Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252022v35n313rc

GREEN MANURE AND *Pochonia chlamydosporia* FOR *Meloidogyne javanica* CONTROL IN SOYBEAN[1]

LUIZA EDUARDA STRAMBAIOLI GARCIA ALVES[2], LAÍS FERNANDA FONTANA[2],
CLAUDIA REGINA DIAS-ARIEIRA[2]*

ABSTRACT - *Pochonia chlamydosporia* (*Pc*) is a nematophagous fungus with saprotrophic activity. However, little is known about the interaction between *Pc* and green manure. This study aimed to investigate the interaction effects of different green manures and *Pc* on the control of *Meloidogyne javanica* in soybean. Two greenhouse experiments were conducted in different periods using a 6 × 2 factorial design, with six replicates. The first factor was green manure application (oat, brachiaria, crotalaria, millet, buckwheat, and untreated control) and the second factor was treatment with *Pc* (in-furrow application and untreated control). Cover crops were grown separately and applied to pots as green manure 15 days before soybean sowing. At 5 days after sowing, soybean was inoculated with 2 000 eggs and juveniles of *M. javanica*. At 60 days after inoculation, nematode and vegetative variables were determined. All green manures reduced nematode population levels, especially oat, crotalaria, and buckwheat. *P*c treatment did not influence nematode population levels. Soybean plants treated with oat or crotalaria green manure had greater height than untreated plants in both experiments. The effects of factors on shoot fresh and dry weights differed between experiments, and green manure application did not affect root development. The findings confirmed the potential of plant residues to control *M. javanica*.

Keywords: Biological control. Nematophagous fungi. Organic matter. Root-knot nematode.

RESÍDUOS DE PLANTAS DE COBERTURA E *Pochonia chlamydosporia* NO CONTROLE DE *Meloidogyne javanica* EM SOJA

RESUMO - *Pochonia chlamydosporia* (*Pc*) em um fungo nematófago, com atividade saprofítica em matéria orgânica. Contudo, pouco é conhecido a respeito da interação entre resíduos de coberturas verdes e o fungo. Objetivou-se avaliar o efeito da associação de resíduos de diferentes espécies de plantas de cobertura com *Pc* no controle de *Meloidogyne javanica* em soja. Dois experimentos foram conduzidos em épocas distintas, em casa-de-vegetação, em fatorial 6 x 2, com a palhada de cinco plantas de coberturas (aveia, braquiária, crotalária, milheto e trigo mourisco) e uma testemunha sem palhada, com e sem *Pc*. Para realização dos experimentos, as palhadas foram produzidas separadamente e aplicadas nos vasos 15 dias antes da semeadura da soja. Após cinco dias da semeadura, a soja foi inoculada com 2000 ovos e juvenis do nematoide. Aos 60 dias da inoculação, foram realizadas avaliações das variáveis nematológicas e vegetativas. Apenas a adição de resíduos promoveu redução na população de nematoides, sendo as menores médias observadas para aveia, crotalária e trigo mourisco. Plantas que cresceram sob palhada de aveia e crotalária apresentaram maior altura em ambos os experimentos. Resultados de massa fresca e seca de parte aérea variaram entre os experimentos, e as coberturas não afetaram o crescimento da raiz. A pesquisa comprovou o potencial dos resíduos vegetais em controlar *M. javanica*.

Palavras-chave: Controle biológico. Fungos nematófagos. Matéria orgânica. Nematoide das galhas.

*Corresponding author
[1]Received for publication in 06/10/2021; accepted in 04/22/2022.
Paper extracted from the Completion of Course Work of the first author.
[2]Universidade Estadual de Maringá, Umuarama, PR, Brazil; luizaeduardasg@gmail.com - ORCID: 0000-0002-3717-1984, laisffontana@hotmail.com - ORCID: 0000-0001-5266-2725, crdarieira@uem.br - ORCID: 0000-0002-1567-816X.

INTRODUCTION

Pant-parasitic nematodes are among the major yield-limiting factors in soybean production. In Brazil, the most damaging species are *Heterodera glycines* Ichinohe, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, *Meloidogyne incognita* (Kofoid & White) Chitwood, and *M. javanica* (Treub) Chitwood (FAVORETO et al., 2019). Within the genus *Meloidogyne*, the species *M. javanica* is the most widespread in soybean fields (MATTOS et al., 2016). Complex interactions between nematodes and host cells cause hypertrophy and hyperplasia in the host's root tissues, leading to the formation of galls, which may vary in size and quantity depending on the susceptibility of the cultivar and the aggressiveness of the nematode population (FERRAZ; BROWN, 2016). Characteristic field symptoms include patches of plants showing stunted growth, yellowish leaves, aborted flowers, and undeveloped pods (FORTI et al., 2015).

Crop rotation, resistant cultivars, and application of chemical or biological nematicides are the most common methods for controlling nematodes (FAVORETO et al., 2019). However, some factors may limit the adoption of these practices, such as high genetic variability in nematodes, occurrence of mixed populations, lack of resistant genotypes, and reduced options of chemical nematicides. These limitations, combined with the increasing interest in more sustainable management methods, have stimulated the growth of the biological control market. According to data from Spark - Strategic Intelligence, the Brazilian market of biological nematicides for soybean crops grew by 45% in the 2018/2019 season compared with 2017/2018 (PORTAL DO AGRONEGÓCIO, 2019).

Biological control of nematodes is defined as the modulation of nematode population density by the action of living organisms (KÖHL; KOLNAAR; RAVENSBERG, 2019). A bionematicide with great potential in the control of *Meloidogyne* spp. is the chitinolytic fungus *Pochonia chlamydosporia* (*Pc*) Zare & Gams (MANZANILLA-LÓPEZ et al., 2013; MEDEIROS et al., 2015; MONTEIRO et al., 2018). The fungus can parasitize nematode eggs as well as sedentary females and, in the absence of nematodes, can live as a saprophyte, growing in organic residues in the soil (DALLEMOLE-GIARETTA et al., 2011; MANZANILLA-LÓPEZ et al., 2013; MEDEIROS et al., 2015). Furthermore, *Pc* produces chlamydospores, asexual survival structures that persist in soil under adverse conditions (KERRY; HIRSCH, 2011; FERNANDES et al., 2017). Yet another advantage of this fungal agent is its endophytic behavior; the fungus can live in plant tissues, promoting plant growth (ZAVALA-GONZALEZ et al., 2017) and inducing resistance to pathogenic microorganisms (MANZANILLA-LÓPEZ et al., 2013).

In addition to biological control, crop rotation with cover crops is an important strategy in the management of root-knot nematodes (DALLEMOLE-GIARETTA et al., 2011; DONG et al., 2013; NASCIMENTO et al., 2020). Cover crops help reduce nematode population density because they accumulate organic matter, which can stimulate soil biological activity, favoring the development of antagonistic microorganisms (DONG et al., 2013; KARLEN et al., 2013). Furthermore, during decomposition, plants can release nematicidal compounds into the soil (CHITWOOD, 2002).

Research has shown that biological agents and green manure are effective against nematodes; however, little is known about the effects of green manure on biological agents. It is hypothesized that organic residues from cover crops can serve as substrates to improve plant colonization by saprophytic nematophagous fungi. On the other hand, chemical compounds released during organic matter decomposition may impair the establishment of biological agents. This study aimed to assess the interaction effects of different green manures and *Pc* on *M. javanica* control and soybean development.

MATERIAL AND METHODS

Experiments were performed in a greenhouse. A completely randomized design arranged in a 6 × 2 factorial was used to assess the interaction between green manure treatments (five green manures and an untreated control) and *Pc* treatment (treated and untreated plants), with six replicates. The experiment was conducted from August 2019 to January 2020 (Experiment 1) and repeated from January to June 2020 (Experiment 2) to confirm the results.

Seeds of *Urochloa ruziziensis* Germain & Evrard (=*Brachiaria ruziziensis*), *Crotalaria spectabilis* Roth, *Pennisetum americanum* (L.) Leeke (millet) 'ADR 300', *Avena sativa* L. (common oat) 'IPR Afrodite', and *Fagopyrum esculentum* Moench (buckwheat) 'IPR 92 Altar' were sown in pots containing 5 L of soil and grown for 60 days to obtain sufficient green manure biomass. During this phase, the mean minimum and maximum temperatures were 17.2 and 28.5 °C in Experiment 1 and 25.6 and 32.6 °C in Experiment 2.

After 60 days, the aerial parts of green manure crops were harvested, weighed, and chopped into pieces of about 1 cm. Green manure was applied to the soil surface at a rate of 5.6 t ha^{-1} in Experiment 1 and 1.1 t ha^{-1} in Experiment 2. This difference in application rate was due to differences in green manure production between experimental periods. Pots without green manure were used as control.

Experimental units consisted of polystyrene pots containing 500 cm^3 of a 2:1 (v/v) mixture of soil and sand previously sterilized in a vertical autoclave at 120 °C for 2 h. Seven days before green manure application, the substrate was amended with 0.4 g of limestone (85% relative neutralizing power) and fertilized with 0.24 g of NPK (14-14-14) fertilizer per experimental unit. During this phase of the experiment, the mean minimum and maximum temperatures were 21.9 and 30.8 °C in Experiment 1 and 20.6 and 28.2 °C in Experiment 2.

Fifteen days after green manure application, crop residues were deposited on the soil surface. After a further five days, one seed of soybean 'M6210 IPRO' was sown in each pot and half of the experimental units received an in-furrow application of *P. chlamydosporia* Pc-10 (Rizotec®, 5.2×10^7 chlamydospores g^{-1}) at a dose equivalent to 2.5 kg product ha^{-1} and a spray volume equivalent to 400 L ha^{-1}. At 5 days after sowing, 2 000 eggs and eventual second-stage juveniles (J2) of *M. javanica* were inoculated in two holes, about 3 cm deep, made in the soil around each plant, which were covered with soil after inoculation.

The inoculum was obtained from a pure population of *M. javanica* maintained on soybean in a greenhouse. Nematodes were extracted according to the method of Hussey and Barker as modified by Boneti and Ferraz (1981). Eggs and J2 were counted by using a Peters' counting slide under a photonic microscope, and the inoculum was adjusted to 1 000 eggs + J2 mL^{-1}.

At 60 days after inoculation, soybean plants were removed from the pots and separated into shoots and roots. The roots were carefully washed, weighed, and subjected to nematode extraction by the above-mentioned method. Nematodes were counted in a Peters' slide under a photonic microscope. Then, the total nematode number was divided by the root fresh weight to obtain the number of nematodes per gram of root (population density). Shoots were evaluated for height, fresh weight, and dry weight. For dry weight determination, samples were dried in a forced-air oven at 65 °C for 3 days.

Data were subjected to analysis of variance, and means were compared by the Scott–Knott test at the 5% significance level. When necessary, original data were transformed to $\sqrt{(x+0.5)}$ to meet normality assumptions, which were assessed using the Shapiro–Wilk test. Statistical analyses were performed using Sisvar software (FERREIRA, 2014).

RESULTS AND DISCUSSION

There were no significant interaction effects ($p < 0.05$) of green manure and fungal inoculation on nematode variables. In both experiments, only the total mean values of green manure treatments were significant ($p < 0.05$) (Table 1). In Experiment 1, all green manures reduced total nematode number and nematode population density compared with the control (Table 1). Reductions in total nematode number ranged from 56 to 76% in soybean with millet and crotalaria green manure, respectively. These treatments also led to 57–77% reductions in nematode population density. In Experiment 2, buckwheat, oat, and crotalaria green manures differed from the control, reducing total nematode number and nematode population density by 44–56% and 34–55%, respectively (Table 1). Many studies have demonstrated the importance of cover crops in the control of root-knot nematodes (CHITWOOD, 2002; DALLEMOLE-GIARETTA et al., 2011; DONG et al., 2013; NASCIMENTO et al., 2020); however, in most studies, nonhost or antagonistic plants are cultivated in nematode-infested soil, which makes it difficult to isolate the effects of cover crops from those of cover crop residues. Pigeon pea (*Cajanus cajan* (L.) Millsp.) and forage turnip (*Raphanus sativus* L.) were more effective in controlling *P. brachyurus* when used as green manure than when grown in rotation with soybean (VEDOVETO et al., 2013).

Soil amendment with organic matter is an important strategy for the control of nematodes, as it provides numerous benefits to crop systems, including improvements in soil microbiota and plant nutrition (OKA, 2010; NASCIMENTO et al., 2020). Plant residues can release nematicidal substances during decomposition. *Crotalaria* spp., for instance, synthesize secondary metabolites that have nematicidal effects, such as the pyrrolizidine alkaloid monocrotaline (CHITWOOD, 2002; COLEGATE et al., 2012). In buckwheat, this effect is attributed to the flavonoid rutin (KREFT; FABJAN; YASUMOTO, 2006). Members of the family Poaceae (oat, brachiaria, and millet) produce the cyclic hydroxamic acids 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), which have nematicidal activity (BUCHMANN et al., 2007).

The fact that millet and brachiaria green manures were not effective in controlling *M. javanica* in Experiment 2 might be due to the lower amount of amendment used. In the field, the amount of green manure may vary according to region, cultivar, and, particularly, weather conditions (CAMARGO; PIZZA, 2007). Furthermore, Poaceae species have high carbon/nitrogen (C/N) ratios, resulting in slow decomposition (PACHECO et al., 2013) and thereby reducing the release rate of chemical compounds and nutrients.

Table 1. Total number and population density (eggs + second-stage juveniles per gram of root) of *Meloidogyne javanica* in soybean treated or not with *Pochonia chlamydosporia* (*Pc*) and different green manures in two experimental periods.

Green manure	Experiment 1			Experiment 2		
	Without *Pc*	With *Pc*	Mean	Without *Pc*	With *Pc*	Mean
Total number of nematodes						
Untreated control	12 095	9 188	10 642 a	14 446	12 426	13 436 a
Millet	5 047	4 243	4 645 b	9 208	12 346	10 777 a
Brachiaria	4 583	3 522	4 052 b	14 485	13 837	14 161 a
Buckwheat	2 738	4 788	3 763 b	5 196	6 493	5 845 b
Oat	4 868	2 017	3 442 b	7 592	7 508	7 547 b
Crotalaria	2 648	2 547	2 597 b	9 271	9 344	9 305 b
Mean	5 330 A	4 384 A		10 073 A	10 325 A	
CV (%)	34.25			31.99		
Nematode population density (nematodes g^{-1} root)						
Untreated control	970	815	892 a	3 712	2 092	2 902 a
Millet	403	369	386 b	2 266	2 033	2 150 a
Brachiaria	404	307	356 b	2 123	2 615	2 369 a
Buckwheat	174	368	271 b	1 261	1 324	1 292 b
Oat	346	184	265 b	1 701	1 763	1 734 b
Crotalaria	210	194	202 b	2 023	1 780	1 910 b
Mean	418 A	373 A		2 189 A	1 934 A	
CV (%)	32.10			30.37		

Means followed by the same lowercase letter within a column or the same uppercase letter within a row are not significantly different at $p < 0.05$ by the Scott–Knott test. Original data were transformed to $\sqrt{(x + 0.5)}$ before analysis. Only the overall mean of each factor was subjected to statistical analysis because there were no significant interaction effects between factors. CV, coefficient of variation.

It was expected that green manure and *Pc* treatments would exert additive effects, as the fungus is saprophytic (MANZANILLA-LÓPEZ et al., 2013) and has proven effective in controlling root-knot nematodes, affording reductions of up to 70% (MEDEIROS et al., 2015; BARBOSA et al., 2019; DALLA PASQUA et al., 2020). However, no interaction effects were found in the current study. Dallemole-Giaretta et al. (2011) obtained similar results in evaluating the effects of cover crop (millet and forage turnip) and *Pc* on nematode control. A study on the association between *Pc* and pumpkin seed flour showed that the isolated use of organic material did not differ from the combined use of plant residues and fungi for *M. javanica* control (DALLEMOLE-GIARETTA et al., 2010). The combined use of *Trichoderma*- and *Bacillus*-based products with agricultural wastes, such as poultry litter, filter cake, coffee husk, and rice hull, had a negative effect on nematode control, demonstrating the deleterious effects of organic residues on biocontrol agents (HERNANDES et al., 2020). Nevertheless, in the same study, such combinations were also shown to exert positive or neutral effects on plants, corroborating the findings of Chen, Abawi, and Zuckerman (2000).

There are some hypotheses for the lack of additive effects between treatments. One is that green manure might have exerted a major effect because of the high amount used, suppressing the secondary effect of the fungus. Time and re-inoculation may also be determinant, especially under field conditions. There is also the possibility that green manure served as a food source for fungi, decreasing nematode parasitism. These hypotheses are supported by the facts that plant organic matter is effective in controlling nematodes (DALLEMOLE-GIARETTA et al., 2010; DALLEMOLE-GIARETTA et al., 2011; MELO; SERRA, 2019; NASCIMENTO et al., 2020) and that fungi and bacteria are sensitive to chemicals produced by plants (CHEN; ABAWI; ZUCKERMAN, 2000; VILCHIS-MARTÍNEZ et al., 2013; PAGNUSSATT et al., 2013). *Crotalaria ochroleuca* G.Don, for instance, exerts suppressive effects on *Pc* (VILCHIS-MARTÍNEZ et al., 2013).

These findings need to be carefully analyzed, as the use of biological control agents in combination with cover crops is a widely recommended strategy for integrated nematode management. Regardless of the lack of effect or suppressive activity of cover crops on *Pc*, fungal treatment may lead to different results from those observed here, necessitating further research. Furthermore, the dose of organic material should be investigated; at small doses, plant residues may function as carriers, representing a source of nutrients for the initial growth of biocontrol agents (CHEN; ABAWI; ZUCKERMAN, 2000).

The factors green manure and *Pc* did not show significant interaction effects on plant height ($p < 0.05$), and green manure was the only factor to significantly influence this variable in both experiments (Table 2). Oat and crotalaria green manures afforded higher plant heights in both experiments. Soybean development was stimulated by buckwheat green manure in Experiment 1 and by brachiaria green manure in Experiment 2.

Table 2. Plant height and root fresh weight of soybean treated or not with *Pochonia chlamydosporia* (*Pc*) and different green manures in two experimental periods.

Green manure	Experiment 1			Experiment 2		
	Without *Pc*	With *Pc*	Mean	Without *Pc*	With *Pc*	Mean
			Plant height (cm)			
Untreated control	51.20	52.23	51.72 b	26.20	22.47	22.48 b
Millet	45.93	48.95	47.44 b	20.66	26.54	23.60 b
Brachiaria	54.30	53.85	54.07 b	29.01	27.86	28.43 a
Buckwheat	62.05	59.52	60.78 a	20.17	25.20	22.68 b
Oat	57.00	65.27	61.13 a	26.28	24.64	25.40 a
Crotalaria	63.03	64.27	63.58 a	26.20	26.64	26.41 a
Mean	55.59 A	57.32 A		24.13 A	25.56 A	
CV (%)		15.19			16.50	
			Root fresh weight (g)			
Untreated control	11.85	11.28	11.57 a	4.36	5.95	5.15 a
Millet	13.13	11.10	12.12 a	4.48	6.11	5.45 a
Brachiaria	11.75	11.82	11.78 a	6.80	5.34	6.07 a
Buckwheat	16.15	12.97	14.56 a	4.09	4.83	4.62 a
Oat	13.93	11.38	12.66 a	4.48	4.62	4.56 a
Crotalaria	12.52	13.45	12.98 a	4.85	5.05	4.94 a
Mean	13.22 A	12.00 B		4.09 A	5.32 A	
CV (%)		20.01			32.12	

Means followed by the same lowercase letter within a column or the same uppercase letter within a row are not significantly different at $p < 0.05$ by the Scott–Knott test. Only the overall mean of each factor was subjected to statistical analysis because there were no significant interaction effects between factors. CV, coefficient of variation.

The effect of buckwheat, oat, and crotalaria green manures on soybean development might be related to their rate of decomposition; this variable is known to differ across plant species (MENEZES; LEANDRO, 2004). Nutrient release is directly proportional to the time needed for decomposition (HENTZ et al., 2014). The degradation time of green manure is related to biomass composition and C/N ratio. Species belonging to the family Poaceae (e.g., oat, millet, and brachiaria) have high C/N ratios, resulting in slower decomposition than species of the family Fabaceae (e.g., crotalaria) and Polygonaceae (e.g., buckwheat), which mineralize more rapidly because of the low C/N ratio (PACHECO et al., 2013; HENTZ et al., 2014).

Similar to the observed for plant development, there were no interaction effects on root fresh weight in either experiment (Table 2). Green manure did not influence this variable ($p < 0.05$). On the other hand, in Experiment 1, plants treated with *Pc* had a lower root fresh weight (12.00 g) than plants not treated with fungi (13.22 g). Such findings may be due to the fact that *Pc* activates natural defense mechanisms in host plants, including expression of polyphenol oxidases and peroxidases (MEDEIROS et al., 2015), leading to energy expenditure and, consequently, temporarily compromising plant development. Another possibility is that the endophytic activity of fungi might have induced the activation of jasmonic acid-mediated defense mechanisms in plants (ZAVALA-GONZALEZ et al., 2017). This relationship is similar to that observed for mycorrhizal interactions. Mycorrhizal fungi must overcome the host's basal defenses by temporarily inhibiting salicylic acid-dependent signaling pathways and defense compound production (KLOPPHOLZ; KUHN; REQUENA, 2011).

Green manure and fungal treatment had a significant interaction effect ($p < 0.05$) on shoot fresh weight in Experiment 1 (Table 3). In the absence of *Pc*, soybean treated with buckwheat or crotalaria green manure had higher shoot fresh weight, whereas, in the presence of fungi, all treatments afforded higher shoot fresh weights than the control. In comparing the effects of fungal inoculation within each green manure treatment, we observed that soybean amended with buckwheat green manure had greater shoot growth in the absence of *Pc*. In Experiment 2, only green manure treatment significantly ($p < 0.05$) influenced shoot fresh weight, with higher values in plants amended with brachiaria green manure (Table 3).

The results of shoot dry weight were similar to those of shoot fresh weight. Significant interaction ($p < 0.05$) effects were observed in Experiment 1 (Table 3), with the highest shoot dry weights in plants not treated with fungi and amended with buckwheat or crotalaria green manure (Table 3). On the other hand, in the presence of fungi, only crotalaria green manure treatment differed from the control, affording higher values than the other treatments. As observed for shoot fresh weight, in plants treated with buckwheat green manure, fungal inoculation compromised development. In Experiment 2, green manure was the only factor to influence shoot dry weight. Soybean treated with oat green manure had lower shoot dry weight than plants treated with other green manures (Table 3).

Table 3. Shoot fresh and dry weights of soybean treated or not with *Pochonia chlamydosporia* (*Pc*) and different green manures in two experimental periods.

Green manure	Experiment 1*			Experiment 2†		
	Without *Pc*	With *Pc*	Mean	Without *Pc*	With *Pc*	Mean
			Shoot fresh weight (g)			
Untreated control	21.00 bA	17.88 bA	19.44	7.44	7.57	7.51 b
Millet	25.25 bA	28.63 aA	26.94	7.52	8.40	7.96 b
Brachiaria	23.60 bA	24.45 aA	24.02	12.09	9.64	10.86 a
Buckwheat	36.05 aA	25.70 aB	30.87	6.06	7.90	6.98 b
Oat	24.15 bA	29.43 aA	26.79	6.52	4.04	5.18 b
Crotalaria	30.02 aA	33.12 aA	31.57	8.66	7.96	8.34 b
Mean	26.53	26.68		8.10 A	7.59 A	
CV (%)		21.03			40.12	
			Shoot dry weight (g)			
Untreated control	5.35 bA	4.87 bA	5.11	2.29	1.90	2.09 a
Millet	6.10 bA	6.85 bA	6.47	1.77	2.19	1.98 a
Brachiaria	6.13 bA	6.15 bA	6.14	2.87	2.29	2.58 a
Buckwheat	8.45 aA	5.72 bB	7.08	1.92	1.77	1.85 a
Oat	6.30 bA	6.33 bA	6.32	1.61	0.96	1.26 b
Crotalaria	7.35 aA	8.47 aA	7.91	2.09	1.79	1.95 a
Mean	6.61	6.39		2.10 A	1.81 A	
CV (%)		22.10			33.84	

Means followed by the same lowercase letter within a column or the same uppercase letter within a row are not significantly different at $p < 0.05$ by the Scott–Knott test. *Main and interaction effects were analyzed. †Only the overall mean of each factor was subjected to statistical analysis because there were no significant interaction effects. CV, coefficient of variation.

A previous study on the effects of organic matter amendment and *Pc* on *M. javanica* control reported different results for shoot fresh weight (MACHADO et al., 2013). In the referred study, plants were treated with *Pc* + bovine manure, resulting in greater production of shoot biomass. Such results were attributed to the release of nutrients during organic matter decomposition and the ability of fungi to improve nutrient absorption in plants, particularly that of phosphorus (CALONEGO et al., 2012; MONTEIRO et al., 2018; GOUVEIA et al., 2019). The effects of treatments on vegetative development can be variable, as observed in both experiments of the current study and in previous research (DALLEMOLE-GIARETTA et al., 2011; MACHADO et al., 2013).

In contrast to previous reports showing that organic matter amendment and fungal treatment can potentiate nematode control when combined (PARIHAR et al., 2015), in the current study, we did not observe additive effects between control strategies. Knowledge of the factors affecting the activity of biocontrol agents and their interaction is very important. Thus, further studies are needed to confirm the interaction effects of *Pc* and green manures on the control of *M. javanica* in soybean and to determine optimal doses and application timing. Nevertheless, this research confirms the indisputable importance of crop management for reducing root-knot nematode population levels.

CONCLUSION

Organic matter amendment reduced nematode population levels in soybean, with a mean reduction in population density ranging from 35 to 67% in different experiments, but showed no additive effects with *Pc*. The fungus did not contribute to soybean development.

ACKNOWLEDGEMENTS

We thank the Brazilian National Council for Scientific and Technological Development (CNPq) for granting a research productivity fellowship to CRDA (grant no. 303269/2020-0).

REFERENCES

BARBOSA, R. T. et al. *Pochonia chlamydosporia* no controle do nematoide de galhas em bananeira. **Nematropica**, 49: 99-106, 2019.

BONETI, J. I. S.; FERRAZ, S. Modificações do método de Hussey and Barker para extração de ovos de *Meloidogyne exigua* em raízes de cafeeiro. **Fitopatologia Brasileira**, 6: 553-553, 1981.

BUCHMANN, C. A. et al. Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy -1,4-benzoxazin-3-one (DIBOA), two naturally occurring benzoxazinones contained in sprouts of Gramineae are potent aneugens in human-derived liver cells (HepG2). **Cancer Letter**, 246: 290-299, 2007.

CALONEGO, J. C. et al. Persistência e liberação de nutrientes da palha de milho, braquiária e labe-labe. **Bioscience Journal**, 28: 770-781, 2012.

CAMARGO, R.; PIZZA, R. J. Produção de biomassa de plantas de cobertura e efeitos na cultura do milho sob sistema de plantio direto no município de Passos, MG. **Bioscience Journal**, 23: 76-80, 2007.

CHEN, J.; ABAWI, G. S.; ZUCKERMAN, B. M. Efficacy of *Bacillus thuringiensis*, *Paecilomyces marquandii*, and *Streptomyces costaricanus* with and without organic amendments against *Meloidogyne hapla* infecting lettuce. **Journal of Nematology**, 32: 70-77, 2000.

CHITWOOD, D. J. Phytochemical based strategies for nematode control. **Annual Review of Phytopathology**, 40: 221-249, 2002.

COLEGATE, S. M. et al. Dehydropyrrolizidine alkaloids, including monoesters with an unusual esterifying acid, from cultivated *Crotalaria juncea* (sunn hemp cv. 'Tropic Sun'). **Journal of Agricultural and Food Chemistry**, 60: 3541-3550, 2012.

DALLA PASQUA, S. et al. Combined application of *Pochonia chlamydosporia* and solid by-product of the wine industry for the control of *Meloidogyne javanica*. **Applied Soil Ecology**, 147: 1-6, 2020.

DALLEMOLE-GIARETTA, R. et al. Efeito da farinha de sementes de abóbora e de *Pochonia chlamydosporia* no controle de *Meloidogyne javanica*. **Nematologia Brasileira**, 34: 91-97, 2010.

DALLEMOLE-GIARETTA, R. et al. Cover crops and *Pochonia chlamydosporia* for the control of *Meloidogyne javanica*. **Nematology**, 13: 919-926, 2011.

DONG, Z. et al. Response of soil nematodes to elevated temperature in conventional and no-tillage cropland systems. **Plant and Soil**, 373: 907-918, 2013.

FAVORETO, L. et al. Diagnose e manejo de fitonematoides na cultura da soja. **Informe Agropecuário**, 40: 18-29, 2019.

FERNANDES, R. H. et al. Survival of *Pochonia chlamydosporia* on the soil surface after different exposure intervals at ambient conditions. **Revista Iberoamericana de Micologia**, 34: 241-245, 2017.

FERRAZ, L. C. C. B.; BROWN, D. J. F. **Nematologia de Plantas: fundamentos e importância**. 1. ed. Manaus, AM: Norma Editora, 2016, 251 p.

FERREIRA, D. F. Sisvar: A Guide for its Bootstrap procedure in multiple comparisons. **Ciência e Agrotecnologia**, 38: 109-112, 2014.

FORTI, V. A. et al. *Meloidogyne javanica* infection of soybean plants: plant response, seed quality and green seeds occurrence. **Seed Science and Technology**, 43: 409-420, 2015.

GOUVEIA, A. S. et al. Understanding how *Pochonia chlamydosporia* increases phosphorus availability. **Geomicrobiology Journal**, 36: 747-751, 2019.

HERNANDES, I. et al. Biological products in association with organic matter to control *Meloidogyne javanica* in tomato. **European Journal of Horticultural Science**, 85: 14-21, 2020.

HENTZ, P. et al. Ciclagem de nitrogênio em sistemas de integração lavoura-pecuária. **Ciência e Natura**, 36: 663-676, 2014.

KARLEN, D. L. et al. Thirty-year tillage effects on crop yield and soil fertility indicators. **Soil & Tillage Research**, 130: 24-41, 2013.

KERRY, B. R.; HIRSCH, P. R. Ecology of *Pochonia chlamydosporia* in the rhizosphere at the population, whole organism and molecular scales. In: DAVIES, K.; SPIEGEL, Y. (Eds.). **Biological control of plant -parasitic nematodes: building coherence between microbial ecology and molecular mechanisms**. London, UK: Springer, 2011. v. 1, cap. 11, p. 171-182.

KLOPPHOLZ, S., KUHN, H., REQUENA, N. A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. **Current Biology**, 21: 1204-1209, 2011.

KÖHL, J.; KOLNAAR, R.; RAVENSBERG, W. J. Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. **Frontiers in Plant Science**, 10: 1-19, 2019.

KREFT, I; FABJAN, N.; YASUMOTO, K. Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. **Food Chemistry**, 98: 508-512, 2006.

MACHADO, J. C. et al. Controle de *Meloidogyne javanica* com *Pochonia chlamydosporia* e esterco bovino. **Bioscience Journal**, 29: 590-596, 2013.

MANZANILLA-LÓPEZ, R. H. et al. *Pochonia chlamydosporia*: Advances and challenges to improve its performance as a biological control agent of sedentary endo-parasitic nematodes. **Journal of Nematology**, 45: 1-7, 2013.

MATTOS, V. S. et al. *Meloidogyne* spp. populations from native Cerrado and soybean cultivated areas: Genetic variability and aggressiveness. **Nematology**, 18: 505-515, 2016.

MEDEIROS, H. A. et al. Induction of resistance in tomato against *Meloidogyne javanica* by *Pochonia chlamydosporia*. **Nematoda**, 2: 10015-10022, 2015.

MELO, T. A.; SERRA, I. M. R. S. Materiais vegetais aplicados ao manejo agroecológico de *Meloidogyne incognita* em tomateiro. **Summa Phytopathologica**, 45: 97-103, 2019.

MENEZES, L. A. S.; LEANDRO, W. M. Avaliação de espécies de coberturas do solo com potencial de uso em sistemas de plantio direto. **Pesquisa Agropecuária Tropical**, 34: 173-180, 2004.

MONTEIRO, T. S. A. et al. Nematophagus fungi increasing phosphorus uptake and promoting plant growth. **Biological Control**, 123: 71-75, 2018.

NASCIMENTO, D. D. et al. Crotalaria and millet as alternative controls of root-knot nematode infecting okra. **Bioscience Journal**, 36: 713-719, 2020.

OKA, Y. Mechanisms of nematode suppression by organic soil amendments- a review. **Applied Soil Ecology**, 44: 101-115, 2010.

PACHECO, L. P. et al. Ciclagem de nutrientes por plantas de cobertura e produtividade de soja e arroz em plantio direto. **Pesquisa Agropecuária Brasileira**, 48: 1228-1236, 2013.

PAGNUSSATT, F. A. et al. Promising antifungal effect of rice (*Oryza sativa* L.), oat (*Avena sativa* L.) and wheat (*Triticum aestivum* L.) extracts. **Journal of Applied Biotechnology**, 1: 1-5, 2013.

PARIHAR, K. et al. Role of oil cakes and *Pochonia chlamydosporia* for the management of *Meloidogyne javanica* attacking *Solanum melongena* L. **Journal of Plant Pathology and Microbiology**, 2015: 1-5, 2015.

PORTAL DO AGRONEGÓCIO. **Estudo da Spark aponta tendência de crescimento na adoção de produtos biológicos e registra avanço de ‘nematicidas’**. 2019. Disponível em: https://www.portaldoagronegocio.com.br/noticia/estudo-da-spark-aponta-tendencia-de-crescimento-na-adocao-de-produtos-biologicos-e-registra-avanco-de-nematicidas-186636. Acesso em: 26 fev. 2020.

VEDOVETO, M. V. V. et al. Adubos verdes no manejo de *Pratylenchus brachyurus* em soja. **Nematropica**, 43: 226-232, 2013.

VILCHIS-MARTÍNEZ, K. et al. Effect of the addition of crude plant extracts on the parasitism of *Pochonia chlamydosporia* var. *chlamydosporia* on *Meloidogyne incognita*. **Nematropica**, 43: 254-260, 2013

ZAVALA-GONZALEZ, E. A. et al. *Arabidopsis thaliana* root colonization by the nematophagous fungus *Pochonia chlamydosporia* is modulated by jasmonate signaling and leads to accelerated flowering and improved yield. **New Phytologist**, 213: 351-364, 2017.

This work is licensed under a Creative Commons Attribution-CC-BY https://creativecommons.org/licenses/by/4.0/