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Short communication

Interaction between arbuscular mycorrhizal fungi and biofertilizer in cotton crops

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Abstract

The use of biofertilizers and biological solutions to meet the nutritional needs of plants has grown significantly in agriculture as a way to replace mineral fertilizers, which in turn contribute to increasing the production costs for farmers. Therefore, it is essential to conduct studies to prove the effectiveness of these methods and assist producers in their decision-making. The objective of this study was to evaluate the effect of seed treatment with biofertilizer and inoculation with Rhizophagus clarus on cotton growth. The experiment was carried out in an experimental area in Rolândia-PR in a randomized block experimental design, with four treatments and four replicates: T1: (T) control (not inoculated), T2: (M) mycorrhiza (Rhizophagus clarus), T3: (B) biofertilizer of plant extracts of Aloe vera (Aloe solo) and T4: (M+B) mycorrhiza + Aloe solo. Cotton seeds of cultivar TMG 81 were treated according to the treatments described above. Sowing was carried out with 12 plants per linear meter with spacing of 90 cm between rows. Chemical management practices were carried out to control weeds and pests. Chemical fertilization was carried out at sowing in the furrow, where 310 kg per hectare of the formula 12-17-11 and 90 kg of calcium carbonate per hectare were deposited. Top dressing fertilization was applied 25 days after sowing with 125 kg ha^{-1} of NPK formula in the proportion 20-05-20, 63 kg ha^{-1} of urea and 75 kg ha^{-1} of potassium chloride, in addition to 0.625 L ha^{-1} of Boron 10 via foliar application. Plants from two 2-meter rows of each plot were evaluated for the following parameters: yield, plant height, number of open and closed bolls and nos per plant. Leaves were collected at the time of full flowering for leaf analysis. Data were analyzed using Tukey's test at 5%. The combined treatment of biofertilizer and mycorrhiza showed the best results, with a significant increase in all production components, including an increase of 525 kg per hectare compared to the control. Inoculation with mycorrhizal fungi and the application of biofertilizers contributed to higher yield and vegetative parameters of cotton.

Keywords: *Gossypium hirsutum*; symbiotic association; root system; phosphorus solubilization; arbuscular mycorrhizal fungi; production cost.

Introduction

Cotton (*Gossypium hirsutum* L. r. *latifolium* Hutch) belongs to the Malvaceae family, with a shrubby appearance and can reach up to four meters in height. However, in commercial cultivation, it is desirable that the height be a maximum of 1.5 meters, facilitating phytosanitary management and avoiding problems with diseases and rot in the lower area.

The raw material generated by the cotton plant has a wide range of uses, with its fiber being used in the textile industry and the seeds being used in the cosmetics industry, vegetable oil, animal feed, among others. This makes the crop responsible for an extensive production chain in Brazil, which ranks fourth in the global production ranking, being the largest producer of dryland cotton cultivation (Associação Brasileira dos Produtores de Algodão [ABRAPA], 2021). Cotton cultivation areas in Brazil total just over 1.670 million hectares, responsible for an average cotton lint yield of 1,727 kg per hectare. The states of Mato Grosso and Bahia are the largest producers (Companhia Nacional de Abastecimento [CONAB], 2025). Despite the good average Brazilian yield, Paraná still leaves much to be desired in terms of yield, reaching an average yield of 1,053 kg per hectare in the 2019/2020 harvest (CONAB, 2025).

Currently, the rise in fertilizer prices is contributing to an increase in agricultural production, and producers are seeking alternatives to supply plants with nutrients in a more viable and economical way. Arbuscular mycorrhizal fungi (AMF) are part of these alternatives, being able to associate symbiotically with the root system and solubilize phosphorus from the labile layer of the soil, reducing or even eradicating the need for phosphate fertilization.

AMF can contribute to increased absorption of phosphorus from the soil, increasing the agronomic interest in these microorganisms, since, according to Carvalho (2006), phosphorus is the fifth most required nutrient by the crop, with 13 to 25 kg of P2O5 being necessary to produce 1000 kg of seed cotton. This requirement can be partially met by inoculation with AMF, with the possibility of a severe reduction in the use of phosphate fertilizers, since mycorrhization is only efficient when there are low levels of P in the soil. Otherwise, the plant inhibits colonization, since it can be autonomous in the absorption of P (Moreira & Siqueira, 2006).

Furthermore, when analyzing cotton cultivation areas in Brazil, concentrated in the cerrado, we observed soils that present notorious nutrient restriction. In addition to the immobilization of phosphate fertilizers in clay mineral fractions, which triggers the low yield of phosphorus corrections in these soils, this makes AMF an excellent alternative for the adequate supply of P to cotton and to achieve higher yield.

One way to increase crop yield is to use beneficial microorganisms, also known as Plant Growth Promoting Microorganisms MPCPs, as microbiais inoculants or biofertilizers (Laishram et al., 2025). Some studies have been conducted using growth-promoting microorganisms such as those of the genus Bacillus sp, Pseudomonas sp and Azospirillum sp to improve the yield and technological characteristics of cotton fiber (Abdulla & Karademir, 2019). Although a large number of recent studies have confirmed that AMF can improve plant growth, productivity, quality and phosphorus acquisition, the effect of these fungi on these economic and agronomic traits in cotton is largely unknown (Gao et al., 2020).

Given the above, the objective of this study was to evaluate the influence of mycorrhization in cotton and its synergy when used together with biofertilizers based on plant extracts.

Materials and Methods

An experiment was conducted in the experimental area of the municipality of Rolândia-PR, Brazil, geographically located at 23°19'50.95"S and 51°18'34.62"W, with an altitude of 603 m and a predominant climate of the subtropical (Cfa) type, according to the Koppen classification. The soil in the area is classified as a typical Eutroferric Red Latosol, clayey texture, with gentle undulating relief (Santos et al., 2018).

The design used was randomized blocks, with four treatments and four replicates: T1: (T) control (not inoculated), T2: (M) mycorrhiza (Rhizophagus clarus), T3: (B) biofertilizer of Aloe vera plant extracts (Aloe solo), and T4: (M+B) mycorrhiza + biofertilizer of Aloe vera plant extracts, both treatments carried out via seed treatment. The plots with dimensions of 12.5 x 2.7 m were divided into four sowing lines.

The AMF were prepared with the species Rhizophagus clarus, acquired from the Microbial Ecology Laboratory of the State University of Londrina. AMF were applied with a volume of 50 $g.kg^{-1}$ of seed. For the inoculation of AMF, a sugar solution with a concentration of 10% was prepared. 3 ml of the sugar solution was added, with the aid of a syringe, to a plastic bag with 1 kg of cotton seeds, and then homogenized. Then, 50 g of the peat inoculant was added to the seed with sugar solution and homogenized.

To prepare the biofertilizer (Aloe Solo), 20 ml of liquid Aloe vera plant extract biofertilizer were added to a plastic bag with 1 kg of seeds and then homogenized at a dose of 1 liter for 100 kg of seeds. In the mycorrhiza + biofertilizer treatment, the AMF preparation process was repeated with the addition of 50 g of peat inoculant to 1 kg of seed treated with biofertilizer; in this treatment, the biofertilizer replaced the sugar solution.

The cotton cultivar TMG81 was used with chemical treatment with Thiamethoxam + Azoxystrobin + Metalaxyl-M + Fludioxonodil + Abamectin for weed and weed management. The seeds were sown totaling a plant population of 133,333 plants ha^{-1}, that is, 12 plants per linear meter and 90 centimeters between rows. Sowing was under a direct seeding system on wheat straw on 11/22/2021. Weed management was carried out with Glyphosate (3 L ha^{-1}), Carfetrazone-ethyl (125 ml ha^{-1}) and S-metolachlor (1 L ha^{-1}).

The four-row seeder was adapted with popcorn-type seed buckets to facilitate cleaning when changing treatments. During sowing, fertilization was carried out in the sowing furrow, where 310 kg per hectare of the 12-17-11 formula and 90 kg of calcium carbonate per hectare were deposited. Topdressing was carried out 25 days after sowing (DAS) with 125 kg ha^{-1} of NPK formula in the proportion 20-05-20, 63 kg ha^{-1} of urea and 75 kg ha^{-1} of potassium chloride, in addition to 0.625 L ha^{-1} of Boron 10 via foliar application.

For pest management, five applications of malathion were performed on the border (1 L ha^{-1}) to prevent the entry of weevils, and six applications of Malathion (1 L ha^{-1}) + Ethiprole (1 L ha^{-1}) in the entire plot. For thrips management, two applications of acetamiprid (100 g ha-1) were performed, and for Spodoptera sp., two compound applications were performed with Methomyl (1.45 L ha^{-1}), Flubendiamide (125 ml ha^{-1}) and Lufenuron (200 ml ha^{-1}). In addition, for brown stink bug management, Beauveria bassiana + Metarhizium anisopliae (0.7 kg ha^{-1}) was used. Weed management was performed during the cycle with Glufosinate ammonium (2 L ha^{-1}) and Clethodim (0.8 L ha^{-1}). The growth regulator used was Mepiquat chloride (0.7 L ha^{-1}) and for defoliation Diuron+Thidiazuron (0.2 L ha^{-1}) was applied.

The following parameters were evaluated: yield, height, number of open and closed bolls and number of nodes per cotton plant. They were evaluated in the plants present in two central lines of two meters in the plot. For productivity analysis, all seed cotton was manually removed from the samples, which was subsequently weighed and then calculated equivalently for kilograms per hectare. For the analysis of the number of open bolls, number of closed bolls, number of nodes and plant height, subsamples were taken from 10 plants of each sample, to measure the production components.

The sampling time for the nutritional content of cotton leaves was at full flowering (90-100 days after sowing) when the fourth and fifth leaves were collected from the tip of the main stem, in a sample composed of 20 leaves per plot.

For statistical analysis, analysis of variance was performed according to the model below:

$$y_{ij} = \mu + t_i + \beta_j + e_{ij}$$

Where:
y_{ij} observed value in the plot that received treatment i in block j;
t_k effect of the i-th treatment (i = 1, 2, 3, 4);
β_j effect of the j-th block (j = 1, 2, 3, 4);
e_{ij} is the error

Analysis of variance was performed for each variable to detect statistically significant differences among treatments. Means were then compared using Tukey's test at a 5% significance level ($p \leq 0.05$).

Averages were compared using Tukey's test at a 5% probability level when necessary, following a preliminary analysis of assumptions (Banzatto & Kronka, 2008). Data analysis was performed using R software (R Core Team, 2024). Bar graphs were used to present the results, with different letters on the bars indicating statistically distinct groups according to Tukey's test.

Results and Discussion

Cotton yield after inoculation and/or application of biofertilizers can be seen in Figure 1. The highest cotton yield was observed in the treatment where FMAs were inoculated with biofertilizer application, obtaining a yield of 271 kg ha^{-1} (4,056 kg ha^{-1}). This yield exceeded the Paraná average, which is around 200 @ ha^{-1} (CONAB, 2025).

Treatment B+M did not differ statistically from the control, but was superior to treatments B and M. Even so, there was a difference of 525 kg ha^{-1}. These results report a synergistic relationship between the inoculation of R. clarus and biofertilizer from Aloe vera extracts in the yield component, since the inoculation of R. clarus and seed treatment with biofertilizer, when evaluated separately, did not differ statistically from the control, but were less productive.

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The higher cotton yield results in the treatment with the mixture of AMF and biofertilizer can be explained by the composition of the biofertilizer. Amino acids, proteins, and vitamins in its composition may have functioned as a nutrient solution and colonization stimulant for AMF, providing better conditions for maintaining AMF viability while there was still no root tissue susceptible to infection. As already studied by Salgado et al. (2017), where the application of colonization stimulants together with mycorrhization in cotton plants promoted increased initial growth, better development, and increased plant dry matter. Similar results were also obtained by Leoni, Loconsole, Cristiano and Lucia (2019), where the use of biofertilizer together with AMF inoculation in chrysanthemum plants promoted better development of the root system and improved nutrient absorption, culminating in yield gains.

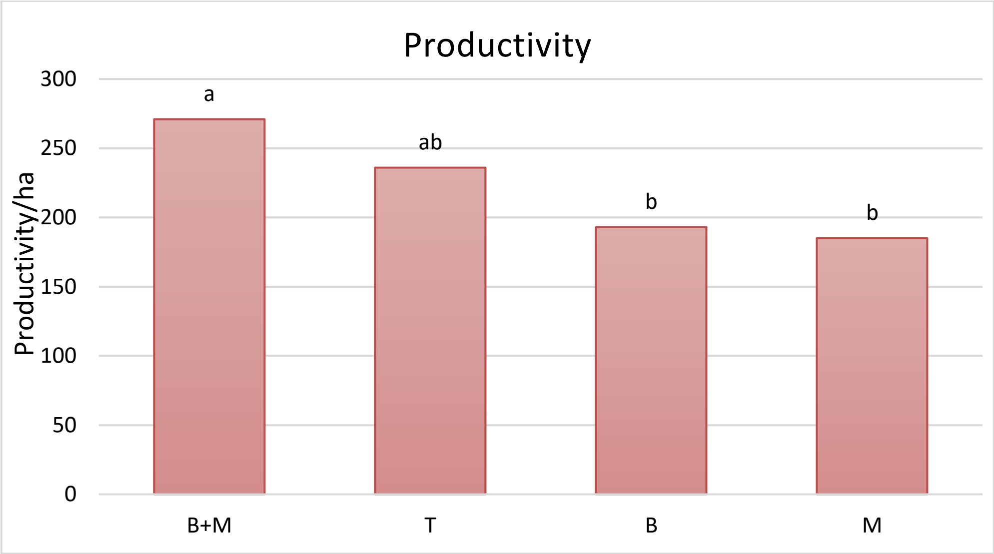

Figure 1. Cotton yield in kg ha-1 after inoculation with AMF and/or application of biofertilizer. Means followed by the same letter do not differ from each other by the Tukey test at 5%. Where: B+M = Biofertilizer+mycorrhiza; T = Control; B = Biofertilizer and M = Mycorrhiza.

Khater, Abd-Allah and Shafay (2020) carried out studies with seed treatment with biofertilizer and this practice promoted an increase in cotton yield. It was observed by Arya and Buch, 2013, that the inoculation of AMF also increased cotton yield. The height of cotton plants after inoculation and/or application of biofertilizers can be seen in Figure 2.

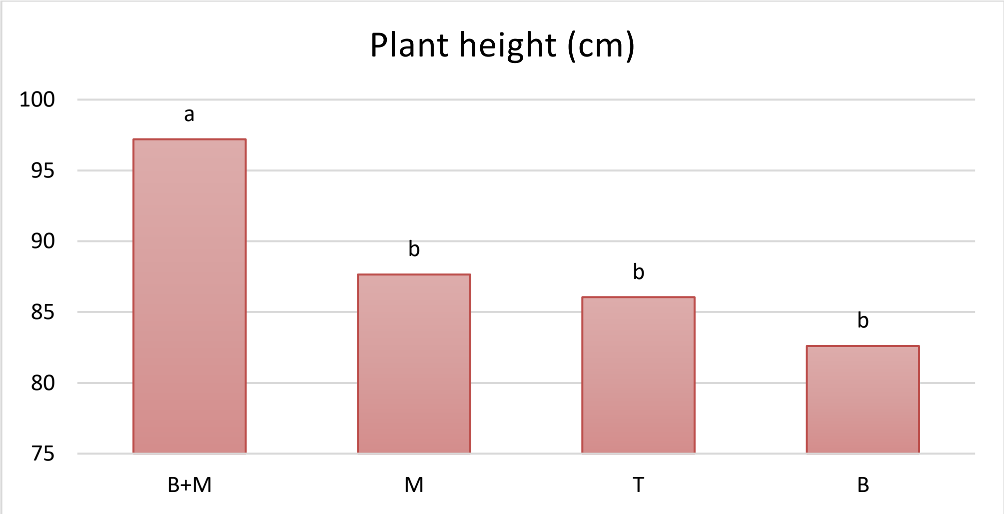

Figure 2. Height of cotton plants in kg ha-1 after inoculation with AMF and/or application of biofertilizer. Means followed by the same letter do not differ from each other by the Tukey test at 5%. Where: B+M = Biofertilizer+mycorrhiza; M = Mycorrhiza; T = Control and B = Biofertilizer.

Regarding plant height, the greatest contribution was observed from the B+M treatment, with a height of 97.2 centimeters, statistically different from the other treatments. Plant height may be linked to the mycorrhizal association, which can be explained by the production of phytohormones from the mycorrhizae together with the auxins present in the biofertilizer, a result opposite to that obtained by Cely et al. (2016), where the inoculation of R. clarus in G. hirsutum did not significantly influence plant height. The number of open cotton bolls per plant after inoculation and/or application of biofertilizers can be seen in Figure 3.

The highest number of open bolls per cotton plant was observed in the treatment with AMF inoculation and biofertilizer application, with an average of 14.6 open bolls per plant. AMF inoculation, together with seed treatment with biofertilizer, achieved similar results to a study already carried out by Moosavi (2019), in which the inoculation of AMF + biostimulant increased the number of bolls, as well as in a study by Gao et al. (2020), where plants inoculated with AMF showed a higher number of bolls per plant. However, treatment M did not differ statistically from the control, differing from the results obtained in Gao et al. (2020). The number of closed cotton bolls per plant after inoculation and/or application of biofertilizers can be seen in Figure 4.

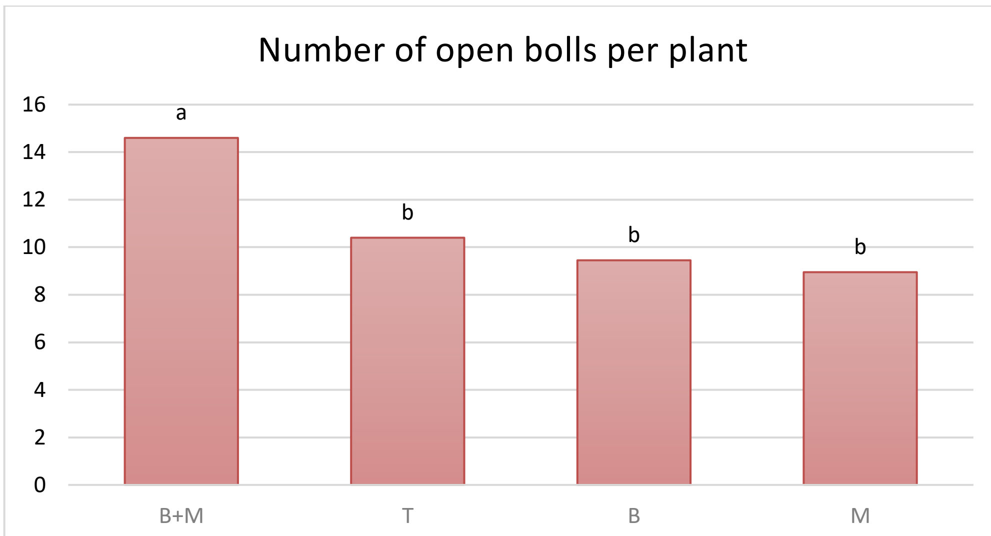

Figure 3. Average number of opened bolls per cotton plant after inoculation with AMF and/or application of biofertilizer. Averages followed by the same letter do not differ from each other by the Tukey test at 5%. Where: B+M = Biofertilizer+mycorrhiza; T = Control; B = Biofertilizer and M = Mycorrhiza.

The highest number of closed bolls per cotton plant was observed in the treatment with FMas inoculation and biofertilizer application, with an average of 2.65 closed bolls per plant. This result expresses superiority in the number of apples produced, because, although this treatment contains a greater number of closed bolls that, in turn, are not used in the harvest, it presented the highest number of open bolls, differing statistically between the treatments (Figure 3). Similar results were obtained in Moosavi (2019), where mycorrhiza inoculation together with a biostimulant increased the number of apples per square meter. The number of nodes per cotton plant per plant after inoculation and/or application of biofertilizers can be seen in Figure 5.

The highest number of nodes per cotton plant was observed in the treatment with AMF inoculation and biofertilizer application with an average of 13.1 nodes. This result can be attributed to the presence of auxins in the biofertilizer composition and also to the ability to promote mycorrhiza growth through the production of phytohormones and better absorption of water and nutrients.

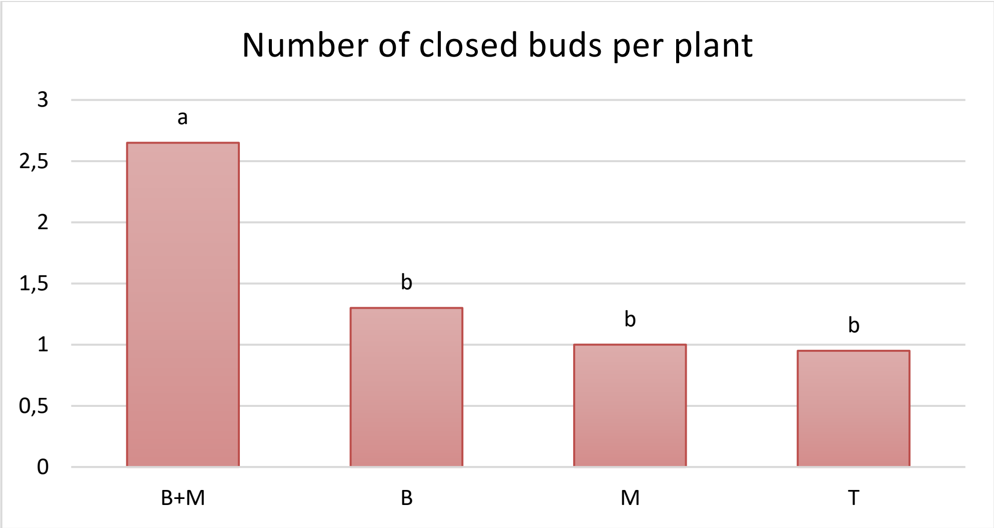

Figure 4. Average number of closed bolls per cotton plant after inoculation with AMF and/or application of biofertilizer. Averages followed by the same letter do not differ from each other by the Tukey test at 5%. Where: B+M = Biofertilizer+mycorrhiza; B = Biofertilizer; M = Mycorrhiza and T = Control.

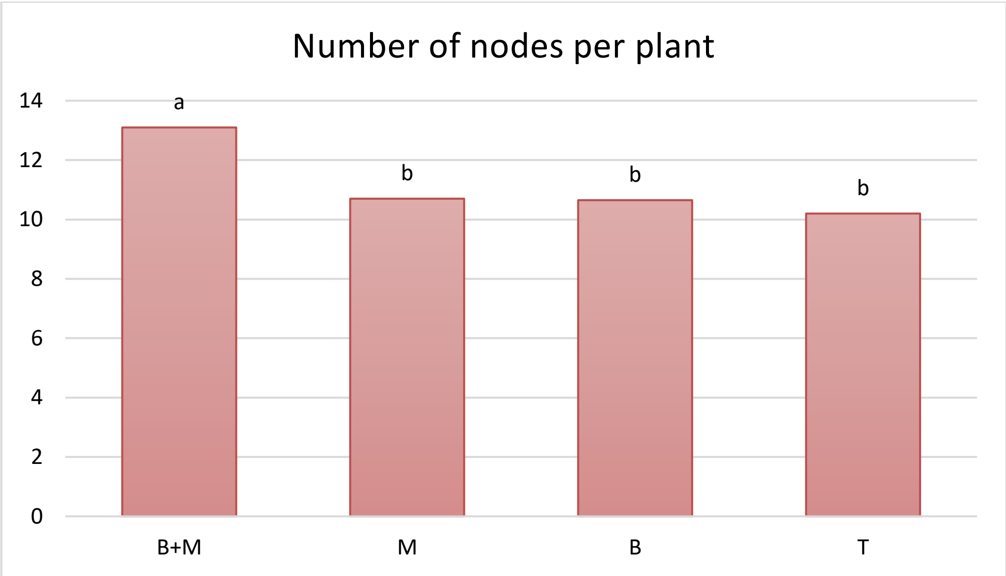

Figure 5. Number of nodes per cotton plant after inoculation with FMAs and/or application of biofertilizer. Means followed by the same letter do not differ from each other by the Tukey test at 5%. Where: B+M = Biofertilizer+mycorrhiza; M = Mycorrhiza; B = Biofertilizer and T = Control.

The treatments that were inoculated with AMF and biofertilizer presented a greater number of nodes, a result already confirmed in a study by Gao et al. (2020), where the inoculation of AMF promoted an increase in the number of nodes in *Gossypium hirsutum* plants, as well as a study conducted by Arif et al. (2019), which found that cotton plants treated with biofertilizers based on plant extracts obtained statistical superiority in the number of nodes when compared to the other treatments.

Conclusions

The inoculation of R. clarus with the application of biofertilizer in the treatment of cotton seeds increased cotton yield by 35 arrobas per hectare, as well as being superior in all production components evaluated.

The association between arbuscular mycorrhizal fungi (AMF) and biofertilizer, via seed treatment, appears promising and can be considered an option for increasing cotton yield.

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