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RESEARCH ARTICLE

TOXICITY OF PLANT EXTRACTS TO MELOIDOGYNE INCOGNITA IN TOMATO PLANTS

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ABSTRACT

The objective of this study was to evaluate the nematicidal and nematostatic effect of nettle (*Fleurva aestuans*), neem (*Azadirachta indica*), castor bean (*Ricinus communis*) and cassava (*Manihot esculenta*) bark extracts prepared through different methods. Tomato seedlings were inoculated in the roots region with a suspension containing 3,000 eggs/juvenile *Meloidogyne incognita*, and after 72 hours 100mL of the plant extracts were added to the soil. After fifty days, plant height, stem diameter, fresh and dry biomass of shoots, fresh and dry biomass of roots, number of galls on roots and number of juveniles in the soil were evaluated. Castor bean and nettle extracts promoted increments in plant height of 48% and 43%, respectively, if compared to water control (positive). Dry biomass of shoots and roots were significantly influenced by the extracts, especially by neem, that provided an increase of 71% and 90% for these variables. The cassava extract, prepared by infusion, provided the greatest reduction in the number of juveniles. Extracts of castor bean and cassava, cooked and prepared by infusion, respectively, reduced the number of galls in 70% and 79%. All extracts regardless the method for preparation, reduced the number of juveniles, especially neem and cassava, which reduced juveniles in 84% and 88%, respectively. These findings look promising for use in sustainable nematode control in tomato crops.

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INTRODUCTION

The tomato (*Lycopersicon esculentum* Mill.) stands out among the most consumed vegetables, both fresh as in industrial preparations. The root-knot nematodes of the genus *Meloidogyne* are considered the most important plant parasitic nematodes worldwide, exhibiting a wide host range and causing economic losses in several crops (Pereira-Carvalho *et al*., 2014). These nematodes are among the biotic factors that most seriously threaten tomato production, by parasitizing the root system of the plant and decreasing absorption of nutrients and water (Vale *et al*., 2013). Upon such aggressiveness, losses caused by *Meloidogyne* sp. present some variations, where are estimated at 12.3% in developed countries and 14.6% in developing countries, the example of Pakistan, where are considered the main barrier in the production of olerícolas (Anwar and McKenry, 2012). Specific estimates point to losses caused by *M. incognita* and *M. incognita* from 24 to 38% for tomatoes in the whole world (Kathy, 2000). In addition to the high aggressiveness of nematoides demonstrated in all phenological phases of plants infested, researches show (Alcanfor *et al*., 2001) That defensive chemicals has limitations of actions in the light of limited permeability in their tegument. Consequently, a wide variety of control methods has been sought to efficiently reduce damages to the crops. Among the most widespread control methods (resistant varieties, crop rotation, crop management, chemicals, etc.), the chemicals have been outstanding (Barros *et al*., 2000). In view of the growing concern with nature, the priority nematode management strategies are those that reduce costs, increase production and do not harm the environment. Nematicides, despite their efficiency, are toxic to the environment and the microbial flora from the soil, are expensive, and may harm the producer and consumer health. Therefore, discussions inherent

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to the nematicides characteristics have led, in several countries, to search for alternatives in the management of phytonematodes (Luiz *et al*., 2007). The disclosure of the adverse effects of chemicals has led consumers to purchase products which are free of these compounds, making increasingly common the need for implementation of alternative methods to control pests and diseases, such the case of plant extracts with nematicidal and/or nematostatic properties (Azam *et al*., 2001; Chitwood, 2002). Besides the positive effects in controlling nematodes, these extracts stand out for several advantages if compared to synthetic products, such as the low cost, easy biodegradation of chemical molecules which are present in the extracts and low toxicity to animals and man.

In addition to being biodegradable, plant extracts commonly used as biocides, hold a great diversity of chemical constituents and may have a broad range of action over microorganisms (Almeida *et al*., 2012). Several substances such as alkaloids, fatty acids, isothiocyanates, cyanogenic glycosides, terpenoids and/or phenolic compounds present in plant species can act directly on the most diverse plant pathogens (Chitwood, 2002). According to Salgado and Campos (2003) and Costa *et al*. (2002), phenolic (eugenol) and glycosidic (ricin) substances exhibit potential as nematicides, being able to affect all stages prior to embryonic development, corresponding to the eggs' hatch and cuticle molt. For the majority of plant species employed for alternative control, it can be noticed that the biocide effect depends on the solvent and the part of the plant used to obtain the extract (Gardiano *et al*., 2008). Another factor that may affect the efficiency of the bioactive is the method of preparation to obtain the extract. Cyanogenic substances such as cyanide, found in cassava, are highly effective against many pathogens, including nematodes (Ponte, 2001), but the molecule can become volatile at high temperatures (Ngudi *et al*., 2003), like the ones used in cooking preparation. On the other hand, Mazaro *et al*. (2008) have found higher concentrations of glyceollin (terpenoid) when prepared by the infusion method if compared to maceration and alcoholic extracts. Therefore, the objective of this work was to evaluate the protective effect of different methods of preparation ~~of~~ from plant extracts originated from barks on *Meloidogyne incognita* in tomato plant crops.

MATERIALS AND METHODS

Experimental site

The test was performed at the experimental area of the Department of Agronomy, Federal University of Rondônia-UNIR, Rolim de Moura - Brazil, within the General Biology Laboratory and in a greenhouse. The studied plant species were collected from the campus of UNIR: nettle (*Fleurva aestuans* L.), neem (*Azadirachta indica* A. Juss), castor bean (*Ricinus communis* L.) and cassava (*Manihot esculenta* Crantz), The experimental design was completely randomized in a factorial scheme (4 x 3 + 2) with four replicates, and the considered factors were the four plant extracts and three preparation methods (cooking, infusion and powdering), with two controls: chemical (carbofuran) and water. Extracts were prepared using only the bark of the plants by collecting fragments in paper bags, drying in a forced air oven at 65 °C until achievement of a constant weight and finally triturating with a mill rotor blade to obtain a homogeneous powder.

Preparation of extracts

Beside powder, two more forms of preparation of plant extracts were considered, by adapting the method described by Ferris and Zheng (1999): a) cooking, consisting in placing 50g of plant bark powder in 500 ml of distilled water and heating until boiling point at 100 ^{o}C; b) infusion, consisting in pouring 500 ml boiling water on 50g of plant bark powder, left to rest until its complete cooling. In order to reduce the excess of impurities, at the end of preparation of the extracts by cooking or infusion, both were filtered using layers of gases, repeating this procedure twice. Before being applied, all extracts were placed in PET bottles (Polyehtylene terephthalate) and stored in refrigerator for a period of 24 hours.

Sowing on tomato

Tomato plants from the variety "Santa Cruz" were sown in polyethylene vases with 4 L capacity, using five seeds per vase filled with a mixture of soil, sand and manure in a ratio 3:2:1 (v/v). This substrate was previously treated with Dazomet (Basamid) at a dose of 50 g/m^2, and then kept under rest for seven days in order to eliminate all possible phytoparasitic nematodes and other microorganisms. Fifteen days after plants emergence, thinning was done, considering the strength and height of the plant and leaving only one plant per vase as for the evaluation of the extracts.

Nematodes extraction and estimation

For the inoculum production, a pure culture of *M. incognita* preserved in tomato plants and grown in a greenhouse was used, obtaining a suspension of eggs, according to the method of Hussey and Barker (1973) modified by Boneti and Ferraz (1981). The procedure consisted in crushing perforated roots in a blender during 20 seconds at low speed in a solution of sodium hypochlorite at 0.5%. To identify the species under study, the key methodology of Mai and Mullin (1996) was used as a parameter. A centrifugal flotation sucrose solution, according to the methodology proposed by Jenkins (1964), was used for extracting nematodes.

Inoculation of tomato nematode

Inoculation of the phytoparasitic nematodes was performed by pouring on each tomato vase a water suspension of 3,000 eggs/juvenile of *Meloidogyne incognita,* previously calibrated in Peters chamber. After inoculation, the plants were irrigated keeping soil next to 80% of the field capacity, for a period of 72 hours, allowing a full parasitic development of the nematodes in the roots. After this period, treatments were performed by means of a single application with 100 mL of the plant extracts in the soil. Untreated control, was represented by distilled water, whereas positive (nematicidal) control, was represented by application of dose of 50 mg a.i. L^{-1} of Carbofuran diluted in water to each treatment (vase). The

temperature inside the greenhouse ranged from 30.2 °C to 35.8 °C.

The evaluations were carried out

The evaluations were performed fifty days after inoculation, corresponding to the period of exposure to the extracts. Plant height, stem diameter, fresh and dry biomass of the aerial part, fresh and dry biomass of root, number of galls per 10 g of roots and number of juveniles in the soil of each treatment were evaluated. The roots were weighed and then 10 g were processed in a centrifuge (Coolen and D'Herde, 1972) to determine the number of juveniles.

Statistical analysis

All data underwent analysis of variance (ANOVA), and the averages of the significant variables were compared by Tukey test ($p \leq 0.05$). To analyze the number of juveniles, data were transformed into square root (x + 1), however untransformed values are shown.

RESULTS AND DISCUSSION

This work aimed at testing the nematicidal potential of four bark extracts obtained by three different methods on *M. incognita* infected tomato plants. Results (Table 1) show that, except for stem diameter, all the other parameters related to tomato growth showed significant differences ($p \leq 0.05$) according to the variable "plant extracts" and that, in particular, plant height, number of galls and *M. incognita* juveniles were highly influenced by the plant extracts, showing a reduction of parasitism by the action of the extracts (Table 4) and, therefore, development of plants. These results corroborate those obtained by Gardiano et al. (2009), who found significant increases in plant height when different plant (castor and neem) extracts were applied to the soil infected with *M. javanica*. Concerning the method of preparation, fresh biomass of the aerial part was the variable which has presented the largest value of F (Table 1), indicating that this factor has great influence on this variable. The method of preparation also influenced variables of fresh and dry biomass of roots, number of galls and number of juveniles. Evaluating the effects of interaction between the extracts and preparation methods (Table 1), the greatest effect was shown on the number of galls and number of juveniles, compared to other variables. Analyzing the extracts obtained from the bark of different species depending on the method for preparation, it appears that powder extract of *Ricinus communis* (castor) and *Fleurva aestuan* (nettle) lead to the greatest increase in plant height, when compared to the untreated control (48% and 43%, respectively) and greater height was also observed when compared to the chemical treatment (Carbofuran) (9% and 5% respectively), although differences were not significant (Table 2). Effectiveness of castor oil extract on reduction *M. javanica* population and inducing increase in plant height were observed by Gardiano *et al*. (2009). Concerning the dry biomass of aerial parts (Table 2), powder extract of *Fleurva aestuan* (nettle) was superior to the other plant extracts in all methods of preparation, providing an increase in biomass of 50% and 45% compared to untreated and chemical controls, respectively. The castor extract prepared by infusion showed the best results also for the fresh biomass of aerial parts (Table 2), determining an increase of 71% and 84%, respectively, compared to carbofuran and water. Similar results were observed by Almeida *et al*. (2012), when extracts from leaves of castor, nettle and neem provided a greater accumulation of dry and fresh biomass in tomato plants. Probably castor extracts act on the physiology of *Meloidogyne* spp., reducing its metabolic activity, as observed by Costa *et al*. (2002) and Rich (1989) who observed that castor oil negatively affected the locomotion and feeding capability of *Meloidogyne* spp. These results therefore confirm those that ricin represents the best nematicidal agent for tomato crops. Similarly to the results obtained on the fresh biomass of the aerial part, the use of extracts of castor, neem and nettle, in powder form, provided the best results for fresh and dry biomass of roots, with increments in this variables of 100% and 85% respectively, compared to the use of water, and 90% and 76% respectively, compared to carbofuran. Similar results on tomato dry and fresh biomass of roots were obtained by Almeida *et al*. (2012) with the application of extracts of cassava leaves and neem.

The greatest accumulation of biomass in roots treated with powder extract of nettle and castor arises as a consequence of the high accretion of dry biomass of the aerial part (Table 2) and the low number of galls formed on roots (Table 4), since a greater dry biomass in aerial part results in greater allocation of photoassimilates to the roots and the lower number of galls results in less reduction of root's growth. Plants infested with *Meloidogyne* sp. show reduction in root growth (Ritzinger and Fancelli, 2006) and thus phytomass formation, although the damage varies according to the reaction of plants and to the environmental conditions. All extracts showed a significant reduction in the number of galls compared to water, especially in the case of castor extracts in powder and prepared by infusion, which reduced the number of galls in 70% and 79%, respectively. The fact that only these two extracts did not differ from chemical control further confirms the nematicidal action on *M. incognita*. These results corroborate researches of Gardiano *et al*. (2009) and Nandal and Bhatti (1987), all the tested extracts significantly reduced the number of juveniles compared to untreated control, with effects comparable to the chemical treatment, except neem infusion extract (Table 4). The best results were obtained with neem and cassava powder extracts which reduced juveniles of 84% and 88%respectively. Similar results were observed by Almeida *et al*. (2012), who noticed a higher efficiency in the reduction of *M. javanica juveniles* applying neem extracts prepared by cooking and by Martinez (2002), who verified the nematicidal effect of neem extract on various species of phytoparasitic nematodes, as *Prathylenchus* sp., *Rotylenchulus reniformis* and *Meloidogyne incognita*. These results indicate that possibly the extracts influenced plants positively whether turning plants unfeasible or turning them less attractive to the nematodes, and so, reducing the parasitism. Other studies (Pandey *et al*., 2000, Stephan *et al*., 2001, Lopes *et al*., 2005) stand out the nematicidal effect of different extracts on various nematodes considered as important agronomic plant parasites such as *M. incognita*, *M. javanica*, *Ditylenchus myceliophagus*, *Xiphinema americanum*, *Longidorus* sp. *Hoplolaimus indicus*, *Pratylenchus* sp. and *Helicotylenchus indicus*.

Table 1. Analysis of variance (F values) for the different parameters evaluated in tomato plants. Rolim de Moura-RO, Brazil

Factors	Plant height	Dry biomass of the aerial part	Fresh biomass of the aerial part	Dry biomass of roots
Plant extracts	12,7**	4,94**	1,34^{ns}	3,34*
Preparation methods	1,21^{ns}	0,79^{ns}	24,9**	5,56**
Extracts x Preparation methods	1,84^{ns}	1,58^{ns}	2,48*	1,40^{ns}
C.V.	12,0	15,9	18,7	28,2
	Fresh phytomass of roots	Stem diameter	Number of galls	Number of juveniles
Plant extracts	3,01*	1,57^{ns}	34,6**	11,8**
Preparation methods	4,69*	0,16^{ns}	3,91*	1,70*
Extracts x Preparation methods	2,16*	0,86^{ns}	5,49**	4,51**
C.V.	28,3	9,69	18,4	24,4

** and * Significant at 1 and 5% probability respectively; ^{ns} – not significant; C.V. – coefficient of variation.

Table 2. Plant height, dry and fresh biomass of the aerial part of tomato plants grown in soil contaminated with the nematode *Meloidogyne incognita* after treatment with plant bark extracts obtained with different preparation methods, compared with water and a nematicidal product. Rolim de Moura-RO, Brazil

Preparation methods	Treatments					
	Fleurva aestuan	*Ricinus communis*	*Azadirachta indica*	*Manihot esculenta*	Carbofuran	Water
	Plant height (cm)					
Cooking	122 Aa	128 Aa	128 Aa	131 Aa	140 Aa	104 Aa
Powder	147 Aa	152 Aa	116 Aab	138 Aa	140 Aa	104 Ab
Infusion	137 Aa	140 Aa	142 Aa	127 Aa	140 Aa	104 Ab
	Dry biomass of the aerial part (g)					
Cooking	31 ABa	27 Aa	32 Aa	33 Aa	29 Aa	28 Aa
Powder	42 Aa	30 Aabc	37 Aab	27 Abc	29 Aabc	28 Ac
Infusion	34 ABa	35 Aa	35 Aa	33 Aa	29 Aa	28 Aa
	Fresh biomass of the aerial part (g)					
Cooking	218 Aa	208 Ba	234 ABa	226 Ba	217 Aa	233 Aa
Powder	309 Aa	228 Bab	225 Bab	186 Bb	217 Aab	233 Aab
Infusion	298 Aab	399 Aa	330 Ab	352 Aab	217 Ab	233 Ab

Means followed by same letter, uppercase for the column and lowercase for the row, do not differ at 5% probability by Tukey test ($P \leq 0.05$).

Table 3. Stem diameter, dry and fresh biomass of the roots of tomato plants grown in soil contaminated with the nematode *Meloidogyne incognita* after treatment with plant bark extracts obtained with different preparation methods, compared with water and a nematicidal product. Rolim de Moura-RO, Brazil

Preparation methods	Treatments					
	Fleurva aestuan	*Ricinus communis*	*Azadirachta indica*	*Manihot esculenta*	Carbofuran	Water
	Stem diameter (mm)					
Cooking	10^{ns}	11^{ns}	11^{ns}	10^{ns}	10^{ns}	10 ^{ns}
Powder	11	10	11	10	10	10
Infusion	10	11	10	11	10	10
	Dry biomass of roots (g)					
Cooking	2,0 Ba	2,4 Ba	2,1 Aa	2,5 Aa	2,1 Aa	2,0 Aa
Powder	3,7 Aa	4,0 Aa	3,4 Aab	2,3 Aab	2,1 Aab	2,0 Ab
Infusion	2,8 ABa	2,5 Ba	2,5 Aa	2,7 Aa	2,1 Aa	2,0 Aa
	Fresh biomass of roots (g)					
Cooking	24 ABa	18 Ba	23 Aa	24 Aa	20 Aa	23 Aa
Powder	39 Aa	42 Aa	28 Aab	28 Aab	20 Ab	23 Ab
Infusion	32 ABa	26 Ba	24 Aa	23 Aa	20 Aa	23 Aa

Means followed by same letter, uppercase for the column and lowercase for the row, do not differ at 5% probability by Tukey test ($P \leq 0.05$).

Table 4. Number of galls and juveniles of *Meloidogyne incognita* detected on roots of tomato plants after treatment with plant bark extracts obtained with different preparation methods, compared with water and a nematicidal product. Rolim de Moura-RO, Brazil

Preparation methods	Treatments					
	Fleurva aestuan	*Ricinus communis*	*Azadirachta indica*	*Manihot esculenta*	Carbofuran	Water
	Number of galls					
Cooking	337 Ab	251 Aa	301 Ab	352 Bb	145 Aa	852 Ac
Powder	295 Ab	249 Ac	563 Bc	294 Bb	145 Aa	852 Ad
Infusion	724 Bc	666 Bb	519 Bc	180 Aa	145 Aa	852 Ad
	Number of juveniles					
Cooking	42 Ba	43 ABa	27 Aa	25ABa	42 Aa	105 Ab
Powder	29 Aa	29 Aab	17 Aa	13 Aa	42 Aa	105 Ac
Infusion	34 Aa	58 Ba	68 Bb	33 Ba	42 Aa	105 Ac

Means followed by same letter, uppercase for the column and lowercase for the row, do not differ at 5% probability by Tukey test ($P \leq 0.05$).

According to several results of studies performed with diverse types of extracts used in the control of species of phytonematodes, many of them with satisfactory results, coupled with their environmental care advantages, makes this an feasible alternative for control in different crops.

Conclusions

In this study, some extracts (neem and cassava) and preparation methods, showed negative effects on nematode growth and gall induction on tomato plants reinforcing the hypothesis that these extracts possibly have metabolites with biocidal activity. However, further studies should be accomplished in the field in a way to complementing the information concerning the extracts evaluated, as well as the identification of compounds that may be acting on nematodes.

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