

# RESEARCH/INVESTIGACIÓN

## USE OF ORGANIC AMENDMENTS TO CONTROL *MELOIDOGYNE INCOGNITA* ON TOMATOES

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### ABSTRACT

Roldi, M., C. R. Dias-Arieira, J. J. Severino, S. M. Santana, T. S. Dadazio, P. M. Marini and D. Mattei. 2013. Use of organic amendment to control *Meloidogyne incognita* on tomatoes. *Nematropica* 43:49-55.

Two experiments were conducted in the greenhouse: the first was aimed at evaluating the effects of varying concentrations of organic fertilizer based on fish waste, and the second, the effects of castor bean cake, bone meal, bokashi and fish waste on the population of *Meloidogyne incognita* in tomato plants. Tomato seedlings were transplanted 15 days after germination into pots inoculated with 4000 *M. incognita* eggs obtained from a pure population maintained on tomato plants (cv. Santa Clara). In the first experiment, three days after inoculation the plants were sprayed with fish waste; a commercial preparation, at concentrations of 0, 1, 2, 4 and 8 mL/L of water. Applications were repeated every week or fortnight until evaluation. In the second experiment, treatments of castor bean cake, bone meal and bokashi were applied in the soil at a rate of 20 g/pot and fish waste was sprayed at a concentration of 2 mL/L of water, three days after inoculation. Sixty days after inoculation, the plants were evaluated in terms of height, fresh and dry shoot weights, fresh root weight and number of galls, eggs/root system and eggs/g of root. Fish waste and bone meal were not efficient in controlling the nematode. Both bokashi and castor bean cake significantly reduced nematode reproduction, but only bokashi improved plant growth.

*Key words:* bokashi, bone meal, phytonematodes, organic matter, fish waste, castor bean cake.

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### RESUMO

Roldi, M., C. R. Dias-Arieira, J. J. Severino, S. M. Santana, T. S. Dadazio, P. M. Marini and D. Mattei. 2013. Uso de matéria orgânica para o controle de *Meloidogyne incognita* em tomateiros. *Nematropica* 43:49-55.

Dois experimentos foram realizados em casa de vegetação: o primeiro objetivando avaliar o efeito de diferentes concentrações do fertilizante orgânico a base de resíduo de peixe, e o segundo o de torta de mamona, farinha de osso, bokashi e resíduo de peixe, sobre a população de *Meloidogyne incognita* em tomateiro. Para isto, plântulas de tomateiro com 15 dias de germinadas foram transplantadas para vasos e inoculadas com 4000 ovos de *M. incognita*, obtido de população pura mantida em tomateiro. No primeiro experimento, três dias após a inoculação, as plantas foram pulverizadas com o resíduo de peixe nas concentrações de 0; 1; 2; 4 e 8 mL/L de água. As aplicações foram repetidas semanal ou quinzenalmente, até o momento da avaliação. No segundo, os tratamentos torta de mamona, farinha de osso e bokashi foram aplicados no solo na dosagem de 20 g/vaso, incorporados superficialmente. O resíduo de peixe foi pulverizado na concentração de 2 mL/L de água. Após 60 dias da inoculação, as plantas foram avaliadas quanto a altura, massa fresca e seca da parte aérea, massa fresca de raiz e número de galhas, ovos/sistema radicular e ovos/g de raiz. O resíduo de peixe e a farinha de osso não foram eficientes em controlar o nematoide. O bokashi e a torta de mamona reduziram significativamente o nematoide e o bokashi promoveu melhor desenvolvimento de plantas.

*Palabras clave:* bokashi, farinha de osso, fitonematoides, matéria orgânica, resíduo de peixe, torta de mamona.

## INTRODUCTION

Root-knot nematodes (*Meloidogyne* Göeldi) are among the main pathogens causing yield losses in economically important crops (Moens *et al.*, 2009). The effects of these pests are further aggravated by the wide range of hosts they affect and their extensive geographical distribution. Since they are difficult to control, it is necessary to adopt practices aimed at integrated management (Ferraz *et al.*, 2010).

The use of organic amendment is cited as one of the main management strategies for reducing nematode populations (Stirling, 1991), and entails applying composts such as pumpkin meal, neem cake, chicken litter and bovine manure (Lopes *et al.*, 2008; Dallemole-Giaretta *et al.*, 2010; Nazareno *et al.*, 2010). Adding organic matter to the soil increases the population of natural enemies and also promotes the release of toxic chemicals during decomposition (Oka, 2010); changing the soil's physical and chemical properties and enhancing plant growth and tolerance to nematode attack (McSorley and Gallaher, 1995).

Various products are currently available in the market as sources of organic matter, especially for small producers. They include castor bean cake, bone meal, and bokashi (organic compost produced by fermenting bran mixtures). Some published reports offer suggestions on how these composts can be used as efficient agents for controlling nematodes (Mashela and Nthangeni, 2002; Dutra *et al.*, 2006; Gomes *et al.*, 2009; Oka, 2010). The use of castor bean waste, mainly in cake form, has proved effective in reducing populations of *Meloidogyne* spp. (Mashela and Nthangeni, 2002; Adegbite and Adesiyun, 2005; Dutra *et al.*, 2006; Lopes *et al.*, 2009), possibly due to the combined activity of ricin and ricinine and the nutritional properties of the compost (Dutra *et al.*, 2006).

Gomes *et al.* (2009) reported a significant reduction in the population of *M. mayaguensis* Rammah and Hirschmann (= *M. enterolobii* Yang and Eisenback) in guava plants after treatment with meat and bone meal; suggesting that the nematicide effect was due to the release of ammonia into the soil during the microbial decomposition process.

For bokashi and fish waste, which are used as organic fertilizers, there is a scarcity of information on their efficiency in controlling root-knot nematodes. Therefore, the objectives of this study were to evaluate the effects of organic fertilizers on the population of *M. incognita* (Kofoid and White) Chitwood and tomato plant (*Solanum lycopersicum* L.) growth.

## MATERIALS AND METHODS

Two experiments were conducted in the greenhouse at the Umuarama Regional Campus of the State University of Maringá, PR, Brazil. The first study was designed to elucidate the effects of varying concentrations of fish waste organic fertilizer and the

second study to determine the effects of castor bean cake, bone meal, bokashi and fish waste, on populations of *M. incognita* in tomato plants.

For both experiments, seeds of tomato plant cv. Santa Clara were sown on trays of polystyrene containing a Plantmax® type substrate. Fifteen days after germination, the seedlings were transplanted into pots containing 1.5 kg of soil (83.95% sand, 3.00% silt, 13.05% clay, pH 4.49 (CaCl<sub>2</sub>), 1.35 g/dm<sup>3</sup> OM) and sand mixture (2:1, v/v), that had been autoclaved at 120°C for 2 hr.

Three days after transplanting, the soil medium in each pot was infested with 4000 eggs of *M. incognita* obtained from a pure population maintained on tomato cv. Santa Clara in the greenhouse. The nematode eggs were extracted from the roots using the method developed by Hussey and Barker (1973). Approximately 1000 eggs in 1 mL water were pipette directly into each of four holes (4 to 5 cm deep) arranged equidistantly in the soil around the plant.

### Experiment 1

Three days after inoculation the plants were treated with fish waste (JK Organic Fertilizer), sprayed onto the leaves at the following concentrations: i) manufacturer's recommendation (2 mL/L of water), ii) half the recommended concentration (1 mL/L of water), iii) two times the recommended concentration (4 mL/L of water), iv) four times the recommended concentration (8 mL/L of water), and v) water; which served as the control. All applications were repeated either weekly or fortnightly up to the termination of the experiment; 60 days after inoculation, totaling eight and four repetitions, respectively.

At the end of the experiment, the plants were removed from the pots, the root systems separated from the shoot systems. The plant height were determined, fresh and dry weights (dried at 70°C until constant weight) of shoots determined.

The roots were carefully washed, placed on absorbent paper to remove excess water, and weighed. The number of galls was then counted directly to obtain galls/root system, and the eggs were extracted using the method previously mentioned. A Peters slide was used to determine the total number of eggs per root system and eggs/g of root.

The data were analyzed to determine the interaction between concentration and application period, and regression analysis run on the concentrations. In the absence of any interaction, the averages were compared using the Tukey test at 5% error probability.

### Experiment 2

The second experiment was completely randomized with five replications and used to evaluate the effects of castor bean cake, bone meal, bokashi and fish waste on nematode control. Untreated plants were used as the

control. The castor bean cake, bone meal and bokashi were applied at a rate of 20 g per pot, incorporated into the soil 10 cm deep, at planting. The fish waste was sprayed onto the plants on the day of inoculation at the concentration recommended by the manufacturer (2 mL/L of water) and applications repeated weekly. The bokashi used in the experiment was of the “bran compost” type, obtained by anaerobic fermentation of a mixture of 6 kg rice bran, 2 kg de castor bean cake, 1.5 kg wheat bran, 0.5 kg bone meal, 15 mL sugar cane molasses, 15 mL effective microorganisms (EM-4) and 1.5 L of water (AAO, 1998).

Sixty days after inoculation, the plants were removed from the pots and evaluated for the same parameters mentioned above. This experiment was repeated at two different times of year (November/2011 to January/2012 [1<sup>st</sup> Evaluation] and March/2012 to May/2012 [2<sup>nd</sup> Evaluation]). The data obtained were subjected to analysis of variance and averages compared using the Tukey test at 5% probability.

## RESULTS

### Experiment 1

Since the interaction between the application periodicity and product concentrations was not significant, the two main effect factors could be

examined independently. Furthermore, none of the regression analysis models evaluated matched the data obtained in the experiment.

In the fish waste treatment, the number of galls/root system was not affected by different concentrations of the product, whether applied weekly or fortnightly. However, when applied weekly, the number of eggs/g of root was significantly lower when the fish waste was applied at 1 mL/L, whereas the other treatments did not differ from the control (Table 1). When applied fortnightly, treatment at 8 mL/L significantly reduced the number of nematodes/root system when compared to treatment at 2 mL/L, but there were no statistical differences between the treatments and the control (Table 1).

Fish waste applied weekly at a concentration of 4 mL/L significantly increased aerial part fresh and dry weight compared to a concentration of 2 mL/L (Table 2), but none of the treatments differed statistically from the control. A concentration of 4 mL significantly increased the root fresh weight compared to the control, but did not differ from the other treatments. Plant height was not affected by product concentration.

When the fish waste was applied fortnightly, only the plant height parameter was significantly affected. Treatment with fish waste at 1 mL/L significantly increased height compared to the treatment at 4 mL/L (Table 2).

Table 1. Number of galls, eggs/root system and eggs/g of root in tomato plants inoculated with *Meloidogyne incognita* and treated with varying concentrations of fish waste, applied at two different periodicities.

Treatment	Galls/plant	Eggs/plant	Eggs/g of root
Weekly application			
Control (0 mL/L)	386.8 <sup>ns</sup>	21860.0 <sup>ns</sup>	4554.8 a
1 mL of waste/L	398.0	16965.0	2819.3 b
2 mL of waste/L	323.5	27630.0	4293.6 a
4 mL of waste/L	372.5	22225.0	3021.1 ab
8 mL of waste/L	327.3	17670.0	3381.9 ab
CV (%)	35.2	50.6	37.5
Fortnightly application			
Control (0 mL/L)	350.0 <sup>ns</sup>	24890.0 ab	4173.6 <sup>ns</sup>
1 mL of waste/L	421.8	25860.0 ab	3865.2
2 mL of waste/L	472.5	43066.7 a	5157.1
4 mL of waste/L	367.0	24590.0 ab	5293.5
8 mL of waste/L	276.0	22095.0 b	3717.0
CV (%)	36.0	38.5	33.3

For each application periodicity, averages followed by the same letter within the columns did not differ in the Tukey test at 5% probability

ns = not significant

CV= Coefficient of Variation

Table 2. Plant height, aerial part fresh weight (APFW), aerial part dry weight (APDW) and root fresh weight (RFW) of tomato plants inoculated with *Meloidogyne incognita* and treated with different concentrations of fish waste applied at two periodicities.

Treatment	Height (cm)	APFW (g)	APDW (g)	RFW (g)
Weekly application				
Control (0 mL/L)	58.3 <sup>ns</sup>	21.7 ab	3.3 ab	4.7 b
1 mL of waste/L	56.0	21.7 ab	2.8 ab	6.0 ab
2 mL of waste/L	55.3	19.1 b	2.6 b	5.8 ab
4 mL of waste/L	57.3	24.0 a	3.5 a	7.7 a
8 mL of waste/L	55.3	22.3 ab	3.2 ab	5.6 ab
CV (%)	8.6	13.6	14.9	31.1
Fortnightly application				
Control (0 mL/L)	58.5 ab	22.6 <sup>ns</sup>	3.6 <sup>ns</sup>	6.0 <sup>ns</sup>
1 mL of waste/L	64.8 a	22.2	3.1	6.7
2 mL of waste/L	62.3 ab	22.4	3.2	8.3
4 mL of waste/L	53.8 b	23.0	3.3	5.3
8 mL of waste/L	61.8 ab	20.7	3.0	6.5
CV (%)	9.4	7.2	14.6	26.6

For each application periodicity, averages followed by the same letter within the columns did not differ in the Tukey test at 5% probability

ns = not significant

CV= Coefficient of Variation

Table 3. Number of galls, eggs/root system (Eggs/RS) and eggs/g of root (Eggs/g) in tomato plants treated with various types of organic matter at 60 days after inoculation with *Meloidogyne incognita*.

Treatment	1 <sup>st</sup> Evaluation			2 <sup>nd</sup> Evaluation		
	Galls	Eggs/RS	Eggs/g	Galls	Eggs/RS	Eggs/g
Control	300.5 a	36380.0 a	4517.8 a	342.8 a	38280.0 a	5857.8 a
Bokashi	73.5 bc	7677.5 b	353.6 b	84.3 b	3220.0 b	251.4 b
Castor bean cake	16.5 c	752.5 b	183.7 b	22.3 b	3260.0 b	273.8 b
Bone meal	260.0 ab	41035.0 a	3940.0 a	261.0 a	24165.0 a	1629.3 ab
Fish waste	366.0 a	55957.5 a	6124.8 a	317.7 a	37220.0 a	4324.1 a
CV (%)	48.7	29.3	21.8	72.5	42.8	38.0

1<sup>st</sup> Evaluation: November/2011 to January/2012; 2<sup>nd</sup> Evaluation: March/2012 to May/2012

Averages followed by the same letter with the column did not differ in the Tukey test at 5 % probability

CV= Coefficient of Variation

### Experiment 2

In the second experiment, fish waste was applied at the manufacturer's recommended dosage in order to confirm previous results, and we observed that this was not effective in reducing the number of galls and nematodes (Table 3), irrespective of the period at which the experiments were conducted, confirming the results obtained in the first experiment (Table 1). Treatment with bone meal also failed to be effective in controlling nematodes, since during both times of year, the number of galls, eggs/root system and eggs/g of root were not

statistically different than those of the control treatment (Table 3).

We verified that the castor bean cake and bokashi treatments reduced the number of galls, eggs/root system and eggs/g of root for the experiments at both times of year (Table 3), with values statistically lower than those of the control. We also noted that there was no statistical difference between castor bean cake and bokashi composts in terms of nematode control.

In regard to plant growth parameters, fish waste was not effective in promoting plant growth in both evaluation periods (Table 4). Bone meal also failed to

Table 4. Plant height, aerial plant fresh (APFW) and dry (APDW) weight and root fresh weight (RFW) of tomato plants treated with different types of organic matter at 60 days after inoculation with *Meloidogyne incognita*.

Treatment	Height (cm)	APFW (g)	APDW (g)	RFW (g)
1 <sup>st</sup> Evaluation				
Control	53.0 b	27.3 b	4.9 b	8.4 b
Bokashi	73.5 a	65.6 a	12.5 a	22.0 a
Castor bean cake	46.8 b	36.5 b	6.3 b	9.1 b
Bone meal	59.3 ab	34.6 b	6.3 b	11.4 b
Fish waste	51.5 b	28.9 b	5.0 b	9.2 b
CV (%)	13.8	10.7	12.9	18.3
2 <sup>nd</sup> Evaluation				
Control	59.5 b	21.1 b	3.6 b	7.2 b
Bokashi	62.0 ab	32.0 a	5.9 a	12.2 a
Castor bean cake	69.5 a	29.2 a	4.9 a	8.9 b
Bone meal	65.0 ab	24.6 ab	3.6 b	14.9 a
Fish waste	65.5 ab	22.1 b	4.1 ab	9.5 b
CV (%)	14.3	19.8	18.2	28.4

1<sup>st</sup> Evaluation: November/2011 to January/2012; 2<sup>nd</sup> Evaluation: March/2012 to May/2012

Averages followed by the same letter within the columns did not differ in the Tukey test at 5 % probability

CV= Coefficient of Variation

produce any significant increase in tomato plant growth, with the exception of an increase in root weight during the second experimental period.

Despite the fact that applying castor bean cake reduces the nematode population (Table 3), plant growth parameters were statistically different from those of the control only in the second experimental period, showing a significant increase in plant height, as well as in aerial part fresh and dry weights (Table 4). The bokashi treatment significantly increased plant growth than the control in terms of height during the first experimental period, and in terms of fresh and dry shoot weights and root fresh weight in both evaluations (Table 4).

## DISCUSSION

Fish waste was not effective in controlling *M. incognita*, irrespective of the experimental time of year and the concentrations used. Furthermore, there are no references in the literature relating to the use of fish waste for controlling nematodes. However, Pinto and Bettioli (2010) verified that fish hydrolysate did not eliminate the problems caused by *Fusarium oxysporum* Schlecht in chrysanthemum.

Bone meal treatment also showed no statistical difference compared to the control in terms of parameters related to nematode multiplication, irrespective of the experimental time of year. These results contradict those obtained by Gomes *et al.* (2009) who reported

that soil amendment with meat and bone meal for guava, had a significant negative correlation between increasing concentrations of meat and bone meal and the number of nematodes, as well as plant height, aerial part fresh weight and root fresh weight. The authors attributed the effect to the release of ammonia during the decomposition process. Gomes *et al.* (2009) also reported that the best results were obtained by applying the meal at a concentration of 3% (v/v), which could explain the difference between the two studies, since the concentration we used corresponds to 1.33% of the substrate volume (1.5 kg). This discrepancy in the results could also be attributed to differences in product preparation and composition.

In regard to castor bean cake, various studies have proved the effectiveness of this product in managing nematodes (Alam *et al.*, 1980; Singh *et al.*, 1988; Mashela and Nthangeni, 2002). Lopes *et al.* (2009) found no reduction in the number of galls when castor bean cake was applied at different concentrations, but they did observe drops of 18 and 48% in the number of *M. incognita* eggs in tomato plants treated with the cake at concentrations of 0.5 and 1.0%, respectively. In addition to the cake, castor bean applied in various forms has proved effective in controlling nematodes. Castor bean root extract inhibited hatching and caused high mortality in juveniles of *Meloidogyne* (Adegbite and Adesiyun, 2005). The decline in the population of *M. arenaria* (Neal) Chitwood was significant when the rate of dry castor bean waste was increased over

a range varying from 2 to 8 g/pot (560 cm<sup>3</sup>), but was ineffective when applied at lower concentrations (1 g/pot) (Ritzinger and McSorley, 1998). It is worth noting that although many plant parts of the castor bean have a positive effect on nematode control, castor bean cake is a by-product of the biodiesel agro-industry. It is estimated that the northeast of Brazil will produce three million metric ton of cake annually over the next few years (Beltrão and Melo, 2002).

Ricin has been shown to be the primary substance in castor bean responsible for nematode control (Dutra *et al.*, 2006). Tests conducted in the laboratory have shown the toxic effect of ricin on *M. incognita* populations; which decline as the concentration of ricin increases (Rich *et al.*, 1989). In addition to the direct effect of ricin, there is also the nutritional action of castor bean cake on the plant (Dutra *et al.*, 2006). The fact that castor bean cake was not effective in increasing the growth of the aerial part of the plant during the first experimental time of year and had no effect on root weight at both times of year could be related to a characteristic of the castor bean skin, which has a high C/N ratio, immobilizing the nitrogen in the microbial biomass and consequently causing nutrient deficiency in the plant (Lima *et al.*, 2008).

Of all the products evaluated in the current study, bokashi was best for reducing the population of *M. incognita* and enhancing plant growth. The effectiveness of bokashi in controlling phytonematodes has already been observed for *Radopholus similis* Cobb in bananas (Elango, 2001). It was also reported that applying 1 kg of bokashi/banana plant significantly increased productivity and root biomass. Zandron *et al.* (2003) confirmed the positive effect of bokashi in increasing tomato yield as a result of the nutrients it contains.

The marked effect of bokashi is possibly due to the mixture of organic matter in its composition, especially castor bean cake, whose effectiveness has already been shown in various studies, as mentioned above. It has also been shown that, despite the effectiveness of applying castor bean cake alone for controlling nematodes, adding another source of organic matter, such as neem cake, enhances nematode control (Saifullah and Gul, 1990).

The data obtained lead to the conclusion that treatment with fish waste and bone meal are not effective in reducing populations of *M. incognita*, and promoting plant growth. However, treatment with bokashi and castor bean cake significantly reduced the number of eggs/g of root and also promoted plant growth. This was especially true of bokashi.

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