Synergism between rhizobium strains and soil bioactivator favor sustainable production of *Arachis pintoi*[1]

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ABSTRACT

The objective of this study was to evaluate the influence of a commercial product, a soil bioactivator, on the establishment and efficiency of symbiosis between rhizobia and *Arachis pintoi* (forage peanut). The experiment was conducted in a greenhouse in 8 dm^3 pots and 12 treatments were used, ten inoculated with different strains of rhizobia and two without inoculation (presence or absence of mineral nitrogen), associated or not with the application of soil bioactivator. The experimental design used was a randomized block design with four repetitions. The experiment was conducted until the time of flowering, when the agronomic characteristics referring to the shoot dry matter (SDM) and root (RDM), relative symbiotic efficiency (RS), number (NN) and nodule dry matter (NMS) and the bromatological characteristics, given by the content of crude protein (CP), neutral detergent fiber (NDF) and acid (ADF) were evaluated. The use of soil bioactivator did not influence the bromatological characteristics, but potentiated the inoculation with rhizobia strains, UNIFENAS 03-16, UNIFENAS 03-31 and UNIFENAS 03-36, effectively contributing to increases in the values of SDM, RS and NN, thus constituting a sustainable alternative for the production of *A. pintoi*.

Keywords: forage legume; animal production; agricultural sustainability.

INTRODUCTION

In recent years, it is observed the growth of livestock activity in all regions of Brazil, with a predominance of animal breeding based on extensive management, a practice that contributes to reducing production costs (Torres Júnior & Aguiar, 2013), but causes degradation of these areas, causing a reduction in the nutritional quality of forage, animal production and producer income (Macedo *et al.*, 2013). Furthermore, the process of pasture degradation is also associated with negative environmental impacts, such as the acceleration of erosion processes, compaction and emission of greenhouse gases (Oliveira *et al.*, 2020), therefore, it is necessary to adopt measures to solve this problem, which are in line with the Sustainable Development Goals (SDGs) and the Low Carbon Agriculture Plan (ABC Plan).

In this sense, the use of forage legumes such as *Arachis pintoi* (forage peanut) is a viable alternative from the economic and environmental point of view to reduce the problems associated with degradation (Terra *et al.*, 2019). The good result of the use of legumes is due to the fact that they establish symbiosis with atmospheric nitrogen (N_2) fixing bacteria, also known as rhizobia, which provide partial or total nitrogen for plant development. Based on this, studies

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have been developed aiming to evaluate the efficiency of rhizobia strains already approved by MAPA for *A. pintoi* (Muniz *et al.*, 2016; Muniz *et al.*, 2017) and also select new strains of rhizobia that may have greater potential to fix N_2, adapted to different soil and climate conditions (Carvalho *et al.*, 2016; Sá *et al.*, 2019).

Besides inoculants with rhizobia strains, the use of other technologies such as bioactivators or soil biostimulants can also contribute to the sustainability of agroecosystems, favoring the improvement of soil quality and plant development (Almeida *et al.*, 2014; Binsfeld *et al.*, 2014; Franco Júnior *et al.*, 2019). These products promote the increase in the density and diversity of soil microorganisms and consequently, favor the realization of beneficial processes, increasing the release of nutrients and promoting greater plant development (Cordeiro Júnior *et al.*, 2019). According to Cataneo *et al.* (2010), the use of these products can also favor physiological processes in plants, such as increasing cell division and elongation, stimulating chlorophyll synthesis and photosynthesis, and reducing effects caused by biotic and abiotic stresses. Franco Júnior *et al.* (2019) analyzing the effects of bioactivator use on soil biological properties, found that they contribute significantly to the reduction of phytopathogenic nematodes and increase in the population of diazotrophic and phosphorus and potassium solubilizing bacteria.

Based on this information, the importance of studying the joint action of rhizobia strains and bioactivator in the cultivation of *A. pintoi* is verified, since they can act synergistically contributing to the development of plants and consequently, with the sustainability of pasture areas, enhancing the use of this legume. In this context, the objective of this study was to evaluate the influence of a commercial product, a soil bioactivator, on the establishment and efficiency of symbiosis between rhizobia and *Arachis pintoi* (forage peanut).

MATERIAL AND METHODS

Identification of the strains used in the experiment, inoculant and soil biactivator preparation

The bacterial strains used in the experiment were from the collection of the Soil Microbiology Laboratory of the Professor Edson Antônio Velano University - UNIFENAS, isolated by Florentino *et al.* (2014). Table 1 shows the identification and morphological characteristics in medium 79 (Fred & Waksman, 1928).

All strains showed rapid growth on culture medium, regular shaped colonies, and exopolysaccharide of gummy consistency. To prepare the inoculants, the strains were grown in Petri dishes containing culture medium 79 for 48 hours, enough time for isolated colony formation and confirmation of purity. Subsequently, these were transferred to liquid culture medium 79, and cultured for the same period, the time needed to reach the log phase containing approximately 10^8 cells mL^{-1}. Inoculation occurred at the time of transplanting, where two mL $plant^{-1}$ was applied. The bioactivator used was soil bioactivator at a dose of 600g ha^{-1}, according to the manufacturer's recommendation (PENERGETIC®, 2019). The product was weighed on a precision scale and then applied to the soil one day before transplanting.

Pot preparation and experimental design

The soil used was from the 0-20 cm layer of an experimental area of UNIFENAS, with the chemical characteristics described in Table 2. For the preparation of the soil was performed phosphate fertilization at a dose of 60 kg ha^{-1}, according to the recommendation for the crop described by Carvalho (1999).

The experimental design used was in randomized blocks, in a factorial scheme 12 (ten bacterial strains, one negative control and one positive control) x 2 (use or not of bioactivator), with four repetitions per treatment. The negative control consisted of growing *A. pintoi* without the use of rhizobium inoculation and mineral nitrogen. For the positive control, mineral nitrogen was used in the form of ammonium nitrate, divided into two applications of 35 $mg.L^{-1}$ $N\text{-}NH_4\ NO_3$, totaling 70 $mg.L^{-1}$ $N\text{-}NH_4\ NO_3$ and rhizobium inoculant was not used.

Transplanting, conduct and evaluation of the experiment and statistical analysis

The transplanting of *A. pintoi* was done vegetatively, using 20 cm long segments of branches containing four buds, which were buried about ¾ of the seedling, at a depth of five to eight cm, according to the guidelines of Carvalho (1999). Four seedlings were planted per pot, and after the stolons had set and emerged, only two plants were left in each pot, and the inoculation was performed with two mL of bacterial suspension next to the stolons. In this same period, 35 mg L^{-1} de $N\text{-}NH_4NO_3$ was added to the positive control treatment.

Table 1: Identification and morphological characteristics of bacterial strains grown on culture medium 79 using bromothymol blue indicator isolated from root nodules of *Gliricidia sepium*

Bacterial strains	Morphological characteristics in médium "79"		
	pH	Production of exopolysaccharides	Colony Color
UNIFENAS 03-10	Ácid	Low	Yellow
UNIFENAS 03-13	Neutral	Medium	Cream
UNIFENAS 03-16	Ácid	Low	Yellow
UNIFENAS 03-23	Alkaline	Low	Cream
UNIFENAS 03-24	Alkaline	Low	Cream
UNIFENAS 03-25	Alkaline	Low	White
UNIFENAS 03-27	Neutral	Medium	Cream
UNIFENAS 03-31	Ácid	Medium	Cream
UNIFENAS 03-35	Ácid	Medium	Yellow
UNIFENAS 03-36	Neutral	Low	Yellow

Soil moisture was maintained at 70% of its field capacity throughout the experimental phase by weighing the pots and replenishing the water. The experiment was conducted until flowering. The experiment was conducted in a greenhouse, in pots containing eight dm^3 of soil, during the period from May to September 2019.

The agronomic characteristics referring to the shoot dry matter (SDM) and root (RDM), relative symbiotic efficiency (RS), number (NN) and nodule dry matter (NDM) were evaluated. To evaluate the SDM, RDM and NDM, an air oven at 55 ºC for 72 hours was used.

The RS was calculated following the formula adapted from Bergersen *et al.* (1971).

$$RS\% = \frac{\text{SDM inoculated}}{\text{SDM control}} * 100 \quad (1)$$

For the bromatological characteristics, the crude protein (CP) content was evaluated by the micro-Kjeldahl method (Association of Official Analytical Chemist, 2005), neutral detergent fiber (NDF) and acid (ADF) with the aid of a Tecnal® apparatus, using the method of Van Soest et al. (1991).

The data were submitted to variance analysis, using the statistical analysis Sisvar program, version 5.3. The means of the treatments were compared by the Scott-Knott test at 5% probability level ($p < 0.05$) (Ferreira, 2019).

RESULTS AND DISCUSSION

The association of inoculation of rhizobia strains and use of bioactivator contributed to the agronomic parameters SDM, RDM, RS, NN and NDM. For the bromatological characteristics, there was no contribution of this association.

Table 3 shows the values of shoot dry matter (SDM) and root (RDM), relative symbiotic efficiency (RS) of *A. pintoi* inoculated with different rhizobia strains, associated or not with bioactivator.

Analyzing the SDM data, it was observed that the positive control treatment showed higher values when compared to the negative control, indicating the response of *A. pintoi* to the application of mineral nitrogen. Also, the efficiency of the inoculation of bacterial strains was verified, which promoted SDM values similar to the positive control.

Table 2: Results of the chemical analysis of the soil from the 0-20 cm layer in the experimental area of UNIFENAS

pH	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	CEC	V	OM
H_2O	mg dm^{-3}				$cmol_c$ dm^{-3}				%	g dm^{-3}
5,8	8	133	2,0	1,0	0,2	3,4	3,3	6,7	49	20

P: Phosphorus; K: Potassium; Ca^{2+}: Calcium; Mg^{2+}: Magnesium; Al^{3+}: Aluminum (Exchangeable acidity); H+Al: Potential acidity; SB: Sum of base cations; CEC: Cation exchange capacity; V: base saturation; OM: Organic Matter.

Regarding the use of bioactivator, it was observed that it had no influence on the positive and negative control treatments and treatments inoculated with UNIFENAS strains 03-10, 03-35 and 03-27. For the other treatments, UNIFENAS 03-13, 03-16, 03-23, 03-24, 03-25, 03-31, 03-36, it was observed that the bioactivator acted differently among the bacterial strains.

These results demonstrate the influence of the use of soil bioactivator on the efficiency of the rhizobia population, thus presenting great practical importance. The fact that the use of bioactivator favors or not the performance of a particular rhizobium strain may be related to the occurrence of ecological interactions between soil microorganisms (Manhães & Francelino, 2013), which can be variable according to the rhizosphere of a particular plant species (Coelho *et al.*, 2007). However, in general, the use of bioactivator potentiated the use of most bacterial strains, contributing to the promotion of higher SDM values. Different results were observed by Almeida *et al.* (2014) in which the use of a commercial product with similar characteristics to that used in this study did not influence the development of the aerial part of bean plants.

Table 3: Values of shoot dry matter (SDM) and root (RDM), relative symbiotic efficiency (RS) of *Arachis pintoi* inoculated with different rhizobia strains, associated or not with the use of soil bioactivator

Treatments	SDM (g $planta^{-1}$)		RDM (g $planta^{-1}$)		RS (%)	
	WITH BIO	WITHOUT BIO	WITH BIO	WITHOUT BIO	WITH BIO	WITHOUT BIO
UNIFENAS 03-10	14,30 Ba	15,33 Ba	4,85 Da	5,18 Ca	82,93 Ba	87,59 Ba
UNIFENAS 03-13	14,51 Bb	15,09 Aa	4,12 Da	5,28 Ca	84,11 Ba	86,17 Ba
UNIFENAS 03-16	17,45 Aa	15,15 Bb	6,99 Ba	5,41 Ca	101,16 Aa	86,55 Bb
UNIFENAS 03-23	16,74 Aa	15,68 Ba	7,94 Ba	6,41 Ca	97,05 Ba	89,55 Ba
UNIFENAS 03-24	16,08 Aa	15,21 Ba	9,82 Ca	9,44 Ba	93,21 Ba	86,87 Ba
UNIFENAS 03-25	12,55 Bb	16,48 Aa	10,23 Ca	9,34 Ba	72,78 Bb	94,12 Ba
UNIFENAS 03-27	16,99 Aa	16,07 Aa	11,64 Aa	9,25 Bb	98,52 Ba	91,80 Ba
UNIFENAS 03-31	17,81 Aa	13,43 Bb	5,27 Da	4,38 Ca	103,27 Aa	76,74 Bb
UNIFENAS 03-35	14,64 Ba	13,85 Ba	12,14 Aa	3,14 Cb	84,87 Ba	79,14 Ba
UNIFENAS 03-36	18,19 Aa	13,46 Bb	4,29 Da	5,90 Ca	105,45 Aa	76,89 Bb
Positive control	17,25 Aa	17,51 Aa	6,34 Bb	12,53 Aa	100,00 Aa	100,00 Aa
Negative control	14,07 Ba	14,39 Ba	5,65 Db	10,32 Ba	81,59 Ba	82,21 Ba
CV%	9,01		19,00		8,97	

* Means followed by different letters, uppercase in the column and lowercase in the row, differ by the Scott Knott test at 5% probability, (WITH BIO = Bio (+); WITHOUT BIO = Bio (-); Positive control = Without rhizobium inoculation and with mineral nitrogen; Negative control = Without rhizobium inoculation and mineral nitrogen; CV = coefficient of variation).

Regarding the RDM parameter, it can be seen that the control treatments (negative and positive) were negatively influenced by the use of soil bioactivator. As for the treatments inoculated with bacterial strains, it was possible to observe that the use of bioactivator contributed to greater root development in the treatments inoculated with UNIFENAS strains 03-27 and 03-35, different results from those observed for the SDM, in which the use of bioactivator had no influence. The increased root development is important from an agronomic point of view, due to the main function of the roots in absorbing water and nutrients, reinforcing the importance of the data obtained in this study.

Literature data report the contribution of rhizobia strains and soil bioactivator acting alone on root development. In the case of rhizobia strains, studies have reported their potential to synthesize the phytohormone indoleacetic acid (IAA) (Chagas Júnior *et al.*, 2010), which favors the growth and increase of the number of roots (Muniz *et al.*, 2016). Regarding bioactivator, there are reports of the benefits of its use for soy root growth (Tavares *et al.*, 2007), cotton (Lauxen *et al.*, 2010) and rice (Almeida *et al.*, 2011). However, these authors do not mention the physiological mechanism/process involved.

For the parameter relative efficiency (RS), it can be seen that the bioactivator had no influence on the positive and negative controls, as verified for SDM. For the inoculated treatments, the positive influence of the bioactivator was verified, especially for the strains UNIFENAS 03-16, 03-31 e 03-36. These results are of great relevance, as they indicate the potential of these strains to establish symbiosis and supply nitrogen to meet the metabolic demands of *A. pintoi*, and also for the selection criteria of these strains by using them together with the bioactivator, since the association of these two techniques contributes to higher plant production.

Most studies on biological N_2 fixation in *A. pintoi* report the potential of rhizobia strains that are slow-growing and alkalinize the culture medium, typical characteristics of the *Bradyrhizobium* genus (Carvalho, 1999; Muniz *et al.*, 2016; 2017; Sá *et al.*, 2019). However, this legume establishes symbiosis with a wide diversity of rhizobia, especially those that grow quickly and acidify the medium (Pinto *et al.*, 2004; Ibañez *et al.*, 2008), corroborating the data found in this study, which established effective symbiosis in *A. pintoi*, and may be indicated for selection studies of inoculant strains.

Table 4 shows the values of number (NN) and nodule dry matter (NDM) when inoculated with different rhizobia strains associated or not with the use of soil bioactivator.

Regarding the number of nodules (NN), it can be seen that the treatments suffered a positive influence with the application of bioactivator, with the exception of UNIFENAS strain 03-27.

The increase in NN with the application of bioactivator can be explained by its mechanism of action, which has in its composition organic acids, which stimulate the population of soil microorganisms (Almeida *et al.*, 2014; Silva *et al.*, 2018; Franco Júnior *et al.*, 2019). The formation of nodules in the treatments where there was no inoculation can be explained due to the fact that *A. pintoi* is a highly promiscuous species capable of nodulating with several genera and species of native rhizobia (Santos *et al.*, 2005; Fernandes Júnior & Reis, 2008; Silva *et al.*, 2017).

As for NDM, it was observed that the control treatments (positive and negative) were not influenced by the use of bioactivator. However, significant decreases were observed when bioactivator was used together with UNIFENAS strains 03-23, 03-25 and 03-27. This inference may be related to the stimulation of the activity of native microorganisms present in the soil, generating a competition. Silva *et al.* (2017) highlights that the efficiency of inoculation is related to the ability of the inoculated strain to compete with native soil strains.

In general, it was possible to establish a direct relationship between higher nodule number and nodule dry matter value. This result is of great practical importance, since these attributes are essential for an adequate biological nitrogen fixation process (Hungria *et al.*, 2001).

Regarding the bromatological parameters, no influence of the bioactivator and the bacterial strains was found. The average CP content for the treatments without inoculation was 15,15% (positive) and 13.69% (negative), and the average for the treatments with inoculation was 15,21%. These values, are relatively low compared to those described in the literature (Carvalho *et al.*, 2016; Gondim Filho *et al.*, 2020) and can be explained by the age at which the plant was cut (142 days). CP contents in forages are higher in the early stage of development because of the higher leaf + petiole: stem ratio (Taiz & Zeiger, 1991; Paulino *et al.*, 2012).

The contents of NDF set between 49,60 to 44,55% and those of ADF between 29,22 to 26%, such values are corroborated by Carvalho *et al.* (2016) and Gondim Filho *et al.* (2020).

Table 4: Values of the number (NN) and nodule dry matter (NDM) of A, pintoi inoculated with different rhizobia strains, associated or not with the use of soil bioactivator

Treatments	NN		NDM (mg planta^{-1})	
	WITH BIO	WITHOUT BIO	WITH BIO	WITHOUT BIO
UNIFENAS 03-10	169,00 Ba	75,00 Db	80,00 Ba	60,00 Ca
UNIFENAS 03-13	147,00 Ca	169,25 Aa	90,00 Ba	70,00 Ca
UNIFENAS 03-16	196,00 Ba	146,50 Ab	120,00 Aa	120,00 Ba
UNIFENAS 03-23	219,00 Aa	74,25 Db	110,00 Ab	160,00 Aa
UNIFENAS 03-24	155,00 Ca	126,25 Bb	80,00 Ba	30,00 Db
UNIFENAS 03-25	112,00 Da	135,50 Ba	60,00 Cb	100,00 Ba
UNIFENAS 03-27	95,00 Db	162,50 Aa	60,00 Cb	110,00 Ba
UNIFENAS 03-31	186,00 Ba	66,50 Db	130,00 Aa	50,00 Db
UNIFENAS 03-35	235,00 Aa	104,50 Cb	150,00 Aa	30,00 Db
UNIFENAS 03-36	158,00 Ca	119,25 Bb	110,00 Aa	50,00 Cb
Positive control	178,00 Ba	127,25 Bb	50,00 Ca	60,00 Ca
Negative control	124,00 Da	76,75 Db	60,00 Ca	60,00 Ca
CV%	14,00		19,75	

* Means followed by different letters, uppercase in the column and lowercase in the row, differ by the Scott Knott test at 5% probability, (WITH BIO = Bio (+); WITHOUT BIO = Bio (-); Positive control = Without rhizobium inoculation and with mineral nitrogen; Negative control = Without rhizobium inoculation and mineral nitrogen; CV = coefficient of variation).

CONCLUSIONS

The strains UNIFENAS 03-16, UNIFENAS 03-31 and UNIFENAS 03-36 associated with the bioactivator showed synergism in relation to the shoot dry matter, relative symbiotic efficiency and number of nodules of *A. pintoi*.

The bioactivator and inoculation did not influence the bromatological characteristics.

The use of soil bioactivator potentiated the inoculation with rhizobia strains, and may be a sustainable alternative for *A. pintoi* production.

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