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ARTICLE

Growth and production of poinsettia var. Prestige Red by inoculation of plant growth-promoting rhizobacteria and fertilization doses

Efeito da fertilização e inoculação com bactérias promotoras de crescimento no desenvolvimento da Poinsettia var. Prestige

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Abstract: *Euphorbia pulcherrima* is a plant with colorful bracts sold mainly at Christmas time, and is the most important ornamental container plant in the world. To produce healthy plants with high-quality standards, growers intensively apply pesticides and fertilizers, which increases production costs and promote environmental pollution. Plant growth-promoting rhizobacteria (PGPR) represent an environmentally friendly technological alternative for ornamental production. Therefore, this work evaluated the effect of three bacteria (*Pseudomonas* sp. CPO 2.78, *Enterobacter* sp. CPO 2.5, and *Bacillus megaterium* CPO 2.35) isolated from the rhizosphere of *E. cyathophora* on the vegetative and reproductive growth of *E. pulcherrima* var. Prestige Red in the presence of three doses of multipurpose Ultrasol fertilizer (0, 50% and 100%) after 236 days under greenhouse conditions. At vegetative stage (118 days), bacterial inoculation produced greater leaf area and higher leaf weight, total dry weight, and relative chlorophyll content, especially with the 50% fertilizer combination. At reproductive stage (236 days), inoculating a mixture of the three bacteria combined with 50% fertilizer increased leaf area, chlorophyll content, total dry weight, plant width, and number of branches. Overall, using PGPR plus 50% fertilizer improved growth and production of *E. pulcherrima*, and obtained a plant quality similar to that achieved without inoculation plus 100% fertilization. **Keywords:** *Bacillus, Euphorbia pulcherrima, Enterobacter*, nutrition, *Pseudomonas*.

Key words: Buchnus, Euphorona patener rind, Enterobacter, nutrition, i seauomonas.

Resumo: A *Euphorbia pulcherrima* é uma planta com brácteas coloridas comercializada principalmente no Natal, sendo a mais importante planta ornamental de vaso do mundo. Para produzir plantas saudáveis com altos padrões de qualidade, os produtores aplicam intensivamente defensivos e fertilizantes agrícolas, o que aumenta os custos de produção e causa a poluição ambiental. O uso de rizobactérias promotoras do crescimento de plantas (PGPR) mostra-se como uma nova opção para a produção de plantas devido aos beneficios que proporcionam às plantas, relacionados à nutrição e saúde. Este trabalho avaliou o efeito de três espécies de bactérias (*Pseudomonas* sp. CPO 2.78, *Enterobacter* sp. CPO 2.5 e *Bacillus megaterium* CPO 2. 35) isoladas da rizosfera de *E. cyathophora*, no crescimento vegetativo e reprodutivo de *E. pulcherrima* var. Prestige Red, na presença de três níveis de fertilização multiuso Ultrasol (0, 50% e 100%), sob casa de vegetação por 236 dias. Na fase vegetativa (118 dias), a inoculação bacteriana promoveu o maior incremento de área foliar, peso seco total e foliar e conteúdo relativo de clorofila, especialmente quando combinada com 50% de fertilizante. Na fase reprodutiva (236 dias), a inoculação das três espécies combinadas com 50% de Fertilizante promoveu o aumento da área foliar, o teor de clorofila, o peso seco total, a largura da planta e o número de ramos. Em geral, o uso de PGPR com 50% de fertilizante melhorou o crescimento e a produção de *E. pulcherrima*, cuja qualidade da planta foi semelhante à obtida com 100% de fertilização.

Palavras-chave: Bacillus, Euphorbia pulcherrima, Enterobacter, nutrição, Pseudomonas.

Introduction

Euphorbia pulcherrima Willd. ex Klotzsch is a native Mexican species grown in pots as an ornamental plant (Canul et al., 2018). This species is associated to Christmas festivities worldwide; its annual sale exceeds 100 million dollars in the United States and 700 million pesos in Mexico (Rodríguez-Elizalde et al., 2022). However, due to the lack of regulations and scarce information, tons of fertilizers and pesticides are applied to this ornamental species during its production, potentially inducing environmental pollution (Albiter et al., 2020). One option to minimize agrochemical pollution in ornamental production is using rhizospheric microorganisms. These microorganisms provide nutrients for plant development, favor plant metabolism and physiology, and induce tolerance to biotic and abiotic stress (Raj et al., 2020).

The most studied plant growth-promoting rhizobacteria (PGPR) belong to genera like *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Paenibacillus*, *Pseudomonas*, *Serratia* and *Rhizobium* (Basu et al., 2021). These bacteria stimulate plant growth and development through hormone production, atmospheric nitrogen fixation, and phosphate solubilization (Etemadifar et al., 2022). Most PGPR have been isolated from the rhizosphere of several crops (corn, oats, oak, sugarcane, citrus, etc.), but there is little information about PGPR isolated from wild plant species with ornamental potential. Therefore, the present work

evaluated the effect of inoculating three PGPR previously isolated from the rhizosphere of *Euphorbia cyathophora* (wild plant with ornamental potential) on the growth and production of *E. pulcherrima*, in combination with two doses (50% and 100%) of a commonly applied fertilizer.

Materials and Methods

Plant material, substrates, and containers

Three-month-old rooted cuttings of the Prestige Red variety were cultivated in a mixture of tepojal (porous volcanic rock) and peat moss $(1:2 v v^{-1})$ previously sterilized with steam (80- 90 °C, 8 h). The substrate was placed in 7-inch flexible plastic pots previously disinfected with 80% commercial chlorine and 70% alcohol.

Preparation of the bacterial inoculum

Three bacterial strains were used: *Pseudomonas* sp. CPO 2.78 (atmospheric nitrogen fixer), *Bacillus megaterium* CPO 2.35 (indole producer), and *Enterobacter* sp. CPO 2.5 (phosphate solubilizer). A mixture of the three strains was also inoculated (MIX); this mixture was prepared with 2 mL of each bacterial inoculum. The three strains were previously isolated from the rhizosphere of *Euphorbia cyathophora* and described based on their colonial morphology, physiological activity, and molecular identity (Rodríguez-Elizalde, in preparation).

The three strains were individually propagated in Luria-Bertani liquid medium (g L^{-1} : 10 g Tryptone Bacto, 5 g yeast extract, 5 g NaCl, and 1

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g Tryptophan) at 28 °C for 24 h. At the end of the incubation, 50 mL of inoculum were placed in Falcon tubes and centrifuged at 3,079 g for 15 min. The supernatant was removed, and the sediment (bacterial pellet) was resuspended in sterile distilled water and centrifuged three times to wash the bacterial cells from residues of the nutrient medium. Then, the sediment was placed in 300 mL of sterile distilled water and vortexed. The bacterial suspension was adjusted to an optical density of 0.6 (10⁷ cells m L⁻¹) using a Synergy 2 multimodal microplate absorbance reader Biotek® (Neidhart et al., 1990).

Bacterial inoculation and reinoculation

Plants were inoculated seven days after transplanting (dat), applying 2 mL of the bacterial inoculum directly at the base of the stem with a sterile syringe. Control plants (without bacterial inoculum) received only 2 mL of sterile water. Four months after transplanting, bacteria were re-inoculated for each respective treatment by adjusting the bacterial inoculum as previously described.

Fertilization, pruning, and photoperiod conditions

The fertilizer Ultrasol® Multipurpose 18-18-18 (granular presentation) was applied to plants at 50% and 100% of the dose recommended by the manufacturer. This fertilizer contains 18% N, P, and K; 0.5% Mg; 0.8% S; 400 mg Fe kg⁻¹; 200 mg Mn kg⁻¹; 200 mg Zn kg⁻¹; 100 mg B kg⁻¹; 100 mg Mo kg⁻¹; and 100 mg Cu kg⁻¹. The fertilizer was applied once a week by dissolving it in the irrigation water. Fertilization was conducted under greenhouse conditions for the whole experiment (236 days, May to December).

The first pruning was performed at 76 dat (June), and the second pruning was performed 50 days after the first pruning (August).

To achieve homogeneous pigmentation of bracts and to protect plants from low temperatures, we placed a black ground cover net (93.9% shade) in the upper part of the greenhouse from the first week of September until a homogeneous pigmentation was obtained (November). The net was daily opened at 9 am and closed at 5 pm daily during this period (a total of 82 days out of the 236 days).

Measured variables

Two samplings were performed, the first at the vegetative stage (118 dat) and the second at the reproductive stage, specifically during bract pigmentation (236 dat). In both stages, dry weight was determined with an OHAUS digital balance, leaf area was measured with a LI-COR LI-200 leaf area meter, and chlorophyll content was evaluated with the SPAD-SO2 Plus, Minolta. At 236 days, plant width and branching were evaluated considering mature and expanded leaves. Bract pigmentation was determined using the RHS Color chart (Royal Horticultural Society, 2006) in uniformly colored bracts.

Experimental conditions, experimental design, and statistical analysis

The experiment was carried out in a tunnel-type greenhouse (19°29'W and 98°53'N and at 2,250 masl) with a polyethylene UVII-720 cover and galvanized steel structure and lateral ventilation. The average maximum temperature was 38°C, and the average minimum temperature was 9 °C. The light intensity was 653.43 mmol m^{-2} s⁻¹.

The experimental design corresponded to a 5×3 factorial experiment, with five levels of bacterial inoculation (*Enterobacter* sp. [Enterob], *Pseudomonas* sp. [Pseud], *Bacillus megaterium* [Bmega], a mixture of the three bacteria [MIX], and no bacterial inoculation [SInoc]); and three levels of fertilization (0F, 50F, and 100F), thus, yielding 15 treatments with 15 replicates each. For each sampling date, individual analyses were performed to evaluate the effect of the two independent factors (fertilization and bacterial inoculation) and their interaction. In addition to the analysis of variance, we performed a mean comparison test (Tukey, α =0.05) using SAS software (SAS Institute, 2021).

Results

Leaves and bracts

In the first sampling date (118 dat), the highest leaf area values were observed in the treatment inoculated with Enterob+50F, MIX+50F, and SInoc+50F, and the lowest values were found in Pseud+0F and Enterob+0F. Notably, all treatments inoculated with any bacterial strain and 50% fertilization (Bmega+50F, Pseud+50F, Enterob+50F, and MIX+50F) were significantly superior to the SInoc+100F treatment (Fig. 1A).

Plant leaves and bracts were analyzed separately during the second evaluation (236 dat). Leaf area was significantly higher in plants of the treatments Enterob+50F, MIX+50F, and SInoc+50F; while the lowest values were obtained in the treatments MIX+0F, Pseud+0F, Bmega+0F, and SInoc+0F (Figure 1B). Significantly higher values of bract leaf area were found in the MIX+50F treatment, while the lowest values were observed in the SInoc+0F treatment (Fig. 1B).

Plant weight

At 118 dat, the total dry weight of plants with the MIX+50F, SInoc+50F and Enterob+50F treatments was significantly higher than the SInoc+0F treatment. This same trend was observed for leaf dry weight (Table 1A). For stem dry weight, the Bmega+0F and MIX+0F treatments showed the highest values, while Bmega+100F showed the lowest value (Table 1A).

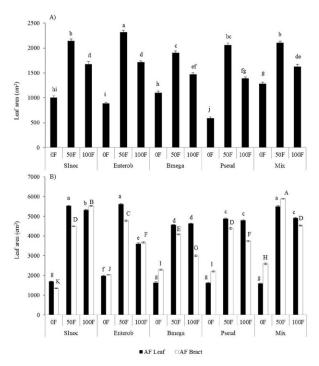


Fig. 1. Leaf area (FA) of *Euphorbia pulcherrima* inoculated with bacterial strains isolated from the rhizosphere of *E. cyathophora*, at 118 (A) and 236 dat (B). Symbology: SInoc = No inoculation, Enterob = *Enterobacter* sp., Bmega = *Bacillus megaterium*, Pseud = *Pseudomonas* sp., MIX = Mixture of the three bacteria. Means \pm standard error. Different letters above the bars show significance between treatments for each variable (Tukey, $\alpha = 0.05$), n = 5.

A) Treatment	Fertilization dose (%)	Leaf	Stem	Root	Т	otal
A) Treatment	rertifization dose (%)	(g)				
SInoc	0	3.34 f	3.49 abc	1.818 ef	6.834 e	
	50	8.1 abc	3.266 abcd	2.434 bcdef	11.366 abc	
	100	6.41 cde	2.612 bcde	3.344 abcd	9.022 cde	
	0	4.732 ef	3.442 abc	2.702 bcdef	7.144 e	
Enterob	50	8.682 ab	3.652 ab	2.028 cdef	12.334 ab	
	100	6.744 bcde	2.618 bcde	1.698 ef	9.362 bcde	
	0	5.49 edf	3.798 a	3.92 ab	9.288 bcde	
Bmega	50	8.43 abc	3.214 abcd	2.438 bcdef	11.644 abc	
	100	5.134 ef	2.064 e	1.556 ef	7.198 de	
Pseud	0	4.126 f	2.93 abcde	3.038bcde	7.056 e	
	50	7.368 abcd	3.004 abcde	2.742bcdef	10.372 abcd	
	100	5.554 def	2.21 de	1.278 f	7.764 de	
MIX	0	5.004 ef	3.75 a	4.75 a	8.754 cde	
	50	9.018 a	3.706 ab	3.448 abc	12.724 a	
	100	6.524 bcde	2.522 cde	1.846 def	9.046 cde	
	LSD	2.21	1.10	1.49	3.19	
B)	Fertilization dose (%)	Leaf	Bract	Stem	Root	Total
Treatment		(g)				
SInoc	0	7.48 e	3.88 g	16.30 fg	26.06 a	27.66 h
	50	10.0(1				
	50	18.96 bc	15.60 ab	20.54 cd	18.99 bc	55.11 b
	100	18.96 bc 18.91 bc	15.60 ab 8.95 de	20.54 cd 16.06 fg	18.99 bc 10.19 e	55.11 b 43.92 de
Enterob	100	18.91 bc	8.95 de	16.06 fg	10.19 e	43.92 de
Enterob	100 0	18.91 bc 8.50 e	8.95 de 6.85 efg	16.06 fg 16.54 f	10.19 e 29.39 a	43.92 de 31.90 gh
Enterob	100 0 50	18.91 bc 8.50 e 19.94 abc	8.95 de 6.85 efg 12.93 bc	16.06 fg 16.54 f 21.55 bc	10.19 e 29.39 a 15.29 cd	43.92 de 31.90 gh 54.44 b
Enterob Bmega	100 0 50 100	18.91 bc 8.50 e 19.94 abc 14.60 d	8.95 de 6.85 efg 12.93 bc 9.19 de	16.06 fg 16.54 f 21.55 bc 12.61 h	10.19 e 29.39 a 15.29 cd 8.83 e	43.92 de 31.90 gh 54.44 b 36.40 fg
	100 0 50 100 0	18.91 bc 8.50 e 19.94 abc 14.60 d 7.82 e	8.95 de 6.85 efg 12.93 bc 9.19 de 6.49 efg	16.06 fg 16.54 f 21.55 bc 12.61 h 15.42 fgh	10.19 e 29.39 a 15.29 cd 8.83 e 20.56 b	43.92 de 31.90 gh 54.44 b 36.40 fg 29.74 gh
	100 0 50 100 0 50	18.91 bc 8.50 e 19.94 abc 14.60 d 7.82 e 17.99 dc	8.95 de 6.85 efg 12.93 bc 9.19 de 6.49 efg 12.36 c	16.06 fg 16.54 f 21.55 bc 12.61 h 15.42 fgh 20.17 cde	10.19 e 29.39 a 15.29 cd 8.83 e 20.56 b 12.42 de	43.92 de 31.90 gh 54.44 b 36.40 fg 29.74 gh 50.52 bcd
	100 0 50 100 0 50 100	18.91 bc 8.50 e 19.94 abc 14.60 d 7.82 e 17.99 dc 19.38 bc	8.95 de 6.85 efg 12.93 bc 9.19 de 6.49 efg 12.36 c 8.61 def	16.06 fg 16.54 f 21.55 bc 12.61 h 15.42 fgh 20.17 cde 14.5 fgh	10.19 e 29.39 a 15.29 cd 8.83 e 20.56 b 12.42 de 8.77 e	43.92 de 31.90 gh 54.44 b 36.40 fg 29.74 gh 50.52 bcd 42.49 ef
Bmega	100 0 50 100 0 50 100 0	18.91 bc 8.50 e 19.94 abc 14.60 d 7.82 e 17.99 dc 19.38 bc 7.38 e	8.95 de 6.85 efg 12.93 bc 9.19 de 6.49 efg 12.36 c 8.61 def 5.85 fg	16.06 fg 16.54 f 21.55 bc 12.61 h 15.42 fgh 20.17 cde 14.5 fgh 17.36 def	10.19 e 29.39 a 15.29 cd 8.83 e 20.56 b 12.42 de 8.77 e 17.22 bc	43.92 de 31.90 gh 54.44 b 36.40 fg 29.74 gh 50.52 bcd 42.49 ef 30.60 gh
Bmega	100 0 50 100 0 50 100 0 50 50	18.91 bc 8.50 e 19.94 abc 14.60 d 7.82 e 17.99 dc 19.38 bc 7.38 e 18.81 bc	8.95 de 6.85 efg 12.93 bc 9.19 de 6.49 efg 12.36 c 8.61 def 5.85 fg 10.73 dc	16.06 fg 16.54 f 21.55 bc 12.61 h 15.42 fgh 20.17 cde 14.5 fgh 17.36 def 25.81 a	10.19 e 29.39 a 15.29 cd 8.83 e 20.56 b 12.42 de 8.77 e 17.22 bc 12.54 de	43.92 de 31.90 gh 54.44 b 36.40 fg 29.74 gh 50.52 bcd 42.49 ef 30.60 gh 55.35 b
Bmega	100 0 50 100 0 50 100 0 50 50 100	18.91 bc 8.50 e 19.94 abc 14.60 d 7.82 e 17.99 dc 19.38 bc 7.38 e 18.81 bc 17.90 dc	8.95 de 6.85 efg 12.93 bc 9.19 de 6.49 efg 12.36 c 8.61 def 5.85 fg 10.73 dc 11.60 dc	16.06 fg 16.54 f 21.55 bc 12.61 h 15.42 fgh 20.17 cde 14.5 fgh 17.36 def 25.81 a 15.94 fgh	10.19 e 29.39 a 15.29 cd 8.83 e 20.56 b 12.42 de 8.77 e 17.22 bc 12.54 de 8.77 e	43.92 de 31.90 gh 54.44 b 36.40 fg 29.74 gh 50.52 bcd 42.49 ef 30.60 gh 55.35 b 45.45 cde
Bmega Pseud	100 0 50 100 0 50 100 0 50 100 0 0	18.91 bc 8.50 e 19.94 abc 14.60 d 7.82 e 17.99 dc 19.38 bc 7.38 e 18.81 bc 17.90 dc 6.79 e	8.95 de 6.85 efg 12.93 bc 9.19 de 6.49 efg 12.36 c 8.61 def 5.85 fg 10.73 dc 11.60 dc 5.84 fg	16.06 fg 16.54 f 21.55 bc 12.61 h 15.42 fgh 20.17 cde 14.5 fgh 17.36 def 25.81 a 15.94 fgh 12.98 gh	10.19 e 29.39 a 15.29 cd 8.83 e 20.56 b 12.42 de 8.77 e 17.22 bc 12.54 de 8.77 e 15.63 cd	43.92 de 31.90 gh 54.44 b 36.40 fg 29.74 gh 50.52 bcd 42.49 ef 30.60 gh 55.35 b 45.45 cde 25.62 h

Tab. 1. Dry weight of *Euphorbia pulcherrima* plants inoculated with bacterial strains isolated from the rhizosphere of *E. cyathophora*, at 118 (A) and 236 (B) dat.

Symbology: SInoc = No inoculation, Enterob = *Enterobacter* sp., Bmega = *Bacillus megaterium*, Pseud = *Pseudomonas* sp., MIX = Mixed bacteria, Different letters in the same column, for each sampling date, show significance between treatments (Tukey, $\alpha = 0.05$), LSD = Least Significant Difference, n = 5.

Regarding root dry weight, the highest value was obtained in MIX+0F, and the lowest value was obtained in Pseud+100F (Table 1A). Fifty percent fertilization favored total and leaf dry weight regardless of bacterial inoculation. Moreover, inoculating the bacterial strains alone or in combination promoted higher root dry weight when no fertilizer was applied. Finally, no specific trends were observed in stem dry weight between treatments (Table 1A).

Total dry weight at 236 dat was statistically higher in the treatments MIX+50F, Enterob+50F, and MIX+100F; the lowest values were recorded in the treatments SInoc+0F, Enterob+0F, Bmega+0F, Pseud+0F, and MIX+0F (Table 1B). The best stem dry weights were found in the treatments Pseud+50F and MIX+50F; while Enterob+100F, Bmega+0F, Bmega+100F, Pseud+100F, and MIX+0F treatments presented the lowest values (Table 1B).

The highest values of root dry weight were observed in Enterob+0F and SInoc+0F, while the lowest values were found in the treatments SInoc+100F, Enterob+100F, Bmega+50F, Bmega+100F, Pseud+50F, Pseud+100F and MIX+100F (Table 1B). For bract dry weight, the treatments MIX+50F and SInoc+50F had the highest values, and SInoc+0F, Enterob+0F, Bmega+0F, Pseud+0F, and the MIX+0F had the

lowest (Table 1B). Importantly, although the treatments Enterob+0F, Bmega+0F, Pseud+0F, and MIX+0F presented similar bract dry weight compared to the SInoc+0F treatment, the plants presented more colorful bracts with a homogeneous distribution, and no phosphorus deficiencies were observed with this treatment as compared to the SInoc+0F.

Chlorophyll content

The relative chlorophyll content (SPAD units) at 118 dat was significantly higher in three treatments (SInoc+100F, Enterob+100F, and Pseud+100F); in contrast, the lowest values achieved at SInoc+0F and Pseud+0F. Importantly, the treatments without fertilization but inoculated with Enterob, Bmega, or MIX had higher relative chlorophyll content than the treatment without inoculation (SInoc+0F) (Fig. 2A).

At reproductive stage (236 dat), the relative chlorophyll content was statistically higher in the MIX+50F and Pseud+100F treatments; while the lowest content was found in the MIX+0F, Pseud+0F, Enterob+0F, Bmega+0F and SInoc+0F treatments (Fig. 2B). When comparing the two samplings, the plants with the Pseud+100F treatment maintained a high chlorophyll content; however, plants with the SInoc+0F and Pseud+0F treatments presented lower chlorophyll content.

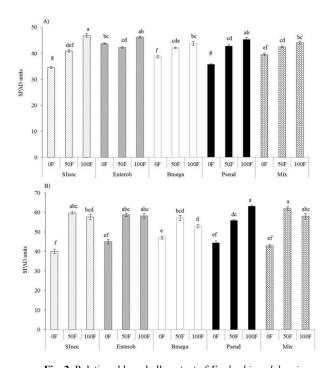


Fig. 2. Relative chlorophyll content of *Euphorbia pulcherrima* inoculated with bacterial strains isolated from the rhizosphere of *E. cyathophora*, at 118 (A) and 236 dat(B). Symbology: SInoc = No inoculation, Enterob = *Enterobacter* sp., Bmega = *Bacillus megaterium*, Pseud = *Pseudomonas* sp., MIX = Mixture of the three bacteria. Means + standard error. Different letters above the bars show significance between treatments for each variable (Tukey, $\alpha = 0.05$), n = 5.

At 236 dat, plant width was significantly higher in the MIX+100F treatment, and the lowest value was obtained in the treatments without inoculation and inoculated without fertilizer application (0F) (Fig. 3A). When plants were inoculated with Pseud, Bmega, Enterob, or MIX, the largest plant width was achieved when fertilized with 50F and 100F; while the lowest values were obtained in plants without fertilization. The SInoc treatment showed statistically similar plant width when fertilized at 50F and 100F (Fig. 3A). Conversely, the number of branches was higher in fertilized plants and plants with rhizobacteria inoculation compared to those with the SInoc+0F and SInoc+50F treatments. Considering only the fertilization dose, more branches were obtained when applying the 100F dose compared to unfertilized plants (0F) (Fig. 3B).

The color of leaves and bracts of *E. pulcherrima* also differed between treatments. The leaves and bracts of the plants without fertilization nor inoculation (SInoc+0F) were classified under a different color category (Green group 146 A and Red group 46 B) compared to inoculated plants without fertilization (Enterob+0F, Bmega+0F, Pseud+0F, and MIX+0F) (Yellow group 147A and Red group 53B). Figure 4 shows a general overview of the characteristics of plants under the different treatments.

Discussion

Leaf area is a good measure of plant growth (Solis et al., 2021). In this study, inoculation of Enterob or MIX with 50F in *E. pulcherrima* produced greater leaf area of both leaves and bracts and improved the quality of the characteristics required for commercialization. Similar results were described for *Calendula officinalis* with the inoculation of an organic source combined with *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus polymyxa*, *B. subtilis*, *Klebsiella pneumoniae*, and *Pseudomonas fluorescens*, showing gradual increases in leaf area, plant height, number of shoots and leaves (Mohsen and Ismail, 2016).

The Enterobacteriaceae family is a heterogeneous group of gramnegative bacteria that receive their name from their localization in the digestive tract as saprophytes. However, these bacteria can also be found in soil, water, and vegetation (Khalifa, 2020). Some species of this family have been implicated in plant growth and productivity because they are highly efficient in phosphate solubilization (Mahdi et al., 2020; Basu et al., 2021), can increase the survival rate of orchids (Kaur and Sharma, 2021), and survive in contaminated media (Etemadifar et al., 2022).

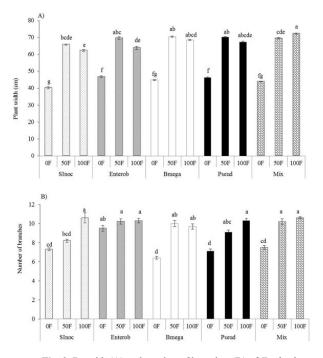


Fig. 3. Breadth (A) and number of branches (B) of *Euphorbia pulcherrima* inoculated with bacterial strains isolated from the rhizosphere of *E. cyathophora*, 236 dat. Symbology: SInoc = No inoculation, Enterob = *Enterobacter* sp., Bmega = *Bacillus megaterium*, Pseud = *Pseudomonas* sp., MIX = Mixture of the three bacteria. Means + standard error. Different letters show significance between treatments (Tukey, $\alpha = 0.05$), n = 10.



Fig. 4. Size aspect of uninoculated *Euphorbia pulcherrima* plants (A), plants inoculated with *Bacillus megaterium* CPO 2.35 (B), plants inoculated with *Enterobacter* sp. CPO 2.5 (C), plants inoculated with *Pseudomonas* sp. CPO 2.78 (D), plants inoculated with the mixture of the three bacterial strains (E), in combination with three fertilization levels (0%, 50% and 100% of Ultrasol® Multipurpose 18-18-18), at 236 dat.

The general trend of dry weight (leaves, stem, and root) showed that plants inoculated with Enterob, Bmega, and the MIX (all with 50F) were superior to the plants with 100% fertilization. Bacteria of the genus *Serratia* sp., *Enterobacter* sp., *Pseudomonas* sp., and *Bacillus* sp. have been reported as growth promoters of ornamental plants, such as *Zantedeschia aethiopica*, *Gaillardia pulchella*, *Petunia* × *hybrida*, *Impatiens walleriana*, among other species (Gupta et al., 2021; South et al., 2021). These observations open the possibility of using PGPR as an environmentally friendly technology in ornamental horticulture.

Relative chlorophyll content at 118 dat was increased in Enterob+100F, Pseud+100F, and SInoc+100F treatments; while at 236 dat, chlorophyll content was higher in MIX+50F, Pseud+100F, Enterob+50F, Enterob+50F, Enterob+100F and SInoc+50F. Chlorophyll content is positively correlated with photosynthesis; thus, a reduction in chlorophyll content may be associated with alterations in the photosynthetic process (reduction in carbon fixation) and a lack of essential elements for the functioning of photosystem I and II (Agathokleous et al., 2020).

Plant width in *E. pulcherrima* is a parameter used to show the area occupied by the plant from an aerial view and to describe new varieties according to the International Union for the Protection of New Varieties of Plants (UPOV) (Mejia et al., 2006). Plants with greater width are more valued among consumers because they are more attractive and robust. In this regard, plants obtained with bacterial inoculation in the present experiment showed higher plant width values than plants only fertilized (Fig. 4). Canul et al. (2018) evaluated two commercial varieties of *Puphorbia pulcherrima* (Prestige red and Freedom red) and a new indoor hybrid (Alondra) performing traditional management without the use of PGPR. The mean plant width values obtained for Prestige red (23.02 cm) were below to those obtained in the present work (72.25, in the MIX+100F treatment). This allows us to conclude that using bacterial strains isolated from the rhizosphere of *E. cyathophora* promoted greater plant width in Poinsettia var. Prestige.

In the present work, the number of branches depended on the pruning, which caused an initial reduction and subsequent increase in branch number. This effect was more evident when the plants were fertilized with 50F and 100F. Of note, no plant losses occurred during the two prunings due to the effect of pathogenic microorganisms, indicating that the PGPR could promote plant health by protecting the poinsettia plants against harmful microorganisms. Jalmi and Sinha (2022) indicated that PGPR produce many chemical compounds with antimicrobial activity (broad-spectrum antibiotics, lactic acid, lysoenzymes, exotoxins, and bacteriocins) used as a defense system.

No information is available on the effect of PGPR on the quality aspects of ornamental plants. However, plants that were inoculated with the bacterial strains and fertilized showed higher leaf and bract color (139A and Red group 53A, respectively) and no chlorosis, compared to the SInoc+0F treatment (146A and Red group 46B, respectively). According to Starkey and Andersson (2000), light intensity (photoperiod) and nitrogen content can influence the development, size, and color of bracts; therefore, plants that did not receive fertilizer were affected, presenting a fainter color.

Overall, our results show that using the three bacterial strains isolated from the rhizosphere of *E. cyathophora* is feasible since they promote plant growth, plant width, number of ramifications, and coloration of the poinsettia plants. In particular, reducing the amount of the multipurpose Ultrasol fertilizer to 50% produced better results than complete fertilization without growth-promoting bacteria.

Conclusions

Inoculating with Enterob, Bmega and/or Pseud isolated from the rhizosphere of *Euphorbia cyathophora* promoted the growth of *Euphorbia pulcherrima* at vegetative stage; plants inoculated and fertilized with 50% doses of Ultrasol® Multipurpose 18-18-18 displayed higher leaf area, total dry weight, leaf dry weight, and chlorophyll content. At reproductive stage, the MIX+50F treatment increased leaf area, chlorophyll content, total dry weight, plant width, and number of branches in the plants. In addition, more intense coloration was achieved in bracts and leaves when inoculated and fertilized with 100F and 50F.

Overall, we conclude that the inoculation of PGPR favors the growth and production of *E. pulcherrima*; in addition, it allows obtaining plants of greater plant width, better quality, and better color with the application of 50% of the dose of Ultrasol[®] Multipurpose 18-18-18, which also allows saving fertilizer application and increasing profits.

Author contribution

MARE and AA: were responsible for study conception, design, and planning. RFC and JJAS: contributed to the field investigation. MARE and MVH: analyzed the data. MARE and AA: wrote the paper. All authors reviewed and approved the manuscript.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability Statement

Data will be available on request.

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