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Reaction of plant species to *Meloidogyne enterolobii* and the efficiency of their aqueous extracts in controlling the pathogen

Reação de espécies vegetais a *Meloidogyne enterolobii* e eficiência de seus extratos aquosos no controle do patógeno

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Abstract

The genus *Meloidogyne* includes species of the most common nematodes to affect crops around the world. The species *M. enterolobii* is notable for affecting and causing serious losses in the production of guava trees and various other economically important crops in Brazil. The aim of this study was to evaluate the susceptibility of 10 plant species to parasitism by *M. enterolobii*, and the *in vitro* and *in vivo* effects of their leaf extracts on the pathogen. Initially seedlings of *Solenostemon scutellarioides*, *Dieffenbachia amoena*, *Spigelia anthelmia*, *Plumbago scandens*, *Ricinus communis, Chenopodium ambrosioides*, *Azadirachta indica, Morinda citrifolia*, *Jatropha curcas* and *Datura stramonium* were inoculated with 5,000 eggs of *M. enterolobii* to evaluate their susceptibility to nematode infection. For the test *in vitro*, a 5% concentration of the leaf extracts were added to Petri dishes, and 50 J2 of *M. enterolobii* were placed in each dish. After 48 hours incubation, the juveniles were evaluated for motility and mortality in the extracts. For the test *in vivo*, leaf extracts were used at the same concentration, however with only the seven most-promising *in vitro* species. This assay included the following sequence: inoculation of 5,000 eggs in autoclaved and moist soil contained in 1L pots; application of 30 ml of extract to the soil after 24 hours; transplanting of 'Santa Clara' tomato seedlings the following day; and reapplying the extract after 7 and 14 days. The results were evaluated 45 days after nematode inoculation. It was seen that the species *D. amoena*, *R. communis*, *A. indica*, *M. citrifolia, J. curcas* and *D. stramonium* displayed highly resistant behaviour; *S. anthelmia, P. scandens* and *C. ambrosioides* were very resistant, whereas *S. scutelarioides* was susceptible to the nematode. With the *in vitro* test, extracts from seven of the 10 species caused 70.4% to 97.4% J2 mortality. Applying the best leaf extracts to the soil was efficient in reducing *M. enterolobii* infestation in roots of the tomato.

Key words: Alternative control. Plant extracts. Root-knot nematode. Susceptibility.

Resumo

O gênero *Meloidogyne* contempla as espécies de nematoides que mais comumente afetam as culturas em todo o mundo. A espécie *M. enterolobii* tem se destacado por afetar e provocar sérias perdas na produção de goiabeiras e diversas outras culturas de importância econômica no Brasil. O objetivo deste trabalho foi avaliar a suscetibilidade de 10 espécies vegetais quanto ao parasitismo pelo *M. enterolobii* e o efeito *in vitro* e *in vivo* de seus extratos foliares sobre o patógeno. Inicialmente mudas das plantas *Solenostemon scutellarioides*, *Dieffenbachia amoena*, *Spigelia anthelmia*, *Plumbago scandens*, *Ricinus communis, Chenopodium ambrosioides*, *Azadirachta indica, Morinda citrifolia*, *Jatropha curcas* e *Datura stramonium* foram inoculadas com 5.000 ovos de *M. enterolobii* para avaliação da sua

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suscetibilidade quanto à infecção pelo nematoide. No ensaio *in vitro,* os extratos foliares na concentração de 5% foram adicionados a placas de Petri, pondo-se em cada placa 50 J2 de *M. enterolobii.* Após 48 horas de incubação, os juvenis foram avaliados quanto à motilidade e mortalidade nos extratos. Para o ensaio *in vivo*, foram também utilizados extratos na concentração final de 5%, contudo, de apenas sete das espécies, as mais promissoras *in vitro*. O ensaio *in vivo* envolveu a seguinte sequência: inoculação de 5.000 ovos em solo autoclavado e umedecido, aplicação de 30 ml de extrato ao solo 24 horas depois; transplantio das mudas de tomateiro 'Santa Clara' no dia seguinte; repetição da aplicação do extrato/vaso após 7 e 14 dias. Os resultados foram avaliados 45 dias contados a partir da inoculação do nematoide. Observaram-se que as espécies *D. amoena*, *R. communis*, *A. indica*, *M. citrifolia, J. curcas* e *D. stramonium* comportaram-se como altamente resistentes. *Spigelia anthelmia, P. scandens,* e *C. ambrosioides* foram muito resistentes, enquanto que *S. scutelarioides* foi suscetível ao nematoide. No teste *in vitro,* extratos de sete das 10 espécies provocaram a mortalidade dos J2 variando de 70,4 a 97,4%. A aplicação desses extratos foliares ao solo*,* foi eficiente para reduzir a infestação de *M. enterolobii* em raízes de tomateiro.
Palavras-chave: Controle alternativo. Extratos vegetais. Nematoide das galhas. Suscetibilidade.

Introduction

Currently more than 100 nematode species of the genus *Meloidogyne* Goeldi 1887 have been reported worldwide, and numerous host plant species are known, most of which are commercially exploited. Root-knot nematodes, in addition to being widely distributed, are extremely harmful to agriculture, causing damage to world production of around 10% and losses of around USD 120 billion (NICOL et al., 2011; OKENDI et al., 2014).

Despite the large number of species included in the genus, those that have caused the greatest losses to world agriculture are *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. In Brazil, in addition to these four species, *M. enterolobii* has recently become prominent due to the damage it causes in guava (*Psidium guajava* L.), often meaning that four years after planting an orchard, it is often no longer viable to grow fruit (PEREIRA et al., 2009). The importance of *M. enterolobii* to the country may increase as infestation of the pathogen increases, not only in areas of guava production, but also in plantations of other crops (CARNEIRO, ALMEIDA, 2001). In the State of Ceará, the nematode was found associated with the roots of the guava tree in every orchard in the regions they were collected (SILVA; SANTOS, 2017).

Controlling phytonematodes with the use of conventional nematocides is expensive and damages the environment. For this reason, researchers have been looking for alternative measures, such as crop rotation, the use of antagonistic plants, biological control, and more recently, the use of natural substances in infested soils, such as plant extracts and essential oils (CARBONI; MAZZONETTO, 2013; MOREIRA et al., 2015; MARTINS; SANTOS, 2016).

The use of plant-based products may be a viable alternative in the control of phytonematodes, since many plant species contain chemical substances and secondary metabolites, such as alkaloids, saponins, tannins, heterosides, flavonoids, steroids and phenols, which have nematocidal action (FERRAZ et al., 2012).

Considering the importance and distribution of *M. enterolobii* in the guava orchards of Ceará, and its occurrence in various crops including vegetables, the aim of this study was to evaluate the susceptibility of 10 plant species to parasitism by *M. enterolobii*, and the *in vitro* and *in vivo* effect of their aqueous extracts in controlling the pathogen.

Material and Methods

The experiments were carried out in the laboratory and under greenhouse conditions at the Department of Plant Science of the Federal University of Ceará (UFC) in Fortaleza in the State of Ceará, Brazil.

The plants and aqueous extracts of 10 species were evaluated: *Solenostemon scutellarioides* L., *Dieffenbachia amoena* Bull, *Spigelia anthelmia* L., *Plumbago scandens* L., *Ricinus communis* L., *Chenopodium ambrosioides* L., *Azadirachta indica* A. Juss., *Morinda citrifolia* L., *Jatropha curcas* L. and *Datura stramonium* L.

The plant species used were selected based on their therapeutic and vermifugal action in animals and humans, and the presence of secondary compounds that may have nematocidal action on *M. enterolobii*.

The plants came from commercially grown seeds or from cuttings obtained from nurseries at UFC and rooted in the greenhouse.

To extract the eggs for the inoculum used in the assays, and for later analysis in which the number of eggs was considered, the Coolen and D'Herde technique (1972) was used.

Evaluation of the susceptibility of the plant species to M. enterolobii

All 10 species were evaluated for susceptibility to *M. enterolobii*. Seeds or plant cuttings were distributed in pots containing previously autoclaved soil and goat manure (2:1 v/v). When the seedlings displayed two pairs of true leaves, they were inoculated with approximately 5,000 eggs of *M. enterolobii* per plant. The inoculum was derived from infected roots of tomato plants. The obtained suspension was calibrated in a Peters chamber under microscopic stereoscopy to determine egg concentration (eggs/ml) for inoculation into 'Santa Clara' tomatoes (positive control).

Sixty days after inoculation, the plants were removed from the pots to evaluate the number of galls (NG), egg mass index (EMI) and reproduction factor (RF), all used to determine species susceptibility. In analysing the NG, a numerical scale from 1 to 5 was applied, as defined by Taylor and Sasser (1978). This scale of numerical values, modified by Hadisoeganda and Sasser (1982), makes it possible to analyse the EMI and its respective classification using assigned concepts. The RF is the result of the calculation, final population/initial population of the pathogen (5,000 eggs), where RF = 0 for immune species, RF <1 for resistant species and RF >1 for susceptible species (OOSTENBRINK, 1966).

The experimental design was completely randomised, comprising 10 treatments and seven replications. Each experimental unit was represented by one pot containing one plant of each species. The results of the data were transformed into $\sqrt{x+1}$, and submitted to analysis of variance, the mean values being compared by Tukey's test at 5% probability using the Assistat v 7.6 beta statistical software (SILVA; AZEVEDO, 2009).

Evaluation of the in vitro effect of the aqueous extracts of the plant species on the mobility and mortality of juveniles of M. enterolobii

The *in vitro* test was performed with leaf extracts of the 10 plant species. The leaves of each species were collected at 60 days and dried in a greenhouse (30 ± 4 ºC) for up to 15 days.

The leaf extracts were obtained following a procedure adopted by Martins and Santos (2016), in which 10 ml of pre-boiled cold water were added to each gram of dry leaf. After standing for 24 hours, the leaves were ground using a pestle and mortar, and the extract filtered through gauze and centrifuged (2,000 rpm for 20 minutes) to obtain a 10% (w/v) or 0.1 g mL^{-1} extract. The extracts of each species were then diluted with an equal proportion of water to obtain the 5% or 0.05 g mL^{-1} extract for immediate use in the tests.

The 0.05g mL^{-1} extracts were distributed in Petri dishes, 3.5 cm in diameter, at a volume of 3 ml per dish. Fifty freshly hatched juveniles (J2) of *M. enterolobii* were added to each dish, which was numbered and closed for observation. After 48 hours, a count was made of active and inactive

juveniles. The juveniles were transferred to a Petri dish containing water to observe any possible recovery of mobility. After 24 hours, a new count of active and inactive juveniles was made. Any that still appeared straight in the water were considered dead. In the assay, the control consisted of Petri dishes containing water and 50 juveniles.

The experimental design was completely randomised, consisting of 11 treatments and one control (water), with six replications per treatment. The data were analysed using the Assistat v 7.6 beta statistical software, with the variances compared by F-test at 5% probability and the mean values compared by Tukey's test at 5% probability.

Evaluation of the efficiency of aqueous extracts applied to the infested soil before and after planting the tomato seedlings

For this assay, leaf extracts at 0.05 g mL^{-1} were obtained as previously described. To set up the experiment, plastic pots with a capacity of 1 kg were used, filled with an autoclaved mixture of soil and manure (2:1 v/v).

The extracts from seven of the 10 plant species were used, which were the most promising in the *in vitro* test. First, the soil in each pot was moistened with running water (100 ml) prior to inoculation. The soil was then infested with a suspension containing approximately 5,000 eggs/J2 of *M. enterolobii*, distributed in two holes. On the following day, 30 ml pot^{-1} of each plant extract was distributed to irrigate the still-moist soil surface. On the third day, 30-day-old 'Santa Clara' tomato seedlings were individually transplanted to the pots. At 7 and 14 days after transplanting the seedlings, another 30 ml of the extracts was applied. Tomato plants, both inoculated and not inoculated with the nematode, were watered and kept as the positive and negative control for the assay, respectively.

Forty-five days after transplanting, the tomato plants were removed from the pots, and the roots washed to determine the number of galls (NG), number of egg masses (NEM) and number of eggs (NE) under microscopic stereoscopy. The percentage population reduction (PR) was also calculated using the formula PR = 100.(1-T/C), adapted from Vizard and Wallace (1987), where PR = population reduction, T = Mean values for NE of the treatment and C = Mean value for the NE of the positive control.

In evaluating the morphological aspects of the tomato plants, the following were considered: leaf colouration, plant height, size and weight of the roots, and fresh and dry biomass. Leaf colouration was observed for cases of phytotoxicity. Plant height and root length (RL) were measured. The aboveground part of each plant was weighed individually, the same being done for the roots. The dried biomass was determined after drying the aboveground part in an oven at 65 °C for 72 hours.

The design was completely randomised, with nine treatments (seven plant extracts and two controls) and seven replications, the experimental unit consisting of one plant in each pot.

To analyse the data for the number of galls, egg masses and eggs, the values were transformed into $\sqrt{x+1}$ for the analysis of variance and the Scott-Knot test of the mean values. Tukey's test at 5% probability was used to compare the mean values of length and weight, using the Assistat v 7.6 beta statistical software (SILVA; AZEVEDO, 2009).

Results and Discussion

Evaluation of the susceptibility of the plant species to M. enterolobii

Due to the large number of zero mean values, adjustment by statistical methods was not possible, the data from this assay was therefore analysed based on the use of scales and classification systems.

The roots of the species *D. amoena, P. scandens, S. anthelmia, R. communis* and *C. ambrosioides*

were small (2 to 3 mm) and had from 3.2 to 24.7 galls with few or even no egg masses. No females were found when scarifying the roots of the *D. amoena* or *R. communis*, suggesting that the pathogen would not be able to complete its development cycle in these two species. There was no gall formation in the *A. indica, M. citrifolia* or *D. stramonium;* whereas in the *S. scutellarioides* and the control, a large number of galls (>200) and females were found in the roots (Table 1).

Table 1. Mean values for the number of galls (NG), number of egg masses (NEM), egg mass index (EMI) and reproduction factor (RF), in the classification of ten plant species as hosts of *Meloidogyne enterolobii*. Fortaleza, Ceará, 2016.

Plant species	NG	NEM	EMI	RF	Classification*
Dieffenbachia amoena	3.2	0	0	0	Highly resistant (HR)
Ricinus communis	9.3	0	0	0	Highly resistant (HR)
Azadirachta indica	0	0	0	0	Highly resistant (HR)
Morinda citrifolia	0	0	0	0	Highly resistant (HR)
Jatropha curcas	0	0	0	0	Highly resistant (HR)
Datura stramonium	0	0	0	0	Highly resistant (HR)
Spigelia anthelmia	24.7	3.5	1.3	0.099	Very resistant (VR)
Plumbago scandens	10.7	2.2	1.2	0.027	Very resistant (VR)
Chenopodium ambrosioides	18	2.2	1.3	0.093	Very resistant (VR)
Solenostemon scutellarioides	215.5	111.3	4.5	3.14	Susceptible (S)
Tomato (Control)	282.5	151.5	4.8	3.27	Susceptible (S)

*Classification as per Taylor and Sasser (1978), modified by Hadisoeganda and Sasser (1982).

According to the Taylor and Sasser scale (1978) and the Hadisoeganda and Sasser classification (1982), the *D. amoena, R. communis, A. indica, M. citrifolia, J. curcas* and *D. stramonium* plants, which had an EMI of zero, were classified as highly resistant. In the *P. scandens S. anthelmia* and *C. ambrosioides* EMI varied from 1.2 to 1.3, the plants being considered very resistant. *S. scutellarioides*, with the greatest EMI (4.5), was defined as susceptible, together with the control (Table 1).

With the classification proposed by Oostebrink (1966), the *D. amoena, R. communis, A. indica, M. citrifolia, J. curcas* and *D. stramonium* plants were classified as immune due to their RF of zero, and the *P. scandens, S. anthelmia* and *C. ambrosioides* plants as resistant, since the RF was less than 1.0. An RF greater than 1.0, which classifies the plant as susceptible, was found only in *S. scutellarioides* (Table 1) and in the control. Despite the low value for RF in *S. scutellarioides* and the tomato, attributed to losses during extraction, the number of galls (215.5 and 282.5) and of external egg masses (111.3 and 151.5) was high, confirming strong multiplication of the pathogen in these plants under the conditions of the assay (Table 1).

There is controversy regarding the systems and variables that should be used in evaluating plant species for reactions of resistance/susceptibility to the root-knot nematode. Carneiro et al. (2012) evaluated 38 species of the Brassicaceae, Euphorbiaceae, Fabaceae and Poaceae families for susceptibility to *M. enterolobii* using the RF, and found that nine of them were considered immune to the pathogen, 26 were resistant and only three were susceptible. Silva and Silva (2009), evaluating the reaction of 11 species of the Poaceae and Fabaceae families to *M. enterolobii*, using the EMI as classification criterion, found that six were resistant and that two,

Crotalaria paulina and mucuna *Mucuna pruriens* (L.) DC reduced the number of galls and egg masses in tomatoes planted in pots in succession to these two species. In this test, comparing the EMI system with the RF system, a similar classification was seen for the 10 species under test.

Several plant species have been reported as antagonistic to *Meloidogyne* spp, as they produce bioactive compounds, such as proteins, alkaloids, tannins and terpenoids that may have a harmful effect on the nematodes. These compounds may be released by the roots or strengthen the plants, hampering penetration by the nematode or interfering in the life cycle and the interaction with the host plant (SOUZA et al., 2011; FERRAZ et al., 2012).

According to Campos et al. (2006), exudates of plants which are antagonistic or bad hosts to the root-knot nematode, as well as affecting the motility of these pathogens in the soil, may interfere in cell division in the nematode while still inside the egg, delaying formation of the second-stage juvenile and of the cuticle being broken by the J2 (hatching), such effects being a result of nematostatic action.

Compounds such as lipids, proteins and alkaloids are reported in *D. amoena* (FERREIRA et al., 2006). The *P. scandens* plant contains naphthoquinones, flavonoids and terpenes (PAIVA et al., 2003). The active principles, actinidin and isoquinoline, are present in *S. anthelmia* (LORENZI; MATTOS, 2002). There is ricin in the *R. communis*, while *A. indica* plants have the presence of triterpenes, more specifically limonoids or tetranortriterpenoids and azadirachtin. *Datura stramonium* presents high rates of hyoscine in the tissue (FERRAZ et al., 2012). Various monoterpenes, alkaloids, saponins, glycosides and flavonoids have been reported in *C. ambrosioides* plants (ALMEIDA et al., 2012). Phenolic components, flavonoids and anthelmintic properties for humans have been reported in the *M. citrifolia*, (DUSSOSSOY et al., 2011). Different species of *Jatropha* sp. present tannins, flavonoids, coumarins, saponins and others (DEVAPPA et al., 2010; SABANDAR et al., 2013). The supposition is that the above compounds might be involved in the resistance reaction to *M. enterolobii* seen in these species; on the other hand, the shikimic acid and flavonoids found in *S. scutellarioides* do not show this antagonistic action (VOLP et al., 2008). The high infestation of the *S. scutellarioides* roots and its easy multiplication through cuttings, together with its little-affected vegetative development, demonstrate that this species can be used for multiplication of the pathogen.

Despite the high degree of polyphagism and aggressiveness seen in *M. enterolobii*, nine non-host or nematode-resistant plant species were found in this research, some of which may contribute to a reduction of the pathogen in the soil, minimising the losses caused in infested areas.

Evaluation of the in vitro effect of the aqueous extracts of the 10 plant species on the motility and mortality of juveniles of M. enterolobii

Leaf extracts from the *D. amoena, D. stramonium, P. scandens, R. communis, C. ambrosioides* and *A. indica* had a pronounced effect on the juveniles (J2) of *M. enterolobii*, with a marked reduction in their motility, which varied from 78.4 to 97.4%. However, extracts of the noni and jatropha had little effect on J2 motility. In turn, the *M. citrifolia* and *J. curcas* had little effect on J2 motility. In turn, the *S. scutellarioides* extract was inefficient, affecting only one in 50 individuals, the same as the control (water) (Table 2). The nematostatic effect was more expressive in the *J. curcas* extract, with 48% of inactive juveniles recovering motility in the water.

Table 2. Mean number of inactive juveniles of *Meloidogyne enterolobii* after 48 hours exposure to leaf extracts at 0.05g mL^{-1} (5%), and dead juveniles after 24 hours recovery in water. Fortaleza, 2016.

Species under study	J2 inactive after 48h in the extracts	Recuperation after 24h in water	J2 dead after 48h in the extracts
Dieffenbachia amoena	*44.8 b (89.6%)	1.0 (2.23%)	43.8 b (87.6%)
Datura stramonium	43.0 b (86.0%)	0.0 (0%)	43.0 b (86.0%)
Plumbago scandens	42.3 b (84.6%)	0.0(0%)	42.3 b (84.6%)
Spigelia anthelmia	42.2 b (84.4%)	7.0 (16.6%)	35.2 c (70.4%)
Ricinus communis	43.2 b (86.4%)	0.0 (0%)	43.2 b (86.4%)
Chenopodium ambrosioides	48.7 a (97.4%)	0.0 (0%)	48.7 a (97.4%)
Morinda citrifolia	21.8 d (43.6%)	2.0 (9.17%)	19.8 d (39.6%)
Azadirachta indica	39.2 c (78.4%)	2.3 (5.9%)	36.9 c (73.8%)
Jatropha curcas	19.2 d (38.4%)	9.3 (48%)	9.9 e (19.8%)
Solenostemon scutellarioides	0.7 e (1.4%)	0.0 (0%)	0.7 f (1.4%)
Water (Control)	0.0 e (0.0%)	0.0 (0%)	0.0 f (0.0%)
CV (%)	8.15	-	9.12

*Average of six replications, 50 juveniles $dish^{-1}$. Mean values followed by the same letter in a column do not differ by Tukey's test at 5% probability.

Placing juveniles in water after a time spent in plant extracts is essential, since the immobility induced by substances with a nematostatic effect can be reversed, i.e. a lack of motility does not imply nematocidal action (NEVES et al., 2008).

As for juvenile mortality, the extracts of *D. amoena, D. stramonium, P. scandens, S. anthelmia, R. communis, C. ambrosioides* and *A. indica* had a high nematocidal effect, causing death in over 70% of the M. enterolobii J2, verified after placing them in water. The most efficient was the extract of *C. ambrosioides*, which caused 97.4% mortality. However, in the assays with *J. curcas, M. citrifolia* and *S. scutellarioides*, the number of dead juveniles in the extracts was less than 40% (Table 2).

Santos (2015), studying the in vitro nematocidal potential of *D. stramonium* leaf extract at a concentration of 0.1 g mL^{-1}, higher than the concentration used in this assay, found 100% mortality in second-stage juveniles of M. enterolobii. This result confirms the toxic action of *D. stramonium* on the nematoid.

The nematocidal effect of *A. indica* leaf extract has been demonstrated in several species of phytonematodes, among them *Pratylenchus* sp., *Rotylenchulus reniformis*, and *M. incognita* (FERRAZ et al,. 2012). *In vitro* tests of *A. indica* extract at a concentration of 0.25 g mL^{-1} caused death in 100% of *M. incognita* J2 (ADEGBITE; ADESIYAN, 2005). In this assay, *A. indica* leaf extract prepared at a lower concentration (0.05g mL^{-1}) resulted in the death of 73.8% of *M. enterolobii* juveniles (Table 2).

Similar data to those obtained in this study were presented by Martins and Santos (2016) for leaf extracts of *S. anthelmia* and *C. ambrosioides,* in which strong nematocidal action on the J2 of *M. incognita* was found for aqueous extracts prepared at 0.1 g mL^{-1} and at 0.05g mL^{-1}.

Aqueous extracts from the aboveground part of the *R. communis* were effective for *in vitro* mortality and the reduction of tumours in the roots of pepper plants infested with the false root-knot nematode, *Nacobbus aberrans* (MAREGGIANI et al., 2005).

A study carried out by Brito and Fernandes (2013), which evaluated the anthelmintic action of aqueous and ethanolic *M. citrifolia* leaf extracts on the bird nematode, *Heterakis gallinarum*, found a nematode mortality of over 90%, demonstrating the high effectiveness of these extracts. However,

in this study, the harmful effect of *M. citrifolia* leaf extracts on the phytonematode, *M. enterolobii*, was low, with a mortality rate of only 39.6% (Table 3).

Table 3. Mean values for plant height, root length (RL), root fresh weight (RFW), shoot fresh weight (SFW), shoot dry weight (SDW) and total fresh weight (TFW) of plants grown in soil inoculated with *Meloidogyne enterolobii* and treated with leaf extracts. Fortaleza, Ceará, 2016.

Treatment	Height	RL	RFW	SFW	SDW	TFW
Spigelia anthelmia	*60.2 ab	22.1 ab	10.7 ab	45.1 bc	5.0 ab	55.8 b
Ricinus communis	64.4 ab	22.7 a	12.3 ab	51.7 ab	6.1 a	64.0 ab
Dieffenbachia amoena	33.9 c	18.2 bc	2.9 c	14.9 d	1.7 cd	17.8 c
Plumbago scandens	38.8 c	17.9 bc	4.2 c	17.6 d	1.4 d	21.7 c
Chenopodium ambrosioides	63.9 ab	25.2 a	9.8 b	40.8 bc	5.2 ab	50.6 b
Datura stramonium	68.4 a	25.3 a	15.4 a	49.2 abc	5.8 a	64.6 ab
Azadirachta indica	66.4 ab	21.9 ab	13.2 ab	45.1 bc	5.3 ab	58.3 ab
Negative control[1]	66.0 ab	21.3 abc	14.3 ab	60.4 a	6.4 a	74.7 a
Positive control[2]	53.6 b	17.2 c	11.3 ab	36.5 c	3.4 bc	47.8 b
CV(%)	12.15	11.26	24.43	19.62	22.94	18.14

*Mean values followed by the same letter in a column do not differ by Tukey's test at 5% probability.
[1] Uninoculated plants with no extract; [2] Inoculated plants with no extract.

There are several studies in the literature that report the use of natural products, such as plant extracts and essential oils from various plant species (condiment, medicinal and toxic), as potential sources of nematocide and nematostatic compounds, especially from the leaves, roots and seeds (NEVES et al., 2008; MOREIRA et al., 2009; FERRAZ et al., 2012).

Studies of plant extracts in the control of phytonematodes are generally carried out *in vitro* or in the greenhouse, and confirmation of the results in the field is necessary.

The information obtained in this assay allowed the selection of promising plants for working with extracts in the greenhouse. The results of this research may encourage testing of the more-effective extracts under field conditions, with the aim of later adopting this practice as an alternative measure of nematode control.

Evaluation of the efficiency of aqueous extracts applied to the infested soil before and after planting the tomato seedlings

In evaluating the variables used in this assay, different action by the seven extracts was seen on plant growth and infection.

The tomato plants grown in soil inoculated with *M. enterolobii* and treated with the leaf extracts of *S. anthelmia, R. communis, C. ambrosioides, A. indica* and *D. stramonium*, showed no difference in height when compared to the negative control (66 cm) (healthy plant with no extract) (Table 3). On the other hand, where the extracts of *D. amoena* and *P. scandens* were used, plant height was reduced (33.9 and 38.8 cm), and was even less than in the tomato plants of the positive control (53.6 cm) (Table 3).

It was found that for the tomato plants treated with extracts of *S. anthelmia*, *R. communis, C. ambrosioides, D. stramonium* and *A. indica*, there was no difference in RL (21.9 to 25.3 cm); this included the negative control. However, in plants treated with extracts of *D. amoena* and *P. scandens*,

root length was smaller (17.9 and 18.2 cm), differing from earlier treatments, but similar to the result obtained with the positive control (Table 3).

It was found that for plants in soil treated with extracts of *S. anthelmia, R. communis, C. ambrosioides, D. stramonium* and *A. indica*, the values for root fresh weight (RFW) were generally similar and did not differ from the results obtained with the controls. However, in the treatments where extracts of *D. amoena* and *P. scandens* were applied to the soil, the plants presented values for RFW well below those of the other treatments (Table 3).

For shoot fresh weight (SFW), the best treatments were found to be those where extracts of *R. communis* and *D. stramonium* were used, and were no different from the negative control, where the average was 60.4 g. In the other treatments, with extracts of *S. anthelmia, C. ambrosioides* and *A. indica*, the values for SFW were no different from the positive control. The plants from soil treated with extracts of *S. anthelmia* and *D. amoena* again had the lowest values related to development (Table 3).

Ferreira et al. (2013), evaluating the aqueous extracts of six species applied to soil infested with *M. incognita*, found that the extracts increased aboveground weight in tomato plants, but found no increase in the fresh weight of the root system. Similarly, in the present study no increase in root development was seen in the tomato plants for any of the treatments.

Evaluation of shoot dry weight (SDW) for each treatment showed that the plants with an application of *S. anthelmia, R. communis, C. ambrosioides, D. stramonium* and *A. indica* did not differ in mean value from the negative control, but did differ from the positive control. This suggests that application of the extracts of these five plant species to the soil may have favoured the biomass of the tomato plants, since in the presence of nematode parasitism, development of the plants is usually affected (Table 3).

Tomato plants from the treatments with *R. communis, D. stramonium* and *A. indica* extract showed no statistical differences in total fresh weight (TFW) when compared to the negative control (70.7 g). Values for TFW in plants treated with extracts of *S. anthelmia* and *C. ambrosioides* were close to that of the positive control. Only the tomato plants treated with *D. amoena* and *P. scandens* had the lowest values for TFW, both less than 22 g (Table 4).

The smaller growth of the plants in soil receiving extracts of *D. amoena* and *P. scandens* may be related to a possible phytotoxic effect, since marked chlorosis was seen in the leaves seven days after the first application of the extracts. This toxic reaction may also have contributed to the lesser development of the treated plants.

Table 4. Mean number of galls (NG), number of egg masses (NEM), number of eggs (NE) and percentage population reduction (PR) in tomato plants 45 days after the inoculation of *Meloidogyne enterolobii* in soil treated with leaf extracts. Fortaleza, Ceará, 2016.

Treatment	NG	NEM	NE	PR
Spigelia anthelmia	51.0 c	25.8 c	1.715 c	91.0
Ricinus communis	90. 0 b	40.3 b	3.093 b	83.77
Dieffenbachia amoena	7.0 e	1.0 f	21.5 d	99.88
Plumbago scandens	10.1 e	1.2 f	19.2 d	99.89
Chenopodium ambrosioides	27.3 d	7.0 e	82.5 d	99.56
Datura stramonium	73.0 b	14.2 d	783.3 c	95.89
Azadirachta indica	17.7 d	3.8 e	352.3 d	98.15
Tomato (control)	270.2 a	112.8 a	19.059 a	-
CV(%)	19.95	23.93	32.47	-

*Mean values followed by the same letter in a column do not differ by Skott-Knot test at 5% probability. The data for NG, NEM and NE were transformed into √x+1 for the statistical analysis. The original values are shown in the table.

The results of evaluating the effect of the seven plant extracts on *M. enterolobii* infestation in tomato plants showed that all treatments differed from the positive control for NG, NEM and NE, with a significant reduction in parasitism, indicating that the extracts were quite effective in controlling the pathogen (Table 4).

Considering the good development of the plant root system, the lowest mean values for NG were seen in treatments involving the extracts of *A. indica* (17.7) and *C. ambrosioides* (27.3), followed by *S. anthelmia, D. stramonium* and *R. communis*, all of which were significantly less than the result of the positive control (270.2) (Table 4).

The NEM in the roots of the treated plants varied from 3.8 in the treatment with *A. indica* extract to 40.3 where *R. communis* extract was applied to the soil. The positive control had a mean value of 112.8 eggs masses.root^{-1}, differing from the other treatments (Table 4).

Soil treated with the extracts of *C. ambrosioides* and *A. indica* had the lowest mean values for NE, of 82.5 and 352.3 respectively. The *D. stramonium, S. anthelmia* and *R. communis* extracts were also effective in reducing nematode infestation in the tomato plants, with even lower values for the number of eggs (783.3; 1,715 and 3,093) than the control with 19,059 eggs (Table 4).

The percentage population reduction (PR) of *M. enterolobii* in the root system of the tomato plants, calculated based on the number of eggs found per root, was greater than 90% in the treatments with *C. ambrosioides, A. indica, D. stramonium* or *S. anthelmia* extract. For the *R. communis,* the PR was still high, with a reduction of 83.77 in egg production per plant (Table 4).

The lower number of galls and reduced parasitism in the tomato plants from the treatments with *D. amoena* (7.0) and *P. scandens* (10.1), as well as the NEM (1.0 and 1.2) and NE (21.5 and 19.2), may be associated with the lesser root development caused by the phytotoxic effect. New studies should therefore be carried out with these extracts, which were efficient *in vitro*, with the aim of evaluating the best concentration for their use in infested soil when it is intended to work with tomato plants.

The marked reduction in the number of eggs in the roots of the tomato plants is associated with the smaller number of females, and consequently of egg masses, in the root system, due to the death of infesting juveniles through the nematocidal action of the leaf extracts. Application of the extracts carried out the day after inoculation, and 7 and 14 days later, were effective in eliminating juveniles of *M. enterolobii* present in the inoculum and in the soil. Repeat applications of the extracts to the soil at seven-day intervals would have affected any hatched J2 through nematocidal action, as seen in the *in vitro* assays. Tests to investigate the *in vitro* effect of the extracts on the hatching of J2 were not carried out in this assay.

Results similar to those of this study were obtained by Almeida et al. (2012) using extracts of *A. indica*, where the authors verified the efficiency of the aqueous extract applied to the soil on *M. javanica*, with a reduction of 92% in the number of galls.

The nematocidal action of *C. ambrosioides* was reported by Rodrigues et al. (2008), who found that the aqueous extract of this species was able to reduce the number of galls of the *M. exigua* coffee nematode by 45.7%.

Ademola et al. (2007) reported from *in vitro* and *in vivo* tests that *S. anthelmia* has an anthelmintic action on sheep nematodes. Martins (2009) demonstrated the efficiency of the aqueous extract of this species on *M. incognita* when applied to the soil, reducing the number of galls in tomato plants by 59.8%.

Santos (2015), cultivating *D. stramonium* and *D. metel* before planting crispy lettuce (*Lactuca sativa* L.), showed a marked reduction (98-100%) in *M. enterolobii* parasitism when compared to the control.

Observations by Gardiano et al. (2009) showed that the aqueous extract of the *R. communis* when applied to the soil, gave a 54.4% and 56.6% reduction respectively in the number of galls and eggs of *M. javanica* in tomato plants.

According to Ferraz et al. (2012), research involving a greater number of botanical families is necessary to select plants with nematocidal potential, and to characterise the active compounds present in those plants, their mode of action, appropriate concentration and best method of application under field conditions.

In this study, the results obtained with leaf extracts were promising for further investigation of nematocidal compounds, aiming at their better use in the effective control of *M. enterolobii* in infested areas.

Conclusions

The plant species, *D. amoena, S. anthelmia, P. scandens, R. communis, C. ambrosioides, A. indica, M. citrifolia, J. curcas* and *D. stramonium* can be recommended for crop rotation in areas infested with M. enterolobii.

Solenostemon scutellarioides can be employed in the multiplication of *M. enterolobii*.

The leaf extracts of *D. amoena, S. anthelmia, P. scandens, R. communis, C. ambrosioides, A. indica* and *D. stramonium* displayed an in vitro nematocidal effect on the juveniles of *M. enterolobii,* with the extract of *C. ambrosioides* being the most efficient.

The extracts of *S. scutellarioides, A. indica* and *J. curcas* had little effect on *M. enterolobii* juveniles *in vitro.*

The leaf extracts of each species effective *in vitro*, were also effective in reducing *M. enterolobii* infestation in the roots of tomato plants.

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