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Crambe Cake to *Meloidogyne javanica* Control in Lettuce

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Abstract

Crambe is an oilseed, which pressing for oil extraction results in the waste called crambe cake. The aforementioned waste may present potential to control nematodes, since it derives from brassica species. The aim of the current study is to assess the best crambe cake application to control *Meloidogyne javanica* in lettuce plants. Five experiments were carried out in a greenhouse by adopting different crambe cake application procedures; each experiment comprised five treatments (0 (control), 5; 10; 15; 20 g crambe cake per 1 L soil). Lettuce seedlings were cultivated in soil treated with crambe cake, and inoculated with 5,000 nematode eggs and occasional juveniles (J2). Nematological and vegetative parameters were assessed 45 days after inoculation. Nematode reduction was observed in the experiment that applied doses close to 15 g crambe cake to the soil surface; nematode control recorded 83 and 68% for eggs and J2 total and per root gram, respectively. The same parameters showed up to 82 and 93% reduction when the cake was incorporated to the first 8 cm deep into the soil. The number of eggs and J2 per root system reduced by 93% when the cake was incorporated to the total soil volume. Overall, the crambe cake did not increase plant development; in some cases, phytotoxicity was observed at the highest doses.

Keywords: organic fertilization, Brassicaceae, organic matter, root-knot nematodes, *Crambe abyssinica*

1. Introduction

Plant parasitic nematodes stand out among the most destructive pathogens found in agriculture, since they lead to significant annual losses in susceptible plants. *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid & White) Chitwood are reported as the nematodes most affecting lettuce crops, mainly in tropical and subtropical countries, where the continuous lettuce cultivation in certain areas leads to significant nematode population increase due to successive pathogen cycles (Pinheiro et al., 2013).

Root-knot nematodes are sedentary endoparasites; females have piriform body and produce, on average, 500 eggs per life cycle, which, under favorable conditions, is completed in four weeks. During the parasitism process, female nematodes induce specific sites for feeding, called giant cells, which present high cellular hypertrophy and hyperplasia (Pinheiro et al., 2013) and lead to the emergence of root nodes, called galls.

Managing these parasites is a complex task due to the small number of cultivars presenting high resistance levels (Fiorini et al., 2007; Dias-Arieira et al., 2012). Crop rotation using non-host or antagonist plants is recommended as management practice (Moraes et al., 2006; Santana et al., 2012); however, producers who intensively use cultivation areas show low acceptability to such practice.

Thus, adding organic matter to the soil is one of the most efficient methods for the sustainable control of nematodes affecting vegetables; different organic wastes showed nematode management potential (Lopes et al., 2009; Nazareno et al., 2010; Roldi et al., 2013; Dias-Arieira et al., 2015). The organic matter addition also presents other benefits such as natural enemy population increase and improvements in the physical and chemical properties of the soil, including base saturation, porosity and water conductivity, which allow plants to develop better, as well as to become more resistant to these pathogens (McSorley & Gallaher, 1995; Oka, 2010). Wastes from animal husbandry and agro-industrial processes stand out among the investigated organic matters. Cakes derived from oilseed pressing for vegetable oil or biodiesel production purposes stand out among agro-industrial wastes. It is worth highlighting the filter cake, which is generated in alcohol production plants, as well as the castor bean cake, which derives from the pressing of grains for biodiesel production purposes; both cake types proved to be effective in nematode management studies (Albuquerque et al., 2002; Lopes et al., 2009; Roldi et al., 2013).

Crambe (*Crambe abyssinica* Hochst) is another crop whose cake presents nematode control potential (Dias-Arieira et al., 2015; Tavares-Silva et al., 2015). The species is an oleaginous brassica, which presents oil conversion efficiency; the crambe oil is characterized by its high erucic acid content—a long-chain fatty acid used to make chemical products (Erikson & Bassin, 1990; Pitol et al., 2010). Similar to other brassica species, crambe is characterized by the production of glycosinolates (Souza et al., 2009; Pitol et al., 2010)—a chemical molecule that is precursor of compounds such as isothiocyanates, cyanotenes and nitriles, which are characteristically toxic and present nematicidal activity (Mayton et al., 1996; Potter et al., 1998; Zasada & Ferris, 2004).

Although crambe cake shows nematode control potential, it may also present phytotoxic effects (Tavares-Silva et al., 2015). In addition, there is lack of information about efficient doses able to help managing these pathogens. Therefore, the aim of the current study was to assess different crambe cake application forms and doses to control *M. javanica* in lettuce plants.

2. Methods

The experiments were conducted in a greenhouse located at the geographic coordinates 23º47′28.46″ S and 53º15′23.46″ W, altitude 430 meters; they followed a completely randomized design, with five treatments (0 (control), 5; 10; 15; 20 g crambe cake/L soil) and five replications. Three experiments were conducted separately; they differed from each other according to the way the cake was applied, namely: superficially, incorporated to the first 8 cm deep into the soil, or incorporated to the total soil volume. The experiments with applied crambe cake applied superficially and incorporated to the first 8 cm were conducted in two different environments: greenhouse with plastic cover and 50% shading screen (Experiment 1) and greenhouse with plastic cover and 75% shading screen (Experiment 2); the last experiment, crambe cake incorporated to the total soil volume, was conducted in a greenhouse with plastic cover and 50% shading screen, only. The experiments were carried out between January and April 2017.

Lettuce seedlings cv. Vera were initially produced in polyethylene trays containing BioPlant® commercial substrate. Plants showing the first fully-expanded pair of leaves were transplanted to pots containing 1 L of soil:sand (2:1) mixture, which was previously autoclaved at 120 ºC for two hours; the soil was characterized as dystrophic Red Latosol.

The crambe cake was applied to the substrate (mixture), on the transplantation day, at the previously mentioned doses and treatment types. A cake sample was subjected to laboratory chemical analysis; results showed 43.40 g/kg, nitrogen, 5.83 g/kg phosphorus, 1.32 g/kg potassium, 0.33 g/kg calcium, 0.08 g/kg magnesium, 8.43 g/kg sulfur, 23.75 g/kg iron, 3.60 g/kg manganese, 0.88 g/kg copper, 4.42 g/kg zinc, 3.67 g/kg boron, 54.37% organic carbon and 93.51% organic matter.

Each plant was inoculated with 5,000 *M. javanica* eggs and eventual second-stage juveniles (J2) three days after transplantation. The herein used inoculum was obtained from a pure nematode population kept in tomato roots (cv. Santa Clara) and it was extracted according to the methodology by Hussey and Barker (1973), adapted by Boneti and Ferraz (1981). The suspension was calibrated for 2,500 eggs and eventual J2/ml, using a nematode count slide (Peters' slide) under light microscope. The inoculum was deposited in two equidistant holes in the soil around the root crown.

Plants were collected 45 days after inoculation; shoots and roots were separated. The root system was carefully washed and placed on absorbent paper to remove water excess; next, the root fresh weight was measured. Subsequently, nematodes were extracted according to the previously mentioned methodology. Finally, the number of eggs and J2 was assessed in Peters' slide, under light microscope; the recorded value was divided by the root weight in order to find the number of eggs and J2 per root gram. The shoot fresh and dry weights were assessed; shoot dry weight was recorded after the shoot was dried in a forced air circulation oven (65 ºC) until it reached constant weight.

Data were subjected to analysis of variance, at 5% probability; in case of significance, doses were assessed through regression analysis using the SISVAR statistical software (Ferreira, 2011).

3. Results

Both experiments wherein the crambe cake was superficially applied to the soil showed positive nematode control—the maximum reduction in the total number of eggs and J2 was recorded at doses close to 15 g (Experiment 1 = 15.37 g; and Experiment 2 = 14.15 g) and showed nematode reduction higher than 83% (Figure 1A). The total reduction of eggs and J2 directly reflected in the number of eggs and J2 per gram of root; the maximum reduction (68% in comparison to the control) was recorded at the dose 17.68 g in Experiment 1, whereas in Experiment 2, it was directly proportional to the applied dose increase (Figure 1B).

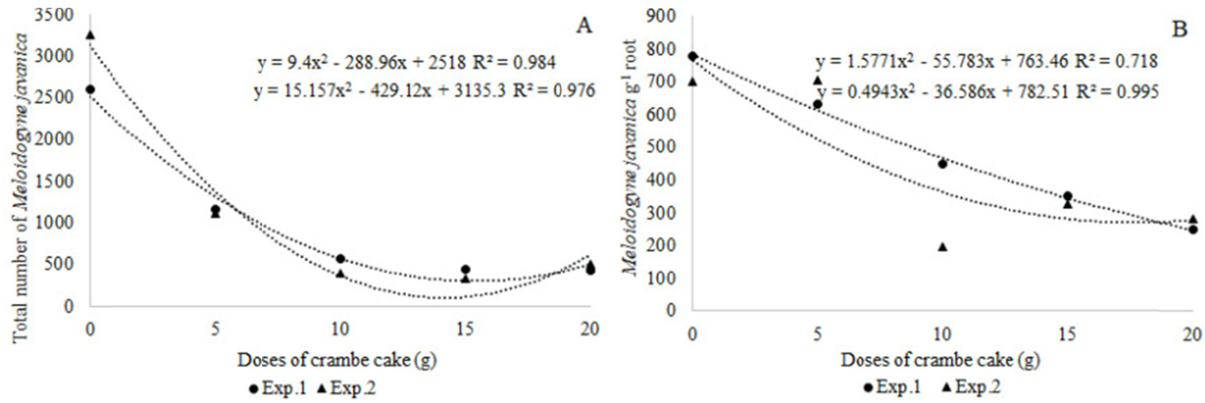


Figure 1. Total number of *Meloidogyne javanica* eggs and J2 (second-stage juveniles) (A), and total number of *Meloidogyne javanica* eggs and J2 per root gram (B) in the root system of lettuce plants treated with increasing crambe cake doses superficially applied to the soil. Experiment 1: greenhouse subjected to 50% shading. Experiment 2: greenhouse subjected to 75% shading

The dose 20 g caused phytotoxicity and plant death when the cake was incorporated to the first 8 cm deep into the soil. However, the other doses promoted significant reduction in the number of eggs and J2 in both experiments. The maximum reduction in Experiment 1 was recorded at the dose 15 g, whereas the best results were observed in Experiment 2 when the crambe cake was applied at the dose 12 g (Figure 2A); reductions ranged from 78 to 82%. The analysis of variance showed significant reduction in number of nematodes per gram of root in Experiment 2, only; the maximum reduction (93%) was recorded when 7.6 g crambe cake was applied per pot (Figure 2B).

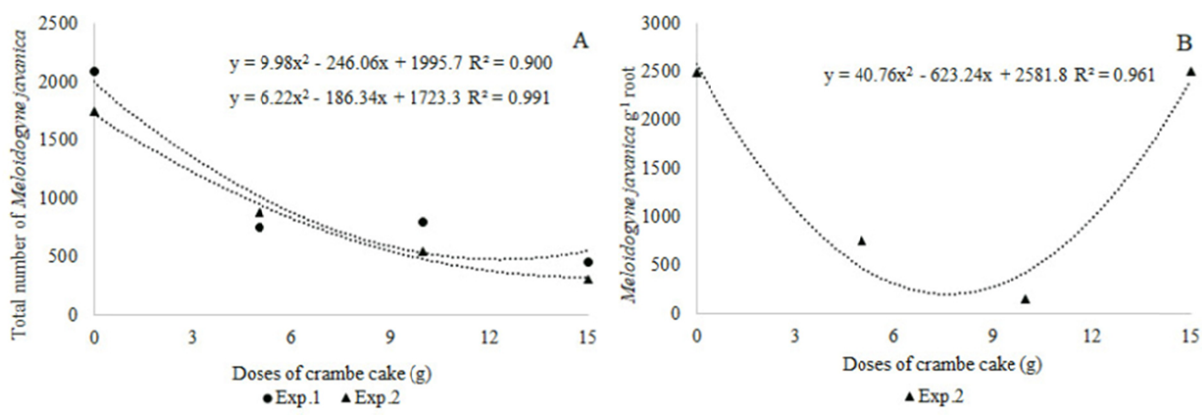


Figure 2. Total number of *Meloidogyne javanica* eggs and J2 (second-stage juveniles) (A), and total number of *Meloidogyne javanica* eggs and J2 per root gram (B) in the root system of lettuce plants treated with increasing crambe cake doses incorporated to the first 8 cm deep into the soil. Experiment 1: greenhouse subjected to 50% shading. Experiment 2: greenhouse subjected to 75% shading

The application of 20 g crambe cake to the total soil volume also led to plant death, fact that made it impossible assessing these plants. The other doses allowed reducing the total number of eggs and the number of eggs per root gram. The application of 12 g crambe cake enabled maximum reduction in the total number of eggs and J2 in the roots (93%) (Figure 3A), as well as in the number of eggs and J2 per root gram (Figure 3B).

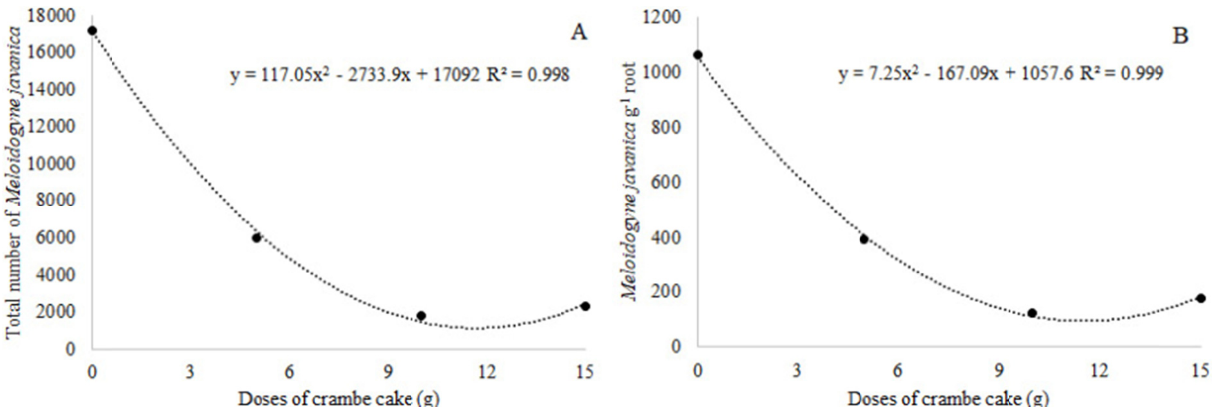
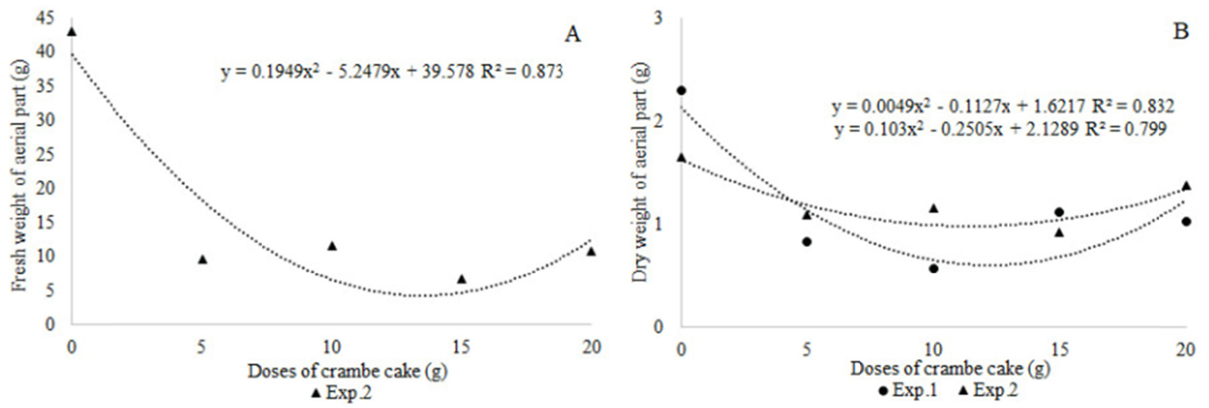


Figure 3. Total number of *Meloidogyne javanica* eggs and J2 (second-stage juveniles) (A), and total number of *Meloidogyne javanica* eggs and J2 per root gram (B) in the root system of lettuce plants treated with increasing crambe cake doses incorporated to 1L soil. Greenhouse subjected to 50% shading

The crambe cake negatively affected lettuce development when it was superficially applied to the soil. There was shoot fresh weight reduction in Experiment 2; it showed minimal development when 13.5 g crambe cake was applied to the soil (Figure 4A). There was shoot dry weight reduction in both experiments; the lowest means were recorded when the cake was applied at doses ranging from 11.5 to 12.2 g (Figure 4B). There was root weight reduction in both experiments; minimum weight was recorded when the cake was applied at doses ranging from 13.0 to 14.5 g (Figure 4C).



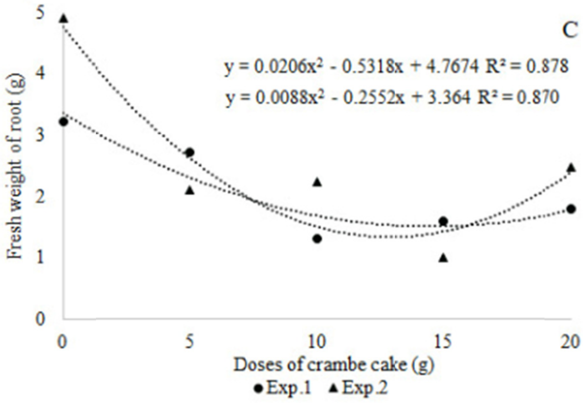


Figure 4. Shoot fresh (A) and dry (B) weight and root fresh weight (C) of lettuce plants treated with increasing crambe cake doses superficially applied to the soil in order to control *Meloidogyne javanica*. Experiment 1: greenhouse subjected to 50% shading. Experiment 2: greenhouse subjected to 75% shading

The root weight was positively affected when the cake was incorporated to the first 8 cm into the soil in Experiment 2; there was root weight increase at the dose 7.6 g (regression equation $y = -0.0428x^2 + 0.6524x + 0.362$ and $R^2 = 59.2\%$). On the other hand, its incorporation to the total soil volume enabled root system reduction directly proportional to the dose increase (equation $y = -0.3664x + 16.783$ and $R^2 = 97.3\%$). The other vegetative parameters were not affected in these experiments (data not shown).

4. Discussion

Results showed that, regardless of the crambe cake application form (superficially, incorporated to the first 8 cm into the soil or to the total soil volume), the treatments enabled significant nematode population reductions, which were expressed both in the total number of nematodes and in the number of nematodes per root gram. Brassicaceae wastes have been investigated for nematode control purposes and recorded satisfactory results in different studies (Mazzola et al., 2001; Reardon et al., 2013; Dias-Arieira et al., 2015; Tavares-Silva et al., 2015). The nematicidal effect recorded in plants belonging to the aforementioned family is related to the plant waste decomposition process, in which glycosinolates are made available to the soil and form bioactive compounds such as isothiocyanates, nitriles and epinitriles, when they react to the myrosinase enzyme (Mayton et al., 1996; Eberlein et al., 1998; Potter et al., 1998; Mazzola et al., 2001).

The crambe cake already showed *M. javanica* control in lettuce plants; reductions varied from 96.9 to 98.9% when 20 g crambe cake was incorporated to 2 L soil (v: v) (Dias-Arieira et al., 2015), and also showed positive results for *M. javanica* and *Pratylenchus brachyurus* (Godfrey) Filipjev & Sch. Stekhoven control in soybean crops (Tavares-Silva et al., 2015). Besides the crambe cake, brown mustard (*Brassica juncea* (L.) Coss), white mustard (*Sinapis alba* L.) and canola (*Brassica napus* L.) cakes also recorded nematode suppression effect (Potter et al., 1998; Mazzola et al., 2001; Reardon et al., 2013); soil biofumigation through brassica plants was effective in controlling *M. javanica* and the nematode reproduction showed more than 46% reduction (Neves et al., 2007).

It is worth highlighting that the high organic matter content in the crambe cake may have contributed to the direct nematode control, as well as indirectly contributed to it through improvements in the chemical, physical and biological features of the soil (Stirling, 1991; McSorley & Gallaher, 1995; Nico et al., 2004). In addition, the nutrients found in the cake may help increasing plant resistance to pathogens and act on different metabolic pathways in the process of inducing plant resistance to nematode attack (Wang et al., 2003; Walters & Bringham, 2007; Lenz et al., 2011). Moreover, the high nitrogen concentration in the waste, whose decomposition may release nitrogen compounds, contributes to pathogen population reduction (Rodríguez-Kábana, 1986), since ammonium nitrate may promote cell plasmolysis in nematodes (Spiegel et al., 1987).

With respect to vegetative parameters, the application of crambe cake to the soil surface reduced plant development. However, only the root weight was positively affected when the crambe cake was incorporated to the first 8 cm deep into the soil, and negatively affected when it was incorporated to the total soil volume, in the other experiments. Thus, it is possible inferring that the crambe cake has toxic effect on lettuce plants. Similar results were recorded when crambe cake application at doses equivalent to 2.5 and 5.0% of soil volume inhibited soybean seed germination (Tavares-Silva et al., 2015). However, they do not corroborate the results recorded by

Dias-Arieira et al. (2015), in which the application of 20 g cake to 2 L soil increased the shoot fresh weight from 27 to 50%, as well as the root weight from 14 to 23%, in lettuce plants. Some factors—such as the chemical composition of the crambe cake, which was not presented in the study by Dias-Arieira et al. (2015), the plant permanence period (which was 60 days in the aforementioned study), and the soil volume used in the experiment—may have contributed to the difference recorded in these results.

Data suggest that it is necessary taking precautions at the time of use high organic compound doses, because high concentrations of some micronutrients, such as manganese and iron, may favor the cellular redox balance, lead to oxidation, and trigger morphological, biochemical and physiological symptoms, which may lead to decreased plant development (Hell & Stephan, 2003). In addition, glycosinolates and their derivatives, which may be responsible for nematode control, can have allelopathic effect on plant development (Eberlein et al., 1998). Thus, the incorporation of *Brassica napus* and crambe wastes reduced germination, delayed seedling emergence, and decreased root length and shoot dry weight in maize (Spiassi et al., 2011). Phytotoxic effect was also observed in tomato plants when 1% (v: v) crambe cake was applied to 0.33 L pots (Walker, 1996). However, the aforementioned author reported decrease in this effect after three weeks of compound reaction to the soil; it led to the hypothesis that the initial crambe cake fermentation process may release toxic compounds, as it was observed in other organic matter sources (Sediyama et al., 2008). In addition, the root fresh weight may have had its growth reduced due to high nutrient availability near the root system, which did not stimulate root growth (Raij, 2011).

It is worth emphasizing that the plant shoot was not affected when crambe cake was incorporated to the first 8 cm deep into the soil or to the total soil volume. In addition, plants grown in commercial areas are often subjected to less stress, because there is no physical constraint to their vegetative development and due to microorganisms able to speed up organic matter decomposition. Thus, complementary studies at field level should be conducted in order to confirm the best crambe cake dose to be applied, as well as to assess the previous fermentation of the material to be used.

In this way, it was concluded that crambe cake reduced the nematode population in the root system, regardless of the application form. The highest doses (20 g in 1 L soil) caused phytotoxicity and compromised the vegetative development of lettuce plants. However, the incorporation of crambe cake to the soil reduced the negative effect on the plant.

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