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Potential of plant ethanolic extracts on *Meloidogyne incognita* control in tomato

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Nematodes are obligatory parasites that compromise agricultural production worldwide. The use of alternative methods to replace the chemical pesticides in the control of this pest is increasingly growing, due to its pollution to the environment. In order to evaluate the biocidal effect of plant extracts, combinations of extracts of Neem (*Azadirachta indica*), croton (*Croton campestris*) and manioc (*Manihot esculenta*) were used in the control of root knot nematode, *Meloidogyne incognita* in tomato plants. The extracts were obtained from dried leaves and exposed to cold extraction with ethanol, under reduced pressure, with the aid of rotaevaporador. The application of the treatments took place 72 h after inoculation of suspension with 4,000 eggs/juveniles in the region of the root. Extracts from croton and cassava promoted plant height in the order of 26.75 and 34.50%, respectively; while neem extract inhibited the numbers of nematode juveniles in the root (51.34%), croton (50.85%), and manioc (41.31%). As for juveniles in the soil, only 47.33% was reduced in cassava. However, the mix of the extracts potentiated the effects on improvement of growth parameters and reduction of nematode parasitism.

Key words: Alternative control, *Meloidogyne incognita*, *Solanum lycopersicum*.

INTRODUCTION

The tomato crop (*Solanum lycopersicum* Mill), cultivated in almost all parts of the world, has high productivity losses by means of the direct interference of phytonematodes on the root system, hindering the absorption of water and nutrients (Cantu et al., 2009). Among the nematodes present in Brazilian production

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fields, the *Meloidogyne* genus and, mainly, the species *Meloidogyne incognita* is considered important not only for the high aggressiveness, but also for parasitizing different crops of agronomic characteristics, which guarantees its greater survival in production areas (Zanella et al., 2005).

Considering the damage potential to crops, the search for innovation of methods other than chemical nematicides is increasing, due to the negative effects on the environment (Ferraz and Freitas, 2004). The increasing information on the impacts caused by pesticides, together with the reduction of management costs, makes it increasingly common to use alternative methods to control pests and diseases (Almeida et al., 2005).

Several researches with plant extracts using the most varied species and distinct parts of plants have been increasingly evident, due to the ability of some plant species to produce antifungal as well as nematotoxic substances (Atti-Santos, 2010; Mateus et al., 2014; Almeida et al., 2016), synergised to the possibility of molecule's rapid biodegradation and low toxicity to animals and humans (Neves et al., 2005).

The vegetable species produce primary and secondary metabolites, which are products of chemical reactions with specific functions in plant physiology. The primary compounds are based on their normal development, taking as example, the phytohormones that act in different phenological stages of the plant (Gardiano et al., 2008). Secondary metabolites, such as alkaloids, fatty acids, isothiocyanates, acyanogenic glycosides, terpenoids and phenolic compounds, directly assure the defense action against the presence of pathogenic invaders that may impede its cycle, as well as attract or repel other organisms, such as phytonematodes (Ferraz et al., 2010; Gardiano et al., 2011).

Leaf extracts of nettle (*Fleurva aestuans*), neem (*Azadirachta indica*), castor bean (*Ricinus communis*) and manioc (*Manihot esculenta*) under preparaton in different forms have demonstrated potential in the control of *M. javanica* (Almeida et al., 2012). Of the possibilities that justify this action, are the release of nematotoxic substances in the soil, such as isothiocyanates and derivatives (Zasada and Ferris, 2004), as well as the induction of resistance to plants when exposed to abiotic substances, such as extracts (Métraux, 2001).

The objective of this study was to evaluate the effect of different ethanolic extracts, separated and combined, applied in the soil for the control of *M. incognita* in tomato.

MATERIALS AND METHODS

Location and experiment conduction

The experiment was carried out in a greenhouse (9°04'45" S and 44°18'46" W) at the Federal University of Piauí, Campus Profa, Cicnobelina Elvas, in Bom Jesus-PI. The average temperature of the greenhouse during the experiment conduction was 29.25°C, with maximum and minimum averages of 33.5 and 25.0°C, respectively.

The substrate used consisted of soil + sand + manure mixture in a ratio of 3:2:1 (v/v), respectively. The substrate was autoclaved before handling at 120°C for 1 h. It was distributed in polypropylene pots of 5 dm^3 capacity and sown with three seeds of tomato cv. "Santa Cruz", which was considered highly susceptible material to *M. incognita*. Fifteen days after sowing, thinning was performed, limiting the experimental unit with one plant per pot.

Subsequently, each plant was inoculated with an aqueous suspension containing 4,000 eggs/juveniles of *M. incognita*, calibrated by Peters's chamber, distributed in three openings 3.0 cm deep, spaced 2.0 cm apart around tomato seedlings. After inoculation, the plants were conditioned only to irrigation in the first three days. Afterwards, the extracts were applied directly in the soil into the pots, using 100 mL of the solution in each treatment at the concentration of 100 g L^{-1}. Two applications were performed at 15-day intervals. As a control, the chemical treatment (Carbofuran®) was considered as a negative control, with 0.2 g of diluted product in water in single application, and the positive control was with water.

Leaf extracts of the plant species, neem (*Azadirachta indica* A. Juss), croton (*Croton campestris* A. St.-Hil) and manioc (*Manihot esculenta* Crantz) were collected from the region in the second half of 2015. The leaves were dried at air temperature and ground in knife mill to obtain 100 g in powder form for each species. Then, they were subjected to the cold extraction with ethanol PA. The solutions were filtered and concentrated under reduced pressure, with the aid of a rotavaporator at 60°C to remove the solvent, obtaining the extracts under viscous appearance, according to the methodology described by Matos (1997). The extracts were prepared 24 h before the applications were performed.

The experimental design was completely randomized, with sixteen treatments with five replications. Fourteen treatments were constituted by different combinations of plant extracts and the nematicide, Carbofuran as follows: Neem, Croton and Manioc's plant extracts; Neem + Croton; Neem + Manioc; Croton + Manioc; Neem + Croton + Manioc; Neem + Carbofuran; Croton + Carbofuran; Manioc + Carbofuran; Neem + Croton + Manioc + Carbofuran; Croton + Manioc + Carbofuran; Neem + Croton + Carbofuran; Neem + Manioc + Carbofuran) and two controls.

Plant development characteristics

The evaluations were carried out sixty days after inoculation, corresponding to the extracts exposure period to the nematodes in tomato plants. The crop growth parameters evaluated were: plant height (PH) performed using a graduated ruler; stem diameter (SD) by digital pachymeter; fresh shoot biomass (FSB), dry shoot biomass (DSB), fresh root biomass (FRB), dry root biomass (DRB), using a semi-analytical balance and root volume (RV), performed by volume difference, using a graduated beaker containing a determined water volume. The root volume was obtained by difference between the initial and final volume.

Parasitism characteristics

For the evaluation of nematode parasitism, the galls's numbers (GN) were determined, using a magnifying glass, manual counter and for egg masses number (EMN), the roots were washed in running water and colored by immersion in 0.015% Floxin B solution for 15 min. To quantify the juvenile number in the soil (JNS), they were extracted from 100 cm^3 of soil by centrifugation and flotation (Jenkins, 1964). Estimation of root juvenile numbers (Coolen and D'herde, 1972) and counting were done using optical

microscope and Peters's blade.

Statistical analysis

Data were subjected to variance analysis and, when significant, the means were compared by Scott-Knott Test at the 5% probability level. In order to satisfy the basic hypothesis of residuals's normal distribution, the absolute values of plots of the NG and EMN variables were transformed using the square root of "x + 1" and, the data processing was performed using the statistical software Assistat (Silva and Azevedo, 2002).

RESULTS AND DISCUSSION

Agronomic characteristics of tomato plants

The use of single and/or combined plant extracts had a positive influence on some characteristics of vegetative development of tomato plants. Most of the treatments differed statistically from positive (water) and negative (chemical) controls.

For plant height (PH), cróton extracts and manioc leaves, when used alone, had increases of 26.75 and 34.50%, respectively, in relation to the positive control, and did not differ statistically from the results observed with the tested chemical. It was also evidenced that extracts in mixture of some plant species presented satisfactory results, in vegetative development for PH, in the order of 28.50% with neem+croton; 30.50% for neem+manioc and 31.50% for neem+croton+manioc (Table 1). It is possible that the extracts, at some time, provide nutrients or even, serve as a tonic to the plants and thus, respond more effectively to the presence of nematodes.

Similar results were obtained by Olabiyi (2008), on the vegetative development of tomato plants, when the aqueous extracts of different plant species, were applied on the soil for *M. incognita* control. Coimbra et al. (2006), using mint leaf extract, obtained 98% immobility with the species *Scutellonema bradys*. Almeida et al. (2012), testing neem, nettle and castor leaves as extracts sources, obtained a reduction of more than 90% in gall numbers of *M. javanica* in tomato plants. According to Gardiano et al. (2009), the plant extracts efficiency that ensure significant plant development is possibly related to the reduction of nematode feeding capacity in the soil, promoted by action of some present compounds.

As for fresh shoot biomass (FSB), only manioc extract applied separately, showed an increase of 14.80%, similar to chemical control (Table 1). For mixtures of plant extracts, a better effect was observed on the combinations with croton+manioc (13.60%), neem+croton (16.00%) and neem+croton+manioc (18.33%). All extracts, when mixed with carbofuran, presented higher efficiency in comparison with the positive control, except for croton+carbofuran. Some researchers (Martinez, 2002; Coimbra et al., 2006) pointed out that the antimicrobial capacity of the extracts, in reducing nematode aggressiveness, is related to the presence of secondary compounds such as alkaloids, fatty acids, isothiocyanates, phenolic compounds, tannins and others, besides the availability of minerals (Ritzinger et al., 2004), making better, the nutrients absorption by the plants, therefore, a greater reaction to the pathogenic effects.

For the fresh root biomass (FRB), only the single croton extract differed from the positive control, with increased for the variable with 67.20%. Among the mixture of extracts, the results were more promising, since neem+croton (77.11%); neem+manioc (83.10%) and nem+croton+manioc (98.32%) had a positive influence on root protection. It is possible that the combinations of the extracts contributed to the release of substances with nematicidal activity which favored a better root system development of tomato plants.

The tomato plants accumulated less dry root biomass (DRB) when treated with single plant extracts. Only the mixtures of neem+croton, neem+manioc and neem+croton+manioc were able to promote greater protection of the plant in a similar way as chemical control. There was no effect of the extracts on root diameter and root volume (Table 1). Ritzinger and Fancelli (2006) pointed out that plants infested by *Meloidogyne* genus exhibited reduction in the root system growth, as a result of less accumulation of phytomass, but these damages vary depending on the reaction power of the plants to the effects of environmental conditions on planting.

Among the extracts applied on the soil, there was variation for some parasitism characteristics in the tomato roots (Table 2). The reduction in gall numbers (GN) was observed in two treatments when the extracts were mixed with carbofuran. However, even with low effect in the reduction of GN, it is noticed that the egg mass numbers (EMN), except for extracts of neem; neem+manioc and nem+croton+carbofuran, differed statistically from the positive control which shows that the presence of galls does not necessarily reflect the high density of viable egg masses.

However, the use of unmixed extracts on the tested chemical croton, manioc, neem+croton and neem+croton+manioc showed the highest reductions of EMN (47.75, 34.83, 48.96 and 45.31%, respectively) of *M. incognita* in the tomato rhizosphere. In turn, some extracts in combination with carbofuran did not show negative influence on the active principle of the product; also, there was no interference of the chemical with the secondary compounds present in the extracts.

Studies on croton species point to the presence of several classes of secondary metabolites including flavonoids, alkaloids, tannins and terpenoids (Payo et al., 2001), as well as essential oils rich in mono and sesquiterpenoids, besides phenylpropanoids (Palmeira-Junior et al., 2006). In the manioc leaves, the secondary

Table 1. Plant height (PH), stem diameter (SD), fresh shoot biomass (FSB), fresh root biomass (FRB), dry shoot biomass (DSB), dry root biomass (DRB) and root volume (RV) of tomato plants inoculated with *Meloidogyne incognita* treated with plant extracts and nematicide.

Treatment	Characteristics/ agronomics						
	PH** (cm)	SD^{ns} (mm)	FSB* (g)	FRB* (g)	DSB**(g)	DRB** (g)	RV^{ns} (mL)
Water	80.00^{b}	0.26^{a}	62.58^{b}	21.50^{b}	12.53^{b}	6.27^{a}	33.40^{a}
Carbofuran	109.60^{a}	0.34^{a}	82.07^{a}	34.20^{a}	27.60^{a}	8.12^{a}	33.00^{a}
Neem	93.00^{b}	0.27^{a}	66.94^{b}	22.60^{b}	13.58^{b}	2.58^{b}	30.00^{a}
Croton	101.40^{a}	0.30^{a}	62.56^{b}	35.94^{a}	15.52^{b}	3.42^{b}	35.00^{a}
Manioc	107.60^{a}	0.28^{a}	71.84^{a}	30.01^{b}	16.70^{b}	1.92^{b}	27.00^{a}
Neem/Croton	102.80^{a}	0.32^{a}	72.59^{a}	38.08^{a}	17.00^{b}	4.54^{a}	34.00^{a}
Neem/Manioc	104.40^{a}	0.30^{a}	64.11^{b}	39.36^{a}	15.26^{b}	4.48^{a}	35.00^{a}
Croton/Manioc	92.80^{b}	0.31^{a}	74.05^{a}	28.64^{b}	14.78^{b}	2.18^{b}	24.00^{a}
Neem/Croton/Manioc	105.20^{a}	0.32^{a}	71.09^{a}	42.64^{a}	16.02^{b}	5.08^{a}	38.00^{a}
Neem/Carbofuran	101.40^{a}	0.30^{a}	67.56^{b}	28.60^{b}	17.90^{b}	3.98^{b}	27.00^{a}
Croton/Carbofuran	90.00^{b}	0.25^{a}	54.93^{b}	25.76^{b}	12.92^{b}	2.20^{b}	24.00^{a}
Manioc/Carbofuran	107.20^{a}	0.34^{a}	75.22^{a}	33.00^{a}	18.98^{b}	2.76^{b}	30.00^{a}
Neem/Croton/Manioc/Carbof	92.40^{b}	0.30^{a}	72.22^{a}	33.90^{a}	15.38^{b}	3.24^{b}	33.00^{a}
Croton/Manioc/Carbof.	115.80^{a}	0.33^{a}	80.15^{a}	32.21^{a}	17.50^{b}	5.26^{a}	45.00^{a}
Neem/Croton/Carbofuran	105.60^{a}	0.24^{a}	80.32^{a}	39.89^{a}	16.97^{b}	3.92^{b}	40.00^{a}
Neem/Manioc/Carbofuran	110.40^{a}	0.33^{a}	84.63^{a}	42.24^{a}	18.36^{b}	4.58^{a}	37.00^{a}
C.V (%)	9.49	20.07	17.31	30.64	26.4	51.83	47.07

*,**Significant at 5 and 1% probability by F test, respectively; ^{ns}not significant. Means followed by the same letters do not differ by Scott Knott test.

Table 2. Gall numbers (GN), egg masse numbers (EMN), hatched juvenile numbers in the root (JNR) and juvenile numbers in the soil (JNS) of *M. incognita* after application via plant extracts and nematicide.

Treatment	Characteristics/parasitism			
	GN (Unt)	EMN (Unt)	JNR (Unt)	JNS (Unt)
Water	12.26^{a}	8.21^{a}	14.16^{a}	12.19^{a}
Carbofuran	4.04^{c}	4.16^{c}	4.14^{c}	5.30^{b}
Neem	12.87^{a}	9.50^{a}	6.89^{c}	8.10^{b}
Croton	12.41^{a}	4.29^{c}	6.96^{c}	8.02^{b}
Manioc	11.42^{a}	5.35^{b}	8.31^{b}	6.42^{b}
Neem/Croton	11.96^{a}	4.19^{c}	6.34^{c}	7.57^{b}
Neem/Manioc	12.07^{a}	8.69^{a}	7.80^{b}	7.94^{b}
Croton/Manioc	11.22^{a}	5.66^{b}	8.94^{b}	8.05^{b}
Neem/Croton/ Manioc	11.58^{a}	4.49^{c}	8.29^{b}	7.78^{b}
Neem/Carbofuran	7.49^{b}	4.26^{c}	4.33^{c}	6.62^{b}
Croton/Carbofuran	7.64^{b}	4.75^{c}	4.31^{c}	7.11^{b}
Manioc/Carbofuran	13.11^{a}	4.03^{c}	6.05^{c}	5.16^{b}
Neem/Croton/ Manioc/Carbofuran	12.90^{a}	6.50^{b}	5.35^{c}	5.59^{b}
Croton/ Manioc /Carbofuran	11.59^{a}	5.45^{b}	5.13^{c}	7.60^{b}
Neem/Croton/Carbofuran	11.35^{a}	8.37^{a}	5.65^{c}	7.99^{b}
Neem/ Manioc /Carbofuran	12.26^{a}	6.10^{b}	8.22^{b}	6.49^{b}
C.V (%)	18.87	13.92	35.02	29.31

*,**Significant at 5 and 1% probability by F test, respectively; ^{ns}not significant. Means followed by the same letters do not differ by Scott Knott test.

metabolites such as phenolic compounds, lectins and trypsin inhibitors are present (Melo et al., 2007).

The presence of these secondary metabolites has been previously confirmed with nematicidal activity (Chitwood, 2002), besides the possibility of these compounds being present in several plant species, in reducing the parasitic activity of different species of nematodes (Mateus et al., 2014).

The use of manioc and croton has been verified in different areas of study to control some microbial species that cause economic losses. Nasu et al. (2010), using “manipueira”, a by-product of manioc flour, verified that when applied on the soil, it reduced *M. incognita* satisfactorily, with a decrease in the gall number and an increase in the biomass of tomato roots. The same authors attributed this efficiency to the presence of cyanogenic compounds, which may have a toxic effect on nematodes. Matias et al. (2010) when evaluating different plant species in the extracts preparation, observed excellent results of antibacterial activity with *Croton campestris*, inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* strains. These results confirm the potential of the compounds present in the plants, to act on different pathogenic microorganisms, hindering their mobility and, consequently, reducing parasitism.

M. incognita parasitism characteristics in tomato plants

All the extracts reduced the juvenile number in the root (JNR) of the tomato plants (Table 2). In the treatments in which the extracts were isolated separately from neem and croton or in mixture with Carbofuran, there was no difference with the chemical treatment and differed statistically from the control (water).

Reductions were observed in the JNR of *M. incognita* in tomato for extracts of plants based on neem (51.34%) and croton (50.85%) as compared to the control (water). Studies with croton extract for the control of phytonematodes are scarce; however, its leaves are used for the treatment of pathogenic organisms (Matias et al., 2010). According to Silva (2007), the croton species are aromatic and are characterized by the essential oils production, which have fumigant action and can be used to combat some pests and substitute other similar product effect. Similarly, *C. campestris* hexanic extracts have demonstrated efficiency in the control of other microbiological agents, mainly by the apolar characteristics of its chemical constituents such as tannins, flavonols and terpenes (Coutinho et al., 2010).

The results obtained with neem extracts corroborate with that of Doihara (2005) who studied the effect of neem oil and other substances on hatching, penetration and reproduction of *M. incognita* in melon plants, and observed after 168 h of exposure to eggs, reduction in the hatched second stage juvenile number in comparison with the control.

The juvenile number in the soil (JNS) was reduced in the presence of the extracts, in single and mixed form, as well as with the nematicide. The extracts applied on the soil is more vulnerable to the extract's chemical compounds effects, showing that these extract molecules do not reduce its viability with immediate efficiency. However, Gobbo-Neto and Lopes (2007) showed some environmental factors (temperature, humidity and precipitation) that may interfere with production rate of plant secondary metabolites, which may have a negative effect on pest’s control.

Even so, several results are promising in the use of plant extracts in phytonematodes control. This practice will become an alternative management for small and medium producers and an economically viable possibility with less toxicity to the environment.

Conclusion

The extracts presented different results when used alone and mixed; potentiated or neutralized the agronomic characteristics and the phytonematodes management. The croton extract has a suppressive effect on egg mass number, with results similar to carbofuran. The number of nematodes in the root reduced substantially with the presence of neem and croton extracts. The extract of the manioc leaf had greater effect on the reduction of soil nematodes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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