Original Research Article

Complementary application of inoculant in post-emergence on soybean crop

ABSTRACT

Objective: Inoculation is an important agricultural practice in soybean cultivation that guarantees high productivity without the external input of nitrogen. The objective of this study was to evaluate if the complementary of liquid inoculant (*Bradyrhizobiumjaponicum*), via leaf, in different times and different application rates, affects the nodulation, plant growth and soybean productivity.
Study design: A randomized complete block design (RCBD) was used, with four replications, in a 5x3 factorial scheme, evaluating the complementary inoculant application, via foliar (0; 75; 150; 225 e 300 mL ha^{-1}), in three stages of vegetative development of the crop (V2, V4 e V6).
Location and duration of the study: The study was performedin a commercial area, in the municipality of Sinop, in the northern of Mato Grosso State, between October 2017 and February 2018.
Methodology: The cultivation was in soil classified as Red-yellow Latosol with of clayey texture, was in a direct seeding system, using the cultivar NS7901RR. The seeds used were treated and inoculated at sowing, with turfous inoculant and liquid. The experimental units received the complementary applications of inoculant, via pulverization, at 15, 21 and 28 days after emergence, according to the treatment.
Results: It was verify that the different times of application of foliar inoculant, does not condition significant differences to the parameters measured in this study. The complementary inoculant application affected, significantly, the leaf chlorophyll index (P<0.05), as well as the dry matter mass of the plants (P<0.001). Up to 300 mL ha^{-1}, a gradual increase in grain yield was verified.
Conclusion: The complementary supply of liquid inoculant (*B. japonicum*), via foliar, in different rates application, affects the nodulation and development of the plants and the productive yield of the crop.

Keywords: nodulation, rhizobium, biological fixation, inoculation

1. INTRODUCTION

Soybean (*Glycine max* L.), as the main source of protein from plants [1], it has the ability to fix atmospheric nitrogen, through nitrogen symbiotic fixation (NSF) by diazotrophic bacteria. This is a complex process, mediated by the chemical communication between the rhizobium and the plant [2], being from the economic and ecological point of view, considered the most important process, including substituting mineral nitrogen fertilization in the soybean crop[3].

In this context, the inoculation is one of the most important agricultural practices in soybean cultivation, and guarantees high productivity without the external input of nitrogen, improving crop yield with low financial risk [4].

However, despite being adopt frequently, plant nodulation failures routinely occur in the field, especially in first-crop areas [5], where normally the survival of the bacteria is compromised, affecting directly the leguminous [6]. This failure is, according to the same authors [5], because to the low quality of the inoculants used and to factors such as inadequate practices (treatment of seeds with incompatible fungicides, misapplication of seeds inoculation) and unfavorable environmental conditions [7], which adversely affect rhizobium survival and infection.

In view of these prerogatives, the need to define efficient and economic alternatives that guarantee productive yield to the crop, this study was realize to evaluate if the complementary supply of liquid inoculant (*B. japonicum*), via foliar, in different times and doses of application, affects the nodulation and development of the soybean crop.

2. MATERIAL AND METHODS

2.1 Experimental localization and soil characteristics

The experiment was conducted between October 2017 and February 2018, in a commercial area in the municipality of Sinop, in the northern of Mato Grosso state, in the following geographical coordinates: 11° 56'10.84"S, 55° 24'11.24"O, and altitude of 372m. According to Koppen-Geiger, the climate is classified as Aw, showing well-defined seasons, being rainy from October to May and drought from May to September. The average annual precipitation and temperature is 1.818 mm and 25ºC, respectively.

The site soil is classified as Red-Yellow Latosol, with clay texture, presenting the following chemical and physical characteristics: pH = 4.6; P = 8.89 mg dm^{-3}; K = 50.00 mg dm^{-3}; Ca = 1.81 $cmol_c$ dm^{-3}; Mg = 0.20 $cmol_c$ dm^{-3}; H + Al = 4.75 $cmol_c$ dm^{-3}; MO = 18.68 g kg^{-1}; Sand = 341 g kg^{-1}; Silt = 167 g kg^{-1}; Clay = 492 g kg^{-1}.

2.2Experimental design and treatments

The experimental design was a randomized block design, with four replications, in a 5x3 factorial scheme, totaling 15 treatments and 60 experimental units. The treatments were constituted by the complementary application of liquid inoculant (*Bradyrhizobiumjaponicum*) via foliar, at the doses of 0; 75; 150; 225 and 300mL ha^{-1} in three stages of vegetative development of the soybean crop, V2, V4 and V6.

2.3 Implantation and experiment conduction

The cultivation was realized in direct sowing, in October 2017, using cultivar NS 7901RR. Before the sowing, the seeds were treated with insecticide in base of Fipronil of the pyrazole group, and the fungicides Piraclostrobina of the Strobilurin group, and Methyl thiophanate of the benzimidazole group, at the dose of 2 mL kg^{-1} of seed.

On the treatments was used the minimum of $5x10^9$ CFU mg^{-1} turf inoculant (*B. japonicum*), at 60g for each 50 kg of seed and liquid inoculant (*B. japonicum*) of minimum concentration of rhizobium 7.2 x 10^9 CFU mL^{-1}, using the 50mL dose for 50 kg of seeds. The strains used were SEMIA 5079 and 5080.

Before sowing, the area received application of 1.5 kg ha^{-1} of glyphosate aiming at the desiccation of weeds. Thirty days after emergence, a further 1.5 kg ha^{-1} of post-emergent glyphosate (granulate) was applied, through a bar sprayer in order to control invasive plants and reduce interference in soybean plants evaluated at the time of this experiment.

The experimental plots consisted of five cultivation lines, measuring 2.5 x 5m, totaling an area of 12.5m²,beingconsidered useful area (6m³), the three central lines, and scattering 0.5m from each. The sowing density was 14.5 seeds per linear meter, with lines spaced at 0.45m, in order to reach a population of around 300,000 ha^{-1} plants.

Four applications of fungicides were realize during the development of the culture, using products based on the chemical groups of Strobilurin and Triazole. A foliar spray with cobalt and molybdenum was performed, on the doses ofthree and 30 g ha^{-1}, respectively.

The complementary applications of foliar inoculant, evaluated in this research, were carried out at three different times, being the first application at 15 days after emergence - DAE (vegetative stage V2); the second application at 21 DAE (vegetative stage V4); and the third application at 28 DAE (vegetative growth in V6). The treatments with complementary inoculation received the inoculant by spraying with a costal spray, using an application volume of about 200 L of ha^{-1}, with inoculant suspended in water. It was used liquid inoculant (*B. japonicum*) with a minimum concentration of rhizobium 7.2 x 109 CFU/mL of the different doses tested in this study.

2.4Variables analyzed

During the reproductive stage R2 (full flowering), it was realized the evaluation of the nodule number (NODN), dry mass of nodules (DMNOD), dry mass of roots (DMR) and aerial part (DMAP), as well as the chlorophyll content index on leaf (CHLOROPHYLL INDEX) and leaf area of plants (LAP). In the reproductive stage R8 (physiological maturity), the mass of 1000 grains (MTG) and the average yield of the treatments (14% moisture) were measured at the time of harvest.

The number and dry mass of nodules were obtained by removing the root system of two plants in the useful area of each experimental unit. A soil sample of approximately 20 x 20 x 20cm was collected, with careful to not damage the root system. In the soil laboratory, the roots were separated and washed. Then, the nodules were separated from the roots, counted and conditioning in paper bags, as well as the roots and aerial part of the collected plants. After drying, in a forced circulation oven at 65ºC until constant mass, the respective values of dry mass of the nodules and root dry mass and aerial part were determined. Chlorophyll contentwere indirectly determined using a portable chlorophyll meter (model CFL-1030), in the medium region of each plant, in the full flowering stage (R2). The leaf area of plants collected in each treatment (cm²) was determined with a leaf area integrator (model LI-3010).

After the plants reached physiological maturity (R8) at 111 DAE, the useful plots were manually collected, in order to determine the grain yield of the different evaluated treatments (14% moisture). The mass of 1000 grains was determined by counting and weighing the samples.

2.5 Statistical analysis of data

The results were submitted to analysis of variance, at the 5% probability level by the F test. For the inoculant doses, regression was used and the R^2 value in the explanatory potential of the models. For the qualitative variables, the Tukey test was applied at 5% probability.

3. RESULTS

Table 1 presents the mean values and analysis of variance for the chlorophyll index, dry mass (aerial and root) and leaf area of soybean plants submitted to different inoculant doses (0, 75, 150, 225 and 300 mL ha^{-1}), in three vegetative stages (V2, V4 and V6).

Table 1. Mean values and variance analysis of the application of different doses of inoculant in three vegetative stages on soybean plants

Treatments	Chlorophyll index	DMAP (g $planta^{-1}$)	DMR (g $planta^{-1}$)	LAP (cm^2)
Period of application				
V2	48.77a	36.94a	6.27a	1,866.70a
V4	48.88a	38.12a	6.10a	1,893.12a
V6	49.36a	34.30b	5.97a	1,924.41a
Inoculant doses				
0 mL ha^{-1}	48.04b	33.45b	5.70a	1,654.11a
75 mL ha^{-1}	49.07ab	37.69a	6.07a	1,835.23a
150 mL ha^{-1}	49.57a	33.35b	6.07a	2,035.80a
225 mL ha^{-1}	49.24a	40.81a	6.34a	2,005.12a
300 mL ha^{-1}	49.13a	37.01ab	6.37a	1,943.45a
Test F				
Periods (P)	2.35	7.01*	1.17	0.10
Doses (D)	4.64*	10.90**	2.23	1.83
P x D	7.44**	8.80**	7.23**	0.58
Block	0.93	0.15	0.31	0.42
CV (%)	1.88	9.04	10.16	20.88

* *P*< 0.05; ** *P*< 0.001by test F; CV = coefficient of variation. Means followed by the same letter is not different by Tukey test. DMAP–dry mass of aerial part; DMR – dry mass of roots; LAP – leaf area of plants.

It can be verified that the different times of inoculant foliar application, in a complementary form of seed inoculation, did not possibility significant differences to the parameters measured by this study. However, it was observed that the aerial dry mass was higher when applied at 15 and 21 DAE, V2 and V4, respectively (*P*<0.05), presenting a decreasing behavior from these stages of development (Table 1). The treatments with complementary application of inoculant, through leaf applicationaffected, significantly, the leaf chlorophyll index (*P*<0.05), as well as the dry matter mass of plants (*P*<0.001).

In a different way, the application of the complementary doses of inoculant, did not influence the mass of the root system and leaf area of the soybean plants, under the conditions of this experiment. However, we observed a quadratic response for the leaf area variable, presenting an increase up to the maximum response dose of 204 mL ha^{-1}, (Figure 1B).

Figure 1 presents the chlorophyll index in the foliar part and the leaf area of soybean plants, in function of five doses of inoculant applied via foliar, in post-emergence, in the soybean crop. The average contents of leaf chlorophyll measured, in this experiment, were close to 49.01. The time of inoculant application did not interfere in these indexes, presenting mean values with low variation between the treatments. However, with the change in the application dose, we detected a growing and quadratic response to the chlorophyll index, so that, the maximum response dose was 185 mL ha^{-1} (Figure 1A). From the dose 185 mL ha^{-1}, a negative influence of the inoculant dose increase was observed, which led to a reduction in the chlorophyll content in the leaf (still superior to the treatment without complementary application), regardless of the application.

Similar responses was observed for leaf area of plants, a quadratic and increasing response was verified, so that the highest mean was obtained with the estimated dose of 204 mL ha^{-1} of inoculant (Figure 1B). As observed in the chlorophyll index in the leaf, the leaf area presents a decrease after the dose mentioned, showing a negative influence of the increase in the supplemental dose of inoculant.

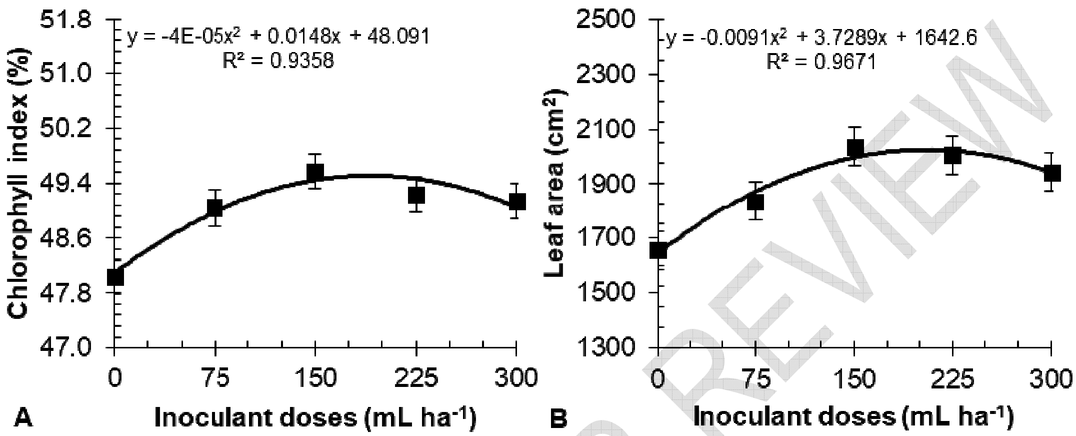

Fig. 1. Effect of different complementary doses of inoculant, via leaf, in the chlorophyll index (A) and foliar area (B), of soybean plants

In relation to the dry mass of the aerial and root parts, different behavior was observed, depending on the application period and the doses used. While the period had an effect on DMAP, higher when application occurred at 15 and 21 days after emergence, V2 and V4, respectively. This factor did not influence DMR. In contrast, as can be seen in figure 2, the dose of inoculant applied in complementary to seed inoculation, influenced the root mass (Figure 2A), but, showing low effect on the shoot mass of the plants (Figure 2B). There was a linear effect of the dose on the root mass of the plants, where the increase of the dose conditioned increment in this variable until the dose of 300 mL ha^{-1}.

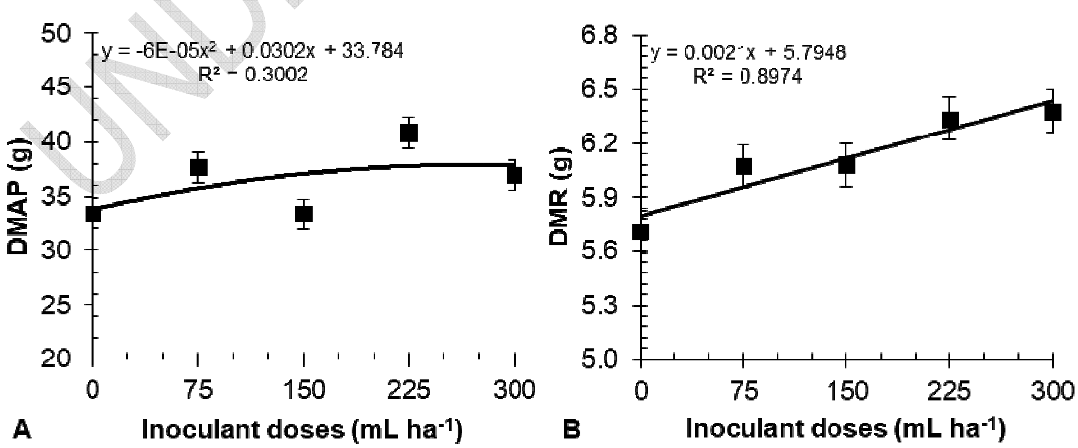

Fig. 2. Effect of different complementary doses of inoculant, via leaf, in the dry mass of aerial part (A) and dry mass of roots (B), in soybean plants

DMAP – dry mass of aerial part; DMR – dry mass of roots.

In the table 2 are the statistical analysis of the number and dry mass of nodules in the flowering, mass of 1000 grains and the average yield of grains in relation about the times and different doses of complementary application of liquid inoculant, via foliar. The time of supply of the liquid inoculant, in isolation, hadn't effect on the number of nodules and dry mass of nodules per plant. Likewise, the mass of 1000 grains and average productivity in each treatment were not significantly affected by the variation in the vegetative stages of application, until V6.

Table 2.Mean values and variance analysis of the application of different doses of inoculant in three vegetative stages on soybean plants

Treatment	NODN ($plant^{-1}$)	DMNOD (g)	MTG (g)	Productivity ($kg\ ha^{-1}$)
Period of application				
V2	171.92a	1.09a	196.12a	4,204.77a
V4	174.34a	1.03a	191.86a	4,224.04a
V6	176.08a	1.07a	200.51a	4,309.47a
Inoculant doses				
0 mL ha^{-1}	150.08b	0.94a	192.15a	3,843.52c
75 mL ha^{-1}	166.55ab	0.97a	194.15a	4,146.34bc
150 mL ha^{-1}	181.41a	1.12a	195.48a	4,271.23ab
225 mL ha^{-1}	181.25a	1.12a	199.33a	4,386.48ab
300 mL ha^{-1}	191.25a	1.17a	199.69a	4,582.89a
Test F				
Periods (P)	0.12	0.45	1.41	0.66
Doses (D)	4.38*	3.09*	0.48	9.87**
P x D	7.60**	3.64*	0.88	2.23*
Block	2.66	0.74	2.19	4.40*
CV (%)	15.27	19.00	8.30	7.19

* P< 0.05; ** P< 0.001 by test F; CV = coefficient of variation. Means followed by the same letter is not different by Tukey test. NODN – number of nodules; DMNOD – dry mass of nodules; MTG – mass of 1000 grains.

About the complementary application of inoculant, up to the dose of 300 mL ha^{-1}, no change was observed in the mass of 1000 grains of the cultivar used in this experiment. Nevertheless, it was verify a tendency to increase as the dose of inoculant rises, with increasing linear behavior.

As observed in the analysis of variance (Table 2), the number and mass of nodules per plant was altered by the applied doses (P<0.05), in a similar manner, there was a significant difference in crop productivity (P<0.05), indicating variability among treatments. The grain yield followed similar tendency to of the 1000 grain mass, for this, the treatments with complementary application of inoculant were expressively superior to the control treatment, only inoculated on the sowing.

Figure 3 presents the effects of treatments with different complementary doses of inoculant, on the number and dry mass of nodules. In summary, there were higher results in both variables when the dose of inoculant sprayed was 300 mL ha^{-1}, gradually increasing with the inoculant dose elevation (Figure 3, A and B). An increase about 21% in the number of nodules and 20% in the mass of nodules was verified with the application of the highest dose of inoculant, in relation to the control (without application). Positive correlation between number of nodules and grain yield (R^2 = 0.95) was detected in these treatments. It can be concluded that the higher number of nodules per plant conditions an increase in productivity in the crop, highly dependent on BNF.

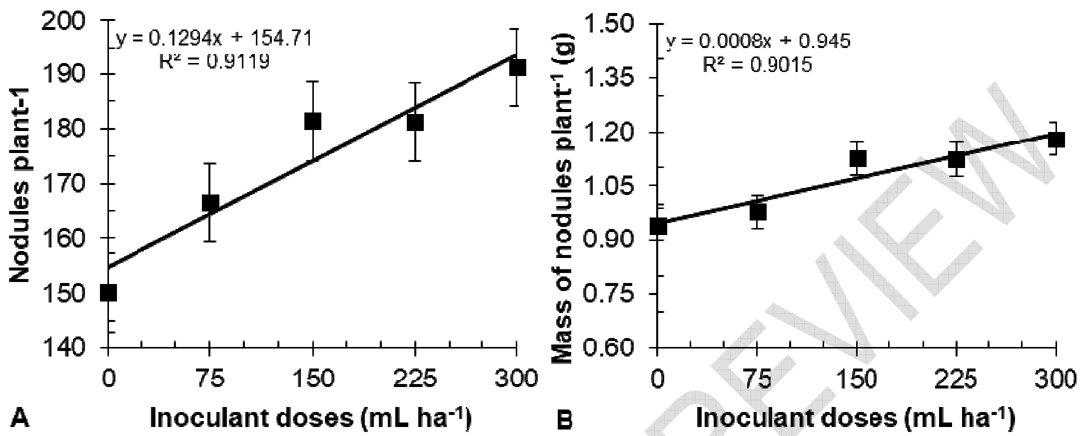

Fig. 3. Effect of different complementary doses of inoculant, via leaf, in the number of nodules per plant (A) and dry mass of nodules (B), in soybean plants

In Figure 4, is presented the average yield of grains and the mass of 1000 grains of the respective treatments, in function of the doses. In all proposed treatments, the yields of grains and the mass of 1000 grains, under complementary inoculation, had an increasing linear behavior. Mean grain yield was positively correlated with the inoculant dose (R^2 = 0.9662), verifying until the dose of 300 mL ha^{-1}, a gradual increase in grain yield (Figure 4A) and a mass of 1000 grains (Figure 4B), although for the last variable, the statistical difference wasn't verified. Increment of slightly more than 16% in productivity was verified with application of 300 mL ha^{-1} of inoculant in cover, in relation to the treatment without inoculation.

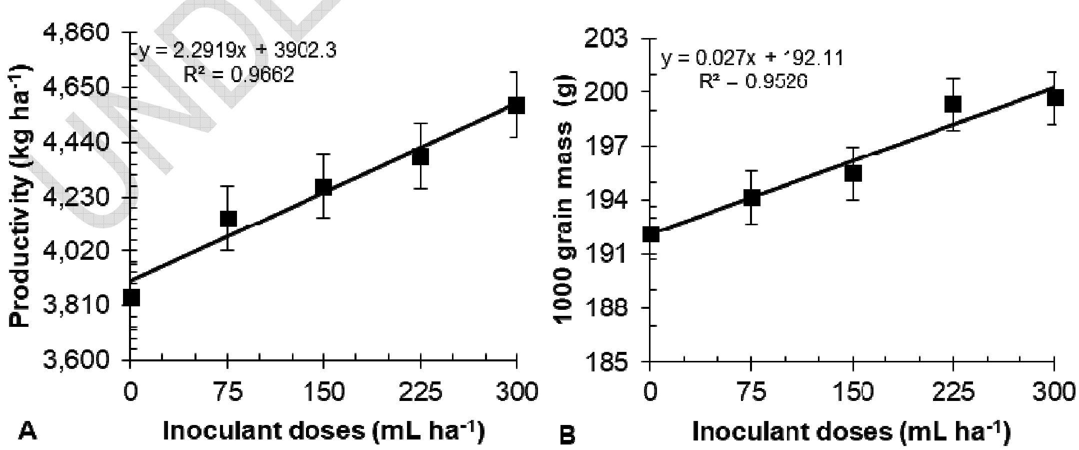

Fig. 4. Effect of different complementary doses of inoculant, via leaf, in the productivity (A) and mass of 1000 grains (B), in soybean plants

As observed in the analysis of variance (Tables 1 and 2), the interaction between period of application and the inoculant dose was significant for some parameters measured. The unfolding of such interactions is shown in table 3.The chlorophyll index in the leaf was one of the variables influenced by the interaction of the factors (*P* <0.001). With the unfolding of the analysis, it was observed that, depending on the dose of inoculant, different responses were obtained in each period. In general, higher values were verified with application in V6 (in the dose of 150 mL ha^{-1}) and V2 (in the dose of 225 mL ha^{-1}), although very close to the other treatments. The lowest chlorophyll indexeswere observed in the treatment without inoculant application. About the dry mass of the aerial part, also affected significantly by the interaction of the factors (*P*<0.001), it was verified increases when applied in the first period (V2), however, a higher dry mass was observed in plants submitted to 225 mL ha^{-1}, in V4 (44.77 g $plant^{-1}$).

For dry mass and number of nodules, the interaction between the period of application and the applied doses, presented similar responses (*P*<0.001 and *P*<0.05, respectively). In general, low change was observed, in each dose, with variation at the period of application. However, higher results are observed with V4 and V6 applications, mainly when using the higher dose (300 mL ha^{-1}).

Table 3.Analysis of the interactions of chlorophyll index, dry mass of aerial part, number of nodules and dry mass of nodules, and the application of different doses of inoculant in three vegetative stages on soybean plants

Period of application	Dose of Inoculant				
	----------Chlorophyll index----------				
	0 mL ha^{-1}	**75 mL ha^{-1}**	**150 mL ha^{-1}**	**225 mL ha^{-1}**	**300 mL ha^{-1}**
V2	46.98bC	49.01bB	48.77bB	50.96aA	48.12bB
V4	49.55aA	48.19abA	49.32abA	47.88bA	49.49abA
V6	47.59bB	49.94aA	50.63aA	48.86bA	49.82aA
	----------Dry mass of aerial part (g $plant^{-1}$)----------				
	0 mL ha^{-1}	**75 mL ha^{-1}**	**150 mL ha^{-1}**	**225 mL ha^{-1}**	**300 mL ha^{-1}**
V2	25.96cB	38.37abA	36.57aA	42.53aA	41.25aA
V4	40.37aA	40.82aA	31.59aB	44.77aA	33.06bB
V6	33.96bA	33.87bA	31.90aA	35.11bA	36.68abA
	----------Number of nodules per $plant^{-1}$----------				
	0 mL ha^{-1}	**75 mL ha^{-1}**	**150 mL ha^{-1}**	**225 mL ha^{-1}**	**300 mL ha^{-1}**
V2	153.25aB	164.67abB	205.25aA	192.11aA	144.33bB
V4	138.50aB	206.25aA	129.50bB	179.78aA	217.67aA
V6	158.50aB	128.75bB	209.50aA	171.89aB	211.75aA
	----------Mass nodules per $plant^{-1}$----------				
	0 mL ha^{-1}	**75 mL ha^{-1}**	**150 mL ha^{-1}**	**225 mL ha^{-1}**	**300 mL ha^{-1}**
V2	1.08aA	1.13aA	1.08abA	1.07aA	1.13aA
V4	0.78aA	1.15aA	0.95bA	1.17aA	1.13aA
V6	0.98aA	0.65bB	1.35aA	1.13aA	1.28aA

Means followed by the same letter, lowercase in the column and uppercase in the line, is not different by Tukey test.

In the figure 5 it can be observed the results for productivity and dry mass of roots. In relation about grain yield, different responses were verified in function of the vegetative stage of application of the inoculant (Figure 5A). Observing an increasing linear behavior in the periods of V2 and V6, in relation to productive yield, and gradual increase with the dose of inoculant applied. Low variation is observed for these periods of application. In V4, the productivity response had quadratic behavior, with low variation between the doses. Possibly this response occurred in function of the availability of water at the time and the pulverization after realized, compromising infection and formation of nodules.

For the dry mass of the root system (Figure 5B), different responses were observed with the variation of the applied inoculant dose, in relation to the different vegetative spray stages. Similar to the results observed for grain yield, in which the application occurred in V2, increased, gradually, in root mass, demonstrating linear behavior (R^2 = .9081). In this condition, the higher explored volume of soil and the higher nodulation, the productive yield was also favored. For this variable, quadratic responses were verified with applications in V4, same observed behavior about the productivity. However, with application in V6, dose the response in function of the doses was different in relation what was observed in productive terms, indicating low variation between treatments.

In general, variations in detriment of the dose of inoculant in cover are observed, and based on the presented figures, application in the vegetative stage V2 presents promising results, without statistic differences between the treatments.

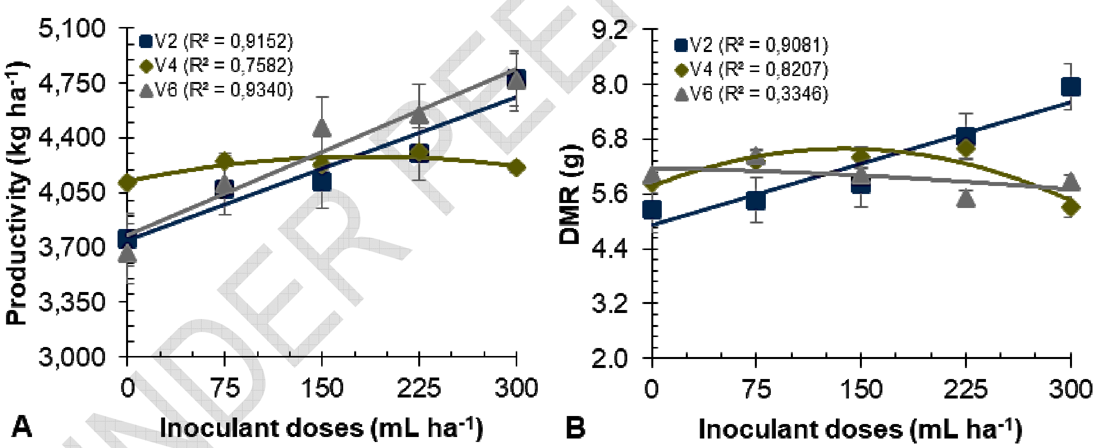

Fig. 5. Effect of different complementary doses of inoculant, via leaf, in the productivity (A) and dry mass of root part (B), in soybean plants, in function of inoculant application in three vegetative stages of the culture (V2, V4 and V6)

DMR = dry mass of root part

4. DISCUSSION

We verified that the application of inoculant, via foliar, independent of the vegetative stage of the culture, can raise the formation of nodules and the mass of the root system of the soybean plants. These variables correlate positively with the average yield of grains. So, we verified that the complementary application of liquid inoculant via foliar provides increases in soybean productivity. These results are corroborate those observed by Pereira et al. [8], in a research evaluating different doses of inoculant in soybean cultivars, in which was verified that the productivity correlated with the formation of nodules and dry mass, showing increasing as a function of inoculant dose.

In the literature, there is low research about coverage inoculations. The researchers are usually directed to the study of inoculation, only in sowing, via seed, or via planting row, but not in cover, including in the inter-row, like in the present study. However, there is a need to conduct this research, because the traditional application, via seed, is not always efficient [5], mainly by the joint application of *Bradyrhizobium* with fungicides, insecticides and micronutrients, as already highlighted by Vargas and Suhet [9],they contribute to toxicity to bacteria, promoting, sometimes, irreversible damage to nodulation.

In relation to the mass of 1000 grains, variable that directly affects the crop yield, it can be observed in the control, lower results in comparation with the treatments with complementary inoculations in cover, evidencing the influence of N fixation on the grain. Thus, it is evident the importance of biological nitrogen fixation on the formation and grain filling, conditioning higher means in the treatments with inoculations in coverage. Possibly, these results were occur because the higher nodulation of the plants, like was verified in the results of this variable. In this case, increasing of rhizobium populations in the soil, it can be observed longer nodulation when compared to inoculation only via seed [10], providing nitrogen in the critical period of demand for this nutrient by the plant [11]. Corroborating these claims, some results in the literature support that coverage inoculation still provides a significant increase in nodule numbers over time, like what was observed by Zilli et al. [5], in measurements realized at 35 DAE and 45 DAE, where was found increase in nodulation with application of cover inoculant (at 15 DAE).

Such increases in nodulation of plants provided differences between the control and the plants with complementary inoculation, regarding the chlorophyll index and leaf area of plants. From the observed results, it can be observed that the index of chlorophyll varies as a function of the leaf area of the plants. It was evidenced that the highest chlorophyll index was obtained at the point of maximum leaf area, showing a tendency to fall from the application of 150 mL ha-1 of inoculant. Due to the effect of nitrogen dilution on plant tissues, as indicated Adell et al. [12], a more pronounced reduction in chlorophyll index can be observed with increasing inoculant dose, increased leaf area and dry mass of plant shoots, variables that had an increase in their values with the increase in inoculant doses.

As mentioned in the research realized by Zilli et al. [5], the strategy of inoculation in soybean crops, by spraying on cover, is not usually recommended, although it is empirically used by farmers, mainly when there is a lack of plant nodulation in the crop and nitrogen deficiency. The results presented after this study confirm the efficiency empirically observed of the complementary inoculant application in post emergence of soybean plants.

In general, regardless of the period of the complementary application (up to V6), soybean plants respond to cover inoculations, however, as several authors have stated [5] [13] [14], there is a need for:high soil moisture, precipitation or irrigation that can favor the contact of the bacterium with the root system of the legume.

However, these same authors [5], in an experiment conducted with nitrogen in cover, inoculation via seed and inoculation in cover to the 14 DAE, verified that inoculation in one dose in the cover, shouldn't be a practice to substitute the traditional inoculation in the seeds, however, that the best result occurred with the standard inoculation. Thus, this practice may be viable as a complementary method, mainly in areas of first soybean cultivation and in soils with low organic matter content.

The observed increase in dry mass and number of nodules is also highlighted in research conducted in Thailand, with a method of inoculation in post emergence, on the soybean planting line. The inoculant application, up to 15 days after planting, resulted in increased nodulation, dry matter mass of the plants and grain yields, significantly equal to those provided by inoculation at planting [14]. These results corroborate those obtained in Australia, with inoculation by irrigation water [13]. In both studies, a significant contribution of post-emergence inoculation to plant nodulation was reported in comparison to treatment without inoculation.

These results of increase in productivity, resulting from the complementary application of inoculant, via foliar, is due to a good association and biological fixation of nitrogen, by the bacterial strains studied. In a complementary manner, the best responses obtained with the V4 application (aerial part dry matter), were due to the appropriate conditions during the development of the crop, such as temperature, pH, water availability, oxygen, nutrients in the soil, among others determinants of nodulation, contributed to the results found.

The best response of the plants to the inoculant to occur when the bacteria are spatially close to the root system of the seedlings, in the first weeks of development. At this stage, the rhizobium captures the molecular signals of the plant and infects the root hairs, which culminates in the formation of nodules [15] [16]. Thus, it was concluded that the cover application was efficient and showed to be able to promote the interaction of the pulverized inoculums and the root system of the plants, increasing the number and mass of nodules in these treatments.

With the cover inoculation, it is observed that the most of the nodules is formed in the secondary roots of the plants, just below the soil surface. This probably occurs because in this root zone there is higher cellular differentiation for root development at the time of inoculation, which allows the exchange of molecular signals with the bacterium [14]. Probably, it was also in this position of the soil profile where the bacteria was concentrated after applied by pulverization.

The dilution of the inoculant in the water, for post-emergence application under soybean cultivation, possibly improved the distribution of rhizobium on the soil surface, which favored by the adequate humidity, promoted an efficient infection of the roots of the plants, especially those more superficial, found in the first layers of soil. It should be noted that higher doses, besides providing a larger number of *B.japonicum* cells, near the adventitious roots [8], promoted an increase in nodulation, and consequently, superior results in productivity, as well as other agronomic characters presented above. Thus, the observed increases in productivity are closely correlated with nodule formation.

In the same way, we verified in this research, that the supply of liquid inoculant in cover is an alternative for higher yields of soybean in soils already inoculated.

5. CONCLUSION

The complementary supply of liquid inoculant (*B. japonicum*), via foliar, in different doses, increases the formation of nodules and consequently the growth and yield of the soybean.The application of leaf inoculant, independent of the vegetative stage of the soybean, increases the nodule formation and the mass of the root part, variables that correlate positively with the average yield of grains.

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