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Assessment of Different PGPR Formulations as a Biological Fertilizer in Cultivation of Poinsettia (*Euphorbia pulcherrima*)

Fazilet Parlakova Karagoz*, Atilla Dursun

Department of Horticulture, Agriculture Faculty, Atatürk University, Erzurum, Turkey

Email address:
f.parlakova@atauni.edu.tr (F. P. Karagoz)

*Corresponding author

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Abstract: Poinsettia (*Euphorbia pulcherrima* Willd.ex Klotzsch.) is one of the most important potted plants. This research was carried out to determine effects of different PGPR formulations, chemical fertilizers and their combinations on plant growth characteristics and nutrient content of growing medium in two different cultivars (Christmas Feelings and Christmas Eve) of poinsettia. The research was conducted in climate controlled research greenhouse between July 2015 and July 2017. The applications were created as formulation 1 (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79), formulation 2 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C), formulation 3 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C), formulation 4 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D), the full amount of commonly used chemical fertilizer (150 g·100L^{-1}) (100% CF) and by combining the reduced amount of chemical fertilizer by (75 g 100L^{-1}) 50% with each bacterial formulation and control. Some plant growth parameters (the first flower (average number of days between flowering of the first plant) (day), total growing time up to marketable commercial size (average number of days between time for the forming red colour of 50% of bracts leaves and the first plant) (day), main flower stalk length (cm) and main flower stalk diameter (mm), total number of leaves (number plant^{-1}), root collar diameter (mm), fresh weight of plant (g), dry weight of plant (g)) and growing medium characteristics were evaluated in the experiment. In addition to the recommended amount of chemical fertilizer application (100% CF) in poinsettia cultivation, BI and BIII bacterial formulation applications were found positive effects on shortening the time until flowering and early flowering. The poinsettia plants grew shortest time marketable commercial size when supplied with BIII+CF application comparing to control. The bacterial viability in the growth medium ranged from 4.91x10^{6} cfu ml^{-1} to 1.80x10^{7} cfu ml^{-1}. The maximum total nitrogen (1008.00%) was obtained from BIV application. The highest solvable phosphorus (12.32 ppm) amount was determined in the BIV+CF while the highest potassium (2.02 cmol kg^{-1}) and calcium (8299.03 mg kg^{-1}) amount were found in the BIII application. The poinsettia plants were absorbed sufficient nutrients from the growth medium in CF, BI, BI+CF, BII, BII+CF, BIII, BIII+CF applications and increased in their plant growth and biomass. The bacterial formulations may be used as efficient PGPR for poinsettia production in farmer's greenhouse to reduce the need for chemical fertilizer and improve plant growth.

Keywords: *Euphorbia pulcherrima* Willd.ex Klotzsch., PGPR Formulations, Flowering, Nutrient

1. Introduction

Poinsettia (*Euphorbia pulcherrima* Willd.ex Klotzsch.) or Atatürk çiçeği (Turkish local name) is one of the most important potted plants grown for their flshy bracts and has been used mainly as a traditional Christmas decoration since the 17th century [1]. For Christmas, the main flowers are poinsettias, consumed especially in the colour red traditional version [2]. Greenhouse of poinsettia production is usually programmed for sales in December [3]. In order to make

poinsettia ready for sale in the Christmas season, the plants are fertilized with each irrigation during the 4-5-month growing period [4-6].

In the production of poinsettia, it is important to produce plants with intensive leaf colour for consumer demand. In order to obtain intense leaf colour, plant nutrition [7] and photoperiod control [8] are known as the most important factors [9]. Inputs or high-performance varieties that provided reduce cost, chemical concentration, fertilizer requirements or facilitated the growth process are attracted great attention of poinsettia producers. There is a growing interest in the ideas of reducing the use of chemicals to protect plant health and reduce production costs. Therefore, the use of bacteria (PGPR) located in the root rhizosphere of plants in agricultural production is increasing in day by day [10-13].

The bacteria, appropriately called rhizobacteria, of the habitat of which is located in a zone surrounding the roots of the plants or rhizosphere are known as plant growth promoting rhizobacteria (PGPR) [10]. PGPR are free-living microorganisms having useful effects on plants fixing N, the synthesis of vitamins and phytohormones, enhanced stress resistance, inhibition of plant ethylene synthesis provided plant nutrient uptake, mineralization of organic phosphate and solubilization of inorganic phosphate [14 -15]. Also, they have used used to prevent or decrease indirectly the detrimental effects of phytopathogens by colonizing in their phyllosphere or rhizosphere [15]. To reduce negative environmental effects by resulting from continuous, use of chemical fertilizers and to improve the physicochemical properties of the growing medium PGPR inoculation may be utilized [13, 14]. There are few studies on the use of PGPRs in ornamental plant cultivation [16-18]. The number of researches about the use of PGPR [19] in poinsettia production is also very limited in the world.

The objective of this work was to determine the effects of different PGPR formulations, chemical fertilizers and their combinations on plant growth characteristics and nutrient content of growing medium in two different cultivars (Christmas Feelings and Christmas Eve) of *Euphorbia pulcherrima* Willd.ex Klotzsch. It was targeted to benefit from these results in cultivation of poinsettia.

2. Materials and Methods

2.1. Experimental Materials and Set-Up

The research was conducted in climate controlled research greenhouse between July 2015 and July 2017 in Erzurum (Turkey). In the study, rooted cuttings of poinsettia [*Euphorbia pulcherrima* Willd. ex Klotzsch cv. Christmas Feelings (CvF) and Christmas Eve (CvE)] were used as plant materials. The cultivation medium was prepared by mixing peat in ratio of 2: 1 (diameter: 3,10 mm) and pumice (diameter: 10-30 mm) as volume (Lineberger 2018). Plants were planted in 3.5 liter plastic pots.

The applications were created as formulation 1 (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79), formulation 2 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C), formulation 3 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C), formulation 4 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D) (Table 1), the full amount of commonly used chemical fertilizer (100% CF=150 g 100 L^{-1}) and by combining the reduced amount of chemical fertilizer (75 g 100L^{-1}) by 50% with each bacterial formulation (Table 2). The bacterial suspensions (measured spectrophotometrically at 600 nm) were properly diluted to $1x10^8$ cfu ml^{-1} in sdH_2O. Bacterial formulations were inoculated in the rooted cuttings (5-8 cm height) of the poinsettia by dipping method for 15 min and they were planted in pots filled with appropriate growing medium. The study was designed as 3 replicates in factorial design with 2 (varieties) x 10 (application) (Table 2) in randomized parcel trial design.

Table 1. *Bacterial isolates used in the study and some biochemical properties [83].*

Isolate No	MIS Diagnosis Result	SIM	Location (in Turkey)	Host	Nitrogen	Phosphate	Siderophore
RK-79	*Pantoea agglomerans*	0.762	Erzurum	Apple	+	+	-
TV-12E	*Paenibacillus polymyxa*	0,551	Van	Poaceae	S+	+	-
TV-17C	*Bacillus subtilis*	0.677	Van	Raspberry	S	W+	-
TV-6D	*Bacillus megaterium*	0.750	Van	Poaceae	+	+	-
TV-42A	*Pseudomonas putida*	0.113	Van	Poaceae	W+	W+	+
TV-91C	*Bacillus megaterium*	0.474	Van	Poaceae	+	W+	-
TV-113C	*Kluyvera cryocrescens*	0.688	Van	Garlic	+	+	-
RK-92	*Pantoea agglomerans*	0.889	Erzurum	Pear	+	S	-

(SIM: Similarity index, S: Strong +, W: Weak +; +: Positive, -: Negative).

After planting of rooted cuttings (one plant per pot) in pots, two different types of fertilizer in a form that can be completely dissolved in water were applied to the pot groups to be applied chemical fertilizer at the determined different doses. These are comprised from "White 15-0-19 + 9CaO + 2MgO + TE, NPK ratio 4: 0: 5" (white composite fertilizer, granule, containing nitrogen, potassium, calcium, magnesium, boron, zinc, iron, copper, magnesium, molybdenum and manganese) and "Blue 18-11-18 + 2.5MgO, NPK ratio 3: 2: 3" (blue composite fertilizer, granule, containing nitrogen, phosphorus, potassium, sulfur, magnesium, boron, zinc, iron, copper, molybdenum and

manganese). These two different chemical fertilizers were given in specified amounts with the irrigation water consecutively [4, 20]. The recommended dose (150 g·100L^{-1}) of these fertilizers for pots, flowerbeds and all covered seedlings were used in this study.

Table 2. *Applications created in the study.*

Code of Application	Applications
Control	Control (Uninoculated)
CF	The full amount of commonly used chemical fertilizer (150 g· 100L^{-1}) (%100 CF)
BI	Formulation 1 (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79)
BII	Formulation 2 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C)
BIII	Formulation 3 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C)
BIV	Formulation 4 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D)
BI+CF	Formulation 1 (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79) + %50 CF [the reduced amount of chemical fertilizer by 50% (75 g 100L^{-1})]
BII+CF	Formulation 2 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C) + %50 CF [the reduced amount of chemical fertilizer by 50% (75 g 100L^{-1})]
BIII+CF	Formulation 3 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C) + %50 CF [the reduced amount of chemical fertilizer by 50% (75 g 100L^{-1})]
BIV+CF	Formulation 4 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D) + %50 CF [the reduced amount of chemical fertilizer by 50% (75 g 100L^{-1})]

2.2. Determinations of Plant Growth Parameters

After 110-120 days from bacterial inoculation, measurements of some plant growth parameters were made on 10 plants per application. These parameters were the date occurring of the first flower (average number of days between flowering of the first plant) (day), total growing time up to marketable commercial size (average number of days between time for the forming red colour of 50% of bracts leaves and the first plant) (day), main flower stalk length (cm) and main flower stalk diameter (mm), total number of leaves (number plant^{-1}), root collar diameter (mm), fresh weight of plant (g), dry weight of plant (g). Diameter was measured to the nearest 0 .01 mm using electronic digital caliper and measuring the root collar diameter (under of the soil line).

2.3. Measurements of Growth Medium Characteristics

The growth medium samples (1g each) were taken from the homogenized rhizosphere growth medium fractions of each experimental unit to estimate total number of bacteria, with colony forming units (cfu). The total number of bacteria in growth medium was determined following the method described by Andrade et al. [21]. Taken growing medium samples were separately dried at 27±2°C for 72 h, and passed through a 1-mm sieve. The growing medium pH was measured on 1:1 extract (Growing medium: Water) [22]. Macronutrients (organic matter (with Smith-Weldon method), P [23], total N (with the Kjeldahl procedure [24]), K, Ca and Mg [25]) and micro contents (Fe, Mn, Zn [26] and B [27]) of growing medium were also determined. Phosphorus, K, Ca, and Mg were determined with an ICP (inductively couples argon plasma) emission spectrometer (Thermo Jarrell Ash Co., Boston, N.Y., USA).

2.4. Statistical Analysis

All data in the present study were processed by SPSS (Statistical Package for Social Sciences, Version 22.0) and the means were separated by Duncan's multiple range tests.

3. Results and Discussion

3.1. Plant Parameters

Days to flowering: In the cultivation of this species, the time of formation of the first flowers in determining the correct planting time is very important as the criterion data. According to the general application averages, the highest mean values with 94.58 day were obtained under the application BIII for days to flowering. The minimum days to flowering was recorded in CF 90.04 day (p<0,001; Figure 1 a). As a result of this research, in addition to the recommended amount of chemical fertilizer application (100% CF) in poinsettia cultivation, BI and BIII bacterial formulation applications were found positive effects on shortening the time until flowering and early flowering. Richmond and Radwan [28] stated that the first flowering date can be used in the measurement of the earliness. It was found that there were differences between varieties and the time until flowering for CvE varieties was shorter. This result can be explained by the genetic characteristics of the varieties [29].

Main flower stalk diameter (mm): There were no significant differences in main flower stalk diameter among treatments for both years (p>0.05). Significant differences in main flower stalk diameter were found among treatments for varieties (p<0.001; Table 3). The main flower stalk diameter of poinsettia ranged from 4.93 mm to 5.15 mm and there was not statistically significant in p<0.05. In the CvF variety while the highest value of main flower stalk diameter was determined to be in BII and BI applications with 4.83 mm and BIII application with 4.81 mm was in the same statistical group with BI and BII applications (p<0.01; Figure 1-b). However, the effect of the all treatments on main flower stalk diameter was not significantly differed (p>0.05). Gurung, et al. [17] documented that the maximum stem thickness was observed in treatment T3 (Azotobacter + PSB + 80% RDF)

in Hydrangea as compared to control. Also, Rezvanypour, et al. [30] found that AMF (Arbuscular Mycorrhizal Fungi) inoculation caused significant increase in floral stem diameter for *Freesia* hybrida plants. The maximum stalk diameter of rose was recorded with Castor cake # 0.8 kg + Azotobacter # 1 ml + PSM # 1 ml + KSB # 1 ml / plant. It might also be due to positively affected by organic manure because chemically, organic manures add an organic compound to the soil. These findings are in conformity with those of [30] in Freesia and [17] in Hydrangea.

Main flower stalk length (cm): When the general average of the study was evaluated, it was determined that effect of applications (p<0.001) and varieties (p<0.05) on main flower stalk length was statistically significant. The main flower stalk length of poinsettia ranged from 20.08 cm to 24.96 cm and showed significantly difference between the treatments. The highest values of main flower stalk length were obtained from CF, BI+CF and BII+CF applications. In the CvE variety, the highest values of main flower stalk length were determined to be in CF and BI+CF applications while BII+CF application was in the same statistical group with CF and BI+CF applications. In the CvF variety, the highest value of main flower stalk length was determined to be in BIV+CF applications and BIV application was in the same statistical group with BIV+CF application (Table 3). De Silva et al. [31] reported that the application of *P. fluorescens Pf5* and *B. pumilus* rhizobacteria increased in the length of the main flower stalk of blueberries. Manju and Subramanian [32] demonstrated that the flower stalk length of gerbera was increased in application of liquid formulation of *B. subtilis* strain BG42 and 48.84 per cent increase according to control. This parameter is directly influenced by day length. The main reason for obtaining the longest main flower length in the CF and BI + CF applications of poinsettia varieties grown under the same conditions is estimated to be the addition of nutrients to the growing medium. It is also thought that PGPR accelerate the decomposition of the present and added nutrients and have characteristics of synthesize growth-promoting substances.

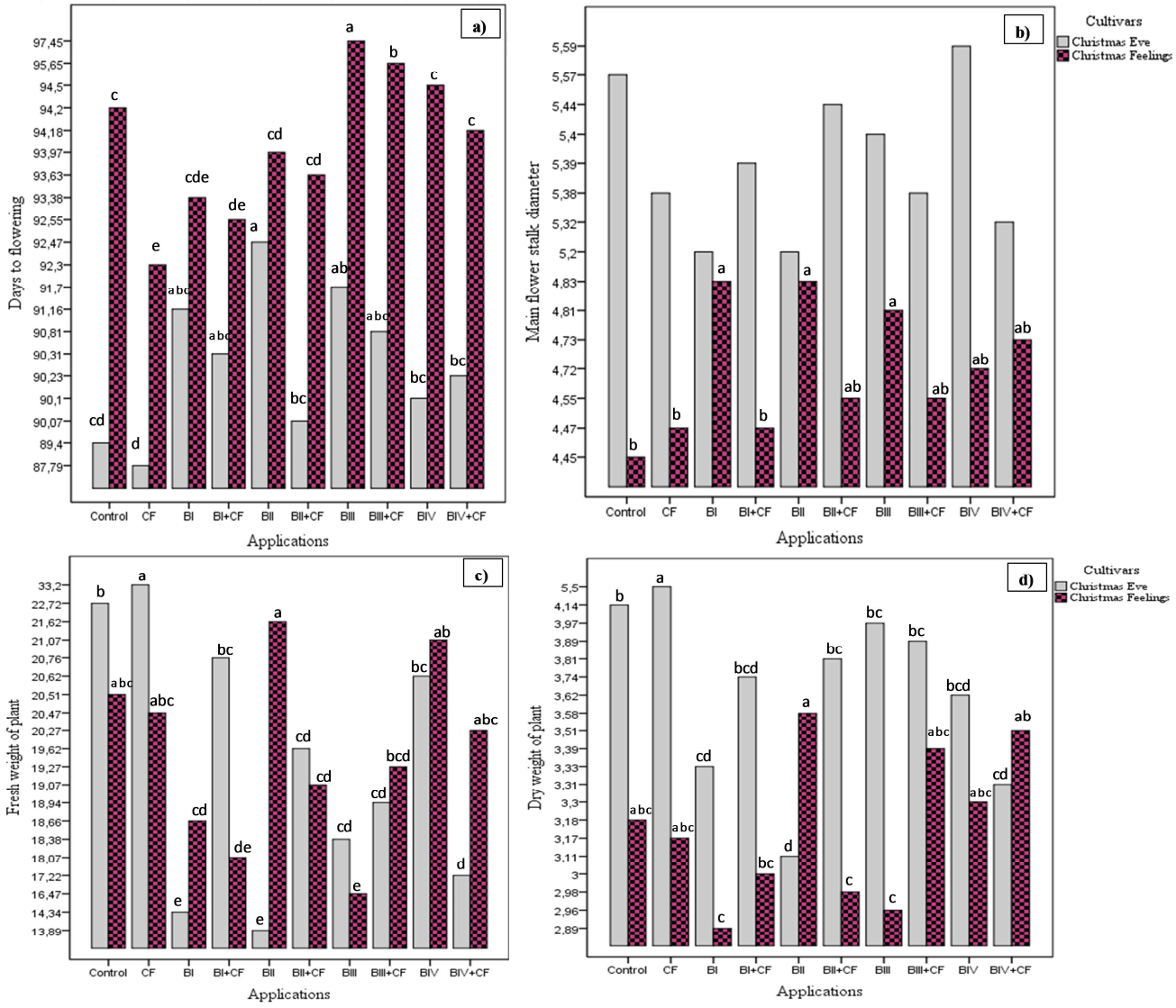

Figure 1. *a) Day to flowering (day); b) Main flower stalk diameter (mm); c) Fresh weight of plant (g/plant); d) Dry weight of plant (g/plant). Bars followed by the same letter do not differ from each other according to Duncan's test (p<0.05).*

Total number of leaves (number/plant): The data indicated significant (p<0.001) influence of applications and varieties on total number of leaves of poinsettia. It is vivid from table that the total number of leave (number plant^{-1}) was recorded with the ranges from 20.86 (BII) to 23.69 (BI+CF) and showed significantly difference between the treatments. Significantly maximum total number of leaves was recorded with treatment BII+CF for both varieties (Table 3). The inoculated PGPRs on *Cistus ladanifer* flower seedlings increased in the number of leaves, in the study conducted by Solano et al. [33]. *Pseudomonas putida* rhizobacteria was effective in increasing the number of leaves of poinsettia [19]. It is thought that the reasons for differences determined in terms of the total number of leaves between applications, in previous studies [34-36] and this study may be related to the species and quantity of microorganisms located in the growing medium and that are transformed into the form that the plant can take of the nutrients.

Table 3. *Effect of different applications of PGPR and chemical fertilizer on growth of poinsettia.*

	Main flower stalk length (cm)									
	Control	**CF**	**BI**	**BI+CF**	**BII**	**BII+CF**	**BIII**	**BIII+CF**	**BIV**	**BIV+CF**
CvE	21.50 cd***	27.04 a	22.49 bcd	26.45 a	20.27 de	24.77 ab	18.46 e	20.04 de	23.24 bc	20.44 de
CvF	22.16 bc***	22.88 bc	22.79 bc	22.65 bc	23.19 bc	23.60 b	21.70 c	23.29 b	24.12 ab	25.07 a
Mean	21.83 C***	24.96 A	22.64 BC	24.55 A	21.73 C	24.18 A	20.08 D	21.67 C	23.68 AB	22.75 BC
Total number of leaves (number plant $^{-1}$)										
CvE	22.95 bc***	23.47 b	23.66 b	25.06 a	20.80 e	23.50 b	21.52 de	22.10 cd	22.72 bc	22.16 cd
CvF	20.76 cd**	22.14 abc	21.43 abcd	22.32 ab	20.92 bcd	22.82 a	21.05 bcd	20.44 d	22.11 abc	22.83 a
Mean	21.85 CD***	22.80 ABC	22.54 BC	23.69 A	20.86 D	23.16 AB	21.28 D	21.27 D	22.42 BC	22.50 BC
Root collar diameter (mm)										
CvE	12.19 a**	11.37 ab	11.30 ab	12.06 a	11.22 ab	10.41bc	11.96 a	10.91abc	9.66 c	11.31 ab
CvF	11.36bcd***	10.56 de	12.52 a	11.30bcd	11.85abc	11.73abc	9.97 e	10.80cde	11.97 ab	11.33bcd
Mean	11.78AB*	10.97 BC	11.91 A	11.68 ABC	11.53 ABC	11.07 ABC	10.97 BC	10.86 BC	10.81 C	11.32 ABC
Total growing time up to marketable commercial size (day)										
CvE	88.43 cd***	87.29 d	90.66 ab	89.81 bc	91.97 a	89.53 bc	91.20 ab	91.34 ab	89.60 bc	89.72 bc
CvF	92.57 ab**	91.80 bc	92.07 abc	91.75 bc	91.89 bc	92.27 ab	92.94 a	92.85 a	91.78 bc	91.31 c
Mean	90.50 B***	89.54 C	91.36 AB	90.78 B	91.93 A	90.90 B	92.07 A	92.09 A	90.69 B	90.52 B

Data points followed by different letters for each parameter are significantly different at *p < 0.05; ** P<0,01 and ***P<0,001 among treatments. Data points followed by ns/NS are not significantly different at p>0.05 among treatments. CF:The full amount of commonly used chemical fertilizer (150g·100L^{-1})]) (%100 CF); BI: *Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79; BII: *Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C*;* BIII*: Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C; BIV: *Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D; BI+CF: *Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79 + *%50* CF [the reduced amount of chemical fertilizer by 50% (75 g·100L^{-1})]; BII+CF: *Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C + %50 CF [the reduced amount of chemical fertilizer by 50% (75 g·100L^{-1})]; BIII+CF: *Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C + %50 CF [the reduced amount of chemical fertilizer by 50% (75 g·100L^{-1})]; BIV+CF: *Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D + %50 CF [the reduced amount of chemical fertilizer by 50% (75 g·100L^{-1})]; CvE: Christmas Eve; CvF: Christmas Feelings.

Fresh and dry weight of plant (g): The study was found that applications factor and variety factor (p<0.01) showed statistically significant in terms of fresh and dry weight of plant. The fresh weight of plant from ranges 16.50 to 26.83 g and the dry weight of plant ranged from 3.11 g to 4.33 g. The highest values of fresh and dry weight of plant were obtained from CF application. Eid et al., [37] noted that even plants grown on the fertilization level produced almost higher dry matter produced by plants grown on the chemical fertilizer treatment. The highest values of fresh and dry weight of plant were determined to be in CF (respectively 33.20 g and 5.50 g) application in the CvE variety and BII (respectively 21.62 g and 3.58 g) application in the CvF variety (Figure 1c, Figure 1d). Jaleel et al. [38] reported that *Pseudomonas fluorescens* had positive effects on the fresh and dry weight of the propellant flower (*Catharanthus roseus*). It was considered the most effective treatment for CF application in the CvE variety and BII application in the CvF variety for increasing in fresh and dry weight/ plant in the present study. The above mentioned results are in harmony with those obtained by Jaleel *et al.* [38] and Eid et al., [37]. Martinetti *et al.* [39] reported that the plant weight ranged from 2.03 to 2.85 g. It can be said that dry weight of poinsettia plants increased in the present study when compared the results of study conducted by Martinetti et al. [39].

Root collar diameter (mm): The results showed that applications factor (p<0.05) was statistically significant, and variety factor (p>0.05) was not statistically significant in terms of root collar diameter of plant. The observation on root collar diameter in poinsettia was presented in Table 3. The data indicated significant influence of, especially, BI treatment on root collar diameter. The root collar diameter (mm) ranged from (BIV application) 10.81 to 11.91 mm (BI

application). In our study, the root collar diameter of inoculation with BI (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79) was greater than of chemical fertilizer, which was greater than that of control treatments. Won, et al., [40] previously reported that root collar diameter of *Pinus thunbergii* Parl. was increased in use of *Bacillus licheniformis* MH48. Also, Li, et al., [41] showed that *B. multivorans* WS-FJ9 significantly promoted growth in root collar diameter of inoculated poplar seedlings compared with controls. These positive results can be explained by the effects of PGPRs on cell expansion and division [42], improving nutrient uptake, and stimulating growth and development of plant organs [43-50].

Total growing time up to marketable commercial size (day): Success in the ornamental plant sector is closely related to the sales time planning required to meet consumer demand and create continuity in the market. When the general averages of the study are examined, it was determined that influence of applications and varieties was statistically significant in terms of total growing time up to marketable commercial size in $p<0.001$ level. The poinsettia plants grew shortest (92.09 day) time marketable commercial size when supplied with BIII+CF application, comparing with other application. BIII+CF application was in the same statistical group with BII and BIII applications. The plants of CvE variety achieved shortest (87.29 day) time marketable commercial size with CF application. In the CvF variety, the time until the longest up to marketable commercial size was determined to be in BIII and BIII + CF applications while the shortest period was determined in BIV+CF (91.31 day) (Table 3). Enhanced yield and marketable grade yield have been reported inoculation of other crops with PGPR in previous studies [51-52]. Eid et al., [37] also, demonstrated that *Bacillus* and *Azotobacter* bacterial strains significantly enhanced the early plant growth of *Matthiola incana* L. plants.

Growth medium parameters

Total number of bacteria in the growth medium (cfu ml^{-1}): The study was found that applications factor ($p<0.001$) was showed statistically significant, and variety factor ($p>0.05$) was not statistically significant in terms of the total number of bacteria in the growing medium. According to the averages of the values of both years of the experiment, the total number of bacteria in the growing medium ranged from $4.91x10^6$ cfu ml^{-1} to $1.80x10^7$ cfu ml^{-1}. BIV+CF application resulted in an increase in the number of bacteria by approximately 267% in the growth medium compared to the control. When the effects of applications for both varieties were separately examined, the maximum number of bacteria in the growth medium was obtained from BIV+CF application (Table 4; Figure 2). The total number of colony formation of Azospirillum brasilense and *Glomus intraradices* was determined as $5.0x10^6$ cfu g^{-1} in total [53]. *Azospirillum brasilense* was inoculated into lettuce seeds and bacteria population was determined as $3.1x10^6$ cfu g^{-1}, 60 days after planting [54]. Wheat seed was inoculated with *Azospirillum brasilense* and 40 days after planting and the total bacterial colony number was determined as $1x10^{12}$ cfu g^{-1} [55]. Pérez García et al. [53] interpreted that the total amount of bacterial colonies was affected by the plant species. Accordingly, determining the difference in the number of total bacteria among the varieties in this study can be explained by the literature.

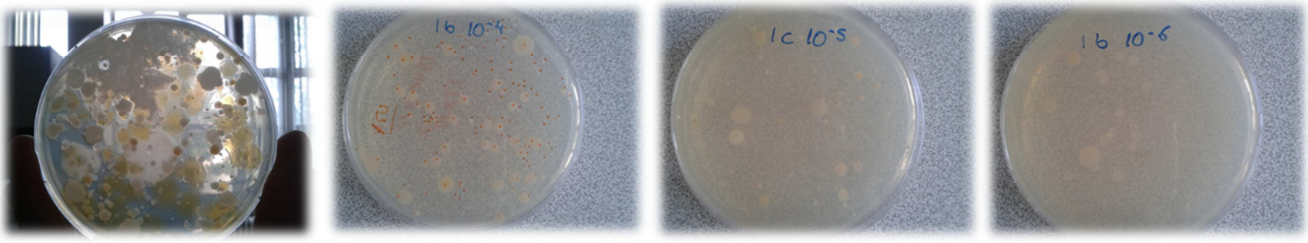

Figure 2. *View from the results of total number of bacteria in the growth medium.*

Growth medium reaction (pH): On analyzing effect of different treatment of chemical fertilizer and PGPR on pH in the growth medium of poinsettia, it was observed that applications factor ($p<0.001$) and variety factor ($p<0.01$) showed statistically significant. According to the average of the data obtained in every two years, the highest pH value was obtained from control application with 7.53. The highest average pH value was found in CvE variety of Poinsettia. The pH values determined in this study ranged from 7.33 to 7.53 (Table 4). It is said that pH values of the most suitable growing medium are 5.5-6.5 for the commercial cultivation of poinsettia [6]. It can be said that the pH levels determined in the present study can be tolerated by different varieties of poinsettia and that more than 6.5 of these values do not lead to major problems in cultivation of these varieties.

Organic matter (%): At the end of the experiment, the study was found that applications factor and variety factor ($p<0.001$) showed statistically significant in terms of organic matter of the growth medium. In the present study, the highest organic matter value was obtained from BIV application; the rate of organic matter in samples of the growth medium was found between 1.67% and 1.91% according to the general average of the applications. The highest organic matter was obtained from BIV application in every two poinsettia cultivar. The lowest organic matter was obtained from the control application in the CvE cultivar and it was determined in control and CF applications in the CvF cultivar (Table 4). The organic matter content of the growing medium in the cultivation of potted poinsettia is very important. Reasons for the increasing in percentage of organic matter of the BIV bacteria formulation in this study may be

nutrients containing more decomposed by bacteria and that these bacteria have more activities and numbers [56]. Prasad [57], Cakmakçi *et al.* [58] and Chen [59] said that bacteria decompose the nutrients connected in organic matter and thus they have played a very important role in making the plant available. Explanation of the results of this study and previous studies are possible to do with other studies [60, 45, 61-62, 58] that stated improvement of plant nutrient uptake, increasing in plant growth through phytohormone and vitamin synthesis.

Total nitrogen (%): The data indicated significant (p<0.001) influence of applications and varieties on total nitrogen in the growth medium of poinsettia. In the present study, the highest total nitrogen was obtained from BIV application, according to the general average of the applications. BIV application increased in the total nitrogen by approximately 12.75 % in the growth medium when compared to the control (Table 4). Orhan et al. [63] and Singh and Chauhan [64] reported that bacterial applications affect on the total nitrogen content of the growth medium. PGPRs are known to play an active role in ensuring that plants receive better nutrients and in ensuring that plant growth, development and quality with hormonal activity and suppressing harmful microorganisms naturally found in the plant root [62]. It was stated that PGPR strains such as *Azoarcus* sp., *Klebsiella pneumoniae*, *Beijerinckia* sp., *Rhizobium* sp. and *Pantoea agglomerans* stabilize atmospheric N_2 in the soil [65] and make them available to the plant [66]. In this study, taking place of *Pantoea agglomerans* bacteria strain in the bacterial formulation in which the highest total nitrogen was obtained, was found to be compatible with the previous study results.

Available phosphorus (ppm): The data explained significantly (p<0.001) difference between applications and varieties on available phosphorus in the growth medium of poinsettia. When the averages of the two years of study and both varieties of poinsettia were examined and the highest amount of phosphorus was obtained from BIV+CF application with 12.32 ppm, and the lowest amount of phosphorus with 9.44 ppm was obtained from the control application (Table 4). The soil samples of all PGPR combined fertilizer applications in the study done by Das and Singh [67] were contained higher N, P and K than the fertilizer applications without PGPR. In addition, Singh and Subba Rao [68], Prasad and Chandra [69] and Gunasekaran et al. [70] found that *Bacillus megaterium* increases in the available P content of soil. As a result of the study conducted by Arab et al. [71], depending on the activity of *Pseudomonas* bacteria, accessing to the nitrogen and phosphorus elements in the soil was increased. The fact that PGPRs can quickly dissolve the phosphates used by the plants promotes in plant growth [72].

Table 4. *Effect of the treatments on total number of bacteria in the growth medium, pH, organic matter, total N, P, K, Ca and Mg at different treatment growth medium of Euphorbia pulcherrima Willd.ex Klotzsch.*

Total number of bacteria in the growth medium (x10^6 cfu ml^{-1})										
	Control	**CF**	**BI**	**BI+CF**	**BII**	**BII+CF**	**BIII**	**BIII+CF**	**BIV**	**BIV+CF**
CvE	5.62 g***	9.18 ef	9.15 ef	10.07 de	7.93 f	11.07 d	8.05 f	13.10 c	15.75 b	17.37 a
CvF	4.20 g***	11.78 c	9.85 d	14.92 b	7.87 e	14.45 b	6.37 f	6.95 ef	12.18 c	18.68 a
Mean	4.91 G***	10.48 D	9.50 E	12.49 C	7.90 F	12.76 C	7.21 F	10.03 DE	13.97 B	18.03 A
pH										
CvE	7.60 a***	7.31 e	7.27 f	7.53 b	7.44 d	7.47 cd	7.50 bc	7.47 cd	7.21 g	7.29 ef
CvF	7.47 a***	7.44 a	7.39 b	7.46 a	7.33 c	7.38 b	7.29 d	7.33 c	7.39 b	7.45 a
Mean	7.53 A***	7.37 EF	7.33 G	7.49 B	7.38 DEF	7.42 C	7.39 DE	7.40 D	7.30 H	7.37 F
Organic Mater (%)										
CvE	1.82 e***	1.78 de	1.90 bcd	1.98 ab	1.89 cd	1.96 abc	1.95 abc	1.83 de	2.01 a	1.85 de
CvF	1.60 c***	1.56 c	1.70 b	1.70 b	1.66 b	1.66 b	1.69 b	1.68 b	1.82 a	1.72 b
Mean	1.71 EF***	1.67 F	1.80 BCD	1.84 B	1.77 CD	1.81 BC	1.82 BC	1.76 DE	1.91 A	1.78 CD
Total Nitrogen (%)										
CvE	0.0938 f***	0.0913 ef	0.0978 bcde	0.1020 ab	0.0968 cde	0.1010 abc	0.1003 abcd	0.0942 ef	0.1033 a	0.0953 def
CvF	0.0850 c***	0.0850 c	0.0900 bc	0.0900 bc	0.0883 bc	0.0883 bc	0.0917 b	0.0917 b	0.0983 a	0.0933 b
Mean	0.0894 CD***	0.0882 D	0.0939 B	0.0960 B	0.0926 BC	0.0947 B	0.0960 B	0.0929 B	0.1008 A	0.0943 B
P (ppm)										
CvE	9.54 d***	10.05 d	11.35 c	10.07 d	11.06 c	10.27 d	12.17 ab	11.53 bc	11.17 c	12.62 a
CvF	9.04 e***	9.20 e	10.15 d	9.88 d	10.75 bc	9.95 d	11.16 b	11.04 b	10.43 cd	11.92 a
Mean	9.29 G***	9.63 FG	10.75 D	9.98 EF	10.90 CD	10.11 E	11.67 B	11.29 BC	10.80 D	12.27 A
K (cmol kg^{-1})										
CvE	2.07 cd***	1.93 e	2.08 cd	2.15 abc	2.14 bcd	2.07 cd	2.22 ab	2.26 a	2.01 de	2.08 cd
CvF	1.71 cd***	1.61 e	1.74 bc	1.79 ab	1.78 ab	1.67 d	1.82 a	1.76 bc	1.79 ab	1.82 a
Mean	1.89 DE***	1.77 F	1.91 CDE	1.97 ABC	1.96 ABCD	1.87 E	2.02 A	2.01 AB	1.90 CDE	1.95 BCD
Ca (cmol kg^{-1})										
CvE	12.67 c***	13.71 b	14.76 a	13.32 bc	14.01 b	13.27 bc	15.32 a	13.94 b	14.97 a	13.97 b
CvF	14.48 cd***	14.04 d	15.23 b	14.45 cd	15.08 b	14.63 c	16.11 a	14.32 cd	15.14 b	14.02 d
Mean	13.58 E***	13.88 DE	14.99 B	13.89 DE	14.54 C	13.95 DE	15.71 A	14.13 DE	15.05 B	14.00 D
Mg (cmol kg^{-1})										
CvE	2.25 c***	2.24 c	2.36 b	2.22 c	2.43 b	2.36 b	2.39 b	2.35 b	2.53 a	2.23 c
CvF	2.07 f***	2.05 f	2.28 cd	2.18 e	2.38 b	2.23 d	2.50 a	2.38 b	2.34 bc	2.15 e
Mean	2.16 E***	2.14 E	2.32 CD	2.20 E	2.41 AB	2.30 D	2.45 A	2.36 BC	2.43 A	2.19 E

Available potassium (cmol kg^{-1}): The data indicated significant (p<0.001) influence of applications and varieties

on available potassium in the growth medium of poinsettia. The highest amount of available *potassium* was obtained from BIII application with 2.02 cmol kg^{-1} and the lowest amount of available potassium with 1.77 cmol kg^{-1} was obtained from the CF application. The highest potassium was obtained from the BIII+CF application in the CvE cultivar while it was determined in BIII and BIV+CF applications in the CvF cultivar. The lowest potassium was obtained from the CF for every two varieties (Table 4). In the present study, the amount of available potassium was increased in the growth medium by using PGPR applications. This increase may be due to the production of carboxylic acids such as citric, tartaric and oxalic acid [73-75].

Available calcium (*cmol kg^{-1}*): The data indicated significant (p<0.001) influence of applications and varieties on available calcium in the growth medium of poinsettia. It was reported that poinsettia requires high levels of nitrogen and potassium [76], and also a high percentage of calcium, magnesium and molybdenum are needed [77]. In the present study, the highest amount of available calcium was obtained from BIII application, according to the general average of the applications. The lowest amount of available calcium was obtained from the control and BI applications. The highest calcium was obtained from the BI, BIII and BIV applications in the CvE cultivar while it was determined in BIII application in the CvF cultivar (Table 4). According to McAvoy and Bible [78], the nutrition of calcium (Ca), molybdenum (Mo) and boron (B) is critical to produce quality poinsettia. During this study, no signs of deficiency were observed in the poinsettia plants. As can be seen from the results, the calcium nutrition needed by the plant was sufficient in the present study.

Available magnesium (*cmol kg^{-1}*): The results showed significant (p<0.001) influence of applications and varieties on available magnesium in the growth medium of poinsettia. When the averages of the two years of study were examined; the highest amount of available magnesium was obtained from BIII and BIV applications. The highest magnesium was obtained from the BIV application in the CvE cultivar and it was determined in BIII application in the CvF cultivar (Table 4). In the research, the bacterial formulations were increased in the amount of available magnesium. Reason of this increase may be due to the role of PGPRs in the synthesis of growth hormones such as auxin and other mechanism properties [60-62, 58].

Available iron (mg kg^{-1}): Overall, there were significant differences among (p<0.001) applications and varieties factors on available Fe in the growth medium of poinsettia. According to the averages of the two years of study, the highest amount of available iron was obtained from BIV applications as 3.22 mg kg^{-1}. The highest iron was obtained from the BIV application in the CvE cultivar while it was determined in BIII application in the CvF cultivar according to control application (Table 5). Rhizosphere bacteria, by releasing siderophore compounds, increase in their competitive potential by preventing the growth of other microorganisms, pathogens, and limiting existing iron. They improve the nutrition of the plant directly and indirectly [79-80].

Available manganese (mg kg^{-1}): The data indicated significant (p<0.001) influence of applications and varieties on available Mn in the growth medium of poinsettia. In the present study, the highest amount of available manganese was obtained from BIV application as 5.06 mg kg^{-1} according to the general average of the applications (Table 5). Manganese is taken up by plants from the soil very quickly. It does not bind to insoluble organic ligands or root tissues. In addition, the toxicity of Mn varies according to the plant factors [81]. In this study, no deficiencies or toxic effects related to Mn were observed in poinsettia varieties.

Table 5. *Effect of the treatments on Fe, Mn, Zn and B at different treatment growth medium of Euphorbia pulcherrima Willd.ex Klotzsch.*

	Fe (mg kg^{-1})									
	Control	**CF**	**BI**	**BI+CF**	**BII**	**BII+CF**	**BIII**	**BIII+CF**	**BIV**	**BIV+CF**
CvE	2.76 e***	3.23 d	3.65 ab	3.38 cd	3.58 abc	3.30 d	3.60 abc	3.22 d	3.77 a	3.44 bcd
CvF	1.91 g***	2.26 f	2.53 cd	2.45 de	2.63 bc	2.42 e	2.80 a	2.41 e	2.67 b	2.45 de
Mean	2.33 F***	2.74 E	3.09 B	2.92 CD	3.10 AB	2.86 CDE	3.20 AB	2.81 DE	3.22 A	2.94 C
Mn(mg kg^{-1})										
CvE	4.17 e***	5.32 ab	5.68 a	4.93 bc	4.98 bc	4.93 bc	4.66 cd	4.29 de	5.68 a	5.14 b
CvF	3.64 de***	3.98 bcd	4.08 abc	3.91 bcde	4.13 ab	3.92 bcde	4.21 ab	3.58 e	4.44 a	3.73 cde
Mean	3.90 D***	4.65 BC	4.88 AB	4.42 C	4.55 C	4.43 C	4.43 C	3.93 D	5.06 A	4.43 C
Zn (mg kg^{-1})										
CvE	1.79 g***	2.57 de	3.20 b	2.49 de	2.62 de	2.26 f	2.66 d	2.42 ef	3.46 a	2.98 c
CvF	1.25 d***	1.36 cd	1.68 ab	1.53 bcd	1.49 bcd	1.45 bc	1.88 a	1.56 bcd	1.87 a	1.52 bcd
Mean	1.52 F***	1.97 DE	2.44 B	2.01 DE	2.05 D	1.86 E	2.27 C	1.99 DE	2.66 A	2.25 C
B (mg kg^{-1})										
CvE	0.47 d***	0.46 d	0.53 d	0.50 d	0.61 c	0.64 c	0.72 b	0.86 a	0.73 b	0.71 b
CvF	0.35 d***	0.39 d	0.48 bc	0.45 c	0.50 bc	0.49 bc	0.52 b	0.52 b	0.62 a	0.61 a
Mean	0.41 E***	0.43 E	0.51 D	0.47 D	0.55 C	0.57 C	0.62 B	0.69 A	0.67 A	0.66 AB

Available zinc (mg kg^{-1}): The differences among applications and cultivars were statistically significant (p<0.001) in terms of available zinc in the growth medium of poinsettia. When the averages of the two years of study were examined; the amount of available zinc ranged from 1.52 mg kg^{-1} to 2.66 mg kg^{-1}. While the highest amount of available zinc was obtained from BIV application, the lowest amount of available zinc was found in control application. The highest zinc was obtained from the BIV application in the CvE cultivar and it was determined in BIII and BIV

applications in the CvF cultivar according to control application (Table 5). In the present study, the amount of chemical fertilizer applied for intensive leaf colour production can be reduced by the use of BIV bacterial formulation and the highest amount of zinc was determined in the same application. The results of this study were consistent with the results of the study by Balashouri [82].

Available boron (mg kg^{-1}): The results indicated significant (p<0.001) influence of applications and varieties factors on available B in the growth medium of poinsettia. While the highest amount of available boron was obtained from BIII+CF (0.69 mg kg^{-1}) and BIV (0.67 mg kg^{-1}) applications, the lowest amount of available zinc was found in control (0.41 mg kg^{-1}) and CF (0.43 mg kg^{-1}) applications (Table 5). Micro elements such as Ca, Mo and B play an important role in the growth of poinsettia [7]. In this study, no deficiencies or toxic effects related to B were observed in poinsettia varieties.

4. Conclusions

BI and BIII bacterial formulation applications as well as the recommended amount of chemical fertilizer application (100% CF) in poinsettia cultivation were found positive effects on shortening the time until flowering and early flowering. The poinsettia plants grew shortest time marketable commercial size when supplied with BIII+CF application. The poinsettia plants were absorbed sufficient nutrients from the growth medium in CF, BI, BI+CF, BII, BII+CF, BIII, BIII+CF applications and increased in their plant growth and biomass. In contrast, maximum plant nutrients in the growth medium samples have especially determined in the BIV application. The present study showed that BI, BI+CF, BII, BII+CF, BIII, BIII+CF applications as an alternative to CF application can be considered for a next level of evaluation as a promising application due to its good performance in terms of plant growth promotion in poinsettia cultivation. The bacterial formulations may be used as efficient PGPR for poinsettia production in farmer's greenhouse to reduce the need for chemical fertilizer and improve plant growth.

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