academicJournals

Vol. 12(12), pp. 1037-1044, 23 March, 2017
DOI: 10.5897/AJAR2016.11624
Article Number: 87842D963302
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Extract of *Persea* americana (Mill.) used for the control of *Meloidogyne incognita* in tomato plant

Carla Rosane Kosmann*, José Renato Stangarlin, Odair José Kuhn, Katia Kabroski Andrioli, Laura Cristiane Nascimento Freitas, Daiana Karoline Kaiser, Eloisa Lorenzetti, Cristiane Cláudia Meinerz, Danielle Mattei, Francielle Fiorentin Peters, Anderson Luis Heling, Jeferson Carlos Carvalho and Jaqueline De Araújo Barbosa

State University of West Paraná, Brazil.

Received 29 August, 2016; Accepted 19 January, 2017

The study aimed to evaluate the control of *Meloidogyne incognita* in resistant and susceptible tomato genotypes treated with hydrogel containing avocado extracts (*Persea americana* Mill.). The methanolic extract of avocado seeds was evaporated and re-suspended in distilled water containing Tween 80 (0.6%). The extract was prepared with the following concentrations: 100, 200, 400, 800 and 1000 mg L^{-1}. The hatching, motility and mortality of juvenile were evaluated, *in vitro*, and from these results, we selected the most nematocide concentration which was incorporated into different hydrogel doses (0.1, 0.25, 0.5, 0.75 and 1.0 g pit^{-1}), for *in vivo* testing. The hydrogel containing avocado extract was added to the pits of tomato plants during transplanting, and after three days, *M. incognita* was inoculated. After 30 days, the relative chlorophyll content was assessed, along with the total volume of root, the number of galls and egg masses, viability of the egg mass, and number of eggs and juveniles per root system in 100 cm^3 of soil. From these assessments, the most effective dose in the control of nematodes was tested again with the extract concentrations of 1000, 2000, 4000, 6000 and 8000 mg L^{-1} in the hydrogel, which was added to the pits at the time of transplantation. After 30 days of inoculation the same evaluations were performed. *In vitro*, the concentration of 1000 mg L^{-1} of the avocado extract was the most effective in reducing the hatched juveniles, while motility and mortality were not influenced. *In vivo*, 1.0 g pit^{-1} dose in the first test had greater control of nematodes in susceptible plants for all variables assessed. It was incorporated into the pits of tomato plants for the realization of the second test. In this, the concentration of 8000 mg L^{-1} was the most effective in controlling *M. incognita*. Therefore, the avocado seed extract served in hydrogel has the potential to control *M. incognita* in tomato plants.

Key words: Avocado tree, alternative control, plant extract, hydrogel, motility, mortality, galls nematode.

INTRODUCTION

The galls nematodes, belonging to the genus *Meloidogyne*, are pathogens of major agricultural importance, because they cause direct and indirect damage to plants. Direct damage occurs in the root system. This is time when the juveniles penetrate the plant, the second stage of the nematodes, which entails the formation of gall that clogs the conducting vessels of the xylem and phloem compromising thus the absorption of water and nutrients. From this, the indirect symptoms arise, such as chlorosis and wilt in the shoot and

formation of patch contour in the useful area (Ferraz and Monteiro, 2011).

When nematodes are present in a specific area they are hardly eradicated, because they may remain viable for long periods in the soil. For this reason, a number of measures must be employed in order to decrease the inoculum, including: The use of crop rotation, flood, solarization, genetic resistance, synthetic nematicides and biological and alternative controls (Dias-Arieira et al., 2013).

The use of plant extracts as an alternative control has been widely studied in order to reduce their environmental impact (Salgado and Campos, 2003a). There are several plants which contain secondary metabolites with nematotoxic properties. But many of these plants have not been studied, such as avocado (*Persea americana* Mill.), whose seeds contain large quantities of phenolic compounds (Daiuto et al., 2014), providing greater antioxidant activity, and consequently, may be involved in plant defense against pathogens (Soares, 2002).

These antioxidant compounds are generally sensitive to ultraviolet radiation and therefore must be conveyed in formulations such as hydrogel in order to prevent them from damage by radiation (Souza et al., 2013). Moreover, it has as an advantage, which is the slow release of the toxic substances present in the extract around roots infected with the nematode (Byrne et al., 2002).

The aim of this study is to evaluate the effect of different concentrations of crude extracts of avocado seeds on hatching, motility and mortality of second stage juveniles of *M. incognita* and the control of this nematode in tomato susceptible and resistant to the pathogen, using extract of formulated hydrogel.

MATERIALS AND METHODS

Experimental design

The experimental design used for all tests was totally randomized. For the *in vitro* assays (hatching, motility and mortality of second stage juveniles (J2)) the factorial used was 2×7, considering two types of avocado extracts (red color extract and brown color extract) and seven concentrations (0, 100, 200, 400, 600, 800 and 1000 mg L^{-1}), with five repetitions, each represented by a plastic flask (70 ml) with lid.

In a greenhouse, two experiments were performed, both in factorial 2×6, with two tomato genotypes (Santa Cruz Kada and Ivety, susceptible and resistant to *M. incognita*, respectively), and six doses of hydrogel or six extract concentrations. For the first experiment, six hydrogel doses were used (0, 0.1, 0.25, 0.5, 0.75 and 1.0 g pit^{-1}) containing avocado extract that was more nematotoxic in the experiment *in vitro*. For the second experiment, the dose of the hydrogel + extract which gave greater control in the first test was used adding different concentrations of the avocado extract (0, 1000, 2000, 4000, 6000 and 8000 mg L^{-1}). Each treatment consisted of five repetitions, represented by a 2 L vessel containing a tomato plant.

Acquisition of the inoculum of *M. incognita*

Populations of *M. incognita* were obtained from okra plants presenting symptoms of galls and identified by perineal configuration technique proposed by Hartman and Sasser (1985). This population was multiplied in tomato Santa Cruz Kada, in a greenhouse, to serve as inoculum. The eggs and J2 for inoculation were obtained by the method of Coolen and D'Herde (1972).

Preparation of the avocado seed extracts

Crude extracts were prepared from fresh avocado seeds. For the preparation of each extract, an avocado seed with about 75 g each was used, totalizing two seeds in the experiment. The seeds were cut into pieces of two sizes to obtain two types of extracts: In one smaller pieces were cut (0.5 cm^3), shown with a red color after cutting, being called red color extract (RE), and in another, the pieces were larger (1.5 cm^3) and had brown color, calling the brown coloring extract (BE).

These seed pieces were placed in flasks containing 100 mL of methanol P.A., so as to cover the seed. The vials were sealed and protected from light, remaining under stirring for 24 h at 150 rpm. After this period, the solution passed through two filters, being first on paper filter (8 µm of pore diameter) and then on filter membrane (0.45 µm of pore diameter).

The solution obtained in the second filtration was evaporated in a rotary evaporator at 45°C and 60 rpm for 4 h to obtain the crude extract. For each extract was added 600 µm of Tween 80 (0.6%), to promote homogenization of the solutions. From this, the different concentrations were prepared: 100, 200, 400, 600, 800 and 1000 mg L^{-1} of crude extracts of avocado seeds. Control used was distilled water containing Tween 80 (0.6%).

In vitro tests – nematotoxic activity

To test the hatching of *M. incognita* juveniles, 1 ml of suspension containing 500 eggs and 5 mL of the treatments were incubated for 15 days at 25°C in the dark in a plastic container (70 ml) with lid. After this period, there was an evaluation under the light microscope, counting the first 100 eggs, and thereby determining the percentage of hatching (Costa et al., 2001).

For the motility and mortality tests, the juveniles were obtained through a hatching chamber by the Baermann funnel methodology (Baermann, 1917).

In each plastic container, 5 mL of the treatments and 1 ml of the suspension containing 500 juveniles of *M. incognita* were placed. The assessment of motility, conducted to check the nematostatic activity of the extracts was performed after 24 h, measuring the percentage of apparently immobile juveniles. Subsequently, the juveniles were transferred to a sieve of 400 Mesh, replacing the treatments of distilled water and collecting the suspension. This remained in the containers for more 24 h. Juveniles that remained motionless, straight or slightly bent were considered dead, thus

*Corresponding author. E-mail: carlakosmann@hotmail.com.

proving the nematicide activity of the extracts. The evaluations of the tests were performed in Peters slides and observed under an optical microscope (Rocha et al., 2005).

Hydrogel preparation containing avocado extract

With the results of the *in vitro* tests, it was determined that the concentration of one of the extracts that was more active against against *M. incognita* be used for the first assay in the greenhouse. In this test, the hydrogel containing avocado seed extract was placed in holes made for the transplanting of the tomato seedlings.

For the preparation of this mixture hydrogel + extract, 6 L of the chosen extract and 6 g of the hydrogel were used. After its homogenization, the mixture remained in the oven at 50°C for five days, until complete drying of the material. This dry and impregnated extract hydrogel was crushed with the aid of a mortar and a pistil to obtain a powder. Soon after, the different hydrogel doses containing the extract: 0.1, 0.25, 0.5, 0.75 and 1.0 g pit^{-1} were prepared. As control, only the hydrogel without extract incorporation was used.

Sowing of tomato plants and inoculation of *M. incognita*

Susceptible tomato seeds (Santa Cruz Kada) and resistant (Ivety) were placed in trays of 128 cells containing commercial substrate. After 25 days, the seedlings were transplanted into 2 L pots containing a mixture of soil, sand and organic material (2:2:1, v:v:v), pre-autoclaved at 120°C and 1 atm for 1 h.

At the time of the transplantation, the different doses of hydrogel containing avocado seed extract were placed on each pit made. After three days of transplanting, the seedlings were inoculated with a suspension containing 2067 eggs and juveniles per plant, remaining in a greenhouse for 30 days.

Evaluation of different doses of hydrogel per pit containing avocado extract

At 30 days after the inoculation, the relative chlorophyll content (SPAD index) in the leaves of tomato plants susceptible and resistant to *M. incognita* was assessed, using a portable measurer SPAD 502 *Plus Daminolta*. Five readings were carried out for each repetition, and subsequently made the means, which were expressed as chlorophyll content in cm^3.

After this evaluation, plants of tomato plants were removed from pots and the roots were washed in water and dried on absorbent paper to obtain the total volume of the root (TVR). The number of galls (NG) and egg masses (NEM) in the root system were counted, the latter being performed according to Taylor and Sasser methodology (1978).

To determine the total volume of the root (TVR), it was placed in a graduated cylinder containing a known volume of distilled water (450 mL), thereby measuring the displacement of the water column. Through the difference obtained, the root volume was expressed in cm^3.

For the viability analysis of eggs produced by the pathogen in the treated plants, the masses were removed and placed in wells containing 250 µl of distilled water, on ELISA plates. These were sealed with plastic film, keeping them in the dark at 25°C for 15 days. Viability was determined by assessing the number of hatched juveniles. For each treatment, five repetitions were performed, each in duplicate.

The determination of the population of *M. incognita* was performed by quantifying the number of eggs and juveniles on the root system (Coolen and D'Herde, 1972) of the susceptible tomato plants resistant to nematodes, and in 100 cm^3 of soil. The latter was carried out according to the procedure described by Jenkins (1964).

Evaluation of different avocado extracts concentrations in the hydrogel

From the results of the first test, where it was determined the best dose of the hydrogel + extract placed in the pits, the second trial testing different avocado extract concentrations was carried out (1000, 2000, 4000, 6000 and 8000 mg L^{-1}) with this hydrogel dose.

At the time of the transplanting of the tomato seedlings, on each pit held we placed the different avocado extract concentrations in the hydrogel, and three days later, the seedlings were inoculated with a suspension containing 2184 eggs and juveniles per plant. They stayed in a greenhouse for 30 days later for measurement of chlorophyll content, total volume of the root (TVR), counting of the number of galls (NG) and egg mass (NEM) and determination of the population of *M. incognita* on the root system.

Statistical analysis

The data obtained from *in vitro* assays and the two experiments in the greenhouse were subjected to analysis of variance at 5% probability, with the help of statistical program SISVAR 5.3 (Ferreira, 2011). Checking a significant interaction between the factors, the developments were carried out. The average comparison was made for the types of extracts or genotypes using Tukey test at 5% probability; while regression analysis was done for the doses or concentrations.

RESULTS

With respect to the *in vitro* tests, for the variable juvenile hatching, it was observed that the increase of the avocado extract concentration afforded a linear decrease in hatching; 1000 mg L^{-1} concentration was more effective in this reduction compared to the others, regardless of the type of extract used (Figure 1). When analyzing the extracts (Table 1), it is possible to note statistical differences only in the range of concentrations between 400 and 800 mg L^{-1}, showing minor hatching of juveniles in red color extract compared to brown coloring extract.

With regard to motility and juvenile mortality, none of the treatments were significant ($p>0.05$). It is possible to infer that there was no nematostatic and nematicide effect of the avocado seed extract on *M. incognita*.

These results obtained from the in vitro evaluations indicated the concentration of 1000 mg^{-1} of the red extract of the avocado seed as the most efficient against *M. incognita*, therefore, being chosen to be incorporated into the hydrogel, for tests in a greenhouse.

For the variable chlorophyll content (SPAD index), there was significant difference ($p<0.05$) only for the tomato genotypes, with averages for the susceptible and resistant of 35.46 and 39.05 cm^3, respectively (data not shown). With regard to the variable total volume of the root, none of the factors were significant ($p>0.05$), indicating that regardless of the genotype of tomato and the extract concentration used, the root volume is not

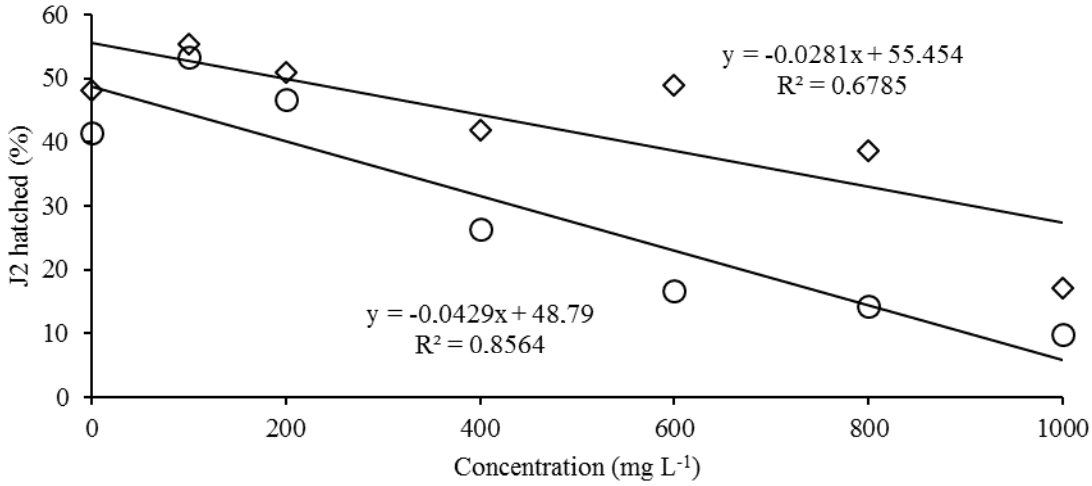

Figure 1. Hatch of second stage juveniles (J2) of *Meloidogyne incognita* in the different concentrations of avocado extracts, after 15 days of incubation. RE: Red color extracts (○); BE: Brown color extract (◊).

Table 1. *In vitro* hatching of second stage juveniles (J2) of *M. incognita* in different concentrations of two kinds of avocado seed extracts.

Extract	Concentration (mg L^{-1})							Average
	0	100	200	400	600	800	1000	
RE	41.40[a]	53.40[a]	46.60[a]	26.40[a]	16.60[a]	14.20[a]	9.80[a]	29.77[a]
BE	48.20[a]	55.40[a]	51.00[a]	41.80[b]	49.00[b]	38.60[b]	17.06[a]	43.09[b]

The averages followed by the same letter in the columns do not differ statistically by Tukey test ($p \leq 0.05$). Average values obtained of five repetitions of each treatment, expressed as a percentage. RE: Red color extracts; BE: Brown color extract.

interfered with (data not shown).

For the variables number of galls, egg masses and hatched juveniles, the interaction between the factors was significant ($p<0.05$), indicating that the increase in the hydrogel dose containing avocado extract promotes a reduction of these variables in tomato roots susceptible to nematode and the dose of 1.0 g pit^{-1} that showed the lowest means. This, however, was not observed in resistant tomato plants (Figure 2A, B and C).

Regarding the genotypes (Table 2), for the variables number of galls, masses of eggs and hatched juveniles, there were statistical differences in all concentrations, with lower values for the resistant genotype.

The number of eggs and juveniles per root system was not influenced by different doses, only the factor genotype was significant ($p>0.05$), with averages of 560.5 and 8.4 + juvenile eggs per root system, for the susceptible and resistant genotypes, respectively (data not shown).

With regard to the number of eggs and juveniles in the soil, the interaction between the factors was significant ($p<0.05$), being only the susceptible genotype influenced by different hydrogel concentrations containing the extract. Therefore, with this increase, there is an increase in the number of eggs and juveniles up to a certain concentration, followed by a reduction of these nematodes in soil up to a dose of 1.0 g pit^{-1}. In this, the susceptible plant behaves like the resistant, not showing significant difference between them (Table 2D and Figure 2).

From the data resulting from this test, the dose of 1.0 g pit^{-1} of the hydrogel containing the avocado extract showed the highest reduction of *M. incognita*, in comparison to the other tested doses and, therefore, chosen to perform the second test.

Similar to the results found in the first test, for the variable total volume of root, none of the factors were significant ($p>0.05$), and the variable chlorophyll content (SPAD index), only the tomato genotypes were influenced, with averages of 39.60 cm^3 for the susceptible and 42.62 cm^3 for the resistant (data not shown).

For the variables number of galls, egg masses and eggs and juveniles in the root system, the interaction between the factors was significant ($p<0.05$), because with the increase in avocado extract concentrations in hydrogel there was a linear decrease of these variables. 8000 mg L^{-1} concentration was the most efficient in this reduction. Regarding resistant tomato roots, there was no

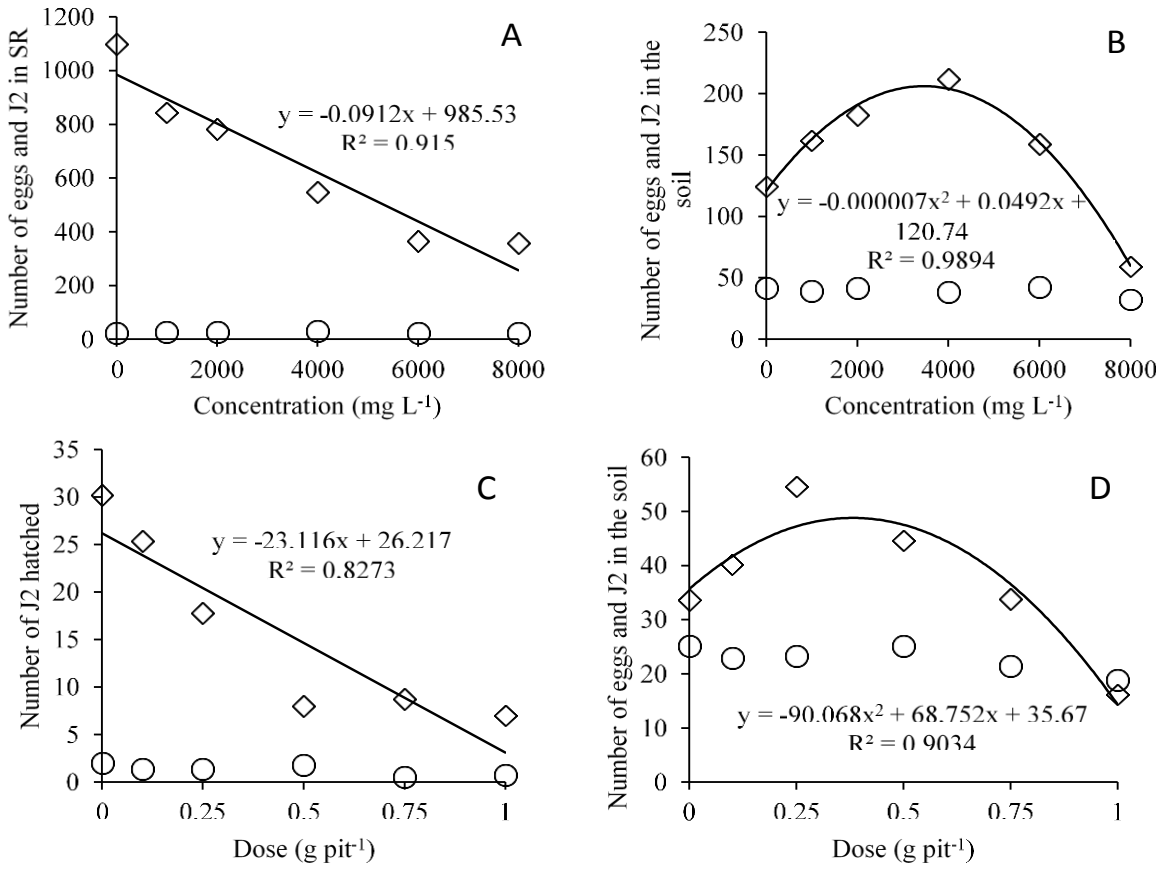

Figure 2. Effect of different doses of the hydrogel containing the red color of avocado extract (RE) in susceptible tomato plants (◊) (Santa Cruz Kada) and resistant (○) (Ivety) to *M. incognita*. Variables analyzed: number of galls (NG) (A); number of egg masses (NEM) (B); viability of egg masses assessed by the number of second stage juveniles (J2) hatched (C); number of eggs and second stage juveniles (J2) in 100 cm^3 of soil (D).

Table 2. Average of the variables number of galls (NG), number of egg masses (NEM), viability of egg masses assessed by the number of second stage juveniles (J2) hatched, and number of eggs and second stage juveniles (J2) in 100 cm^3 of soil, on susceptible tomato genotypes (S) (Santa Cruz Kada) and resistant (R) (Ivety) to *M. incognita*, when subjected to various doses of hydrogel containing avocado seed extracts.

Dose (g pit^{-1})	NG[1]		NEM[1]		J2 hatched[1]		Eggs and J2 – soil[1]	
	S	R	S	R	S	R	S	R
Witness	276.4^b	9.8^a	260.2^b	8.2^a	30.2^b	2.0^a	33.6^b	25.2^a
0.1	275.4^b	10.6^a	236.0^b	7.8^a	25.4^b	1.4^a	40.2^b	23.0^a
0.25	272.4^b	8.8^a	225.2^b	6.6^a	17.8^b	1.4^a	54.6^b	23.4^a
0.5	269.4^b	8.0^a	223.8^b	5.0^a	8.0^b	1.8^a	44.6^b	25.2^a
0.75	246.6^b	7.0^a	226.0^b	5.2^a	8.8^b	0.6^a	33.8^b	21.4^a
1.0	238.0^b	7.8^a	220.0^b	4.8^a	7.0^b	0.8^a	16.2^a	18.8^a
CV(%)	5.05		6.56		22.02		17.93	

The averages followed by the same letters in the lines, for each variable, do not differ statistically by Tukey test (p≤0.05);
[1]Average value obtained from five repetitions of each treatment for each variable. Hydrogel incorporated with avocado seed extract in concentration of 1000 mg L^{-1} of extract.

statistical difference between the treatments used in the study (Figures 3A, B and C).

With relation to the variable number of eggs and juveniles in the soil, similar behavior was observed in the first test, by providing an increased number of nematodes as the concentration increased. 4000 mg L^{-1} concentration

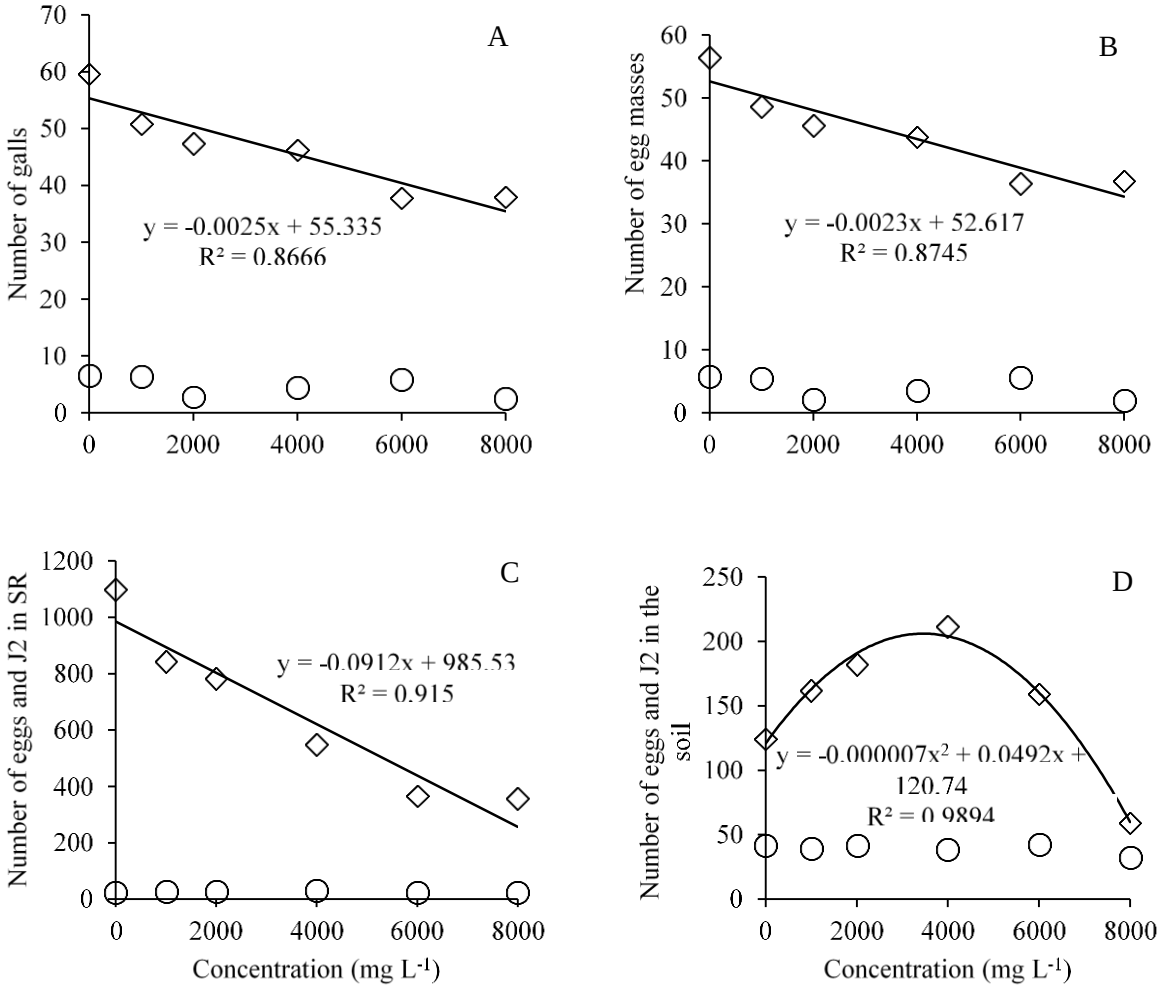

Figure 3. Effect of different concentrations of the red color of avocado extract (RE) in the hydrogel, used at a dose of 1.0 g pit^{-1}, in susceptible tomato plants (◊) (Santa Cruz Kada) and resistant (○) (Ivety) to *M. incognita*. Variables analyzed: number of galls (NG) (A); number of egg masses (NEM) (B); number of eggs and second stage juveniles (J2) on the root system (SR) (C) and 100 cm^3 of soil (D).

had the highest number of eggs and juveniles. From this, the final population of *M. incognita* decreases until it reaches the maximum concentration (8000 mg L^{-1}) used in the study, in which there was 52.57% of reduction of this nematode compared to the control (Figure 3D).

Evaluating the genotypes of tomato (Table 3), for the variable number of galls, mass of eggs, eggs and juveniles in the root system and in the soil, it is possible to observe significant differences in all tested concentrations, being the resistant genotype, in all cases, which had the lowest averages.

DISCUSSION

The lowest hatching of the juveniles seen in red color extract (RE) compared to the brown color extract (BE) shows a possible toxic effect on these nematodes and this toxicity can be due to the presence of substances in the extract that inhibit the hatching or affect phases of cell proliferation, embryonic development and change of the cuticle of the nematode (Salgado and Campos, 2003a). The avocado seed is rich in phenolic compounds such as catechins, hydroxybenzoic acids, hydroxycinnamic acids, flavanols and procyanidins, which give it a greater antioxidant power (Rodríguez-Carpena et al., 2011).

The difference in inhibition of juveniles hatching observed among extracts is probably due to the size of the cuts made in each of the seeds. For the RE, which was obtained from smaller seed pieces, a larger surface area was exposed for the occurrence of lipid oxidation process and the formation of potentially toxic compounds, such as free radicals, and due to this, the number of phenolic compounds released would be increased, in order to sequester these radicals, thus inhibiting the oxidation. As for the BE obtained from larger seed

Table 3. Averages of the variables number of galls (NG), number of egg masses (NEM), and number of eggs and second stage juveniles (J2) on the root system (RS) and 100 cm^3 of soil in susceptible tomato genotypes (S) (Santa Cruz Kada) and resistant (R) (Ivety) to *M. incognita*, when subjected to different avocado seed extract concentrations contained in the hydrogel.

Concentration	NG[1]		NEM[1]		Eggs and J2 – RS		Eggs and J2 – soil[1]	
(mg L^{-1})	S	R	S	R	S	R	S	R
Witness	59.6[b]	6.6[a]	56.4[b]	5.8[a]	1099,2[b]	25.2[a]	124.4[b]	41.6[a]
1000	50.8[b]	6.4[a]	48.6[b]	5.4[a]	844.0[b]	29.2[a]	162.0[b]	39.6[a]
2000	47.4[b]	2.8[a]	45.6[b]	2.2[a]	782.4[b]	28.8[a]	182.0[b]	41.8[a]
4000	46.2[b]	4.4[a]	43.8[b]	3.6[a]	549.2[b]	29.6[a]	211.6[b]	38.6[a]
6000	37.8[b]	5.8[a]	36.4[b]	5.6[a]	366.0[b]	25.6[a]	159.2[b]	43.0[a]
8000	38.0[b]	2.6[a]	36.8[b]	2.4[a]	358.0[b]	24.2[a]	59,0[b]	32.4[a]
CV (%)	17.57		17.47		5.48		8.25	

The averages followed by the same letters in the lines, for each variable, do not differ statistically by Tukey test ($p \leq 0.05$);
[1]Average value obtained from five repetitions of each treatment for each variable. The hydrogel containing the different concentrations of avocado seed extract was used at a dose of 1.0 g pit^{-1}.

pieces, a minor number of phenolic compounds may have been released because of the smaller area subject to oxidation.

In the case of motility and mortality of juveniles, the concentration range used in this study may not have shown a deleterious effect on the juvenile already formed (Salgado and Campos, 2003a), or, according to Chitwood (2002), due to the fact that the cuticle of the nematode may be impervious to many organic molecules such as phenolics.

The results of the chlorophyll content, obtained in two trials in the greenhouse, were expected due to the fact that the susceptible plant directs a greater amount of its assimilates to the formation of galls on the root system, thereby compromising the direction for other plant structures.

For the root volume, the fact that this variable was not influenced by the different varieties and concentrations of extract used indicates that the experimental period cannot have been enough for the vegetative recovery of plants after application of the extracts to reduce nematodes (Salgado and Campos, 2003b).

The reduction in the number of galls, egg masses, hatched juveniles and eggs and juveniles in the root system can be due to the higher concentration of tannins in the avocado seeds, as the extract concentrations are increased. This substance, present in large quantities in avocado seeds (Soares et al., 2012), when applied prior to transplantation or at planting, has as function the disorientation of nematodes, hindering thus the location of the root systems and, consequently, reducing the damage caused to plants (Maistrello et al., 2010).

Associated with this, the presence of organic matter in the soil solution can suppress nematodes due to the release of toxic metabolites such as phenolic compounds from decomposition. Furthermore, the increase of the population of parasitic microorganisms and/or predators of nematodes may also assist in reducing the population of *M. incognita* (Ritzinger and Fancelli, 2006).

When evaluating the number of eggs and juveniles on the ground in both tests in the greenhouse, the slight increase in initial doses may be due to the presence of minerals or some compound found in avocado extract, which may have been released after its application into the soil, favoring population increase in the nematode (Salgado and Campos, 2003b). Along with this increase, there has been a sharp drop in the number of eggs and juveniles in the soil, probably due to increased concentration and release of toxic substances contained in the extract.

These substances can often be considered as repellents to nematodes, promoting, according to Morillo and Silva (2015), modifications of the chemical composition of the exudates, thus affecting the reception of stimuli by the chemoreceptors of the juveniles. This behavior causes that the juvenile continually moves in the soil in order to find a host, depleting its energy reserves, and committing thus their penetration ability (Rocha et al., 2008).

For all variables assessed in the greenhouse, the susceptible genotype showed averages higher than the resistant genotype, highlighting that the latter has more effective defense mechanisms than the susceptible, preventing or delaying the nematode entrance into their root systems.

Conclusion

In the *in vitro* assays, the concentration of 1000 mg L^{-1} obtained from avocado red color extract seems to be more effective in reducing hatched juveniles of *M. incognita*. Motility and juvenile mortality were not affected by the extracts and their concentrations. The dose of 1.0 g of hydrogel per pit, impregnated with avocado seed extract at a concentration of 8000 mg L^{-1} was the most

efficient combination in the control of *M. incognita* in susceptible tomato.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

To CAPES for the financial support.

REFERENCES

Baermann G (1917). Eine einfache methode zur auffindung von Ankylostomum (Nematoden) larven in erdproben. Nederlandsch Indie Batavia 57:131-137.

Byrne ME, Park K, Peppas NA (2002). Molecular imprinting within hydrogels. Adv. Drug Deliv. Rev. 54(1):149-161.

Coolen WA, D'Herde CJ (1972). A method for the quantitative extraction of nematodes from plant tissue. Ghent: State Agriculture Research Centre, 77 p.

Costa MJN, Campos VP, Oliveira DF, Pfenning LH (2001). Toxidade de extratos vegetais e de estercos a *Meloidogyne incognita*. Fitopatol. Bras. 279(2):245-250.

Chitwood DJ (2002). Pahytochemical based strategies for nematode control. Annu. Ver. Phytopathol. 40(1):221-249.

Daiuto ER, Tremocoldi MA, Alencar SM, Vieites RL, Minarelli PH (2014). Composição química e atividade antioxidante da polpa e resíduos de abacate 'Hass'. Rev. Bras. Frutic. 36(2):417-424.

Dias-Arieira CR, Santana-Gomes SM, Puerari HH, Fontana LF, Ribeiro LM, Mattei D (2013). Induced resistance in the nematodes control. Afr. J. Agric. Res. 8(20):2312-2318.

Ferraz LCCB, Monteiro AR (2011). Nematoides. In: Amorin L, Rezende JAM, Bergamin Filho A (Eds.). Manual de fitopatologia: princípios e conceitos. 4. ed. Piracicaba: Agronômica Ceres, cap. 13:277-305.

Ferreira DF (2011). Sisvar: a computer statistical analysis system. Ciênc. Agrotec. 35(6):1039-1042.

Hartman KM, Sasser JN (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: Barker KR, Carter CC. eds. *An advanced trestise on Meloidogyne*. Raleigh: North Carolina State University Grahics 1:95-112.

Jenkins WR (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48(9):692.

Maistrello L, Vaccari G, Sasanelli N (2010). Effect of chestnut tannins on the root-knot nematode *Meloidogyne javanica. Helminthol.* Bratislava 47(1):48-57.

Morillo SRC, Silva GS (2015). Efeito antagônico de feijão-de-porco sobre *Meloidogyne enterolobii* em tomateiro. Summa Phytopathol. 41(4):305-310.

Ritzinger CHSP, Fancelli M (2006). Manejo integrado de nematoides na cultura da bananeira. Rev. Bras. Frutic. 28(2):331-338.

Rocha FS, Campos VP, Silva EH, Rocha SPS, Carli MC, Costa LSAS (2008). Migração de *Meloidogyne incognita* para as raízes de soja *in vitro*. Nematol. Bras. 32(4):294-302.

Rocha FS, Campos VP, Souza RM (2005). Efeito de extrato de calos de diversas plantas na adesão de endósporos de *Pascoradateuria penetrans* e na infectividade e parasitismo de juvenis do segundo estádio de *Meloidogyne incognita*. Summa Phytopathol. 31(1):46-51.

Rodríguez-Carpena JG, Morcuende D, Andrade MJ, Kylli P, Estévez M (2011). Avocado (*Persea americana* Mill.) phenolics, *in vitro* antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. J. Agric. Food Chem. 59(10):5625-5635.

Salgado SML, Campos VP (2003a). Eclosão e mortalidade de *Meloidogyne exigua* em extratos e em produtos naturais. Fitopatol. Bras. 28(2):166-170.

Salgado SML, Campos VP (2003b). Extratos naturais na patogenicidade e reprodução de *Meloidogyne exígua* em cafeeiro e de *Meloidogyne incognita* raça 3 em feijoeiro. Nematol. Bras. 27(1):41-48.

Soares SE (2002). Ácidos fenólicos. Rev. Nut. 15(1):71-81.

Soares SE, Miranda MS, Mancini Filho J (2012). Composição química e perfil de ácidos fenólicos de semente de abacate das variedades Wagner e Prince. Diálogos Ciênc. 10(32):233-237.

Souza FP, Campos GR, Packer JF (2013). Determinação da atividade fotoprotetora e antioxidante em emulsões contendo extrato de *Malpighia glabra* L.- acerola. Rev. Ciênc. Farm. Básica Apl. 34(1):69-77.

Taylor AL, Sasser JN (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* especies). North Carolina State University Graphics, 110 p.