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ARTICLE

Sustainable zinnia cultivation: influence of rhizobacteria inoculation on emergence and biometric traits

Cultivo sustentável de zínia: influência da inoculação de rizobactérias na emergência e nos parâmetros biométricos

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Abstract
Plant growth-promoting rhizobacteria establish beneficial symbiotic interactions with plants, exerting a positive and sustainable impact on the growth and development of various plant species. The adoption of new sustainable technologies in ornamental plant cultivation can enhance competitive market advantages. This study investigated the effects of rhizobacteria on seedling emergence and growth in *Zinnia peruviana* L., chosen due to its commercial importance in the ornamental plant industry. The aim was also to evaluate whether reapplications of rhizobacteria are necessary throughout the plant cycle. The experiment had two phases. In phase 1, six treatments were used, corresponding to five rhizobacteria (*Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *B. megaterium*, *B. pumilus*, *B. subtilis*) and the absence of rhizobacteria - control; assessing seedling emergence percentage and Speed Index. In phase 2, a 6 x 2 factorial design was used with the same treatments from phase 1, combined with either 1 or 2 applications, to evaluate plant growth and development. Results showed that rhizobacteria did not affect the emergence rate, but *B. amyloliquefaciens* and *B. subtilis* accelerated seedling emergence. Additionally, *B. subtilis* promoted superior growth, development, and flowering. Importantly, there was no need for reapplications during the plant cycle, highlighting the practical benefit of reducing the frequency of treatments, which can lower costs and minimize environmental impact in ornamental plant production.
Keywords: *Azospirillum brasilense, Bacillus amyloliquefaciens, Bacillus megaterium, Bacillus pumilus, Bacillus subtillis, Zinnia peruviana* L.

Resumo
As rizobactérias promotoras de crescimento em plantas estabelecem interações simbióticas benéficas com as plantas, exercendo um impacto positivo e sustentável no crescimento e no desenvolvimento de diversas espécies vegetais. A adoção de novas tecnologias sustentáveis no cultivo de plantas ornamentais pode aprimorar as vantagens competitivas no mercado. Este estudo investigou os efeitos das rizobactérias na emergência e no crescimento das mudas de *Zinnia peruviana* L., escolhida como espécie modelo devido à sua importância comercial na indústria de plantas ornamentais. O objetivo foi também avaliar se reaplicações de rizobactérias são necessárias ao longo do ciclo da planta. O experimento foi conduzido em duas fases. Na fase 1 foram utilizados seis tratamentos correspondendo a cinco rizobactérias (*Azospirillum brasilense, Bacillus amyloliquefaciens, B. megaterium, B. pumilus, B. subtillis*) e ausência de rizobactéria - controle; analisou-se a porcentagem e o Índice de Velocidade de Emergência de plântulas. Na fase 2, foi utilizado um esquema fatorial 6 x 2 com os mesmos tratamentos, combinados com uma ou duas aplicações, para avaliar o crescimento e o desenvolvimento das plantas. Os resultados mostraram que as rizobactérias não afetaram a taxa de emergência, mas *B. amyloliquefaciens* e *B. subtilis* aceleraram a emergência das mudas. Além disso, *B. subtilis* promoveu crescimento, desenvolvimento e floração superiores. Importante destacar que não foi necessária reaplicação durante o ciclo da planta, evidenciando o benefício prático de reduzir a frequência dos tratamentos, o que pode reduzir custos e minimizar o impacto ambiental na produção de plantas ornamentais.
Palavras-chave: *Azospirillum brasilense, Bacillus amyloliquefaciens, Bacillus megaterium, Bacillus pumilus, Bacillus subtillis, Zinnia peruviana* L.

Introduction

Microorganisms with plant growth-promoting mechanisms play a vital role in plant development and production, becoming a viable alternative in the search for sustainable agriculture (Queiroz and Oliveira, 2023). Plant growth-promoting rhizobacteria stand out among these microorganisms, being capable of positively interfering in the growth and development of plants in several ways, including producing phytohormones, relieving stress due to water deficit, mitigating the stressful effects of salinity, acting in the phytoextraction of heavy metals, nutrient supplementation and/or pathogen biocontrol (Dias and Santos, 2022).

Through several rhizobacteria biological processes, nitrogen fixation is important, which reduces the need for synthetic nitrogen fertilizers by converting atmospheric nitrogen into a form that plants can use (Cassán et al., 2020). Furthermore, by solubilizing phosphorus, rhizobacteria assist plants in obtaining phosphorus by dissolving its insoluble forms in the soil. They release organic acids like gluconic and citric acids, which dissolve insoluble phosphorus compounds like calcium phosphate and reduce the pH around the roots. By transforming phosphorus into an absorbable form, this mechanism improves nutrient intake and fosters plant development, a nutrient essential to plant growth; rhizobacteria increases nutrient availability (Santoyo et al., 2021). Additionally, auxins, cytokinins, and gibberellins are plant growth hormones that affect root elongation and shoot growth, and rhizobacteria regulate flowering. Because of these hormonal changes, plants can produce more, grow stronger, and have more extensive root systems (Aloo et al., 2022)

Among the many existing rhizobacteria, the genera *Azospirillum* and *Bacillus* stand out. The bacterium *Azospirillum brasilense* has a good capacity for biological nitrogen fixation, phosphate solubilization, and desirable characteristics of proliferation in the root system, and its main mechanisms are the production of phytohormones, especially auxin, which promotes plant growth and development (Cassán et al., 2020). Similarly, *Bacillus amyloliquefaciens* has shown excellent results in promoting plant growth, even under environmental stress conditions. It is considered an excellent biofertilizer and disease suppressor biocontrol, in addition to stimulating the production of phytohormones and forming volatile organic compounds and siderophores that increase the availability of nutrients in the soil (Luo et al., 2022).

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Another important bacterium, *Bacillus megaterium*, is resistant to environmental stresses and exhibits strong potential for biotechnology. It solubilizes inorganic phosphorus, improving nutrient availability for plants while also enhancing plant resistance to pathogens and abiotic stress (Nascimento et al., 2020). Additionally, *Bacillus pumilus* helps increase the production of growth-promoting hormones such as auxins, cytokinins, and gibberellins. It also provides indirect protection against phytopathogenic agents and contributes to nitrogen fixation (Dobrzyński et al., 2022).

Finally, the *Bacillus subtilis* species is widely known for its biocontrol actions in agriculture, preventing the colonization of some pathogens, the action of pests and the effects of other agents in the soil and helping the health of the plant rhizosphere, increasing phosphate solubilization, production of siderophores and indoleacetic acid, which, consequently, contributes to more significant and healthier plant growth and greater tolerance to stress (Arnaouteli et al., 2021).

Several studies have been carried out, demonstrating the success of rhizobacteria in the growth and development of plants, such as those carried out for corn - *Zea mays* L. (Guimarães et al., 2021), white oat - *Avena sativa* L. (Santos et al., 2021), watermelon- *Citrullus lanatus* [Thunb.] Matsum and Nakai (Silva et al., 2022), açaí tree - *Euterpe oleracea* Mart. (Campos et al., 2023) and *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos (Campos et al., 2024).

Belonging to the Asteraceae family, *Zinnia peruviana* L., traditionally medicinal, gained popularity when introduced into the ornamental world, being an erect annual herbaceous plant with elliptical or oval leaves, 70 to 100 cm tall, most frequently found in Peru and Argentina (Echenique et al., 2020). Its greatest prominence in floriculture was mainly due to the ease and speed of cultivation with the exuberant result of its colorful solitary flowers and its use as a cut flower (Gomma et al., 2019). As it is a relatively easy plant to grow and can adapt to the most varied soil types, it became commercially important, where production requires qualitative attributes (Zulfiqar et al., 2023). In addition, it is a plant widely used in traditional medicine, mainly in the cure of malaria, stomach pains, and other illnesses (Mattana et al., 2016).

The hypothesis of the present study is based on the premise that the application of rhizobacteria in ornamental plants, such as *Zinnia peruviana* L., promotes better growth, development, and flowering, making it possible to guide more in-depth studies on the efficiency of different rhizobacteria in the sustainable cultivation of ornamental plants.

Seeking new technologies that can increase benefits in the commercial production of *Z. peruviana*, this work aimed to verify if some rhizobacteria that promote growth in plants affect the emergence of seedlings and the cultivation of this ornamental and whether there is a need for reapplication during the crop cycle.

Materials and Methods

The study was conducted at the Experimental Nursery of Ornamental and Forestry Plants, the Horticultural Plant Seeds Laboratory and the Soil Microbiology Laboratory, both belonging to the Department of Agricultural Production Sciences of the Faculty of Agricultural and Veterinary Sciences (UNESP/FCAV), Jaboticabal Campus, São Paulo, Brazil, under coordinates 21°15’2” latitude, 48°16’47”, longitude and at 600 meters altitude. The climate classification of the region is subtropical type Cwa - humid tropical with dry winter and rainy summer (Andre and Garcia 2015). The climatic data regarding maximum temperature, minimum temperature, average temperature, and relative humidity during the experiment period (August to October 2023) are shown in Fig. 1 (UNESP, 2024).

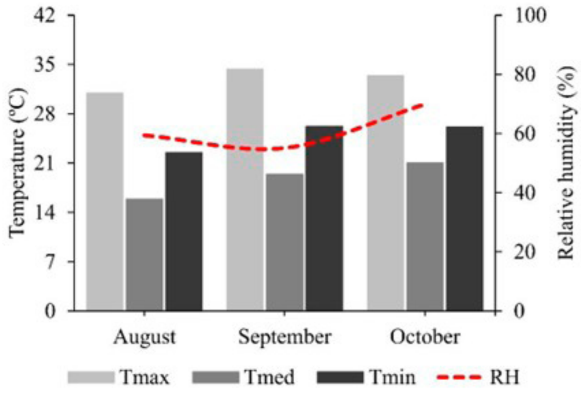

Fig. 1. Climatic data from August to October 2023. Maximum temperature - Tmax, Minimum temperature - Tmin, Average temperature - Tmed, and Relative humidity – RH.

The experiment was carried out in two phases, adopting a completely randomized experimental design. Phase 1: emergence phase, with six treatments corresponding to microorganisms (*Azospirillum brasilense, Bacillus amyloliquefaciens, Bacillus megaterium, Bacillus pumilus, Bacillus subtillis* and a control treatment - absence of rhizobacteria), with four replications and 36 seeds per plot. Phase 2: Plant growth and development phase, with 12 treatments in a 6 x 2 factorial scheme. The factors tested in this phase were five rhizobacteria (*Azospirillum brasilense, Bacillus amyloliquefaciens, Bacillus megaterium, Bacillus pumilus,* and *Bacillus subtillis*) and the absence of rhizobacteria – control, combined with 2 applications on both phases, four replications and three plants per plot. A two-level approach enables a more in-depth analysis of the effects of rhizobacteria at different stages of the plant cycle, providing valuable information for sustainable practices in the production of ornamental plant seedlings.

The rhizobacteria used are part of the collection of the Soil Microbiology Laboratory at UNESP/FCAV, where they were produced separately in a nutrient broth environment for seven days in bottles kept in B.O.D. (Eletrolab, model 347 F, Brazil). At a temperature of 25 °C. After incubation, the bacteria were centrifuged separately at 10.000 rpm for 10 min at 28 °C (Novatecnica, model MLW K24, Brazil). The inoculum concentration was standardized as recommended by Barry and Thornsberry (1991) and Sahm and Washington II (1991) at 1×10^7 UFC mL^{-1} with the aid of a spectrophotometer (Micronal, model B382, Brazil) at an absorbance of 695 nm.

Seeds obtained from the market were sown on August 7, 2023, in trays of cells (three seeds per cell) containing the commercial substrate Carolina Soil®, composed of peat, vermiculite, roasted rice husk, calcined dolomitic limestone, NPK fertilizer (14-16-18) and micronutrients (information obtained from data on the packaging), which was maintained at 100% water retention capacity. The trays were placed in a covered greenhouse with sides protected by a black screen that allowed 50% of the light to pass through, and there was also a transparent plastic cover above the screen cover. The substrate was not autoclaved since it was purchased commercially, therefore it is assumed that it has already undergone manufacturing processes that minimize the presence of significant contaminants. Furthermore, it becomes a strategy to maintain a more balanced microbial environment, favoring beneficial interactions between the applied rhizobacteria and other microorganisms present in the substrate.

At sowing, 200 µL of each rhizobacteria solution was inoculated per cell, according to the treatment, using a mechanical micropipette (VF-1000, Digipet®). The cells containing seeds for the control treatment were not inoculated.

In the experiment's first phase, the number of emerged seedlings was observed daily until emergence stabilized, which occurred 20 days after sowing. From the data collected, the following were calculated: Emergency Percentage and Emergency Speed Index. After the emergence and counting of the seedlings, thinning was carried out, leaving one seedling per cell, using the most vigorous and most centralized seedling as a criterion.

On August 28, 2023, seedlings with a length of 2 cm ± 0.5 cm were transplanted into separate containers with a volumetric capacity of 280 cm³, containing soil, manure, and sand (1:1:1 v:v:v) as substrate, placed suspended on metal mesh benches 70 cm from the ground, in the same greenhouse where phase 1 was carried out. Irrigation of the seedlings was carried out using automatically activated micro-sprinklers twice daily, lasting 15 min each, with a flow rate of 30 L h^{-1}. After transplanting, 1 mL per plant was inoculated with each solution containing the rhizobacteria, maintaining the treatments on the plants where the application was carried out at the time of sowing. A new application of rhizobacteria was carried out 30 days after the first half of the seedlings in each plot (treatments with two applications); the other half (treatments with one application) was not reapplied. The application was directed to the substrate close to the stem with a mechanical micropipette (VF-1000, Digipet®). The seedlings destined for the control treatment were not inoculated.

The evaluation was carried out on October 15, 2023, in all treatments, as soon as 50% of the plants in most treatments had fully opened and formed flowers (without damage). The plants were removed from the containers and, still in the nursery, rinsed to remove excess substrate from the roots and then taken to the Horticultural Plant Seeds Laboratory at UNESP/FCAV, where a new wash was carried out to remove any remaining residue. The substrate on the roots and then the shoot length - SL (cm) was evaluated, obtained from the level of the substrate to the apex of the last leaf, with the aid of a millimeter ruler; the stem diameter - SD (mm), determined at the substrate level, using a digital caliper with an accuracy of 0.01 mm (Western® PRO DC-6); the number of fully expanded leaves - NL, verified by visual counting of fully expanded leaves; leaf area (cm^2), using an electronic leaf area measurer (Li-3100C, LI-COR®, Lincoln, Nebraska, USA); the number of flowers (open and buds) - NF, the shoot dry mass - SDM (g) and roots - RDM (g), obtained after drying in a kiln with forced air circulation at 70 ºC until reaching a constant weight, and then weighed on a scale precision of 0.001g (SHIMADZU®, model AY220) and the total dry mass - TDM (g) was obtained by adding up the dry masses of the aerial part and roots.

The data obtained were subjected to analysis of variance and the means compared by the Tukey test ($p \leq 0.05$), using the AgroEstat Software version 1.1.0.712 (Barbosa and Maldonado Junior 2015). In addition to evaluating the main effects of the rhizobacteria treatments, the interaction between the rhizobacteria and the number of applications was also analyzed to assess whether the effect of rhizobacteria on plant growth and development varied with the frequency of application. A correction analysis was also carried out between the variables.

Results

The interaction between rhizobacteria and the number of applications was insignificant for the Emergency Percentage or the Emergency Speed Index. In phase 1 of the experiment, no significant difference was found in the percentage of emergence seedlings with or without inoculation of rhizobacteria, which was, on average, 38%. However, zinnia seeds emerged faster in the presence of rhizobacteria of the genus *Bacillus*, particularly for the species *B. amyloliquefaciens* and *B. subtilis*, which presented the highest averages (5.19 and 6.05, respectively, when compared to the bacteria *Azospirillum brasilense* and to the control (Table 1).

Table 1. Emergence (E) and Emergence Speed Index (ESI) of seedlings, shoot length (SL), stem diameter (SD), number of leaves (NL), leaf area (LA), number of flowers (NFlo), root length (RL), root dry mass (RDM), shoot dry mass (SDM), and total dry mass (TDM) of zinnia (*Zinnia peruviana* L.) treated or not (control), with and without reapplication the rhizobacteria *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Bacillus pumilus*, and *Bacillus subtilis*.

Treatments	E (%)	ESI	SL (cm)	SD (mm)	NL -	LA (cm^2)
A. brasilense	38.33 a	1.41 c	42.38 b	2.81 b	12.75 a	84.46 bc
B. amyloliquefaciens	34.58 a	5.19 a	44.20 ab	3.05 ab	13.84 a	100.98 b
B. megaterium	35.08 a	4.41 ab	43.08 ab	2.22 c	12.04 a	64.36 c
B. pumilus	36.67 a	4.64 ab	45.37 ab	2.77 b	14.00 a	93.92 b
B. subtilis	41.25 a	6.05 a	49.85 a	3.40 a	13.96 a	129.54 a
Control	40.35 a	2.83 bc	45.42 ab	3.04 ab	13.92 a	109.32 ab
without reapplication	-	-	46.97 a	2.96 a	13.81 a	101.39 a
With reapplication	-	-	43.13 b	2.81 a	13.03 a	92.80 a
CV (%)	15.47	10.72	10.04	12.64	11.22	18.07
	NFlo	RL (cm)	RDM (g)	SDM (g)		TDM (g)
				without [1]	With[2]	
A. brasilense	2.25 ab	24.66 a	0.169 b	1.348 abA	0.774 cB	1.23 ab
B. amyloliquefaciens	2.46 ab	23.97 a	0.186 ab	1.668 aA	1.019 abcB	1.53 ab
B. megaterium	2.00 b	22.52 a	0.121 b	1.010 bA	0.909 bcA	1.08 b
B. pumilus	2.42 ab	22.04 a	0.179 ab	1.265 abA	1.344 abA	1.48 ab
B. subtilis	2.75 a	25.89 a	0.239 a	1.718 aA	1.518 aA	1.67 a
Control	2.33 ab	23.83 a	0.147 b	1.297 abA	1.297 abcA	1.44 ab
without reapplication	2.47 a	24.54 a	0.1877 a	-	-	1.57 a
with reapplication	2.26 a	23.10 a	0.1595 a	-	-	1.24 b
CV (%)	15.79	10.96	33.55	20.18		27.09

Means followed by the same lowercase letter in the column and uppercase letter in the row (SDM) do not differ from each other according to Tukey's test ($p \leq 0.05$). [1]without reapplication, [2]with reapplication.

Analyzing the results of phase 2, it was observed that the interaction between rhizobacteria and the number of applications was insignificant for the characteristics studied, except for the dry mass of the aerial part.

For the characteristics where the interaction was non-significant, no differences were observed between the treatments regarding the number of leaves and root length. For the other characteristics, the rhizobacteria *B. subtilis* stood out, presenting the highest averages, although not differing from the control and other bacteria (Table 1).

There was no significant difference between one or two applications for most of the characteristics studied, except shoot length and total dry mass. For these characteristics, the averages were lower when two applications were carried out (Table 1).

For the dry mass of the aerial part, where the interaction between rhizobacteria and the number of applications was significant. The bacteria *A. brasilense* and *B. amyloliquefaciens* showed lower averages when applied twice. In the other treatments, there was no difference between the number of reapplications. When only one application was carried out, *B. amyloliquefaciens* and *B. subtilis presented* the highest averages (1.668 g and 1.718 g, respectively), with no significant difference between the control and the rhizobacteria *A. brasilense* and *B. pumilus*. When two applications were carried out, *B. subtilis* stood out with the highest average (1.518 g), not differing significantly from the control or the rhizobacteria *B. amyloliquefaciens* and *B. pumilus* (Table 1).

According to the Pearson Correlation Matrix (Fig. 2), significant and positive correlations were found between most of the variables analyzed. When only one application of rhizobacteria was carried out (Fig. 2A), it was observed that the highest correlation coefficients were between total dry mass and dry mass of roots (r = 0.94), shoot dry mass and root dry mass (r = 0.91), shoot dry mass and leaf area (r = 0.81) and total dry mass and leaf area (r = 0.81). It is also noted that the root length variable was the least correlated with the others when only one application of rhizobacteria was carried out, with a correlation only with the root diameter (r = 0.42).

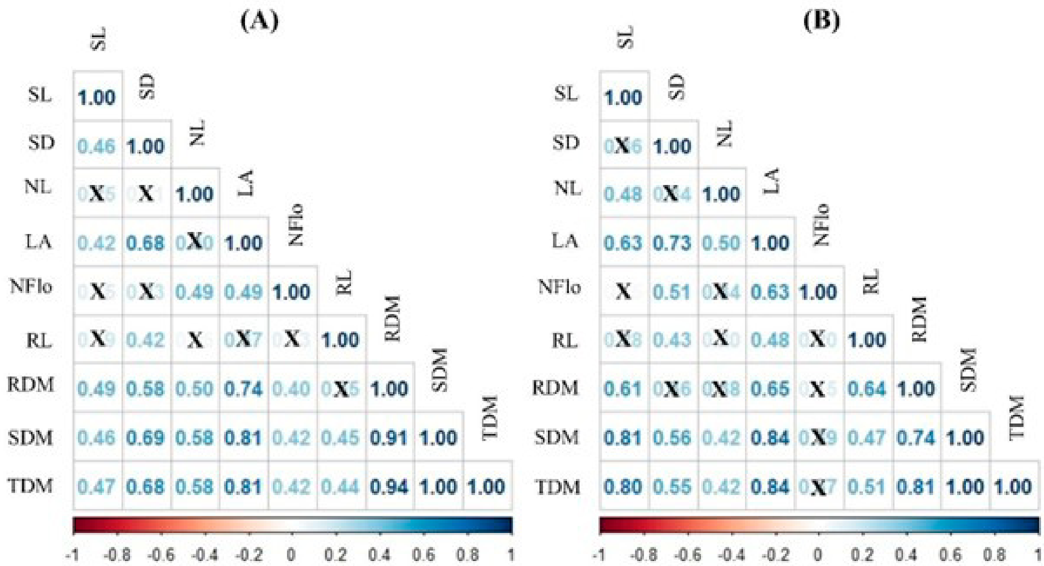

Figure 2. Pearson correlation matrices between the analyzed variables of marigold seedlings (*Tagetes erecta*) with one application (A) and two applications (B) of the rhizobacteria *Azospirillum brasilense*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Bacillus pumilus*, and *Bacillus subtilis*. Significant at 5% probability. An "**X**" over the values indicates no significant correlation between the variables. Where: SL = shoot length; SD = stem diameter; NL = number of leaves; LA = leaf area; NFlo = number of flowers; RL = root length; RDM = root dry mass; SDM = shoot dry mass; TDM = total dry mass.

When two applications of rhizobacteria were carried out (Fig. 2B), the highest coefficients were observed between the total dry mass and the dry mass of the aerial part (r = 1.00), the leaf area, the dry mass of the aerial part and the dry mass total (r = 0.84), the dry mass of the aerial part and the length of the aerial part (r = 0.81), the total dry mass and the dry mass of roots (r = 0.81) and between the total dry mass and shoot length (r = 0.80). The variable that was least correlated with the others was the number of flowers when two applications of rhizobacteria were carried out.

Discussion

Rhizobacteria did not influence the percentage of emergence, which was, on average, 38%. However, seeds emerged more quickly in the presence of rhizobacteria of the genus *Bacillus*, especially the species *B. amyloliquefaciens* and *B. subtilis* (Table 1). Campos et al. (2024) observed that inoculation of *B. amyloliquefaciens* efficiently produced *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos seedlings, although the influence on seedling emergence was not evaluated.

Studies have demonstrated the superiority of rhizobacteria of the genus *Bacillus*, especially *B. subtilis*, in seed germination and initial seedling growth. Silva et al. (2022), when investigating the effect of *B. subtilis* on seed germination and initial growth of watermelon cv. Sugar Baby observed that, although the microorganisms did not influence the germination percentage, they promoted faster germination of the seeds.

As with zinnia, other research has also demonstrated that the application of *B. subtilis* contributes to increased growth and development of seedlings and plants of various plant species such as corn - *Zea mays* L. (Guimarães et al., 2021), white oat - *Avena sativa* L. (Santos et al., 2021), watermelon - *Citrullus lanatus* [Thunb.] Matsum & Nakai (Silva et al., 2022), açaí tree - *Euterpe olerace*a Mart. (Campos et al., 2023) and *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos (Campos et al., 2024).

Furthermore, applying *B. subtilis* to *Arabidopsis thaliana* increased root surface, phosphorus solubilization, and greater nitrogen availability through biological fixation, promoting rapid growth expression in plants (Oleńska et al., 2020). Also, wheat seedlings inoculated with *B. subtilis* produced large amounts of exopolysaccharides, significantly increasing the osmotic potential, water potential, and chlorophyll content. These effects increased plant growth and development (Vocciante et al., 2022). These results reinforce the efficiency of these rhizobacteria as plant growth promoters.

Higher values of leaf area and number of leaves indicate a greater capacity to absorb sunlight, which, in turn, is associated with better photosynthetic capacity of the seedlings. This increase in photosynthetic capacity allows the development of other organs to be more accelerated (Taiz et al., 2017). Although no significant difference in the number of leaves was observed, seedlings inoculated with *B. subtilis* showed superior leaf area, reflected in other characteristics (Table 1). This suggests that this bacterium acted more efficiently in plant metabolism (Ngalimat et al., 2021), resulting in higher-quality plants with more flowers (Table 1).

No significant differences observed between treatments for root length (Table 1). However, it is well established that root length measurements can be problematic when using containers, as roots often grow along the surface of the pots. The physical limitations of containers can interfere with accurate root length assessments, as roots are constrained in their natural growth patterns. In pots, roots may not expand as freely as they would in field conditions, where they have greater freedom to explore and develop within the soil. Consequently, the use of containers can hinder the proper expansion of the root system, potentially leading to measurement inaccuracies and limiting the results' applicability to real-world agricultural or ecological settings (Gallegos et al., 2020).

The inoculation of *Bacillus megaterium* in zinnia resulted in lower averages for most of the analyzed characteristics (Table 1). However, this rhizobacteria demonstrated efficacy when associated with other plant species, such as bean (*Phaseolus vulgaris* L. cv. 'Elkoca-05'), which presented satisfactory results regarding biological nitrogen fixation and nodulation (Oleńska et al., 2020). Furthermore, the authors also observed increased plant growth when inoculated with this microorganism.

The unsatisfactory effect observed in the inoculation of *B. megaterium*, despite its positive effect on bean plants (Oleńska et al., 2020), may be related to several factors. One of them is a limitation of the container used, the temperature that may not have been ideal for the development of this bacterium, and, mainly, a possible lack of effective interaction with the plant species, as observed by Campos et al. (2023) on the growth and development of açaí seedlings.

The possibilities of interference in microbial life are diverse and wide-ranging. Abiotic factors, such as temperature, nutrient availability, pH, salinity, energy sources, and the presence of toxic elements, as well as biotic factors, represented mainly by microbial genetics and interactions between microorganisms, exert a significant influence on the survival and activity of microorganisms (Furtak and Galazka, 2019; Cavalcante et al., 2022).

In addition to the preferential interaction between rhizobacteria and the plant species, another factor that may have influenced the action of bacteria in the growth and development of zinnia would be the related environmental conditions, mainly the temperature and relative humidity of the air at the location. The conditions of the zinnia experiment (Fig. 1) were similar to those of the study carried out by Campos et al. (2023) with açaí seedlings, where they observed that the rhizobacteria that stood out in promoting the growth of the evaluated seedlings were also *B. subtilis*.

Rhizobacteria have been highlighted in increasing the growth and development of several plant species. In this study, the results demonstrated that the bacteria *B. subtilis* was effective in accelerating the emergence of seedlings and promoting the growth, development, and production of zinnia flowers sustainably (Table 1). The rhizobacteria *B. amyloliquefaciens* also proved efficient for the growth and development of zinnia.

The correlation between biometric variables and zinnia production showed significant associations, of which it was possible to verify positive correlations, with no negative correlations noted (Fig. 2). In the present study, positive associations between shoot dry mass, stem diameter, and leaf area are expected since the leaves represent these variables, and the stem is used to measure these characteristics (SDM). Furthermore, with both one application and two of the rhizobacteria, there were associations between total dry mass and dry mass of roots, indicating that this variable (RDM) has a direct influence on the accumulation of biomass of this species, in which, with its reduction, there is a decrease in the allocation of the total dry mass of zinnia plants.

It was also possible to note that when two applications of rhizobacteria were carried out (Fig. 2B), the variable number of flowers showed a positive correlation only between the diameter of the stem and leaf area, with no association with the other variables analyzed. Therefore, it can be inferred that these variables (SD and LA) are directly associated with flower production for this species. Thus, with the increase of these two variables, there will be a more outstanding production of flowers per zinnia plant.

Despite the advances achieved, the study presented important limitations that deserve to be highlighted. Some rhizobacteria, such as *Azospirillum brasilense*, *Bacillus megaterium* and *Bacillus pumilus*, did not show significant results in promoting the emergence, growth or development of *Zinnia peruviana* L. seedlings, limiting their effectiveness in comparison to *Bacillus subtilis* and *Bacillus amyloliquefaciens*. Furthermore, the absence of significant differences between one and two applications for all rhizobacteria suggests that reapplications may be unnecessary in certain conditions, which raises questions about the cost-effectiveness of these practices in other contexts. The evaluation was conducted under controlled conditions, which may not fully reflect the behavior of rhizobacteria in field scenarios, where environmental factors may influence their effectiveness.

For future studies, it would be interesting to investigate the application of these rhizobacteria in other ornamental species to verify if the results can be generalized across the species, as well as conducting experiments in varied environmental conditions, simulating field situations in order to evaluate the performance of rhizobacteria under different climate and soil influences. These studies could contribute to a better understanding of the potential of these biotechnologies in the sustainable cultivation of ornamental plants.

Conclusions

Rhizobacteria do not affect the percentage of emergence. However, zinnia seedlings emerge more quickly when inoculated with the rhizobacteria *Bacillus amyloliquefaciens* and *B. subtilis*. The rhizobacteria *B. subtilis* provides more notable growth, development, and flowering of zinnia, and there is no need for reapplication during the plant cycle.

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Author's contribution

MMS: Conceptualization, Data Curation, Investigation, Supervision, Writing - Original Draft. **ACB:** Data Curation, Formal Analysis, Investigation. **MCL:** Data Curation, Formal Analysis, Investigation. **AMBS:** Data Curation, Methodology, Software, Validation, Writing - Review & Editing. **TSC:** Formal Analysis, Methodology, Software, Validation, Writing - Review & Editing. **CHBS:** Formal Analysis, Investigation, Supervision. **ECR:** Conceptualization, Methodology, Resources, Visualization. **KFLP:** Conceptualization, Methodology, Project Administration, Validation, Visualization, Writing - Review & Editing.

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 made available on request.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that AI and AI-assisted technologies were not applied in the writing process.

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