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Yield and morphology of *Nopalea cochenillifera* under N fertilization and biological inoculation

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Abstract. The objective was to evaluate the effect of inoculation with *Azospirillum brasilense* and levels of nitrogen fertilization on the productive aspects of the spineless cactus (*Nopalea cochenillifera*). The experiment was carried in Ribeirão do Largo, Southwest region of the state of Bahia, from April 2019 to April 2020. The experiment was carried out in a 2×4 factorial, in a randomized block design with eight treatments and four replications. The treatments consisted of the absence or inoculation with *Azospirillum brasilense* and nitrogen fertilization levels 0, 50, 100 and 150 kg^{-1} of N ha^{-1}. There was a positive effect for the use of the bacteria on cladode area index, height, number of secondary, tertiary and total cladodes, total weight for cladodes of all orders and also for final weight per ha^{-1}. There was a significant effect of the use of the inoculant for the levels of total soluble sugars and starch, where there was a decrease in the values for the treatments submitted to the use of the bacteria. There was a quadratic effect for the weight of the tertiary cladodes, number of tertiary cladodes and total of spineless cactus when submitted to nitrogen fertilization levels. There was a linear test for cladodes area index when nitrogen fertilization was used. The use of *Azospirillum brasilense* is positive for the cultivation of spineless cactus, improving development and increasing crop productivity.

Key words: *Azospirillum brasilense*, cladodes, microorganisms, morphometric characteristics, spineless forage cactus.

INTRODUCTION

Pasture growth in the Brazilian semiarid region is limited by the low water availability in the region, which means that pasture production does not meet the needs of the herd during the drought, directly impacting zootechnical indexes, requiring an alternative to meet this demand for food.

The spineless cactus is an alternative for cultivation in drier regions, because the plant can produce even water restriction situation, besides having a high roughness and the ability to produce forage (Silva et al., 2014). Higher spineless cactus productivity can be achieved when the crop is subjected to nitrogen fertilization, a fact that can be justified by the increase in the emission of cladodes, which in turn reflects directly on the production (Souza & Fernandes, 2006; Cunha et al., 2012).

With the growing demand for nitrogen fertilizers, an alternative has been sought to reduce costs and meet the nitrogen demands of plants, which can be achieved with the use of growth-promoting bacteria, which biologically fix atmospheric nitrogen, making it available for the culture with which it is associated. In addition to biological nitrogen fixation, these bacteria can stimulate plant growth by releasing phytohormones (Hungria, 2011).

Among the growth promoting bacteria we have *Azospirillum brasilense*, which has been bringing positive results in the agricultural scenario, being used in the cultivation of corn, wheat and pastures, increasing productivity and reducing production costs of these crops (Guimarães et al., 2022).

In order to characterize bacterial isolates in giant and spineless cactus (*Opundia* spp. and *Nopalea* spp.), using a partial gene, Silva et al. (2015), managed to isolate twelve types of batteries, among them the *Azospirillum* genus. Bacteria of the genus *Azospirillum* are free living organisms, considered as growth promoters of plants, capable of fixing atmospheric nitrogen in a usable form for plants, these organisms can live in a way associated with the rhizosphere of plants internally or externally (Okon & Lacerda Gonzales, 1994).

The mode of action of the bacterium is associated with nitrogen fixation and also the secretion of phytohormones, which interfere with the development of the plant, acting mainly on the root system, making it have a greater capacity for absorbing nutrients, the result being better development of the plant, the combination of factors (Bashan &Levanony, 1990; Okon & Itzigsohn, 1995).

The nitrogen fixing diazotrophic bacteria, a group to which the *genus Azospirillum spp.* belongs, can perform biological nitrogen fixation, fixing N2 in NH3, with FBN being the main form of entry of atmospheric nitrogen into natural systems (Hungria, 2011; Freitas et al., 2015). These bacteria can be used as an alternative to supply nitrogen to the spineless cactus and provide for its growth, however, no work has addressed this subject.

These bacteria can be used as an alternative to supply nitrogen to the spineless cactus and provide for its growth Almeida et al. (2012), report that the practice of nitrogen fertilization in palm guarantees a significant increase in productivity, regardless of the cultivar, showing positive responses to nitrogen fertilization in conjunction with other nutrients.

The objective of this work was to evaluate the effect of inoculation with *Azospirillum brasilense* and levels of nitrogen fertilization on the productive aspects of the spineless cactus.

MATERIALS AND METHODS

The experiment was conducted on a farm in the district of Rio do Curral, Encruzilhada, BA, the geographical coordinates in which the experimental area is located are 15° 33’35” south latitude, 40º 43’39” west longitude, and with an approximate altitude of 890 m. The region is characterized by a tropical climate, with an average annual rainfall of 777 mm, an average annual temperature of 22.2 °C according to the Köppen classification.

The treatments were carried out in a randomized block design, composed of 8 treatments arranged in a 2×4 factorial scheme, with four replications, totaling thirty-two experimental units. The first factor was constituted by the presence or absence of the bacteria (*Azospirillum brasilense*) in the dosage of 1 L ha^{-1}, with the administration of the amount divided in two moments, the second factor was constituted in levels of nitrogen fertilization (0, 50, 100 e 150 kg ha^{-1} of N) so that it can be verified whether there will be a linear increase in productivity. The experimental plot had its stipulated dimensions of 8 m × 4 m, and a useful area of 6 m × 3 m.

With the defined area, 5 soil samples were collected at a depth of 0 to 20 cm, which after being mixed were sent for analysis at the Laboratory of the Department of Agricultural and Soil Engineering at UESB, Vitória da Conquista Campus, the chemical analysis of the soil revealed the following characteristics: pH (water) = 4.8; p = 1.0 mg dm^{-3}; K^+ = 0.10 cmolc dm^{-3}; Ca^{2+} = 1.3 cmolc dm^{-3}; Mg^{2+} = 1.0 cmolc dm^{-3}; Al^{3+} = 0.7cmolc dm^{-3}; H^+ = 6.5 cmolc dm^{-3}; and organic matter (OM) = 18.0 g dm^{-3}. The recommendation for fertilizing and liming was carried out according to Ribeiro et al. (1999), with a calculated dose of 4.09 ton ha^{-1} of limestone, after ninety days of the application of the limestone, phosphorus was applied in the amount of 181,8 kg ha^{-1} of P_2O_5, using the simple superphosphate as the source of the nutrient, and potassium in the amount of 282,48 kg ha^{-1} of K_2O using potassium chloride as the nutrient source. The planting furrows were made, with the aid of a furrower, for planting spineless cactus. The seedlings obtained were removed from the mother plants, selected in the field, and after this process, they were left to rest in the shade for a period of fifteen days for the wilt and healing of the wounds arising from the harvest and selection process.

The crop was implanted at 0.10 m × 2 m spacing, aiming at a population of 50,000 plants ha^{-1}. The cladodes were deposited in the planting furrows on April 29, 2019, burying them to a depth of up to one-third the length of the cladode. The first application of the bacteria (*Azospirillum brasilense*) was carried out shortly after planting the cladodes, on April 29, at a dose of 500 mL ha^{-1}, or 1.6 mL per plot, the strains used come from selection research carried out in Brazil by Embrapa (Brazilian Agricultural Research Corporation), with strains AbV5 (CNPSo 2083) and AbV6 (CNPSo 2084) which are currently used as inoculants for agricultural crops such as corn (*Zea mays L.*) for pastures planted with Urochloa spp. (*Brachiaria spp.*) and can also be used in coinoculation of soybean (*Glycine max L.*) (Hungria et al., 2010; Hungria et al., 2013; Hungria et al., 2016). At the beginning of the water season, on October 29, the second dose of the bacteria (500 mL ha^{-1}, or 1.6 mL in each experimental unit) was applied. The microorganism was applied to the plant cladodes in a sprayed manner, diluting 1.6 mL of the solution with *A. brasilense* in 500 mL of distilled water. At the beginning of the water period, the treatments submitted to nitrogen fertilization received the first fertilization dose, which was divided into 50 kg ha^{-1} of N or 355 g of urea per experimental unit, for each month, during the months of October, November and December, respecting the pre-established quantities for each treatment, which were applied in installments. Nitrogen fertilization in cover was carried out by hand. Weed control was performed through manual weeding, without using phytosanitary products.

In April 2020, quarterly collections of morphometric data were carried out as a monthly increase in the width and length of the cladodes of the plants. The final data collection took place on April 29, 2020, where morphological data were collected, such as width, length and thickness of all cladodes of spineless cactus plants, with the aid of a caliper and an invar basimeter, also the height and number of cladodes data. In possession of these values, the cladodes area indices were calculated according to the methodology proposed by Pinheiro et al. (2015). The cladodes collected from the plots were weighed in the field, with a precision scale to determine the production of green mass. The cladode samples were pre-dried in a forced ventilation oven at 55 °C for 72 hours, then they were weighed again to determine the dry mass production. Total soluble sugars and starch were quantified using the Antrona method (Dische, 1962).

The results were subjected to analysis of variance and, when significant, determined whether the regression to nitrogen levels and the F Test to compare the means of treatment without and with inoculation T adopted the 5% level of probability and used the statistical software SAS.

RESULTS AND DISCUSSION

The variables enlargement rate of secondary cladodes, elongation rate of primary cladodes and elongation rate of secondary cladodes (Enlar C2, Elong C1 and Elong C2), were influenced by inoculation ($P < 0.05$), with higher rates being demonstrated when use of bacteria, showing that inoculated plants have a higher rate of increase in the dimensions of cladodes (Table 1). It can also be observed the quadratic behavior for the Elong C2 variable, with maximum value 138.9 kg ha^{-1} of N.

Table 1. Enlargement and elongation rate of cladode the spineless cactus (cm month^{-1}) depending on the application of *Azospirillum brasilense* and nitrogen fertilization

Items	BAC		Nitrogen (kg ha^{-1})				CV	*P*-Value		
	With	Without	0	50	100	150		BAC	N	BACxN
Enlar C1	0.77A	0.76A	0.73	0.74	0.81	0.79	10.57	0.671	0.173	0.347
Enlar C2	0.77A	0.72B	0.71	0.72	0.76	0.79	7.43	0.010	0.055	0.068
Elong C1	2.04A	1.86B	1.96	1.87	2.00	1.98	10.38	0.027	0.562	0.424
Elong C2	2.11A	2.04B	$\hat{Y} = 1.96 + 0.002x - 0.000009x^2$; $R^2 = 0.99$				3.66	0.022	< 0.001	0.714

Enlar C1: Primary cladode enlargement rate; Enlar C2: secondary cladode enlargement rate; Elong C1: primary cladode elongation rate; Elong C2: secondary cladode elongation rate; BAC: bacteria; N: nitrogen. Means followed by the same capital letter, for bacteria, do not differ by the *F* test ($P > 0.05$).

For the total soluble sugars and starch, the effect of inoculation was verified ($P < 0.05$), with lower levels for the inoculated plants (Table 2). Such behavior is related to productivity, in the situation that plants in constant growth accumulate less reserve and sugars, because they are in constant use, as can be seen, inoculated plants obtained greater growth and presented lower levels of total soluble sugars and starch.

Table 2. Total soluble sugars and starch of the spineless cactus cladodes (mg g^{-1}) depending on the application of *Azospirillum brasilense* and nitrogen fertilization

Items	BAC		Nitrogen (kg ha^{-1})				CV5	*P*-Value		
	With	Without	0	50	100	150		BAC	N	BACxN
TSS	80.09B	103.74A	85.16	85.70	93.38	103.41	23.02	0.004	0.300	0.952
STARCH	13.47B	17.29A	13.86	15.58	15.55	16.54	25.23	0.011	0.588	0.658

TSS: Total soluble sugars; STARCH: Starch Content; BAC: bacteria; N: nitrogen; Means followed by the same capital letter, for bacteria, do not differ by the *F* test ($P > 0.05$).

Low levels of TSS and starch in plants with higher growth are justified, because plants in full vegetative growth, constantly consume soluble sugars for their development, and only store these carbohydrates when they slow their growth (Watt et al., 2013).

The inoculation factor had a significant influence ($P < 0.05$) for the variables of primary, secondary cladodes and average total length (LPC, LSC and LTTC) and also for the variables of secondary and tertiary cladodes, average total width (WSC, WTC, WTTC) (Table 3), where there were higher values for the variables when the inoculation was used. For the variable mean total thickness of cladodes, there was no difference between the factors studied ($P > 0.05$).

Table 3. Dimensions of the spineless cactus cladodes (cm) depending on the application of *Azospirillum brasilense* and nitrogen fertilization.

Items	BAC		Nitrogen (kg ha^{-1})				CV	*P*-Value		
	With	Without	0	50	100	150		BAC	N	BACxN
LPC	24.55A	22.43B	23.62	22.45	24.08	23.81	10.38	0.022	0.562	0.424
LSC	25.32A	24.52B	$\hat{Y} = 23.64 + 0.029x - 0.0001x^2$; R^2=0.99				3.66	0.020	< 0.001	0.710
LTC	23.32A	20.45A	19.29	23.22	21.26	23.76	23.53	0.129	0.317	0.659
LTTC	24.42A	23.11B	23.24	23.43	23.91	24.96	6.01	0.017	0.346	0.324
WPC	9.35A	9.20A	8.87	8.89	9.57	9.78	10.57	0.671	0.173	0.347
WSC	9.30A	8.64B	8.58	8.64	9.49	9.18	7.43	0.010	0.055	0.068
WTC	8.61A	7.25B	7.37	8.34	7.82	8.20	20.00	0.024	0.624	0.803
WTTC	9.05A	8.56B	8.53	8.49	9.23	8.98	6.34	0.020	0.051	0.506
TTC	1.68	1.43	1.63	1.41	1.66	1.52	30.71	0.165	0.707	0.351
PHEI	65.97A	60.04B	58.77	62.91	63.84	66.49	12.71	0.049	0.310	0.528

SPC: Length of primary cladodes; LSC: length of secondary cladodes; LTC: length of tertiary cladodes; LTTC: length of total cladodes; WPC: width of primary cladodes; WSC: width of secondary cladodes; WTC: width of tertiary cladodes; WTTC: width of total cladodes; TTC: thickness of total cladodes; PHEI: plant height; BAC: bacteria; N: nitrogen. Means followed by the same capital letter, for bacteria, do not differ by the F test ($P > 0.05$).

The length of secondary cladodes variable showed quadratic behavior when the nitrogen fertilization levels were evaluated, presenting the highest length cladode (25.8 cm) when the plot was subjected to application of 147 kg ha^{-1} of N. The width of primary cladodes variable did not show any difference for no factor.

The relationships between the morphological characteristics of the plant, such as width, length and height, are closely linked to the area index of cladodes, and consequently to the yield of the plant (Neder et al., 2013; Pinheiro et al., 2014).

Factors such as cladodes area index and height indicate the quality of fertilizer management on the crop, as they will directly correspond to productivity, in addition to estimates of forage accumulation (Costa et al., 2012; Litke et al., 2018; Hourani, 2023).

The interaction between inoculation with the bacterium *Azospirillum brasilense* and nitrogen fertilization was not significant ($P > 0.05$) for the cladodes area index (CAI), however, there was an isolated effect of the factors studied (Fig. 1) for CAI, obtaining higher values when they were inoculated with the bacteria.

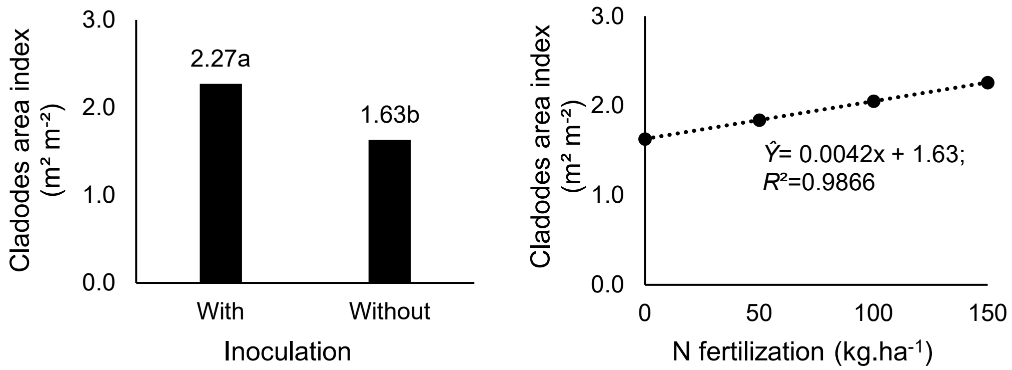

Figure 1. Cladodes area index of spineless cactus depending on the application of *Azospirillum brasilense* and nitrogen fertilization.

Averages followed by the same capital letter, for bacteria, do not differ by the *F* test ($P > 0.05$).

The variable CAI showed an increasing linear response to nitrogen fertilization levels, and it can be observed that as the nitrogen fertilization dose increases, the cladode area index has a positive response, presenting 2.29 when submitted to a dose of 150 kg ha^{-1} of N. Dubeux Júnior & Santos (2005) present in their work results that the spineless cactus extracts 9 kg of nitrogen for each ton of dry matter produced, emphasizing the importance of nitrogen fertilization for culture.

Lemaire & Chapman (1996), show that the appearance and development of leaves has a fundamental role in morphogenesis, and directly influences the structure of the pasture. As with the monthly increment values, the elongation and enlargement rates had significant increases when the plants were subjected to inoculation, which directly reflected in the height and CAI variables, also showing higher values with the inoculation, making the use of the same be beneficial for the development and growth of the spineless cactus.

Significant increments ($P < 0.05$) were obtained for the variables total average weight of primary cladodes, total average weight of secondary cladodes, total average weight of tertiary cladodes, total weight of green mass of cladodes and production of matter dry (TWPC, TWSC, TWTC, TWE and DMP). The plants that did not receive inoculation with the bacteria were the least productive (Table 4).

Table 4. Total weight of spineless cactus (kg ha^{-1}) depending on the application of *Azospirillum brasilense* and nitrogen fertilization

Items	BAC		Nitrogen (kg ha^{-1})				CV	*P*-Value		
	With	Without	0	50	100	150		BAC	N	BACxN
TWPC	108,638A	85,572B	93,154	94,133	96,752	104,379	21.47	0.005	0.702	0.103
TWSC	131,087A	85,626B	90,656	109,027	113,294	120,450	20.86	0.001	0.085	0.576
TWTC	50,3800A	28,103B	$\hat{Y}$ = 32,58 -66.06x +1.32x²; R^2=0.99				34.74	0.001	0.025	0.303
TW	292,480A	200,453B	217,960	237,067	251,437	279,402	18.01	0.001	0.070	0.181
DMP	23,813A	16,695B	18,537	19,301	20,971	22,203	18.97	0.001	0.249	0.229

TWPC: Total weight of primary cladodes; TWSC: total weight of secondary cladodes; TWTC: total weight of tertiary cladodes; TW: total weight of cladodes; DMP: dry matter production; Averages followed by the same capital letter, for bacteria, do not differ by the F test ($P > 0.05$).

For the variable total weight of tertiary cladodes (TWTC), when submitted to nitrogen fertilization levels, it presented a quadratic behavior, where the lowest weight of cladodes, 32,585 kg, can be identified, when nitrogen fertilization with values of 24.9 kg ha^{-1} of N was used.

Plants inoculated with *Azospirillum brasilense*, show greater development and production, as this bacterium can fix atmospheric nitrogen and produce phytohormones, inhabiting the rhizosphere of the plants, favoring the development of the same (Okon & Labandera Gonzalez, 1994; Bashan et al., 2004; Fiori et al., 2011).

Phytohormones such as cytokinin and auxin act directly on the growth and development of the plant, being transported to all parts of the plants through the xylem (Hungria et al., 2011). Cytokinin stimulates cell division, which can be called cytokinesis and, together with the auxin that regulates growth, providing greater vegetative development (Radwan et al., 2004; Moreira et al., 2010; Taiz & Zeiger, 2017).

Such development may be justified by the direct link with the production of phytohormones and biological nitrogen fixation, since they are growth promoters and act in different physiological processes, both in the root system and in the aerial part of the plant, therefore, justifying the largest length and width of the spineless cactus for treatments submitted to inoculation with *Azospirillum brasilense* (Dobbelaere et al., 2002; Hungria, 2011).

The auxins (indolacetic acid, indolbutyric acid and naphthalene acetic acid) and cytokinins (benzylaminopurine, isopentenyladenine and zeatin), will act on organs and tissues, being transported in the plant, where it is possible to identify the cytokinin in greater quantity, as the main responsible for favor the development of the aerial part of plants, which together with auxins will regulate plant growth (Moubayidin et al., 2009; Hungria et al., 2011; Nakhooda et al., 2012).

Greater development, production and vegetative growth were observed in inoculated plants, as can be seen by the value of total weight of cladodes (TWE), which reflect the result of the variables that indicate a better growth and development of spineless cactus plants that received the application of *Azospirillum brasilense*, attributing the bacterium the results.

CONCLUSIONS

The use of *Azospirillum brasilense* contributes positively to the development and productivity of the spineless cactus.

The use of nitrogen fertilization did not promote significant effects on crop productivity in the doses used.

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