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Enhancing growth and quality of *Handroanthus impetiginosus* (Mart. ex DC.) Mattos (Bignoniaceae) seedlings by rhizobacteria inoculation

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Abstract

Background: *Handroanthus impetiginosus* (lavender trumpet tree) is valued for its construction, medicinal, and ecological uses. However, its slow initial growth and weak root system hinder seedling production. Rhizobacteria from the genera *Bacillus* and *Azospirillum* enhance plant growth and resilience. This study evaluated their effects on *H. impetiginosus* seedling development.

Methods: The experiment was conducted in a greenhouse at the Experimental Nursery of Ornamental and Forestry Plants - College of Agricultural and Veterinary Sciences (UNESP/FCAV), Jaboticabal, Brazil, during the summer 2022/23. A completely randomised design included five treatments: *Bacillus subtilis*, *B. megaterium*, *B. amyloliquefaciens*, *Azospirillum brasilense*, and a control (no inoculation). Each treatment had four replicates of 20 plants. Seeds were sown in 280 cm^3 tubes with a commercial substrate, composed of peat, vermiculite, roasted rice husk, calcined dolomitic limestone, NPK 14-16-18, and micronutrients. The rhizobacteria were inoculated at 30 and 60 days after sowing. Growth parameters (shoot height, stem diameter, root length, and biomass) were assessed at 107 days. Photosynthetic performance and microbiological colonisation were also evaluated. Data were analysed using ANOVA, Tukey's test and Pearson correlation.

Results: *Azospirillum brasilense* significantly enhanced growth, with the highest averages for shoot height (13.9 cm), stem diameter (1.72 mm), shoot dry mass (0.172 g), and total dry mass (0.686 g). It also improved seedling quality indices, including the Dickson Quality Index and shoot height-to-stem diameter ratio. Photosynthetic efficiency increased when inoculated with *Azospirillum brasilense*, as indicated by greater leaf area and chlorophyll content. Colony Forming Units (CFU) analysis showed higher bacterial colonisation in the substrate, roots, and aerial parts of *A. brasilense*-treated plants, with strong correlations between colonisation and plant growth.

Conclusions: *Azospirillum brasilense* was the most effective rhizobacterium promoting *H. impetiginosus* seedling growth and quality. Its use could enhance reforestation and nursery production efficiency, accelerating seedling establishment. These findings highlight the potential of rhizobacteria to improve seedling vigour and adaptation in early growth stages.

Keywords: *Azospirillum*; *Bacillus*; forest nurseries; ipê-roxo; photosynthesis; plant growth promoting microorganisms.

Introduction

The lavender trumpet tree or ipê-roxo (*Handroanthus impetiginosus* Mart. ex DC. Mattos), a member of the Bignoniaceae family, is a towering evergreen tree that can reach up to 30 metres in height. Notable for its striking magenta-coloured flowers, this species is commonly found in dry seasonal tropical forests throughout Central and South America (Lorenzi 2020).

The *H. impetiginosus* species has diverse applications: its wood is utilised in furniture manufacturing and in civil and naval construction due to its superior technological quality, high mechanical strength, and long-lasting durability (Silva-Junior et al. 2018). Additionally, its bark, flowers, and leaves are valued in phytopharmaceuticals for their bioactive compounds with anti-cancer, anti-inflammatory, antioxidant, antibacterial, and anti-leishmanial properties (Valle et al. 2023; Ahmad et al. 2020; Mariano et al. 2022; Santos et al. 2023). Beyond these uses, *H. impetiginosus* is also recommended for rehabilitating degraded areas (Viana et al. 2019) and for ornamental purposes, thanks to its aesthetic appeal during the flowering period, making it suitable for landscaping projects and urban afforestation (Lorenzi 2020).

During the initial growth stage of the lavender trumpet tree species, slow development and a weak root system are common, adversely affecting height growth and overall plant quality (Lima et al. 2014). These conditions can significantly undermine plant survival in later stages (Viana et al. 2019). Therefore, producing and ensuring the availability of robust, high-quality seedlings is crucial for the successful utilisation of this species.

One promising approach for developing more resistant and healthier plants in less time is the use of plant growth-promoting bacteria. Numerous studies have shown that specific species of bacteria, known as rhizobacteria, can colonise plant roots and provide direct stimuli or confer benefits that enhance growth, development, and resistance to both biotic and abiotic stressors in various plant species (Castro et al. 2020; Kondo et al. 2020; Alexandre et al. 2021; Campos et al. 2023; Rodrigues et al. 2023; Tarh et al. 2023).

In the rhizosphere, which extends from the root surface to a depth of 1 to 3 mm, a substantial portion of the root surface—approximately 7% to 15%—is occupied by a diverse array of bacteria. This region is critical for plant development and is characterised by significant microbiological activity involving several key species (Santoyo et al. 2016). Rhizobacteria can colonise extracellular spaces within the root tissues of various plants, where they promote vegetative growth by producing specific signals or substances, such as plant growth hormones (Olanrewaju et al. 2017).

Within the group of rhizobacteria, the genus *Bacillus* plays a significant role in influencing plant growth and development through the synthesis and release of phytohormones, such as cytokinin, abscisic acid, and gibberellins (Miljaković et al. 2020). Furthermore, these rhizobacteria have potential as biological agents against plant diseases and exhibit resistance to water, saline, and thermal stresses (Hashem et al. 2019).

Bacteria of the genus *Azospirillum* are highly beneficial to plants due to their unique ability to fix atmospheric nitrogen (N_2), thus making it more accessible for plant use (Fukami et al. 2018). These bacteria also can stimulate the production and release of phytohormones, including auxins, gibberellins, and cytokinins (Cassán et al. 2020).

The symbiotic association between plants and bacteria of the genera *Bacillus* and *Azospirillum* offers numerous benefits for plant growth and health. The ability of the *Handroanthus impetiginosus* to interact with naturally endophytic bacteria was confirmed by Yarte et al. (2022), who collected leaves and roots from naturally distributed adult trees. They identified native endophytic bacteria belonging to the genera *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, *Rummeliibacillus*, and *Methylobacterium*. Many of these strains demonstrated significant potential to promote plant growth and enhance resistance to challenging conditions, such as high salinity. Llacsa et al. (2019) also investigated the microbial diversity in the rhizosphere of *H. chrysotrichus* and *Handroanthus billbergii* (Bureau & K. Schum.) S.O.Grose, identifying more than 546 bacterial species and 154 fungal species. The success of *in vitro* propagation involving the interaction between *H. impetiginosus* and *A. brasilense*, was indicated by Larraburu & Llorente (2015 a,b). Their findings showed that bacterial colonisation enhanced cell division, secondary stem growth, and root density compared to non-inoculated plants. This understanding of symbiotic associations and bacterial diversity is essential for advancing rhizobacterisation technology in the Bignoniaceae family. However, given that this interaction has not been tested *ex vitro*, the extent to which these benefits can be consistently reproduced in natural environments remains uncertain.

Given the above, this study aimed to evaluate the effect of plant growth-promoting rhizobacteria on the nursery production of *H. impetiginosus* seedlings.

Methods

Study site

The experiment was conducted from September 2022 to January 2023 at the Experimental Nursery of Ornamental and Forestry Plants - College of Agricultural and Veterinary Sciences (UNESP/FCAV), Jaboticabal Brazil (21°14’45” S, 48°18’58” W, at an elevation of 595 m). The climate of the microregion, classified as type Aw (tropical savannah) according to the Köppen-Geiger system, features dry winters. The climatic data regarding maximum temperature, minimum temperature, average temperature, and relative humidity during the experiment period (September 2022 to January 2023) are shown in Figure 1 (UNESP, 2023).

The Experimental Nursery was equipped with black screens on the sides to filter light, allowing 50% of the light to pass through, and a transparent plastic cover on top of the screen. Irrigation was managed using micro-sprinklers, automatically activated three times per day

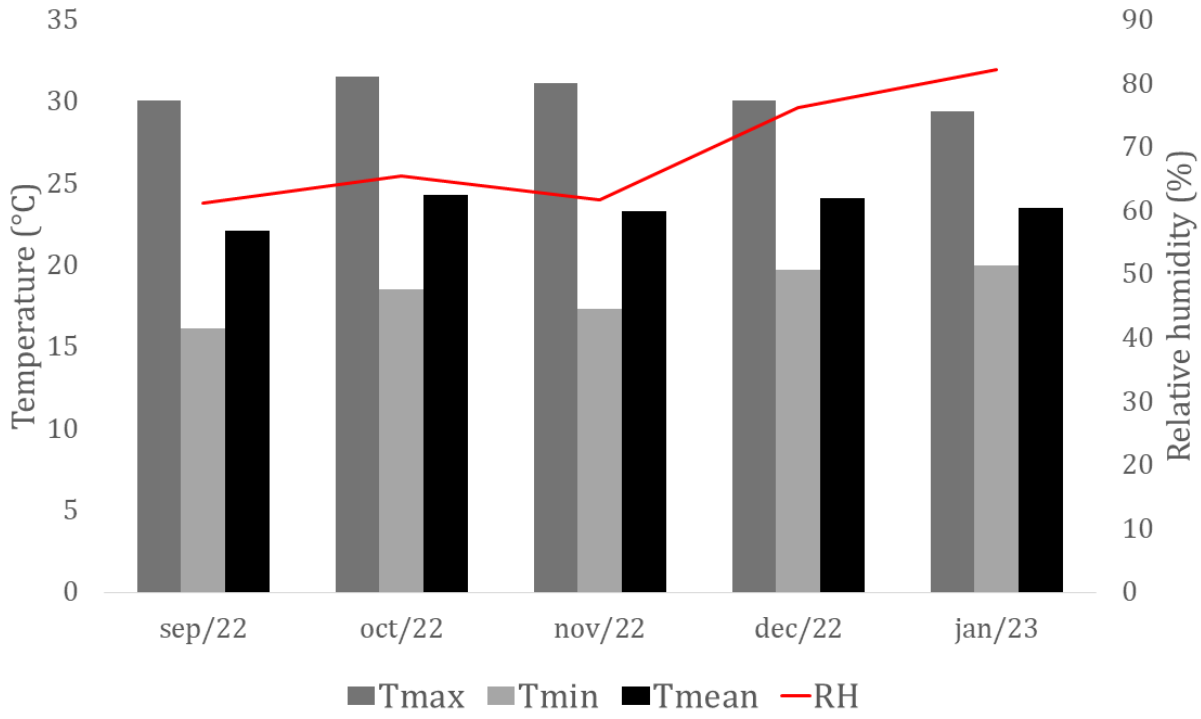

FIGURE 1: Climatic data from September 2022 to January 2023. Maximum air temperature - Tmax, Minimum air temperature - Tmin, Average air temperature - Tmean, and Relative humidity - RH.

for 15 minutes per session. Based on this configuration, the irrigation rate corresponded to approximately 4.17 L/m^2/day. The trays used in the nursery measured 43.5 cm by 62 cm and held 54 tubes each, resulting in a planting density of approximately 200 seedlings per square metre.

Inoculum production

The microorganisms used in the study are part of the collection from the Soil Microbiology Laboratory of the Department of Plant Production at UNESP-FCAV, Jaboticabal Campus. They were individually cultured in nutrient broth for seven days in bottles maintained in a B.O.D. incubator (Eletrolab, model 347 F, Brazil) at 25 °C. Following incubation, the microorganisms were centrifuged separately at 10,000 rpm for 10 minutes at 28 °C (Novatecnica, model MLW K24, Brazil). The inoculum concentration was standardised to 1 x 10^7 CFU mL^{-1}, as per the recommendations of Barry & Thornsberry (1991) and Sahm & Washington (1991), using a spectrophotometer (Micronal, model B382, Brazil) with an absorbance measurement at 695 nm.

Experimental design

The experimental design was completely randomised, consisting of five treatments (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloliquefaciens*, *Azospirillum brasilense*, and a control - absence of rhizobacteria), with four replicates consisting of twenty plants each.

Seeds of *H. impetiginosus* collected in September 2021 from existing matrices at the Experimental Nursery of Ornamental and Forestry Plants at the College of Agricultural and Veterinary Sciences (UNESP/FCAV) were used for the experiment. The seeds were sown in tubes with a volumetric capacity of 280 cm^3, which were then placed in polypropylene trays holding 54 containers each. The trays contained Carolina Soil® as the commercial substrate, which is composed of peat, vermiculite, roasted rice husk, calcined dolomitic limestone, and enriched with NPK 14-16-18 fertiliser, also includes micronutrients, with the following concentrations: Fe (150 ppm), Mn (90 ppm), Zn (60 ppm), Cu (25 ppm), B (20 ppm), and Mo (0.9 ppm), according to the product packaging. These trays were positioned on metal mesh benches 70 cm above the ground within a nursery. The seeds were planted in each tube, and thinning was performed five days after emergence, leaving only the most vigorous and centrally located seedling per container.

Substrate rhizobacterisation

Microorganism inoculation was performed twice: first at 30 days after sowing and again at 60 days. A 1.0 mL solution of the inoculum was applied to the substrate near the stem using a mechanical micropipette (VF-1000, Digipet®). Seedlings designated for the control treatment were not subjected to inoculation.

Growth, quality and photosynthetic variables

When 50% of the roots began to emerge at the lower base of the tubes, corresponding to 107 days after sowing, the experiment was evaluated for all the tested variables (Table 1).

TABLE 1: Variables assessed in *H. impetiginosus* seedlings at 107 days after sowing

Variable	Description	Method/Instrument
Shoot Height (cm)	Measured from substrate level to the apex of the last leaf.	Millimetric ruler
Stem Diameter (mm)	Measured at substrate level.	Digital calliper (Western® PRO DC-6)
Number of Fully Expanded Leaves	Counted manually.	Manual counting
Root Length (cm)	Measured as the length of the longest root.	Millimetric ruler
Shoot Dry Mass (SDM)	Obtained by drying at 70ºC until reaching a constant weight.	Precision scale (SHIMADZU®, model AY220)
Root Dry Mass (RDM)	Obtained by drying at 70ºC until reaching a constant weight.	Precision scale (SHIMADZU®, model AY220)
Total Dry Mass (TDM)	Calculated as the sum of shoot and root dry masses.	Derived from SDM and RDM results
Shoot Height / Stem Diameter (SH/SD)	Ratio calculated between shoot height and stem diameter.	Calculated from shoot height and stem diameter
Dickson Quality Index (DQI)	Calculated based on the dry mass and height-to-diameter ratio.	Calculated using standard formula
Leaf Area (cm^2)	Measured using an electronic leaf-area meter.	Li-3100C, LI-COR®
Chlorophyll Content (SPAD)	Measured to assess chlorophyll levels in leaves.	ClorofiLOG device, model CFL1030, FALKER®
Minimum Fluorescence (Fo)	Fluorescence measurement during photosynthesis assessment.	OS30p Fluorometer (Opti Science)
Maximum Fluorescence (Fm)	Fluorescence measurement during photosynthesis assessment.	OS30p Fluorometer (Opti Science)
Maximum Photochemical Efficiency (Fv/Fm)	Measuring photosystem II photochemical efficiency.	OS30p Fluorometer (Opti Science)

The Dickson Quality Index (DQI) was determined using the formula proposed by Dickson in 1960, as described in Souza et al. (2023):

DQI = TDM (g)/(SH/SD) + SDM (g)/RDM (g)

Colony Forming Units (CFU) variable

To observe the mobilisation of rhizobacteria after inoculation, bacteria were counted in the substrate, roots, and aerial parts (Table 2) of plants where rhizobacteria were applied.

Statistical analyses

The data obtained were analysed using analysis of variance (ANOVA), and mean differences were compared with the Tukey test at a 5% significance level, using AgroEstat Software (Barbosa & Maldonado Junior, 2015). Pearson correlations were performed using the R software, version 4.4.1 (R Core Team, 2024).

Results

The rhizobacteria *Azospirillum brasilense* showed the highest averages for shoot height (13.9 cm), stem diameter (1.72 mm), shoot dry mass (0.172 g), and total dry mass (0.686 g) (Figure 2). When compared to *Bacillus subtilis*, *A. brasilense* showed no difference in root dry mass, with averages of 0.503 g and 0.443 g, respectively (Figure 2E). In terms of root length, the rhizobacteria did not differ from each other, with an average of 19.98 cm, with all of them, except *B. amyloliquefacens*, superior to the control (Figure 2C).

Azospirillum brasilense exhibited the highest average values for both the shoot height-to-stem diameter ratio (SH/SD) (8.82) and the Dickson Quality Index (DQI) (0.089). These values were significantly higher than those observed with other rhizobacteria and the control, representing an increase of 18.8% for the SH/SD ratio and 60.36% for the DQI compared to the control (Figure 3).

Although root length did not show a significant increase (Figure 3C), there was a notable enhancement in dry mass due to an increase in secondary root formation (Figure 3E). This improvement in root development is evident in the morphological appearance of the lavender trumpet tree seedlings at 107 days after sowing (Figure 4E). The observed gains are reflected not only in the root system but also in other vegetative parts of the seedlings. Figure 4 provides a comparative view of the morphological improvements achieved across different treatments.

TABLE 2: Methodology for bacterial quantification in substrate and plant tissues

Step	Process	Details
Substrate Bacteria Counting	Preparation	10 g fresh material + 95 mL 0.1% sodium pyrophosphate saline solution in an Erlenmeyer flask.
	Shaking	Shake for 1 hour.
	Serial Dilutions	Prepare dilutions from flask contents as per Wollum (1983).
	Plating	Plate 100 µL from each dilution onto nutrient agar (triplicates).
	Incubation	Incubate plates at 30°C in a B.O.D. chamber for 24, 48, and 72 hours.
	CFU Counting	Count Colony Forming Units (CFUs) after incubation (Vieira & Nahas 2005).
	Comment	Does not account for Viable but for Non-Culturable (VBNC) or slow-growing bacteria.
Plant Tissue Bacteria Counting	Preparation	Separate aerial parts and roots, wash with running water.
	Disinfection	1 g vegetative tissue (leaves and roots) soaked in: 70% ethanol (1 min), 2-2.5% sodium hypochlorite (3 min), 70% ethanol (30 sec), washed 3x with distilled water (Araújo et al. 2002).
	Maceration	Aseptically macerate tissues using flask and pestle.
	Plating	Plate 100 µL from tissue dilutions onto tryptone soy agar plates.
	Incubation	Incubate plates at 30°C for 24 hours.
	CFU Counting	Count CFUs after 24, 48, and 72 hours.
	Comment	Does not account for Viable but for Non-Culturable (VBNC) or slow-growing bacteria.

The photosynthetic parameters of lavender trumpet tree seedlings (Figure 5), including leaf area, SPAD index, and the maximum photochemical efficiency of photosystem II, highlighted the impact of the rhizobacterium *A. brasilense*. The average values for this treatment were 32.43 cm^2 of leaf area, 18.86 SPAD units, and a maximum photochemical efficiency (Fv/Fm) of 0.738. Similarly, *B. subtilis* showed values of 28.22 cm^2 of leaf area, 18.14 SPAD units, and a Fv/Fm of 0.705. For the number of leaves, the average was 4, not varying between treatments.

Regarding Colony Forming Units (CFU) attribute (Figure 6), the inoculation of the rhizobacteria *A. brasilense* resulted in significantly higher bacterial colonisation in the substrate (6,071,262 CFU/mL), roots (98,628 CFU/mL), and aerial parts (6,636 CFU/mL) compared to the other rhizobacteria inoculation.

When analysing the Pearson correlation matrix (Figure 7), significant positive and negative correlations were found among the variables. The correlations between growth quality parameters ranged from –0.47 (between root length and DQI) to 0.99 (between root dry mass and total dry mass) (Figure 7A). All growth quality and colony-forming parameters exhibited good positive correlation among themselves, except from root length, that did not have a strong correlation with any variable. The highest correlation among growth quality and colony-forming variables were observed between shoot height and substrate CFU (0.72), shoot CFU (0.68), and root CFU (0.80). Additionally, stem diameter and DQI also showed strong correlations with colony-forming variables. Pearson correlations between photosynthetic and colony-forming variables varied from –0.14 (between Fv/Fm and substrate CFU) to 0.88 (between leaf area and shoot CFU) (Figure 7B). Shoot and root CFU variables exhibited strong positive correlations with photosynthetic parameters, whereas substrate CFU showed weak correlations with the other variables.

Discussion

The results obtained in the lavender trumpet tree seedling production experiment via inoculation of rhizobacteria in substrate showed a significant effect ($P<0.05$) for all parameters evaluated except the number of leaves. The observed improvements in shoot height, stem diameter, and total dry mass in seedlings inoculated with *A. brasilense* and *B. subtilis* suggest that these rhizobacteria enhance overall plant growth. However, to better understand whether the plants were simply larger or if the root system was delivering relatively more resources to the aerial components, it is essential to evaluate shoot-to-root ratios and root efficiency metrics.

The root system plays a critical role in water and nutrient uptake, directly influencing shoot development. In this study, *A. brasilense* inoculation resulted in a 50.2% increase in root dry mass and a 45.9% increase in shoot dry mass, while *B. subtilis* increased root dry mass

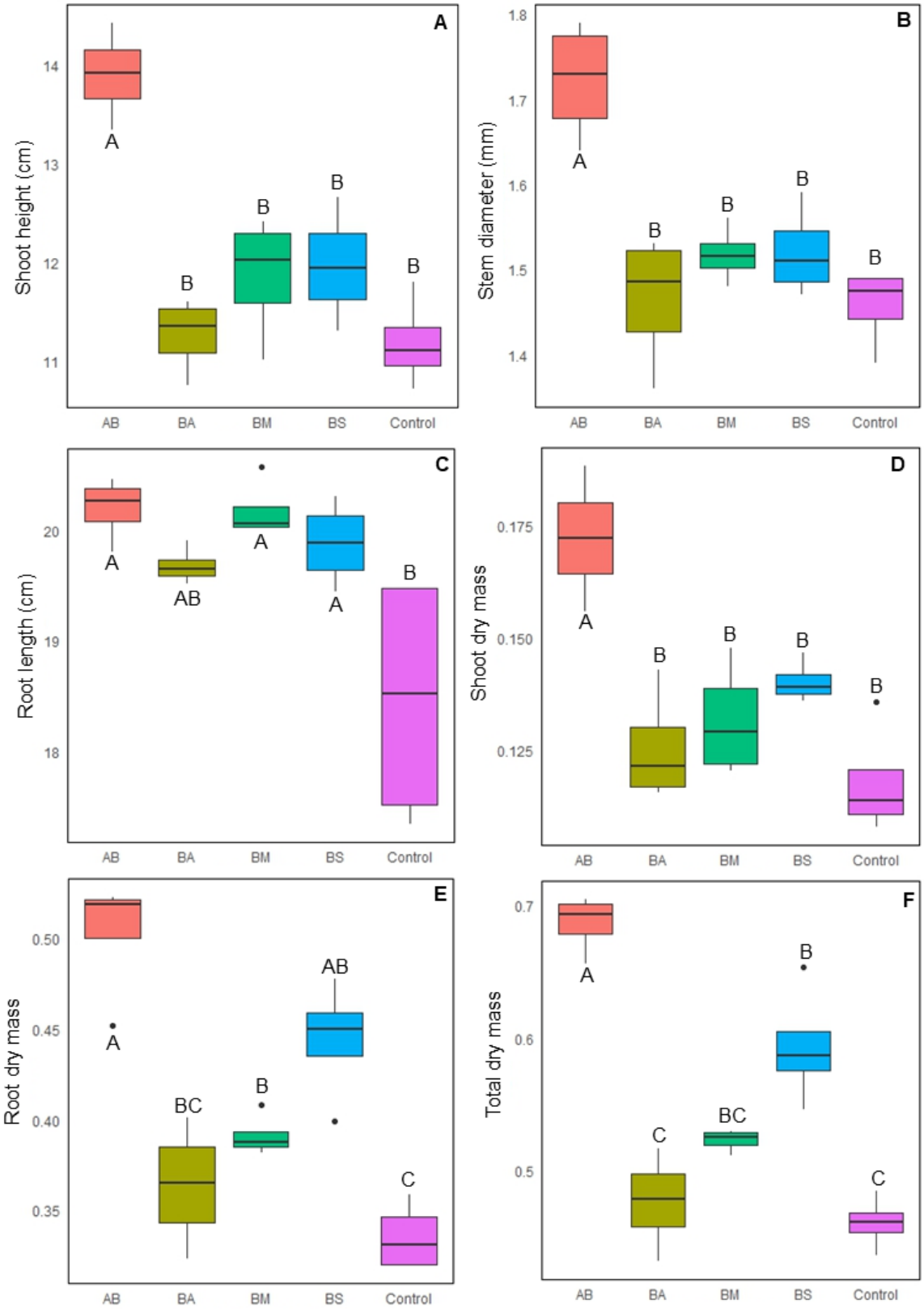

FIGURE 2: Boxplots of growth evaluation of *Handroanthus impetiginosus* seedlings with and without inoculation of rhizobacteria. A – shoot height (cm), B – stem diameter (mm), C – root length (cm), D – shoot dry mass (g/plant), E – root dry mass (g/plant), F – total dry mass (g/plant). Means followed by the same letter do not differ by Tukey's test ($p \leq 0.05$); n = 4 replicates per treatment. *Azospirillum brasilense* (AB), *Bacillus amyloliquefaciens* (BA), *Bacillus megaterium* (BM), *Bacillus subtilis* (BS) and without inoculation (Control).

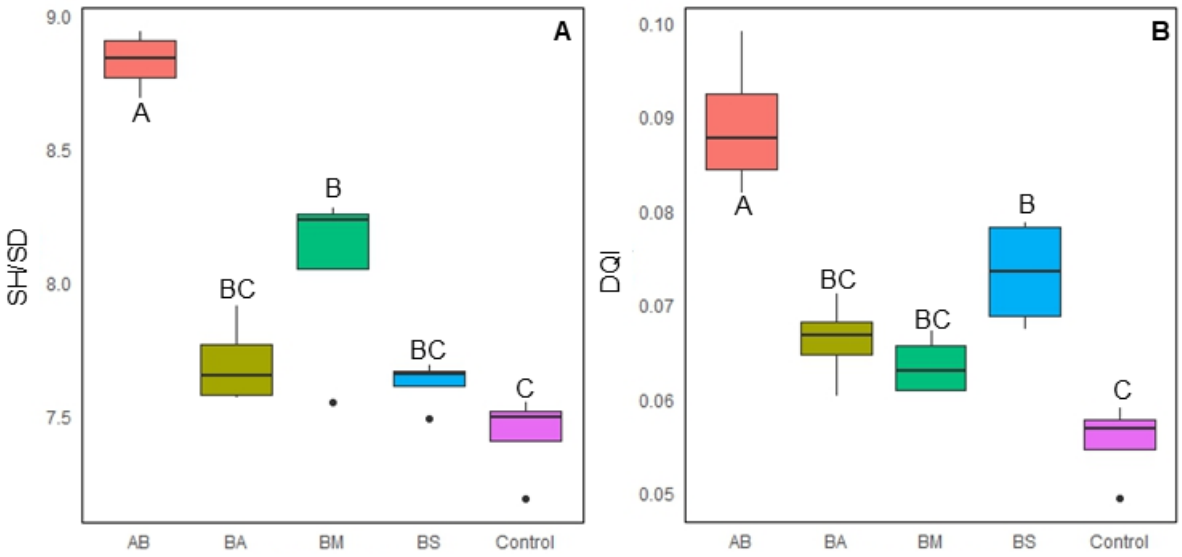

FIGURE 3: Boxplots for quality evaluation of *Handroanthus impetiginosus* seedlings with and without inoculation of rhizobacteria. A - shoot height/stem diameter ratio (SH/SD), B - Dickson Quality Index (DQI). Means followed by the same letter do not differ by Tukey's test ($p \leq 0.05$); n = 4 replicates per treatment. *Azospirillum brasilense* (AB), *Bacillus amyloliquefaciens* (BA), *Bacillus megaterium* (BM), *Bacillus subtilis* (BS) and without inoculation (Control).

FIGURE 4: *Handroanthus impetiginosus* seedlings 107 days after sowing. Treatments: A - Control, B - *Bacillus amyloliquefaciens* (BA), C - *Bacillus megaterium* (BM), D - *Bacillus subtilis* (BS) and E - *Azospirillum brasilense* (AB).

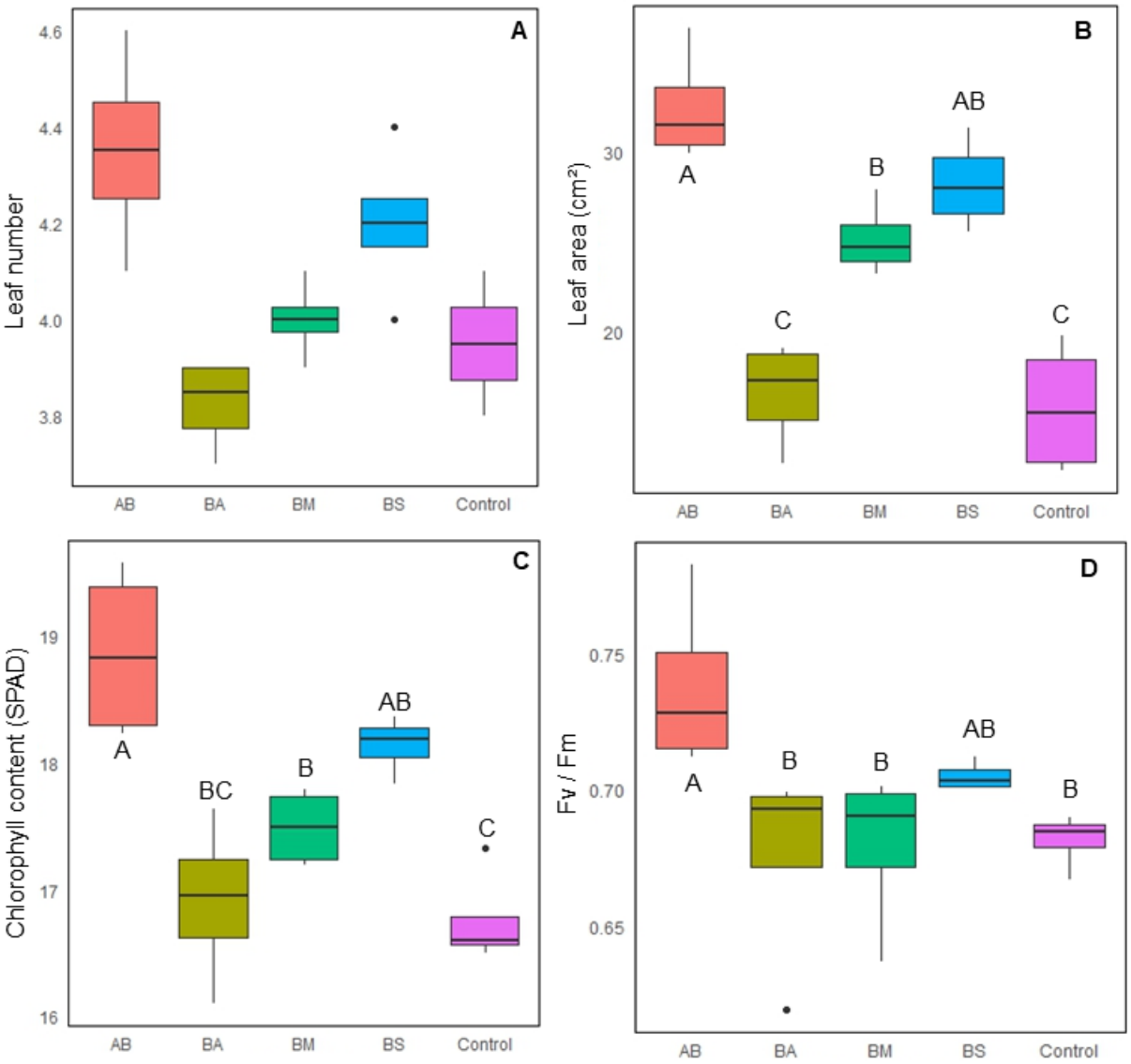

FIGURE 5: Boxplot photosynthetic performance evaluation of *Handroanthus impetiginosus* seedlings with and without inoculation of rhizobacteria. A – leaf number, B – leaf area (cm^2), C – chlorophyll content (SPAD index), D - maximum photochemical efficiency of photosystem II (Fv/Fm). Means followed by the same letter do not differ by Tukey's test ($p \leq 0.05$); n = 4 replicates per treatment. *Azospirillum brasilense* (AB), *Bacillus amyloliquefaciens* (BA), *Bacillus megaterium* (BM), *Bacillus subtilis* (BS) and without inoculation (Control).

by 32.52% and shoot dry mass by 18.93%, compared to the control. The greater proportional increase in root dry mass relative to shoot dry mass in *A. brasilense*-treated plants suggests that this rhizobacterium not only promotes larger root systems but also enhances resource allocation efficiency. This aligns with findings from

Rodrigues et al. (2023), where microbial inoculation led to significant biomass accumulation in *H. impetiginosus* seedlings compared to non-inoculated controls. They found that inoculating with isolates of the fungus *Trichoderma asperellum* resulted in increases in height by 12.30 cm, stem diameter by 4.08 mm, and total dry mass to 0.84 g, measured 60 days after inoculation, which was conducted 14 days post-germination. Rodrigues et al. (2023) showed that microbial inoculation significantly improved biomass accumulation in *H. impetiginosus* seedlings. Inoculation *with Trichoderma asperellum* increased seedling height by 12.30 cm, stem diameter by 4.08 mm, and total dry mass to 0.84 g, measured 60 days after inoculation, compared to non-inoculated controls.

Furthermore, the seedling quality index (DQI), a metric that integrates shoot and root parameters to assess overall plant vigour, increased by 60.36% with *A. brasilense* and 31.94% *with B. subtilis* compared to the control. A higher DQI suggests that the plants were not merely larger but had a well-balanced development, with sufficient root mass to sustain aboveground growth. Additionally, the height-to-stem diameter ratio (SH/SD),

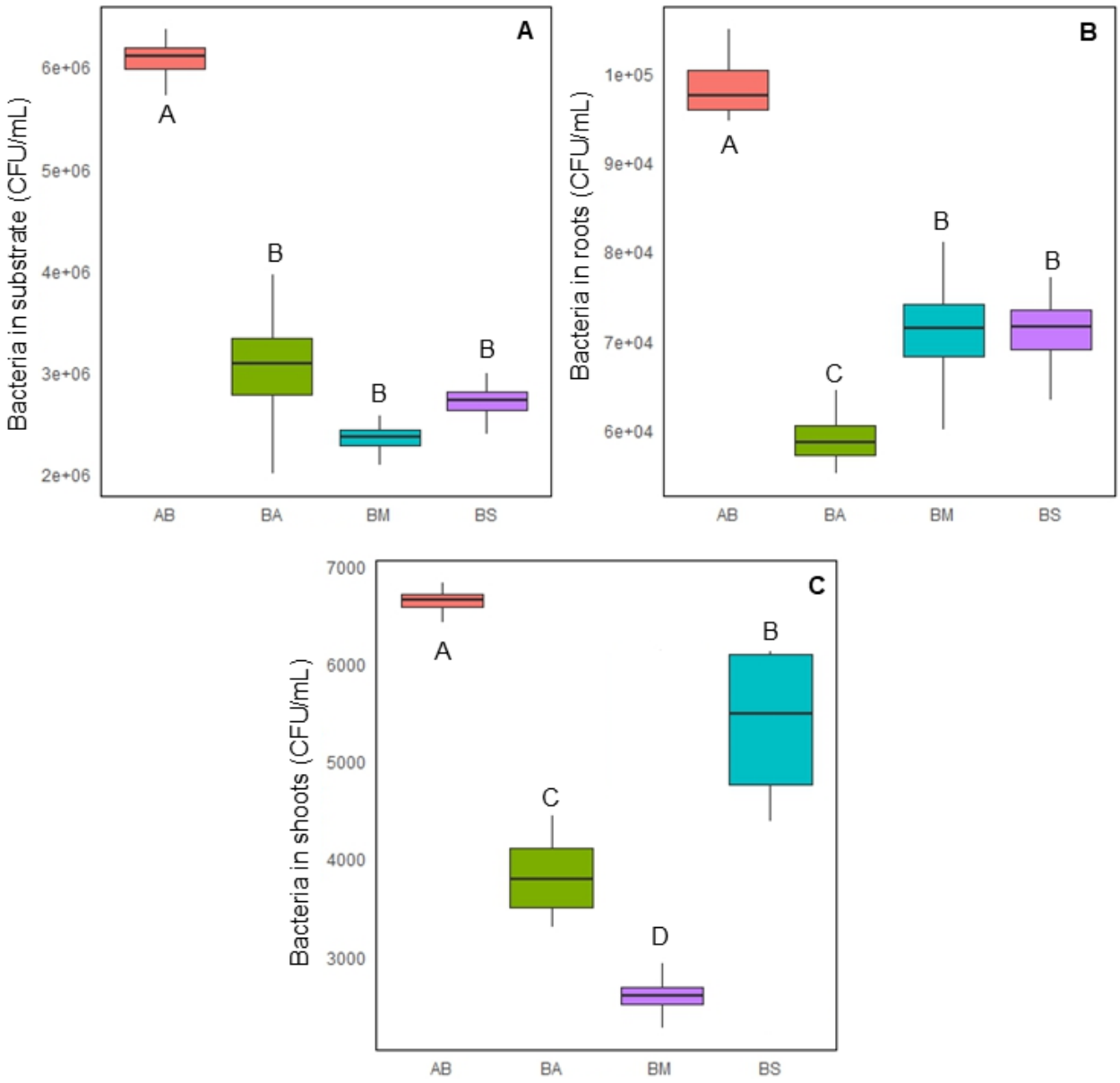

FIGURE 6: Boxplots of colony-forming units per millimetre (CFU/mL) in *Handroanthus impetiginosus* seedlings inoculated with rhizobacteria. A – CFU in the substrate, B – CFU in the roots, and C – CFU in the shoots. Means followed by the same letter do not differ by Tukey's test ($p \leq 0.05$); n = 4 replicates per treatment. *Azospirillum brasilense* (AB), *Bacillus amyloliquefaciens* (BA), *Bacillus megaterium* (BM), *Bacillus subtilis* (BS).

which serves as an indicator of structural stability and resource allocation, increased by 18.76% in *A. brasilense*-inoculated plants and only 2.55% in *B. subtilis*-treated plants compared to the control. This suggests that *A. brasilense* not only promoted shoot growth but also contributed to improved plant architecture, likely by enhancing root nutrient absorption and water uptake. Similarly, Inoculation of *Azospirillum* sp. also improved the growth and quality of seedlings in *Soymida febrifuga* (Tarh et al. 2023) and *H. chrysotrichus* (Campos et al. 2024), leading to increases in height, stem diameter, total leaf area, seedling dry mass, and the quality index of seedlings (DQI).

Variables such as the number of leaves, leaf area, and SPAD index are indicative of the plant's photosynthetic potential, as they represent the surface area available for capturing light energy and, consequently, the carbon assimilation potential (Taiz et al. 2017). In this study, the best results were achieved with the inoculation of *A. brasilense* followed by *B. subtilis*.

These findings support the observed improvement in photosynthetic efficiency, as reflected by the Fv/Fm values. *Azospirillum brasilense* and *B. subtilis* were particularly effective in enhancing this parameter, with values of 0.738 and 0.705, respectively, representing an average increase of 8.25% compared to the control

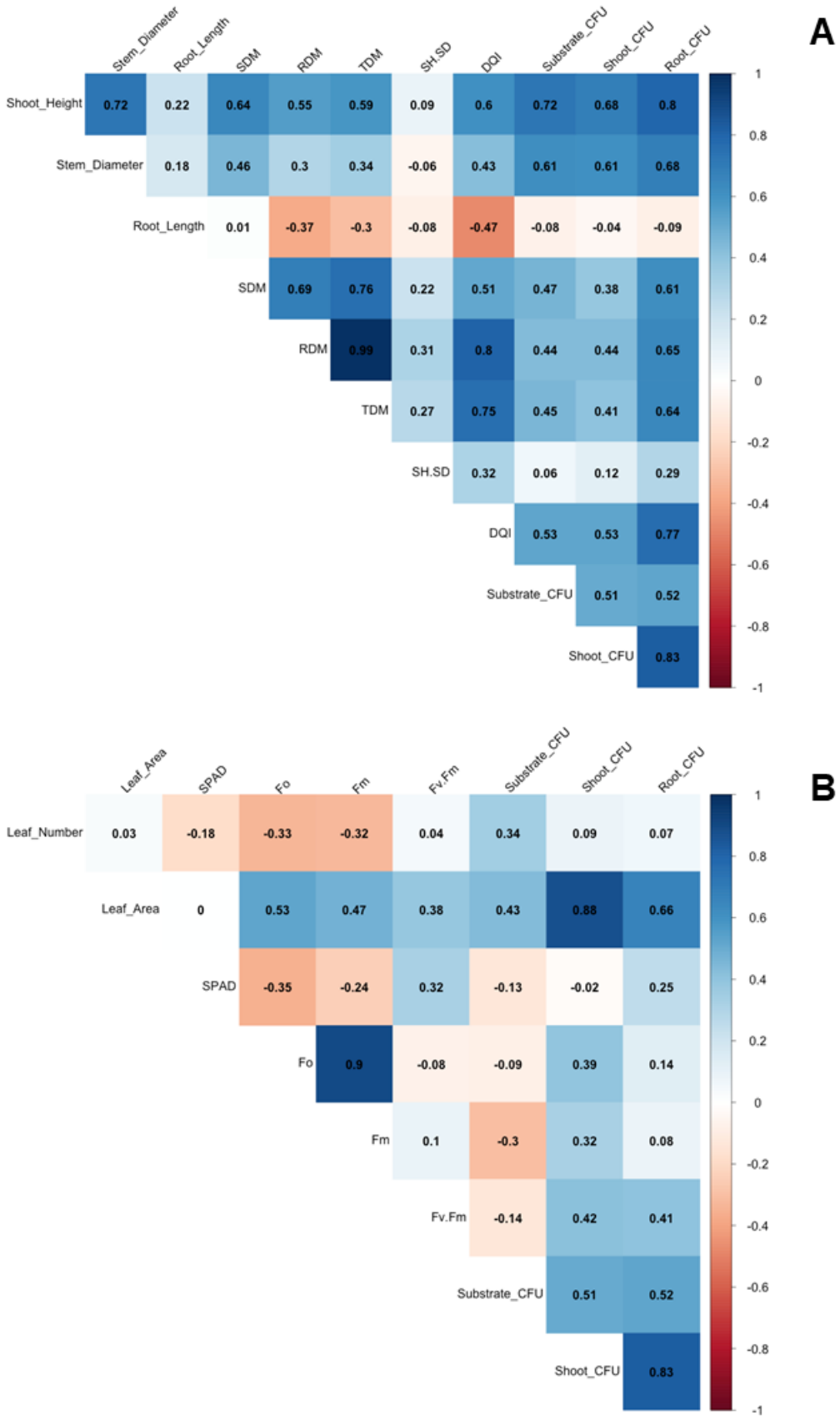

FIGURE 7: Pearson correlation matrix between the analysed. A – growth quality and colony-forming variables and B - photosynthetic and colony-forming variables of *Handroanthus impetiginosus* seedlings production without and with inoculation of rhizobacteria.

(Figure 5D). Under optimal physiological conditions, the Fv/Fm ratio typically ranges from 0.83 to 0.85 (Stirbet et al., 2018). Deviations from this range can occur due to factors such as non-photochemical quenching, degradation of the D1 protein, or inactivation of Photosystem II (PSII) reaction centers, which are key indicators of plant oxidative stress (Kalaji et al., 2016; Stirbet et al., 2018). Therefore, the elevated Fv/Fm values observed in inoculated seedlings indicate the maintenance of active PSII centers and a greater capacity for energy conversion. This suggests a positive adaptive response to photo-inhibitory environmental conditions (Kalaji et al., 2016).

However, length of the study evaluations and experimental conditions are crucial to observe the differences between treatments when applying growth-promoting microorganisms. In a study by Rodrigues et al. (2023), the *H. impetiginous* seedlings evaluated at 60 days after planting showed no difference in chlorophyll content (SPAD) between *T. asperellum* and the control. In studies involving the interaction of plants with microorganisms, the biology and life cycle of the specific rhizobacteria in question must be considered (Santoyo et al. 2016). Therefore, it is imperative to ensure that the length of the study is adequate to observe the expected responses, with more extended experiments offering a more comprehensive understanding of the adaptations and responses of rhizobacteria.

As plants develop, their demand for water and nutritional resources increases. When these resources are scarce, plants rely on their energy reserves, leading to higher metabolic costs to ensure survival reducing photosynthesis by closing stomata, limiting CO_2 intake and lowering glucose production, which heightens metabolic stress (Taiz et al. 2017; Yang et al. 2021). The root microbiota can help to mitigate abiotic stress in plants by optimizing water and nutrient uptake. Additionally, the root microbiota support plants by boosting antioxidant defences and influencing hormone levels (Hashem et al. 2019). These actions collectively contribute to better photoprotection, allowing plants to handle high light conditions more effectively.

The use of rhizobacteria also enhanced photosynthetic capacity in *Euterpe oleracea*, as demonstrated by a study where a consortium of *B. subtilis* + *Burkholderia* sp. + *B. safensis* + *Pseudomonas fluorescens* resulted in increased SPAD values and improved PSII efficiency (Castro et al. 2020). Similarly, in the case of *Corylus avellana* seedlings inoculated with the bacterial consortium *Pseudomonas putida* + *B. subtilis* + *Enterobacter cloacae*, revealed an increase in the SPAD index and leaf area (Rostamikia et al. 2016). Results from this study support the findings of Khan et al. (2021), which highlight the potential of rhizobacteria to help plants withstand abiotic stresses and improve growth, either by enhancing root architecture or by producing various metabolites, including phytohormones, exopolysaccharides, siderophores, antioxidant enzymes, and volatile compounds.

The colonisation by *A. brasilense* promoted gains of 125% for CFU in the substrate, 47% for CFU in the root, and 68.6% for CFU in the aerial part compared to the other rhizobacteria (Figure 6). Rhizobacteria play a key role in biological nitrogen fixation, root growth stimulation, improved nutrient absorption, and water use efficiency (Meena et al. 2016; Fukami et al. 2018). Their presence in the rhizosphere not only strengthens plants resistance to water, saline, and thermal stresses, but also enhances resistance to soil pathogens, as they act as biological control agents (Olanrewaju et al. 2017; Ferreira et al. 2019). Furthermore, these bacteria produce growth-promoting compounds, such as auxins and cytokinins, which positively impact plant development (Cassán et al. 2020; Miljaković et al. 2020).

The evaluated variables accurately characterised the effects of the rhizobacterisation treatments. Such treatments led to an increase in the number of leaves and leaf area, significantly influencing the growth and quality of the seedlings. These results can be attributed to the favourable interaction between *H. impetiginosus* and rhizobacteria.

The benefits of microbial inoculation extend beyond biomass accumulation, as a well-developed root system optimises nutrient uptake efficiency, allowing plants to better withstand environmental stresses. Previous studies have demonstrated that rhizobacteria improve water-use efficiency and nutrient acquisition (Meena et al. 2016; Fukami et al. 2018), and the significant increases in shoot and root dry mass observed in this study support the hypothesis that *A. brasilense* and *B. subtilis* enhance resource allocation efficiency rather than merely promoting overall plant size. Additionally, several studies have shown that rhizobacteria improve growth and seedling quality in forest species (Missio 2016; Kondo et al. 2020; Campos et al. 2023; Rodrigues et al. 2023). These findings highlight the varying effectiveness of microbial inoculation across different species, reinforcing the need for species-specific research to optimise the use of beneficial microorganisms in plant production.

Taking together, these results indicate that rhizobacterisation with *A. brasilense* and *B. subtilis* contributes to improved root efficiency, leading to more effective support for shoot development. The ability of these bacteria to promote root expansion and improve plant stability suggests that their use could be a valuable strategy for seedling production in forestry and restoration projects.

Conclusions

The use of rhizobacteria-inoculated substrate is recommended to produce *Handroanthus impetiginosus* seedlings*. Azospirillum brasilense* was the most effective rhizobacterium in promoting vegetative growth, improving seedling quality, and enhancing photosynthetic efficiency, primarily due to its high colonisation capacity and positive interaction with the plants.

List of abbreviations

SDM – short dry mass
RDM – root dry mass
TDM – total dry mass
SH/SD – shoot height and stem dimeter ratio
DQI – Dickson Quality Index
Fo – minimum fluorescence
Fm – maximum fluorescence
Fv/Fm – maximum efficiency of photosystem II
CFU – Colony Forming Units
AB – *Azospirillum brasilense*
BA – *Bacillus amyloliquefaciens*
BM – *Bacillus megaterium*
BS – *Bacillus subtilis*

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TSC and AMBS conducted the experiments, collected the data and wrote the manuscript. GRV and ACB conducted the experiments. CHBS analysed the data and supervision of the experiment. ECR carried out the methodological design and reviewed the manuscript. KFLP obtained the funds to support the study and reviewed the manuscript. All authors read and approved the final manuscript.

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