

***Pratylenchus brachyurus* Suppression by Organic Fertilizers and the Development of Soybean Plants**

Supressão de *Pratylenchus brachyurus* por Fertilizantes Orgânicos e Desenvolvimento de Plantas de Soja

Supresión de *Pratylenchus brachyurus* Mediante Fertilizantes Orgánicos y Desarrollo de Plantas de Soja

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Abstract

The root lesion nematode, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, has been caused significant losses in soybean crops in Brazil. The addition of organic fertilizers may suppress the population of these plant-parasitic nematodes. The aim of this work was to evaluate the action of cattle manure, cattle vermicompost, cattle compost, and slaughterhouse swine compost on the reduction of *P. brachyurus* population and the development in soybean plants (cv. NR 5909). The experiment was conducted for 90 days after *P. brachyurus* inoculation (DAI) in the pots, in greenhouse. At 90 DAI, shoots, roots and soil were collected from all experimental units to determine shoot dry matter, root fresh mass and nematodes quantification present in roots and soil. All the organic fertilizers promoted the increase of shoot dry matter and root fresh mass in relation to control, even under nematode infestation

in soybean plants (cv. NR 5909). However, organic fertilizers did not promote a suppressive effect on *P. brachyurus* population in soybean plants.

Keywords: *Glycine max*; Nematode alternative control; Organic agriculture; Organic waste; Root lesion nematodes; Teaching.

Resumo

O nematóide das lesões radiculares, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, tem causado perdas significativas na cultura da soja no Brasil. A adição de fertilizantes orgânicos pode suprimir a população desses nematoides parasitas de plantas. O objetivo deste trabalho foi avaliar a ação de esterco bovino, vermicomposto bovino, composto bovino e composto suíno de matadouro na redução da população de *P. brachyurus* e no desenvolvimento de plantas de soja (cv. NR 5909). O experimento foi conduzido por 90 dias após a inoculação de *P. brachyurus* (DAI) nos vasos, em casa de vegetação. Aos 90 DAI, a parte aérea, as raízes e o solo foram coletados de todas as unidades experimentais para determinação da matéria seca da parte aérea, massa fresca das raízes e quantificação dos nematoides presentes nas raízes e no solo. Todos os fertilizantes orgânicos promoveram aumento da matéria seca da parte aérea e massa fresca da raiz em relação à testemunha, mesmo sob infestação de nematoides em plantas de soja (cv. NR 5909). No entanto, os fertilizantes orgânicos não promoveram efeito supressor sobre a população de *P. brachyurus* em plantas de soja.

Palavras-chave: *Glycine max*; Controle alternativo de nematoides; Agricultura orgânica; Resíduos orgânicos; Nematoides das lesões radiculares; Ensino.

Resumen

El nematodo de las lesiones de la raíz, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, ha causado pérdidas significativas en los cultivos de soja en Brasil. La adición de fertilizantes orgánicos puede suprimir la población de estos nematodos parásitos de plantas. El objetivo de este trabajo fue evaluar la acción del estiércol vacuno, vermicompost vacuno, compost vacuno y compost porcino de matadero sobre la reducción de la población de *P. brachyurus* y el desarrollo de plantas de soja (cv. NR 5909). El experimento se realizó durante 90 días después de la inoculación de *P. brachyurus* (DAI) en las macetas, en invernadero. A los 90 DAI, se recolectaron brotes, raíces y suelo de todas las unidades experimentales para determinar la materia seca de los brotes, la masa fresca de raíces y la cuantificación de nematodos presentes en raíces y suelo. Todos los fertilizantes orgánicos promovieron el aumento de materia seca aérea y masa fresca de raíz en relación al control, incluso bajo infestación de nematodos en plantas de soja (cv. NR 5909). Sin embargo, los fertilizantes orgánicos no promovieron un efecto supresor sobre la población de *P. brachyurus* en las plantas de soja.

Palabras clave: *Glycine max*; Control alternativo de nematodos; Agricultura orgánica; Residuos orgánicos; Nematodos lesionadores de raíces; Enseñanza.

1. Introduction

The root lesion nematode, *Pratylenchus brachyurus* (Godfrey), has caused significant losses in several crops in Brazil, including soybean (Dias et al. 2010; Schmitt et al., 2020). The edaphoclimatic conditions and intensive cultivation of susceptible crops provide for these nematode favorable conditions for the development, dissemination and survival in the soil and roots of plants (Costa et al. 2012, Abd-Elgawad et al., 2021). *Pratylenchus* is a genus of nematode that has a migratory endoparasitic habit and, due to the feeding mode, movement, and injection of toxins inside the root tissues, results in the reduction of the vegetative canopy and grain yield (Ferraz & Brown 2016). In the case of soybean cultivation, production losses can reach 30% (Dias et al. 2010).

In order to reduce losses and achieve the sustainability of agricultural production, it is necessary to use a set of strategies that aim to reduce population nematodes levels and stimulating crop production and root development in coexistence with these parasites (Costa et al. 2012, Trentin et al., 2021). These strategies mainly include cultural practices, resistant cultivars, biological and chemical control. Therefore, due to the absence of resistant cultivars associated to the inconsistency of the results achieved with the use of chemical nematicides, the alternative control with the addition of organic material to the soil has been presented as a promising strategy for the management of *P. brachyurus* (Silva, 2016).

In cultural practices, organic material added to the soil could perform several functions, such as cycling and nutrient availability, fertilizer solubilization, toxic metal complexation, soil buffering power, gas flow into the atmosphere and soil aggregation (Stevenson 1994, Soares et al. 2008, Leite et al., 2019). Brazil is among the world's largest producers and

exporters of beef, poultry, and pork meat (FAO, 2017) resulting in the generation of organic waste. The organic waste could be used in agriculture as biofertilizers; however, there is a great lack of studies on its suppressive potential in the soil, whether they are used for composting (Schmitt et al., 2020) and/or vermicomposting (Steffen et al. 2017). Thus, there is a need to evaluate the waste management as to its potential for use in agriculture, especially in the suppression of plant-parasitic nematodes. Therefore, the objective of this study was to evaluate the effect of organic fertilizers, fresh, composted and vermicomposted, in reducing the population of *P. brachyurus* and the development of soybean plants (cv. NR 5909).

2. Methodology

Organic fertilizers, soil and *Pratylenchus brachyurus* used in experiments

The treatments tested in the study were four organic fertilizers: cattle manure (CM); cattle vermicompost (CV) (Vione et al. 2018), cattle compost (CC) (Ramires et al. 2019) and slaughterhouse swine compost (SSC). The cattle manure residue was obtained from the dairy cattle sector of the Federal University of Santa Maria and the slaughterhouse swine residue was obtained from a slaughterhouse located in the northern region of Rio Grande do Sul, Brazil. Cattle manure residue was previously dried and chemically analyzed, and the other fertilizers were previously treated, stabilized by aerated composting (CC and SSC) and by vermicomposting (CV). Organic fertilizers were chemically characterized (Table 1). Mineral fertilization (MF) was used as a control treatment.

Table 1. Chemical analysis of organic fertilizers, cattle manure (CM); cattle vermicompost (CV); cattle compost (CC); Slaughterhouse swine compost (SSC), used in this study for fertilization of soybean plants under greenhouse conditions.

Variables	CM	CV	CC	SSC
pH - H ₂ O	7.1	7.2	6.5	4.4
C-organic % (m/m)	17.4	17.0	12.0	32.0
N % (m/m)	1.2	1.1	1.1	4.7
C/N	14.0	15.4	11.0	7.0
P % (m/m)	0.7	1.6	0.8	1.6
K % (m/m)	1.2	0.3	0.7	0.3
Ca % (m/m)	1.1	4.1	1.2	4.1
Mg % (m/m)	1.5	0.5	0.6	0.5
Na % (m/m)	0.1	0.04	0.1	0.2

C-organic: organic carbon; N: nitrogen; C/N: carbon/nitrogen ratio; P: phosphor; K: potassium; Ca: calcium; Mg: magnesium; Na: sodium. Source: Authors (2022).

The soil used in the experimental units, classified as typical Ultisol (Soil Survey Staff 2014), was chemically characterized and autoclaved for one and a half hours at 120°C, together with the sand used in the mixture (2:1) for the experiments. The values obtained in the chemical analysis of the soil are: pH (1:1) 4.9, Ca²⁺ (cmol_c dm³) 1.7, Mg²⁺ (cmol_c dm³) 0.5, Al³⁺ (cmol_c dm³) 23.3, H+Al (cmol_c dm³) 6.9, CTC pH7 (cmol_c dm³) 9.2, base saturation (%) 25.3, SMP Index 5.6, SOM (%) 1.5, P-Mehlich (mg dm⁻³) 13.1, and K (mg dm⁻³) 44.0.

The inoculum of the nematode (*P. brachyurus*) used in the experiments, consisted of a pure population. Nematode multiplication was performed continuously in a greenhouse using sorghum (*Sorghum bicolor* L.) and soybean (cv. NR 5909) previously inoculated with *Bradyrhizobium elkanii*. After three months, the nematodes were extracted from the roots according to Coolen & D'Herde (1972) and the nematode suspension was calibrated for inoculation of the experiment.

Liming and organic fertilization of the soil

The fertilization and liming of the experimental units were performed to obtain the maximum yield of soybean, following preliminary analysis of soil and organic fertilizers (CQFS-RS/SC 2016). To satisfy the nutritional needs of soybean crop, it was necessary 0,0; 95,0 and 105,0 kg.ha⁻¹ of N, P and K, respectively. The amounts of organic fertilizer required for each treatment were converted to grams per kilogram of soil and the dose was calculated according CQFS-RS/SC (2016).

Evaluation of *Pratylenchus brachyurus* reproduction in soybean plants

To evaluate the reproduction factor of *P. brachyurus* in greenhouse conditions, soybean plants (NR 5909, Nidera) were used. The experimental units were 3 dm³ pots containing 3 kg of a soil/sand mixture (2:1). The seeds used were previously inoculated with *Bradyrhizobium japonicum* and sown in the pots containing the mixture of soil and fertilizer. The quantities of organic fertilizers were balanced according to the most demanding nutrient from the crop, in this case potassium (CQFS-RS/SC 2016) and the amount of soil in the experimental unit. Thus, the experimental units received organic fertilizers in the doses of 24.5; 24.0; 80.6 and 64.0 grams of CM, CV, CC and SSC, respectively. The control treatment received 0.2 grams of mineral fertilizer (K₂HPO₄).

The experiment was conducted in a completely randomized design with five treatments and six replications. Fifteen days after germination of soybean plants, about 8.000 *P. brachyurus* specimens were inoculated per pots, through three holes near to the plant.

The experiment was conducted for 90 days after *P. brachyurus* inoculation (DAI) in the pots. At 90 DAI, shoots, roots and soil were collected from all experimental units to determine shoot dry matter, root fresh mass and quantification of nematodes present in roots and soil. The shoot dry matter was determined by weighing, after drying in an oven at 65°C for 24h. Fresh root mass was determined by direct weighing immediately after sample collection. To quantify the number of nematodes present in soybean roots was used the methodology of Coolen & D'Herde (1972) and to quantify the number of nematodes in the soil was used the method of centrifugal flotation in sucrose solution (Jenkins 1964), using 250 cm³ of soil.

The Reproduction Factor (RF) of the nematode was calculated according to the proposed by Oostenbrink (1966), where $RF = FP / IP$ (final population)/IP (initial population). As the evaluated nematode is considered a migratory endoparasite, the total number of nematodes found in both roots and soil of each treatment was used to calculate the PF. Also, the number of nematodes per gram of root was calculated.

Statistical analysis

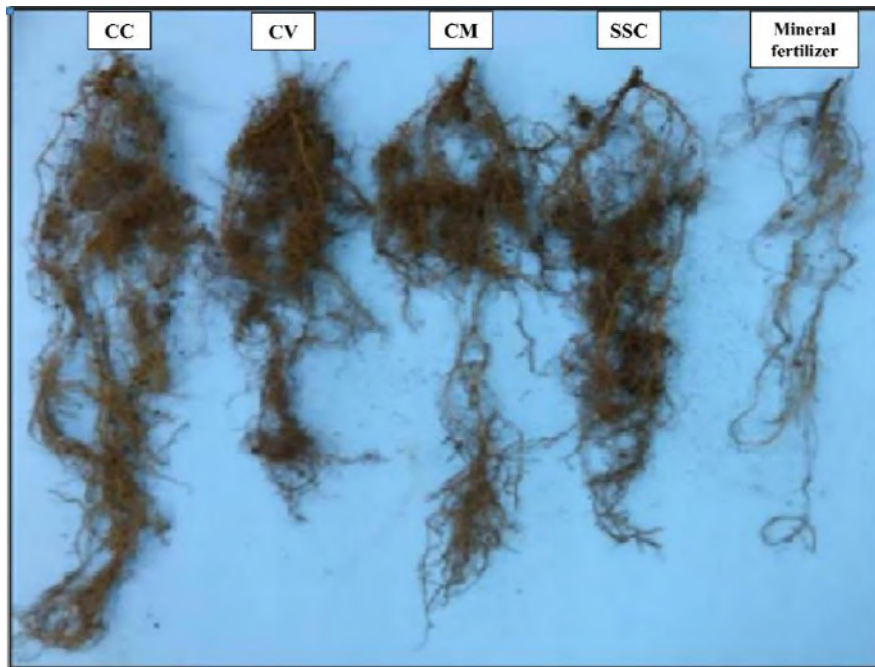
The data were submitted to the Shapiro-wilk normality test (5% probability). The data considered non-normal were transformed by the formula $\sqrt{x + 1}$. Subsequently, the analysis of variance (ANOVA) was performed and, complementary orthogonal contrast tests using the Dunnett test ($p \leq 0.05$). We compared treatments versus control treatment and treatments versus treatments, using the multcomp package (Ferreira et al. 2018) in the software R (Team & Core 2019).

3. Results and Discussion

All treatments with organic fertilizers promoted an increase of shoot dry matter and fresh root mass in relation to the mineral fertilization after 90 DAI in soybean plants (Table 2 and 3; Figure 1). This effect on plant development is possibly related to the presence of humic and fulvic acids in the organic fertilizers used. The humic substances are related to the increase in nutrient absorption, due to the influence on cell membrane permeability and chelating power, as well as photosynthesis, ATP formation, amino acids and proteins (Vaughan et al. 1985, Gerke 2018). These affect directly the

biochemical metabolism of plants and, consequently, may influence their growth and development (Façanha et al. 2002, Ferdous et al. 2018).

Figure 1. Soybean plants (cv. NR 5909) submitted to different organic fertilizers, 90 days after inoculation of *Pratylenchus brachyurus* in the greenhouse conditions. CC – cattle compost; CV- cattle vermicompost; CM- cattle manure; SSC- slaughterhouse swine compost.



Source: Authors (2022).

Table 2. Shoot dry matter (SD), fresh root mass (FR), number of nematodes present in the soil (NNS), number of nematodes present in the roots (NNR), final population (FP = NNS + NNR), number of nematodes per gram of roots (NNGR) and reproduction factor (RF) of the treatments tested in the suppression, 90 days after inoculation of *Pratylenchus brachyurus* in soybean plants (NR 5909) under greenhouse conditions.

Treatments	SD (g)	FR (g)	NNS	NNR	FP	NNGR	RF
Mineral fertilizer	3.7	2.9	2.400.0	21,000.0	23,400.0	8,979.0	2.9
Cattle Manure	5.8	7.3	19.100.0	5,750.0	24,850.0	935.0	3.1
Cattle Compost	7.6	8.6	16.600.0	3,167.0	19,767.0	503.0	2.5
Cattle Vermicompost	6.5	9.5	11.700.0	5,333.0	17,033.0	631.0	2.1
Slaughterhouse swine compost	5.1	5.8	23.200.0	14,667.0	37,867.0	3,500.0	4.7

SD – shoot dry matter; FR – fresh root matter; NNS – number of nematodes in soil; NNR – number of nematodes in roots; FP – final population; NNGR – number of nematodes to root gram and RF- reproduction factor. Source: Authors (2022).

Table 3. Analysis of orthogonal contrasts between the treatments tested in the suppression, 90 days after inoculation of *Pratylenchus brachyurus* in soybean (cv. NR 5909) under greenhouse conditions. (MF- Mineral fertilizer (control treatment); CM- cattle manure; CC – cattle compost; - CV- cattle vermicompost and SSC- slaughterhouse swine compost)

Contrasts*	SD	FR	NNS	NNR	FP	NNGR	RF
MF × CM	<i>S</i>	<i>ns</i>	<i>s</i>	<i>s</i>	<i>ns</i>	<i>s</i>	<i>ns</i>
MF × CC	<i>S</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>ns</i>	<i>s</i>	<i>ns</i>
MF × CV	<i>S</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>
MF × SSC	<i>ns</i>	<i>ns</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>
CM × CC	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
CM × CV	<i>ns</i>	<i>ns</i>	<i>s</i>	<i>ns</i>	<i>s</i>	<i>ns</i>	<i>s</i>
CM × SSC	<i>ns</i>	<i>ns</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>
CC × CV	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
CC × SSC	<i>S</i>	<i>ns</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>
CV × SSC	<i>ns</i>	<i>ns</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>

**s* - significant; *ns* - not significant through orthogonal contrasts using the Dunnett test ($p \leq 0,05$). SD – shoot dry matter; FR – fresh root matter; NNS – number of nematodes in soil; NNR – number of nematodes in roots; FP – final population; NNRG – number of nematodes to root gram and RF- reproduction factor. Source: Authors (2022).

About the number of nematodes present in the soil, all organic fertilizers differed significantly when contrasted against the MF (control treatment), with higher averages. However, for the number of nematodes present in the soybean roots, all organic fertilizers were significantly lower when compared to the control treatment (Table 2 and 3). This suppression of plant pathogens generally occurs indirectly due to improvements in the chemical, physical and biological properties of soils (Castillo & Vovlas 2007, Coutinho et al. 2009), including changes in pH, nutrient availability, release of compounds during decomposition, moisture, aeration, and soil structure, resulting in addition or increased of antagonist microorganisms (Oka 2010). Furthermore, higher levels of organic matter and potassium in the soil were related to reduce the number of root-penetrated *P. brachyurus* in soybean plants and the possible increase of plant resistance (Freitas et al. 2017). In addition, potassium increases the thickness of the epidermal cell wall, and in this case, a greater stiffness of the plant root tissue may have hampered nematode action (Rocha et al. 2007).

In relation final population data, the treatments SSC and CV differed significantly when contrasted against the control treatment, in which SSC presented superior means, and the CV presented inferior mean values. The other treatments (CM and CC) did not differ significantly from the control treatment. For the number of nematodes per gram of roots, all the organic fertilizers had significantly lower averages compared to control treatment (mineral fertilization) (Table 2 and 3). Regarding to the reproduction factor, the SSC and CV differed significantly from the control treatment, in which the SSC presented higher averages, and the CV lower mean values. When organic fertilizers were compared to each other, using orthogonal contrasts (Table 2), the CC showed significant differences when compared to the SSC, and in relation to the vegetative variable SD, the average obtained was higher (Table 2).

According to these results, the influence of organic material added to the soil is dependent on the composition of the material used. This could favor the growth and development of antagonistic species in the soil (e.g., fungi, bacteria, free-living nematodes), and the supply of different metabolites released by their decomposition, either for the suppression of phytoparasites or for plant nutrition (Akhtar & Malik 2000). Therefore, well-nourished plants generally have greater root system development, allowing them to be more tolerant to pathogen attack, reducing the impact of yield losses (McSorley & Gallaher 1995, McSorley 1998, Bridge 2000, Ritzinger et al. 2006). The same occur to the others nematological variables, such as the number of nematodes present in the soil, nematodes present in soybean roots, final population, number of nematodes per

gram of roots and reproduction factor, the averages found were lower than the SSC (Tables 2 and 3). And the CV and CM had significantly higher averages than SSC only in the physiological variables, shoot dry matter and fresh root matter (Tables 2 and 3).

The SSC has large amounts of N in its composition, about 75% more N than cattle manure-based fertilizers (Table 1) Ammonia, one of the major substances released during N microbial decomposition, has often been associated with plant-parasitic nematode control due its relationship in inducing plasmolysis (Ferraz et al. 2010). The amount of ammonia produced varies according to the N content of the residue (Rodríguez-Kábana 1986). However, SSC showed no reduction in the incidence of *P. brachyurus* in soybean plants, due to C/N ratio ($C/N = 7$). Thus, this C/N ratio favor soil N accumulation, causing phytotoxicity to plants and losses by leaching and volatilization (Stirling 1991). In addition, organic compounds of animal origin, when incorporated into the soil, may have a suppressive or conductive effect depending on its C/N ratio, with the optimum range being between 14 and 20 (Pereira et al. 1996). Thus, the cattle manure fertilizers have better action potential in the plant-parasitic nematode complex, due the C/N ratio great ($C/N = 14$).

Therefore, knowledge of the nutritional and pollutant potential of organic materials is important for the sustainability of the production system. In addition, it is known that after the establishment of a population of nematodes in a given area, its eradication is practically impossible. Finally, the use of combined strategies, such as integrated management, from the use of resistant cultivars, crop rotation, biological control, and the reuse of organic materials, among others, is necessary within the production system where there is a presence of plant-parasitic nematodes.

4. Conclusion

According to the results obtained in this work with reuse of organic materials further studies should be carried out regarding the effect of SSC on *P. brachyurus* population dynamics in soybean plants. The organic fertilizers, cattle manure, cattle compost, cattle vermicompost and slaughterhouse swine compost, promoted the increase of shoot dry matter and root fresh mass in relation to conventional mineral fertilization in soybean plants (cv. NR 5909). However, the organic fertilizers not promoted suppressive effect on *P. brachyurus* population to these soybean plants.

This work contributed to the search for alternative substrates that can be used to control *P. brachyurus*, minimizing the use of chemical fertilizers and nematicides, enabling a more sustainable use of the environment. However, there is a need for further studies involving the potential of using different organic fertilizers, of animal, plant and agro-industrial origin, as well as analyzes of the nematicidal compounds released by this material.

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