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Emprego de *Calotropis procera* no controle de *Meloidogyne incognita* raça 2 em tomateiro

Use of *Calotropis procera* to control *Meloidogyne incognita* race 2 in tomato

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A B S T R A C T

The aim of this study was to investigate the potential of using *Calotropis procera* leaves *in vitro* and *in vivo* to control of *Meloidogyne incognita* race 2 in tomato (*Solanum lycopersicum*). *In vitro* tests consisted of evaluating the effect of the aqueous extract of leaves of *C. procera* (5 and 10%) on the hatching and mortality of juveniles of second stage considering the residence time of the J2 in the extract for 24 and 48 hours. *In vivo* tests consisted of evaluating the application of the extract to the soil and the incorporation of fresh leaves of *C. procera* (50 and 100 g/kg of soil) in pots with soil infested with *M. incognita* to control the nematode. After 48 hours, the 10% aqueous extract caused greater inhibition of hatching and mortality of J2. Results of *in vivo* tests demonstrated that the application of the aqueous extract to the infested soil caused a reduction in the number of galls, number of eggs masses, number of eggs and the reproduction factor of de *M. incognita* in tomato, and the incorporation of fresh leaves of *C. procera* into the soil promoted the eradication of *M. incognita* race 2.

R E S U M O

O objetivo deste estudo foi investigar o potencial do uso de folhas de de *Calotropis procera* no controle *in vitro* e *in vivo* de *Meloidogyne incognita* raça 2 em tomateiro (*Solanum lycopersicum*). Os ensaios *in vitro* consistiram em avaliar o efeito do extrato aquoso de folhas de *C. procera* (5 e 10%) sobre a eclosão e mortalidade de juvenis de segundo estádio (J2), considerando o tempo de permanência dos J2 no extrato durante 24 e 48 horas. Nos ensaios in vivo foi avaliada a aplicação do extrato ao solo e a incorporação de folhas frescas de *C. procera* (50 e 100 g/kg de solo) em vasos com solo infestado com *M. incognita*, para controle do nematoide. Após 48 horas, o extrato aquoso a 10% provocou maior inibição da eclosão e mortalidade de J2. Os resultados dos testes *in vivo* demonstraram que a aplicação do extrato aquoso ao solo infestado provocou uma redução do número de galhas, número de massas de ovos, número de ovos e do fator de reprodução de *M. incognita* em tomateiro, e a incorporação de folhas frescas de *C. procera* ao solo promoveu a erradicação de *M. incognita* raça 2.

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Introduction

Root-knot nematodes belonging to the genus *Meloidogyne* Goeldi are one of the most damaging plant parasites that compromise various agricultural crops (Caballero-Luque et al., 2021). The tomato (*Solanum lycopersicum* L.) is one of the vegetables of greatest economic importance worldwide, susceptible to a wide range of plant pathogens, including root-knot nematodes (Bergougnoux, 2014), particularly the *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood e *M. arenaria* (Neal) Chitwood species (Charchar & Lopes, 2005).

The application of nematicides in agriculture is considered the most effective control practice of plant-parasitic nematodes but has been reduced in recent decades due to its high toxicity, persistence in the soil and adverse effects on man, animals, and the environment, in addition to the high cost it represents for small and medium agricultural producers (Onkendi et al., 2014).

Alternative measures have been studied as a way of replacing the use of conventional nematicides such as the use of bionematicides and antagonistic plants. Plants produce several types of organic compounds, such as secondary metabolites, which can act to protect against microorganisms, among them nematodes (Ferraz et al., 2012).

The nematicidal effect is attributed to several plant species, such as *Calotropis procera* (Ait.) R. Br (Agatha & God, 2018). This plant is considered an invasive exotic species belonging to the Apocynaceae family, known by various names like Sodom apple, sea apple, swallow wort, and milk weed (Ibrahim et al., 2015). This species is widely distributed geographically throughout the world, especially in tropical and subtropical regions, and is originally from the African and Asian continent (Fabricante et al., 2013; Mello et al., 2001). Commonly found in urban environments, roadsides, coastal areas, and pasture areas, the species *C. procera* is considered a ruderal species since it occupies environments modified by man (Rangel & Nascimento, 2011).

Several studies have been carried out with *C. procera*, with different purposes, and in the most diverse segments. Can be used in animal feed (Costa et al., 2009), in the production of drugs with anti-inflammatory effects (Parihar et al., 2011), analgesic (Mello et al., 2001), anti-bacterial (Ali et al., 2014), nematicide (Cavalcante et al., 2020), insecticide (Begum et al., 2010), and allelopathic (Gulzar & Siddiqui, 2017).

Research involving the chemical characterization of the constituents of *C. procera* has been carried out to control pests and plant diseases (Cavalcante et al., 2016), due to the presence of several secondary metabolites responsible for plant defense (Mohamed et al., 2015). Considering the economic importance and the damage caused by *M. incognita* to the tomato crop, the present work aimed to investigate the effect *in vivo* and *in vitro* of aqueous extracts and the incorporation of fresh leaves of *C. procera* to control *M. incognita* race 2 in tomato.

Materials and Methods

Four experiments were conducted at the Laboratory of Plant Pathology and greenhouse (3°44'25.6"S 38°34'31.2"W) at the Universidade Federal do Ceará (UFC), Campus of Pici, Fortaleza, CE, Brazil.

Obtaining aqueous extracts

The methodology adopted in the preparation of the aqueous extracts of *C. procera* was an adaptation of the work by Dias et al. (2000). New leaves of *C. procera* were subjected to the drying process in paper bags in a forced air circulation oven at a temperature of 60° C for 72 hours. Then, the dried leaves were immersed in distilled water for 24 hours. After this time, the leaves were crushed in a blender and the extracts were filtered through gauze and centrifuged for 10 minutes at 2.000 rpm. The proportion used was 1 g of dry leaf to 10 mL of water. In this way, the extract was obtained at a 10 % (w/v) dilution.

The concentrations of the extract tested in the *in vitro* and *in vivo* tests were 5 and 10 %. The concentration of 5 % was obtained using a part of the extract at 10 % and an equal part of distilled water. The extracts were prepared 24 hours before the tests were carried out and kept in a conical flask at laboratory room temperature (25 ± 2º C).

Effect of aqueous extract of C. procera leaves on hatching of juveniles of M. incognita

Petri dishes of 3.5 cm in diameter were used as hatching chambers. Three mL of each extract concentration to be tested (5 or 10 %) and 50 *M. incognita* race 2 eggs were distributed in each plate. The control consisted of the suspension of eggs only in distilled water. The eggs of the nematode used in this test were obtained from tomato root using the method of extraction by Bonetti and Ferraz (1981).

The hatching chambers were placed in polyethylene trays lined with moist filter paper, covered with laminated paper, keeping them at a temperature of 25 ± 2º C. Fifteen days after incubation, the number of second-stage juveniles (J2) hatched/plaque in each concentration was evaluated, under stereo microscope.

The adopted statistical design was completely randomized, with three treatments [5% extract; 10 % extract and control (water)] and 10 repetitions. The experimental plot consisted of a hatching chamber with 50 eggs, totaling 1,500 individuals in this trial.

Effect of aqueous extract of C. procera leaves on mortality of juveniles of second stage of M. incognita

Egg masses from tomato roots infected with *M. incognita* race 2 were removed and transferred to Petri dishes containing distilled water. After 24 hours, 50 newly hatched J2 were transferred to Petri dishes 3.5 cm in diameter containing 3 mL of each extract concentration to be tested. The control consisted only of the suspension of J2 in distilled water. After 24 and 48 hours, juvenile mortality and motility were assessed, by counting immovable juveniles and assets, respectively. The immobile juveniles in the extract were transferred to a Petri dish containing distilled water to observe the possible recovery of mobility. A new count of immobile (dead) and active (alive) J2 was performed under a stereo microscope 24 hours after transfer to water, being calculating the juvenile mortality percentage.

The adopted statistical design was completely randomized, in the 2 x 3 factorial scheme, with six treatments (extract 5 % for 24 h; extract 10 % for 24 h; water for 24 h; extract 5 % for 48 h; extract 10 % for 48 h; water for 48 h) and 10 repetitions. The experimental plot consisted of a Petri dish with 50 juveniles, totaling 3,000 individuals.

Effect of the application of aqueous extract of C. procera leaves on soil infested with M. incognita

Initially, aliquots containing 5,000 eggs of *M. incognita* race 2 were distributed in three holes 3 cm deep, in an autoclaved and moist mixture of soil and goat manure in a 2:1 (v: v) ratio, contained in plastic pots with a capacity of 1 L. After the inoculum was distributed, the holes were closed. The day after the soil infestation, 30 ml of the extracts of *C. procera* were applied, using the same dilutions (5 and 10%) of the in vitro tests.

The application of the extracts was carried out in the form of watering over the entire surface of the previously moistened soil, in the late afternoon, to avoid evaporation of the extracts. For the control treatments, 30 ml of distilled water was applied to the soil. One day after applying the extract to the soil, a tomato seedling cv. Carolina with two to three real leaves was transplanted into each pot. Two more applications of 30 mL of the extract were made to the soil, at 7 and 14 days after transplanting the seedlings.

The plants remained in the greenhouse, being irrigated daily. Fifty-five days after transplanting, the plants were removed and the root system separated from the aerial part for evaluation of plant height (PH), root fresh weight (RFW), shoot fresh weight (SFW), shoot dry weight (SDW), number of galls (NG), number of eggs masses (NEM), number of eggs (NE) per tomato plant, and the reproduction factor (RF) was calculated.

The methodology for extracting eggs from the nematodes used was proposed by Bonetti and Ferraz (1981). The counting was performed in a Peters chamber under a stereomicroscope, and later, the reproduction factor (RF = final population/initial population) was calculated.

The experimental design adopted was completely randomized, with six treatments: 1) plants inoculated and treated with 5 % extract; 2) inoculated plants and treated with 10 % extract; 3) plants not inoculated and treated with 5 % extract; 4) plants not inoculated and treated with 10 % extract; 5) negative control (uninoculated plant); 6) positive control (inoculated plant without extract) and 10 repetitions. The experimental plot consisted of a pot with a tomato plant, totaling 60 pots.

Effect of the application of aqueous extract of C. procera leaves on soil infested with M. incognita

Tomato seedlings cv. Carolina with two to three real leaves were transplanted to plastic pots with a capacity of 1 L, containing an autoclaved and moist mixture of soil and goat manure in a 2:1 (v: v) ratio. Two days after transplantation, 5,000 eggs of *M. incognita* race 2 were inoculated in each pot containing a tomato seedling. Sixty days after inoculation, the plants were removed and fresh and crushed leaves of *C. procera* were incorporated into the infested the soil contained in each pot until a homogeneous mixture was obtained. The addition of the leaves was also made in pots with uninoculated tomato plants.

After the incorporation of *C. procera* leaves into the infested soil, the pots were kept in a greenhouse for 30 days, being watered daily. Thirty days after incorporation, a tomato seedling cv. Carolina with two to three real leaves was transplanted into each pot. In the control treatment, tomato plants were grown in infested and non-infested soil, and in both cases, there was no incorporation of *C. procera* leaves into the soil.

Fifty-five days after transplanting, plants were removed from the pots to evaluation of plant height (PH), root fresh weight (RFW), shoot fresh weight (SFW), shoot dry weight (SDW), number of galls (NG), number of eggs (NE) and the reproduction factor (RF) was calculated, per tomato plant.

The experimental design adopted was completely randomized, with six treatments: 1) infested soil and incorporation of 50 g of leaves/pot; 2) infested soil and incorporation of 100 g of leaves/pot; 3) non-infested soil and incorporation of 50 g leaves/pot; 4) non-infested soil and incorporation of 100 g leaves/pot; 5) negative control (soil not infested without leaf incorporation); 6) positive control (soil infested without leaf incorporation) and 10 repetitions. The experimental plot consisted of a pot with a tomato plant, in a total of 60 pots.

Statistical analysis

The data obtained in all tests were submitted to analysis of variance and Tukey's mean comparison test at 5 % probability. Statistical analysis was performed using the statistical program Sisvar® version 5.7.

Results and Discussion

Effect of aqueous extract of C. procera leaves on hatching of juveniles of M. incognita

It was observed that the aqueous extracts of *C. procera*, in both dilutions, 5 and 10%, differed significantly ($p<0.05$) from the control treatment. The 10 % extract caused the lowest percentage of hatching of J2 with mean of 6.6 hatched J2/plate which corresponds to a percentage of 13.2 % hatching in this treatment. The 5 % extract allowed mean hatching of 15.1 individuals/plate indicating a percentage of 30.2 % hatching (Table 1).

Table 1. Means values and percentages of *in vitro* hatching of *Meloidogyne incognita* race 2 of juveniles second stage (J2) after 15 days of exposure to aqueous extract of *Calotropis procera* at 5 and 10 %.

Treatments	Hatched J2
5% Extract	15.1* b (30.2%)
10% Extract	6.6 c (13.2%)
Control (water)	47.7 a (95.4%)
CV (%)	18.81

*Mean of ten repetitions with 50 eggs/plate; Means followed by the same lowercase letter in the column do not differ statistically between by the Tukey test ($p <0.05$); CV: coefficient of variation.

Similar results to this work were reported by Barros (2004), who, when evaluating the effect of the *C. procera* leaf extract on the hatching of *M. javanica*, observed a hatching percentage of 12 %. Sharma and Trivedi (2002) also reported nematicidal properties in *C. procera* leaf extracts against *M. incognita*, noting a reduction in the hatching of juveniles of up to 93.14 %.

In a study by Chedekal (2013), the hatching of *M. incognita* eggs in treatments with aqueous extract of fresh *C. procera* leaves were reduced by 99.83 %. However, unlike the results observed in the present work, Joshi et al. (2019) reported a hatching percentage of *M. javanica* eggs of 47 and 66 % after 72 and 96 h of exposure to aqueous extract *of C. procera* to the concentration of 5 %, respectively.

Few studies have been reported dealing with the action of *C. procera* on *M. incognita*. However, high contents of active compounds have already been found in *C. procera* leaves,

such as cardiac glycosides, alkaloids, terpenes, resins, lipids, flavonoids, tannins, steroids, and saponins (Mohamed et al., 2015), chemical groups found in several plants that have known nematicidal activity (Ferraz et al., 2012).

Effect of aqueous extract of C. procera leaves on mortality of juveniles of second stage of M. incognita

The results presented in Table 2 show that there was a significant interaction between the factors time and extract concentration, indicating that the dilution of the aqueous extract of *C. procera* and the permanence time of second-stage juveniles (J2) in the extracts influence each other, causing a nematicide effect on juveniles of *M. incognita*.

Table 2. Means and percentages of immobility and mortality of second stage juveniles (J2) of *Meloidogyne incognita* race 2 in aqueous extracts of *Calotropis procera* at 5 and 10 % counted after 24 and 48 hours of exposure.

Treatments	Immobility		Mortality[1]	
	24 h	48 h	24 h	48 h
5% Extract	33.90* bB (67.8%)	44.30 aA (88.6%)	29.50* bB (59%)	43.10 aA (86.2%)
10% Extract	46.10 aA (92.2%)	43.90 aA (87.8%)	46.10 aA (92.2%)	41.20 aB (82.4%)
Control (water)	0.40 cA (0.8%)	0.50 bA (1.0%)	1.40 cA (2.8%)	1.20 bA (2.4%)
CV %		8.93		11.14

*Mean of ten repetitions with 50 juveniles/plate; Means followed by the same lowercase letter in the column and uppercase in the row do not differ statistically between by the Tukey test ($p < 0.05$); CV: coefficient of variation; [1]J2 dead after transfer to water.

It was observed that the J2 that remained for 24 and 48 h in the 10 % extract had high averages of immobile J2 per plate, 46.10 and 43.90, corresponding to immobility percentages of 92.2 and 87.8 %, respectively (Table 2). In the 5% extract, the average of J2 immobile was 33.9 J2/plate (67.8 %) in 24 h of exposure, increasing to 44.3 J2/plate (88.6 %) within 48 h in the extract. In the control treatment, an average of 0.4 and 0.5 J2 immobile/plate was observed, whose percentages of immobility were 0.8 and 1.0%, respectively (Table 2).

After the J2 were transferred from the leaf extracts to water, a reduced reduction in mobility was observed, revealing the nematicidal, and non-nematostatic, the action of the *C. procera* extracts. Mean mortality of 29.5 J2/plate (59 %) and 43.1 J2/plate (86.2 %) was recorded for the 5 % extracts for 24h and 48h, while the mortality of the 10 % extracts was 46.1 J2/plate (92.2 %) and 41.2 J2/plate (82.4 %) after 24h and 48h, respectively. In the control treatment, the means of J2 mortality after 24 and 48 h, respectively, were 1.4 to 1.2 J2/plate, corresponding to less than 3 % of juvenile death (Table 2).

The passage of juveniles to water after remaining in extracts is necessary, since some plant species may have substances with only a nematostatic effect, thus masking the nematicide action (Dias et al., 2000).

Santos (2015) verified the *in vitro* nematicidae effect of the aqueous extract of *C. procera* on second-stage juveniles of *M. enterolobii* at a dilution of 10 and 20 %, causing 100 % mortality of juveniles after 48 h in the extract. A percentage mortality of second stage *M. incognita* juveniles of 60.33 % were obtained by Chedekal (2013), by testing the *in vitro* effect of the aqueous extract of fresh *C. procera* leaves after 72 h time of exposure. Kumar et al. (2018) observed the nematicide activity of extracts from bio-guided latex fractionation of *C. procera*, in the *in vitro* assay against second stage juveniles of *M. javanica*.

Knowledge of the chemical composition of extracts and essential oils can lead to the synthesis of molecules giving rise to natural nematicides, replacing conventional nematicides (Silva, 2011). The promising results obtained for the *in vitro* control of *M. incognita* with *C. procera* extracts reinforce the need for further studies and research that explore the potential nematicide action of this species, given the scarcity of work with this plant species.

Effect of the application of aqueous extract of C. procera leaves on soil infested with M. incognita

The means of shoot fresh weight (SFW) and dry weight (SDW) of tomato plants treated with *C. procera* extracts did not differ (p<0.005) from each other (5 and 10 %) and from the negative control (healthy plants and without extract). However, these two variables differed from the positive control (inoculated plant, without extract), which presented the lowest means (Table 3).

Table 3. Shoot fresh weight (SFW), shoot dry weight (SDW), plant height (H) and root fresh weight (RFW) of tomato plants cv. Carolina grown in soil infested with *Meloidogyne incognita* race 2 and treated with aqueous extract of *Calotropis procera*.

Treatments	SFW (g)	SDW (g)	Height (cm)	RFW (g)
Inoculated plant + 5% extract	25.9 a	4.2 a	51.6 a	7.3 a
Inoculated plant + 10% extract	28.4 a	4.5 a	49.4 ab	7.5 a
Uninoculated plant + 5% extract	31.4 a	4.6 a	53.5 a	7.9 a
Uninoculated plant + 10% extract	26.7 a	3.9 a	46.2 ab	7.6 a
Negative control[1]	27.5 a	3.9 a	40.8 b	4.8 b
Positive control[2]	13.2 b	1.9 b	31.9 c	7.4 a
CV (%)	17.87	15.93	10.74	16.35

Means followed by the same lowercase letter in the column do not differ statistically between by the Tukey test (p <0.05); CV: coefficient of variation; [1]Plant not inoculated with extract; [2]Plant inoculated without extract.

The plant height (H) of the four treatments with an application of both extract dilutions of *C. procera* to the soil, with or without nematode inoculation, had significantly higher mean

heights, both to the positive control (inoculated) and to the negative control (healthy) plants (Table 3). It is noteworthy that the extracts of *C. procera* did not cause any phytotoxicity symptoms in tomato plants.

For the variable root fresh weight (RFW), there was no significant difference between plants treated with extract, inoculated or not with the nematode, and the positive control, however, all these differed from the negative control that had the root with the lowest fresh weight (Table 3).

Agatha and God (2018) evaluating the effect of applying the crude aqueous extract of *C. procera* to the soil on the growth and production of tomato plants infected with *M. javanica*, observed that plants treated with extracts showed significantly higher growth and yield, in addition to causing a reduction in the nematode population.

Regarding the action of the extracts on the infectivity of juveniles, it was observed that the application of 5 and 10% aqueous extract of *C. procera* reduced the number of galls (NG), number of egg masses (NEM), number of eggs (NE) in the roots and, consequently, the reproduction factor (RF) in relation to the positive control, indicating the efficiency of the application of this plant extract in the control of *M. incognita* in the form of soil irrigation (Table 4).

Table 4. Means values of number of galls (NG), number of egg masses (NEM), number of eggs (NE) and reproduction factor (RF) of tomato plants cv. Carolina grown in soil infested with *Meloidogyne incognita* race 2 and treated with aqueous extract of *Calotropis procera*.

Treatments	NG	NME	NE	RF
Inoculated plant + 5% extract	259.7 a (-62.5 %)	29.2 a	1.483.3 a	0.29 a
Inoculated plant + 10% extract	126.4 a (-81.7 %)	12.6 a	1.624.4 a	0.33 a
Positive control[1]	692.7 b (10.0 %)	105.7 b	7.433.2 b	1.48 b
CV (%)	32.47	42.44	41.61	41.48

Means followed by the same lowercase letter in the column do not differ statistically between by the Tukey test ($p < 0.05$); CV: coefficient of variation; [1]Inoculated plant without extract.

The means of NG and NEM in plants, whose soil was treated with *C. procera* extract (5 and 10 %), were similar to each other but differed significantly from the positive control. The percentage of reduction in the number of galls on tomato roots was 62.5 and 81.7 %, for treatments with extract at 5 and 10 %, respectively. The positive control had a mean NG of 692.7 galls/root (Table 4). The mean NEM in roots of plants with soil treated with 5 and 10 % extracts were 29.2 and 12.6 egg masses/root, respectively, while in the positive control the mean NEM was 105.7 egg/root pasta (Table 4).

Regarding NE, there was no difference between treatments with application to the soil of *C. procera* extracts either with 5 or 10%, being observed means, respectively, of 1,483.3 and

1,624.4 eggs/root, differing from the positive control, whose mean NE (7,433.2 eggs/root) was much higher than the treatments with applications of leaf extracts (Table 4). The plants, whose soils were treated with 5 and 10 % plant extracts, had RF means of 0.29 and 0.33., respectively, values that differ from the mean observed in the positive control tomato plants (1.48). These values represent a reduction in the reproduction factor of 80.4 and 77.7 %, respectively (Table 4).

Franzener et al. (2007) evaluated the protective effect of aqueous extract of *T. patula* leaves, flowers, and roots on tomato cv. Santa Cruz Kada on *M. incognita* parasitism observed that the pure extract of *T. patula* flowers when applied to the soil promoted a reduction of 62.2 % of NG and 52.8 % of NE, compared to the control (inoculated tomato without application of extract). The extracts of *C. procera* used in this work showed satisfactory results in reducing galls and the number of eggs when compared to other studies conducted with *M. incognita*.

According to Quarles (1992), botanical extracts have some advantages over synthetic pesticides in the control of pests and diseases because they have new compounds that are not easily inactivated, they are easily biodegradable, have multiple modes of action, and are derived from renewable resources.

Effect of the application of aqueous extract of C. procera leaves on soil infested with M. incognita

Tomato plants, whose soils of the pots were incorporated with leaves of *C. procera* (50 or 100g), had SFW mean similar to each other, regardless of the presence of the nematode, also not differing from that obtained in the negative control (soil not infested and without incorporation of leaves) (Table 5).

Table 5. Means values of Shoot fresh weight (SFW), shoot dry weight (SDW), height (H) and root fresh weight (RFW) of tomato plants cv. Carolina grown in soil infested with *Meloidogyne incognita* race 2 and incorporated with fresh leaves of *Calotropis procera*

Treatments	SFW (g)	SDW (g)	Height	RFW
Infested soil + incorporation of 50 g leaves/pot	33.8 ab	3.4 a	59.5 ab	2.1 a
Infested soil + incorporation of 100 g leaves/pot	37.3 a	3.7 a	68.8 a	2.7 a
Uninfested soil + incorporation of 50 g leaves/pot	33.3 ab	2.8 a	57.4 ab	2.6 a
Uninfested soil + incorporation of 100 g leaves/pot	29.1 ab	3.1 a	57.0 ab	2.7 a
Negative control[1]	33.9 ab	3.3 a	56.7 ab	2.7 a
Positive control[2]	27.6 b	2.8 a	52.5 b	2.3 a
CV (%)	14.75	24.86	14.79	34.96

Means followed by the same lowercase letter in the column do not differ statistically between by the Tukey test ($p < 0.05$); CV: coefficient of variation; [1]Soil not infested without incorporation of leaves; [2]Infested soil without incorporation of leaves

The means of SDW and RFW did not differ significantly from between treatments (Table 5). Similar behavior to that observed for the SFW was observed for the variable H, in which, except for the treatment whose soil was infested with *M. incognita* and incorporated with 100 g of *C. procera* leaves, the other treatments that received incorporation did not differ statistically from the positive control, which had its growth reduced and had the lowest mean of H (52.50 cm). Regarding the variable root fresh weight (RFW), there was no statistical difference between the means of all treatments (Table 5).

Lopes et al. (2008) reported that the incorporation of dry leaves of *C. procera* into the soil, in an experiment to control *M. javanica*, promoted an increase in the fresh mass of roots of tomato cv. Santa Cruz Kada. Silva et al. (2017) verified that the incorporation of dry biomass of *C. procera* to the soil in beds cultivated with radish (*Raphanus sativus* L.), caused an increase in plant height, with an increase proportional to the amounts of biomass incorporated.

It was observed the absence of symptoms of galls on the roots of tomato plants cv. Carolina cultivated in pots whose soils were infested with *M. incognita* and treated with the incorporation of *C. procera* leaves in both quantities (50 and 100g) (Figure 1).

Figure 1. Root systems of tomato cv. Carolina incorporation of fresh leaves of *Calotropis procera* in soil infested with *Meloidogyne incognita* race 2. (A) 50g of incorporated leaves, (B) 100g of incorporated leaves (C) positive control (plant in soil infested without incorporation).

The suspensions from the nematode extraction process from the roots of these tomato plants did not contain any juvenile stage or eggs of *M. inocgnita*, indicating that the infection did not occur due to the probable total elimination of the pathogen from the soil. On the other hand, the plants of the positive control (infested soil without leaf incorporation) had a mean NG of 166.8 per root, NE of 2086.7 eggs/root, and RF of 2.4.

These results demonstrated that the elimination of the nematode from the infested soil was due to the incorporation of *C. procera* leaves and not due to the absence of hosts in the pots during the 30 days. The presence of active compounds with nematicidal action on the leaves of C. *procera* may have caused the inhibition of hatching and/or death of weed juveniles in the soil, resulting in the effective control of this nematode.

A result similar to that obtained in this work was reported by Santos (2015) who, when incorporating leaves of *C. procera* in soil infested with *M. enterolobii,* found that tomato plants cv. Santa Clara cultivated in the soil after incorporation, did not show gall symptoms. However, Lopes et al. (2008), evaluating the incorporation of dry leaves of *C. procera* into the soil, did not observe the eradication of *M. javanica* in tomato cv Santa Cruz Kada. The authors recommended the use of incorporated leaves associated with other management procedures, such as solarization and biological control.

Hussain et al. (2011), when studying the incorporation of dry leaves of *C. procera* in the control of *M. incognita*, verified that there was a reduction in the number of eggs and nematode reproduction factor in okra (*Abelmoschus esculentus* (L.) Moench) cv. Punjab.

The incorporation of organic residues into the soil may act on plant-parasitic nematodes through direct action of nematicide substances released and produced during the decomposition process in the soil, or indirectly by stimulating the antagonistic microbial population present in the soil. However, Oka (2010) speculated that this form of phytonematode control is influenced by the type of organic waste, plant species used, time of year, amount of material incorporated, soil type, pathogen-host interactions, environmental conditions, population level of the pathogen in the soil, among others.

Among the researches that have been carried out to develop and improve cultural methods for the control of nematodes, the incorporation of plant residues into the soil is one of the methods that have generated several positive results. The reduction of production costs and the low impact on agroecosystems are additional advantages of this control method, which can be adopted in sustainable agriculture systems as well as in conventional systems.

Calotropis procera can be considered a promising plant species for the control of *M. incognita*, both with the application of leaf extracts to the soil and with the incorporation of fresh leaves to the soil for decomposition. Both forms can be considered low-cost control practices, since *C. procera* is a ruderal species, grows quickly, and is easily found in tropical and subtropical regions. Field trials with this Apocynaceae should be carried out to confirm the efficiency under commercial garden conditions to control the root-knot nematode in infested areas.

Conclusion

Leaf extracts *C. procera* inhibit hatching and cause the mortality of juvenile *M. incognita* race 2 *in vitro*. The application of leaf extract of *C. procera* to the infested soil reduce parasitism of *M. incognita* race 2 in tomato roots cv. Carolina. The incorporation of fresh leaves of *C. procera* into the soil eradicates this nematode.

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REFERENCES

AGATHA, C. A.; GOD, V. L. T. The nematicidal property of aqueous crude extract of *Calotropis procera* on tomatoes (*Lycopersicum esculentus*). ***Journal of Biological Control***, v. 32, n. 4, p. 275-278, 2018.

ALI, A. et al. Antibacterial potential of *Calotropis procera* (flower) extract against various pathogens. ***Pakistan Journal of Pharmaceutical Sciences***, v. 27, n. 5, p. 1565-1569, 2014.

BARROS, R. F. X. Efeito de *Calotropis procera* sobre a eclosão e mortalidade de *Meloidogyne javanica* in vitro. **Monografia** (Graduação em Agronomia), Universidade Estadual de Montes Claros, Janaúba-MG, 2004.

BEGUM, N. et al. Evaluation of insecticidal efficacy of *Calotropis procera* and *Annona squamosa* ethanol extracts against *Musca domestica*. ***Journal of Biofertilizers & Biopesticides***, v. 1, n. 1, p. 1-6, 2010.

BERGOUGNOUX, V. The history pf tomato: from domestication to biopharming. ***Biotechnology Advances***, v. 32, n. 1, p. 170-189, 2014.

BONETTI, J. I. S.; FERRAZ, S. Modificações do método de Hussey & Barker para extração de ovos de *Meloidogyne exigua* em raízes de cafeeiro. ***Fitopatologia Brasileira***, v. 6, p. 553, 1981.

CABALLERO-LUQUE, T. M. et al. Characterisation of root-knot nematode (*Meloidogyne* spp.) and frequency of nematodes associated with artichoke crops in the "Irrigación Majes", Arequipa, Peru. ***Journal of Plant Diseases and Protection***, v. 128, p. 627-632, 2021.

CAVALCANTE, G. S. et al. Chemical composition and *in vitro* activity of *Calotropis procera* (Ait.) latex on *Haemonchus contortus*. ***Veterinary Parasitology***, v. 226, p. 22-25, 2016.

CAVALCANTE, G. S. et al. Chemical constituents of *Calotropis procera* latex and ultrastructural effects on *Haemonchus contortus*. ***Brazilian Journal of Veterinary Parasitology***, v. 29, n. 2, p. 1-12, 2020.

CHARCHAR, J. M.; LOPES, C. A. Nematoides. In: LOPES, C. A.; ÁVILA, A. C. (Ed.). **Doenças do Tomateiro**. Brasília: Embrapa Hortaliças. p. 95-100, 2005.

CHEDEKAL, A. N. Effect of four leaf extracts on egg hatching and juvenile mortality on root knot nematode *Meloidogyne incognita*. ***International Journal of Advanced Life Sciences***, v. 6, n. 1, p. 68-74, 2013.

COSTA, R. G. et al. Perspectivas de utilização da flor-de-seda *(Calotropis procera)* na produção animal. ***Revista Caatinga***, v. 22, n. 1, p. 276-285, 2009.

DIAS, C. R. et al. Efeito de extratos aquosos de plantas medicinais na sobrevivência de juvenis de *Meloidogyne incognita*. ***Nematologia Brasileira***, v. 24, n. 2, p. 203-210, 2000.

FABRICANTE, J. R. et al. Aspecto da ecologia de *Colatropis procera* (Apocynaceae) em uma área de Caatinga alterada pelas obras do projeto de Integração do Rio São Francisco em Mauriti, CE. ***Rodriguésia***, v. 64, n. 3, p. 647-654, 2013.

FERRAZ, S. et al. **Manejo sustentável de fitonematoides**. Viçosa: Editora UFV, 2012. 304p,

FRANZENER, G. et al. Proteção de tomateiro a *Meloidogyne incognita* pelo extrato aquoso de *Tagetes patula*. ***Nematologia Brasileira***, v. 31, n. 1, p. 27-36, 2007.

GULZAR, A.; SIDDIQUI, M. B. Allelopathic effect of *Calotropis procera* (Ait.) R. Br. on growth and antioxidant activity of *Brassica oleracea* var. botrytis. ***Journal of the Saudi Society of Agricultural Sciences***, v. 16, n. 4, p. 375-382, 2017.

HUSSAIN, M. A. et al. Efficacy evaluation of *Azadirachta indica*, *Calotropis procera*, *Datura stramonium* and *Tagetes erecta* against root-knot nematodes *Meloidogyne incognita*. ***Pakistan Journal of Botany***, v. 43, p. 197-204, 2011.

IBRAHIM, S. R. M. et al. Calotroposides H–N, new cytotoxic oxypregnane oligoglycosides from the root bark of *Calotropis procera*. ***Steroids***, v. 96, p. 63–72, 2015.

JOSHI, V. et al. Efficacy of botanicals on mortality of second stage juveniles and egg hatching of *Meloidogyne javanica* L: An eco-friendly management approach. ***Journal of Pharmacognosy and Phytochemistry***, v. 8, n. 3, p. 710-714, 2019.

KUMAR, L. et al. Isolation of biocidal compounds of calotropis latex and their *in vitro* and *in vivo* effect on the mortality of root knot nematode (*Meloidogyne javanica*) in Brinjal and Chilli. ***International Journal of Current Microbiology and Applied Sciences***, v. 7, n. 4, p. 3361-3372, 2018.

LOPES, E. A. et al. Efeito da incorporação da parte aérea de quatro espécies vegetais sobre *Meloidogyne javanica*. ***Nematologia Brasileira***, v. 32, n. 1, p. 76-80, 2008.

MELLO, M. M. et al. Estudo fitoquímico da *Calotropis procera* Ait., sua utilização na alimentação de caprinos: efeitos clínicos e bioquímicos séricos. ***Revista Brasileira de Saúde e Produção Animal***, v. 2, n. 1, p. 15-20, 2001.

MOHAMED, N. H. et al. Cytotoxic cardenolides from the latex of *Calotropis procera*. ***Bioorganic & Medicinal Chemistry Letters***, v. 25, n. 20, p. 4615-4620, 2015.

OKA, Y. Mechanisms of nematode suppression by organic soil amendments - A review. ***Applied Soil Ecology***, v. 44, n. 2, p. 101–115, 2010.

ONKENDI, E. M. et al. The threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: a review. ***Plant Pathology***, v. 63, n. 4, p. 727-737, 2014.

PARIHAR, G. et al. Anti inflammatory effect of *Calotropis procera* root bark extract. ***Asian Journal of Pharmacy & Life Science***, v. 1, n. 1, p. 29-44, 2011.

QUARLES, W. Botanical pesticides from *Chenopodium*. ***IPM Practitioner***, v. 14, p. 1-11, 1992.

RANGEL, E. S.; NASCIMENTO, M. T. Ocorrência de *Calotropis procera* (Ait.) R. Br. (Apocynaceae) como espécie invasora de restinga. ***Acta Botanica Brasilica***, v. 25, n. 3, p. 657-663, 2011.

SANTOS, M. L. L. Emprego de plantas com princípios tóxicos no controle de *Meloidogyne enterolobii*. **Dissertação** (Mestrado em Fitotecnia), Universidade Federal do Ceará, Fortaleza-CE, 2015.

SHARMA, N.; TRIVEDI, P. C. Screening of leaf extracts of some plants for their nematicidal and fungicidal properties against *Meloidogyne incognita* and *Fusarium oxysporum*. ***Asian Journal of Experimental Sciences***, v. 16, p. 21-28, 2002.

SILVA, A. F. A. et al. Desempenho agronômico do rabanete adubado com *Calotropis procera* (Ait.) R. Br. em duas épocas de cultivo. ***Revista Ciência Agronômica***, v. 48, n. 2, p. 328-336, 2017.

SILVA, G. S. Métodos alternativos de controle de fitonematoides. ***Revisão Anual de Patologia de Plantas***, v. 19, p. 81-152, 2011.