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BIOLOGICAL MANAGEMENT OF *Pratylenchus brachyurus* IN SOYBEAN CROPS[1]

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ABSTRACT - The efficiency of biological products combined with biological fertilization for controlling *Pratylenchus brachyurus* in soybean crops, and effects of these treatments on soybean crops were evaluate. A greenhouse experiment was conducted at the Mato Grosso State University, in Brazil, using a randomized block design with a 10×2 factorial arrangement, and four replications. The treatments consisted of *Trichoderma asperellum, B. subtilis*, *Purpureocillium lilacinum, B. subtilis* + *T. asperellum*, *B. subtilis* + *P. lilacinum*, *T. asperellum* + *P. lilacinum*, *B. subtilis* + *T. asperellum* + *P. lilacinum* and abamectin, and Controls with, and without nematodes. Nematode population, plant height, stem base diameter, and soil microbiological characteristics (at 60 and 120 days after sowing - DAS), shoot dry weight (60 DAS), and number of pods per plant, and grain yield (120 DAS) were evaluated. The treatments were efficient for the control of phytonematodes at 60 DAS; the efficiency of treatments with biological products increased when combined with biological fertilization. The biological products were more efficient for controlling the nematodes than abamectin at 120 DAS; and the percentage of control were higher when they were combined with biological fertilization, in both growing periods. The biological treatments resulted in better agronomic characteristics, and higher number of pods per plant, and grain yield, affecting the plants in the first growing period, and significant interaction with the biological fertilizer for these variables in the second growing period. The interaction between treatments and biological fertilization was significant for soil microbiological characteristics in the second growing period.

Keywords: Biological fertilization. *Bacillus subtilis*. Root lesion nematodes. *Purpureocillium lilacinum*. *Trichoderma asperellum*.

MANEJO BIOLÓGICO DE PRATYLENCHUS BRACHYURUS NA CULTURA DA SOJA

RESUMO - Objetivou-se avaliar a eficiência de produtos biológicos associados à adubação biológica no biocontrole de *Pratylenchus brachyurus* na cultura da soja, além do impacto destestratamentos sobre o desenvolvimento da cultura. O experimento foi conduzido em casa de vegetação, na UNEMAT, Campus de Tangará da Serra, com delineamento experimental em blocos casualizados, esquema fatorial 10X2, com quatro repetições. Foram avaliados: testemunha com e sem nematoides, *Trichoderma asperellum, Bacillus subtilis, Purpureocillium lilacinum, B. subtilis+ T. asperellum, B. subtilis+ P. lilacinum, T. asperellum+ P. lilacinum, B. subtilis+ T. asperellum+ P. lilacinum* e abamectina. Avaliou-se o nível populacional de *P. brachyurus,* altura, diâmetro do colo das plantas e características microbiológicas do solo aos 60 e 120 DAS; massa seca da parte aérea aos 60 DAS; número de vagens/planta e produtividade aos 120 DAS. Observou-se que aos 60 DAS os tratamentos promoveram eficiência no controle do fitonematoides, quando associados a adubação biológica somente os tratamentos biológicos melhoraram sua eficiência. Aos 120 DAS os produtos biológicos apresentaram maior eficiência no controle de *P. brachyurus* que a abamectina, aumentando sua eficiência quando adicionada a adubação biológica (nas duas épocas de semeadura). As características agronômicas, número de vagens/planta e produtividade submetidas aos tratamentos biológicos apresentaram melhor desempenho, havendo interação significativa entre tais tratamentos na primeira época de semeadura e interação com à aplicação do adubo biológico para estas variedades nas segunda época de semeadura. Com relação ás características microbiológicas do solo, houve interação entre os tratamentos somente com a aplicação da adubação biológica na segunda época de semeadura.

Palavras-chave: Adubação biológica. *Bacillus subtilis.* Nematoide das lesões radiculares. *Purpureocillium lilacinum. Trichoderma asperellum.*

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INTRODUCTION

Grain production has contributed positively to the Brazilian economy, with a production that can reach 229.5 million Mg in the 2017/2018 harvest. Mato Grosso is the largest grain producer state of Brazil, accounting for 23.66% of the national production. Soybean represents more than half of the grains produced in Brazil, which is the second largest world producer, after the United States (CONAB, 2018).

Technological advances in agriculture and valorization of commodities caused an expansion of soybean crop areas in Brazil, and a significant increase in soybean production. However, farmers still face several challenges to maintain grain yield of crops due to pathogen attack, such as nematodes, which reduces grain yield and significantly increases production costs (ALMEIDA; SOUZA; ARAÚJO, 2016).

Different nematode species cause serious problems to soybean (*Glycine max* L.) crops in different regions of the world, including Brazil. The root lesions nematode *Pratylenchus brachyurus* (Godfrey) Filipjev & S. Stekhoven can cause losses in grain production of up to 50% (GOULART, 2008).

Pratylenchus is the second most important nematode genus for agriculture in Brazil, after *Meloidogyne* (SANTOS et al., 2015). *P. brachyurus* is the main nematode species that causes damages to soybean crops in Brazil (ALVES, 2015). This species is widely distributed and parasitizes various commercial crops, and weeds. It is a polyphagous nematode, thus, the selection of non-host crops for rotation programs is difficult (DIAS et al., 2010).

Controlling *P. brachyurus* in soybean crops is difficult (GOULART, 2008). This nematode directly affects plant growth and crop yield because it causes necrosis in the roots of the plants, decreasing plant nutrient absorption (LIMA et al., 2015).

Brazilian farmers have control root lesion nematodes using chemical nematicides (AGROFIT, 2018). However, these products persist in the soil, contaminate groundwater, is a risk to human health and fauna, and have high cost and temporary efficiency (COSTA, 2015).

Biological control is an alternative to chemical control; it is cheaper, easier to apply, does not cause environmental imbalances (NUNES; MONTEIRO; POMELA, 2010), improves soil microbiota, and is selective, as opposed to the chemical control, which is not selective (VAZ et al., 2011). Moreover, biological control can be carried out through seed treatments, with inoculation of seeds with fungi and bacteria (GOULART, 2008).

Several microorganisms contribute to reduce phytopathogenic nematode populations. They are generally found associated with the root system of plants, in soils with high organic matter content (RITZINGER; FANCELLI, 2006). In addition, the presence and development of these microorganisms in the soil favors plant residue degradation and nutrient cycling (STIEVEN et al., 2009).

Therefore, practices that favor beneficial microorganisms in the soil are necessary, and the use of biological products, and biological fertilization are alternatives to balance the soil microfauna, and minimize productive, financial, and environmental damages. In this context, the objective of this work was to evaluate the efficiency of biological products based on *Bacillus subtilis*, *Trichoderma asperellum*, and *Purpureocilium lilacinum* Luangsa-ard, Houbranken and Van Doorn (2011), Sin. *Paecilomyces lilacinus* combined with biological fertilization for the control of *P. brachyurus* in soybean crops.

MATERIAL AND METHODS

A *P. brachyurus* population supplied by the Mato Grosso Seed Producer Association (APROSMAT) was multiplied in greenhouse on okra plants (*Abelmoschus esculentus* L. Moench cv. Santa Cruz 47), grown in pots with sterilized soil, and maintained until inoculation of the soybean seeds. The inoculum extraction was carried out according to the methodology described by Coolen and D'Herde (1972), at the phytopathology laboratory of the Center for Research, Studies and Agro-Environmental Development of the Mato Grosso State University, Tangará da Serra campus, Brazil.

The experiment was conducted in a randomized block design in 10×2 factorial arrangement consisted of 10 nematode control products with, and without biological fertilization, with four replications. The experiment was conducted in two growing periods to confirm the effect of the treatments on the nematode population, the first during the crop season, and the second during the fallow season.

The substrate used for the soybean plants consisted of 8 kg of soil, sand, and poultry litter (3:2:0.2), which was packed in high-density polyethylene bags and autoclaved (120ø, 1 atm) for one hour. Subsequently, chemical fertilizer (500 kg ha^{-1} of the 0-18-18 NPK formulation) was added, and the substrate was placed into 8L plastic pots.

The products used for the treatments consisted of a biological fertilizer (Microgeo®; 150mL ha^{-1}), four products marketed as agricultural nematicides, one chemical product (Abamectin 18%; 277 mL ha^{-1}), and biological products containing *Bacillus subtilis* (Rizos®; 100 mL ha^{-1}), *Trichoderma asperellum* (Quality®; 25 g ha^{-1}), and *Purpureocillium lilacinum* (Nemat®, 600 g ha^{-1}). The treatments consisted of Abamectin; *T. asperellum*; *B. subtilis*; *P. lilacinum*; *B. subtilis* + *T. asperellum*; *B. subtilis* + *P. lilacinum*; *T. asperellum* + *P. lilacinum*;

B. subtilis + *T. asperellum* + *P. lilacinum*, and Controls with and without nematodes. These treatments were applied with, and without biological fertilization.

A susceptible soybean cultivar to root lesions nematode (TMG 132RR) was used (TMG, 2018). The tested products were applied through seed treatment together with the inoculation of nitrogen fixing bacteria (*Bradyrhizobium elkanii* SEMIA 587, and *Bradyrhizobium japonicum* SEMIA 5079). Five seeds were sown per pot, and the seedlings were thinned after emergence, leaving one plant per pot. *P. brachyurus* was inoculated at five days after emergence (DAE), using 500 specimens per plant. The nematode suspension was placed into three orifices with depth of approximately 2 cm around the stem base of the seedling, using a pipette. Biological fertilization was applied by spraying the soil at 15 DAE.

The pots were maintained in a greenhouse with irrigation and temperature control; the system was activated when the internal temperature reached 28 ºC. The control of pests and diseases, especially soybean rust, was carried out according to the incidence of pests and diseases, since the experiment was also conducted during the fallow period.

The final *P. brachyurus* population in the roots and soil, crop agronomic characteristics (plant height, stem base diameter, and shoot dry weight), and soil microbiological variables (basal respiration, and soil microbial biomass carbon) were evaluate to determine the efficiency of the treatments. These evaluations were performed at 60 and 120 days after sowing (DAS), except for shoot dry weight (only at 60 DAS), and grain yield, and number of pods (only at 120 DAS).

The nematodes were extracted from the soil and roots using the methodologies of Jenkins (1964);Coolen and D'Herde (1972), respectively. The nematodes were quantified under optical microscope, using Peter's slides. The *P. brachyurus* populations found in the soil and roots were extrapolated to the root and soil volume of each sample, and then summed and presented as number of nematodes per sample. The reproduction factor (RF; Final Population / Initial Population) of each replication was calculated for the different treatments (OOSTENBRINK, 1966).

Plant height (cm), and stem base diameter (mm) were measured using a tape measure, and a caliper ruler, respectively.

The plant shoot material was dried in an oven at 70±2 °C for 48 hours, or until constant weight. Then, the material was weighed to obtain the shoot dry weight (EMBRAPA, 2012), and the results were expressed in grams (g).

The soybean pods were harvested, counted, threshed, and weighed to evaluate grain yield per plant of each treatment; the number of pods and grain weight were multiplied by the amount of plants in one hectare with the standard soybean population of the variety (320 thousand per hectare) (EMBRAPA, 2003). The results were presented in kilograms per hectare (kg ha^{-1}).

The microbiological variables evaluated were soil microbial biomass carbon (SMBC), and basal respiration. Samples of 300 g of soil per treatment were collected and passed through 2 mm mesh sieves, and stored in identified plastic bags under refrigeration (4 °C) until analysis. SMBC was determined through the fumigation-incubation method proposed by Jenkinson and Powlson (1976) - soil samples were fumigated with chloroform for two days and then incubated with sodium hydroxide (NaOH) for five days. The released CO_2 was determined by titration (ALEF, 1995) and the results were expressed in milligrams of carbon dioxide per gram of soil per day (mg CO_2 g^{-1} day^{-1}). The basal respiration was estimated together the SMBC by the amount of CO_2 released from a non-fumigated soil during the five days of incubation, and the results were expressed in milligrams of carbon per gram of soil (mg C g^{-1}).

The data were subjected to analysis of variance and significant means were compared by the Scott Knott test ($p \leq 0.05$) using statistical software Sisvar (FERREIRA, 2011).

RESULTS AND DISCUSSION

In the first growing period, the treatments were significant for the *P. brachyurus* reproduction factor (RF) (Table 1), plant height and stem base diameter (Table 3), number of pods, and grain yield at 120 DAS (Table 4). The interaction between the treatments and biological fertilization was significant only for the variables RF, and plant height. The treatments had no effect on the soil microbiological variables in none of the growing periods (Table 5).

In the second growing period, the application of biological fertilizer was significant for the variables shoot dry weight at 60 DAS, plant height and stem base diameter at 60, and 120 DAS, and number of pods and grain yield at 120 DAS (Table 2). The interaction between the treatments and biological fertilization was significant only for the RF at 60, and 120 DAS (Table 1), and significant differences in soil microbiological variables were found between treatments only at 120 DAS (Table 5).

In both growing periods and evaluation times, the biological products had a nematicidal effect (lower RF) when compared to the control with inoculation of nematodes in both evaluation periods, except the one with P. lilacinum at 60 DAS (Table 1). Similar results were found by Nunes, Monteiro and Pomela (2010) with seeds treated with P. lilacinum for the control of Meloidogyne incognita (Kofold & White); they observed decreases in eggs and juveniles, and increase in root dry weight. Costa (2015) also observed a satisfactory nematicidal effect of Trichoderma species on M. incognita, Heterodera glycines, and P. brachyurus in cotton, soybean, and maize crops, with high control efficiency for M. incognita, H. glycines, and moderate control efficiency for P. brachyurus. Araújo and Marchesi (2009) evaluated the use of B. subtilis and observed satisfactory control of root knot nematodes, and increase in shoot biomass in tomato plants.

Table 1. Reproduction factor (RF) and percentage of control (%) of Pratylenchus brachyurus at 60, and 120 days after sowing (DAS) with different treatments with (WBF), and without (NBF) biological fertilization.

Treatments	First growing period							
	60 DAS				120 DAS			
	RF WBF	% WBF	RF NBF	% NBF	RF WBF	% WBF	RF NBF	% NBF
Control with nematode	1.8 Ca	0.0	2.7 Fb	0.0	12.7 Ea	0.0	14.5 Gb	0.0
Abamectin	0.3 Aa	83.3	0.4 Aa	85.2	4.5 Da	64.6	6.2 Fb	57.2
T. asperellum	0.8 Ba	55.5	1.8 Db	33.3	1.9 Ca	85.0	6.3 Fb	56.5
B. subtilis	0.4 Aa	77.8	0.7 Bb	74.1	1.7 Ca	86.6	4.4 Eb	69.5
P. lilacinum	1.7 Ca	5.5	2.1 Eb	22.2	1.0 Ba	92.1	1.9 Cb	86.9
T. asperellum + B. subtilis	0.5 Aa	72.2	0.9 Bb	66.7	0.7 Aa	94.5	1.5 Bb	89.6
T. asperellum + P. lilacinum	0.5 Aa	72.2	0.8 Bb	70.4	1.2 Ba	90.5	2.7 Db	81.4
B. subtilis + P. lilacinum	1.0 Ba	44.4	1.3 Cb	51.8	0.4 Aa	96.8	1.0 Ab	93.1
T. asperellum + B. subtilis + P. lilacinum	0.4 Aa	77.8	1.9 Db	29.6	0.7 Aa	94.5	2.6 Db	82.1
CV (%)	9.5				6.5			

Treatments	Second growing period							
	60 DAS				120 DAS			
	RF WBF	% WBF	RF NBF	% NBF	RF WBF	% WBF	RF NBF	% NBF
Control with nematode	1.7 Ba	0.0	4.78 Bb	0.0	5.7 Da	0.0	8.2 Db	0.0
Abamectin	0.4 Aa	73.3	0.5 Aa	89.5	1.8 Ca	68.4	3.1 Cb	62.2
T. asperellum	0.7 Aa	53.3	1.3 Ab	72.8	0.8 Aa	86.0	1.8 Ab	78.0
B. subtilis	0.4 Aa	73.3	0.6 Ab	87.4	0.7 Aa	87.7	2.4 Bb	70.7
P. lilacinum	0.9 Aa	40.0	1.2 Ab	74.9	0.6 Aa	89.5	1.6 Ab	80.5
T. asperellum + B. subtilis	0.5 Aa	66.7	0.9 Ab	81.2	0.7 Aa	87.7	1.2 Ab	85.4
T. asperellum + P. lilacinum	0.7 Aa	73.3	1.0 Ab	79.1	1.2 Ba	78.9	2.1 Ba	74.4
B. subtilis + P. lilacinum	0.5 Aa	66.7	0.8 Ab	83.3	0.8 Aa	86.0	2.1 Bb	74.4
T. asperellum + B.s subtilis + P. lilacinum	0.5 Aa	66.7	0.9 Ab	81.2	0.7 Aa	87.7	2.0 Bb	75.6
CV (%)	36.3				17.8			

Means followed by the same uppercase letter in the columns, and lowercase letter in the rows do not differ statistically by the Scott-Knott test at 5% probability. CV = coefficient of variation.

At 120 DAS, the biological products presented greater efficiency for the control of nematodes than the chemical product. When combined with biological fertilization, they had greater control efficiency than the control with inoculation of *P. brachyurus* and application of biological fertilization (Table 1). The seed treatment with abamectin lost its control efficiency over time as also found by Gonçalves Junior et al. (2013), who reported a residual effect of abamectin of approximately 25 days followed by gradual decreases after its application. Contrastingly, biological treatments remain in the soil, mainly in the rhizosphere, increase its population and, consequently, the nematicidal effects (VAZ et al, 2011). Bortolini et al. (2013) evaluated biological and chemical control of *P. brachyurus*, and found that biological treatments of soybean seeds with *P. lilacinum* + *Arthrobotrys* spp., and *T. viride* had similar nematicidal efficiency to the chemicals imidacloprid + thiodicarb, pyraclostrobin + thiophanatomethyl + fipronil, and abamectin.

The nematicidal efficiency of the chemical treatment (abamectin) used in the present work did not increase when combined with biological fertilization at 60 DAS. However, after the end of its residual effect, this combination was efficient for the control of *P. brachyurus* at 120 DAS. It was also observed in the inoculated control; the nematodes on plants treated with biological fertilization had lower RF, denoting the nematicidal effect of the biological fertilization.

The use of biological products to control phytonematodes is a viable alternative; these products cause little or no damage to the environment, are selective, and have low cost (ARAÚJO; MARCHESI, 2009). Farmers who use biological products are closer to a sustainable agriculture, which requires strategies to increase food production without harming the environment, or human health. Microorganisms are a viable alternative for seed treatment, they are easy to apply, and some fungi and bacteria are native to the soil and do not interfere in the ecological balance (MARIANO et al., 2004).

The biocontrol of the root lesion nematode in soybean found in the present work was related to direct actions of the microorganisms on the pathogen. Fungi and bacteria with direct action on *P. brachyurus* were chosen because these biological agents have greater potential for agricultural uses and have different modes of action for nematode control (SANTIAGO, 2015).

P. lilacinum and *T. asperellum* are promising fungi for biocontrol of nematodes. *P. lilacinum* is a facultative parasite of eggs and females of sedentary and opportunistic nematodes, with little host specificity; it is a very competitive fungus in field conditions, which adapts to a wide soil pH range and can grow in various substrates (ALMEIDA; SOUZA; ARAÚJO, 2016). This microorganism penetrates eggs of nematodes, destroys the embryo and reduces the reproductive capacity of sedentary females, which are later killed (SANTIAGO et al., 2006), and has a toxic effect on adult nematodes (DEVRAJAN; SEENIVASAN, 2002). The biological control mechanisms of *Trichoderma* species are antibiosis, mycoparasitism, and enzymatic hydrolysis. This fungus penetrates cysts and nematode eggs, resulting in the death of juveniles (CARVALHO, 2017). *T. asperellum* produces toxic compounds and parasitizes eggs of phytopathogenic nematodes (COSTA, 2015).

Bacteria present two main nematicidal mechanisms, direct obligate parasitism, and indirect effects. Bacteria of the genus Bacillus produce proteases, which destroy the cuticle of nematodes; B. subtilis produces endotoxins in the soil that affect the reproductive cycle of nematodes, affecting mainly the oviposition and hatching of juveniles (CARVALHO, 2017), causing difficulties for the nematodes to locate the roots because of the production of toxins that alter root exudates (SANTIAGO, 2015).

The RF was expected to be higher at 120 DAS than at 60 DAS because of the time of exposure of the plant to the nematodes, but the treatment based on the fungus P. lilacinum in both growing periods, and the combination of the products based on P. lilacinum + T. asperellum in the first growing period resulted in lower RF at 120 DAS. This denotes the efficiency of these microorganisms on the control of P. brachyurus (Table 1). Santiago et al. (2006) found a reduction in the population of Meloidogyne paranaenses in tomato plants from seeds treated with P. lilacinum isolates. A greenhouse study to evaluate the control of H. glycines with the bacteria B. subtilis showed reduced egg hatching, inhibition of juvenile nematode migration to the plant, and reduced number of females in soybean roots in treated soils and seeds (D'AGOSTINO; MORANDI, 2009).

The interaction between the microorganisms and the biological fertilizer was beneficial for the control of P. brachyurus. The combination of microorganisms decreased the nematode population. These results can be explained by the combination of the nematicidal action of the microorganisms and the plant physiology.

The treatments were significant for the agronomic variables plant height, and stem base diameter in the first growing period (Table 3). In the second growing period, the agronomic characteristics number of pods and grain yield had significant differences only when using the biological fertilization, showing its benefits to the plant (Table 2).

Table 2. Agronomic variables, number of pods per plant, and yield of soybean plants subjected to treatments for the control of Pratylenchus brachyurus, with (WBF), and without (NBF) biological fertilization in the second growing period at 60, and 120 DAS.

Variables	60 DAS			120 DAS		
	WBF	NBF	CV (%)	WBF	NBF	CV (%)
Shoot dry weight (g)	18.6 A	16.0 B	25.52	-	-	
Plant height (cm)	31.0 A	23.4 B	35.40	38.4 A	32.9 B	30.22
Stem base diameter (mm)	5.4 A	4.7 B	16.62	6.3 A	5.4 B	13.93
Number of pods	-	-		39.8 A	23.5 B	41.9
Grain yield (Kg ha^{-1})	-	-		2694.8 A	2199.2 B	38.48

Means followed by the same letter in the rows do not differ statistically by the Scott-Knott test at 5% probability. CV = coefficient of variation.

The biological fertilization had no effect on the plant height at 60 DAS in the first growing period, however, the plant height increased compared to the control without nematode when it the three biological products were combined. At 120 DAS, the use of biological products resulted in higher plant heights compared to the inoculated control, and the chemical treatment; and when combined with biological fertilization, the treatments had different efficiencies, with plant heights smaller than those of the control with nematodes in some treatments (Table 3). According to Wehr (2014), even if the biological fertilizer is applied to the soil, concentrations above 20% can cause stress and physiological damages to plants. This may have affected the plant heights found in the present work.

The plant heights found can be explained by the high amount of microorganism interfering in biological processes of the plants, harming their development. Rhizobacteria associate with plants and colonize their roots; it can be beneficial, deleterious, or neutral to the plants, since some beneficial bacteria propagate in the root system and promote plant growth (rhizobacteria), and deleterious bacteria hinder plant development by causing hormonal imbalances (DUTRA et al., 2014).

The stem base diameter of infected soybean plants with P. brachyurus in the first growing period had significant differences between treatments only at 120 DAS, with the biological products resulting in higher stem base diameter than the control and the chemical treatment; however, no significant difference in stem base diameter was found for the interaction between the treatments and the biological fertilization (Table 3). This increase can be attributed to the plant growth induced by the microorganisms tested. B. subtilis is a plant growth promoter bacterium because the action of its metabolites increases plant development through phytohormones and antibiotics production (ARAUJO, 2008). B. subtilis presents mutualistic interaction with plants (JONK et al., 2014), and assists in nitrogen fixation, solubilization of nutrients, and improvement of soil conditions. Thus, the association of soybean plants with B. subtilis may have increased metabolites production, enhancing the root system sensitivity to external conditions, facilitating the absorption of nutrients (LANNA FILHO; FERRO; PINHO, 2010).

Harman, Taylor and Stask (1989) observed increases in the growth of maize plants inoculated with Trichoderma spp. According to Brotman, Gupta and Viterbo (2010), Trichoderma species promote plant growths of up to 300%. Menezes (1992) evaluate common bean and soybean plants with Trichoderma spp. and observed increases in germination percentage, and plant growth.

The higher plant heights found when using the biological fertilizer were due to the action of the biological fertilizer on soil fertility. Biological fertilizers improve organic matter percentage and cation exchange capacity, reduce exchangeable aluminum contents, favor important organic acids for the solubility of minerals, and nutrient cycling and mobility, and contribute to water infiltration, improving soil attributes and available phosphorus (CARNEIRO et al., 2016). These benefits favor plant development and nutrition.

Table 3. Plant height (cm) and stem base diameter (mm) of soybean plants subjected to treatments for the control of *Pratylenchus brachyurus*, with (WBF), and without (NBF) biological fertilization in the first growing period at 60, and 120 DAS.

Plant height (cm)			
Treatments	60 DAS	120 DAS	
		WBF	NBF
Control without nematode	60.2 B	63.5 Aa	73.0 Aa
Control with nematode	54.5 B	69.2 Aa	44.5 Bb
Abamectin	52.9 B	60.7 Aa	55.0 Ba
T. asperellum	56.7 B	61.5 Aa	50.7 Ba
B. subtilis	56.2 B	61.2 Aa	59.5 Aa
P. lilacinum	56.1 B	59.5 Aa	53.7 Ba
T. asperellum + B. subtilis	49.6 B	52.2 Ba	60.0 Aa
T. asperellum + P. lilacinum	56.4 B	62.2 Aa	60.7 Aa
B. subtilis + P. lilacinum	53.0 B	43.2 Ba	52.2 Ba
T. asperellum + B.s subtilis + P. lilacinum	71.5 A	42.5 Bb	64.2 Aa
CV (%)	16.5	16.5	

Stem base diameter (mm)	
Treatments	120 DAS
Control without nematode	10.4 B
Control with nematode	10.4 B
Abamectin	9.7 B
T. asperellum	11.2 A
B. subtilis	11.2 A
P. lilacinum	11.9 A
T. asperellum + B. subtilis	9.7 B
T. asperellum + P. lilacinum	12.5 A
B. subtilis + P. lilacinum	10.2 B
T. asperellum + B.s subtilis + P. lilacinum	11.1 A
CV (%)	13.9

Means followed by the same uppercase letter in the columns, and lowercase letter in the rows do not differ statistically by the Scott-Knott test at 5% probability. CV = coefficient of variation.

Plants of the first growing period had greater pod production and grain yield in some treatments with biological products (Table 4), but the interaction between the treatments and biological fertilization was not significant. All treatments resulted in more pods than the control with nematode and the treatment with abamectin, except the treatments *P. lilacinum*, and *T. asperellum + P. lilacinum*. The treatments T. asperellum, B. subtilis, and T. asperellum + P. lilacinum resulted in the highest grain yields. The biological products improved grain yield; most biological treatments resulted in higher grain yield than the national average (3,208 kg ha^{-1}) (CONAB, 2018). Increases in grain yield were also observed in studies with *Trichoderma* spp. applied in the furrows at planting, and at piling in potato crops, with increases of more than 20% in the yield, and improved tuber quality by reducing stains caused by Rhizoctonia spp., and *Streptomyces scabies* (POMELLA; RIBEIRO, 2009).

In the second growing period, the shoot dry weight at 60 DAS, plant height and stem base diameter at 60 and 120 DAS, and number of pods per plant, and grain yield at 120 DAS presented significant differences only when the biological fertilizer was used, which generated the best results (Table 2). The biological treatments result in better development of the soybean plants because of direct and indirect stimuli to the plants caused by the microorganisms. (MARTINS et al., 2015) evaluated common bean plants treated with biological fertilization and found increases in pod formation and production due to the better use of nutrients. Increases were also found in shoot dry weight, and grain yield of cherry tomatoes (GOMES JÚNIOR et al., 2011), in the growth and production variables of maize plants (BEZERRA et al., 2008), and in the production of sweet pepper (ARAÚJO et al., 2007) with the application of biofertilizers.

Table 4. Number of pods, and grain yield of soybean plants subjected to different treatments to control *Pratylenchus brachyurus*, at 120 DAS in the first growing period.

	Number of pods per plant	Grain yield (Kg ha^{-1})
Treatments	120 DAS	120 DAS
Control without nematode	121.0 A	5.100.4 A
Control with nematode	86.2 B	2.911.6 B
Abamectin	74.7 B	3.502.4 B
T. asperellum	117.1 A	4.468.0 A
B. subtilis	124.6 A	6.674.4 A
P. lilacinum	99.7 B	3.872.8 B
T. asperellum + B. subtilis	130.7 A	2.464.4 B
T. asperellum + P. lilacinum	90.3 B	5.576.4 A
B. subtilis + P. lilacinum	106.6 A	2.718.0 B
T. asperellum + B.s subtilis + P. lilacinum	115.7 A	3.880.8 B
CV (%)	32.6	38.5

Means followed by the same letter in the columns do not differ statistically by the Scott-Knott test at 5% probability. CV = coefficient of variation.

Biological fertilizers are composed of living cells of different microorganisms that convert nutrients in the soil to available forms to plants. It is a low cost, and environmentally sustainable alternative to promote nutrient cycling and improve soil chemical, physical and biological properties (SILVA et al., 2007). Biological fertilizers are also used as stimulator of proteosynthesis, insect repellent, and for disease control (GONÇALVES; SCHLEDECK; SCHWENGBER, 2009).

Considering the soil microbial biomass carbon (SMBC), significant differences were found only in the second growing period (Table 5). The interaction between treatments and biological fertilization was not significant probably due to the sterilization of the soil used in the experiment, and the inoculation with microorganisms in all treatments. Most treatments with biological products had high SMBC; the best result was found with the treatment B. subtilis + P. lilacinum at 60 DAS; and with the control without nematode, B. subtilis*, and* B subtilis + P. lilacinum at 120 DAS. In general, all treatments increased the SMBC at 120 DAS, denoting that the longer the microorganisms grow and the greater their population in the soil, the greater the SMBC. According to Mercante et al. (2004), a high SMBC generates greater temporary immobilization of nutrients and consequently lower nutrient losses in the soil-plant system.

Table 5. Soil microbial biomass carbon (mg CO_2 g^{-1} day^{-1}) as a function of different treatments for the control of *Pratylenchus brachyurus*, at 60 and 120 days after soybean sowing (DAS) in the second growing period.

Soil microbial biomass carbon (mg CO_2 g^{-1} day^{-1})		
Treatments	60 DAS	120 DAS
Control without nematode	1.0 B	1.4 A
Control with nematode	0.4 C	0.5 B
Abamectin	0.5 C	0.8 B
T. asperellum	0.5 C	0.9 B
B. subtilis	1.0 B	1.8 A
P. lilacinum	0.5 C	0.3 B
T. asperellum + B. subtilis	1.1 B	0.7 B
T. asperellum + P. lilacinum	0.3 C	0.5 B
B. subtilis + P. lilacinum	1.5 A	1.8 A
T. asperellum + B. subtilis + P. lilacinum	0.7 C	0.5 B
CV (%)	57.3	80.4

Means followed by the same letter in the columns do not differ statistically by the Scott-Knott test at 5% probability. CV = coefficient of variation.

SMBC represents the amount of carbon that the soil microbial biomass immobilizes in its cells, and shows possible soil imbalances (ALVES et al., 2011). Soil is a living and heterogeneous system, composed of microbial associations that are sensitive to physical and chemical changes that affect its balance (GODOY et al., 2013). Even using a sterilized soil, the biological treatments seem to have favored the bacterium and fungi development in the soil throughout the crop cycle, potentializing the control of the root lesion nematodes, unlike the chemical treatment, whose nematicidal potential reduced over time. Silva et al. (2016) used a substrate with 50% sterilized content and found reduction in SMBC; this can be explained by the reduction of the biotic potential of the native microbiota due to the autoclaving of the natural soil.

Soil microbiota richness is also related to SMBC, as observed in studies on soil organic matter accumulation. According to Alves et al. (2011), soils with high organic matter content have high SMBC. Moreover, soils with native vegetation have higher SMBC than agricultural soils using conventional soil preparation system, denoting the soil microbiological richness and balance (GODOY et al., 2013).

CONCLUSION

Biological products based on *T. asperellum, B. subtilis,* and *P. lilacinum* have potential for the control of *P. brachyurus*. These products showed greater efficiency for the control of this nematode than the chemical treatment with abamectin at 120 days after sowing. The percentage of control were higher when they were combined with biological fertilization, denoting that biological fertilization is also a potential tool for integrated management of phytonematodes.

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