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Abstract

Nematodes are of great importance in soybean cultivation, especially the *Pratylenchus brachyurus* known as root lesion nematode. Its attack on plant roots causes less efficiency in the absorption of water and nutrients, in addition to damaging the plant's development. There is still no fully efficient method to control this phytopathogen, however, some products are available on the market, including biological control. Thus, the aim of this study was to evaluate commercial biological products in the efficiency of reducing the nematode population in soybean crop in Goiás, Brazil. The design was completely randomized in a 2x4 factorial scheme, the first factor being two soybean genotypes (Brasmax *Bônus* and Nidera NS 8383) and the second factor the treatments consisting of different dosages in an association of three commercial products: No-Estio[®], Bio-fertility[®] and Radic[®]. The treatments used were: T1 control - without application of the products; T2 half the recommended dose; T3 the recommended dose and T4 a dose and a half that recommended by the manufacturer. Plant evaluation was carried out after 75 days of nematode inoculation. The results obtained showed that both cultivars hosted *P. brachyurus*, however, the treatments using the products had a lower population density of this nematode. It was concluded that the two soybean cultivars are hosts of *Pratylenchus brachyurus*. The agronomic character plant height was more affected when there was no application by the biological method. The association of No-Estio[®], Bio-fertility[®] and Radic[®] products reduced the population density of nematodes in infected plants.

Keywords: Biological control; *Glycine max* L.; Root lesion nematodes; *Pratylenchus brachyurus*.

Resumo

Os nematoides possuem grande importância na cultura da soja, destacando-se o *Pratylenchus brachyurus* conhecido como nematoide das lesões radiculares. O seu ataque nas raízes das plantas provoca menor eficiência na absorção de água e nutrientes, além de danificar o desenvolvimento do vegetal. Não existe ainda um método com total eficiência para o controle desse fitopatógeno, no entanto, alguns produtos estão disponíveis no mercado, entre eles, de controle biológico. Assim, objetivou-se com o presente estudo avaliar produtos biológicos comerciais na eficiência de redução da população de nematoides na cultura de soja em Goiás, Brasil. O delineamento foi inteiramente casualizado em esquema fatorial 2x4, sendo o primeiro fator, dois genótipos de soja (Brasmax Bônus e Nidera NS 8383) e o segundo fator os tratamentos constituídos de diferentes dosagens em uma associação de três produtos comerciais: No-Estio[®], Bio-fertility[®] e Radic[®]. Os tratamentos utilizados foram: T1- testemunha- sem aplicação dos produtos; o T2 meia dose recomendada; o T3 a dose recomendada e o T4 uma dose e meia da recomendada pelo fabricante. A avaliação das plantas foi realizada após 75 dias de inoculação dos nematoides. Os resultados obtidos demonstraram que ambas as cultivares foram hospedeiras de *P. brachyurus*, porém, os tratamentos utilizando os produtos apresentaram menor densidade populacional desse nematoide. Concluiu-se que as duas cultivares de soja são hospedeiras de *Pratylenchus brachyurus*. O caractere agrônomo altura de planta foi mais prejudicado quando não houve a aplicação pelo método biológico. A associação dos produtos No-Estio[®], Bio-fertility[®] e Radic[®] diminuíram a densidade populacional dos nematoides nas plantas infectadas.

Palavras-chave: Controle biológico; *Glycine max* L.; Nematode das lesões radiculares; *Pratylenchus brachyurus*.

Resumen

Los nematodos son de gran importancia en el cultivo de la soja, especialmente el *Pratylenchus brachyurus* conocido como nematodo lesionador de raíces. Su ataque a las raíces de las plantas provoca una menor eficiencia en la absorción de agua y nutrientes, además puede perjudicar el desarrollo de lo vegetal. Todavía no existe un método totalmente eficaz para controlar este fitopatógeno, sin embargo, algunos productos están disponibles en el mercado, incluido el control biológico. Así, el objetivo de este estudio fue evaluar los productos biológicos comerciales en la eficiencia de reducir la población de nematodos en el cultivo de soja en Goiás, Brasil. El diseño fue completamente al azar en un esquema factorial 2x4, siendo el primer factor dos genotipos de soja (Brasmax Bônus y Nidera NS 8383) y el segundo factor los tratamientos consistentes en diferentes dosis en una asociación de tres productos comerciales: No-Estio[®], Bio-fertility[®] y Radic[®]. Los tratamientos utilizados fueron: T1- control - sin aplicación de los productos; T2 la mitad de la dosis recomendada; T3 la dosis recomendada y T4 una dosis y media de la recomendada por el fabricante. La evaluación de la planta se llevó a cabo después de 75 días de inoculación de nematodos. Los resultados obtenidos mostraron que ambos cultivares hospedaron *P. brachyurus*, sin embargo, los tratamientos con los productos tuvieron una menor densidad poblacional de este nematodo. Se concluyó que los dos cultivares de soja son hospedadores de *Pratylenchus brachyurus*. La altura de la planta de carácter agronómico se vio más afectada cuando no hubo aplicación por el método biológico. La asociación de los productos No-Estio[®], Bio-fertility[®] y Radic[®] redujo la densidad de población de nematodos en plantas infectadas.

Palabras clave: Control biológico; *Glycine max* L.; Nematodo lesionador de raíces; *Pratylenchus brachyurus*.

1. Introduction

Soybean (*Glycine max* L.) is an oleaginous plant species belonging to the Fabaceae family, originally from China, with a determined and indeterminate growth habit, standing out for being one of the most important economic crops in the world (Villalva, 2008; Zhao et al., 2018). The cycle of this plant can vary, showing early to late cultivars, in addition to containing different resistance to phytopathogens (Sediyama, 2009).

Soybean is one of the main agricultural commodities exported by Brazil. Production in the 2020/21 crop was approximately 136 million tons (Conab, 2021). This oilseed is used for various purposes, mainly in animal nutrition and human food. This culture increases regional economic development, agricultural industrialization, machinery, inputs and feed, and immense use in the production process (Mello & Brum, 2020).

The culture needs specific handling and care for high production. Some obstacles can affect its productivity, among them are the occurrence of undesirable pests and diseases in the area (Torkamaneh et al., 2021). The lack or deficit of health, negatively affects the final production of the grain and its quality, when not properly managed (Hartman et al., 2015). Among the phytopathology's, phytopathogens of bacterial, fungal, viral origin and also caused by different groups of nematodes stand out (Morais et al., 2020). Crop damage can be greater with the use of monoculture, expansion to new areas, in addition to the

use of direct planting in the areas (Embrapa, 2004; Sharma et al., 2020).

In commercial terms, the problems caused by nematodes in the soybean crop generate losses of over 80 billion dollars. In Brazil, this loss is over 35 billion for agriculture, especially for the soybean crop. of 16.2 billion a year (Rosa et al., 2021). Among the main causes of phytopathologies and losses in soybean production, nematodes are directly responsible for the significant damage of this oleaginous plant, even in the development of root and aerial parts. These microorganisms have great economic importance as they are highly aggressive phytopathogens. The main nematodes in the soybean crop are the soybean cyst, *Heterodera glycines*, the reniform nematodes, *Rotylenchulus* sp., the root-knot nematodes *Meloidogyne javanica* and root lesion nematodes *Pratylenchus brachyurus* (Dias et al., 2010; Rosa et al., 2021).

Among the most important nematodes, *P. brachyurus* (Godfrey) Filipjev & S. Stekhoven stands out as a polyphagous nematode, mainly associated with poaceas such as corn, rice and sugar cane. Although they also can parasitize other plants such as cotton and soybean (Goulart, 2008). In Brazil, the Midwest region has the highest recorded rate of root lesions in soybean plants, with productivity losses in up to 50% of the total infested area (Franchini et al., 2014; Brida et al., 2017). *P. brachyurus*, according to Antonio et al. (2012) and Santos et al. (2019), show a considerable loss of more than 21% on grain production. The species *P. brachyurus* is an endoparasitic phytonematode; that is, it lives inside the roots of plants, causing damage to the roots due to its internal movement, feeding on tissues and injecting toxins into the roots, directly affecting the metabolism of the plants (Goulart, 2008; Bellé et al., 2017; Santana-Gomes et al., 2019).

Biological control, different from chemical control that uses synthetic molecules, is considered an efficient method to reduce and balance phytopathogens and pests. This control model combined with good agricultural practices and resistant cultivars present advantages for the production system. This is due to its easy application, no toxicity and no negative interaction with the environment, conserving and maintaining the sustainability of the *on-site* production system (Cardoso & Araújo, 2011).

The biological element present in the soil is of great importance to limit or stabilize nematode populations and attacks through antagonistic effects and also through competition and parasitism mechanisms (Lopes et al., 2007; Castanheira et al., 2020); biochemical processes carry them out between microorganisms (Pimentel et al., 2009). With the introduction of microorganisms, the induction of resistance may also occur during the inoculation and treatment of components based on solutions containing microorganism cells (Bonaldo et al., 2005). There are numerous products based on dispersive solutions of microorganisms for use in the control and elimination of various pests, thus having the ability to perform, in some cases, an antagonistic effect to phytopathogenic microorganisms (Federici et al., 2007, 2010; Oliveira et al., 2021).

The use of several integrated control methods has demonstrated greater efficiency. The application of microorganisms that support the good development of plants has been studied as an alternative form of integrated nematode management. These microorganisms, when introduced into an area, can promote balance on the soil fauna and can also form symbiotic systems with plant roots, thus contributing, mainly to the absorption of water and nutrients, presenting a form of defense and recovery on the nematode attack (Gerdemann, 1968; Rhodes; Gerdemann, 1975; Leite et al., 2019).

Thus, this study aimed to evaluate commercial biological products in reducing the population of *Pratylenchus brachyurus* in soybean crop planting areas in the state of Goiás, Brazil.

2. Material and Methods

The experiment was conducted under greenhouse conditions located on the experimental campus of the Evangelical Faculty of Goianésia (FACEG). The seeds used in the experiment are indicated for the Midwest region and were acquired from rural producers in Goianésia, Goiás, Brazil.

The design used was completely randomized, in a 2x4 factorial scheme, with the first factor being two soybean cultivars (Brasmax Bônus and Nidera NS8383) and the second factor, four levels of association of commercial products (No-Estio[®], Bio-fertility[®] and Radic[®]) and five repetitions, each pot corresponded to a parcel. The sowing of soybean seeds was carried out with 10 seeds in each plastic pot with a capacity of 5 L, in which they were filled with a substrate composed of autoclaved soil and sand in a 2:1 ratio. The vases were placed on pallets, not allowing direct contact with the ground, thus eliminating possible contamination. The planting fertilization consisted of 3 g of the 04-30-10 formula for each pot. After germination of the seeds, a thinning was performed, leaving only two plants per pot.

For the treatment of soybean seed with the products No-Estio[®], Bio-fertility[®], and Radic[®], 250 g of seed were separated in properly identified plastic bags. Also, at the time of seed treatment, 1 L beakers were used for the mixtures. For the control (Treatment 1 – T1), no commercial product was used. Liquid products were measured with the aid of a graduated pipette, and solids were weighed on a precision balance. After measuring the products, 5 mL of distilled water were placed in the beakers and then the mixture was homogenized according to the recommendations of each treatment.

The second level (treatment 2 -T2) was performed with half the recommended dosage of the products, the third level (treatment 3-T3), the recommended dosage of the products was: 1 g for 1 kg⁻¹ of No-Estio[®] seed; 2.5 mL for 1 kg⁻¹ of Bio-fertility[®] seed and 2.5 mL for 1 kg⁻¹ of Radic[®] seed. The fourth level (treatment 4-T4) was applied one and a half of the recommended dose.

The inoculation of *P. brachyurus* phytonematodes was carried out 20 days after sowing the soybean seeds. Inoculation of nematodes was performed by inserting 150 g of soil naturally infested with nematodes close to the seedling neck. The infested soil used came from pots with corn for the multiplication of nematodes. After inoculation, cultural treatments and fertilization were carried out according to the needs of the culture. After 30 days of sowing the seeds, a top dressing was carried out with the formula 20-00-20.

After 75 days of installing the experiment and consecutive insertion of the infested soil in the pots, the evaluation was carried out. The following agronomic traits were evaluated: root mass (g), root length (cm), plant height (cm), green mass (g), and pod mass (g). The variables were evaluated by measuring the mass and length of agronomic characters. The plants were separated and the roots kept in plastic bags for the extraction of nematodes, where they were transported to the Soil Microbiology laboratory at FACEG.

In laboratory procedures, the roots were washed and their mass determined (g); the same was done for the length (cm) measured using a millimeter ruler. After the measurements, the roots were cut into pieces with stainless-steel scissors measuring approximately 2 cm and were then ground in a domestic processor with 250 mL of distilled water for 30 s. For the collection of these roots, a 100-mesh sieve was used, superimposed on a 400-mesh sieve. The remains in the 100 "mesh" sieve were discarded, and those retained in the 400-mesh sieve were collected and transferred to beakers. The samples were placed in plastic bottles for the quantification of nematodes. For their identification and counting, an optical microscope and a Peters camera were used. Soon after the quantification of microorganisms, the population density of nematodes was calculated. The population density in each cultivar was calculated by the ratio between the final population present in the Peters' chamber.

The data obtained in the experiment were submitted to normality and homogeneity of variance tests. According to the statistical presuppositions, analyzes of variance (ANOVA) and a mean test were performed, with a 5% probability level for the analyzed variables, and if there was a difference, the Tukey test with 5% significance was applied, using the SASM-Agri statistical program (Canteri et al., 2001).

3. Results and Discussion

The results obtained after the mean test (Table 1) showed that both cultivars were hosts of *P. brachyurus*; it is observed that in all four treatments, infestation caused by this phytopathogen was observed. Although, the treatments where commercial products were used had the lowest population densities compared to the control treatment. It was impossible to observe statistical differences between the products' levels used according to the applied statistical tests. Thus, the association of the three products reduced the population density of *P. brachyurus* in soybean roots.

Table 1. Population density of *P. brachyurus* (10 g of the root) in two soybean cultivars.

Grow crops	T1	T2	T3	T4
Nidera NS8383	2221.80b	578.20a	561.00a	592.00a
Brasmax BÔNUS	3074.00b	1075.80a	508.20a	1245.20a

Note: Levels of associated commercial products: T1: control, T2: half the recommended dose, T3: the recommended dose, and T4: one and a half of the recommended dose.
Source: Authors.

The two cultivars showed lower amounts of nematodes in plant roots with the application of biological products; this may have happened because the microorganisms present in biological products compete directly with nematodes in the roots of affected plants, as well as helping root development as well as described in the study by Trentin (2016) on soybean crop with root-lesion nematodes. According to Vos et al. (2012), the researchers also reported that the mycorrhizal fungi *Glomus mosseae* considerably reduced the population of the nematodes *P. brachyurus* penetrans in the tomato crop.

Several studies have been carried out to know the action of these microorganisms in association with plant roots. Elsen et al. (2003) evaluated banana trees in Belgium with the interaction of the mycorrhizal fungus *Glomus mosseae* with some species of nematodes with *Radopholus similis* e *Pratylenchus coffeae*. The researchers observed that the association of fungi with the roots stimulated plant growth even with these phytopathogens.

The microorganisms interact in the roots together with the nematodes, reducing, increasing, or even having no effect on the life of these infective forms that cause phyto-diseases that cause the plant's death. However, currently, there is evidence of greater resistance by plants with the mycorrhizal fungi present, thus decreasing their reproduction in various crops of agricultural and economic interest (Moreira & Siqueira, 2002; Kath et al., 2017; Miamoto et al., 2017).

Plants colonized by mycorrhizal fungi or beneficial bacteria may have better development and growth, thus demonstrating greater resistance and tolerance to these pathogens (Shreenivasa et al., 2007). Comparing the agronomic characters for the treatments used: PH (plant height), RL (root length), PM (pod mass), GM (green matter), RM (root mass), it can be observed that there was a different statistic between dosages and control in the item height of plants according to (Table 2).

Table 2. Agronomic characteristics of soybean after four levels of application of commercial biological products (No-Estio[®], Bio-fertility[®], and Radic[®]) in the greenhouse.

Agronomic Characters	Witness	Half dose of products	A dose of the products	One and a half dose of the products
Plant height (PH)	45.85a	50.25ab	55.10b	49.70ab
Root length (RL)	34.25a	39.00a	31.75a	32.95a
Pod mass (PM)	1.81a	4.62a	4.33a	5.37a
Green matter (GM)	16.29a	19.06a	20.76a	16.27a
Root mass (RM)	2.78a	3.30a	3.88a	4.00a

Note: Means followed by the same letter in the lines do not differ by Tukey test with 5% probability. Source: Authors.

It is observed that the treatment with a half dose, one dose, and one and a half dose did not statistically differ from the control with the agronomic character composed by the plant height (cm). It was then verified that the use of the recommended dose obtained a greater statistical difference when compared to the other doses in plant height (cm). It is suggested that the use of microorganisms may have increased and provided a beneficial effect on the development and height of plants due to their harmonic interaction with the roots. It is observed that the agronomic characters' root length, pod mass, green matter, and root mass did not differ from each other about the control as observed by the applied statistical test.

The control action mechanisms are not yet fully elucidated and may act in isolation or synergism (Elsen et al., 2001; Elsen et al., 2003; Vos et al., 2012b). The antagonistic effects of these microorganisms have great variability, depending on several factors, such as the species of microorganisms, the phytopathogen, the host plant, and environmental conditions (Dong; Zhang, 2006).

Kamunya et al. (2008) discussed the use of cultivars with genetic resistance, which is the most efficient option. The researchers still argue in the study about the technique of handling nematodes that is environmentally correct and has a low production cost to control phytonematodes. However, it is essential to use other techniques such as crop rotation and biological control to provide better results and to diversify the methods, thus providing a low resistance effect (Xiang et al., 2018; Melo et al., 2021).

In general, the recommendation of biological control for phytoparasitic nematodes deserves caution since the microorganisms used as antagonists can suffer different environmental influences, such as soil temperature, pH, humidity, and the presence of chemical residues, which can derail the potential effects of biological control (Lopes et al., 2020; Oliveira et al., 2021).

According to the cultivars evaluated in the experiment, it is observed that both were hosts for *P. brachyurus*, thus limiting their use in areas of high infestation. However, studies must be carried out to find genes that have resistance as an important characteristic on the root-lesion nematode so that the plant can maintain its agronomic characters desired by producers, which is a way to minimize production losses that impact negatively on production and economic gain.

4. Conclusion

Soybean cultivars Brasmax Bônus and Nidera NS8383 hosted root-lesion nematodes *Pratylenchus brachyurus*.

The agronomic character plant height (cm) was more affected when the biological method was not applied.

The association of No-Estio[®], Bio-fertility[®], and Radic[®] reduced the population density of nematodes *P. brachyurus*.

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