

How do rhizobacteria species influence the growth and yield of soybean in a tropical environment?

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Abstract. The application of rhizobacteria has gained space in agricultural production, given the demand for more sustainable systems. However, most of the results obtained are related to soil or seed application, leaving a gap in relation to the foliar application of these microorganisms. The objective of this work was to evaluate the impact of foliar application of different types of growth-promoting rhizobacteria on morphological aspects and production components of soybean. For this, the foliar application of four rhizobacteria (*Serratia* sp.; *Bacillus subtilis*; *Bacillus* sp.; *Pseudomonas fluorescens*) was used, as well as a control without inoculation. Morphological variables of growth and production components were analyzed. The yield ratio of the treatments with rhizobacteria, concerning the control was also calculated. The foliar application with different rhizobacteria in soybean did not affect the vegetative parameters of plant height, stem diameter and dry weight of the canopy. For the number of pods per plant, number of grains per plant and grain yield the use of *Bacillus* sp. was superior to the other treatments, providing an average increase of 27.65%, 20.32% and 28.59%, respectively. Also, the *Serratia* sp., *Bacillus subtilis* and *Pseudomonas fluorescens* application increased the grain yield by 8.49%, 10.73% and 5.71%, respectively. In conclusion, for the condition of the tropical region where this study was conducted, the foliar application with different growth-promoting rhizobacteria in soybean did not interfere in the vegetative development of soybean plants. In addition, considering the factors related to the increase of production in cultivated areas, all rhizobacteria have the potential to improve yield gains when applied as foliar treatment, especially the *Bacillus* sp.

Key words: *Bacillus* sp., biostimulants, biological inputs, *Glycine max*, growth promotion, regenerative agriculture.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is the most economically and nutritionally important legume in the world. Because its grains contain 40% protein and 20% lipids (Saito et al., 2021), being employed also in the animal feed sector and for the production of biofuel, currently, the world's production exceeds 365 million tons (USDA, 2021).

Due to the exponential increase in the number of people in the world, there is a need to increase the production of food and make the best use of technology in agricultural production. The overuse of inputs, such as fertilizers and pesticides, can cause severe damage to health and the environment, leading producers to adopt sustainable measures that decrease the impact of agriculture on the environment (Canellas et al., 2015).

As a result of the growing demand for food as well as the concern to preserve the environment, there is interest in the manipulation of endophytic microorganisms in agricultural practices. Endophytic microorganisms are considered sustainable technologies for agricultural management, reducing dependence on chemical inputs, promoting a decrease in the use of fertilizers and insecticides, and consequently reducing the impact on the environment (Mahanty et al., 2017). In addition, microorganisms can be used in both plant growth promotion and biological control of plant pests and diseases (Ait-El-Mokhtar et al., 2019; Ben-Laouane et al., 2019; Abdel Latef et al., 2020).

Rhizobacteria are a select group of microorganisms that live closely associated with plants, colonizing their roots and providing for the development of their host (Adoko et al., 2021; Vendruscolo & Lima, 2021). This plant development is linked to the ability of rhizobacteria to contribute to the availability of nutrients that are in short supply in the soil, such as iron (Fe) and phosphorus (P), but also contributes by helping the plant in the defense against harmful microorganisms (Raklami et al., 2019; Wu et al., 2020).

Plant growth-promoting bacteria (PGPBs) are a group of microorganisms that are beneficial to plants and can colonize the root surface, rhizosphere, phyllosphere, and internal plant tissues. They can provide a biological alternative of great benefit, increasing crop productivity and reducing the need for fertilizers in agroecosystems (Marques et al., 2010). Thus, the purpose of raising the production of agricultural areas with the use of PGPBs has become an alternative and PGPBs have been interesting research molds to obtain formulation for manufacturing commercial products (Walia et al., 2014; Abdel Latef et al., 2020).

The rhizobacteria promote growth in soybeans, and the stimulation of growth influences other factors such as greater germination in the field, grain production, nutrient absorption, dry weight and height of the cultivars (Adoko et al., 2021; Kalenska et al., 2022). Growth promotion is attributed to the better absorption of nutrients by the roots, which increases the concentration of nutrients translocated to the leaves (Vendruscolo & Lima, 2021). According to Andy et al. (2020), rhizobacteria favor the development of the root zone of different legumes acting as biofertilizers and bioinoculants, because they aim to partially or even completely supply the demand for some development factors, such as nitrogen.

The use of PGPBs in inoculation and co-inoculation is an alternative for sustainable production (Rosas et al., 2009), being used as a technology with the potential to improve the productivity of agricultural systems in the long term and not attack in a harmful way the environment (Naiman et al., 2009; Silva et al., 2020). Thus, Korber et al., 2021; Silva et al., 2023, suggest that the production of inoculants in a sustainable manner with rhizobacteria is an alternative to decrease the environmental risks caused by the inappropriate, and sometimes excessive, use of inputs and pesticides.

In addition to the benefits found with the inoculation of PGPBs during seed treatment or via direct application to the soil, it appears that their use in the form of foliar application also allows gains in terms of the vegetative development of plants and increased productivity. These results are mainly related to the increase in leaf health, as observed for *Bacillus* sp. (Ortiz et al., 2022) and *Serratia* sp. (Nagrle et al., 2023), which can be used as bioproducts for biological control and for ameliorating abiotic stress effects. But also, the effects may be related to metabolic gains and increased nutrient absorption (Mahmood et al., 2022), increased gas exchange activity (Silva et al., 2020) and accumulation of energetic reserves (Asghari et al., 2020).

Thus the use of these rhizobacteria is of fundamental environmental, ecological, and economic importance, as it results in savings for farmers and an improvement in soil fertility. Therefore, due to the use of pesticides, these rhizobacteria can be affected. In light of the above, this work aimed to evaluate the impact of foliar application of different types of growth-promoting rhizobacteria on morphological aspects and production components of soybean.

MATERIALS AND METHODS

The trial was conducted at Fazenda Sozinha, in Leopoldo de Bulhões, belonging to the State of Goiás, with a South latitude of 16° 32'02.4", West longitude of 48° 57'49.3" and an altitude of 1,008 m. The region's climate, according to Köppen, is classified as Aw (tropical with dry season), with a minimum of 18 °C and maximum of 28 °C, with an average temperature of 22 °C, and average annual rainfall of 1,450 mm (Cardoso et al., 2014).

The soil of the property is classified as LATOSSOLO VERMELHO (Oxisol) with a sandy loamy texture. As planting fertilization, 150 kg ha⁻¹ of K₂O and 250 kg ha⁻¹ of P₂O₅ were applied, where the K₂O was applied before planting, in total area, and the P₂O₅ was applied directly in the planting furrow.

The cultivar used was the 'CZ 26B77 IPRO', which has a transgenic related to tolerance to glyphosate herbicide (RR technology), being super-early with excellent yield potential (Cultivar Catalog, 2020). This cultivar belongs to the relative maturity group of 6.7, with indeterminate growth habits, and medium to high fertility requirements, its population recommendation is 300–320 thousand plants ha⁻¹, and its cycle time varies from 100 to 105 days.

The seeds obtained industrial seed treatment (TSI) with Standak®Top at a dosage of 200 mL 100 kg⁻¹ of seed. Inoculation was performed with *Bradyrhizobium elkanii*, with the commercial name Gelfix 5® at a dosage of 200 mL ha⁻¹.

Sowing was performed on November 4, 2020, with a sower-tractor set, where an area of 400 m² was planted, divided into 40 rows spaced at 0.50 m, and a length of 20 m.

The experimental design was a randomized block with five treatments, one control and all four repetitions. Each repetition was a 4.0 m wide by 4.0 m long plot, and the six central lines were evaluated, totaling a usable area of 9.0 m². The spacing between blocks was 0.5 m and between plots 0.5 m, totaling 300,000 seeds ha⁻¹.

The treatments used were with biological products, being the bacterial strains *Serratia* sp. and *Bacillus subtilis* from the Collection of Microbial Isolates of the Agrolab Laboratory, and the strains of *Pseudomonas fluorescens* from the Collection of Multifunctional Microorganisms of Embrapa Rice and Beans. The initial bacterial concentration of all biological products used was 1×10⁹ CFU mL⁻¹, being: T1 - *Serratia* sp.; T2 - *Bacillus subtilis*; T3 - *Bacillus* sp.; T4 *Pseudomonas fluorescens*; T5 - fresh water (control). The treatments were applied using a knapsack sprayer, with an application speed of 1.0 m s⁻¹ and a syrup volume of 140 L ha⁻¹, at the V2 and V5 stages in the vegetative phase, and R1 and R5.1 in the reproductive phase. The biological materials were concentrated in an aqueous solution and were subsequently diluted in fresh water for application in the aforementioned concentrations.

For the evaluations, 10 random plants were collected from the four central rows of the plot and evaluated at the R1 stage: canopy fresh weight, aboveground height, and stem diameter. For height a tape measure graduated in centimeters was used, for the diameter of the stem a digital caliper was used and the measurement in millimeters. In the canopy fresh weight of ten plants was used, then only the aerial part was selected, and the fresh weight was estimated using a precision balance.

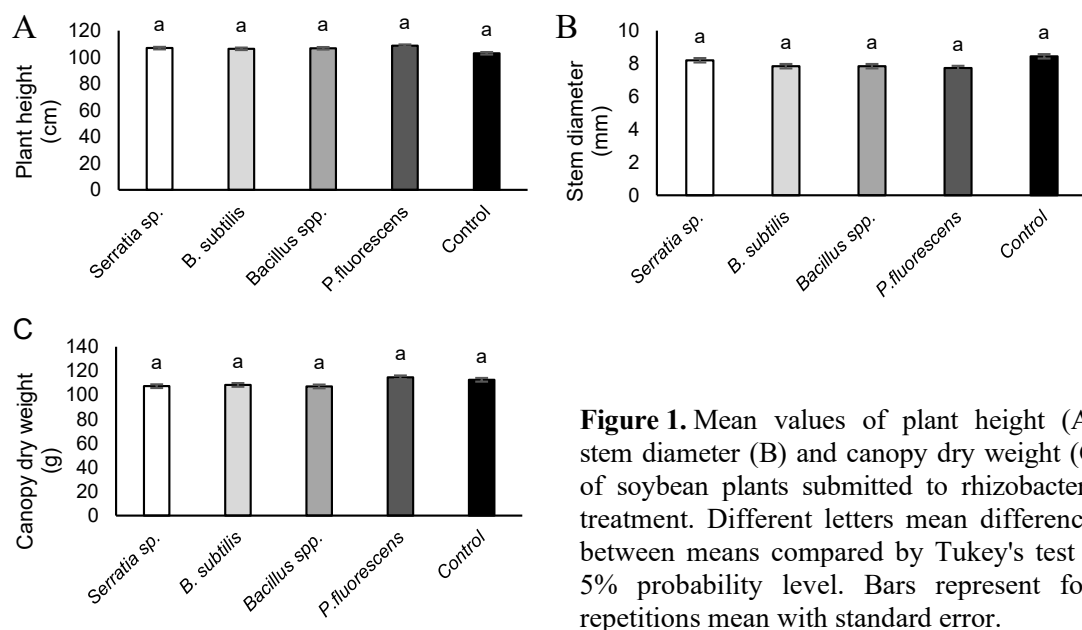
At the R9 stage, 10 plants per plot were collected and the following parameters were evaluated: number of pods per plant, number of grains, average number of grains per pod, and grain yield per hectare of each plot. The percentage yield gain of the treatments over the control treatment was also calculated.

The mean data of vegetative development and those related to grain production were submitted to analysis of variance and the means were compared using the Tukey test at 5% probability, except for the percentage gain in grain yield, which was expressed as a percentage. The software SISVAR 5.6 (Ferreira, 2014) was used for the statistical analyses.

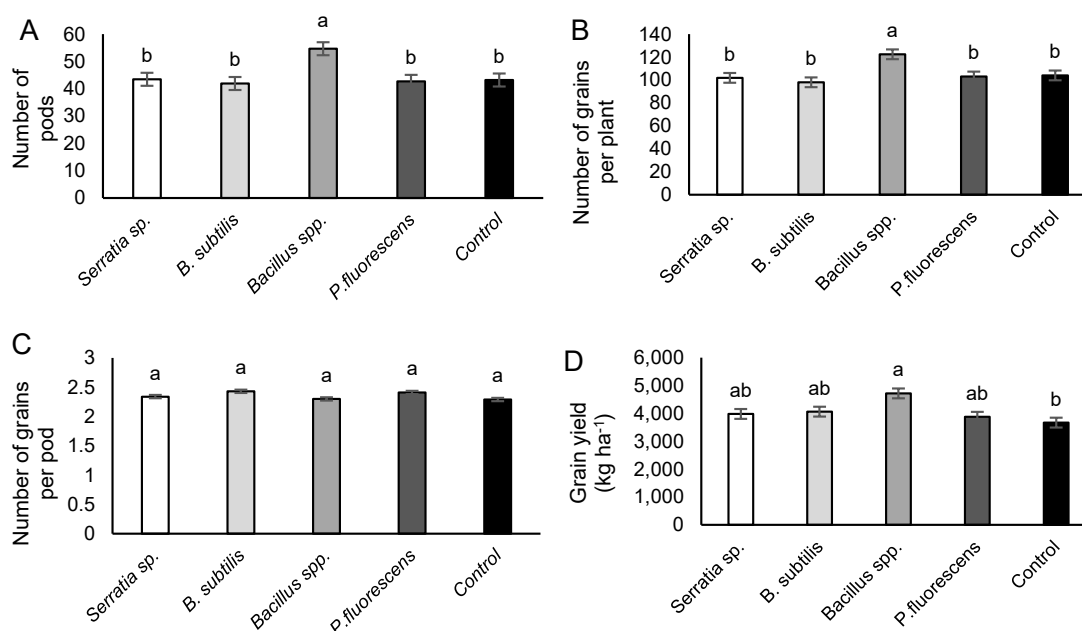
RESULTS AND DISCUSSION

It was found that for plant height, stem diameter, and aboveground fresh weight there were no significant effects of the treatments with the rhizobacteria (Fig. 1).

The results related to the vegetative growth (Fig. 1), found in the present experiment, do not corroborate with those observed by Paula et al., (2021), who state that inoculation with rhizobacteria is viable for stimulating the development of soybean plants. But these authors also showed that different bacterial isolates can act differently concerning soybean plants, influencing the production of phytohormones, nutrient uptake, or even interacting antagonistically with possible pathogens that may attack the crop. In addition, there is also the possibility that the genetic material of the chosen cultivar does not respond to the stimulation during vegetative growth. Environmental conditions may also have masked the initial effects of the rhizobacteria's action, given that the plants developed properly and without health problems.



For the number of pods per plant (Fig. 2, A), the use of *Bacillus* sp. bacteria was superior to the other treatments, providing an average increase of 27.65%. The other treatments showed no difference among themselves or concerning the control treatment.



Also, for the parameter of the number of grains per plant (Fig. 2, B), the treatment with *Bacillus* sp. showed a significant difference from the other treatments, with average superiority of 20.32%, equivalent to 20.67 grains per plant.

There was no difference between treatments concerning the number of grains per pod (Fig. 2C). However, it was found that there was an increase in grain yield when the treatment with *Bacillus* sp. was performed, but without a significant difference to the other treatments composed by the application of rhizobacteria (Fig. 2D).

Compared to the control treatment, inoculation with *Bacillus* sp. promoted the greatest gain in grain yield, about 28.59%, equivalent to more than 1,048 kg ha⁻¹. In sequence, the treatments with *Serratia* sp., *Bacillus subtilis* and *Pseudomonas fluorescens* also resulted in yield gains of 8.49%, 10.73% and 5.71%, respectively (Fig. 3).

The beneficial effect of applying *Bacillus* sp. is largely related to its ability to act protectively against the attack of diseases common to soybean cultivation, such as Asian rust (*Puccinia pachyrhizi*), downy mildew (*Peronospora manshurica*), target spot (*Corynespora cassiicola*), brown spot (*Septoria glycines*) and Anthracnose (*Colletotrichum truncatum*). By helping to control these diseases, the application of *Bacillus* reduces the loss of leaf area

of soybean plants, which is directly linked to the plant's productive capacity, while it appears that when managing the application of the bacteria together with fungicides chemicals, it is possible to obtain superior results (Santos et al., 2022).

Inoculation via foliar application with *Bacillus* sp., alone or in combination with other microorganisms, also results in a higher photosynthetic rate of plants, accompanied by greater root development and greater accumulation of nutrients in plant organs (Silva et al., 2020; Solanki et al., 2023), which is positively related to the productive capacity of soybean plants (Moretti et al., 2020). However, as observed in the present study, it appears that these effects have little interference in relation to vegetative growth (Solanki et al., 2023).

The positive effects of applying *Bacillus* sp. on reproductive characteristics are verified for other cultivated plant species, such as tomato in an organic cultivation system, for which the application of *B. megaterium* increased the productive capacity, in addition to improving the quality of the harvested fruits (Yagmur & Gunes, 2021). In addition, the application of *B. Amyloliquefaciens* was efficient for the control of Botrytis in cucumber plants grown in a protected environment, also contributing to the increase in fruit productivity of this species (Nakkeeran et al., 2020).

Despite the inferior results obtained with other microorganisms, in relation to *Bacillus* sp., it appears that there is potential for its use (Fig. 3). In this sense, new studies should explore different application management, verifying better environmental conditions and also taking into account the genetic material used. Other studies have shown that the application of *Serratia* sp. has a positive effect on alleviating abiotic

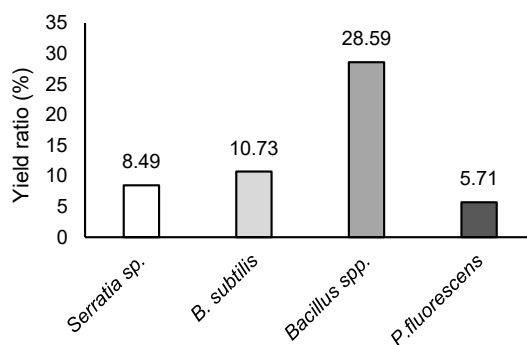


Figure 3. Yield ratio of soybean plants subjected to rhizobacteria treatment.

(Moon et al., 2023) and biotic (Ortiz & Sansinenea, 2023) stresses. In addition, similar effects were observed for *Pseudomonas fluorescense*, verifying its ability to act against pathogens of cultivated species (Vicentini et al., 2022) and increasing leaf nutrient content, as well as lentil grain productivity (Erdemci, 2020).

Considering the results, the present study brings a new perspective on the use of rhizobacteria in soybean production, showing that foliar application can have a positive effect on the characteristics linked to grain production. There is also a need for future studies that point to better management of this application, taking into account the possibility of combinations between the studied materials and other inputs, such as nutrients, beneficial elements, bio-inputs, hormones and chemical products conventionally used in the cultivation of soybean.

CONCLUSIONS

For the condition of the tropical region where this study was conducted, the foliar application with different growth-promoting rhizobacteria in soybean did not interfere in the vegetative development of soybean plants. In addition, considering the factors related to the increase of production in cultivated area, all rhizobacteria have the potential to improve yield gains when applied as foliar treatment, especially the *Bacillus* spp.

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