



Development of a Macaúba Biochar-Based Inoculant as a Carrier for *Rhizobium tropici*

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Abstract

Peat remains a commonly utilized carrier in the formulation of rhizobial inoculants, particularly for common bean (*Phaseolus vulgaris* L.) cultivation. Given that peat is a finite resource, there is a significant demand for sustainable alternatives. This study aimed to assess the potential of using Macaúba biochar as a carrier for *Rhizobium tropici*. The shelf life of inoculants based on peat and Macaúba biochar was evaluated over a period of 180 days. Parameters like number of nodules (NN), nodule dry weight (NDW), root dry weight (RDW), root volume (RV), and shoot dry weight (SDW) were measured in common bean under in vitro and greenhouse conditions. Treatments included Peat-based inoculant (P), Macaúba biochar-based inoculant (B), Macaúba biochar-based inoculant with pH adjusted using HCl (B+HCl), Macaúba biochar-based inoculant supplemented with glycerol as a carbon source (B+Gl), and Macaúba biochar-based inoculant with both pH adjustment and glycerol supplementation (B+HCl+Gl). Control treatments were non-inoculated (ni) and inoculated (i) with shelf life tested at 15 (15) and 180 days (180). The B+HCl+Gl and B+Gl inoculants exhibited a similar shelf life to the P inoculant. In the in vitro assays, the Bi-180, Bi+HCl-180, and Bi-HCl+Gl-180 inoculants resulted in comparable NN and NDW in common bean to those observed with Pi-180. In the greenhouse experiments, the Bi-180 inoculant showed similar NN and NDW as the Pi-180, while the Bi+HCl-180 inoculant showed similar RV. Additionally, the Bi-180, Bi+HCl-180, and Bi-HCl+Gl-180 inoculants produced RDW and SDW values akin to those of the Pi-180. These findings suggest that Macaúba biochar is a viable alternative to peat in microbial inoculant formulation, providing an environmentally friendly and sustainable option.

Keywords Biochar · *Acrocomia aculeata* · Organic residues · Inoculant

Introduction

Peat-based inoculants are considered the optimal carrier for rhizobia due to the high organic matter content in peat, which provides essential nutrients for bacterial growth [15]. However, peat is a non-renewable natural resource, taking millions of years to form, and thus may become scarce in

the future [18]. Consequently, there is a pressing need to identify alternative carriers for rhizobia.

The utilization of organic waste or biomass has seen a significant increase globally in recent years. Biomass, encompassing all organic matter derived from plants, animals, and processed natural products, holds substantial potential for conversion into value-added products. This is particularly relevant for agricultural, livestock, and forestry residues, as well as by-products from the food and wood processing industries [3, 9].

Thermochemical conversion processes, such as pyrolysis, convert biomass into valuable products like biochar, bio-oil, and synthesis gas (syngas) [17]. Biochar, the solid residue from pyrolysis, is a carbonaceous material produced by the thermal decomposition of organic matter. It serves as a solid form of carbon derived from organic waste. Biochar has garnered significant attention for its role in environmental

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and agricultural applications. Its primary uses include soil conditioning, metal immobilization, reducing water deficiency, fertilization, and soil regeneration, largely due to its high water-holding capacity and ability to raise soil pH [12, 20, 25].

Biochar is known to positively influence soil physical and chemical properties, thereby enhancing microbial activity [2, 26]. However, the literature lacks information on the use of biochar as a carrier for diazotrophic bacteria in inoculant formulations, indicating the need for research into alternatives that can enhance agricultural productivity while minimizing environmental impact. Therefore, the objective of this study was to characterize and evaluate Macaúba biochar as a carrier for *R. tropici* SEMIA 4077 strain, proposing it as a sustainable alternative to peat.

Materials and Methods

Obtaining, Characterizing, and Conditioning Macaúba Biochar

The macaúba palm (*Acrocomia aculeata*) is a species well adapted to semi-arid environments, particularly in the Brazilian Cerrado. It is tolerant to drought and low temperatures and has a wide geographic distribution, occurring mainly in the states of Goiás, Mato Grosso, and Mato Grosso do Sul. Macaúba produces a fruit known as the “macaúba nut,” from which two types of oil are extracted. Oil from the pulp is primarily used for biodiesel production, whereas oil from the kernel is used for human consumption and cosmetic applications. The industrial processing of macaúba nuts generates large amounts of biomass residues, which represent a valuable feedstock for biochar production.

The macaúba biochar used in this study was supplied by Embrapa Agroenergy. Biochar was produced by carbonizing macaúba residues at 350 °C for 12 min. Peat was used as a reference material (control) for comparison with biochar-based treatments. The physico-chemical characteristics of both carriers are presented in Supplementary Table 1.

Biochar pH was determined by weighing 5 g of each sample into Falcon tubes in triplicate, followed by the addition of 25 mL of distilled water. The suspensions were homogenized, and pH was measured using a calibrated pH meter.

Particle size distribution was assessed by dry sieving using eight mesh sizes (0.037, 0.12, 0.14, 0.17, 0.25, 0.55, 0.71, and 0.84 mm). Samples were manually shaken until no further particle movement was observed. The mass retained on each sieve was recorded, and the percentage and cumulative distribution of each size fraction were calculated [1].

Structural characteristics and microbial colonization of peat and biochar were analyzed by scanning electron

microscopy (SEM). Samples were air-dried, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h at 4 °C, rinsed, and post-fixed in 1% osmium tetroxide for 1 h. After dehydration through a graded ethanol series (30–100%), samples were mounted on aluminum stubs with carbon tape and analyzed in secondary electron mode to visualize surface morphology.

Preparation of Inoculants and Shelf-Life Test

The *R. tropici* SEMIA77 strain, obtained from the Collection of Phytopathogenic and Multifunctional Microorganisms of Embrapa Rice and Beans, was used for inoculant preparation. The bacterium was cultivated in nutrient broth under agitation (155 rpm) at 28 °C for 48 h. Cell concentration was adjusted to 1×10^9 CFU mL⁻¹ using sterile saline solution (0.85%).

For biochar-based inoculant preparation, pH correction and carbon source supplementation were performed. Biochar pH was adjusted to 6.9 by adding 4.5 µL of 1 M HCl per gram of biochar. Glycerol was added as a carbon source at a rate of 20 µL g⁻¹. The treatments consisted of peat (P), biochar (B), biochar with pH correction (B+HCl), biochar supplemented with glycerol (B+Gl), and biochar with both pH correction and glycerol supplementation (B+HCl+Gl).

Polypropylene bags containing 10.5 g of each previously sterilized carrier material received 4.5 mL of bacterial suspension (1×10^9 CFU mL⁻¹), followed by manual homogenization. The inoculants were stored in a BOD incubator at 25 °C until use.

Bacterial survival was evaluated at 0, 30, 60, 90, 120, 150, and 180 days. For each evaluation, 1 g of sample was suspended in 9 mL of sterile saline (0.85% NaCl). Serial decimal dilutions were prepared up to 10⁻⁷ using successive transfers of 100 µL aliquots [33]. Dilutions from 10⁻⁵ to 10⁻⁷ were plated in triplicate on Yeast Extract Mannitol Agar (YEMA) [21] and incubated at 28 °C for 72 h. Bacterial concentration was calculated as the arithmetic mean of triplicate plates and expressed as CFU mL⁻¹ [33].

Evaluation of Biochar Efficiency as an Inoculant

In Vitro Assay

The in vitro experiment was conducted in a completely randomized design with 13 treatments and four replications, as described in Table 1.

Common bean (*Phaseolus vulgaris* L.) seeds, cultivar BRS FC415, were surface-disinfected for 16 h using chlorine gas generated by mixing 3.5 mL of hydrochloric acid (HCl 12 N) with 100 mL of commercial bleach (5.25% sodium hypochlorite) [30]. Seeds were inoculated with 24 g

Table 1 Treatments used in in vitro and greenhouse experiments with inoculants presenting 180 and 15 days of shelf life, containing the strain SEMIA 4077 of *Rhizobium tropici*

Treatments ^a	Carrier	pH correction	Glycerol	Shelf life	CFU
Absolut control	-	-	-	-	-
Pni	Peat	Yes	No	-	-
Bni	Biochar	No	No	-	-
Bni+Gl	Biochar	No	Yes	-	-
Bni+HCl+Gl	Biochar	Yes	Yes	-	-
Pi-15	Peat	Yes	No	15	1.5×10^9
Bi-15	Biochar	No	No	15	9.1×10^9
Bi+Gl-15	Biochar	No	Yes	15	5.2×10^9
Bi+HCl+Gl-15	Biochar	Yes	Yes	15	6.3×10^9
Pi-180	Peat	Yes	No	180	8.2×10^8
Bi-180	Biochar	No	No	180	3.0×10^6
Bi+Gl-180	Biochar	No	Yes	180	1.3×10^8
Bi+HCl+Gl-180	Biochar	Yes	Yes	180	3.0×10^8

^aTreatments are composed of peat (P) and biochar (B), inoculated (i) and non-inoculated (ni), subjected to pH correction (HCl) and with glycerol (Gl) supplementation as a carbon source. CFU Colony forming unit

of each inoculant per 48 seeds, using 0.3 mL of 20% (w/v) polyvinylpyrrolidone (PVP) as an adhesive.

Seeds were mixed with the inoculant in Petri dishes until uniformly coated and transferred to a humid chamber for germination.

Germinated seeds (seedlings) were placed in 700 mL polypropylene cups containing 200 mL of Norris semi-solid medium [29], with one seedling per cup. After planting, another cup was placed over the cup and the bottom was covered with a paper bag to prevent light from reaching the roots [7]. Following germination, plants were grown under controlled conditions in an incubation chamber at 25 °C with a 12/12 h photoperiod.

At 40 days after sowing, plants were harvested. Nodules were detached and counted to determine the number of nodules (NN). Nodule activity (NA) was assessed visually by dissecting nodules and observing the presence of reddish coloration. Shoots and nodules were dried in a ventilated oven (65 °C for 72 h) to determine shoot dry weight (SDW) and nodule dry weight (NDW) [21].

Greenhouse Assay

The greenhouse experiment followed a randomized complete block design with four replications. Seed disinfection, inoculation procedures, and treatments were identical to those used in the in vitro assay. Soil showing pH=5.4 and organic matter content=20 g kg⁻¹ was collected from Embrapa Rice and Beans (Santo Antônio de Goiás, GO) sieved, mixed with sand at a 1:1 ratio, autoclaved, and placed into 3 kg pots. The chemical characteristics of the

soil used to make the potting mixture are presented in Supplementary Table 2.

Six inoculated seeds were sown per pot, and thinning was performed at 7 days after emergence, leaving two plants per pot. Subirrigation was carried out by adding autoclaved water to the trays beneath the pots [34]. Plants were harvested at 40 days after sowing to evaluate nodulation and growth parameters.

NN, NDW, and SDW were determined as described for the in vitro assay. Root volume (RV) was measured by water displacement using a 500 mL graduated cylinder. Roots were immersed in 250 mL of water, and the displaced volume was recorded as RV (cm³) [6]. Roots were then dried at 65 °C for 72 h to determine root dry weight (RDW).

Statistical Analysis

The data were subjected to tests of normality and homogeneity of variances for each variable and then to analysis of variance (ANOVA). When a statistically significant value was confirmed in the F-test ($p \leq 0.05$), the means were compared using Tukey's test at a 5% significance level using SISVAR[®] software [14].

Results

Physical-Chemical Characteristics of the Carriers

Peat and biochar exhibited distinct physicochemical properties. The pH values of peat and biochar were 6.5 and 7.8, respectively. Their moisture content was 11.1% for peat and 4.05% for biochar, both of which were adjusted to 40%. The C and N content of the peat was 42.15% and 1.92%, respectively, while the biochar showed values of 74.62% of C and 0.47% of N. Due to the alkaline nature of biochar, its pH was adjusted to 6.8 using 1 M HCl to improve suitability as a microbial carrier.

Particle size analysis revealed marked differences between the two materials (Fig. 1). In peat, approximately 50% of the particles ranged from 0.25 to 0.149 mm, and nearly 28% of the particles were smaller than 0.037 mm (Fig. 1A), indicating a predominance of fine particles. In contrast, biochar exhibited a coarser texture, with more than 70% of particles between 0.84 and 0.177 mm and only about 20% smaller than 0.149 mm (Fig. 1B). These differences in particle size distribution may influence aeration, moisture retention, and microbial colonization capacity.

Scanning electron microscopy (SEM) analysis further highlighted structural differences between peat and biochar (Fig. 2). Peat displayed amorphous aggregates of varying sizes with limited porosity (Fig. 2A). In contrast, biochar

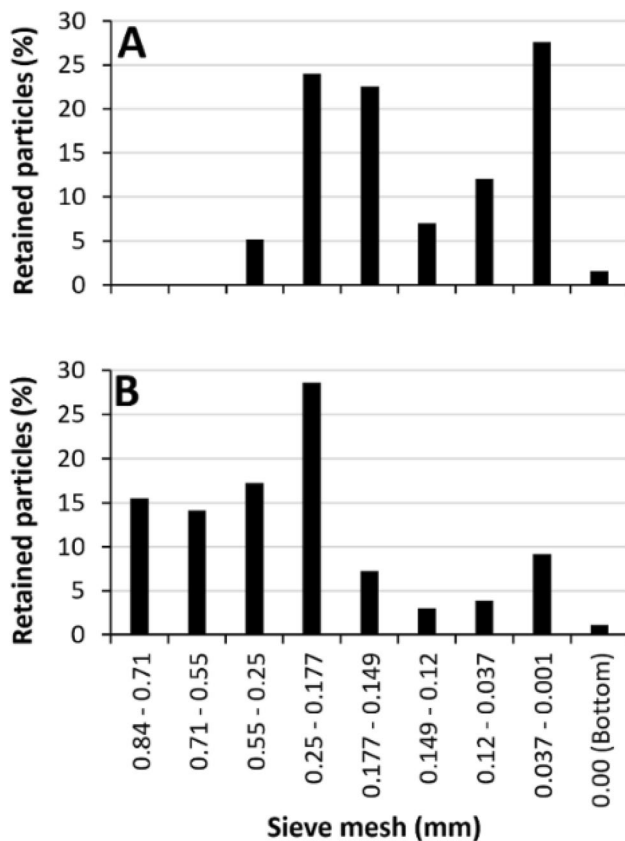


Fig. 1 Percentage distribution of peat (A) and biochar (B) particles by sieve mesh size

exhibited a complex and highly porous structure, characterized by interconnected pores of different sizes and shapes (Fig. 2B). These features favored microbial attachment and colonization, allowing the SEMIA 4077 strain of *R. tropici* to colonize both the surface and internal pore spaces of the biochar, as indicated by red arrows in Fig. 2.

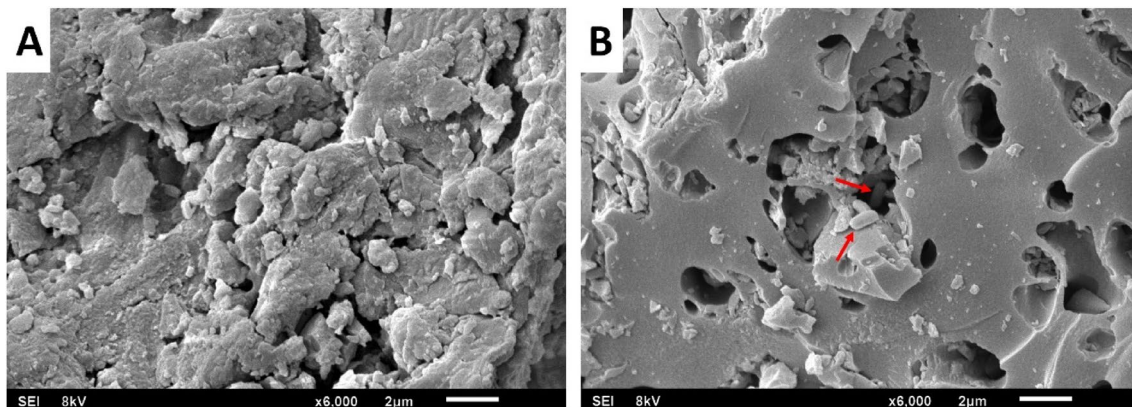


Fig. 2 Scanning electron micrographs of peat (A) and biochar (B) showing the structural difference between the materials. Red arrows indicate the presence of the SEMIA 4077 strain of *Rhizobium tropici* adhering to the surface and within the pore of the biochar

Evaluation of Inoculant Shelf Life

The survival of the SEMIA 4077 strain of *R. tropici* varied among treatments over the 180-day storage period (Fig. 3). Regression analysis indicated that the biochar (B), biochar with glycerol supplementation (B+Gl), and biochar with pH correction plus glycerol supplementation (B+HCl+Gl) treatments were best described by linear regression models, with coefficients of determination (R^2) of 0.94, 0.90, and 0.81, respectively. In contrast, peat (P) and biochar with pH correction only (B+HCl) followed quadratic regression models, with R^2 values of 0.82 and 0.67, respectively.

A reduction in viable cell counts was observed in all treatments over time, except for peat, which showed a slightly higher bacterial population at the end of the storage period compared with the initial count (Fig. 3). At 180 days, peat exhibited the highest viable cell concentration, reaching 8.2×10^8 CFU g^{-1} . This was followed by the B+HCl+Gl and B+HCl treatments, which maintained populations of 3.0×10^8 and 1.3×10^8 CFU g^{-1} , respectively (Fig. 3). These results indicate that biochar-based carriers, particularly those supplemented with glycerol and/or subjected to pH correction, were able to sustain bacterial viability at levels comparable to peat over extended storage periods.

Effect of Different Inoculants on Nodulation and Growth of Common Bean

In Vitro Assay

Biochar-based inoculants carrying the SEMIA 4077 strain of *R. tropici* successfully promoted nodulation and early development of common bean plants, as well as nodule activity, evidenced by the reddish coloration inside nodules (Fig. 4). No nodules were detected in the absolute control or non-inoculated treatments (Pni, Bni, Bni+HCl, and

Fig. 3 Regression analysis for the colony forming unit (CFU) of *Rhizobium tropici* strain SEMIA 4077 during the 180-day shelf-life test. The treatments are: peat (P), biochar (B), biochar with pH corrected using HCl (B+HCl), biochar supplemented with glycerol (B+Gl) and biochar with pH corrected using HCl and supplemented with glycerol (B+HCl+Gl)

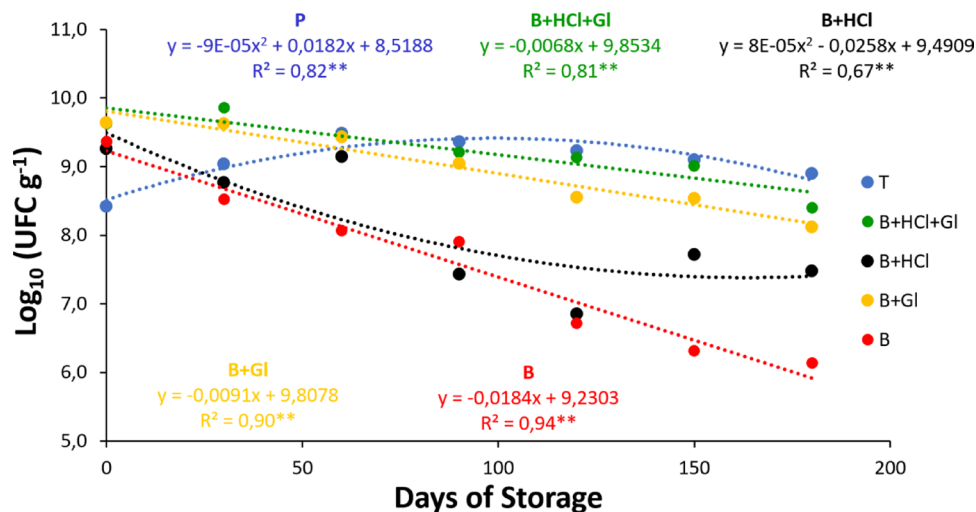
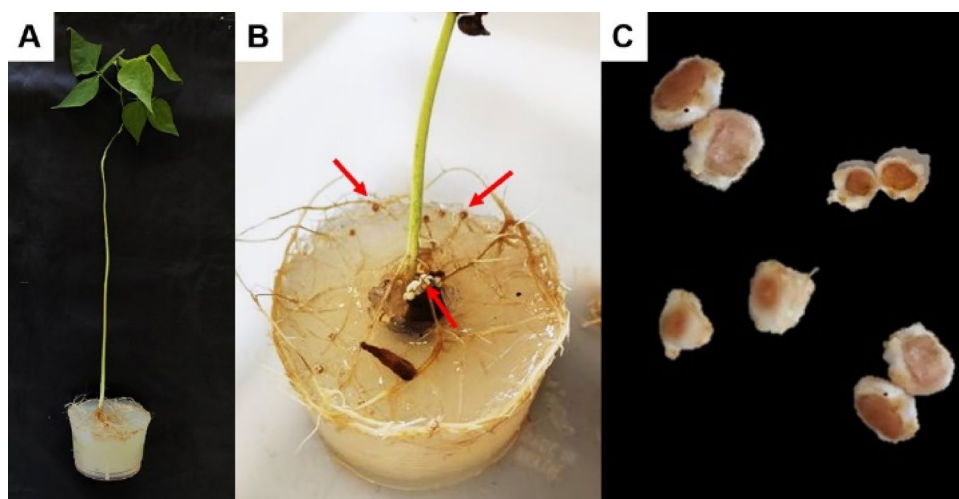


Fig. 4 Common bean plant inoculated with the strain SEMIA 4077 of *Rhizobium tropici* in an in vitro test, grown in semi-solid Norris medium (A), showing nodulation on the roots, indicated by red arrows (B), and reddish coloration inside the nodules (C) indicating nitrogenase activity



Bni+HCl+Gl), confirming the absence of contamination (Table 2).

No significant differences in the number of nodules (NN) were observed among inoculated treatments. However, the inoculated peat stored for 180 days (Pi-180) resulted in low nodulation (16 nodules per plant), which was statistically similar to the absolute control and non-inoculated treatments. In contrast, the Bi+Gl-15 and Bi+HCl+Gl-15 treatments exhibited approximately 40 nodules per plant, indicating high nodulation efficiency. Importantly, no significant differences in NN were detected between inoculants stored for 15 and 180 days, demonstrating that both peat and biochar carriers preserved the nodulation capacity of the SEMIA 4077 strain over time (Table 2).

For nodule dry weight (NDW), only the Bi+Gl-15 treatment differed statistically from the non-inoculated treatments and the absolute control. Although differences among the remaining treatments were not statistically significant, Bi+HCl+Gl-15 and Pi-180 showed relatively high NDW values of 8.6 and 7.6 mg plant⁻¹, respectively (Table 2).

Shoot dry weight (SDW) was not significantly affected by the treatments, indicating similar shoot biomass accumulation under in vitro conditions.

Greenhouse Assay

Under greenhouse conditions, visual differences among treatments were evident. Plants from the absolute control and non-inoculated biochar treatments (Bni and Bni+HCl+Gl) exhibited smaller shoots and root systems compared with inoculated treatments, regardless of whether the inoculant had been stored for 15 days (Fig. 5A) or 180 days (Fig. 5B).

As observed in the in vitro assay, nodules were absent in the absolute control and all non-inoculated treatments, confirming experimental sterility (Table 2). The highest number of nodules was recorded in the Bi+Gl-15 treatment, with more than 126 nodules per plant. However, this value did not differ statistically from those observed in Bi-15, Bi+HCl+Gl-15, Pi-180, and Bi-180 (Table 3), indicating

Table 2 Effect of different biochar and peat-based treatments carrying the strain SEMIA 4077 of *Rhizobium tropici* on the number of nodules (NN—n° planta⁻¹), nodule dry weight (NDW—mg planta⁻¹) and shoot dry weight (SDW—g planta⁻¹) of common bean plants (BRS FC415)

Treatments	NN	NDW	SDW
Absolute control	0.0b	0.0b	1.26A
Pni	0.0b	0.0b	1.70a
Bni	0.0b	0.0b	1.32a
Bni+HCl	0.0b	0.0b	1.33a
Bni+HCl+Gl	0.0b	0.0b	1.91a
Pi-15	20.5a	2.0ab	1.77a
Bi-15	21.0a	1.7ab	1.82a
Bi+Gl-15	40.0a	9.2a	1.84a
Bi+HCl+Gl-15	40.0a	8.6ab	1.74a
Pi-180	16.0ab	7.9ab	1.75a
Bi-180	19.8a	5.1ab	1.72a
Bi+HCl-180	17.3a	3.5ab	1.79a
Bi+HCl+Gl-180	24.0a	5.6ab	1.73a
CV (%)	34.01	0.18	24.93

The treatments are composed by sole and/or combination of peat (P), biochar (B), inoculated (i), non-inoculated (ni), with pH corrected using HCl (HCl), supplemented with glycerol (Gl), with pH corrected with HCl and supplemented with glycerol (B+HCl+Gl), stored for 15 days (15) and stored for 180 days (180). Means followed by different letters are significantly different by Tukey's test ($p \leq 0.05$)

that both peat and biochar carriers maintained rhizobial effectiveness even after 180 days of storage.

Nodule dry weight followed a similar trend. The highest NDW values were observed in the Bi+Gl-15 treatment, although only Bi+Gl-15 and Bi-15 differed significantly

Table 3 Effect of different biochar and peat-based treatments carrying the strain SEMIA 4077 of *Rhizobium tropici* on the number of nodules (NN—n° planta⁻¹), nodule dry weight (NDW—mg planta⁻¹), root volume (RV—cm³ planta⁻¹), root dry weight (RDW—g planta⁻¹) and shoot dry weight (SDW—g planta⁻¹) of common bean plants (BRS FC415)

Treatments	NN	NDW	RV	RDW	SDW
Absolute control	0.0c	0c	8.8d	0.53c	1.65b
Pni	0.0c	0c	12.9 cd	0.76abc	2.07ab
Bni	0.0c	0c	11.9 cd	0.65bc	1.54b
Bni+HCl	0.0c	0c	11.5 cd	0.70bc	2.11ab
Bni+HCl+Gl	0.0c	0c	12.0 cd	0.41c	2.72ab
Pi-15	19.1b	18bc	11.3 cd	0.74abc	2.20ab
Bi-15	74.0ab	66ab	20.0abc	1.20ab	3.07a
Bi+Gl-15	126.3a	87a	27.8a	1.42a	3.58a
Bi+HCl+Gl-15	48.8ab	39abc	14.4 cd	0.92abc	3.09ab
Pi-180	47.6ab	36abc	25.0ab	1.05abc	2.90ab
Bi-180	40.8ab	30abc	13.0 cd	1.17ab	2.76ab
Bi+HCl-180	33.4b	21abc	15.6bcd	1.06abc	2.82ab
Bi+HCl+Gl-180	32.4b	34abc	15.0 cd	0.96abc	2.84ab
CV (%)	47.62	12.40	25.9	32.68	28.19

The treatments are composed by sole and/or combination of peat (P), biochar (B), inoculated (i), non-inoculated (ni), with pH corrected using HCl (HCl), supplemented with glycerol (Gl), with pH corrected with HCl and supplemented with glycerol (B+HCl+Gl), stored for 15 days (15) and stored for 180 days (180). Means followed by different letters are significantly different by Tukey's test ($p \leq 0.05$)

from the absolute control and non-inoculated treatments (Table 3).

Root volume (RV) was also influenced by the treatments. The highest RV was observed in Bi+Gl-15; however, this

Fig. 5 Visual appearance of common bean plants inoculated with the strain SEMIA 4077 of *Rhizobium tropici* in different carriers. The treatments are composed by sole and/or combination of peat (P), biochar (B), inoculated (i), non-inoculated (ni), with pH corrected using HCl (HCl), supplemented with glycerol (Gl), with pH corrected with HCl and supplemented with glycerol (B+HCl+Gl), stored for 15 days (15) and stored for 180 days (180)

value was not statistically different from those recorded for Bi-15 and Pi-180 (Table 3). Notably, the Bi+HCl-180 treatment showed RV values comparable to Pi-180.

For root dry weight (RDW), only the Bi+Gl-15 treatment differed significantly from the absolute control and most non-inoculated treatments, except for Pni, which showed statistically similar RDW (Table 3). Biochar-based inoculants stored for 15 days (Bi-15, Bi+HCl-15, and Bi+HCl+Gl-15) and 180 days (Bi-180, Bi+HCl-180, and Bi+HCl+Gl-180) did not differ significantly and exhibited mean RDW values of 1.18 and 1.06 g plant⁻¹, respectively.

Shoot dry weight (SDW) was significantly higher in the Bi-15 and Bi+Gl-15 treatments compared with the absolute control and Bni treatments (Table 3). Although these treatments did not differ statistically from peat, biochar-based inoculants stored for 15 days produced SDW values ranging from 3.07 to 3.58 g plant⁻¹, whereas peat (Pi-15) resulted in 2.20 g plant⁻¹. After 180 days of storage, SDW values for peat and biochar treatments converged, ranging from 2.76 to 2.90 g plant⁻¹.

Discussion

Biochar exhibits a range of physicochemical properties that directly influence its suitability as a microbial inoculant carrier. Particle size is a key factor, as excessively large particles may adhere poorly to seeds, whereas very fine particles are more susceptible to leaching through the soil profile [8]. Consequently, particle size analysis provides not only a physical characterization of the material but also important insights into its effectiveness in inoculant formulations.

Another defining characteristic of biochar is its high porosity (Fig. 2), which results in a large specific surface area. This structural feature enhances the retention of water and nutrients and creates a favorable microhabitat for beneficial microorganisms within its pore network. By occupying these pores, microbial cells are protected from environmental stressors such as drought, unfavorable pH, and soil toxins [10, 31]. These properties make biochar particularly advantageous for sustaining microbial viability under variable soil conditions.

Both specific surface area and porosity strongly influence bacterial adhesion (cell–surface interactions) and cohesion (cell–cell interactions). However, these physical properties alone do not guarantee effective microbial colonization. Colonization efficiency also depends on the chemical characteristics of the biochar surface, including the presence and distribution of functional groups [22]. In addition, pore size must be compatible with the dimensions of the microbial cells. Optimal colonization generally occurs when pore diameters are approximately two to four times larger than

the cell size, allowing adequate protection while maintaining accessibility to nutrients and oxygen [32].

The ability to preserve microbial viability over extended storage periods is a critical requirement for any inoculant carrier. In this study, shelf-life evaluations demonstrated that macaúba biochar maintained at least 1.3×10^8 CFU g⁻¹ of the SEMIA 4077 strain of *R. tropici* after 180 days of storage. In comparison, a study evaluating different biochars reported populations of approximately 1×10^6 CFU g⁻¹ of *R. leguminosarum* after 84 days [19]. Another investigation using *R. phaseoli* observed bacterial survival of up to 10^9 CFU g⁻¹ over 180 days [16]. Although these studies differ in rhizobial species and biochar sources, they consistently demonstrate the protective and nutritional role of biochar in maintaining microbial populations [13].

While shelf life is an essential parameter, preserving the symbiotic functionality of the bacterium—particularly its ability to form nodules—is even more critical. Positive nodulation is confirmed by the presence of at least one nodule in the root system of the test plant [7]. In our in vitro assay, biochar-based inoculants stored for 180 days produced between 17 and 24 nodules per plant in vitro, while greenhouse experiments resulted in 32 to 40 nodules per plant. These results indicate that macaúba biochar-based inoculants effectively preserved the nodulation capacity of *R. tropici* in common bean plants.

Comparable findings have been reported in other studies. For example, wheat biochar used as a carrier for *R. leguminosarum* in pea (*Pisum sativum* L.) resulted in nodule number (NN) and nodule dry weight (NDW) values similar to those obtained with commercial inoculants [19]. In the present work, NN and NDW values for biochar-based treatments were statistically equivalent to those observed with peat-based inoculants in both in vitro and greenhouse assays. These results confirm the effectiveness of macaúba biochar as a carrier for *R. tropici*. Additionally, plant growth parameters—including root volume, root dry mass, and shoot dry mass—were similar between peat- and biochar-based treatments, even after 180 days of storage. Similar increases in plant biomass following biochar-based inoculation have been reported previously [19].

Despite differences among studies in plant species, bacterial strains, and biochar feedstocks [4, 13, 16, 19], the overall effectiveness of biochar as a microbial carrier is well supported. These findings highlight biochar's potential as a sustainable alternative to peat and emphasize its relevance within environmentally responsible agricultural systems aligned with circular economy principles.

According to Ajeng et al. [4] bacteria in biochar-based inoculants can enhance plant growth by increasing nutrient availability, improving soil structure, and enhancing soil water and air retention. These improvements create a

favorable environment for root development and beneficial microbial activity while suppressing pathogenic organisms involved in nutrient cycling. In addition, biochar contributes to carbon sequestration due to its high stability, allowing carbon to remain stored in soil for centuries rather than being released into the atmosphere. Thus, the use of biochar in sustainable agriculture supports both crop productivity and climate change mitigation [5, 23, 24].

Replacing peat with alternatives such as macaúba biochar also reduces the environmental impacts associated with peat extraction. This substitution aligns with the objectives of Brazil's National Solid Waste Policy [7], which seeks to minimize environmental damage resulting from inadequate waste management. In this context, biochar represents a viable and ecologically sustainable alternative that valorizes agribusiness residues. In Brazil alone, approximately 200 million tons of biomass waste are generated annually [27]. This biomass could be converted into biochar for use as an ecological fertilizer in sustainable agriculture [11] or as a carrier in microbial inoculant formulations [28].

This study provides novel and pioneering evidence supporting the use of macaúba biochar as a carrier for the SEMIA 4077 strain of *R. tropici* in common bean inoculation. To our knowledge, no previous studies have specifically investigated macaúba biochar for this purpose. Our findings demonstrate that replacing peat with macaúba biochar is a viable and sustainable alternative, with the potential to reduce environmental impacts and contribute meaningfully to the circular economy associated with macaúba production.

Conclusions

The results demonstrate that macaúba biochar-based inoculants present agronomic performance comparable to the peat-based inoculant. The formulations B+HCl+Gl and B+Gl exhibited similar shelf life to peat, indicating adequate microbial survival during storage. In both in vitro and greenhouse assays, biochar-based inoculants with 180 days of shelf life promoted nodulation, nodule dry weight, root volume, and shoot and root biomass in common bean at levels equivalent to the peat-based inoculant.

These findings indicate that macaúba biochar is a technically viable carrier for microbial inoculant formulation. Therefore, its use is recommended as a sustainable and ecological alternative to peat, a non-renewable and increasingly scarce resource.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40003-026-00974-8>.

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Data Availability The data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest The authors declare no conflict of interest.

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