

## Effects of PGPR Formulations, Chemical Fertilizers, and Their Combinations on Physiological Traits and Quality of Bracts of Poinsettia

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### ABSTRACT

This study was carried out to determine the effects of different PGPR formulations, chemical fertilizers, and their combinations on some color characteristics and nutrient content of bract leaf of two cultivars of *Euphorbia pulcherrima* Willd. ex Klotzsch in a research greenhouse between July 2015 and July 2017. The treatments included bacterial formulations: (1) BI: *Paenibacillus polymyxa* TV-12E+*Pseudomonas putida* TV-42A+*Pantoea agglomerans* RK-79, (2) BII: *Bacillus megaterium* TV-91C+*Pantoea agglomerans* RK-92+*Bacillus subtilis* TV-17C, (3) BIII: *Bacillus megaterium* TV-91C+*Pantoea agglomerans* RK-92+*Kluyvera cryocrescens* TV-113C, and (4) BIV: *Bacillus megaterium* TV-91C+*Pantoea agglomerans* RK-79+*Bacillus megaterium* TV-6D). Also, fertilizer treatments included the full amount of commonly used Chemical Fertilizer (CF= 150 g 100 L<sup>-1</sup>) and combination of the reduced amount of chemical fertilizer by 50% with each bacterial formulation, and control. The first red leaves, life of bracts, color properties (L, a\* and b\*), content of anthocyanin, chlorophyll content in green leaf, macro and micronutrient contents of bracts were evaluated in the experiment. CF and BII+50%CF applications encouraged the coloring of bract leaves early (4.01%). It was determined that CF (7.76%), BI+50%CF (6.03%) and BII+50%CF (5.27%) applications significantly increased chlorophyll content of poinsettia bract when compared to the control. Darker colored bracts were obtained from BI and BIV applications compared to the control. The highest total nitrogen amount (3.69%), soluble phosphorus (4,285.33 mg kg<sup>-1</sup>), potassium (28,132.45 mg kg<sup>-1</sup>) and calcium (8,299.03 mg kg<sup>-1</sup>) amount were found in the BII+50%CF application. It was determined that bacterial formulations BI, BIV, BIV+50%CF and BII+50%CF had positive effects on some plant aesthetic, quality characteristics, and nutrient content of bract of poinsettia and can be used in poinsettia production stage as one of the biological products. Thus, bacterial formulations may replace or reduce use of chemical fertilizers in poinsettia production.

**Keywords:** Bract colored, Pot ornamental plant, Rhizobacteria formulation.

### INTRODUCTION

The sector of ornamental plants, which is a branch of agriculture, has turned into an industry all over the world due to an increase in commercial demand (Chavada *et al.*, 2017). Potted plants have an important place in the classification of ornamental plants and are the group of plants that decrease air pollution in indoor, and can preserve natural leaf and flower aesthetic

properties throughout the life (Jones, 1999; Lamont and Elliott, 2016). Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) or Atatürk Çiçeği (Turkish name) is one of the most important potted plants grown for its fleshy bracts and has been used mainly as a traditional Christmas decoration from the 17<sup>th</sup> century (Karunananda and Peiris, 2011). For Christmas, the main flowers are poinsettias, sold especially in the traditional

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red color version (Junqueira and Peetz, 2017).

It is extremely important to produce plants with the intensive bract leaf color for consumer demand in poinsettia cultivation. Control of plant nutrition and photoperiod are the most important factors in order to obtain intense bract leaf color. Poinsettia needs to be cultivated with high nutrients to produce a quality flower. For this reason, adjustments including weekly fertilization and/or other chemical applications are required in the cultivation of poinsettia (Lineberger, 2018). Kofranek *et al.* (1963) and Khandan-Mirkohi *et al.* (2015) also stated that fertilization was made with every irrigation to the plants in cultivation. The superior performance varieties, or inputs that can reduce fertilizer requirements, costs, chemical amount, and make the growth process even easier attract the great interest of the producers of the poinsettia plant. There is a growing interest in the ideas of reducing the use of chemicals to protect plant health and reduce production costs. The use of bacteria in the rhizosphere of plants is one of the important alternatives and its use in agriculture is increasing day-by-day in ornamental plants (Zulueta-Rodriguez *et al.*, 2014; Arab *et al.*, 2015; Parlakova Karagöz *et al.*, 2016), vegetables (Bahadır *et al.*, 2018), and fruits (Seema *et al.*, 2018). This study was undertaken to determine the effects of different PGPR formulations, chemical fertilizers, and their combinations on some color characteristics and nutrient content of bract leaf in two different cultivars of *Euphorbia pulcherrima* Willd. ex Klotzsch (Christmas Feelings and Christmas Eve). The aim was to benefit from these results in the cultivation of poinsettia.

## MATERIALS AND METHODS

### Experimental Materials and Set-Up

This study was conducted under climate-controlled research greenhouse from July

2015 to July 2017 in Erzurum (Turkey) with an elevation of about 2,000 m above sea level. 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 and pumice (diameter: 10-30 mm) in the ratio of 2:1 by volume (Lineberger, 2018). Plants were planted in 3.5-liter plastic pots.

The treatments included bacterial formulations: (1) *Paenibacillus polymyxa* TV-12E+*Pseudomonas putida* TV-42A+*Pantoea agglomerans* RK-79, (2) *Bacillus megaterium* TV-91C+*Pantoea agglomerans* RK-92+*Bacillus subtilis* TV-17C, (3) *Bacillus megaterium* TV-91C+*Pantoea agglomerans* RK-92+*Kluyvera cryocrescens* TV-113C, and (4) *Bacillus megaterium* TV-91C+*Pantoea agglomerans* RK-79+*Bacillus megaterium* TV-6D (Table 1). Also, fertilizer treatments included Chemical Fertilizer (100% CF) and combination of the reduced amount of chemical fertilizer by 50% with each bacterial formulation (Table 2). Absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm. The bacterial suspensions were properly diluted to  $1 \times 10^8$  CFU mL<sup>-1</sup> in sdH<sub>2</sub>O. Approximately, 0.2 g of sucrose (10 mg mL<sup>-1</sup>) was put in each Erlenmeyer flasks. Bacterial formulations were inoculated in the rooted cuttings of the poinsettia by a dipping method (Karthikeyan *et al.* 2010; Ipek *et al.*, 2014) and they were planted in pots filled with the appropriate growing medium (by mixing peat and pumice in ratio of 2:1 by volume). The study was designed as 3 replicates in randomized design.

After planting the rooted cuttings in pots, two different types of completely soluble fertilizers were applied to the pot groups to supply chemical fertilizers at the determined different doses. These comprised of "White 15-0-19+9 CaO+2 MgO+TE, NPK ratio 4:0:5" (white composite fertilizer, granule,

**Table 1.** Bacterial isolates used in the study and some biochemical properties (Kotan *et al.*, 2014).<sup>a</sup>

Isolate No	MIS Result	Diagnosis	SIM	Location (in Turkey)	Host	Nitrogen	Phosphate	Siderophore
RK-79	<i>Pantoea agglomerans</i>		0.762	Erzurum	Apple	+	+	-
TV-12E	<i>Paenibacillus polymyxa</i>		0.551	Van	Poaceae	S+	+	-
TV-17C	<i>Bacillus subtilis</i>		0.677	Van	Raspberr y	S	W+	-
TV-6D	<i>Bacillus megaterium</i>		0.750	Van	Poaceae	+	+	-
TV-42A	<i>Pseudomonas putida</i>		0.113	Van	Poaceae	W+	W+	+
TV-91C	<i>Bacillus megaterium</i>		0.474	Van	Poaceae	+	W+	-
TV-113C	<i>Kluyvera cryocrescens</i>		0.688	Van	Garlic	+	+	-
RK-92	<i>Pantoea agglomerans</i>		0.889	Erzurum	Pear	+	S	-

<sup>a</sup> SIM: Similarity Index, S: Strong +, W: Weak +; +: Positive, -: Negative.

**Table 2.** Treatments used in the study.

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

containing N, K, Ca, Mg, B, Zn, Fe, Cu, Mo and Mn) and "Blue 18-11-18+2.5 MgO, NPK ratio 3:2:3" (blue composite fertilizer, granule, containing N, P, K, S, Mg, B, Zn, Fe, Cu, Mo and Mn). These two different chemical fertilizers were given in specified amounts with the irrigation water consecutively (Kofranek *et al.*, 1963; Khandan-Mirkohi *et al.*, 2015). The recommended dose (150 g 100 L<sup>-1</sup>) of these

fertilizers for pots, flower beds, and all covered seedlings were used in this study.

#### Determinations of Some Plant Parameters

After 110-120 days from bacterial inoculation, some plant growth parameters were measured on 10 samples from each application. These parameters included duration to first bract appearance (day), life of bracts (day) (Serek and Reid, 2000), color properties of bracts (L\*, a\* and b\*) (Minolta



CR-400 Colorimeter (Minolta Camera Co., Ltd., Ramsey, NJ)), content of anthocyanin in bracts (Slatnar *et al.*, 2013), chlorophyll content in green leaf (chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan)). The color of each bract was recorded using the CIE (Commission Internationale d'Eclairage) L\* a\* b\* uniform color space [by using Madeira *et al.* (2003) method].

### Determinations of Macro- and Micro Nutrients in Bracts

Macronutrients (N, P, K, Ca and Mg) and micro nutrient contents of bracts (Fe, Mn, Zn and B) were also determined according to Parlakova Karagöz *et al.* (2016) and by using the Mertens (2005) method.

### 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.

## RESULTS AND DISCUSSION

### Plant Parameters

The data indicated significant ( $P \leq 0.001$ ) effect of bacterial applications  $\times$  varieties interaction on the duration to first bract appearance of poinsettia. The shortest duration to the first bract appearance (day) was in the variety Christmas Eve and CF (63.57 days) followed by BII+CF (66.30 days). The durations to the first bract appearance (day) in Christmas Feelings variety were ranked as CF < BI+CF < BII+50%CF < BIV+50%CF < BIII+50%CF < BII < BI, in the increasing order (Table 3). While the color of the bract leaves is an important aesthetic feature for consumers,

the colorful residence time and period of coloring of the bract leaves is important for growers (Medina-Ortega, 2011). The results of our study encouraged the coloring of bract leaves early. Factors such as genetic makeup, environmental conditions, and nutrient play a role in determining the degree of effectiveness of the pigments in plants (Jaakola, 2003). Larson *et al.* (1978) stated that poinsettia is very sensitive to photoperiod and temperature. In short-day conditions, the upper leaves (bracts) of poinsettia change color due to the accumulation of anthocyanin pigment and chlorophyll retention (Kannangara and Hansson, 1998). According to the results of the study, it is thought that nutrition, especially in CF, BI+50%CF and BII+50%CF treatments, is the cause of early coloring of bract leaves due to increase in nitrogen and phosphorus contents in the growing medium.

The study found that bacterial and fertilizer applications  $\times$  varieties interaction ( $P \leq 0.05$ ) were statistically significant in terms of life of bracts (day). In the present study, it was determined that the applications were effective in maintaining a longer period of colors of red bract leaf. The longest life of bracts was obtained in CF treatment (Table 3). The life of bracts was determined as 130-141 days in our study. The reason for this can be explained by nutrition and the climate data of the study region.

It was observed that bacterial and fertilizer applications  $\times$  variety interaction ( $P \geq 0.05$ ) was not statistically significant in terms of chlorophyll content in the green leaves. The highest average value of chlorophyll content (SPAD value) was in CF, BI+50%CF and BII+50%CF treatments. The lowest chlorophyll content was obtained from the control. Christmas Eve variety had higher chlorophyll content than the Christmas Feelings variety (Table 3). Qasim *et al.* (2014) stated that PGPR increased in chlorophyll contents of *Gladiolus grandifloras* L. leaves. It is known that there is an increase in germination of seedlings,

**Table 3.** The effects of treatments on some phenological and plant growth characteristics of poinsettia. <sup>a</sup>

Treatments	Days to first bract appearance			Life of bracts (Day)		
	CvE	CvF	Overall mean	CvE	CvF	Overall mean
Control	69.07 d***	75.00 a***	72.03 B***	134.09 b**	133.14 cde***	133.61 BCD***
CF	63.57 f	70.30 b	66.93 E	144.75 a	138.17 a	141.46 A
BI	72.34 bc	71.81 b	72.07 B	132.67 b	137.42 ab	135.04 BC
BI+ 50%CF	70.15 cd	70.60 b	70.38 C	136.53 b	134.58 cd	135.56 BC
BII	73.81 ab	71.42 b	72.61 B	131.83 b	132.92 de	132.38 CD
BII+ 50%CF	66.30 e	70.83 b	68.57 D	135.22 b	135.31 bc	135.26 BC
BIII	75.44 a	73.90 a	74.67 A	132.84 b	128.11 f	130.47 D
BIII+ 50%CF	68.78 d	71.32 b	70.05 C	132.67 b	134.14 cd	133.40 BCD
BIV	72.22 bc	73.62 a	72.92 B	138.20 ab	131.00 e	134.60 BC
BIV+ 50%CF	68.17 de	71.08 b	69.63 CD	137.06 b	136.92 ab	136.99 B
Mean	69.98 ***	71.99		135.58 <sup>NS</sup>	134.17	
Treatments	Anthocyanin content (mg 100 g <sup>-1</sup> )			Chlorophyll content (SPAD value)		
	CvE	CvF	Overall mean	CvE	CvF	Overall mean
Control	65.93 d***	68.75 f***	67.34 E***	49.03 bc***	46.97 d**	48.00 D***
CF	72.13 c	74.18 e	73.16 D	54.14 a	49.94 abc	52.04 A
BI	79.08 b	84.32 cd	81.70 C	48.73 bc	47.34 cd	48.03 D
BI+50% CF	78.75 b	85.95 c	82.35 C	50.97 b	51.20 a	51.08 AB
BII	77.98 b	83.89 cd	80.94 C	48.82 bc	48.62 abcd	48.72 D
BII+50% CF	87.08 a	93.17 a	90.13 A	51.06 b	50.27 ab	50.67 ABC
BIII	77.98 b	83.25 cd	80.62 C	49.44 bc	48.35 bcd	48.90 CD
BIII+50% CF	79.22 b	81.92 d	80.57 C	49.77 bc	48.00 bcd	48.88 CD
BIV	83.97 a	89.35 b	86.66 B	47.92 c	48.78 abcd	48.35 D
BIV+50% CF	86.78 a	94.48 a	90.63 A	48.89 bc	50.32 ab	49.60 BCD
Mean	78.89 ***	83.93		49.88 *	48.98	

<sup>a</sup> a-e and A-E: In each column, there is no difference between the means shown with the same letter at  $P \leq 0.05$  significance level. \* Significant at  $P \leq 0.05$ ; \*\* Significant at  $P \leq 0.01$ ; \*\*\* Significant at  $P \leq 0.001$ , ns: Non-significant at  $P \geq 0.05$ . CvE: Christmas Eve, CvF: Christmas Feelings.

plant height, shoot weight, nutrients and chlorophyll content when ornamental plants (Zulueta-Rodriguez *et al.*, 2014; Arab *et al.*, 2015), forest trees, vegetables (Bahadır *et al.*, 2018) and agricultural products (Seema *et al.*, 2018) are inoculated with PGPR (Saharan and Nehra, 2011).

The red pigment on the bracts of poinsettia is identified as anthocyanin (Lawrence *et al.*, 1939; Bennett *et al.*, 2008; Tanaka *et al.*, 2008). The content of anthocyanin in poinsettia is determined by the lack of intensity of red color in bracts (Bennett *et al.*, 2008). We found significant ( $P \leq 0.001$ ) influence of bacterial and fertilizer applications×varieties interaction on the anthocyanin content. The highest anthocyanin content was obtained from

BIV+50%CF application compared to the control application. According to the overall mean data, it was determined that the content of the anthocyanin in the treatments ranged from 67.34 to 90.63 mg 100 g<sup>-1</sup> (Table 3). *Pseudomonas putida* rhizobacteria has been effective in increasing pigmentation of anthocyanins and the coloring of the bract leaves of poinsettia variety (Zulueta-Rodriguez *et al.*, 2014). The finding that the amount of chemical fertilizer applied for dense leaf color can be reduced by the use of BIV bacterial formulation is an important result of our study.

Assorted colors and color tones of bract leaf colors in poinsettia directly affected the desired properties in the market of poinsettia.



In general, studies of variety development are directed towards this. In the present study, bacterial and fertilizer applications×variety interaction ( $P \geq 0.05$ ) was not statistically significant in terms of  $L^*$  value. The darkest red bract leaf color was in BI and BIV applications where the lowest  $L^*$  value (Medina-Ortega, 2011) was obtained. The highest mean value for the bract leaf  $L^*$  value was in the control and BII+50%CF applications (Table 4). Zulueta-Rodriguez *et al.* (2014) stated that *P. putida* was effective in increasing the pigmentation of anthocyanins and coloring of the bract leaves of poinsettia plants. Obtaining darker colored bracts in BI and BIV applications according to control. PGPRs may be one of the biological products that can be used in the production of this kind of plant.

All  $a^*$  values taken from the bracts are positive (+). The applications×variety interaction ( $P \leq 0.001$ ) was statistically significant in terms of  $a^*$  value. The darkest red bract leaf color was recorded in CF application where the highest  $a^*$  value (Medina-Ortega, 2011) was obtained (Table 4). CF and BII+50%CF applications were included in the same statistical group. According to this result, it is concluded that BII+50%CF application can be used instead of CF application.

All  $b^*$  values obtained in this study were positive numbers. The values of the  $b^*$  parameter represent yellow color (Medina-Ortega, 2011). The applications×variety interaction ( $P \leq 0.01$ ) was statistically significant in terms of  $b^*$  value. Accordingly, the lowest  $b^*$  value was in BIV application. This is also the application with the least amount of yellowness (Table 4). In addition, the value in this and other applications were not found to be high enough to increase the yellowness level.

### Macro and Micro Nutrients in Bract Leaf

The data indicated significant ( $P \leq 0.001$ ) effect of applications×varieties interaction on N, P, Ca, Mg, Zn, Fe and B in bracts. While the applications×variety interaction was statistically significant at  $P \leq 0.01$  level for Mn, it was statistically significant at  $P \leq 0.05$  level for K. It was determined that the highest total N amount, soluble P, K and Ca amount determined in the bract leaf samples were in the BII+50%CF application. It was concluded that the total amount of nitrogen in the bract leaf samples varied between 3.11 and 3.90% depending on the applications. The amount of soluble P, K and Ca varied between 3,592.10 and 4,425.64 mg kg<sup>-1</sup>; 2,3305.12 and

**Table 4.** The effects of treatments on the color properties of bracts of poinsettia.

Treatments	$L^*$			$a^*$			$b^*$		
	CvE	CvF	Overall mean	CvE	CvF	Overall mean	CvE	CvF	Overall mean
Control	29.66 <sup>ns</sup>	30.58 <sup>a***</sup>	30.12 A*	51.69 <sup>bc***</sup>	48.21 <sup>ns</sup>	49.95 <sup>CDE***</sup>	15.28 <sup>abc***</sup>	13.97 <sup>ns</sup>	14.62 CD**
CF	29.38	29.95 ab	29.66 AB	53.98 a	50.03	52.01 A	15.85 a	14.73	15.29 A
BI	28.43	28.96 bc	28.69 B	51.22 c	49.8	50.51 BCD	14.97 c	14.53	14.75 ABCD
BI+50% CF	29.83	29.00 bc	29.41 AB	51.33 c	48.89	50.11 BCD	15.02 bc	14.26	14.64 BCD
BII	29.12	29.42 b	29.27 AB	49.11 d	49.67	49.39 CDE	14.90 c	14.21	14.55 CD
BII+50%CF	30.29	30.02 ab	30.15 A	53.13 abc	49.73	51.43 AB	15.74 ab	14.62	15.18 AB
BIII	29.5	29.52 ab	29.51 AB	48.60 d	49.83	49.22 DE	15.06 bc	14.53	14.79 ABCD
BIII+50%CF	30.22	28.13 c	29.17 AB	53.54 ab	47.83	50.69 ABC	15.90 a	14.01	14.95 ABC
BIV	27.88	29.18 bc	28.53 B	49.24 d	48.04	48.64 E	14.19 d	14.43	14.31 D
BIV+50%CF	29.22	30.03 ab	29.62 AB	51.78 bc	49.64	50.71 ABC	15.23 abc	14.51	14.87 ABC
Mean	29.35 <sup>ns</sup>	29.48		51.36 ***	49.17		15.22 ***	14.38	

\* Significant at  $P \leq 0.05$ ; \*\* Significant at  $P \leq 0.01$ ; \*\*\* Significant at  $P \leq 0.001$ , ns: Non-significant at  $P \geq 0.05$ .

**Table 5.** Effects of the treatments on soluble N, P, K Ca, Mg, Zn, Fe, Mn and B contents of bract leaf of poinsettia.

Treatments	N (%)		P (mg kg <sup>-1</sup> )		K (mg kg <sup>-1</sup> )	
	CvE	CvF	Overall mean	CvE	CvF	Overall mean
Control	2.94 b***	3.28 d***	3.11 D***	3212.76 e***	3669.83 d***	3441.30 E***
CF	2.93 b	3.65 bc	3.29 C	3867.10 b	4262.85 bc	4064.97 BCD
BI	3.04 b	3.68 bc	3.36 C	3599.75 cd	4163.71 bc	3881.73 D
BI+50% CF	3.04 b	4.09 a	3.56 B	3495.13 cd	4432.36 ab	3963.75 CD
BII	3.01 b	4.13 a	3.57 B	3666.18 bc	4583.13 a	4124.65 AB
BII+50% CF	3.69 a	4.11 a	3.90 A	4285.32 a	4565.95 a	4425.64 A
BIII	3.61 a	3.81 b	3.71 B	4225.72 a	4114.71 c	4170.21 B
BIII+50% CF	3.64 a	3.78 b	3.71 B	4132.71 a	4132.20 c	4132.46 AB
BIV	2.99 b	3.69 bc	3.34 C	3632.51 bc	4121.52 c	3877.01 D
BIV+50% CF	2.98 b	3.45 cd	3.21 CD	3378.71 de	3805.50 d	3592.10 E
Mean	3.19 ***	3.77		3749.59 ***	4183.17	
Treatments	Ca (mg kg <sup>-1</sup> )		Mg (mg kg <sup>-1</sup> )		Zn (mg kg <sup>-1</sup> )	
	CvE	CvF	Overall mean	CvE	CvF	Overall mean
Control	7815.38 b***	7315.16 d***	7565.27 D***	3669.78 bc*	4241.34 ab***	3955.56 A***
CF	7165.18 c	8933.01 a	8049.10 BC	3579.65 bc	3649.60 de	3614.62 B
BI	7544.51 bc	8763.64 ab	8154.07 BC	3852.31 ab	3965.96 bc	3909.13 A
BI+50% CF	7696.70 b	8963.17 a	8329.93 AB	3641.70 bc	3545.60 de	3593.65 B
BII	7715.83 b	8897.14 a	8306.48 AB	3566.48 c	3642.72 de	3604.60 B
BII+50% CF	8299.03 a	8848.50 a	8573.76 A	3683.63 bc	3486.65 e	3585.14 B
BIII	8305.86 a	8489.61 abc	8397.73 AB	3970.38 a	3992.91 bc	3981.64 A
BIII+50% CF	8284.64 a	8470.28 abc	8377.46 AB	3800.72 abc	3821.24 cd	3810.98 A
BIV	7840.37 b	8219.38 bc	8029.88 BC	3638.57 bc	4358.13 a	3998.35 A
BIV+50% CF	7759.39 b	7943.51 c	7851.45 CD	3813.28 abc	4062.11 bc	3937.70 A
Mean	7842.69 ***	8484.34		3721.65 ***	3876.63	
Treatments	Fe (mg kg <sup>-1</sup> )		Mn (mg kg <sup>-1</sup> )		B (mg kg <sup>-1</sup> )	
	CvE	CvF	Overall mean	CvE	CvF	Overall mean
Control	164.23 d***	263.21 b***	213.72DE***	31.76 c***	38.74 d***	35.25 E***
CF	169.19 d	218.17 c	193.68 F	34.31 c	40.62 cd	37.46 DE
BI	202.58 b	234.90 c	218.74 CD	39.67 b	41.25 bcd	40.46 BC
BI+50% CF	185.73 bcd	216.97 c	201.35 EF	37.76 b	46.76 a	42.26 AB
BII	186.35 bcd	221.72 c	204.03 DEF	38.21 b	45.29 ab	41.75 AB
BII+50% CF	242.27 a	218.16 c	230.22 BC	39.49 b	45.02 ab	42.25 AB
BIII	259.55 a	239.13 c	249.34 A	38.61 b	38.81 d	38.71 CD
BIII+50% CF	256.82 a	233.36 c	245.09 AB	43.83 a	43.09 abc	43.46 A
BIV	173.62 cd	292.47 a	233.04 BC	38.93 b	44.17 abc	41.55 AB
BIV+50% CF	195.77 bc	265.93 b	230.85 BC	40.50 b	46.41 a	43.46 A
Mean	203.61 ***	240.4		38.31 ***	43.02	

\* Significant at P ≤ 0.05; \*\* Significant at P ≤ 0.01; \*\*\* Significant at P ≤ 0.001, ns: Non-significant at P ≥ 0.05.



28,800.40 mg kg<sup>-1</sup>; and 7,565.27 and 8,573.76 mg kg<sup>-1</sup>, respectively, in the bract leaf samples (Table 5). İbrikçi *et al.* (1994) reported that the adequacy level of total nitrogen was 2.50-3.20% in the leaf. Pritts and Handley (1998) stated that total nitrogen was 2.0-2.8% for strawberry. In our findings, the total amount of nitrogen in the bract leaf samples was found to be higher. It was concluded that these values were sufficient for the development of the poinsettia. Thus, the amount of chemical fertilizer can be reduced by using BI, BII, and BII+50%CF applications in the cultivation of poinsettia. The result of this study is parallel with the finding that the amount of P absorption by the plant is dependent on the nitrogen content of the soil (Esquivel, 2013). It is estimated that the bacterial strains (*Bacillus megaterium*, *Pantoea agglomerans*, *Bacillus subtilis*) present in BII+CF application may have fixed more N, P and K in the growth medium due to the N<sub>2</sub> fixation and the ability to dissolve P (Arab *et al.*, 2015). In the present study, the amount of phosphorus increased in bacterial formulations applications where *Bacillus* were found. These bacteria increased in the initial substrate of reactions associated with the production of secondary metabolites. Thus, it is estimated that this increase is achieved by providing more nitrogen intake by the plant. Potassium acts as a growth regulator when the presence of nitrogen is high (Mikkelsen, 2005). Since the total nitrogen was the highest in BI, BII, and BII+CF applications, potassium may have acted as a plant growth regulator in these applications.

Similar findings have been reported in previous studies with some PGPR strains confirming our data in this study. PGPR applications stimulated the uptake of macro and micro elements (N, Ca, P, Fe, K, Mg, Mn, Cu and Zn) in raspberry (Orhan *et al.*, 2006), sweet cherries (Esitken *et al.*, 2006), lettuce (Lai *et al.*, 2008), and tomatoes (Adesemoye *et al.*, 2010). The findings of our study were parallel with all these findings (Sundra *et al.*, 2002; Shen *et al.*, 2004). The reason for these increases can

also be explained by the differences in the production of IAA and cytokines of PGPR strains and/or the differences in the Ca-bicarbonate dissolving capacity and/ or colonization capacity, the production of organic acids by plants and bacteria in the rhizosphere (Gupta *et al.*, 2015).

According to the general application averages, the highest mean values for soluble magnesium and zinc were in the BIV application (Table 5). It was reported that PGPR strains, namely, *Pseudomonas* and *Acinetobacter* increase uptake of Fe, Zn, Mg, Ca, K and P by plants (Esitken *et al.*, 2006). The amount of soluble zinc in the bract leaf varied between 44.09 and 60.85 mg kg<sup>-1</sup> in our study (Table 5). The Zn contents of the plants are normally between 5-100 mg kg<sup>-1</sup> and the toxicities usually begin after 400 mg kg<sup>-1</sup> (Marschner, 2002). According to these findings, the amount of zinc in the bract leaves obtained from this study was adequate or in appropriate range. The amount of soluble zinc in the bract leaf was statistically different according to the varieties ( $P \leq 0.001$ ). Zinc intake efficiency of plants can vary in a plant variety, different genotypes of the same variety (Karaman *et al.*, 2012). In general, increases in zinc determined in BIII and BIV bacterial formulation applications may have occurred by the effects of some organic compounds. Fasim *et al.* (2002) found that bacteria dissolved the insoluble Zn compounds. In this context, the results obtained in the present study can be explained with this finding.

According to the general application averages, the highest mean values for the amount of soluble iron was determined in the BIII application. The amount of soluble iron varied between 193.68 and 249.34 mg kg<sup>-1</sup> (Table 5). Pritts and Handley (1998) reported that the total amount of iron in the plant's leaf should be between 60-250 mg kg<sup>-1</sup>.

The highest mean values for soluble manganese was in the BIII+50%CF and BIV+50%CF applications in the bract leaf samples (Table 5). Karakurt and Aslantas



(2010) reported that PGPR applications increased Mn content in apple leaf. Orhan *et al.* (2006) reported that PGPR applications increased Mn content in raspberry leaves. The solubility of manganese microelement in the plant-growing medium varies according to the properties of soil water, microorganism content and activity, and soil reaction (Anaç and Esetlili, 2011). The reason for the increase in manganese content by combination applications of the bacterial formulation with 50% reduced chemical fertilizer in the present study may be explained by these expressions and the production of organic acids by plants and bacteria in the rhizosphere (Sharma *et al.*, 2013; Gupta *et al.*, 2015).

The highest amount of soluble boron was in the BIV+50% CF application in the bract leaf samples. The lowest amount of soluble boron was in the control and BI applications (Table 5). In general, the amount of boron nutrients required for the development of many plants is between 6 and 60 ppm (Epstein and Bloom, 2005). Microelements such as Ca, Mo and B play an important role in the bract pigmentation and growth of poinsettia (Ayala Arreola *et al.*, 2008). Accordingly, the highest anthocyanin content was in BIV+50% CF application in this study. In addition, BIV+50%CF application significantly increased in amount of boron in the bract leaf of the Christmas Feelings variety. In present results were same with Ayala Arreola *et al.* (2008) findings.

## CONCLUSIONS

According to the results, appearance of the first red leaves (bracts) of poinsettia started in the second week of October and completed in December under the natural light in Erzurum. BII+50% CF application was found to be effective in shortening the duration to the first bract appearance of poinsettia. It is also thought that BII+50%CF application affected on sale quality of poinsettia. In the Christmas Feelings variety,

BI and BIV bacterial formulations were found to positively affect the length of the life of bract leaves. In particular, it was concluded that the BI formulation could be used in the cultivation of this variety to reduce the chemical fertilizer input. It was determined that the CF, BI+50% CF and BII+50% CF applications had positive effects on the chlorophyll content of the poinsettia. It was concluded that the BI and BIV bacterial formulations were the most effective in obtaining darker colored bracts. Thus, it was concluded that the amount of chemical fertilizer required for dense leaf color can be reduced by the use of BIV bacterial formulation. In summary, it is concluded that the PGPRs could give a positive answer to the search for economically, socially, and ecologically acceptable fertilization alternatives in poinsettia cultivation. Thus, bacterial formulations may allow reducing the use of chemical fertilizer in poinsettia cultivation.

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## اثر فرمولاسیون PGPR، کود شیمیایی، و ترکیب آنها روی صفات فیزیولوژیکی و کیفیت برگواره های بنت قنسل (Poinsettia)

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### چکیده

هدف از اجرای این پژوهش تعیین اثرات فرمولاسیون PGPR، کود شیمیایی و ترکیب آن ها روی برخی ویژگیهای رنگ و محتوای عناصر غذایی برگواره های دو کوتیوار *Euphorbia pulcherrima* Willd. ex Klotzsch در یک گلخانه تحقیقاتی در طی دوره ژوئیه ۲۰۱۵ تا ژوئیه ۲۰۱۷ بود. تیمارهای فرمولاسیون های مختلف باکتریایی شامل موارد زیر بود:

(1) BI: *Paenibacillus polymyxa* TV-12E+*Pseudomonas putida* TV-42A+*Pantoea agglomerans* RK-79, (2) BII: *Bacillus megaterium* TV-91C+*Pantoea agglomerans* RK-92+*Bacillus subtilis* TV-17C, (3) BIII: *Bacillus megaterium* TV-91C+*Pantoea agglomerans* RK-92+*Kluyvera cryocrescens* TV-113C, and (4) BIV: *Bacillus megaterium* TV-91C+*Pantoea agglomerans* RK-79+*Bacillus megaterium* TV-6D).

همچنین، تیمارهای کود شیمیایی شامل بود بر مصرف مقدار کامل کود معمول (CF=۱۵۰ گرم در ۱۰۰ لیتر) و ترکیبی از مقدار کود در حد ۵۰٪ همراه با هریک از فرمولاسیون های کود باکتریایی) و تیمار شاهد. اندازه گیری های آزمایش عبارت بود از زمان اولین برگ قرمز رنگ، دوره زندگی، خواص رنگ ها (L، a\*، و b\*) محتوای آنتوسیانین، محتوای کلروفیل در برگ سبز، و عناصر غذایی ماکرو و میکرو در برگواره ها (bracts). تیمارهای CF و BII+CF موجب تسریع در افزایش رنگ برگواره ها (۴/۰۱٪) شد. مشخص شد که در مقایسه با تیمار شاهد، تیمار (۷/۷۶٪) CF+BI و (۶/۳۰٪) BII+CF، و (۵/۲۷٪) افزایش معنی دار روی مقدار کلروفیل در برگواره های بنت قنسل داشتند. تیمارهای BI و BIV در مقایسه با تیمار شاهد، برگواره های تیره رنگ تری داشتند. بیشترین مقدار نیتروژن کل (۳/۶۹٪) فسفر محلول (۴۲۸۵/۳۳ میلی گرم در کیلوگرم)، پتاسیم (۲۸۱۳۲/۴۵ میلی گرم در کیلوگرم) و کلسیم (۸۲۹۹/۰۳ میلی گرم در کیلوگرم) در تیمار BII+CF مشاهده شد. نیز، مشخص شد که فرمولاسیون های باکتریایی BI، BIV، CF+، و BII+CF اثر های مثبت روی برخی ویژگی های زیبایی و کیفی گیاه مزبور و عناصر غذایی در برگواره های بنت قنسل داشتند و می توان

در تولید این گیاه به عنوان محصولات زیستی از آنها استفاده کرد. از این قرار، در تولید بنت قنسول، فرمولاسیون های باکتریایی می توانند به جای کود شیمیایی یا برای کاهش مصرف آنها به کار روند.