CONTROL OF *MELOIDOGYNE JAVANICA* AND *PRATYLENCHUS BRACHYURUS* WITH CRAMBE PRESSCAKE

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ABSTRACT

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The nematicidal effect of crambe, *Crambe abyssinica* Hochst, has not yet been investigated. Crambe cake is a byproduct of the extraction of oil from seeds of crambe. The present study aimed to investigate the effect of crambe cake on *Meloidogyne javanica* and *Pratylenchus brachyurus*. Soybean seedlings were transplanted and inoculated with *M. javanica* or *P. brachyurus*. Sixty (60) days later, the aerial part was discarded, and the treatments (0, 0.5, 1, 2.5, and 5% of crambe cake per pot (% weight/weight) were applied, and soybean was sown again. Sixty days after seed germination, vegetative and nematological parameters were assessed. For each nematode, the experiment was conducted in two different periods. The treatment with 5% of crambe cake inhibited seed germination in all the experiments. Higher doses favored plant growth. Crambe cake effectively controlled *M. javanica* in one of the experiments when 1% was applied per pot containing 2 L of soil, and the increase in crambe cake doses promoted the control of *P. brachyurus* in both experiments.

Key words: management, organic matter, root lesion nematode, root-knot nematode.

RESUMO

Tavares-Silva, C. A., C. R. Dias-Arieira, F. Rogerio, H. H. Puerari, D. Mattei, T. R. B Silva, e O. Ferrarese-Filho. 2015. Controle de *Meloidogyne javanica* e *Pratylenchus brachyurus* com torta de crambe. Nematropica 45:215-221.

Os efeitos nematicidas do crambe, *Crambe abyssinica* Hochst, ainda não foram investigados. A torta de crambe é um subproduto da extração do óleo das sementes de crambe. O presente estudo objetivou avaliar o efeito de doses da torta de crambe sobre a população de *Meloidogyne javanica* e *Pratylenchus brachyurus*. Plântulas de soja foram transplantadas e posteriormente inoculadas com *M. javanica* ou *P. brachyurus*. Passados 60 dias, a parte aérea das plantas foi descartada e aplicaram-se os tratamentos: 0, 0,5, 1, 2,5, e 5% de torta de crambe por vaso (% peso/peso), realizando-se em seguida nova semeadura de soja. Após 60 dias da germinação, foram estudados os parâmetros vegetativos e nematológicos. Para cada nematoide, o experimento foi conduzido em duas épocas distintas. O tratamento com 5% de torta inibiu a germinação das sementes em todos os experimentos. Altas dosagens favoreceram o crescimento da planta. A torta de crambe foi eficiente em controlar *M. javanica* em um dos experimentos quando aplicado na quantidade de 1% por vaso, contendo 2 litros de solo; o incremento de torta de crambe promoveu o controle de *P. brachyurus* em ambos os experimentos.

Palavras-chave: Manejo, matéria orgânica, nematoide das lesões radiculares, nematoide das galhas.

INTRODUCTION

Phytonematodes have been identified as a limiting factor on soybean (*Glycines max* (L.) Merr.) yield. The no-till planting system with soybean has resulted in considerable increases in nematode populations, specifically *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Steckhoven (Ferraz, 2006). This nematode is considered a major pest in Brazil's soybean-producing regions, with high population densities causing losses in yield ranging from 30 to 50% (Ferraz, 2006; Inomoto, 2011). Another phytonematode of importance in soybean is *Meloidogyne javanica* (Treub) Chitwood. This nematode is found in most growing areas of Brazil and has been reported to cause yield loss of soybean (Yorinori, 2000).

In an attempt to reduce nematode populations in soybean, a number of control methods have been researched with a view to integrating techniques in order to render production more rational, efficient, and economical. Recommended strategies include the use of resistant cultivars, incorporation of organic matter, use of antagonistic plants, crop rotation with non-host plants, and application of nematicides (Oliveira *et al*., 2005). The use of organic matter has some additional benefits compared to other control methods since it increases the population of nematode-antagonistic microbes and consequently changes the physical and chemical properties of soil (Kaplan *et al*., 1992; McSorley and Gallaher, 1995).

Various sources of organic matter for controlling nematodes may be available locally or regionally. Oilseed presscakes made from castor bean, neem, peanut, mustard, cotton, soybean, flax, or other plant species have been suggested (Lopes *et al*., 2008; Umar and Simon, 2008; Lopes *et al*., 2009; Mohan, 2011; Tiyagia *et al*., 2011). Plant biomass resulting from the green manure (Vedoveto *et al*., 2013; Costa *et al*., 2014; Rodrigues *et al*., 2014) may also be used. Animal waste (chicken litter and cow manure, fish-cleaning waste, bone meal, and chitin) (Dourado *et al*., 2013; Roldi *et al*., 2013a; Santos *et al*., 2013) and agroindustrial waste from the industrialized production of tea, cotton, cassava, sugarcane bagasse, cellulose and sawdust, as well as chaff from coffee, rice, and groundnut (Dourado *et al*., 2013; Roldi *et al.*, 2013b) can be good sources of organic matter, as can urban waste from water treatment (Araújo and Gentil, 2010; Dourado *et al*., 2013) in some locales.

The focus of this study was crambe presscake, a byproduct of oil extraction by mechanical pressing of seeds of *Crambe abyssinica* Hochst. The antagonistic effect of presscakes of some plants on nematodes is mainly through the release of different forms of nitrogen into the soil (Rodríguez-Kábana, 1986; Kaplan *et al*., 1992). The use of oilseed presscakes has been reported to control a number of nematode species and extensive research has been conducted on neem presscake (Lopes *et al*., 2008; Umar and Simon, 2008; Mohan, 2011), castor bean presscake (Dutra *et al.*, 2006; Lopes *et al.*, 2009; Dinardo-Miranda and Fracasso, 2010), and sunflower and linseed presscake (Tiyagia *et al*., 2011).

Crambe presscake may present a further possible alternative (Souza *et al*., 2009; Oka, 2010). Crambe is an oleaginous winter cruciferous plant and a member of the Brassicaceae (Machado *et al*., 2007). According to Cole (1976), brassica plant waste contains glucosinolates, which, when hydrolyzed by myrosinase, form bioactive compounds such as isothiocyanates, organic cyanides, and nitriles. *Brassica* seedcakes differ in their effects on a range of organisms in the soil and may act as suppressants of a varying spectrum of organisms over different periods of time (Mazzola *et al*., 2007).

The aim of this study was to assess the potential of crambe presscake for control of *M. javanica* and *P. brachyurus* in soybean, and to determine the effect of crambe presscake on the nematodes and on soybean plant growth.

MATERIALS AND METHODS

Experiments were conducted in a greenhouse at the Umuarama Regional Campus of the State University of Maringá, Brazil. For each of the nematodes studied, two experiments were conducted over different periods: experiment 1 (2011) from October 2011 to February 2012; and experiment 2 (2012) from February to June 2012. The experimental design was fully randomized, with five treatments and six replications for each treatment.

Seeds of soybean cultivar 'CD 234RR' were sown into trays containing commercial substrate Plantmax®. Seedlings were transplanted (2 seedlings per pot) 25 d after germination into pots containing 2 L of soil, classified as typical sandy-textured dystrophic Red Latosol soil (Embrapa, 2013). Prior to use, the soil was autoclaved for 2 hr at a constant temperature of 120ºC. Two days after transplanting, the seedlings were inoculated separately with phytonematodes. For *M. javanica* in experiment 1, plants were inoculated with a suspension of 3,000 eggs plus second-stage juveniles (J2), and in experiment 2 with 1,000 eggs plus J2. For *P. brachyurus*, inoculations consisted of 3,000 nematodes in experiment 1 and 1,000 nematodes in experiment 2. Nematode inoculum

was obtained from populations maintained on tomato (*M. javanica*) and maize (*P. brachyurus*). *Meloidogyne javanica* eggs were extracted from the tomato roots using the method proposed by Hussey and Barker (1973) and *P. brachyurus* were extracted from the maize roots by the technique described by Coolen and D'Herde (1972).

Sixty days after inoculation, the aerial part of the soybean plant was discarded and crambe presscake applied at rates of 0, 0.5, 1, 2.5, and 5% (weight/weight), corresponding to 0, 0.9, 1.8, 4.5, and 9 tons of crambe cake per hectare, incorporating it into the surface layer of soil (15 cm). The crambe presscake was obtained from R M Industria, Campo Grande, Mato Grosso do Sul, Brazil.

After incorporating the crambe presscake, the pots were reseeded with one soybean seed per pot. Sixty days after germination, the plants were removed from the pots and the following vegetative growth parameters were assessed: aerial and root fresh weights and aerial dry weight (after drying in a fan oven for three days at 65ºC). For both nematode species, the number of nematodes in roots was determined and divided by the root weight to obtain the number of nematodes per gram of root. Nematodes were extracted from the roots for quantification using the methods described above. For *P. brachyurus*, nematodes were also extracted from 100 cm^3 soil using centrifugal flotation (Jenkins, 1964).

The data were subjected to Tukey's test at 1%. To meet the assumption of normality, data concerning nematodes per gram of root in experiment 1 for *M. javanica*, and in soil for *P. brachyurus* were transformed by $\sqrt{(x+0.5)}$ prior to analysis.

RESULTS AND DISCUSSION

Soybean seeds did not germinate after treatment with 5% of crambe cake per pot in any of the experiments, nor with 2.5% of crambe cake in experiment 2 with *M. javanica*.

In experiment 1 with *M. javanica* (Table 1), the use of crambe cake had a positive influence on vegetative growth of soybean plants. The best results were obtained with doses of 1 and 2.5% for plant fresh weight and plant dry weight. Root fresh weight was lower for plants that did not receive the crambe cake in the pots. The means observed in experiment 2 corroborated the positive influence of crambe cake on vegetative growth of soybean.

For *P. brachyurus* (Table 2), the crambe cake also resulted in increased weight of aerial part and roots of soybean plants. In 2011, there was significant difference between the dose of 2.5% cake and other treatments. In 2012, the plant dry weight and root fresh weight did not differ significantly. Only the plant fresh weight of soybean was influenced by the application of crambe cake when compared to the control.

The population of *M. javanica* was not influenced by the addition of crambe cake in experiment 1 (Table 3). However, in experiment 2, the population of nematodes in the pots that received 1% of crambe

Table 1. Means of fresh and dry plant weight and fresh root weight of soybean inoculated with *Meloidogyne javanica* after soil amendment with crambe cake, 2011-2012.

Crambe cake (% w/w)	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)
	2011[z]		
0	4.46 b	1.48 b	3.56 b
0.5	3.83 b	1.20 b	8.59 a
1	9.91 a	4.16 a	13.00 a
2.5	11.50 a	5.41 a	11.04 a
CV (%)	39.8	65.0	47.8
	2012[z]		
0	2.16 b	0.81 b	4.56 b
0.5	6.33 b	1.77 b	6.73 ab
1	11.14 a	2.83 a	8.27 a
CV (%)	50.9	41.2	29.7

CV = Coefficient of variation
[z]Means followed by the same letter in the columns within a year do not differ by Tukey test at 1% probability.

Table 2. Means of fresh and dry plant weight and fresh root weight of soybean inoculated with *Pratylenchus brachyurus* after soil amendment with crambe cake, 2011 and 2012.

Crambe cake (% w/w)	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)
	2011[z]		
0	6.14 c	1.95 b	7.83 b
0.5	14.63 b	4.47 b	14.82 b
1	14.86 b	4.88 b	16.89 b
2.5	35.00 a	10.17 a	38.81 a
CV (%)	35.4	37.4	43.2
	2012[z]		
0	6.62 b	1.24 a	15.08 a
0.5	15.86 a	3.46 a	12.87 a
1	22.55 a	4.85 a	12.30 a
2.5	16.02 a	2.79 a	9.48 a
CV (%)	47.39	58.43	32.29

CV = Coefficient of variation
[z]Means followed by the same letter in the columns within a year do not differ by Tukey test at 1% probability.

Table 3. *Meloidogyne javanica* eggs + second-stage juveniles (J2) per gram of soybean root after soil amendment with various rates of crambe cake.

Crambe cake (% w/w)	Eggs + J2/g root	
	2011	2012
0	5,339[y,z]	13,571 a
0.5	8,347	12,716 a
1	24,548	1,686 b
2.5	21,418	-
CV (%)	33.2	47.8

[y]Means followed by the same letter in the columns do not differ by Tukey test at 1% probability.
[z]Original data transformed by equation √(x+0.5) for statistical analysis.
CV = Coefficient of variation

cake was significantly lower compared to the treatments with 0 and 0.5% of crambe cake (Table 3).

The application of crambe cake reduced the number of *P. brachyurus* per gram of root in both experiments. (Table 4). However, it did not affect the population of *P. brachyurus* in the soil in experiment 1.

The negative effect of high doses of crambe cake on the germination of soybean seeds could be due to the fact that *Brassica* species synthesize large quantities of glucosinolates, which are converted into various potentially allelopathic substances (Eberlein, 1998). This is the case for rapeseed (*B. napus* L.), colza (*"B. napus* subsp. *napus*), and crambe. Incorporation of crambe mulch reduced the speed of emergence and the final number of corn seedlings, in addition to reducing the length of the shoot and root and suppressing the dry mass of the aerial part of the seedlings (Spiassi *et al.*, 2011). Walker (1996) observed phytotoxic effect of crambe cake and rapseed in tomato plants when applied doses of 1% (v / v) in containers 0.33 L of and seeding immediately. The effect was reduced when seeding occurred 3 wk after application of the soil cakes. Also, the fermentation process caused by the incorporation of fresh crambe cake may have contributed to the negative effect (Sediyama *et al*., 2008).

On the other hand, in our study lower doses of crambe cake (0.9 and 1.8 tons of crambe cake per hectare) increased the vegetative growth of soybean as has been reported by others. According to Pereira *et al*. (2014), the application of 0 to 5 tons of crambe cake per hectare inhibited the rate of seed germination in sunflower plant, but the use of lower doses, 1 or 2 tons per hectare, increased plant growth. The beneficial effect of different cakes on various plant species has also been corroborated in other studies (Lopes *et al.*, 2008; Lopes *et al.*, 2009; Roldi *et al*., 2013a).

Regarding nematological parameters, the difference observed in the control of *M. javanica* with the addition of crambe cake has been previously addressed by Oka (2010), who noted that one problem with the use of this control method is the inconsistency of the results, which can be highly influenced by changes in soil type, temperature, and water conditions.

Despite the lack of information in the literature on the effect of crambe cake on plant-parasitic nematodes, the use of plant residues of *Brassica* species reduced the population of *Pratylenchus neglectus* (Rensch) Filipjev and Sch. Stekhoven (Potter *et al*., 1998). The nematicidal activity was also demonstrated by the cakes obtained from *Brassica* seeds where the use of cakes of brown mustard (*Brassica juncea* (L.) Coss), white mustard (*Sinapis alba* L.), and turnip seed at rates of 0.1, 1.0, or 2.0% (vol/vol), effectively controlled *P. penetrans* (Cobb) Chitwood and Oteifa and *Rhizoctonia solani* Khun in an apple orchard, and brown mustard suppressed the pathogens (Mazzola

Table 4. *Pratylenchus brachyurus* per gram of soybean roots and 100 cm^3 soil amendment with various rates of crambe cake.

Crambe cake (% w/w)	2011[z]		2012[z]	
	Root	Soil	Root	Soil
0	779.0 b	43.5 a	1275.1 a	86.0 a
0.5	1117.1 a	65.7 a	437.2 a	18.75 b
1	604.4 b	28.5 a	42.1 b	0.0 b
2.5	85.4 c	0.0 a	25.9 b	0.0 b
CV (%)	49.1	69.6	72.1	81.2

[z]Means followed by the same letter in the columns within a year do not differ by Tukey test at 1% probability.
CV = Coefficient of variation

et al., 2001). Subsequently, the potential of cake of brown mustard seed in the control of nematodes was corroborated by Reardon *et al*. (2013). Mustard oil of *B. campestres* L. has also promoted the effective control of *M. incognita* (Kofoid and White) Chitwood (Akhtar and Mahmood, 1993) and *M. javanica* (Neves *et al*., 2007).

During the process of decomposition of the tissues of the brassica, sulfur compounds called glucosinolates are released in the soil. When glucosinolates react with the enzyme myrosinase, they form compounds such as isothiocyanates (ITC), nitriles, thiocyanates, and epinitrilas (Mayton *et al*., 1996). Some of these compounds have nematicidal effects, such as the allyl isothiocyanate against *Globodera rostochiensis* (Wollenweber) Behrens (Ellenby, 1951) and 2-phenylethyl glucosinolate against *P. neglectus* (Potter *et al*., 1998). Nevertheless, it should be stressed that the amount of nematicidal substances produced differs among the *Brassica* species, which may interfere with the findings. Zasada and Ferris (2004) reported differences in the concentrations of ITC between *Brassica* species and observed that the glucosinolates in broccoli and mustard (*B. hirta* Moench) were insufficient to control the pathogens. Regarding crambe, there are no comparative studies with other species, and further studies are needed to assess the viability of use of crambe cake in field conditions.

Our findings showed that the dose of 5% of crambe cake of soil or 9 ton ha^{-1} of crambe cake inhibited the germination of soybean seeds and the same occurred in the treatment with 2.5% of cake, or 4.5 ton ha^{-1} of crambe cake in one of the experiments conducted while lower application rates tended to promote vegetative growth of soybean and reduced the population of nematodes. Therefore, the use of crambe has the potential to control these parasites, especially considering the growing demand for renewable energy sources, such as biodiesel, which will increase the growing of oil seeds, resulting in the availability of seed cakes for agricultural use.

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