Elements	Applications			
	Control	MF-1	A-16	M-3
Total N (%)	0.062±0,0006 ^{d}	0.067±0,0004 ^{c}	0.068±0,0002 ^{b}	0.072±0,0006 ^{a}
P (%)	0.14±0.006 ^{a}	0.10±0.002 ^{b}	0.06±0.002 ^{d}	0.07±0.004 ^{c}
K (%)	0.08±0.011 ^{a}	0.08±0.004 ^{a}	0.05±0.003 ^{b}	0.07±0.004 ^{a}
Ca (%)	0.69±0.03 ^{a}	0.60±0.009 ^{b}	0.55±0.007 ^{c}	0.60±0.007 ^{b}
Mg (%)	0.17±0.006 ^{a}	0.15±0.001 ^{b}	0.17±0.002 ^{a}	0.15±0.001 ^{b}
Fe (%)	0.13±0.003 ^{b}	0.12±0.002 ^{c}	0.19±0.002 ^{a}	0.13±0.001 ^{b}

*Mean values in the same column by the same letter are not significantly different from the HSD Tukey's multiple comparison test. ($P < 0.05$), NS: Statistically insignificant result.

According to the results of the nutrient element analysis in the growing medium, it was determined that there were statistically significant differences in total N, K, P, Ca, Fe and Mg elements. It was determined that the results of the total N determination in the growing medium were parallel to the total nitrogen ratio in the plant leaf. Total N element in the growing medium was found to be 17% higher in the M-3 isolate application, 9% in the A-16 isolate application and 3% in the MF-1 isolate application compared to the control application. It is thought that this situation is due to the ability of the bacterial isolates to fix nitrogen in the growing medium and to increasing its uptake into the plant at the right rate. It has been stated that PGPB bacteria can fix symbiotic or asymbiotic nitrogen, mineralize organic phosphate and other nutrient elements, and dissolve mineral phosphate and iron

(Çakmakçı et al. 2007; Karagöz et al. 2016a; Dadaşoğlu et al. 2017). It is thought that P, K, Ca and Mg elements were taken up from the soil to the plant at higher rates in bacterial applications compared to the control application and therefore they were low in the growing medium. In addition, the same situation is valid for Fe element for MF-1 and M-3 isolates. Compared to the control application in the growing medium, A-16 isolate decreased P element by 57%, K element by 37.5%, Ca element by 20%, and MF-1 and M-3 isolates decreased Mg element by 11% compared to the control application. It is known that bacteria applied to the soil also contribute significantly to the decomposition of organic matter in the soil and facilitating their uptake by the plant. It has been reported that PGPB applications increased the uptake of nutrient elements such as N, P, K, Mg and Fe (Khan; 2005; Karlıdağ et al. 2007; Karthikean, et al. 2010). Orhan et al. (2006) stated that bacterial applications significantly affected the available Mg in the soil.

CONCLUSION

Ornamental plant cultivation is increasingly turning to alternative methods to reduce dependency on chemical inputs. PGPBs, one of these alternatives, have significant potential in effectively controlling environmental and soil pollution. It was determined that MF-1 *P. chlororaphis*, A-16 *A. radiobacter* and M-3 *B. megaterium* isolates used in this study had highly positive effects on plant height, leaf number, leaf area, leaf fresh weight, leaf dry weight, stem diameter, flower stem, plant width and number of flowers opened in begonia plants compared to control application. Especially, M-3 bacterial isolate application had high total N element ratio in plant leaves and growing medium and showed the best result in many of the plant growth parameters, while MF-1 isolate application increased P, K, Mg, Fe elements in plant leaves, A-16 isolate application increased Ca element, Mg and Fe elements in plant leaves in growing medium and positively affected plant quality. According to these results, it is anticipated that the bacterial strains could serve as highly effective microbial fertilizers, particularly for begonia and other ornamental plants. There are limited studies in the literature regarding the use of such bacterial applications on plant growth parameters and the quality traits of potted ornamental plants. This highlights the value of the findings from the current study and underscores their potential contribution to future sustainable agriculture research.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author declare no competing interests.

Author contribution

The author conducted the experiments, recorded and processed the data, designed tables and graphs, prepared the draft manuscript, revised and edited the final manuscript, developed and discussed and interpreted the results.

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