



# Growth, development and nitrogen nutrition of colored cotton cv. BRS Topázio under sustainably-constructed soil

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Received in: 28/03/2022

Accepted in: 25/10/2022

## Abstract

Agroecological practices such as seed inoculation with diazotrophic bacteria and biofertilizers use have contributed to the development of sustainable agriculture. Therefore, this work aimed to study the effect of biofertilizers and the bacterium *Azospirillum brasilense* on the growth, development, and mineral nutrition of colored cotton cv. BRS Topázio. Thus, we conducted an experiment with the following treatments: 1) control; 2) inoculation of cotton seeds with nitrogen-fixing bacterium *A. brasilense* ( $N_{\text{fixed}}$ ); 3) treatment of seeds with biofertilizer (Bio) + subsequent applications; 4) ( $N_{\text{fixed}}$ ) + subsequent applications of Bio; and 5) mineral fertilization. The results showed that agroecological practices increase plant growth; the root:aerial ratio increases with conventional agricultural practices such as mineral fertilization, which also provides a lower reproductive mass:vegetative mass ratio of the aerial parts; plants fertilized with mineral sources concentrate more nitrogen (N), crude protein, magnesium (Mg), and sulfur (S) in their tissues; plants from seeds inoculated with *A. brasilense* accumulated more S when they received anaerobic biofertilizer compared with those that did not receive it; and agroecological practices improve soil fertility.

**Keywords:** *Gossypium hirsutum*. Nitrogen. Biofertilizers. Diazotrophic bacteria. *Azospirillum*.

## Introduction

BRS Topázio is a colorful cotton cultivar with light brown, uniform, resistant, and soft fiber, launched by Embrapa Algodão in 2005 due to the demand of small industries that work with colored cotton. This cultivar has the advantage of having high fiber yield (43.5% on average). Along with other colored cotton cultivars already available (BRS Marrom, BRS Verde, BRS Safira, and BRS Rubi), BRS Topázio has been favoring the creativity of industries in the manufacture of new collections of clothing and handicrafts (VIDAL NETO *et al.*, 2010; QUEIROZ *et al.*, 2012; TELES; FUCK, 2016).

Biofertilizers are one of the sustainable practices used in cotton cultivation of (CAVALCANTE *et al.*, 2019; MAHAPATRA *et al.*, 2022). According to Guazzelli *et al.* (2012), biofertilizers are liquid organic fertilizers that undergo fermentation. They can be made with any type of fresh organic matter and are used

in planting and/or cover fertilization. There are numerous ways to make biofertilizers; mostly, manure tea is used, but fresh vegetable leftovers as well as other products can also be used.

Another use for biofertilizers is the treatment of seeds before sowing (KHALEQUZZAMAN, 2015; KALITA *et al.*, 2019). According to Ferreira *et al.* (2007), biofertilizers can provide seeds with nutrients, plant growth regulators, amino acids, and vitamins during germination. According to the authors, they are also compounds that promote the metabolic balance of seedlings, favoring the expression of their genetic potential and stimulating roots development.

Several researchers have studied the effect of biofertilizers on the germination and the initial development of seedlings of various crops. Dan *et al.* (2014), studying the effect of the application of the biostimulant Stimulate on maize seeds, observed an improvement in their physiological quality, with consequent increase in germination

index. Albrecht *et al.* (2014) observed positive results of biostimulant application on germination, emergence, vigor, and length of pea seedlings. Rampim *et al.* (2012) found that biostimulants significantly improved the initial development of wheat seedlings.

Another practice that is part of ecologically-based agriculture is the use of free bacteria in the soil or associated with the roots of plants that fixate atmospheric nitrogen ( $N_2$ ), transforming it into amino acids. Those bacteria from genera *Rhizobium* and *Bradyrhizobium* are the most used in agriculture; By symbiosis with the roots of legumes, these bacteria supply them with nitrogen (N), avoiding the use of nitrogen fertilizers (BARROS-CARVALHO *et al.*, 2019; ARACHCHIGE *et al.*, 2020).

Other genera have been studied for the supply of N in cultivated plants. Bacteria of the genus *Azospirillum* can fix  $N_2$  when associated with grasses (FUKAMI *et al.*, 2018). Its efficiency in supplying N and in increasing crop yield has already been demonstrated in maize, wheat, and millet (HUNGARY, 2011). The use of all these genera of N-fixing bacteria consists in wetting the seeds and innocuous with bacteria before sowing, leaving them available to symbiosis with the newly formed radicles during germination (FELIX *et al.*, 2021). In cotton, Fayez and Daw (1987) inoculated the seeds with *Azospirillum brasilense* before planting under subtropical conditions in Egypt and found an increase in dry matter production and N uptake.

Therefore, this work aimed to study the effect of biofertilizers and the  $N_2$ -fixing bacteria *A. brasilense* on the growth, development, and nitrogen nutrition of colored cotton cv. BRS Topázio.

## Material and methods

The experiment was conducted outdoors at the *Centro Nacional de Pesquisa do Algodão* (Brazilian Cotton Research Center), municipality

of Campina Grande, Paraíba State, Brazil, whose geographic coordinates are 7°13'50"S latitude and 35°52'52"W longitude, with 600 m of altitude and AWI climate (rainy tropical), according to the Köppen classification, from May 12 to August 26, 2015.

The experimental design adopted was completely randomized, with five treatments and five replications, totaling 25 experimental plots. Each plot was represented by a polyvinyl chloride tube (PVC) with a diameter of 20 cm, height of 50 cm, and volume of 16 dm<sup>3</sup>, or 16 L, filled with the substrate (which varied according to treatment), and containing two plants.

Table 1 shows the treatments used. The standard substrate used in experiments with Embrapa Algodão consists of three parts of soil for one of organic matter. The soil came from the *Estação Experimental da Empresa Estadual de Pesquisa Agropecuária da Paraíba* (Experimental Station of the Paraíba State Enterprise for Agricultural Research – Emepa-PB), Lagoa Seca/PB, whose chemical characteristics are: pH (in H<sub>2</sub>O) = 5.9; phosphorus (P) = 10.6 mg dm<sup>-3</sup>; potassium (K) = 18.4 mg dm<sup>-3</sup>; aluminum (Al<sup>+3</sup>) = 0.00 cmol<sub>c</sub> dm<sup>-3</sup>; calcium (Ca(Ca<sup>+2</sup>)) = 2.08 cmol<sub>c</sub> dm<sup>-3</sup>; magnesium (Mg<sup>+2</sup>) = 1.22 cmol<sub>c</sub> dm<sup>-3</sup>; and organic matter (OM) = 12.87 g kg<sup>-1</sup>. The organic matter used in the manufacture of the substrate consisted of composted and sieved bovine manure.

The biofertilizer used in Treatments 3 (t<sub>3</sub>) and 4 (t<sub>4</sub>) was made in the Assentamento Queimadas, municipality of Remígio, state of Paraíba, based on a mixture of 200 L of water; 10 kg of pure fresh manure; 5 L of milk; 5 crumbled bricks of black *rapadura*; rock dust; bone powder; and leftovers of fruits, vegetables, weeded bush, and prepared foods, all subjected to anaerobic fermentation in a properly sealed plastic drum for 45 days.

Mineral fertilization with nitrogen, phosphorus, and potassium fertilizer (NPK) in

**Table 1** – Treatments used in the experiment growth, development, and nitrogen nutrition of colored cotton cv. BRS Topázio under sustainably constructed soil. Campina Grande, PB, 2015.

	Treatment				
	1*	2	3	4	5
Conventional substrate used in Embrapa Algodão experiments: soil + manure in 3:1 ratio	X	X	X	X	
Inoculation of cotton seeds with the N <sub>2</sub> -fixing bacterium <i>Azospirillum brasilense</i>		X		X	
Treatment of cotton seeds with biofertilizer			X		
Later applications of the biofertilizer extract			X	X	
Fertilization with NPK fertilizers according to soil analysis					X

\*Control; N: nitrogen; NPK: nitrogen, phosphorus, and potassium.

treatment 5 (t<sub>5</sub>) was performed according to soil analysis (CARVALHO et al., 2007). A total of 15 kg ha<sup>-1</sup>N were applied at sowing and 60 kg ha<sup>-1</sup> 30 days after germination (DAG); and P and K, 90 and 40 kg ha<sup>-1</sup>, respectively, at sowing. The sources of N, diphosphorus pentoxide (P<sub>2</sub>O<sub>5</sub>), and potassium oxide (K<sub>2</sub>O) were, respectively: ammonium sulfate (SA, 21% N), simple superphosphate (SSP, 18% P<sub>2</sub>O<sub>5</sub>), and potassium chloride (KCl, 60% K<sub>2</sub>O).

Before sowing, the soil was irrigated until it reached the field capacity point, with excess water being properly drained. Then, eight seeds of colored cotton cv. BRS Topázio were sowed by PVC tube. Germination occurred about four days later. The first thinning was performed at 9 DAG, leaving three seedlings per tube (the ones that established more vigorously). At 18 DAG, a second thinning was performed, leaving the two more vigorously seedlings in the tubes.

Before sowing, the seeds of Treatments 2 (t<sub>2</sub>) and 4 (t<sub>4</sub>) were inoculated with the N<sub>2</sub>-fixing bacterium *A. brasilense*. The seeds used in t<sub>3</sub> were treated with biofertilizer. For seed inoculation with N-fixing bacterium, *A. brasilense*, strains Abv5 and Abv6, was used, and inoculation was performed in the laboratory using sterilized flasks with a solution of bacteria of 2 × 10<sup>8</sup> viable cells mL<sup>-1</sup> diluted in 30 mL of distilled water, properly homogenized. This solution was sprinkled on the seeds to obtain a uniform application. The seeds

treated with biofertilizer were immersed in the product and left for approximately 5 min before sowing. Biofertilizer application in t<sub>3</sub> and t<sub>4</sub> was performed at 20, 41, 61, and 82 DAG, applying 175 mL per PVC tube.

Plots were irrigated whenever necessary, trying to keep the soil always at field capacity, with the excess water being properly drained. For the control of the cotton plant aphid (*Aphis gossypii*), tobacco juice was sprayed on all plots at 30 and 32 DAG, applying 175 mL per PVC tube.

At 78 DAG (approximately at the beginning of flowering), leaves were collected and taken to the *Laboratório de Solos e Nutrição de Plantas* (Soil and Plant Nutrition Laboratory – LSNP) of Embrapa Algodão to determine macronutrient contents. Those leaves completely expanded from the apex were collected. They were then packed in labelled paper bags and placed to dry in a greenhouse with air circulation at 65°C for 48 hours, or until constant mass was obtained. Later, they were ground in a Wiley mill, and the material was stored in properly closed glass and labelled for analyses.

At LSNP, the samples were subjected to sulfuric (for the determination of N, P, and K) and nitro-perchloric (for the determination of Ca, Mg, and sulfur – S) digestions. N and P were determined by UV-visible spectrophotometry, K

by flame photometry, Ca and Mg by titration, and S by turbidimetry. The crude protein (CP) content was obtained by  $N \times 6.25$ .

At 96 DAG, the following variables were taken: plant height (Hgt), stem diameter ( $\emptyset$ ), N content in *in situ* leaves, and chlorophyll *a*, *b*, and total contents. The plant height was determined with a ruler.  $\emptyset$  was measured 10 cm above the root crown with a digital caliper. *In situ* N contents were determined using the N-Pen N100, a portable device that quickly and non-destructively measures the content of this nutrient in leaves by reflectance, at field level. As there is a close relationship between N and chlorophyll, this device can also be used to estimate the latter. Chlorophyll *a* and *b* contents were determined with a portable chlorophyll meter, ClorofiLOG CFL 1030 (Falker). This portable meter has a sensor that can measure the chlorophyll index present in the leaves based on the energy absorbance at 650 and 940 nm wavelengths. The readings were performed on the fifth leaf completely expanded from the apex. By the sum of chlorophyll *a* and *b* contents, the total chlorophyll content was obtained.

The experiment was closed at 102 DAG, in which the variables aerial part length (APL) and root length (RL) were taken, and the plants were separated into aerial part and root, with the former being subdivided into vegetative and reproductive. The three parts of the same plant were packed in paper bags labelled for the plot and part. These bags were then taken to an air oven at 65°C for 48 hours, or until constant mass was reached. Then, they were weighed to obtain the following variables: root dry matter (RDM), vegetative aerial dry matter (VADM), reproductive aerial dry matter (RADM), total dry matter (TDM), root:aerial part ratio (R:AP), and reproductive part:vegetative part ratio (R:Ve) of the aerial part.

Then, soil samples were collected in the PVC tubes, and these samples were sent to LSNP

for routine analysis: pH, Ca, Mg, Na, S (sum of bases), hydrogen (H) + Al, cation exchange capacity (T), base saturation percentage (V), Al, P, and OM.

All data obtained were subjected to statistical analysis using the Statistical Analysis System (SAS) program version 9.2. All variables were subjected to analysis of variance (ANOVA) and, when significant by the *F*-test at 5% probability, the following contrasts were tested using the same level:  $t_1$  vs.  $t_2$ ;  $t_1$  vs.  $t_5$ ;  $t_3$  vs.  $4 t_{(t1, t_2, t_3, t_4)}$  vs.  $t_5$ ;  $t_1$  vs. ( $t_2, t_3, t_4$ ); and  $t_2$  vs.  $4 t_{(t1, t_2, t_3, t_4)}$ . All contrasts were adopted with one degree of freedom, and were chosen a priori as they were of practical interest.

## Results and discussion

Table 2 shows the mean of the treatments and the contrasts of practical interest, chosen a priori, that were significant by the *F*-test. ANOVA test resulted in significant *F*-test for Hgt,  $\emptyset$ , root length, VADM, RADM, RDM, TDM, R:AP ratio, and R:Ve ratio of the aerial part (Table 2).

As Table 2 shows, among the contrasts of practical interest, only  $t_1$  vs.  $t_5$  and ( $t_1, t_2, t_3, t_4$ ) vs.  $t_5$  were significant by ANOVA. That is, there was only a significant difference between the control and the mineral fertilization and between the mean of Treatments 1 ( $t_1$ ) to 4 and the mineral fertilization.

Any materials made from biological products used in this experiment promoted better results in growth variables regarding mineral fertilization. Natural resources such as manures, biofertilizers, and diazotrophic bacteria, with biofertilizers and N-fixing bacteria being used in seed treatment before sowing or via sprinkling in the course of plant growth, are important tools in building sustainable soil fertility, and are therefore widely accepted in agroecologically based agricultures (SENEVIRATNE, 2008; VARGAS *et al.*, 2012; SANTOS *et al.*, 2014).

**Table 2.** Mean and summary ANOVA of the a priori chosen contrasts concerning the effect of biofertilizer, the atmospheric nitrogen-fixing bacterium *Azospirillum brasilense*, and the mineral fertilization on the variables related to the growth of colored cotton cv. BRS Topázio. Campina Grande, PB, May to August 2015.

Treatment	Hgt	Ø	RL	VADM	RADM	RDM	TDM	R:AP	R:Ve
(Unit)	cm	mm	cm	g plant <sup>-1</sup>	g plant <sup>-1</sup>	g plant <sup>-1</sup>	g plant <sup>-1</sup>	-	-
1	69.85	7.78	31.75	48.16	15.31	12.32	75.81	0.19	0.33
2	66.40	7.76	25.15	41.82	8.48	13.10	63.40	0.26	0.20
3	76.25	8.98	35.16	61.41	8.91	20.59	90.92	0.29	0.15
4	72.35	8.29	34.99	54.75	15.23	15.93	85.91	0.22	0.30
5	31.03	4.66	6.60	8.03	0.35	3.67	11.81	0.49	0.03
Contrasts	Hgt	Ø	RL	VADM	RADM	RDM	TDM	R:AP	R:Ve
t <sub>1</sub> vs. t <sub>2</sub>	ns	ns	ns	ns	ns	ns	ns	ns	ns
t <sub>1</sub> vs. t <sub>5</sub>	3767.9** <sup>(1)</sup>	24.36**	1580.55**	4024.84*	320.83**	187.06*	10230.40**	0.22**	0.22**
t <sub>3</sub> vs. t <sub>4</sub>	ns	ns	ns	ns	ns	ns	ns	ns	ns
(t <sub>1</sub> , t <sub>2</sub> , t <sub>3</sub> , t <sub>4</sub> ) vs. t <sub>5</sub>	6459.5**	50.27**	2531.90**	7570.22**	246.30*	558.85**	18047.50**	0.25**	0.18**
t <sub>1</sub> vs. (t <sub>2</sub> , t <sub>3</sub> , t <sub>4</sub> )	ns	ns	ns	ns	ns	ns	ns	ns	ns
t <sub>2</sub> vs. t <sub>4</sub>	ns	ns	ns	ns	ns	ns	ns	ns	ns

Hgt: Plant height; Ø: Stem diameter; RL: Root length; VADM: Vegetative aerial dry matter; RADM: Reproductive aerial dry matter; RDM: Root dry matter; TDM: Total dry matter; R:AP: Root:aerial part ratio; R:Ve: Reproductive part:vegetative part of the aerial part ratio; ns: non-significant

\*\*,\*Significant at 1% and 5% level, respectively, by the *F*-test.

(1)Mean squares of the contrasts

In a study conducted by Annadurai and Nelson (2018), a mixture of organic fertilizers — vermicompost, cattle manure, and neem cake — was tested alone or combined with biofertilizer applications. The mixture of manure combined with biofertilizer provided better germination, growth, and production of protein, amino acid, total sugar, and starch contents in the cotton plants, probably due to the potentiation effect of organic materials by the biocomposite.

Arif *et al.* (2018), studying the effect of biofertilizer enriched with rock dust compared with the use of SSP in cotton cultivation, found that the former significantly surpassed the latter regarding plant growth, cotton seed production and efficiency in the use of P. They attributed these results to the natural phosphate-solubilizing bacteria in the biofertilizer.

The highest R:AP ratio was obtained at t<sub>5</sub> (mineral fertilization, Table 2). A higher value of this variable means a substrate less favorable

to both root and aerial development, causing plants to reallocate a higher proportion of photo-assimilates for root development as a way to better explore the limiting soil, while decreasing the development of the vegetative part, saving on photo-assimilates (IDSO *et al.*, 1988; MIN *et al.*, 2014; WANG *et al.*, 2020).

On the other hand, the lower R:Ve ratio, which was also obtained in the treatment with mineral fertilization, shows that the substrates in which agroecological materials were used developed their floral structures better, which can directly reflect in the production of colored cotton cv. BRS Topázio (Table 2). Wang *et al.* (2004), working with 11 cotton cultivars, showed that the R:Ve ratio has a direct correlation with the net rate of carbon dioxide (CO<sub>2</sub>) assimilation, the average mass, and the production of bolls.

ANOVA obtained significant *F* for N, CP, Mg and S (Table 3). The other nutrients analyzed in the laboratory, the *in situ* N content, and the

chlorophyll *a*, *b*, and total contents did not differ among treatments. Table 3 shows the contrasts of practical interest, chosen a priori, which were significant to the *F*-test.

As Table 3 shows, the contrasts of practical interest chosen a priori,  $t_1$  vs.  $t_2$  and  $t_1$  vs.  $t_5$  were significant, by ANOVA, for N and CP;  $t_1$  vs.  $t_2$ ;  $t_1$  vs.  $t_5$  and  $(t_1, t_2, t_3, t_4)$  vs.  $t_5$  were significant for N, CP, and Mg; and,  $t_2$  vs.  $t_4$ , for S.

There were significant differences between the substrates only for nutrients N, Mg, and S, and for CP (Table 3). The inoculation of cotton seeds with the N<sub>2</sub>-fixing bacterium *A. brasilense* and its use in the conventional substrate caused a significant increase in N and, consequently, CP levels in the leaves, clearly demonstrating the nutritional advantage regarding the N element of the inoculated plants (Table 3). Guerrero-Molina *et al.* (2014) found in strawberry plants that the diazotrophic bacterium *A. brasilense* makes the roots more able to absorb essential mineral nutrients. Brusamarello-Santos *et al.* (2017),

studying the metabolic profile of two maize strains inoculated with *A. brasilense*, concluded that inoculation increased mannitol, trehalose, isocitrate, and asparagine levels, metabolites that are indicators of nitrogenase activity, an enzyme that reduces N<sub>2</sub>, and can be used as genetic markers of the plant-diazotrophic bacteria interaction.

Table 3 shows that  $t_5$  (mineral fertilization) had higher leaf concentration of N, CP, and Mg in relation to the mean of all other treatments (agroecological substrates). This fact is due to the concentration effect of the nutrient. With the highest growth in plant from treatments  $t_1$  to  $t_4$  (Table 2), these factors were diluted, concentrating more on chemically fertilized plants ( $t_5$ ). Because of the dilution effect, those plants that grow and produce better have lower content of essential nutrients, Krüger *et al.* (2021) recommend replacing this variable for the total nutrient content in the leaves. These authors concluded this by studying the mineral nutrition of trees such as beech, oak, fir, and pine.

**Table 3.** Summary of ANOVA of the a priori chosen contrasts of the effect of biofertilizer, atmospheric nitrogen-fixing bacterium *Azospirillum brasilense*, and mineral fertilization on the mineral nutrition of colored cotton cv. BRS Topázio. Campina Grande, PB, May to August 2015.

Treatment	N	CP	Mg	S
----- % -----				
1	0.550	3.454	0.214	0.466
2	0.644	0.022	0.286	0.504
3	0.600	3.766	0.246	0.400
4	0.580	3.654	0.310	0.388
5	1.034	6.478	0.492	0.528
Contrasts	N	CP	Mg	S
$t_1$ vs. $t_2$	0.022 <sup>*(1)</sup>	0.807*	ns	ns
$t_1$ vs. $t_5$	0.585	22.861**	0.193**	ns
$t_3$ vs. $t_4$	ns	ns	ns	ns
$(t_1, t_2, t_3, t_4)$ vs. $t_5$	0.776**	30.34**	0.208**	ns
$t_1$ vs. $(t_2, t_3, t_4)$	ns	ns	ns	ns
$t_2$ vs. $t_4$	ns	ns	ns	0.034*

N: nitrogen; CP: crude protein; Mg: magnesium; S: sulfur; ns: non-significant

\*\*,\*Significant at 1% and 5% level, respectively, by the *F*-test.

(1)Mean squares of the contrasts

Another significant contrast was the  $t_2$  vs.  $t_4$  for S (Table 3). Cotton plants from seeds inoculated with *A. brasilense* concentrated in their leaves more S in relation to those that, in addition to inoculation, received later applications of liquid biofertilizer (Table 3). However, in a study conducted in the el-Saff region, El-Giza province, Egypt, where Awad *et al.* (2011) inoculated onion (*Allium cepa L.*, "Giza 20") seeds with  $N_2$ -fixing bacteria, there was no increase in the plant-available  $SO_4^{2-}$  content in the soil.

Table 4 presents the mean of the treatments and the contrasts of practical interest, chosen a priori, that gave significance by the *F*-test. ANOVA obtained significant *F* for pH, Ca, Mg, K, S, H + Al, T, V, Al, P, and OM. Among the contrasts of practical interest, only  $t_1$  vs.  $t_5$  and  $(t_1, t_2, t_3, t_4)$  vs.  $t_5$  was significant by ANOVA (Table 4). Therefore, there was only a significant difference between the control and the mineral

fertilizer, and between the mean of the treatments  $t_1$  to  $t_4$  and the mineral fertilizer.

Significant differences were observed between substrates for all soil fertility indicators (Table 4). The use of bovine manure in the substrate, the inoculation of cotton seeds with the  $N_2$ -fixing bacterium *A. brasilense*, and the application of anaerobically-made biofertilizer caused a significant improvement in the indicators of soil acidity, availability of essential mineral nutrients, and organic matter compared with poor soil fertilized with chemical fertilizers, as also found in other studies (SHARMA; VASUDEVA, 2005; LIMA *et al.*, 2007; SCHWEINSBERG-MICKAN; MÜLLER, 2009; OBIA *et al.*, 2015; GALINDO *et al.*, 2017; SILVA *et al.*, 2019; ZHU *et al.*, 2022). Moreover, diazotrophic bacteria also contribute to increase the soluble P available to plants in the soil (SILVA *et al.*, 2006; BUSATO *et al.*, 2012).

**Table 4.** Average of the effect of biofertilizer, atmospheric nitrogen-fixing bacterium *Azospirillum brasilense*, and mineral fertilization on the fertility of a soil cultivated with colored cotton cv. BRS Topázio. Campina Grande, PB, May to August 2015.

Treatment	pH	Ca	Mg	K	S	H+Al	T	V	Al	P	OM
(Unit)	----- mmol <sub>c</sub> dm <sup>-3</sup> -----							%	mmol <sub>c</sub> dm <sup>-3</sup>	mg dm <sup>-3</sup>	g kg <sup>-1</sup>
1	7.36	44.44	33.46	3.86	87.62	0.00	87.62	100.00	0.00	606.94	30.42
2	7.46	47.76	30.34	5.48	91.60	0.00	91.60	100.00	0.00	572.16	27.98
3	7.52	48.16	33.14	5.42	93.66	0.00	93.66	100.00	0.00	601.84	28.16
4	7.54	44.94	29.24	6.76	87.72	0.00	87.72	100.00	0.00	643.60	25.74
5	4.60	7.52	6.98	0.94	19.24	55.48	74.68	25.60	8.40	41.58	12.68
Contrast	pH	Ca	Mg	K	S	H+Al	T	V	Al	P	OM
$t_1$ vs. $t_2$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$t_1$ vs. $t_5$	19.04** <sup>(1)</sup>	3407.72**	1752.98**	21.32*	11689.56**	7695.08**	418.61*	13838.40**	176.40**	799079.82**	786.77**
$t_3$ vs. $t_4$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$(t_1, t_2, t_3, t_4)$ vs. $t_5$	32.95**	6023.31**	2413.76**	78.85**	20112.91**	12312.12**	957.28**	22141.44**	282.24**	1274889.39**	948.02**
$t_1$ vs. $(t_2, t_3, t_4)$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$t_2$ vs. $t_4$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Ca: Calcium; Mg: Magnesium; K: Potassium; S: Sum of bases; T: Cation-exchange capacity; V: Percentage of saturation by base; Al: Aluminum; P: Phosphorus; OM: Organic matter; ns: non-significant

\*\* , \*Significant at 1% and 5% level, respectively, by the *F*-test.

(1) Mean squares of the contrasts

Agroecological practices of organic farming contribute to building soil fertility over time (GALÁN et al., 2012; ALBUQUERQUE et al., 2015), especially in *Caatinga* soils (TEIXEIRA et al., 2019; LIRA et al., 2012), as verified by the data in Table 4.

## Conclusions

1. Agro-ecological practices, such as incorporating bovine manure into the soil, inoculating cotton seeds with the N<sub>2</sub>-fixing bacterium *A. brasilense*, and applying anaerobically brewed biofertilizer increase cotton plants growth.
2. The R:AP ratio increases with conventional agriculture practices, such as mineral fertilization.
3. Mineral fertilization provides lower R:Ve ratio of the aerial part.
4. Chemically fertilized plants concentrate more N, P, Mg, and S in their tissues.
5. The agroecological practices used in this study improve soil fertility.

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