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CONVENTIONAL INOCULANTS AND BIOLOGICAL PROTECTOR, CO-INOCULATION AND NITROGEN FERTILIZATION IN SOYBEAN

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ABSTRACT - There is little information that supports the new technology of inoculants with biological protectors and indecates the results in soybean crop. In order to evaluate the morphophysiological responses of soybean crop to conventional inoculants, inoculants with protectors, co-inculation with *Azospirillum brasilense* and at increasing doses of N. The treatments consisted of: Control; *Bradyrhizobium elkanii* + biological protector; *Bradhyrizobium elkanii* + *Bradhyrizobium japonicum* + biological protector; *Bradyrhizobium elkanii*; *Bradyrhizobium elkanii* + co-inoculation with *Azospirillum brasilense; Bradyrhizobium elkanii* + *biological protector* + co-inoculation with *Azospirillum brasilense*; *Bradyrhizobium elkanii* + *Bacillus amyloliquefaciens* + biological protector; Dose 20 kg ha^{-1} of N; Dose N 40 kg ha^{-1} and Dose N 60 kg ha^{-1} of N. Nodulation, chlorophyll *a*, *b* and total content, nitrogen content in leaf tissue and grains were evaluated thousand grain mass and productivity were evaluated. The biological agents tested in the experiment provided an increase in the number of nodules and an increase in the mass of 1,000 grains when compared nitrogen fertilization. The treatments with nitrogen fertilization, compared to the treatments with inoculation, were higher for chlorophyll *a*, *b* and total contents. Long-life inoculants, in the absence and presence of coinoculation in seed treatment, demonstrated efficiency in number of nodules and in the supply of N in soybean crop, with superior responses on the non-inoculated control, even in soil with initial concentration 1,510 10^{-4} bacteria cells per gram of soil. Long-life inoculants offer greater practicality to the rural producer and demonstrated efficiency in the supply of N to soybean crop.
Keywords: *Glycine max* (L.) Merril., inoculation, *Azospirillum, Bradyrhizobium*.

INOCULANTES CONVENCIONAIS E PROTETORES BIOLÓGICOS, COINOCULAÇÃO E ADUBAÇÃO NITROGENADA NA CULTURA DA SOJA

RESUMO - São escassas as informações que dão suporte a nova tecnologia de inoculantes com protetores biológicos e evidenciam os resultados na cultura da soja. Objetivou-se avaliar as respostas morfofisiológicas da cultura da soja à inoculantes convencionais, inoculantes com protetores, coinoculção com *Azospirillum brasilense* e à doses crescentes de N. Os tratamentos consistiram em: testemunha, *Bradyrhizobium elkanii* + protetor biológico, *Bradhyrizobium elkanii* + *Bradhyrizobium japonicum* + protetor biológico, *Bradyrhizobium elkanii*, *Bradyrhizobium elkanii* + coinoculação com *Azospirillum brasilense*, *Bradyrhizobium elkanii* + protetor biológico + coinoculação com *Azospirillum brasilense*, *Bradyrhizobium elkanii* + *Bacillus amyloliquefaciens* + protetor biológico, 20 kg ha^{-1} de nitrogênio (N), 40 kg ha^{-1} de N e 60 kg ha^{-1} de N. Foram avaliadas a nodulação, teor de clorofila *a*, *b* e total, teor de nitrogênio em tecido foliar e grãos, massa de mil grãos e produtividade. Os agentes biológicos testados no experimento proporcionaram aumento número de nódulos e aumento na massa de mil grãos, quando comparados à fertilização nitrogenada. Os tratamentos com adubação nitrogenada, em comparação aos tratamentos com inoculação, foram superiores para teores de clorofila *a*, *b* e total. Os inoculantes longa vida, na ausência e presença de coinoculação em tratamento de sementes, demonstraram eficiência em número de nódulos e fornecimento de N na cultura da soja, com respostas superiores sobre a testemunha não inoculada, mesmo em solo com concentração inicial 1,510 10^{-4} células de bactérias por grama de solo. Inoculantes longa vida oferecem maior praticidade ao produtor rural e demonstraram eficiência no fornecimento de N à cultura da soja.
Palavras-chave: *Glycine max* (L.) Merril., inoculação, *Azospirillum, Bradyrhizobium*.

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INTRODUCTION

The dynamics of nitrogen (N) in the environment makes the process of nitrogen fertilization a challenge in obtaining high productivity in Brazilian agriculture (SOUZA CÂMARA, 2014). Currently, incomes of up to 5.000 kg ha^{-1} occur in soybean crops in different regions of the country and require high demand of this nutrient for the crop (PEREIRA et al., 2016).

The nitrogen (N) supply in the soil is carried out by the decomposition of soil organic matter (SOM), by electrical discharges of lightning, nitrogen fertilizers and atmospheric nitrogen biological fixation (NBF) process (SILVA et al., 2018). The N reservoir present in SOM is limited with rapid depletion in crops, and electrical discharges occur in an uneven manner, with consequent variability in glebes (SIQUEIRA NETO et al., 2010). Similarly, NBF's ability to meet N requirements has been questioned for high yields in soybeans, in order to induce that the crop should receive additional mineral nitrogen fertilization, in order to supply the supposed deficiency of the symbiont system (FONTOURA et al., 2015).

To perform inoculation at the time of sowing, the scarcity of labor required and the lack of technical knowledge, demand for new technologies that increase the use and efficiency of inoculants (SCHNEIDER et al., 2017). In this sense, there is a need for inoculants that can remain in the seeds a few weeks before cultivation. Technologies such as the use of biological protectors used in industrial seed treatments, become alternative solutions as they reduce the risk to operators and decreases the deleterious effects of fungicides and insecticides on bacteria of the genus *Bradyrhizobium* (MERTZ et al., 2009).

In order to achieve high productivity of soybean crop and raise the productive level of the region, the use of microorganisms to improve and increase the use of N, is one of the technological measures that has been evidenced in agricultural experimentation works (MUNDIM et al., 2018). Among these microorganisms, *Azospirillum brasilense* is one of the most studied, because, in addition to providing NBF in grasses, it is able to increase growth-promoting hormones in plants, and stimulate production of natural defenses in some species (BULEGON et al., 2015).

Oxidative enzymes and contents of photosynthetic pigments, which are destroyed under water deficit, can be increased in crops by inoculation with *Azospirillum brasilense* (BULEGON et al., 2016). When comparing *Azospirillum brasilense* with *Herbaspirillum sp.*, Radwan et al. (2004), concluded that *Azospirillum brasilense* proved to be much more efficient in the production of growth-promoting metabolites such as indoes and auxins in rice and corn crops.

When comparing the treatment of soybean seeds, with five doses of *Azospirillum brasilense* (0, 0.5, 1.0, 1.5 and 2.0 mL kg^{-1}) and two doses of *Bradhyrizobium japonicum* (0 and 3.0 mL kg^{-1}) in a completely randomized design in pots grown in greenhouse, Zuffo et al. (2015), found no significant responses to plant height, chlorophyll and nitrogen content in leaf tissue. Another study showed a positive response in the productive performance of the BMX Turbo genotype, in response to coinoculation of *B. japonicum* with *A. brasilense* (BULEGON et al., 2015).

Rios et al. (2018), when studying the effects of inoculant doses of 0, 2.5, 5, 10, 15 and 20 mL 100 kg^{-1} of seeds of *Bacillus amyloliqufaciens* (CEPA MBI 600^{-1}) seed treatment in soybean crop, in four municipalities in northern Paraná, found positive effects on production components such as: stand dry mass, shoot dry mass, number of nodules and productivity. Research with doses of 0, 10, 20 and 40 kg ha^{-1} N and two doses of *Bradhyrizobium japonicum* (3.0 and 6.0 mL kg^{-1}) and its effects on soybean crop, showed that there was no significant difference for productivity in dystrophic Yellow Red Latosol, in first cultivation area (SILVA et al., 2011).

Little are the studies that seek to associate two different genera of NBF, formulations and presence and absence of biological protectors, in soybean crops compared to increasing doses of N. In this context, inoculation and coinoculation of soybean crop deserve attention because in addition to the supply of N to the plant, the plant-microorganism interaction can lead to increases in productivity and greater resistance of plants to biotic and abiotic stresses. In view of the above, the objective of this study was to verify the effects of commercial inoculants available in the market, with and without biological protectors, coinoculation and increasing doses of N on leaf and grain nitrogen contents, chlorophyll content and soybean crop yield.

MATERIAL AND METHODS

The present work was carried out in the field from November to February, of the agricultural year 2016/2017, at the Agro Schimi Experimental Station - Agronomic Research and Consulting, in Corbelia (PR). The Experimental Station is located under geographic coordinates of 24°48'30,32"S and 53°17'03,52"O at 637 m. The climate of the region, according to Köppen, is *Cfa*, with warm summer and tendency to concentration of rains, winter of infrequent frosts without defined season, the average annual precipitation is around 1,500 mm, with average temperature above 20ºC in the summer and below 18ºC in the winter (IAPAR, 2017). Rainfall was monitored throughout the experiment (Figure 1).

The experiment was conducted in gleba with slope of 1.5%, with no-tillage for more than 10 years and in a rotation system of soybean crops in summer and wheat, second crop corn and black oat in winter, fertilized exclusively with mineral fertilizers, according to the needs of crops. At the sowing of the experiment, the soil cover consisted of corn residue second crop. The soil was classified as typical Dystrophic Red Latosol (EMBRAPA,

2013). Initially, soil sampling was performed at a depth of 0-0.20 m, with the samples forwarded to the chemical analysis laboratory of the Unithal (Campinas, SP). The data from the chemical analyses are presented in Table 1.

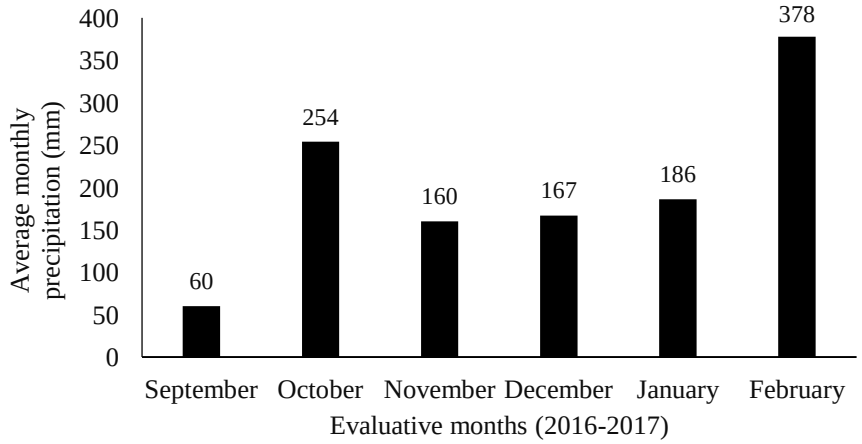

FIGURE 1 - Average monthly precipitation (mm) occurred during the experimental evaluation months (September/2016 to February/2017).

TABLE 1 - Chemical attributes of the soil before the installation of the experiment. Agro Schimi Experimental Station, Corbelia (PR).

Depth	P	OM	pH $CaCl_2$	Ca^{+2}	Mg^{+2}	K^{+}	H + Al	Al^{+3}	CTC	SB	Ca/Mg
m	mg dm^{-3}	g dm^{-3}		---------- $cmol_c$ dm^{-3} ----------							
0 – 0.20	5.00	4.80	4.10	3.30	0.90	0.10	8.80	0.90	13.10	4.30	3.70

Depth	V	Al	Ca	Mg	K	$S\text{-}SO_4^{-2}$	B	Mn	Zn	Cu	Fe
		% saturation in the CTC									
m	---------- % ----------					---------- mg dm^{-3} ----------					
0 – 0.20	32.82	6.90	25.20	6.90	0.80	18.60	0.40	6.00	2.00	3.00	48.00

P (phosphorus), K^{+} (potassium), Cu (copper), Zn (zinc), Fe (iron) and Mn (manganese) = $Mehlich^{-1}$, Ca^{2+} (calcium), Mg^{2+} (magnesium) and Al^{3+} (aluminium) = KCl (potassium chloride), OM (organic matter) = Walkey Black, pH = calcium chloride ($CaCl_2$), H + Al = buffer SMP (Shoemaker, Mac lean and Pratt), $S(SO_4)^{-2}$ = phosphate monocalcium, B = barium chloride ($BaCl_2$) (LANA et al., 2016).

The amount of bacteria in the soil of the experiment at the time of sowing was quantified in 1,510 x 10^{-4} bacteria cells/g of soil. The determination was carried out in the Technology Prospecting and Evaluation Sector (TPES) of EMBRAPA soybean, Londrina (PR). The methodology used for this determination is the one in the Analytical Methods approved in the normative instruction, by means of the most likely number of cells method, which consists of inoculation of serial dilutions of the samples, in specific testing plants (such as soybean), grown under aseptic conditions, with evaluation of the formation of nodules.

The experimental design used was randomized blocks, containing 4 replications. The experimental plots measured 3.5 m wide by 10 m in length (35 m^2). The treatments used are described in Table 2, with the appropriate products applied and concentrations of bacteria. At the time of sowing, the repetitions were homogenized, generating a sample composed of 6 kg, used in sowing. The supply of 2 g ha^{-1} cobalt (Co) a.i. and seed treatment with Fipronil (25 g ha^{-1} of i.a.) + Piraclostobine (2.5 g ha^{-1} of i.a.) + Thiophanate - methyl (2.25 g ha^{-1} of i.a.), were held on September 5, 2016. The soybean cultivar used was Pioneer 95Y52, in spacing of 0.50 m and population of 14.5 seeds per linear meter, obtaining a stand of 13 plants per meter.

The T2 treatment was performed on September 23, 2016 and stored in the absence of luminosity and average temperature of 21°C. Treatments T3 and T7 were performed on October 31, 2016 and stored in the same conditions of luminosity and average temperature as T2. Sowing was performed on November 7, 2016, therefore, T2 was

performed 40 days before sowing and T3 and T7, 8 days before sowing. Treatments T4 and T5 were performed at the time of sowing. T6 treatment was performed with the leftover T2 samples, receiving application of *Azospirillum brasilense* only at the time of sowing.

Treatments T8, T9 and T10 received 50% of the n dose in urea form at the time of sowing and the remainder 27 days after emergence. The application was performed manually in the plots and, at the time of sowing, the application of N was located, approximately 0.02 m from the seeds.

TABLE 2 - Description of treatments, with the appropriate products applied and concentrations of bacteria.

Treatments	Products applied	Concentration of Bacteria	Protector in ST	Amount of N (kg ha^{-1})
T1	Witness	-	Out	-
T2	*Be* + protective	5 x 10^9 UFC mL^{-1}	S30®	-
T3	*Be* + *Bj* + protective	5 x 10^9 UFC g^{-1}, 5 x 109 UFC g^{-1}	Present	-
T4	*Be* liquid	5 x 10^9 UFC mL^{-1}	Out	-
T5	*Be* + *Azo*	5 x 10^9 UFC mL^{-1}, 2 x 10^8 UFC mL^{-1}	Out	-
T6	*Be* liquid + peaty + *Azo*	5 x 10^9 UFC mL^{-1}, 2 x 10^8 UFC mL^{-1}	S30®	-
T7	*Be* + *Ba* + protective	5 x 10^9 UFC, 2 x 10^{10} UFC mL^{-1}	Extender®	-
T8	20 kg of N	-	Out	20
T9	40 kg of N	-	Out	40
T10	60 kg of N	-	Out	60

T1 = absence of inoculation and nitrogen (N) in cover, T2 = 2 mL kg^{-1} of *Be* (*Bradyrhizobium elkanii* SEMIA 587 and SEMIA 5019) + 3 mL kg^{-1} protective S30® (polymer concentrate) + 1.2 g kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019, T3 = 2 mL kg^{-1} of *B. elkanii* SEMIA 5019 + *Bj* (*B. japonicum* SEMIA 5079) + biological protectors not specified by the company, T4 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 liquid, T5 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + coinoculation with 0.83 mL kg^{-1} of *Azo* (*Azospirillum brasilense*), strains Abv5 and ABv6, T6 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + 3 mL kg^{-1} protective S30® (polymer concentrate) + 1.2 g kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + coinoculation with 0.83 mL kg^{-1} of *Azo* (*Azospirillum brasilense*), strains Abv5 and ABv6, T7 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + 0.1 mL kg^{-1} of *Ba* (*Bacillus amyloliquefaciens* CEPA MBI 600) + 0.6 mL kg^{-1} protective Extender® (polymer concentrate). ST = seed treatment.

The applications of phosphorus (P), calcium (Ca) and sulfur (S) were performed with a 7-line seeder, spaced at 0.50 m, using as fertilizer the super simple, at a dose of 80 kg ha^{-1}, providing 15.2 kg ha^{-1} of P_2O_5 soluble, 12.8 kg ha^{-1} of Ca and 6.4 kg ha^{-1} of S. Potassium was applied on September 23, in a total area of the Research Station, at a dose of 250 kg ha^{-1} of KCl (potassium chloride), providing 150 kg ha^{-1} of K_2O. The application of 20 g of molybdenum was performed foliarly, in application in the V3 stage of soybean crop development. The other cultural treatments were carried out taking into account the level of economic damage for weeds, pests and diseases.

Soybean crop evaluations initially consisted of determining the number of nodules found in the root system of 10 random plants, v6 stadium. At harvest, the height (m) of 10 plants was evaluated, determined from the measurement of the distance between the soil and the last pod emitted.

To determine the leaf content of pigments (chlorophyll *a* and *b*), performed in the phytopathology laboratory of the State University of Western Paraná (Unioeste), *Campus* of Marechal Cândido Rondon (PR), 0.1 g of leaves and subsequently placed in vials containing 10 mL of 80% acetone. Such vials were kept in orbital shaker at 100 rpm temperature (25°C) for seven days. Next, the spectrophotometer was read at 663 nm and 645 nm, obtaining chlorophyll *a* and chlorophyll *b* respectively, according to Equations 1, 2 and 3, according to Arnon's methodology (1949).

$$\text{Chlorophyll } a \text{ (mg gpf}^{-1}) = [12.7 \times (A_{663}) - 2.69 \times (A_{645})] \times V/(1000 \times W)$$ (Equation 1)

$$\text{Chlorophyll } b \text{ (mg gpf}^{-1}) = 22.9 \times (A_{645}) - 4.68 \times (A_{663}) \times V/(1000 \times W)$$ (Equation 2)

$$\text{Total Chlorophyll (mg gpf}^{-1}) = [20.2 \times (A_{645}) + 8.02 \times (A_{663})] \times V/(1000 \times W)$$ (Equation 3)

Where:

A = absorbance in the wavelength used (mg chlorophyll/g of fresh weight),

V = final volume of the extract (mL),

W = Weight (g) and

Total Chlorophyll = obtained from the sum of chlorophyll *a* and *b* values.

For the determination of N contents, 10 leaves were collected with petiole, of soybean at random by subplot, detached from the 3rd or 4th leaf fully open, from the apex to the base, at the R2 stage (EMBRAPA, 2014), with determination of the contents in leaf tissues and grains, at the time of harvest, based on the methodology described by Lana et al. (2016). The mass of one thousand grains per subplot was determined with an analytical scale and grain yield was determined with manual harvest (13% of five central lines of the plots (7.5 m linear length and useful area of 18.75 m^2).

Statistical analysis of the results obtained was performed with the aid of the system for statistical analysis SAEG software, (RIBEIRO Jr., 2001), so that the data were submitted to unfolding, according to orthogonais contrast scheme. For comparison between treatments, 9 orthogonais contrasts have been formulated, described below.

The comparison 1 (C1) was performed with the mean result of the control, against the average of the other treatments of the experiment: (T1) *vs*. (T2, T3, T4, T5, T6, T7, T8, T9 and T10). Comparison 2 (C2), performed with the effects of nitrogen fertilization on soybean crop, versus the effects of biological agents: (T8, T9 and T10) *vs*. (T2, T3, T4, T5, T6, T7). Comparison 3 (C3), with the effects of nitrogen fertilization (20 kg ha^{-1}) against nitrogen fertilization (40 and 60 kg ha^{-1}): (T8) *vs*. (T9, T10). Comparison 4 (C4) with nitrogen fertilization (40 kg ha^{-1}) *versus* 60 kg ha^{-1}: (T9) *vs*. (T10). Comparison 5 (C5), with the added effects of biological agents isolated from the genus *Bradyrhizobium* versus effects of biological agents: *Bradyrhizobium*, *Azospirillum* and *Bacillus amyloliquefaciens* (T2, T3, T4) *vs*. (T5, T6, T7). Comparison 6 (C6), with the effects of the use of biological protectors against the absence of protector in seed treatment: (T2, T3) *vs*. (T4). Comparison 7 (C7), with the effects of *Bradhyrizobium elkanii*, in liquid formulation, combined with peat formulation, in the presence of biological protector, against *B. elkanii*, *B. japonicum* in the absence of biological protector: (T2) *vs.* (T3). Comparison 8 (C8), with the use of coinoculation of *B. elkanii* + *Azospirillum brasilense* (in the absence of biological protector), against coinoculation of *B. elkanii* + *A. brasilense* in the presence of biological protector, with coinoculation of *B. elkanii* + *B. amyloliquefaciens*: (T5) *vs.* (T6, T7). Comparison 9 (C9), with coinoculation of *B. elkanii* + *A. brasilense* in the presence of biological protector against coinoculation of *B. elkanii* + *B. amyloliquefaciens* in the presence of biological protector: (T6) *vs*. (T7). In case of significant effect, a Tukey test was used, at 1 and 5% probability of error, to differentiate the means.

RESULTS AND DISCUSSION

Table 3 shows the mean squares and the F-test for contrasts between treatments for number of nodules (nodulation), chlorophyll *a*, chlorophyll *b* and total chlorophyll, leaf nitrogen (N) and N contents in the grains. For the number of nodules, the influence of treatments on C2 was verified ($p<0.01$). There was an increase of 11.98 nodules, with application of biological agents, compared to nitrogen fertilization. In this sense, a relationship between the use of N fertilization is evidenced in reducing the number of nodules, with lower averages, when compared to treatments with biological agents.

Studies carried out by Stephens and Neyra (1983), in soybean plants with the addition of increasing doses of nitrogen (in the form of KNO_3), the authors found a reduction in nitrogenase activity of +50%, due to nitrate and nitrite behavior when accumulating in the nodule region, decreasing the availability of energy to the bacteroid.

When analyzing contrast (C6), there was a high impact on the increase in crop nodulation, compared to treatment with absence of biological protector. Microorganisms and the ratio of biological protectors used together with the tested species demonstrate a positive interaction between the species in increasing nodulation. In C6, similarly, there was a difference regarding the use of biological protectors vs. absence of protector, in the treatment of seeds, in the order of 13.25 more nodules, in relation to the absence of protector.

Coinoculation has interesting reports as a management technique, in order to increase nodulation in soybean crop, as evidenced by Rios et al. (2018), with positive effects and interaction between *Bacillus amyloliquefaciens* and *Bradhyrizoubium japonicum* and positive responses in the number of nodules at 14 days after emergence (DAE), as well as at 35 DAE, with linear and quadratic effect, evidenced, respectively. In C7, when confronting *Bradhyrizobium elkanii* in peat and liquid formulations, + biological protector *vs*. *B. elkanii* and *B. japonicum* + biological protector, it was evidenced that the use of these formulations obtained a higher result, with 10.40 more nodules.

With regard to chlorophyll *a* levels, C2 and C6 positively influenced, in the order of 1.98 and 1.74 mg kg^{-1}, respectively. In C7, they were influenced in the order of -0.32 mg kg^{-1}. For chlorophyll *b*, C2 and C6 showed significant influence, together with C7.

This technology, which consists of inoculating days or weeks before sowing, associated with biological protectors, has been tested in Brazil, but they are still incipient. These studies are important due to the need for protection of bacteria, which, has its survival negatively influenced when in contact with chemical pesticides in the treatment of seeds (ZILLI et al., 2010).

For both chlorophyll *a*, chlorophyll *b*, and total chlorophyll, nitrogen fertilization resulted in higher content, with values of 1.98, 0.18 and 2.16 mg kg^{-1} more, respectively, in relation to treatments with biological agents, evidenced by C2. Similarly, the use of biological protectors provided higher chlorophyll levels, with 1.74 mg kg^{-1} more

chlorophyll *a* and 0.82 more chlorophyll *b*, compared to treatments without the use of protectors, verified in C6. Thus, it was possible to evidence, with the analysis of chlorophyll content, that biological agents in association with biological protectors were responsible for the increases in values.

In the present experiment, chlorophyll *b* contents were higher when compared to chlorophyll *a*. This fact can be explained because the experimental period was characterized by one month with cloudy weather, at the beginning of culture development, in December 2016 (Figure 2).

Chlorophyll is the most abundant pigment in nature and has as main function to absorb sunlight and convert to chemical energy during photosynthesis, green plants mainly contain chlorophyll *a*, which corresponds to about 75% of the total green pigments, and chlorophyll *b*, is a supplementary pigment, at the approximate ratio of 3:1, and can be between 2.5 and 4.0, with variation according to growth and environmental factors, as an example, plants that grow on shading, have high amounts of chlorophyll *b*, due to their light absorption properties (GROSS, 1991).

TABLE 3 - Estimates of nodulation, chlorophyll *a*, chlorophyll *b* and total chlorophyll, due to the applied products and increasing doses of N and their contrasts.

Treatments	Products applied	Nodulation	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll
		(unit)	---------- mg kg^{-1} ----------		
T1	Witness	22.25	0.92	5.43	6.35
T2	*Be* + protective	42.20	5.34	7.68	13.03
T3	*Be* + *Bj* + protective	31.80	5.67	7.71	13.39
T4	*Be* liquid	23.75	3.77	6.87	10.64
T5	*Be* + *Azo*	22.38	3.29	6.72	10.02
T6	*Be* liquid + peaty + *Azo*	32.70	3.71	7.02	10.73
T7	*Be* + *Ba* + protective	28.10	2.26	6.41	8.67
T8	20 kg of N	18.00	3.66	6.51	10.18
T9	40 kg of N	16.50	3.22	6.31	9.53
T10	60 kg of N	20.00	11.10	8.93	20.04
Contrasts					
C1	(T1) *vs*. (T2...T10)	-3.90^{ns}	-3.75^{ns}	-1.69^{ns}	-5.45^{ns}
C2	(T8, T9 and T10) *vs*. (T2...T7)	-11.98**	1.98**	0.18**	2.16*
C3	(T8) *vs*. (T9, T10)	-0.25^{ns}	-3.50^{ns}	-1.10^{ns}	-4.61^{ns}
C4	(T9) *vs*. (T10)	-3.50^{ns}	-7.88^{ns}	-2.62^{ns}	-10.50^{ns}
C5	(T2, T3, T4) *vs*. (T5, T6, T7)	4.85^{ns}	1.83^{ns}	0.70^{ns}	2.54^{ns}
C6	(T2, T3) *vs*. (T4)	13.25**	1.74**	0.82**	2.56^{ns}
C7	(T2) *vs*. (T3)	10.40*	-0.32*	-0.02*	-0.35^{ns}
C8	(T5) *vs*. (T6, T7)	-8.02^{ns}	0.31^{ns}	0.00^{ns}	0.31^{ns}
C9	(T6) *vs*. (T7)	4.60^{ns}	1.45^{ns}	0.61^{ns}	2.06^{ns}

T1 = absence of inoculation and nitrogen (N) in cover, T2 = 2 mL kg^{-1} of *Be* (*Bradyrhizobium elkanii* SEMIA 587 and SEMIA 5019) + 3 mL kg^{-1} protective S30® (polymer concentrate) + 1.2 g kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019, T3 = 2 mL kg^{-1} of *B. elkanii* SEMIA 5019 + *Bj* (*B. japonicum* SEMIA 5079) + biological protectors not specified by the company, T4 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 liquid, T5 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + coinoculation with 0.83 mL kg^{-1} of *Azo* (*Azospirillum brasilense*), strains Abv5 and ABv6, T6 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + 3 mL kg^{-1} protective S30® (polymer concentrate) + 1.2 g kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + coinoculation with 0.83 mL kg^{-1} of *Azo* (*Azospirillum brasilense*), strains Abv5 and ABv6, T7 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + 0.1 mL kg^{-1} of *Ba* (*Bacillus amyloliquefaciens* CEPA MBI 600) + 0.6 mL kg^{-1} protective Extender® (polymer concentrate). ^{ns} = not significant, *significant at 5% probability of error by f-test, **1% error probability, by F test.

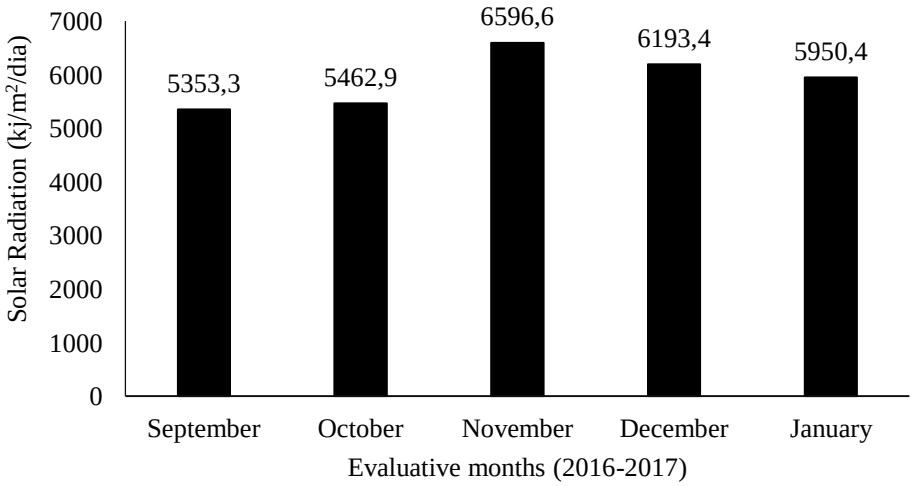

FIGURE 2 - Solar radiation occurred during the conduct of the experiment, from September 2016 to February 2017.

About high productivity in soybean crop, Morrison et al. (1999) by studying the physiological behavior of 58 soybean cultivars in Canada, showed that the cultivars that produced higher chlorophyll content, consequently showed higher productivity.

For N levels in leaf tissue, there was a significant effect for treatments, with values of -6.10 and -5.46 g kg^{-1}, in C1 and C4, respectively (Table 4). In this sense, treatments using biological agents showed superior results regarding the supply of N for the crop. However, for the N contents in the grains, the treatments did not present significant differences between them. Therefore, it is possible to say that N supply to soybean crop did not differ statistically between inoculants and co-inoculation tested.

For the mass of 1,000 grains, there were significant differences between the treatments tested. The treatments with nitrogen fertilization provided a greater increase in compared to biological agents, in the order of 3.53 g more, evidenced by C2. In relation to N fertilization, the recommended dose for culture (20 kg ha^{-1}), provided the highest mass of 1,000 grains, of the order of 4.97 g more than, compared to doses of 40 and 60 kg ha^{-1} (C3). In C5, only treatments inoculated with *Bradyrhizobium* were superior to those with coinoculation with *Bradyrhizobium, Azospirillum* and *Bacillus amyloliquefaciens*, in the order of 9.06 g more.

According to embrapa's recommendation (2014), the increase in nitrogen rates via mineral fertilizer in soybean crop causes a reduction in nodulation and the efficiency of biological nitrogen fixation, besides not favoring the increase in productivity in the presence of nitrogen fertilization, both in planting and covering. The use of fertilizers for soybean crop, which have nitrogen in their formulation, should be done provided that it has a lower cost than those without N and which do not exceed 20 kg ha^{-1}.

For productivity, it was found that the amount of bacteria in the soil was quantified in 1.510 x 10^{-4} bacteria cells/g of soil, where C1 demonstrates that the technologies used in the experiments exceeded productivity by 431.40 kg ha^{-1}. Even in the absence of a significant response to productivity in other comparisons, treatments T2, T3, T5, T6 and T10 exceeded the productivity of 5,200 kg ha^{-1}. In this sense, in order to supply N in the crop to be observed as efficient by the treatments tested, nitrogen fertilization in soybean crop is discarded, corroborated Libório et al. (2015), which did not verify beneficial responses to productivity and biometric variables in soybean crop in Dystrophic Red Latosol.

TABLE 4 - Estimates of leaf nitrogen (N), Grain N, thousand grain mass (TGM) productivity (PROD), due to the applied products and increasing doses of N and their contrasts.

Treatments	Products applied	Leaf N (g kg^{-1})	Grain N (%)	TGM (g)	PROD (kg ha^{-1})
T1	Witness	55.65	5.50	137.30	4,756.94
T2	*Be* + protective	63.36	5.42	135.06	5,437.50
T3	*Be* + *Bj* + protective	63.18	5.75	138.84	5,291.67
T4	*Be* liquid	59.82	5.82	134.20	4,891.67
T5	*Be* + *Azo*	59.28	5.94	129.17	5,237.50
T6	*Be* liquid + peaty + *Azo*	61.50	5.67	124.25	5,321.50
T7	*Be* + *Ba* + protective	60.18	5.91	127.49	5,006.94
T8	20 kg of N	63.36	5.18	138.35	5,097.22
T9	40 kg of N	59.82	5.40	129.75	5,006.94
T10	60 kg of N	65.28	5.38	137.00	5,404.17
Contrasts					
C1	(T1) *vs*. (T2...T10)	-6.10*	-0.11^{ns}	-1.37^{ns}	-431.40*
C2	(T8, T9 and T10) *vs*. (T2...T7)	1.60^{ns}	-0.43^{ns}	3.53*	-28.35^{ns}
C3	(T8) *vs*. (T9, T10)	0.80^{ns}	-0.20^{ns}	4.97*	-108.33^{ns}
C4	(T9) *vs*. (T10)	-5.46*	0.01^{ns}	-7.25**	-397.23^{ns}
C5	(T2, T3, T4) *vs*. (T5, T6, T7)	1.80^{ns}	-0.17^{ns}	9.06*	18.30^{ns}
C6	(T2, T3) *vs*. (T4)	3.45^{ns}	-0.23^{ns}	2.75^{ns}	472.91^{ns}
C7	(T2) *vs*. (T3)	0.17^{ns}	-0.32^{ns}	-3.78^{ns}	145.83^{ns}
C8	(T5) *vs*. (T6, T7)	-1.55^{ns}	0.15^{ns}	3.30^{ns}	73.28^{ns}
C9	(T6) *vs*. (T7)	1.31^{ns}	-0.23^{ns}	-3.24^{ns}	314.56^{ns}

T1 = absence of inoculation and nitrogen (N) in cover, T2 = 2 mL kg^{-1} of *Be* (*Bradyrhizobium elkanii* SEMIA 587 and SEMIA 5019) + 3 mL kg^{-1} protective S30® (polymer concentrate) + 1.2 g kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019, T3 = 2 mL kg^{-1} of *B. elkanii* SEMIA 5019 + *Bj* (*B. japonicum* SEMIA 5079) + biological protectors not specified by the company, T4 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 liquid, T5 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + coinoculation with 0.83 mL kg^{-1} of *Azo* (*Azospirillum brasilense*), strains Abv5 and ABv6, T6 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + 3 mL kg^{-1} protective S30® (polymer concentrate) + 1.2 g kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + coinoculation with 0.83 mL kg^{-1} of *Azo* (*Azospirillum brasilense*), strains Abv5 and ABv6, T7 = 2 mL kg^{-1} of *B. elkanii* SEMIA 587 and SEMIA 5019 + 0.1 mL kg^{-1} of *Ba* (*Bacillus amyloliquefaciens* CEPA MBI 600) + 0.6 mL kg^{-1} protective Extender® (polymer concentrate). ^{ns} = not significant, *significant at 5% probability of error by f-test, **1% error probability, by F test.

CONCLUSIONS

The biological agents tested in the experiment provided an increase in the number of nodules and an increase in the mass of 1,000 grains.

The treatments with nitrogen fertilization, compared to the treatments with inoculation, were higher for chlorophyll contents *a*, *b* and total.

Long-life inoculants, in the absence and presence of coinoculation in seed treatment, demonstrated efficiency in number of nodules and n supply in soybean crop, with superior responses on non-inoculated control, even in soil with initial concentration 1.510 10^{-4} bacteria cells g^{-1} of soil.

Long-life inoculants offer greater practicality to the rural producer and demonstrated efficiency in supplying N to soybean crop.

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