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RESEARCH ARTICLE

EFFECT OF CO-INOCULATION WITH RHIZOBIUM TROPICI AND AZOSPIRILLUM BRASILENSE IN OFF-SEASON COWPEA CULTIVATION IN WESTERN BAHIA

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Abstract

Beans represent an important food source throughout the world, being a staple in diet due to their ease of cultivation and management. In the North and Northeast of Brazil, the consumption of cowpeas (*Vigna unguiculata* L. Walp) is very significant. Through this work, sought to demonstrate the effect of coinoculation on cowpea using *Azospirillum brasilense* and *Rhizobium tropici* in second crop cultivation in the western region of Bahia. The experimental design was in randomized blocks with four treatments and four replications. The experiment was carried out in the field, using the Pingo de Ouro landrace variety with the following treatments: T1 without inoculant, T2 with *Rhizobium tropici*, T3 with *Azospirillum brasilense* and T4 with both. The variables of quantity and weight of nodules, root length and weight (cm), weight of fresh leaves (g), weight of dry leaves (g) and stem weight (g) were analyzed in V5 and R1, as well as such as the analysis of plant tissue in V5 and R1, and bromatological analysis of harvested grains. From the results obtained, it was observed that T4 presented greater productivity and performance in relation to the other treatments in terms of quantity and weight of nodules, root length and dry mass of leaves. Macro and micronutrient contents were also higher in T4, both in V5 and R1, especially for N, P and K and Zn and Fe which were higher in R1. The study concluded that the use of the co-inoculation process in cowpea seeds was efficient and sustainable.

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Introduction: -

Common bean represents a dietary staple for various populations worldwide. Its importance stems from being a nutritious food source, containing several minerals and vitamins essential for human development, alongside its ease of cultivation for smallholder farmers and traditional communities. Furthermore, it plays a fundamental role as a sustainable tool for acquiring atmospheric nitrogen, serving as an alternative to mineral fertilizers. Cowpea (*Vigna unguiculata* (L.) Walp.) is a grain legume widely and diversely used in human nutrition, acting as one of the primary

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protein sources for populations facing food insecurity across several regions of Latin America and Africa. Within the Brazilian context, cowpea is a species of fundamental importance, not only as a source of nutrients and vitamins but also regarding the economic and social aspects of the crop. This is because it is an easy-to-manage and easy-to-cultivate plant, especially in regions with scarcer rainfall patterns, such as the North and Northeast regions of the country.

According to data from the Companhia Nacional de Abastecimento (CONAB), the states of Ceará, Bahia, and Piauí are the main producers, respectively, with yields ranging from 152 kg/ha to 679 kg/ha in highly technified areas. Regarding total Brazilian productivity for the 2024/2025 and 2025/2026 crop years, there was an 18% increase, which can be attributed to technical improvements in crop management and favorable environmental conditions, particularly the consistent rainfall index throughout all critical growth stages of the crop (CONAB, 2026). Due to its high adaptability to elevated temperatures and low rainfall, cowpea is extensively cultivated in Western Bahia, especially during off-season windows. Cultivar selection relies not only on broad agronomic guidelines, but primarily on genotypes adapted to local conditions. Therefore, factors including resilience to biotic and abiotic stresses, climate, soil type, planting date, and phytosanitary management are decisive for optimal crop performance (Oliveira, 2024).

Biological nitrogen fixation (BNF) plays a central role in the sustainability of legume production systems in Brazil. This process incorporates high levels of atmospheric nitrogen into the production chain particularly for soybean exempting farmers from purchasing mineral nitrogen fertilizers. Consequently, this makes soybean highly productive and competitive in both national and international markets, while also remaining fundamental for the maintenance and preservation of natural ecosystems. The co-inoculation process occurs when two bacterial species specifically symbiotic bacteria and Plant Growth-Promoting Bacteria (PGPB) are inoculated together onto the seed to promote greater root growth and development, linked to increased efficiency in nodule formation and atmospheric nitrogen absorption. Therefore, this study aimed to evaluate the field-scale effects of co-inoculating cowpea seeds with *Azospirillum brasilense* and *Rhizobium tropici* in the Bahian Cerrado. The objective was to increase crop yield through the synergistic application of these two bacterial strains during an off-season period marked by elevated temperatures and low precipitation. The study evaluated parameters such as nodule number, and fresh and dry leaf weight, as well as nutrient content in leaf tissue and bromatological analysis of the grains, in order to measure the gains achieved through co-inoculation. Consequently, the increase in yield was determined by quantifying the harvested grains and evaluating the Thousand-Seed Weight (TSW).

Materials and Methods: -

Location and Characterization of the Experimental Area:-

The present study was conducted at Horizontina Farm, located in the Bela Vista Community, rural zone of Luís Eduardo Magalhães, Bahia, Brazil, at latitude 12°12'16"S and longitude 46°16'24"W (Figure 1). According to the Brazilian Soil Classification System (SiBCS, 5th edition), the soil is classified as a Latossolo Amarelo Eutrófico (Yellow Oxisol), with a sandy loam texture.

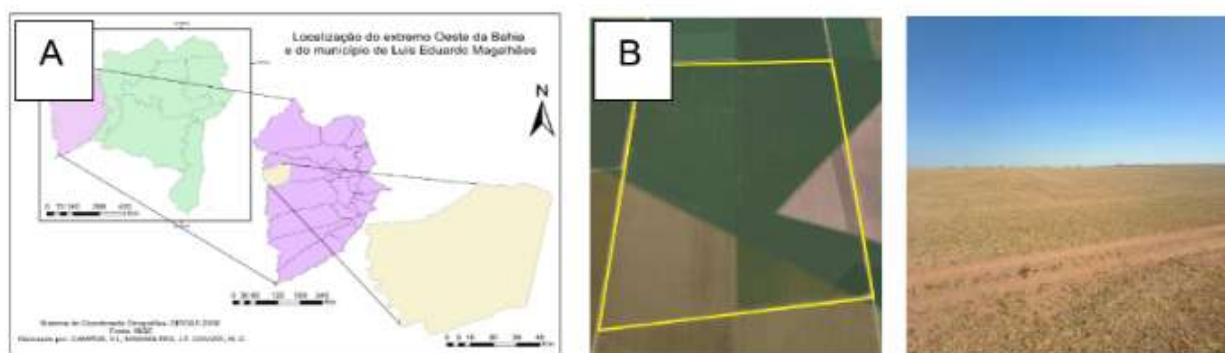


Figure 1.(A) Geographic location of Luís Eduardo Magalhães, Bahia and (B) Location of the field plot where the experiment was conducted. Source: Google Earth (2023), (Italiano, 2023; Magalhães et al., 2017).

The trial was conducted during the off-season period, a timeframe characterized by scarcer rainfall patterns, where the established crops such as grain sorghum and cowpea are positioned to withstand water deficits and high temperatures. Cowpea was specifically selected due to its shorter growth cycle. In the experimental area, soybean had been previously planted as the summer crop. According to the Köppen climate classification, the region's climate features Aw (tropical savanna) and Cwa (humid subtropical with dry winters and hot summers) types, with an average annual temperature of 24°C. The average annual precipitation ranges from 900 to 1000 mm, characterized by torrential rainfall patterns that lead to severe flooding in the summer, contrasting with periods of scarcity during the winter. The occurrence of low-intensity and poorly distributed rainfall, combined with daily temperatures around 32°C, induced water restriction in the crops.

Trial Establishment: -

The experimental area was managed under a No-Till System (NTS) without soil tillage under rainfed conditions without supplemental irrigation. Soil correctives were applied biennially, while fertilizers were applied annually at variable rates broadcast across the soil surface and as topdressing. During the off-season, no additional fertilizers were applied, utilizing only the residual fertility remaining from the primary crop fertilization. Sowing was performed mechanically using a 25-row John Deere® planter (model DB40), with a sowing speed ranging from 6.8 to 7 km/h. The 'Pingo de Ouro' cowpea landrace was used for the study because it is a traditional variety in the region that has not undergone breeding processes, thereby retaining its genetic robustness and hardiness. The planting density was 11 to 12 seeds per linear meter, with a row spacing of 50 cm, representing a population of 220,000 plants/ha.

Experimental Design and Treatments: -

The experimental design was a randomized complete block design (RCBD) with four treatments distributed across four replications, arranged as follows: T1, uninoculated control; T2, inoculated with *Rhizobium tropici*; T3, inoculated with *Azospirillum brasilense*; and T4, co-inoculated with both *Rhizobium tropici* and *Azospirillum brasilense*.

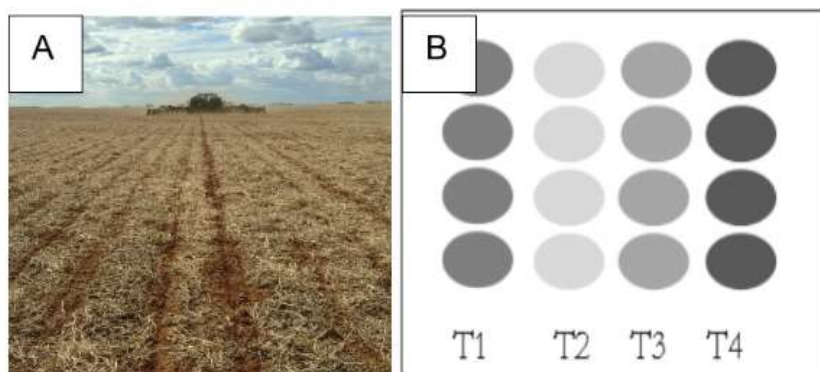


Figure 2. (A) Sowing of the experiment and (B) Field layout scheme of the experimental area showing treatments and replications (Italiano, 2023).

The inoculation process was carried out using two commercial liquid inoculants. *Rhizobium tropici* (strains SEMIA 4077) was applied at a concentration of 1×10^9 cells mL⁻¹ at the recommended rate of 2.5 doses per 100 kg of seeds. The second inoculant comprised the plant growth-promoting, nitrogen-fixing bacterium *Azospirillum brasilense* (strains Ab-V5 and Ab-V6) at 4×10^8 cells mL⁻¹, administered at the same rate of 2.5 doses per 100 kg of seeds.

At the V5 and R1 growth stages (32 and 58 days after emergence, respectively), five plants were harvested from each plot and each replication, totaling 80 plants evaluated. The following variables were measured: number of nodules per plant, individual nodule weight (g), root length (cm), root fresh weight (g), leaf fresh weight (g), leaf dry weight (g), and stem fresh weight (g). In addition, five plants were randomly collected from each treatment and taken to the laboratory for plant tissue chemical analysis. Plant tissue analysis determines the concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), boron (B), and manganese (Mn) present in roots, stems, and leaves, enabling the identification of the plants' nutritional status across different phenological stages.

Pre-harvest desiccation was performed at 82 days after emergence (DAE) using the non-selective, contact herbicide Diquat (bipyridylum group) at 1.5–2.0 L ha⁻¹ (300–400 g a.i. ha⁻¹), according to manufacturer recommendations. Seven days after desiccation, cowpea grains were manually harvested from a 10 m long by 2 m wide area in each plot, across 4 replications. Seed count and grain weight per plant were determined to evaluate final yield. No plant loss from failed germination or mortality occurred. Grain weighing was conducted following the Rules for Seed Analysis (Brasil, 2023) via Thousand Seed Weight (TSW), after which samples were transported to the laboratory for bromatological analysis.



Figure 3. Experimental stages: (A) Seed selection, (B) Planting, (C) Nodulation, (D) Flowering, (E) Sample weighing and (F) Harvest (Italiano, 2023).

Results and Discussion: -

Plant Analysis at V5 and R1 Growth Stages: -

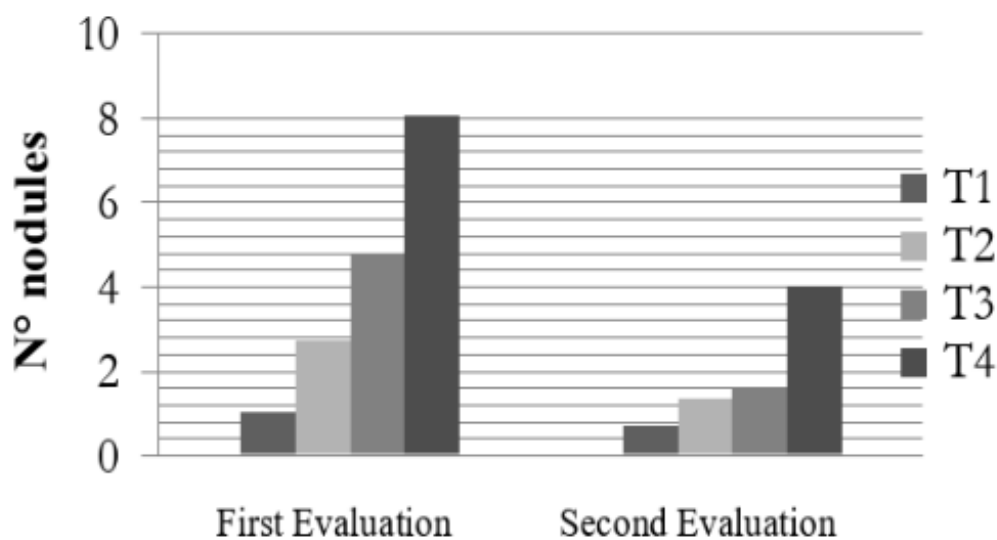
The collected data were subjected to statistical procedures to describe, relate, and interpret the productive variables presented in the following tables. Subsequently, Pearson's correlation coefficient was used to evaluate the linear relationships among cultivated area, total production, and yield. To illustrate the data and facilitate the understanding of plant response patterns, all statistical analyses were performed in R software (R Core Team, 2023) using the agricolae package (Mendiburu, 2023). Data were subjected to Analysis of Variance (ANOVA), with mean comparisons evaluated via Tukey's test at a 5% probability level ($p < 0.05$).

Table 1. Number of nodules (NN) and nodule weight (NW) at V5 and R1 growth stages of cowpea subjected to co-inoculation.

Treatments	NN (V5)	NN (R1)	NW (V5) (g)	PN (R1) (g)
Uninoculated control	1,05 c	0,75 b	0,0175 c	0,005 c
<i>Rhizobium tropici</i>	2,75 c	1,4 b	0,0245 bc	0,0255 bc
<i>Azospirillum brasilense</i>	4,80 b	1,6 b	0,0465 b	0,0845 b
<i>Rhizobium tropici</i> + <i>Azospirillum brasilense</i>	8,10 a	4,05 a	0,0955 a	0,2575 a
CV (%)	3,73%	3,40%	2,58%	3,14%

Means followed by the same letter do not differ significantly by Tukey's test at a 5% probability level. CV (%): Coefficient of variation. Co-inoculation with *Rhizobium tropici* and *Azospirillum brasilense* (T4) markedly enhanced cowpea nodulation at both evaluated phenological stages (Figure 4). Such substantial increases underscore the high efficacy of co-inoculation in optimizing nodule formation and biomass accumulation. A study conducted by Prando et al. (2022) reported an average nodulation on the main root of soybean plants of 14.5 nodules per plant with co-inoculation and 11.2 nodules per plant without inoculation, values higher than those observed in the present study. Thus, the application of *Rhizobium tropici* combined with *Azospirillum brasilense* via seed treatment positively influenced the development of a higher number of nodules in cowpea plants.

Standalone Biological Nitrogen Fixation (BNF) is often insufficient to fully meet the total nitrogen demand of common bean crops. Therefore, co-inoculation with *Rhizobium* and *Azospirillum* strains represents a sustainable management strategy that promotes enhanced nutrient absorption, greater salinity tolerance, and improved drought resilience, particularly under off-season cultivation. According to research conducted by Galindo et al. (2020) on common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp), co-inoculation promotes root exudation of flavonoids, which are responsible for inducing root hair formation and growth, resulting in a considerable increase in the nodulation process and favoring crop yield potential. The second evaluation took place at the R1 stage, where the total number of nodules was lower than that observed in the first evaluation at V5, with a 50% reduction in T4. This pattern was previously noted by Costa et al. (2006), who reported that the highest number of nodules in cowpea occurs from seven days after emergence (DAE) until the beginning of pod formation, after which older nodules undergo senescence and subsequent decomposition, typically occurring around 56 days.

**Figure 4. Number of nodules obtained at V5 and R1 growth stages in cowpea plants (Italiano, 2023).**

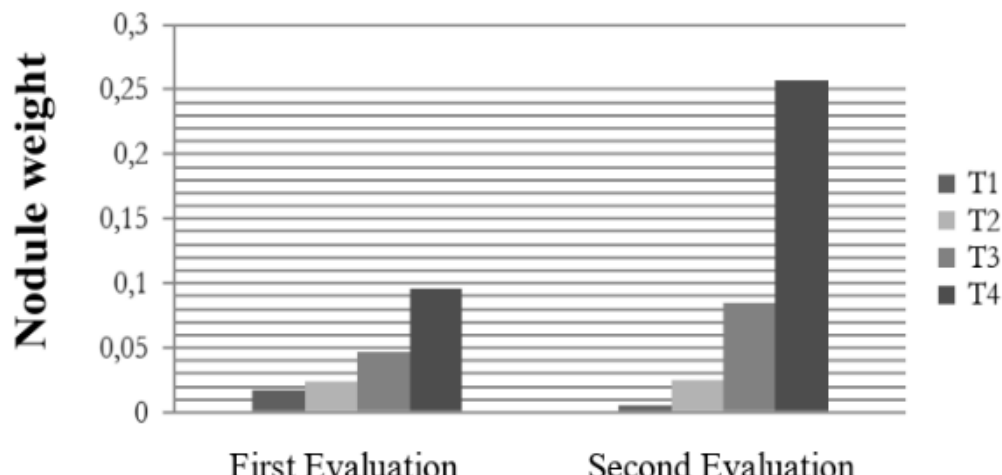


Figure 5. Nodule weight obtained at V5 and R1 growth stages in cowpea plants. Source: (Italiano, 2023).

Data presented in Table 2 and Figure 6 demonstrate the marked superiority of the co-inoculation treatment, which outperformed all other treatments at both phenological stages by increasing root length by 50.55% (V5) and 35.21% (R1). Such substantial enhancement of root architecture plays a fundamental role in crop physiology by expanding soil exploration volume, optimizing water and nutrient acquisition, and enhancing tolerance to drought stress.

Table 2. Root length (RL) and root weight (RW) at V5 and R1 growth stages of cowpea subjected to co-inoculation.

Treatments	CR (V5)	CR (R1)	RW (V5) (g)	RW (R1) (g)
Uninoculated control	5,37 c	16,3025 b	0,64675 c	2,075 c
<i>Rhizobium tropici</i>	6,03 bc	17,725 b	0,9175 b	2,7705 b
<i>Azospirillum brasilense</i>	7,0725 b	16,76 b	0,8715 b	2,9415 b
<i>Rhizobium tropici</i> + <i>Azospirillum brasilense</i>	10,86 a	25,1625 a	1,6365 a	3,51 a
CV (%)	3,55%	4,46%	2,49%	2,58%

This enhanced root development occurred due to the action of plant growth-promoting bacteria present in the *A. brasilense*-based inoculant. According to research by Benmride (2023), One of the key physiological mechanisms driving this cooperative association is the production of phytohormones, particularly auxins, which stimulate root growth, increase root hair density, and enhance water and nutrient uptake. This expanded root architecture subsequently provides more infection sites for the rhizobia-legume interaction. Co-inoculation of sole-cropped soybean with the IAA-producing strain *Azospirillumbrasilense* Az39 (ipdC +) boosted the efficiency of the *Bradyrhizobium*-soybean symbiosis, underscoring the vital role of microbially synthesized IAA in sustaining this symbiotic partnership. Furthermore, a more developed root system provides a better nodulation process and, consequently, greater nitrogen fixation through FBN, increasing both pod and seed yield (Hungria, Nogueira, and Araújo, 2015).

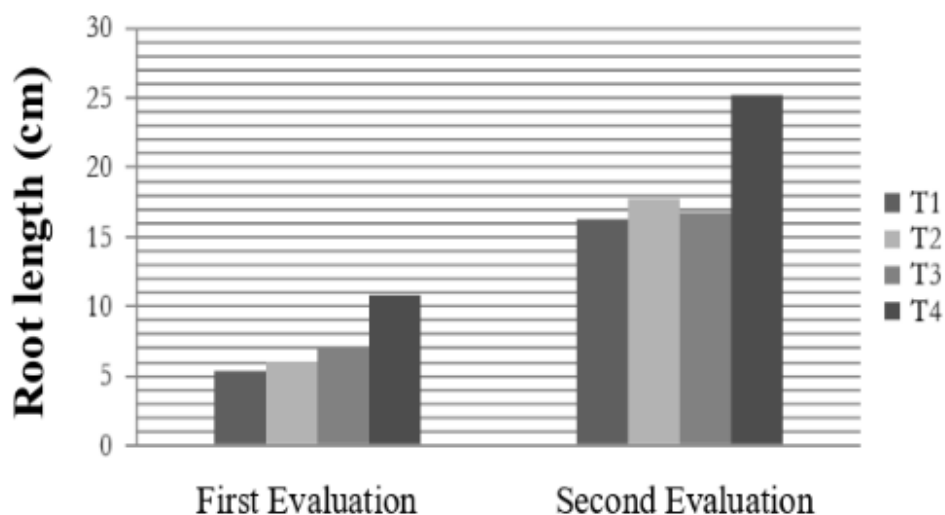


Figure 6. Root length in cowpea plants at V5 and R1 growth stages (Italiano, 2023).

Table 3 presents the values for stem weight and thousand-seed weight. It can be observed that at V5 and R1, the results obtained by plants originating from the co-inoculation process were statistically higher, representing an increase of 63.99% and 47.77% in stem weight, respectively. Regarding the thousand-seed weight variable, the increase obtained was 26.90% when comparing the *Rhizobium tropici* + *Azospirillum brasilense* treatment with the control treatment.

Table 3. Stem weight (SW) and thousand-seed weight (TSW) at V5 and R1 growth stages of cowpea subjected to co-inoculation. Luís Eduardo Magalhães, Bahia, Brazil, 2023.

Treatments	SW (V5) (g)	SW (R1) (g)	TSW
Uniclutated Control	3,3065 d	16,315 c	12,85 c
<i>Rhizobium tropici</i>	4,236 c	37,624 a	16,2875 ab
<i>Azospirillum brasilense</i>	4,993 b	19,1825 b	15,65 b
<i>Rhizobium tropici</i> + <i>Azospirillum brasilense</i>	9,1665 a	31,2415 a	17,5875 a
CV (%)	4,45%	3,99%	12,79%

Source: (Italiano, 2023).

Crop yield data (Table 4) highlight the agronomic efficiency of co-inoculation in cowpea. Treatment T4 promoted a substantial 32.25% increase in grain weight per plant compared to the uninoculated control (T1). This physiological gain directly converted into an additional yield of 3.66 bags per hectare (a 13.78% increase in overall grain yield relative to T1). These findings underscore the technical viability and high efficacy of co-inoculation technology as a low-cost, sustainable management strategy to enhance yield potential in cowpea cropping systems. A comprehensive meta-analysis by Castro Pias et al. (2022) across numerous studies on common beans under no-till management demonstrated that soil organic matter, inoculation, and previous crop species significantly contribute to reducing mineral nitrogen fertilization requirements and improving crop productivity, thereby mitigating environmental damage

Table 4. Cowpea yield harvested in the experiment.

Treatments	Average Weight of Grains + Pod*	Average Weight of Grains*	Grain Weight per plant	Productivity (ha)	Final Productivity (ha)
T1	910, 25 g	216, 50 g	2,1 g	462,0 kg	7,7 60-kg bags
T2	975, 50 g	235, 25 g	2,5 g	549,6 kg	9,16 60-kg bags
T3	835, 42 g	203, 60 g	2,7 g	594,0 kg	9,9 60-kg bags
T4	1055, 73 g	274, 22 g	3,1g	681,6 kg	11,36 60-kg bags

*Grains harvested manually along 10 linear meters

Findings by Luna (2023) align with the data presented herein, indicating that co-inoculation treatments exhibit superior performance under field conditions in the Brazilian Cerrado. This highlights the applicability of the practice, owing to the low cost of biological inputs and its environmental benefits as an eco-friendly technology. The macronutrients and micronutrients most extracted by cowpea are N, K, Ca, Fe, B, and Mn (Fonseca et al., 2010). The nutritional content from plant tissue analyses of cowpea collected at the V5 and R1 phenological stages are shown in Tables 5 and 6:

Table 5. Results of leaf analysis conducted at V5 growth stage.

Sample description	Macronutrients g/kg						Micronutrients mg/kg (ppm)				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
T01	38,0	4,0	19,5	16,9	6,2	2,1	26,2	5,0	78,1	17,4	30,2
T02	41,1	4,0	18,4	16,2	5,1	2,1	27,3	5,4	89,1	18,4	32,9
T03	42,8	4,5	20,1	17,0	6,0	2,3	28,2	5,8	80,3	14,8	30,1
T04	44,2	4,5	22,4	17,3	6,5	2,4	28,9	8,4	83,0	34,0	36,2

Source: (Italiano, 2023).

Table 6. Results of leaf analysis conducted at R growth stage.

Sample description	Macronutrientsg/kg						Micronutrientsmg/kg (ppm)				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
T01	40,0	3,1	18,9	17,6	3,1	1,9	30,6	4,0	93,6	26,4	25,9
T02	45,1	3,5	19,4	18,1	3,3	1,9	32,5	4,7	103,0	53,6	52,1
T03	43,2	3,6	19,5	17,0	3,4	2,0	31,6	4,3	94,8	42,5	40,4
T04	48,6	4,2	21,0	19,5	3,9	2,3	34,1	5,2	124,2	56,4	53,9

Source: (Italiano, 2023).

It was observed that there was an increase in both macronutrients and micronutrients present in the leaves across both phenological stages. Starting with Nitrogen at the V5 stage, a 14.02% increase was observed in T4 compared to the control (T1), a fact directly related to the greater number of nodules observed with co-inoculation. For the R1 phenological stage, there was a 17.69% increase in N content in T4 compared to T1, demonstrating nodulation efficiency. Associated with this finding, an increase of 26.19% in P uptake was observed when comparing T4 and T1. This occurs because co-inoculation promotes root system development due to the action of plant growth-promoting rhizobacteria (PGPR)—in this case, *Azospirillum*—thereby enhancing the absorption of available soil P. Gavilanes et al. (2021) observed in their research that the practice of co-inoculation using *A. brasilense* was responsible for increasing N and P levels in the leaves. In most soils where cowpea is commercially cultivated, potassium deficiency is prevalent (Sousa & Zonta, 2022). Although the optimal range is 10 to 24 g kg⁻¹ (Embrapa, 2009), the concentrations remained within established levels, ranging in V5 from 19.5 g kg⁻¹ for T1 to 22.4 g kg⁻¹ for T4, representing a 12.94% increase in co-inoculated plants. Meanwhile, in R1, a 10% difference was observed when comparing the same T1 and T4 treatments. The supply of micronutrients to plants occurs through soil reserves, as cowpea demands them in small amounts (Embrapa, 2003); for this reason, soil reserves are usually capable of meeting plant requirements.

Bromatological Analysis of Grains: -

Following laboratory analysis, the cowpea grains presented the macronutrient and micronutrient values shown in Table 7.

Table 7. Results of the bromatological analysis of grains.

Sample description	Macronutrients g/kg						Micronutrients mg/kg (ppm)				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
T01	11,67 1,12	2,15	10,08	3,09	1,23		21,25	6,02	25,35	35,62	15,41
T02	13,25 1,25	2,41	11,32	3,65	1,52		18,52	5,85	28,25	41,25	13,25
T03	15,25 1,31	2,35	10,14	4,11	1,41		19,32	5,62	27,45	38,25	16,32
T04	16,32 1,88	2,85	12,12	3,85	3,85		23,47	6,91	30,31	36,35	18,74

Source: (Italiano, 2023).

Co-inoculation (T4) significantly enhanced grain nutritional quality, yielding the highest macronutrient and micronutrient concentrations among all treatments. Both single and combined inoculation treatments increased phosphorus (P) content by up to 24.56% compared to the uninoculated control (T1). For potassium (K), treatment T4 achieved the highest concentration 12.12g kg⁻¹. All Ca concentrations were below the range recommended by IAC (2022), where the optimal range is 10 to 25 g kg⁻¹; the highest Ca concentration in the grains was found in T3 at 4.11 g kg⁻¹, which received *Azospirillum* inoculation alone. This finding reflects the bacterium's role in promoting root growth and development, thereby enhancing soil exploration and increasing nutrient uptake (Fachinelli & Ceccon, 2020). Co-inoculation (T4) also enhanced magnesium (Mg) and boron (B) uptake in grains. Magnesium levels varied, accounting for a 68.05% increase. Regarding boron, treatment T4 recorded the highest grain concentration 23.47 mg kg⁻¹.

Copper (Cu) concentrations in the grains ranged from 6.02 mg kg⁻¹ in T1 to 6.91 mg kg⁻¹ in T4, representing a 12.87% increase in Cu content, whereas from T2 to T3 there was an increase of only 3.93%. Grain manganese (Mn) concentrations remained within the reference levels established by IAC (2022) 15 to 90 mg kg⁻¹. Regarding zinc (Zn), for which optimal levels in cowpea range from 18 to 50 mg kg⁻¹, only the co-inoculation treatment (T4) met the adequate threshold, reaching 18.74 mg kg⁻¹. Overall, these findings demonstrate that co-inoculation with *Rhizobium tropici* and *Azospirillumbrasilense* (T4) substantially enhances the nutritional quality of cowpea by optimizing essential grain macro- and micronutrient accumulation. Beyond boosting grain yield and expanding root architecturethereby conferring greater resilience and performance against adverse climatic events such as

drought this clean biological technology stands out as a highly sustainable, low-cost management strategy to mitigate environmental impacts and promote sustainable agriculture.

Conclusion: -

This study demonstrates that the synergistic effects of microorganisms substantially enhance crop performance during the off-season. It is concluded that co-inoculation technology using *Azospirillum brasilense* and *Rhizobium tropici* in landrace cowpea seeds is of fundamental importance for increasing crop yield, in addition to contributing to the efficient absorption of macro- and micronutrients in a sustainable and accessible manner for farmers in Western Bahia. As a clean, low-cost biological technology, co-inoculation optimizes fertilizer management and stands out as an essential strategy under climate change scenarios. Its adoption ensures crop development and enhances grain nutritional quality, particularly under the high temperatures and severe water deficit typical of the off-season. Ultimately, advancing this sustainable practice strengthens traditional cowpea cultivation in the region, promoting agronomic resilience, food security, and environmental efficiency.

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