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Bacterial consortium enhances nodulation and yield of legumes grown in degraded soils

Consorcio bacteriano mejora la nodulación y el rendimiento de leguminosas cultivadas en suelos degradados

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ABSTRACT

Microbial consortia are a sustainable alternative to improve crop performance in degraded soils; however, evidence of their effectiveness in legumes under tropical conditions remains scarce. This study evaluated the effect of a multispecific bacterial consortium on three legume species grown in a Ferrallitic Red Lixiviated soil with moderate physical and chemical degradation. Between 2022 and 2024, at the Institute of Fundamental Research in Tropical Agriculture "Alejandro de Humboldt," Havana, Cuba, three compatible plant growth-promoting bacterial strains (*Rhizobium pusense*, *Bacillus thuringiensis* and *Bacillus velezensis*) were selected and applied as a consortium to two cultivars each of chickpea (*Cicer arietinum*), common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*). A randomized complete block design and analysis of variance followed by Duncan's test (α = 0.05) were used to compare four treatments: consortium, consortium + 50% mineral fertilization, 100% NPK fertilization and an untreated control. The consortium significantly increased nodule number and biomass, as well as pod number and weight in all three crops, outperforming both the control and the 100% fertilized treatment, with significant differences among cultivars. Nacional-29 (chickpea), Güira-89 (common bean) and INIFAT-94 (cowpea) showed the greatest response to inoculation. These findings confirm the potential of the bacterial consortium of *R. pusense* R3, *B. thuringiensis* B3 and *B. velezensis* B8, as an effective biofertilizer to enhance legume productivity in degraded soils and reduce the use of chemical fertilizers in tropical agroecosystems.
Keywords: biofertilizers, soil degradation, biological nitrogen fixation, legumes.

RESUMEN

Los consorcios microbianos son una alternativa sostenible para mejorar el rendimiento de cultivos en suelos degradados, pero aún existe poca evidencia de su eficacia en leguminosas bajo condiciones tropicales. Este estudio evaluó el efecto de un consorcio bacteriano multiespecífico sobre tres especies de leguminosas cultivadas en un suelo Ferralítico Rojo Lixiviado con degradación física y química moderada. Entre 2022 y 2024, en el Instituto de Investigaciones Fundamentales en Agricultura Tropical "Alejandro de

Humboldt", La Habana, Cuba, se seleccionaron tres cepas compatibles de bacterias promotoras del crecimiento vegetal (*Rhizobium pusense*, *Bacillus thuringiensis* y *Bacillus velezensis*), que se aplicaron como consorcio a dos cultivares de garbanzo (*Cicer arietinum*), frijol común (*Phaseolus vulgaris*) y frijol caupí (*Vigna unguiculata*). Se utilizó un diseño de bloques al azar y un análisis de varianza seguido de la prueba de Duncan (α = 0.05) para comparar cuatro tratamientos: consorcio, consorcio +50% de fertilización mineral, fertilización 100% NPK y testigo absoluto. El consorcio incrementó significativamente el número y la biomasa de nódulos, así como el número y la masa de vainas en los tres cultivos, superando al testigo y al tratamiento fertilizado al 100%, con diferencias significativas entre cultivares. Nacional-29 (garbanzo), Güira-89 (frijol) e INIFAT-94 (caupí) fueron los que mostraron la mejor respuesta. Estos resultados confirman el potencial del consorcio bacteriano de *R. pusense* R3, *B. thuringiensis* B3 y *B. velezensis* B8, como un biofertilizante eficaz para incrementar la productividad de leguminosas en suelos degradados y reducir el uso de fertilizantes químicos en agroecosistemas tropicales.
Palabras clave: biofertilizantes, degradación del suelo, fijación biológica de nitrógeno, leguminosas.

INTRODUCTION

Natural and anthropogenic activities have generated adverse effects on soils, leading to an increase in their degradation. This situation has resulted in a progressive loss of agricultural biodiversity and imbalances in the availability of essential nutrients for plant development (Zhu *et al.*, 2020; Asad *et al.*, 2022). In this context, it is essential to consider the role of beneficial interactions between plants and microorganisms, as these directly contribute to soil health and fertility. Such interactions not only influence crop growth and development but also shape microbial biodiversity in agroecosystems (Vishwakarma *et al.*, 2020).

Among plant-associated microorganisms, rhizobia represent a key group due to their ability to form root nodules in legumes and fix atmospheric nitrogen under symbiotic conditions (Lindström & Mousavi, 2020). However, nitrogen is not the only limiting nutrient in degraded soils; phosphorus also plays a crucial role, and its low availability can significantly restrict plant growth (Timofeeva *et al.*, 2022). In this regard, several soil microorganisms contribute to phosphorus solubilization, facilitating its uptake by plants (Zhang *et al.*, 2022).

Leguminous species are fundamental in agricultural systems because of their high protein content, contribution of essential amino acids, and ability to enrich the soil with nitrogen (Echevarría *et al.*, 2021). In these crops, phosphorus is indispensable for key physiological processes such as nodulation, amino acid synthesis, and protein production (Etesami *et al.*, 2021). Therefore, ensuring phosphorus availability is critical for establishing efficient symbiotic associations. In this sense, co-inoculation of rhizobia with other plant growth-promoting bacteria (PGPB) has been reported that can promote earlier and more intense nodulation, as well as improved water and nutrient uptake, resulting in greater plant development (Rosabal *et al.*, 2021). These bacteria can colonize nodules and establish synergistic interactions with rhizobia, enhancing plant growth. This is the case of various species of the genus *Bacillus*, known for their roles in growth promotion, phosphorus solubilization, and induction of tolerance to different types of stress (Chaudhary *et al.*, 2022). Furthermore, the functional combination of multiple bacterial strains in microbial consortia has proven to be more effective than the individual use of strains, due to metabolic complementation and synergy in rhizosphere colonization (Devi *et al.*, 2022; Flores-Duarte *et al.*, 2022a).

Despite advances in the use of plant growth-promoting microorganisms, available information on the effects of multispecific microbial consortia on the growth and nodulation of legumes cultivated in degraded soils under tropical conditions remains limited. Moreover, it is not yet clear whether plant species differ in their response to these consortia, an aspect particularly relevant for soil restoration programs using adapted crops. In this context, the present study was conducted in a soil with moderate physical and chemical degradation, characterized by compaction, low organic matter content, and nutrient imbalance, conditions that negatively affect soil structure, aeration, nutrient retention, and microbial functionality. We hypothesized that co-inoculation with a plant growth-promoting bacterial consortium could significantly improve growth and nodulation in legumes cultivated under these soil conditions. Therefore, the objective of this study was to evaluate the effect of applying a microbial consortium on the growth of three legume species: *Cicer arietinum* (chickpea), *Phaseolus vulgaris* (common bean), and *Vigna unguiculata* (cowpea), in this degraded soil.

MATERIAL AND METHODS

The study was conducted in an experimental unit on an agrogenic Ferrallitic Red Lixiviated soil (Hernández *et al.*, 2020), located at the Institute of Fundamental Research in Tropical Agriculture “Alejandro de Humboldt” (INIFAT), Santiago de Las Vegas, Cuba, during the 2022-2024 period.

Soil sampling, characterization, and biological material

A systematic random sampling was performed using a zigzag pattern at five georeferenced points, obtaining duplicate samples from the arable layer (0-20 cm) to form a composite sample (n = 10). Organic matter content was determined by the Walkley and Black wet oxidation method (NC 51, 1999); pH was measured potentiometrically in a soil:KCl suspension (1:2.5) (NC ISO 10390, 1999). Available phosphorus (P_2O_5, mg kg^{-1}) was quantified through extraction with 0.1 mol L^{-1} H_2SO_4 (1:25, NC 52, 1999), and exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+; cmolc kg^{-1}) were determined by extraction with 1 mol L^{-1} NH_4Ac at pH 7 (NC 65, 2000). Ca^{2+} and Mg^{2+} were measured by complexometry, whereas K^+ and Na^+ were quantified by flame photometry (Paneque-Pérez *et al.*, 2010).

The microbial consortium used was composed of *Rhizobium pusense* R3 (CM-CNRG 562) (Ortega-García *et al.*, 2024a), *Bacillus thuringiensis* B3 (CM-CNRG 568), and *Bacillus velezensis* B8 (CM-CNRG 570) (Ortega-García *et al.*, 2024b), strains maintained in the INIFAT Beneficial Bacteria Collection and in the Microorganism Collection of CNRG-INIFAP (CM-CNRG, Mexico). Seeds corresponded to two cultivars per species: chickpea (Nacional-29 and JP-94), common bean (Caujerí 2170 and Güira 89), and cowpea (INIFAT-93 and INIFAT-94).

Inoculum production and seed treatment

The strains were cultivated by submerged fermentation in Nutrient Broth under controlled conditions (200 rpm, 30 °C, 48 h) on an orbital shaker (EDMUD BUHLER). Cultures were harvested upon reaching a density of 10^8 CFU mL^{-1} (McFarland scale). Co-inoculation was performed by immersing seeds in a 1:10 (v:v) dilution of the fermented product in deionized water for 10 min, followed by air-drying at room temperature for 30 min before sowing.

Experimental design, variables, and statistical analysis

The experiment was established under a Randomized Complete Block Design (RCBD) with four replicates per treatment. Each plot (30 m^2) consisted of seven rows (6 × 5 m) with 0.70 m spacing between rows and 0.20 m between plants; the five central rows were considered as the effective plot to minimize border effects.

Four treatments were evaluated: (1) absolute control (no inoculation or fertilization), (2) control fertilized with 100% of the recommended NPK dose, (3) inoculation with the bacterial consortium, and (4) inoculation with the bacterial consortium combined with 50% of the NPK dose. In each plot, ten plants per crop were randomly selected to quantify the number and dry biomass of nodules, as well as the number and fresh biomass of pods (g $plant^{-1}$).

The data obtained were analyzed by ANOVA using the software STATGRAPHICS Plus v.5.0, and means were compared using Duncan’s multiple range test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The evaluated agrogenic Ferrallitic Red Lixiviated soil presented low organic matter levels (2.07%), slightly alkaline pH (7.45), medium available phosphorus content (22.95 mg kg^{-1} P_2O_5), high K^+ values (1.34 cmolc kg^{-1}) and very low Na^+ (0.04 cmolc kg^{-1}), along with a marked imbalance in the Ca^{2+}/Mg^{2+} ratio (20.33/1.30 cmolc kg^{-1}) (Table 1), compared to the optimal range of 2–6 cmolc kg^{-1} (Hernández *et al.*, 2020). This combination of limitations confirms a state of moderate physical and chemical degradation, characterized by low nutrient availability and conditions unfavorable for microbial activity, which can severely restrict the establishment of functional symbioses and crop growth (Hassen *et al.*, 2020; Rosabal *et al.*, 2021).

The low intrinsic fertility of this soil favors positive responses to biofertilization. In nutrient-deficient soils, bioinoculants often increase plant growth due to their ability to mobilize poorly available nutrients and stimulate key physiological processes (Spolaor *et al.*, 2016). *Rhizobium*–legume symbiosis supplies nitrogen, but the incorporation of plant growth-promoting bacteria (PGPB) that enhance the availability of phosphorus and potassium is critical to maximize biological nitrogen fixation and plant development (Tamayo *et al.*, 2020). These effects are particularly relevant in degraded soils where low organic matter concentration and cationic imbalance compromise nutrient uptake (Alemneh *et al.*, 2020). For this reason, in this study, the combination of PGPB with adequate levels of mineral fertilization was evaluated to counteract the limitations of the soil in study.

Table 1. Chemical properties of the agrogenic Ferrallitic Red Lixiviated soil in study

Organic matter (%)	pH	P_2O_5 (mg kg^{-1})	K_2O	K (cmol$_c$ kg^{-1})	Na	Mg	Ca
2.07	7.45	22.95	28.81	1.34	0.040	1.30	20.33

Organic matter: Walkley and Black; pH: Potentiometry; available P_2O_5: extraction with 0.1 N H_2SO_4 (Oniani method); exchangeable cations by extraction with 1 mol L^{-1} NH_4Ac at pH 7 and determination by flame photometry (Na^+ and K^+) and complexometry (Ca^{2+} and Mg^{2+}).

Effect of the consortium on nodulation

Significant differences were observed in the number and biomass of nodules in the three crops, with a clearly positive response to the application of the bacterial consortium (Figure 1). The treatment with the consortium combined with 50% of the mineral fertilization dose showed the highest increases in both indicators, highlighting the potential of coinoculation in degraded soils. These results confirm that the interaction between microsymbionts and the host plant is enhanced when the applied strains are compatible and fulfill complementary functions.

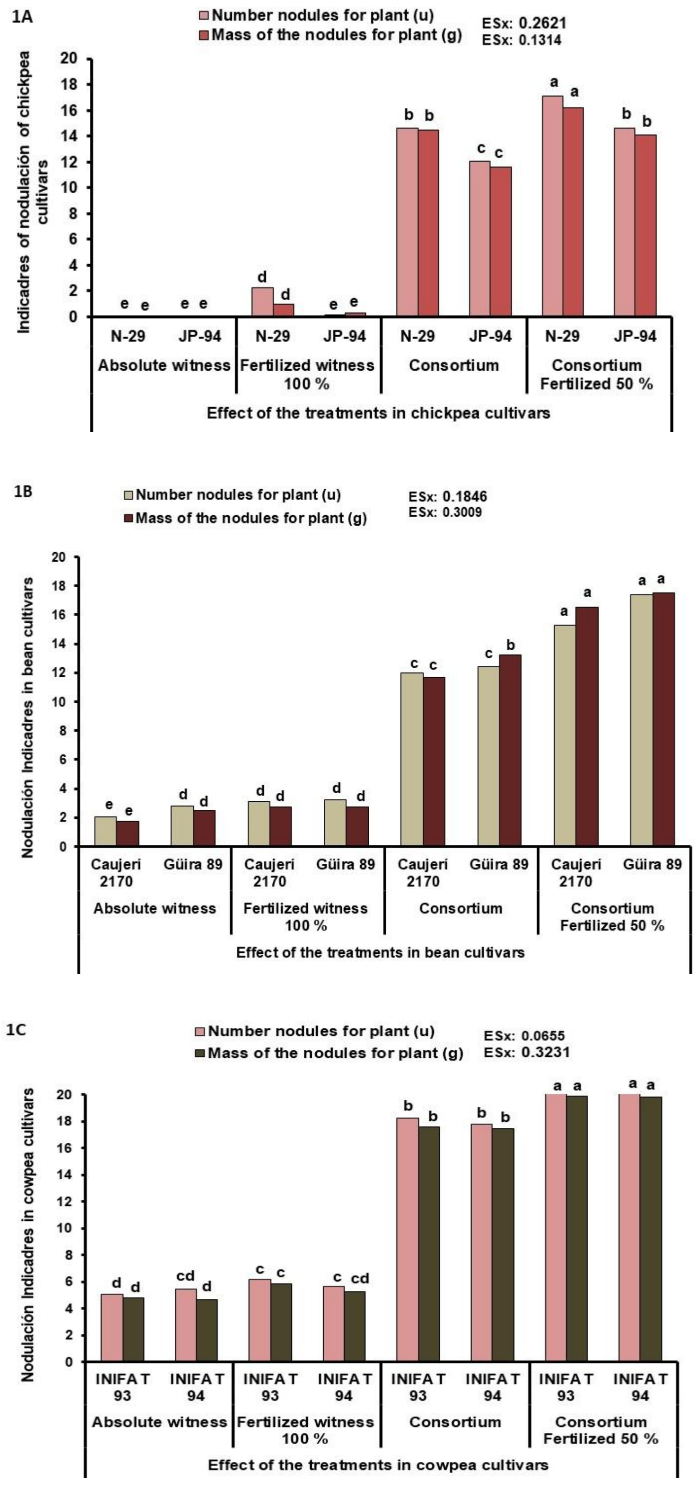

Figure 1. Effect of the consortium on the number and biomass of nodules in chickpea (A), common bean (B), and cowpea (C) in a degraded soil (2022–2024). Identical letters do not differ significantly (Duncan, α = 0.05, n = 10).

In chickpea, the cultivar Nacional-29 outperformed JP-94 in terms of number and biomass of nodules, probably due to genetic differences and root exudate profiles favoring the attraction of *Rhizobium pusense*, a strain originally isolated from this cultivar (Ortega-García *et al*., 2024a). Root exudate composition plays a determining role in colonization by rhizobia and PGPB, as it contains signaling compounds such as flavonoids, amino acids, and sugars that modulate bacterial chemotaxis (Wang *et al*., 2018). This phenomenon could explain the superiority of Nacional-29 over JP-94. The control treatments (absolute and 100% NPK) showed poor nodulation, evidencing the dependence of this crop on inoculation (Bai *et al*., 2022).

In common bean, the cultivar Güira-89 showed the best nodulation values when the consortium plus 50% fertilization was applied, followed by Caujerí 2170. In cowpea, both cultivars (INIFAT-93 and INIFAT-94) responded favorably to inoculation, although without significant differences between them, which may be associated with their known ease of establishing symbiosis with rhizobia. Notably, these are the first studies in Cuba evaluating the association of *R. pusense* with common bean and cowpea in consortium with other bacteria.

The superiority of the consortium treatments can be attributed to the functional complementarity of the strains used, which were selected based on their ability to interact effectively with legumes (Bianco, 2020; Chaudhary *et al*., 2022). *R. pusense* R3 acts as a nitrogen fixer and has been shown to stimulate chickpea growth (Ortega-García *et al*., 2024a), as has been reported with other *R. pusense* strains in crops such as maize (Amezquita-Avilés *et al*., 2022), chickpea (Gopalakrishnan *et al*., 2018), peanut under drought stress (Ramakrishnan *et al*., 2024), and soybean exposed to heavy metals (Saran *et al*., 2020). *B. thuringiensis* B3 is characterized by its ability to solubilize phosphates, exert biocontrol of phytopathogens, and enhance lettuce and tomato growth (Ortega-García *et al*., 2024b), *B thuringiensis* is well known for its use in biocontrol and as a PGPR that promotes root development, improves nutrient uptake, and enhances plant growth and stress tolerance (Azizoglu, 2019). While *B. velezensis* B8 shows similar effects and has demonstrated a positive impact on maize and wheat (Ríos-Rocafull *et al*., 2011). Furthermore, *B. velezensis*, previously classified as *B. subtilis* and *B. amyloliquefaciens* (Bagheri *et al*., 2019; Vahidinasab *et al*., 2019), is notable for producing siderophores, hydrolytic enzymes, and antibiotics that limit the development of phytopathogens (Khan *et al*., 2018; Moreno-Galván *et al*., 2020; Redondo-Gómez *et al*., 2022). In this way, this synergistic interaction may also enhance tolerance to biotic and abiotic stresses, as suggested by Wang *et al.* (2018) and Tewari & Sharma (2020).

Effect on pod production

Regarding pod production, a pattern similar to that observed in nodulation was evident, with significant increases in the number and biomass of pods per plant in the three crops when the consortium was applied, particularly in combination with 50% mineral fertilization (Figure 2). The cultivars Nacional-29 (chickpea), Güira-89 (common bean), and INIFAT-94 (cowpea) were the most productive, which is directly related to potential yield. This increase in productivity confirms the relationship between effective nodulation and greater nutrient availability, especially nitrogen and phosphorus.

The combination of bioinoculants with reduced fertilizer doses has been reported as an efficient strategy to improve productivity and reduce dependence on chemical inputs. In chickpea, Ahlawat *et al.* (2007) and González-Leyva *et al.* (2012) demonstrated the effectiveness of *Rhizobium* and *Bacillus* with partial NPK fertilization, while Escobar-Oña *et al.* (2017) and Apáez-Barrios *et al.* (2020) highlighted the increase in pod production and better nutrient assimilation when using biofertilizers. In common bean, coinoculation with *Rhizobium etli* and mycorrhizae (*Glomus intraradices*) plus reduced fertilization showed similar effects (Apáez-Barrios *et al.*, 2014). Other authors have shown that bacterial consortia increase the number of effective nodules, the accumulation of N and P in plant tissue, and tolerance to drought and salinity in legumes and grasses (Flores-Duarte *et al.*, 2022a; Flores-Duarte *et al.*, 2022b; Flores-Duarte *et al.*, 2022c; Saleem *et al.*, 2021; Redondo-Gómez *et al.*, 2022).

Agronomic implications of the bacterial consortium

The application of the multispecific consortium significantly increased the number and biomass of nodules, as well as yield (pods plant^{-1}), compared to control treatments. These improvements are associated with greater rhizospheric colonization and the formation of effective nodules for biological nitrogen fixation (Beyene *et al.*, 2022), better nutrient uptake (N, P, K, micronutrients) through the solubilizing activity of *Bacillus* spp. (Huang *et al.*, 2020; Khan *et al.*, 2018), and biocontrol and induced resistance effects reported in *B. velezensis* (Bagheri *et al.*, 2019; Redondo-Gómez *et al.*, 2022).

The accumulated evidence indicates that the use of bacterial consortia allows fertilizer doses to be reduced by up to 50% without compromising yield (Ahlawat *et al.*, 2007; Apáez-Barrios *et al.*, 2020), representing a sustainable alternative in low-input systems. These results are consistent with previous studies where bacterial consortia showed advantages over monospecific inoculants in terms of nodulation, nutrient uptake, and tolerance to abiotic stress in legumes and grasses (Flores-Duarte *et al.*, 2022a; Flores-Duarte *et al.*, 2022b; Flores-Duarte *et al.*, 2022c; Saleem *et al.*, 2021; Tewari & Sharma, 2020).

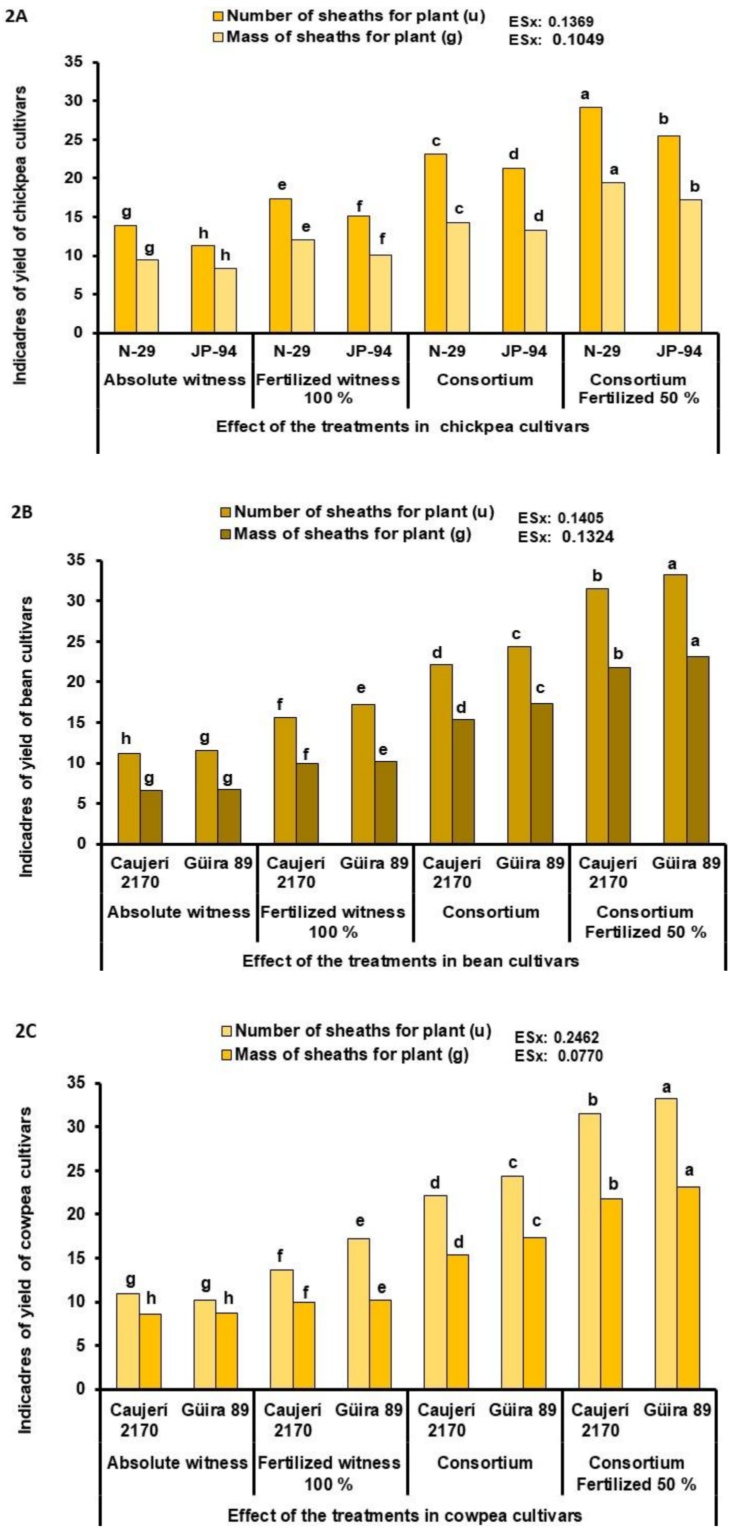

Figure 2. Effect of the consortium on the number and biomass of pods in chickpea (A), common bean (B), and cowpea (C) in a degraded soil (2022–2024). Identical letters do not differ significantly (Duncan, α = 0.05, n = 10).

Finally, the legumes evaluated proved to be strategic crops for degraded agroecosystems, given their ability to associate with rhizobia and improve soil fertility (Hassen *et al.*, 2020). Therefore, biofertilization with bacterial consortia constitutes a viable strategy to increase productivity and reduce the use of synthetic fertilizers in soils with physical and chemical limitations, such as the Ferrallitic Red Lixiviated soil studied here.

CONCLUSIONS

The bacterial consortium composed of *Rhizobium pusense* R3, *Bacillus thuringiensis* B3, and *Bacillus velezensis* B8 significantly promoted nodulation, pod number and biomass, and the potential yield of the three legumes evaluated in a Ferrallitic Red Lixiviated soil with moderate physical and chemical degradation.

The cultivars Nacional-29 (chickpea), Güira-89 (common bean), and INIFAT-94 (cowpea) showed the best response to the consortium, highlighting the importance of compatibility between the microsymbiont and the plant genotype.

These results confirm the potential of the consortium as a multispecific biofertilizer capable of improving legume productivity in degraded soils and allowing a reduction in the use of chemical fertilizers, thereby contributing to the sustainability of agroecosystems.

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