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NODULATION AND VEGETATIVE GROWTH IN SOYBEAN UNDER DIFFERENT WAYS OF APPLYING LIQUID INOCULANT

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ABSTRACT

The inoculation is one of the main cultural practices in leguminous crop such as soybean, providing economy of resources on high doses of mineral nitrogen. The objective of this work was to verify the effect of different ways of applying liquid inoculant on soybean. The experimental design was randomized blocks, in a total of 10 treatments with 5 replications. The treatments were: application of liquid inoculant on the seeds, on the soil, inside de seeds, with the aid of a syringe, besides the application via foliar on the vegetative stages V1, V3, V5 and V8, in addition to treatments with nitrogen fertilization. The treatments did not affect the total number of leaves, chlorophyll index and the number of nonviable nodules. For plant height the inoculation inside the seed, on the soil or via foliar in V3 stage provided the best results. For foliar area and dry mass of aerial part and root the use of mineral nitrogen harmed the crop, it had no inoculation effect compared to the witness treatment. The nitrogen supply harmed the stem diameter and the different ways of inoculation were superior to the witness, except the foliar application in V8 stage. Independent of the way, the inoculation caused an increase in the total number of nodules, being superior to the witness and the use of nitrogen, which did not differ between them. Only the application of mineral nitrogen impaired the number of viable nodules, not having difference of the witness in relation to the treatments with inoculation.

Key words: Biological fixation. Foliar inoculation. Mineral nitrogen. Viable nodules

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1 Introduction

Brazil is today the largest soybean producer in the world, the production in 2018/19 harvest reached 123 million tons, while the second producer, the United States, had a production of 112.95 million tons. Mato Grosso stands out as the largest soybean producer state with a production of 32,454 million tons, reaching almost double of the second largest producer, Rio Grande do Sul, which produced 19,187.1 million tons and Paraná with 16,252.7 million tons [1]. Soybean is a very nutrient-demanding crop, with nitrogen (N) being the most required. In soybean, the biological fixation of N is indispensable, replacing the use of mineral N and reducing the production cost. Thus, the main source of N for soybean is the BFN – Biological Nitrogen Fixation [2].

Generally, for other crops, nitrogen is made available through N fertilizers, which have the fastest assimilated form for plants, but they have two problems: cost and losses, especially when urea is applied, because it has high ammonium volatilization rates due to decomposition by urease. Ammonium volatilization can generate an accumulated total loss ranging from 2 to 50% of the applied N, depending mainly on the physical state in which the fertilizer is applied, the soil moisture and the environmental temperature [3].

Therefore, for soybean, FBN is the most efficient and used form of N supply in Brazil, and many believe they supply all the N needs by soybeans, dispensing the use of chemical N fertilizers [4]. Despite this, some questions still need to be answered about the use of inoculation. One of the problems is the use of fungicides in the seeds treatment, which ends up significantly limiting the population of Bradyrhizobium in the seeds [5].

One of the ways to solve the incompatibility of inoculants and fungicides is the application form, such as inoculation in the sowing furrow, post-emergence inoculation, pre-sowing inoculation and the co-inoculation package [6]. Evaluating different forms of applying inoculant found that the best way is in the furrow and as a peat inoculant, however they highlighted that operationally and in terms of results, liquid application via furrow is the most recommended [7].

Thus, there is still a great need to know the best way to apply inoculants, a way that avoids the contact of seed treatments with the bacteria of the inoculant and, at the same time, come into contact with the plant roots as quickly and intensely as possible. Given the above, the objective of this work was to verify the effect of different ways of applying liquid inoculant, containing bacteria of the genus *Bradyrhizobium*, on soybean culture.

REVISTA TECNO-LÓGICA ISSN 1982-6753

2 Materials and Methods

The experiment was carried out in 2018/2019 harvest, on a screen at Federal University of Mato Grosso nursery, Sinop-MT, whose geographic coordinates are: 11° 51' 49" S and 55° 29' 07 " W. The plants were grown in pots on a plastic-covered screen from November 1, 2018 to January 5, 2019. According to Koppen-Geiger, the Sinop climate is the Am type, with well-defined seasons, with the rainy season from October to May and the dry season from May to September. The average annual precipitation is 1,818 mm, and the average annual temperature is 25ºC.

The soil of the experiment was collected in an area close to the nursery and it was analyzed before full the pots. The results of the physical and chemical analysis performed are shown in Table 1.

The experimental design was randomized complete blocks (RCB) with 10 treatments and 5 replications, totaling 50 plots. The treatments consisted of different ways of applying the liquid inoculant, *B. japonicum* and *B. elkanii*, on the seeds, on the soil and internally on the seeds in addition to the application in post-emergence of the seedlings by leaf spray and mineral nitrogen supply. The treatments were: inside - inside the seed - inoculation inside the seed (injected with the aid of a syringe); seed - inoculation of seeds 4x the dose (100mL / 50kg of seed); soil - inoculation via soil 4x the dose (100mL / 50kg of seed); V1 - inoculation via leaf in V1 4x the dose (100mL / 50kg of seed); V3 - inoculation via leaf in V3 4x the dose (100mL / 50kg of seed); V5 - inoculation via leaf in V5 4x the dose (100mL / 50kg of seed); V8 - inoculation via leaf in V8 4x the dose (100mL / 50kg of seed); witness - without inoculation; 200 kg of N ha^{-1} - control with application of 200 kg of mineral N ha^{-1}; 300 kg of mineral N ha^{-1} - control with application of 300 kg of N ha^{-1}.

Table 1. Results of chemical and physical analysis of the soil, collected at the 0 to 20 cm layer

Macronutrients										
pH (H_2O)	pH ($CaCl_2$)	P	K	K	Ca	Mg	Al	H	H+Al	M.O
		mg/dm³		cmol/dm³						g/dm³
4.40	3.7	1.30	6.00	0.02	0.19	0.06	0.50	5.57	6.07	18.81

Micronutrients + S					
Zn	Cu	Fe	Mn	B	S
mg/dm³					
0.12	0.65	143.50	3.98	0.10	5.98

S (Sum of bases)	T (CTC pH 7)	V (Bases saturation)	m	Sand	Silt	Clay
cmol/dm³		%	%	g/dm³		
0.27	6.34	4.20	65.27	240	167	593

Extractors: pH(H_20) = 1:2.5 pH($CaCl_2$-0,01mol/L)=1:2.5, Ca, Mg e Al = KCl 1N, P; K; Zn; Cu; Fe; Mn = Mehlich 1, H+Al = Calcium acetate pH 7.0, S = Calcium phosphate, B = $CaCl_2$ – 5 mmol/L, M.O = Sodium dichromate

REVISTA TECNO-LÓGICA ISSN 1982-6753

The sowing took place on November 1, 2018 and the cultivar used was CZ 48B32 - BASF®, 6 seeds/pot were sown and after the plants entered the vegetative stage V1 the plants were thinned, leaving only 2 plants/pot. The Masterfix® Soy liquid inoculant was used in all treatments, containing bacteria *Bradyrhizobium elkanii* and *Bradyrhizobium japonicum* in a minimum concentration of $5x10^9$ UFC per mL, using a dose of 100 mL for 50 kg of seeds. The strains used were SEMIA 5019 and SEMIA 5079.

Sowing was carried out in plastic pots, with a volumetric capacity of 8 L, filled with soil taken from an area at UFMT, Sinop campus. After collection, the soil was sieved and its impurities removed. Dolomitic limestone was applied at a dose of 4 tons ha^{-1}, aiming to correct acidity and increase pH, in addition to supply calcium and magnesium to the soil. Then planting fertilization was carried out with potassium chloride (650 kg ha^{-1}) and simple superphosphate (1,250 kg ha^{-1}). Chlorophyll evaluation was performed at 35 days after sowing, sampling 4 points per pot in the 3rd trefoil from the apex of the plant to the soil. The chlorophyll index was obtained with the aid of a portable chlorophyll meter (chlorofiLOG®, CFL 1030) and estimates the green color of the leaves, the results being expressed in Chlorophyll Falker Index, which is dimensionless.

At 66 days after sowing, on January 5, 2019, the plant height (PH), number of leaves (NL), leaf area (LA), as well as the number of viable and non-viable nodules, root dry mass (RDM) and aerial part dry mass (APDM) were evaluated. The plant height was obtained with the aid of a graduated ruler, measuring from the soil to the apical meristem of the plants. The heights of two plants in the plot were taken and after the measurements, the average height per plot was obtained, with the results expressed in centimeters. The stem diameter was obtained by measuring 5 cm from the soil, with the aid of a digital caliper, taking the values of the two plants in the plot and the results were expressed in mm.

After field measurements, with the aid of scissors, the plants were cut close to the ground, packed in paper bags and taken to the UFMT nursery laboratory. In the laboratory, the leaves of the plants were detached and counted, obtaining the number of leaves $plants^{-1}$. Soon after, the leaf area was obtained in cm^2, with the aid of a LICOR leaf area integrator model LI-3010. Finally, the leaf samples were reconditioned in paper bags and placed in an air forced circulation oven at 60ºC, until they reached constant weight, to obtain the aerial part dry mass [8].

The soil with the roots of the plants was removed from the pots through a washing process under running water, and so the soil was separated from the roots. After washing the roots, the nodules were separated from the roots and all nodules were

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counted, obtaining the number of total nodules. Next, the nodules with more than 2 mm were sectioned in half, with the aid of a stylus, and if the pink color was identified they were considered viable, thus obtaining the number of viable nodules, and by difference the number of non-viable nodules. .

After counting the nodules, the roots and nodules were stored separately in paper bags and placed in an air forced circulation oven for 72 hours at 65 °C for drying. Next, they were weighed on a precision scale to obtain root dry mass. After all evaluations, the data were subjected to analysis of variance at the level of 5% probability with the aid of SISVAR® software [9]. As the variables are qualitative the Scott-Knott test was performed, adopting the 5% probability level.

3 Results and Discussion

By the analysis of variance, it was found that only the variables chlorophyll index, nonviable nodules and number of leaves were not significantly influenced by the treatments. The variables related to soybean vegetative growth is presented in Table 2.

Table 2. Means of the variables plant height (PH), aerial part dry mass (APDM), root dry mass (RDM), stem diameter (SD) and leaf area (LA) under different forms and times of applying inoculant.

Treatments	PH (cm)	APDM (kg ha^{-1})	RDM (g)	SD (mm)	LA (m^2)
inside seed	47.30 a	4361.50 a	11.89 a	6.59 a	0.91 a
seed	45.00 b	4207.60 a	11.72 a	6.60 a	0.86 a
witness	44.90 b	4203.74 a	11.72 a	5.85 b	0.83 a
soil	48.20 a	4491.00 a	10.70 a	6.64 a	0.90 a
200 N ha^{-1}	37.20 c	2812.75 b	9.34 b	4.60 c	0.52 b
300 N ha^{-1}	37.20 c	2812.75 b	8.54 b	4.66 c	0.46 b
V1	44.10 b	4278.00 a	12.05 a	6.54 a	0.96 a
V3	48.10 a	4372.00 a	12.40 a	6.83 a	0.98 a
V5	45.40 b	4207.00 a	11.97 a	6.48 a	0.81 a
V8	44.40 b	4245.00 a	11.87 a	5.96 b	0.83 a
C.V.(%)	6.17	8.24	8.66	7.28	14.47

* The averages followed by the same letters do not differ at the 5% probability level by the Scott-Knott test..

For plant height it was found that the treatments that reached the highest heights were: inoculation inside the seed, on the soil and leaf application in V3 stage. The leaf treatments V5, V8, seeds and witness had intermediate behavior and the application of mineral N reached the lowest heights. For stem diameter it was also possible to verify the poorest performance by the treatments with application of mineral N. The witness did not differ of the application by leaf in V8 stage and the other treatments were equivalent. So, the highest values were obtained by the treatments with inoculation inside the seed, on the seed, via soil, and in the stages V1, V3 and V5. This possibly occurred due to the assimilation of N by the bacteria. According to [10], N is an essential nutrient for plant development and, in its absence, variables such as plant height, stem diameter and leaf area are negatively affected.

For leaf area, aerial part dry mass and root dry mass, it is worth mentioning that the application of mineral N harmed the plant, possibly due to the high dose that was applied at once. It is observed then that there was no advantage in the use of N fertilization. Found effect of mineral N applied to a haul up to a dose of 40 kg ha^{-1} on plant height, number of pods and height of the first pod [11]. Also found an effect of the application of 10 kg ha^{-1} of N, however via leaf [12]. Despite the conflicting results of the aforementioned authors, it should be noted that the doses applied in this work were much higher (200 and 300 kg ha^{-1}) and in an isolated form without inoculant. It should emphasize the effects of inoculant application in the soybean and the importance of FBN, which cannot be imagined as unnecessary, but fundamental.

For number of leaves, chlorophyll index and nonviable nodules there was no effect of the treatments (Table 3). For total number of nodules there was no difference among the inoculation forms and times. Only the witness without inoculation and the N mineral application was inferior for this variable. So, independently of the form, the inoculation was efficient to increase the number of nodules in the soybean roots. The number of viable nodules was only affected by mineral N. Regardless the inoculation process or its form and time there was no difference in the number of soybean viable nodules.

Table 3. Means of the variables number of nodules, number of viable nodules, number of nonviable nodules, number of leaves (LN) and chlorophyll index (CI) under different forms and times of applying inoculant.

Treatments	Total nodules	Viable nodules	Non viable nodules	LN	CI
inside seed	80.00 a	73.10 a	6.90 a	31.80 a	33.40 a
seed	77.00 a	73.40 a	6.90 a	30.50 a	35.33 a
witness	61.80 b	65.08 a	6.50 a	28.80 a	32.46 a
soil	88.00 a	77.40 a	6.70 a	32.70 a	34.64 a
200 N ha^{-1}	40.10 b	33.90 b	6.40 a	17.50 a	33.45 a
300 N ha^{-1}	48.20 b	29.80 b	7.00 a	17.60 a	32.96 a
V1	87.80 a	76.80 a	6.80 a	32.20 a	33.76 a
V3	81.20 a	76.50 a	6.80 a	33.40 a	33.83 a
V5	84.00 a	77.60 a	6.80 a	31.60 a	32.44 a
V8	69.90 a	67.10 a	6.60 a	31.90 a	32.94 a
C.V.(%)	20.35	15.96	5.2	14.13	6.23

* The averages followed by the same letters do not differ at the 5% probability level by the Scott-Knott test.

These results corroborate those presented by [13], who found that inoculation via leaf performed 35 and 45 days after emergence cause a significant increase in number of nodules. The post-emergence inoculation technique can be used in place of the standard inoculation that is carried out on the seeds before sowing. The inoculation carried out only via seeds can present flaws due to: the low quality of the inoculant (without the requirements of MAPA), the operational difficulty of this type of application, often done improperly, temperature, low moisture in the soil, contact of the inoculant with seed treatment, what is toxic to bacteria, and expired inoculants [13]. In this way, the use of inoculants after emergence can present itself as a viable alternative being free of these possible flaws and ensuring good yield values [14].

The inoculation inside the seed reached the best results on the evaluated variables, in relation to other forms of inoculation already known, and those already tested, thus being a technique with potential to be explored. The fact that the soil was removed from an area where other experiments with inoculation have already been carried out influenced the nodulation of the plants, since the witness plants, that is, without inoculation, obtained high nodulation numbers. Possibly the soil already had a good number of soybean nodulating bacteria. The lack of response in soils with an established population confirms the results obtained by other authors in the literature.

Despite this, experiments involving the practice of reinoculation have shown great variability on the results, that is, there have been increases in yield, as well as lack of response, because, probably, the populations of Bradyrhizobium existing in the soil had already efficient strains and in adequate numbers [15]. However, due to the low cost of reinoculation practice and events such as stresses of soil and climate, this practice is extremely important for the full establishment of symbiosis.

The application of high doses of mineral N caused burning of the stem and leaves of the seedlings due to the high dosage of urea, which interfered in the results of these treatments. So, it is recommended to split the application of high doses of N in soybean. In addition to the direct effect, urea reduced the nodulation and efficiency of FBN. According to [16] some mineral forms of N in the soil, such as NO^{3-} and NH^{4+}, can inhibit the formation and deteriorate already formed nodules, affecting the N biological fixation (FBN). According to [17], doses above 20 kg of N ha^{-1} can impair nodulation. [18] and [19] found that there is no beneficial effect on the practice of N fertilization in side dressing on soybean yield. According to [20], in certain cases, the effect can be harmful.

The high doses of supplied N inhibited biological fixation, similar to that observed by[21], when testing the effect of N fertilizer and inoculant with *Rhizobium freirei* in different bean cultivars (legumes that perform N biological fixation similarly to soybean culture). Those authors reported a significant reduction in the amount of nodules in treatments in which N was applied.

REVISTA TECNO-LÓGICA ISSN 1982-6753

4 Conclusions

The application of N at doses of 200 and 300 kg ha^{-1}, all at once, at sowing, impairs the development of the crop and the formation of nodules. Inoculation, regardless of the form of application, increases nodule formation and vegetative growth of the crop as a whole. The inoculation carried out inside the seed, in the soil and in the V3 stage reach a better vegetative growth of the plant. Inoculation, regardless of the application method, increases the soybean stem diameter.

NODULAÇÃO E CRESCIMENTO VEGETATIVO EM SOJA SOB DIFERENTES MODOS DE APLICAÇÃO DE INOCULANTE LÍQUIDO

RESUMO: A inoculação é uma das principais práticas culturais em leguminosas como a soja, promovendo economia de recursos em altas doses de nitrogênio mineral. Objetivou-se neste trabalho verificar o efeito de diferentes formas de aplicação de inoculante líquido em soja. O delineamento experimental utilizado foi em blocos casualizados, totalizando 10 tratamentos, com cinco repetições. Os tratamentos foram: aplicação do inoculante líquido sobre as sementes, no solo, aplicação interna às sementes, com o auxílio de uma seringa, além da aplicação foliar nos estádios vegetativos V1, V3, V5 e V8, em adição aos tratamentos com fertilização nitrogenada. Os tratamentos não afetaram o número total de folhas, índice de clorofila e número de nódulos não viáveis. Para altura de planta a inoculação interna à semente, no solo e via foliar em V3 promoveu os melhores resultados. Para área foliar e matéria seca da parte aérea e raiz o uso de nitrogênio mineral prejudicou a cultura, não houve qualquer efeito da inoculação comparado ao tratamento testemunha para essas variáveis. O suprimento de nitrogênio prejudicou o diâmetro de caule e as diferentes formas de inoculação foram superiores à testemunha, exceto a aplicação foliar no estádio V8. Independente da forma, a inoculação causou um aumento no número total de nódulos, sendo superior à testemunha e ao uso de nitrogênio mineral, os quais não diferiram entre si. Apenas a aplicação de nitrogênio mineral comprometeu o número de nódulos viáveis, não havendo diferença da testemunha em relação aos tratamentos com inoculação.

Palavras chaves: Fixação Biológica. Inoculação Foliar. Nitrogênio Mineral. Nódulos Viáveis.

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REVISTA TECNO-LÓGICA ISSN 1982-6753

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