

*Full Length Research Paper*

# **Coinoculation of rhizobia and mycorrhiza in cowpea beans (*Vigna unguiculata* (L.) Walp) in Calixto García Municipality, Cuba**

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The research goal is to evaluate the rhizobia and mycorrhiza application on Cowpea bean (*Vigna unguiculata* L) cv. *Carita Blanco* growth. The experiments were developed at Calixto García municipality, Cuba. It was conducted from April to June 2020, on brown Sialitic soil without carbonate. This design was a random block with three treatments (Control, Rhizobium and Rhizobium + Mycorrhizas). It entailed indicators measurement during ten days and after seeds plantation. The indicators measured were plant length (cm), number of trifoliolate leaves, the diameter of the stem base (mm), number of nodules per plant, as well as yield and its components, at the moment of harvest. Statistic helped to analyze data by one-way analysis of variance, followed by Duncan's multiple range test. Hence, the statistic professional program SPSS, version 20 for Windows was implemented. The Rhizobium and Mycorrhiza co-inoculation showed the best results for growth and yield indicators.

**Key words:** Biofertilizers, rhizobia, mycorrhizas, cowpea beans, inoculation, brown sialitic soils.

## **INTRODUCTION**

In underdeveloped tropical areas, the search for sustainable alternatives to reduce food dependency indicates a group of tropical grain legumes that could be constituents of the protein component needed for human and livestock diets. Current agriculture greatly produces

grain legumes because of their nutritional qualities, their commercial demand, diversity of consumption along with healing properties and economic environmental importance. The cowpea (*Vigna unguiculata* (L.) Walp) is easy to raise and harvest. It naturally adapts to the

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tropical ecosystem. Likewise, it is very nutritional and widely disseminated in the tropics (Boscán, 1987; Labarca et al., 1999; Báez and Hernández, 2016).

This crop acquires more and more importance within the field of research (Saldarriaga, 2015). It has a high content of protein, iron, and zinc. Generally, small farmers cultivate this grain also for economy reasons (Tatis et al., 2017). In Cuba, it is a grain of wide agricultural use, the economic importance of it comprises two fundamental reasons: (a) it constitutes a source of vegetable protein for animal and human consumption and (b) its foliage frequently serves as a green manure (Gómez et al., 2006). The national production of grain legumes still does not satisfy consumer needs, due to the influence of various factors that limit its production. The high price of chemical fertilizers, as well as their high environmental impact, gives room for the search in order to present alternatives that help to satisfy the nutritional requirements of this crop in the country.

In this sense, legume is an option because it is reported as a rustic species that can be found in adverse environments and with tolerance to drought, high temperature, heavy metals, and saline stress. However, it can also yield a considerable amount of nitrogen ( $150 \text{ kg N ha}^{-1}$ ) of symbiotic fixation to the ecosystem (Hernández et al., 2015; Gómez et al., 2017; López-Alcocer et al., 2017; Aguilar et al., 2019). The *Carita Blanco* variety is a prevailing Cuban grain; although, other varieties are grown but on a smaller scale, such as chickpea beans, *Tomeguín* eggs, corn beans, among others (Escalona, 2002). Holguín province has around 4000 ha per year to harvest this crop. Therefore, this province is the main producer of this grain in the latest years. There, Velazco is an area that treasures a very (Domínguez, 2012). On the other hand, the benefits of bio-fertilizers favor the increasing absorption of nutrients and protecting the crop from the attack of pathogens. Additionally, it furthers growth and crop yielding (Otaiku et al., 2019). It is worth mentioning that bacteria of the rhizobia family can induce in the roots of legumes the formation of specialized structures called nodules, within which atmospheric  $\text{N}_2$ , which is very stable and relatively inert. It reduces to ammonium ions ( $\text{NH}_4^+$ ), easily assimilated by most plant species (Llerena, 2017; Thuijsman, 2017). In Calixto García municipality, bean production is generally affected by some problems. For instance, loss of the productive capacity of the soils, inadequate soil management due to excess of chemical fertilizers and the use of water with high salinity for irrigation. Moreover, low yielding in cowpea bean production and limitations in the availability of chemical fertilizers to meet the requirements of the crop play their role. That is why the research aims at the evaluation of the effectiveness of the application of rhizobia and Ecomic (AMF) in the growth of the cowpea bean crop (*V. unguiculata* (L.) Walp), variety of *Carita Blanco* in Calixto García.

## MATERIALS AND METHODS

### Description of the study site

Researchers from the Municipal University Center of Calixto García led the study during the months of April-June, 2020. The study was developed in the rural area of La Alegría, which is located in Buenaventura, Calixto García municipality, Holguín province. It is a place at the eastern end of Cuba (Figure 1), geographically located at  $20^\circ 52' 27'' \text{ N}$  latitude and at  $76^\circ 39' 03'' \text{ W}$  longitude and with an altitude of 104 m above sea level, these data were determined by SAS.PLANET software version 190707.10011 estimated height according to SRTM 3 with a precision of 90 m.

The annual average temperature is  $25.6^\circ\text{C}$  and rainfall ranges between 800 and 1200 mm per year. The behavior of the meteorological variables of rainfalls and high and low temperatures during the development of the research is presented in Figure 2.

### Soil type and fundamental characteristics

The experiment was carried out on a Salty brown soil with carbonates (Hernández et al., 2015) with a pH ( $\text{H}_2\text{O}$ ) of 6.6. The potentiometric method helped to determine it. The method also helped to notice that the soil is slightly acidic to neutral. Therefore, it is considered acceptable for the development and growth of the crop. The phosphorus content is  $6.4 \text{ mg.kg}^{-1}$ . The Oniani method allow the determination of phosphorus. The extraction was simpler with  $\text{H}_2\text{SO}_4$ . Carbon-Nitrogen ratio was of 7.6. The organic matter content rate was of 2.3%; which was determined through Walkley-Black method,  $\text{d}_{15\text{N}}$  10.8% was also noticed. In general terms, the analysis was developed in line with the methodology presented by Paneque et al. (2010). That is why, it is considered to have a too low average fertility. The clay fraction predominates in the soil. Based on the characteristics of the soil, it is considered appropriate for the cultivation of cowpea beans.

### Soil preparation, treatments, and experimental design

Soil preparation was carried out with minimum tillage, breaking, crossing, cultivator pass, and furrowing, with animal traction. The cultivar *Carita Blanco* of the cowpea bean was used, the sowing was carried out at a rate of three seeds per hole at a distance of 0.15 m. When the plants sprouted and had an average height between 10 and 15 cm, each plot was thinned to leave only 2 plants per hole. At 40 days after sowing the crop (DDS), the following growth variables were evaluated: plant height (cm), a measuring tape was used for this; the number of leaves was performed by counting the diameter of the stem base (mm) for which a caliper was used; and the number of total nodules was developed by the plants in each treatment by counting. At the time of harvest, the yield components were evaluated: number of legumes per plant (in 15 plants per plot) by counting, number of grains per legume (mean of 20 legumes) by counting, the average mass of 100 grains (g) for which a digital scale with a capacity of 2610 g and a precision of 0.01 g was used, and the yield per unit area was calculated based on the mass of the grains harvested per plot. The treatments evaluated in the experiments are shown in Table 1.

A randomized block experimental design with three treatments and three replications were used with plots of  $2.80 \text{ m} \times 4.0 \text{ m}$  ( $11.20 \text{ m}^2$ ). The inoculants used on the seeds were Azofert®, *Rizobiumfreirei* PRF-81 strain, with a concentration of  $2.3 \times 10^{10} \text{ CFU mL}^{-1}$  and a dose of  $200 \text{ mL}^{-1}$  for 50 kg of seeds, and EcoMic®, based on arbuscular mycorrhizal fungi (AMF) of the species *Glomus intraradices*, at a dose of 7.5 kg for 50 kg of seed, both



**Figure 1.** The geographical situation of the study area.

supplied by the National Institute of Agricultural Sciences (INCA). The sowing was carried out on June 3, 2020. The rows were 4 m long, with a separation of 0.70 m. Four rows were planted per plot with a total plot area of 11.20 m<sup>2</sup>. A separation of 1 m between blocks and 2 m between treatments was used. Fertilizers were not applied in the experiment, registering a slight attack of *Aphis cracivora* 40 days after sowing, which was controlled as established in the technical instructions for this crop in Cuba. Weeding was carried out at 15 and 45 days after sowing.

#### Growth parameters, yield and their components

"La Jíquima" Meteorological Station located within the boundaries of Calixto García Municipality provided the values of the meteorological variables. The blooming started 38 days later. It was an irregular event in the different blocks of the experiment. At 40 days after sowing (DDS), 10 plants were randomly taken for each replica, the growth variables were evaluated: plant height (cm), number of leaves, the diameter of the stem base (mm), and the number of total nodules developed by plants. One day before harvest, 10 plants were selected per replica with a total of 30 for each treatment in the central rows. The objective was to evaluate the number of legumes per plant, number of grains per legume, and the mass of 100 grains. In the same way, we proceeded to calculate the yield in kg.ha<sup>-1</sup>. Once results came out, an economic assessment was made from the cost-benefit ratio and the profits achieved in each treatment.

#### Statistical analysis of the data

The data corresponding to the growth variables: plant height (cm), number of leaves, the diameter of the stem base (mm) and the number of total nodules of a plant, as well as yielding and its components were subject to a simple classification analysis of variance (ANOVA). A significance level of 5% came out. The statistical program SPSS, version 20.0 for Windows was worth implementing here. With the results of these analyses, tables and

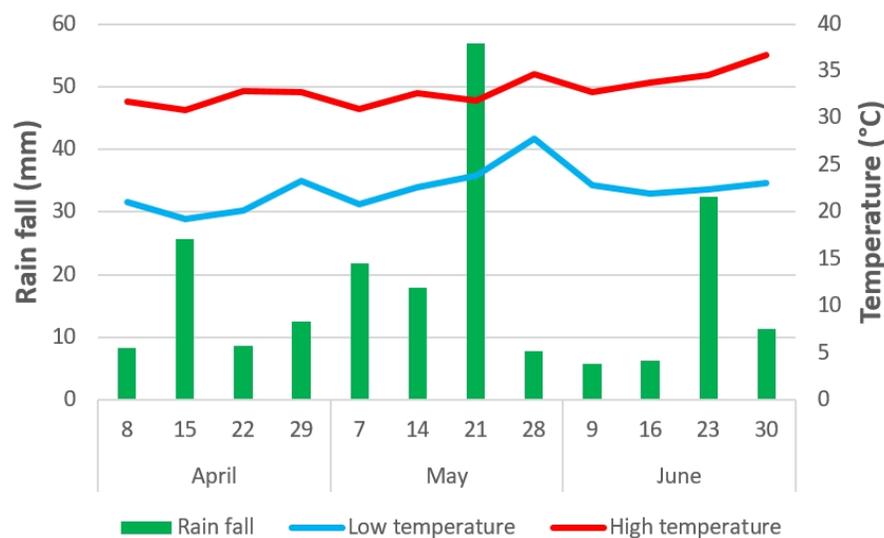
graphs were devised that help to understand the causal relationship of the different inoculums and their combination and meteorological variables with the agricultural yielding of the cowpea bean in Calixto García municipality at the eastern region of Cuba.

## RESULTS AND DISCUSSION

The behavior of the growth variables evaluated at 40 DDS and according to each treatment, is shown in Table 2.

#### Height of the plant

It is observed that the height of the plants shows differences in the treatments that implied the application of Rhizobium and the joint inoculation of Rhizobium and Mycorrhizae. Concerning the control, reaching the treatment II (Rhizobium + Mycorrhizal) the highest values with 63.5 cm. This behavior confirms the importance of the combined use of fungal and bacterial biofertilizers to improve the plant response. These results are similar to those obtained in previous studies carried out when evaluating the inoculation of Rhizobium, Mycorrhizae, and the combination of these inocula in the cultivation of common bean (*Phaseolus vulgaris* L.) in the province of Matanzas (Liriano et al., 2012). Likewise, in studies carried out at the National Institute of Agricultural Sciences (INCA), Corbera and Nápoles (2011) showed significant responses between treatments, standing out, mainly for the harvest stage. This is a treatment where the joint inoculation of biofertilizers was carried out when evaluating the joint application of Rhizobium and



**Figure 2.** The behavior of the rainfall and high and low temperatures during the growing circle.

**Table 1.** Treatments studied in the field experiment.

Treatment	Inoculant treatments used
Control	Without inoculant
Treatment I	Azofert (rhizobia)
Treatment II	Azofert + Ecomic (AMF)

Mycorrhizae in soybean cultivation.

**The number of leaves**

The number of leaves presents significant differences between the evaluated treatments, with 36.1 leaves. The co-inoculation with Rhizobium and Mycorrhizae showed the best results.

These outcomes are a response of the noticeable influence of biofertilizers in the supply of nitrogen and phosphorus to the vegetal. Other aspects that had to do with such outcomes are the increasing content of organic matter of soil and the increasing absorption capacity of the plant.

These results coincide with studies completed in other areas of the country where the highest values in this indicator are reported in the treatment in which the simple inoculation of Rhizobium and Mycorrhizal was carried out when evaluating the effect of these biofertilizers in the cultivation of common beans (Liriano et al., 2012). Similar results were obtained in the region of Saltillo, Coahuila in a study done based on the inoculation with Rhizobium in beans, which reported that in the inoculated plants a

greater number of leaves was noticed along with a better growth in the plant (Aguilar et al., 2019).

**Stem base diameter**

The diameter of the stem base does not show a significant difference between the inoculation with Rhizobium and the co-inoculation of Rhizobium and Mycorrhizae, corresponding to these treatments with 7.6 and 8.1 mm, respectively, the highest values, which differ significantly with the treatment used as a control. These results match those reached in previous investigations about common beans. The highest values in this indicator were obtained in the treatments where the co-inoculation of Rhizobium and Mycorrhizae were used in combination (Liriano et al., 2012). Similar results were achieved by Mexican researchers when evaluating this indicator in bean plants inoculated with Rhizobium in the Saltillo area, Coahuila (Aguilar et al., 2019).

**The number of nodules per plant**

The results obtained for the variable number of nodules

**Table 2.** Effect of treatments on growth variables

Treatment	Plant height (cm)	Number of leaves	The diameter of the stem base (mm)	Number of nodules/plant
Control	45.83 <sup>a</sup>	21.54 <sup>a</sup>	5.73 <sup>a</sup>	2.21 <sup>a</sup>
Azofert (rhizobia)	54.60 <sup>b</sup>	27.38 <sup>b</sup>	7.66 <sup>b</sup>	16.42 <sup>b</sup>
Azofert + Ecomic (AMF)	63.52 <sup>c</sup>	36.15 <sup>c</sup>	8.13 <sup>b</sup>	17.89 <sup>b</sup>
Ex	6.89	4.95	0.87	3.75

Means on with the same letter on the column indicates no significant difference ( $p \leq 0.05$ ) between the treatments.

per plant show significant differences between the control and the rest of the treatments. It reached the highest values where Rhizobium and the combination of Rhizobium with Mycorrhizal were applied with 16.4 and 17.8 nodules per plant respectively; highlighting the marked difference between these treatments and the control in which nodule formation was hardly observed.

The low emission of nodules in the controlling treatment is connected with the lack of a boost for the emission of these structures. It corroborates how beneficial microorganisms are for the improvement and developed of this plantation.

There were no differences between the rhizobia treatment and the combination with AMF. Similar results in this variable have been indicated in the crops of common beans and soybeans when using the inoculation of the seeds with Rhizobium and its combination with arbuscular mycorrhizal fungi (AMF) (Rebeschini et al., 2014, Nápoles et al., 2014). In agricultural soils, the Rhizobium-legume association is considered the most important source of  $N_2$ , since it has been reported that in nodulated legumes, under certain environmental conditions (soils poor in nitrogen), they can fix up to 100 kg  $N_2$ /ha/year, being this mechanism capable of supplying the Nitrogen demand to satisfy the most important nutritional needs of the plant (FAO, 1995; Saidi et al., 2010; Nebiyu et al., 2014; Hernández et al., 2015; Ndalira et al., 2020). Similarly, implemented studies in intercropping systems of cassava with legumes in Badeggi, Nigeria report a significant contribution of this practice on the content of nutrients in the soil; specifically, the content of organic carbon, nitrogen, and available phosphorus (Gbanguba et al., 2020).

The rises triggered by the co-inoculation of de Rhizobium and Mycorrhizal in the evaluated growing variables demonstrate the capacity of the bean plantation to develop a double symbiosis *Rhizobium*-arbuscular mycorrhizal fungi (AMF).

### Yielding and its components

When analyzing the effect of the treatments on the

yielding and yielding component variables (Figure 3), an increase in the results of these variables is generally observed in the treatments where biofertilizers were used concerning the control.

### Number of legumes per plant

In the variables of grain yield and its components, the number of legumes per plant presents significant differences between treatments, observing that the highest results were obtained with the combined inoculation of Rhizobium and Mycorrhizal with 30.2 legumes. It differs significantly from the rest of the treatments, followed by simple inoculation with Rhizobium with 21.1 legumes per plant. There is a significant disparity from each other and the control. These results are similar to those that came out in previous studies where this indicator showed the highest values of legumes per plant in those treatments where soybeans were inoculated with both biofertilizers (Corbera and Napoles, 2011). Other quests found that the number of legumes per plant showed noticeable increases with the application of Rhizobium at the moment of evaluating its application in varieties of common bean under water stress contexts (Estrada et al., 2017).

### The number of grains per legume

This indicator shows that 9.05 comes out from the control with the implementation of Rhizobium 12.15. Concerning the joint co-inoculation of Rhizobium and Mycorrhizal, there is a 14.62 average grains per legume, respectively. These results match those obtained by other studies where an increase in the number of grains per legume in common beans is observed with the application of Rhizobium (Estrada et al., 2017). Likewise, Tatis et al. (2017) got similar outcomes. They evaluated cowpea bean genotypes. They obtained values for the number of seeds per legume that ranged between 10.66 and 13.47 in Colombia.

### Yield and its components

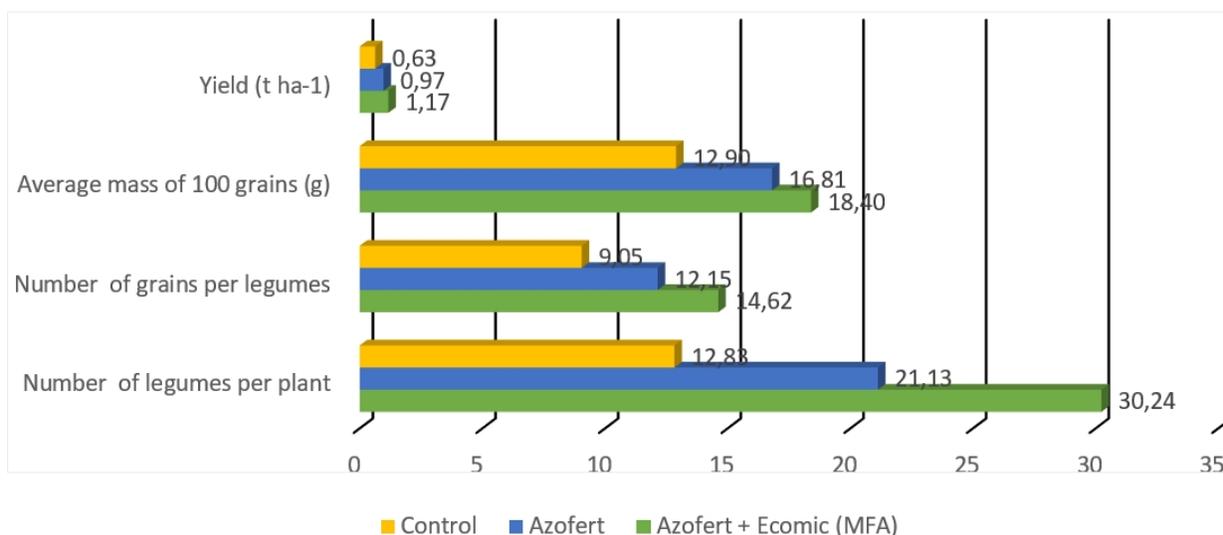


Figure 3. The behavior of the yielding and its components.

#### The average mass of 100 grains

As regard to the average mass of 100 grains, the results indicate that 12.9 g were obtained in the control; with the inoculation of Rhizobium as 16.8 g, while the combination of Rhizobium and Mycorrhizal as 18.4 g, respectively. These results differ from those obtained in earlier research done by Corbera and Napoles (2011). Such results revealed that when evaluating the mass of 1000 grains in the soybean crop, no significant differences were observed between treatments. It indicates that this variable in the crop was little influenced by the application of the evaluated products.

Subsequent studies found that the indicators legume length, legume width, number of seeds per legume, the mass of seeds per plant, and mass of 100 seeds displayed considerable increases with the application of Rhizobium at evaluating its application in varieties of common bean in water stress contexts (Estrada et al., 2017).

#### Yielding

As a result of the growth and development of the crop, the grain yield also indicated conspicuous differences between treatments, highlighting, in the same way, those where Rhizobium and Mycorrhizal were inoculated in a simple and combined way. Hence, it presented increases in yields of 43.06% for the cultivar studied with the control.

The yield shows a meaningful difference between the control and the treatments (simple inoculation with Rhizobium and the combination of Rhizobium and Mycorrhizal). It obtained 0.97 and 1.17 t ha<sup>-1</sup>, respectively, observing a clear tendency to increase the yield when biofertilizers were applied. The control showed the lowest yieldings with 0.63 t ha<sup>-1</sup>. This yielding behavior links itself to the increase in the mass of the grains, the number of legumes per plant, and the number of grains per legume in each treatment. Recent studies in the cultivation of common bean developed in the north of Tanzania corroborated increases in yield by determining the optimal planting density for cultivation under various doses of phosphoric fertilization (Kiriba et al., 2020).

The result obtained in the yielding components shows that the best results appear with the co-inoculation of Rhizobium + Mycorrhizal in the conditions in which the experiment was developed. It presents a close relationship with the rest of the variables analyzed.

Hernández and Cuevas (2003), Hernández (2008) and Ramos et al. (2013) agree that the combined use of Rhizobium and Mycorrhizal optimizes the process of fixation of atmospheric nitrogen, the absorption of nutritive elements, and therefore plant development is stimulated by increasing the productive potential of plants. The joint implementation of Azofert (Rhizobium) along with the arbuscular mycorrhizal *G. intraradices* has an effect on this particular bean plantation. Such effect is revealed in the yielding of grain number as a fundamental variable. It displayed a positive response to the study of context and the assessed plantation. Obviously, there

**Table 3.** An economic evaluation of the results achieved.

Treatments	Yield (t.ha <sup>-1</sup> )	Sale price (CUP.t <sup>-1</sup> )	Total Production Value (CUP/ha.t <sup>-1</sup> )	Nitrogen Fertilizer Price (USD/50kg)	Contribution of N <sub>2</sub> for biological fixation (kg/ha/year)	Cost price of biofertilizer (CUP)	Savings from dispensing with Nitrogen fertilizer (CUP.ha <sup>-1</sup> )
Control	0.63	6820.00	4296.60	40.00	-	-	-
Azofert	0.97	6820.00	6615.40	40.00	150.00	34.00 (200 ml)	366.00
Azofert + Ecomic	1.17	6820.00	7979.40	40.00	150.00	42.00 (20 kg)	306.00

Used conversion factor: 1 USD = 1 CUC = 10 CUP. USD: North American Dollar; CUC: Cuban Convertible Peso; CUP: Cuban Peso.

were increases in the production of this bean. It favors a biotechnological management as an effective nutritional alternative for the development of this plantation.

### Economic valuation

The economic evaluation of the results achieved in each of the treatments is shown in Table 3. To plant a hectare of bean, 50 kg of seeds is required. In order to inoculate this quantity of seeds 200 mL of Azofert is required. Then, 34.00 CUP are needed at the national market. To inoculate the same quantity of seeds with Ecomic, 7.50 kg of this product is required and it takes 8.00-CUP kg<sup>-1</sup> in the national market.

When it comes to analyzing the correlation between investment and benefit in each of the treatments, there is a rise in sales that reports an income of 2 318.80 and 3682.80 CUP, respectively, both using biofertilizers. To attain these levels there are alternatives; for example, the use of nitrogen fertilizer. It requires 40 kg ha<sup>-1</sup> that costs 40.00 CUC at the foreign market, that is to say 400.00 CUP according to the Cuban exchanging rate. However, national biofertilizers produced at the National Institute of Farm Sciences seem to be a better option. It is cheaper

because it only costs 34.00 CUP of Azofert and 42.00 CUP of EcoMic®. The implementation of these biofertilizers reduces the expenses at a 91% for Azofert and 76.5% in a merging of Azofert and Mycorrhizal. Therefore, the combination of both biofertilizers is the most fitting alternative in terms of economy. Thus, the profit is just over 3 588.80 CUP because the production levels are higher. Subsequently, chemical-based fertilizers are disposed of due to the natural and ecofriendly values of biofertilizers.

### Conclusion

The results already presented in this paper suggest an effective plant-microorganism association, considering the simple inoculation and co-inoculation of Azofert and EcoMic® as a viable, sustainable, and ecological alternative for the production of the cowpea bean crop. In general, the co-inoculation of Azofert and EcoMic® showed the best results in the growth variables evaluated, as well as in the performance components.

The use of biofertilizers is an alternative that allows the achievement of better economic results and a reduction in expenses for the purchase of chemical fertilizers.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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