

Control of *Meloidogyne incognita* Using Aqueous Extracts and *Solanum stramonifolium* Jacq. Dialysates

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Abstract

Root-knot nematodes (*Meloidogyne* spp.) considerably affect their plant hosts, causing extensive damage in the world agriculture. The most widely used method to control these pathogens is through the intensive application of nematicides, despite being highly toxic to humans, animals, and the environment. The urgent search for alternative forms of control based on natural resources that are effective, provide a targeted strategy that is less toxic and less harmful to the environment. The species *Solanum stramonifolium* Jacq. (Solanaceae) have been described as resistant to root-knot nematode infection and other diseases, such as fungi and bacteria. Nematotoxic assays here presented demonstrated that aqueous crude seed extract from *S. stramonifolium* is very effective against second stage juveniles (J₂) of *M. incognita* even at very low concentrations such 100µg mL⁻¹ during *in vitro* bioassays. Furthermore, this extract also demonstrated a nematicidal effect after a heating process at 50 °C, killing more than 90% of *M. incognita* J₂. No toxic activity was observed against non-target organisms, like bacteria, and the free-living nematode *Caenorhabditis elegans* at concentrations varying from 25 to 512 µg mL⁻¹. Finally, greenhouse assays showed that external dialysate (ED) can be used to control nematodes in the soil, and that the plants treated with the dialysates display a reproduction factor lower than the synthetic nematicide used as positive control.

Keywords: agriculture, nematicide, nematode, plants, rootknot, Solanaceae

1. Introduction

Root-knot nematodes (RKN) belonging to the genus *Meloidogyne* spp. are phytopathogens with great impact on agriculture due to the damage they cause to crops. Yearly losses of around US\$157 billion rank these nematodes as one of the most severe pathogens affecting food production worldwide (Abad et al., 2008). These nematodes induce galls in the host's root, leading to metabolic and physiological changes that reduce plant vigor and productivity, causing defoliation, stunt growth, and promoting yellowing and wilting of leaves (Abad et al., 2008).

During many years, broad spectrum pesticides such as methyl bromide, organophosphate and carbamate-based have been used as nematicides. However, since 2010 some of those have been banned in Europe and other continents or have had their use restricted. Recently other types of nematicides against RKN, associated with a lower toxicity and lower environmental impact, like fluoroalkenyl thioethers nematicide, have been applied for nematode control in crop species (Kearn, Ludlow, Dillon, O'Connor, & Holden-Dye, 2014).

The use of natural extracts and compounds obtained from plants might be considered as an alternative method for the control of phytoparasitic nematodes. Nonetheless, alternative candidate compounds are still insufficiently commercially explored and the potential effects of those on the environment and human health must be addressed (Timper, 2011; Viaene, Coyne, & Davies, 2013). Studies of plant extracts have shown that the nematotoxic effect can be associated with the presence of phytochemicals such as alkaloids, terpenes, tannins, flavonoids, glucosinolates, isothiocyanates and organic acids (Caboni & Ntalli, 2014; Caboni et al., 2015; Naz et al., 2016; Ntalli & Caboni, 2012; Prakash et al., 2014). Moreover, nematotoxic activity has also been linked with proteins found in seeds, such as CpPRI from *Crotalaria pallida* (Andrade et al., 2010; Colgrave, Kotze, Ireland, Wang, & Craik, 2008).

Further detailed investigation of natural compounds with specific nematotoxic activity is imperative, as those can constitute a promising alternative strategy to control nematode pests in agriculture. Several Solanaceae species showed activity against *M. incognita*, such as *Datura stramonium*, *D. innoxia*, *D. tatula* (Babaali, Roeb, Hammache, & Hallmann, 2017) and *Brugmansia suaveolens* (Nandakumar, Mayil Vaganan, Sundararaju, & Udayakumar, 2017) and *Solanum melongena* L. (Akhter & Khan, 2018). Candidate species for investigation, such as *Solanum stramonifolium* Jacq. (Solanaceae), which can be found in various regions worldwide and is known for its potential to be resistant to RKN nematode infection, other pests, and disease caused by fungi and bacteria (de Mendonça, de Santana, Mattos, & Pinheiro, 2010; Gousset et al., 2005).

Thus, the objective of this work was to evaluate the activity of Solanaceae plant extracts useful to nematodes control and to elucidate their biotechnological potential.

2. Method

2.1 Plant Material

The *S. stramonifolium* seeds (Accession-CNPH19) were provided by the Plant Germplasm Bank from Embrapa Hortaliças-DF/Brazil. The plants were cultivated in Betamax commercial substrate under greenhouse conditions, respecting the natural photoperiod and with a temperature ranging between 19 and 32 °C, at Embrapa Genetic Resources and Biotechnology-DF/Brazil. Twenty days after sowing, seedlings were transplanted to plastic pots of 500g each containing a mixture of 60% ordinary soil, 30% sand and 10% substrate.

2.2 Nematode Material, Egg Collection and Hatching

Pre-parasitic nematodes (J₂s) were generated as described by Hussey (1973). Tobacco (*N. benthamiana*) plants were inoculated with 1500 J₂ under greenhouse conditions, and three months old plant roots were carefully washed in water and homogenized in a blender with a 0.5% aqueous sodium hypochlorite solution. The eggs were placed in a hatching chamber with distilled water for 48 h and nematodes were counted using a Peter's slide under an Olympus BH2 B071 microscope (Dickson & Struble, 1965). For non-targeted organism assay, adults of *Caenorhabditis elegans* were grown on 8P NGM plates as described by (Lu & Goetsch, 1993; Machado et al., 2015).

2.3 Preparation of Crude Seed Extracts

The aqueous crude extracts (ACE) were obtained using *Solanum stramonifolium* seeds extracted with water, as described by Rocha et al. (2017). The supernatants were collected and freeze-dried in a Savant-Super Modulyo (Thermo Fisher, GA, USA) lyophilizer. The dried extracts were subsequently weighed and stored at -20 °C. The ACE was resuspended in distilled water to perform the nematode bioassays.

2.4 Fractionation of Aqueous Extract

The ACE was fractionated as described by Rocha et al. (2017). Fifty grams of *S. stramonifolium* seeds were solubilized in 150 mL of distilled water using gentle agitation during 10 min at 4 °C. The solution was then transferred to dialysis tubing Spectra/Phor 3 with a molecular weight of 3.5 kDa (Spectrum Laboratories inc.) and dialyzed with 5 L of distilled water for 12 h at 4 °C. The *S. stramonifolium* internal dialysate (ID) containing molecules > 3.5 kDa and external dialysate (ED) containing molecules < 3.5 kDa were collected, freeze-dried, weighed, and then stored at -20 °C.

2.5 Nematotoxic Activity of ACE, ID and ED

The nematotoxic activity was evaluated as described by Rocha et al. (2017). The ACE (10 mg mL⁻¹), ID and ED (5 mg mL⁻¹) were diluted each in distilled water. Aliquots of 100 µL from each test solution were then transferred individually to microtubes already containing a suspension of nematodes (100µL) in water with 60 J₂ and completed with distilled water reaching a total final volume 1000 µL. The treatments were replicated three times. The microtubes were incubated for 48 h at 27±1 °C in a dark room. Distilled water and ethyl alcohol 70% (EtOH) were used as negative and positive controls, respectively. After a 48 h exposure period, the mobile and paralyzed J₂ of *M. incognita* were observed and counted microscopically. The paralyzed nematodes were then submitted to a recovery assay. For nematode recovery assay, J₂ displaying a dead posture (paralyzed and stretched form) were transferred to 1.5 mL microtubes and centrifuged at 4000 RPM for 10 min. The supernatant was discarded and the J₂ were carefully re-suspended in 1.5 mL of distilled water. This procedure was repeated three times and after the last wash, the nematodes were left in distilled water for more than 12 h. The juveniles were then counted again to determine nematocidal or nematostatic activity. The statistical analysis of dead and alive J₂ after water treatment was evaluated using ANOVA and Tukey's mean comparison tests, using software PAST v3 (p < 0.05).

2.6 Thermostability Assay of ACE, ID and ED

To certify the thermostability, ACE, ID and ED were incubated in a water bath for 24 h at 50 °C. The ID (100 and 200 µg mL⁻¹) and ED (100 and 300 µg mL⁻¹) were used to confirm the activity against J₂ of *M. incognita*, as previously described. The test was performed in experimental triplicate. ANOVA and Tukey's mean comparison tests were used for statistical analysis using software PAST v3 (p < 0.05).

2.7 Hemolytic Assay

Lyophilized ACE (300 µg mL⁻¹), ID and ED (25, 50 and 100 µg mL⁻¹) were resuspended in 0.9% NaCl, filtered (utilizing a 0.22 µm pore size; Millipore Corp., Bedford, Mass.), sterilized and incubated with bovine red cells obtained from blood samples. The hemolytic activity was monitored using spectrophotometry, measuring absorbance at 567 nm. Distilled water and PBS were used as positive and negative controls, respectively. The ANOVA and Tukey's mean comparison tests were used for statistical analysis using software PAST v3 (p < 0.05).

2.8 Bioassays Using Non-targeting Organisms

The antibacterial assays using ACE were carried out according to Mulder et al. (2015). *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 8739) strains were used to evaluate the non-target activity of *S. stramonifolium* extract. For each assay, chloramphenicol (30 µg mL⁻¹) was used as positive control and LB medium as negative control. To verify the minimum inhibitory concentration (MIC), the *S. stramonifolium* ACE was serially diluted from 2 to 500 µg mL⁻¹ in LB medium. The MIC was determined as the lowest concentration that produced complete growth inhibition (100%) in comparison to the negative control. The ANOVA and Tukey's mean comparison tests were used for statistical analysis using software PAST v3 (p < 0.05).

2.9 Free Living *Caenorhabditis Elegans* Nematode Assay

To verify the non-target activity of ID and ED fractions on *C. elegans*, 20 nematodes were incubated with different concentrations (25 and 200 µg mL⁻¹) of each dialysate for 24 h at 25 °C in *C. elegans* maintenance medium (CeMM) (Lu & Goetsch, 1993). The static nematodes were counted, using a light microscope, and were considered dead. The bioassay was performed in triplicate, using CeMM as negative control. The ANOVA and Tukey's mean comparison tests were used for statistical analysis using software PAST v3 (p < 0.05).

2.10 Greenhouse Evaluation

The ED was evaluated in a greenhouse, as described by Rocha et al. (2017) with modifications. *Nicotiana benthamiana* plant seedlings, 15 days post-germination, were transplanted into plastic pots containin 250 g of sterilized soil (60% ordinary soil, 30% sand and 10% commercial substrate) and grown for 5 days. The nematicide Aldicarb (280 mg kg⁻¹ of soil) and distilled water were used as positive and negative controls, respectively. The ED solution was applied to the soil around each plant arriving at the final concentration of 50 mg kg⁻¹ of soil. One thousand and five hundred *M. incognita* J₂ were added around each plant, 1-2 cm deep. *Nicotiana benthamiana* plants were then maintained under greenhouse conditions at temperatures varying from 26 to 33 °C. After 45 days of the nematode infection, the plants were collected from the plastic pots and the roots removed and washed thoroughly in water, weighed, and submitted to the egg extraction process to obtain the reproduction factor (RF). Experiments were performed in triplicate. The data were submitted to analysis of variance ANOVA and the treatment of the means was compared using the Tukey test (p < 0.05).

3. Results

3.1 ACE, ID and ED Nematotoxic Activity

Aqueous extracts from *S. stramonifolium* CNPH19 seeds were evaluated against *M. incognita* by *in vitro* bioassays, due to low reproduction factor displayed by these plants in previous assays (de Mendonça et al., 2010). *Solanum stramonifolium* aqueous crude seed extract (ACE) showed promising results, paralyzing 100% of J₂. After the recovery assay, 90% of J₂ remained completely static and stretched confirming a strong nematocidal activity for ACE (Figure 1).

To determine the lethal concentration and paralyzing concentration of ACE against *M. incognita*, increasing concentrations of 100, 300, 500 and 1000 µg mL⁻¹ were used in *in vitro* bioassays. All concentrations tested strongly affected the nematodes (> 98% of J₂ was immobile), with significant difference in the results obtained from the control (Figure 1). However, in the recovery assay a bifunctional profile was observed, with more than 90% of the J₂ having recovered mobility at concentrations of 100 and 300 µg mL⁻¹, which expressed the nematostatic profile of these concentrations. On the other hand, for concentrations of 500 and 1000 µg mL⁻¹, 100% of J₂ did not recover their mobility, thus confirming the nematocidal effect of these concentrations (Figure 1).

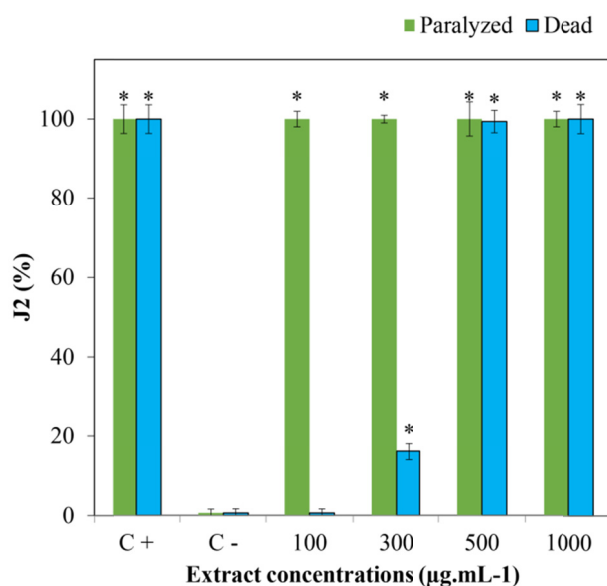


Figure 1. Nematotoxic activity of ACE against *M. incognita* J₂ curve to determine lethal concentration.

Concentrations of 100, 300, 500 and 1000 µg mL⁻¹ of ACE were used. Nematotoxic bioassay (green bar) and recovery assay (blue bar). Distilled water and ethanol 70% were used as negative (C-) and positive control (C+), respectively. The vertical bars represent the standard deviation

To verify if the nematotoxic activity is related to compounds larger or smaller than 3.5 kDa, the ACE was submitted to dialysis and, after this process, the resulting fractions, entitled internal dialysate (ID) and external dialysate (ED). Surprisingly, the *in vitro* bioassays with ID and ED showed that the nematotoxic activity is present in both dialysates, affecting (Paralyzing and/or killing) 100% of *M. incognita* J₂ at all concentrations evaluated (25, 50, 100 and 300 µg mL⁻¹) with significant difference in the results obtained from the control (Figure 2).

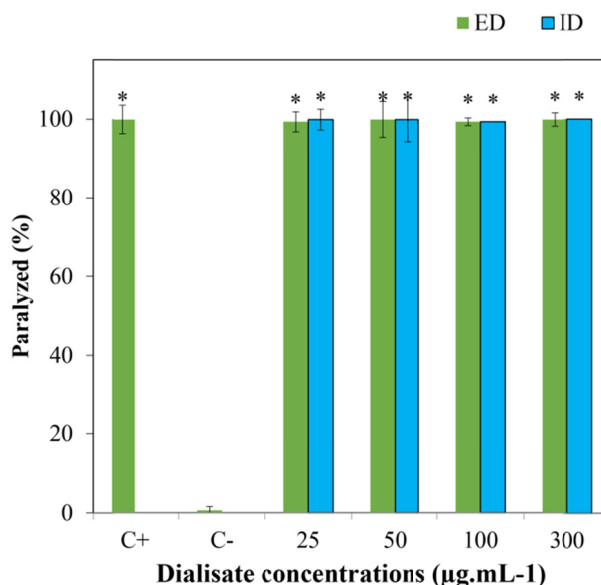


Figure 2. Nematotoxic activity of ID and ED fractions against *M. incognita* J₂ curve to determine lethal concentration. ID (blue bar) and ED (green bar) fractions. The positive control (C+) consisted of 70% ethyl alcohol, while the negative control (C-) consisted of distilled water. The vertical bars indicate the standard deviation

Additionally, the recovery assay revealed that ID is slightly more effective against *M. incognita* than ED. At concentrations of 25 and 50 µg mL⁻¹, ID killed < 1% and 57% of J₂, respectively. The highest ID concentrations (100 and 300 µg mL⁻¹), on the other hand, exhibited a nematocidal activity which killed 100% of J₂ (Figure 3). Whereas, the ED fraction at concentrations of 25, 50 and 100 µg mL⁻¹ killed less than 1% of *M. incognita* J₂, the concentration of 300 µg mL⁻¹ killed 100% of J₂, revealing significant nematocidal activity only at the latter ED concentration (Figure 3). Due to work is focused on small secondary metabolites, ED was then selected to be evaluated in greenhouse conditions while ID was kept for posterior assays.

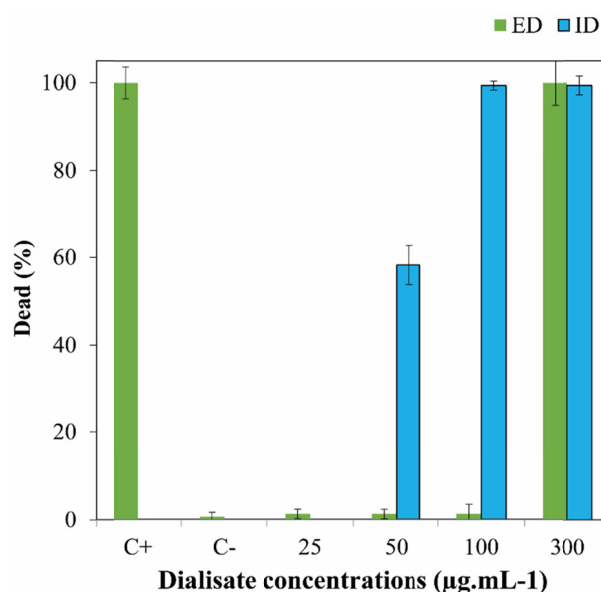


Figure 3. Recovery assay of ID and ED fractions against *M. incognita* J₂ to determine lethal concentration. The concentrations tested were the same as those used in the nematotoxic assay. The positive control (C+) consisted of 70% ethyl alcohol, while the negative control (C-) consisted of distilled water. The vertical bars indicate the standard deviation

3.2 Thermostability Assay of the ACE, ID and ED

Thermostability assay of the ACE were performed at a concentration of 300 $\mu\text{g mL}^{-1}$, ID at 100 and 200 $\mu\text{g mL}^{-1}$ and ED at 100 and 300 $\mu\text{g mL}^{-1}$, after having been heated at 50 °C for 24 h before conducting the bioassay. The nematotoxic assay showed a peculiar and interesting result: the thermal treatment did not decrease the nematotoxic effect in almost all dialysate concentrations and ACE. With exception of ED at 100 $\mu\text{g mL}^{-1}$ (< 1% of activity), 100% of *M. incognita* J₂ in all mentioned treatments were immobile (Figure 4). The recovery assay showed that ACE at concentration of 300 $\mu\text{g mL}^{-1}$, ID at 200 $\mu\text{g mL}^{-1}$ and ED at 300 $\mu\text{g mL}^{-1}$ killed nearly 100% of J₂ (Figure 4) with significant difference for the control.

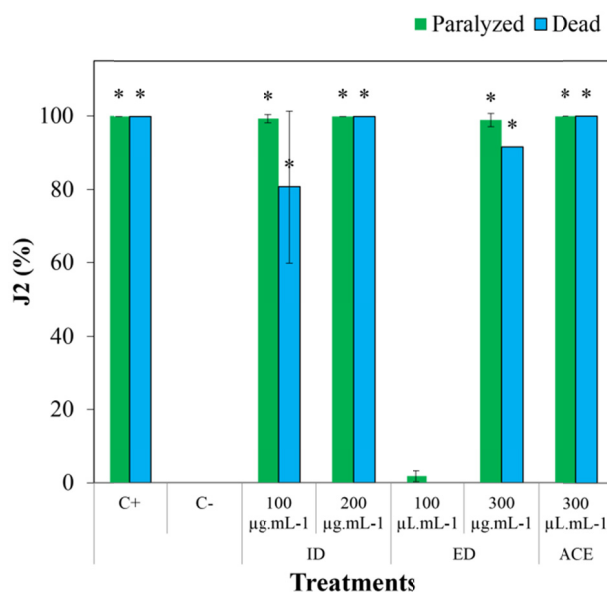


Figure 4. Heating tests of *Stramonifolium* ECA, ID and ED. Nematotoxic activity (green bar) and recovery assay (blue bar) using ACE, ID and ED after 24 h treatment at 50 °C. The negative control (C-) distilled water, and the positive control (C+), 70% ethyl alcohol. The vertical bars indicate the standard deviation

3.3 Hemolytic Assay

An *in vitro* cytotoxic bioassay using a hemolysis test submitted to ACE at a concentration of 300 $\mu\text{g mL}^{-1}$, and to ID and ED at concentrations of 25, 50 and 300 $\mu\text{g mL}^{-1}$ did not show any cytolytic effect on bovine red blood cells.

3.4 Bioassays Using ID and ED Fractions on Non-targeted Organisms

The ID and ED dialysates showed a highly specific activity against *M. incognita* *in vitro* bioassays, as it was innocuous on other non-targeted organisms tested. After 16 h of incubation, *in vitro* bioassays on non-targeted organisms treated with ID and ED diluted from 2 to 512 $\mu\text{g mL}^{-1}$ did not show any inhibitory effect on the growth of Gram-positive *S. aureus* (ATCC 25923) and Gram-negative *E. coli* (ATCC 8739) strains. Moreover, after 24 h of exposition, ID and ED fractions at concentrations of 25 and 200 $\mu\text{g mL}^{-1}$ showed no activity against free-living nematode *C. elegans* (Table 1).

Table 1. Summary table of *in vitro* non-target organism bioassays using different concentrations of ED and ID against nematodes and bacteria. All bioassays were conducted in triplicate

Organisms	Species	ED	ID	Activity
Bacteria (Gram+)	<i>Staphylococcus aureus</i> ATCC 25923	2-512 $\mu\text{g mL}^{-1}$	2-512 $\mu\text{g mL}^{-1}$	None-normal growth
Bacteria (Gram-)	<i>Escherichia coli</i> ATCC 8739	2-512 $\mu\text{g mL}^{-1}$	2-512 $\mu\text{g mL}^{-1}$	None-normal growth
Free-living nematode	<i>Caenorhabditis elegans</i>	25 and 200 $\mu\text{g mL}^{-1}$	25 and 200 $\mu\text{g mL}^{-1}$	None
Nematode	<i>Meloidogyne incognita</i>	25, 50, 100 and 300 $\mu\text{g mL}^{-1}$	25, 50, 100 and 300 $\mu\text{g mL}^{-1}$	Nematotoxic

3.5 Greenhouse Evaluation of ED Activity on *Nicotiana benthamiana* After *Meloidogyne incognita* Inoculation

Nicotiana benthamiana plants treated with ED at a concentration of (1 mg mL⁻¹) of *S. stramonifolium* under greenhouse conditions showed a remarkable decrease of *M. incognita* egg numbers, with results similar to that obtained with the positive control (C+), the synthetic nematicide Aldicarbe® (250 mg kg⁻¹ of soil) . For both treatments, after 45 days of inoculation, only an average of 3 eggs were recorded compared to 4.700 eggs observed in the negative control using *N. benthamiana* plants without treatments and infected with *M. incognita*, with significant difference. ED treatment showed a reproduction factor of 0.01 compared to 3.17 seen in the negative control, confirming ED's high nematicide activity on juvenile nematodes of *M. incognita* (Figure 5).

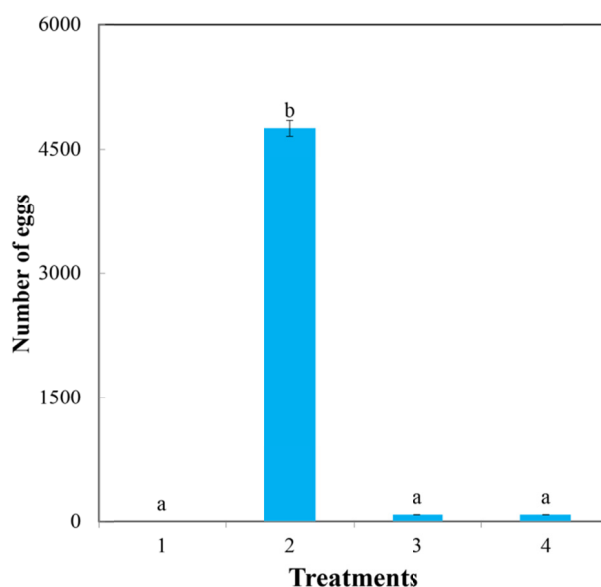


Figure 5. Evaluation of the external dialysate (ED) obtained from *S. stramonifolium* aqueous crude seed extract toward *M. incognita* second stage juveniles under greenhouse conditions. Blue bars represent the number of eggs observed for *N. tabacum* roots after 45 days of infection. The experiment was conducted in triplicate with the following treatments: (1) *N. tabacum* plants without *M. incognita* infection; (2) *N. tabacum* plants infected with *M. incognita*; (3) *N. tabacum* plants treated with the synthetic nematicide (Aldicarb) infected with *M. incognita* and (4) *N. tabacum* plants treated with *S. stramonifolium* ED infected with *M. incognita*

4. Discussion

Solanaceous plants are good host of plant parasitic nematodes (PPN) (Barbary, Djian-Caporalino, Palloix, & Castagnone-Sereno, 2015; Boiteux & Charchar, 1996; Pereira et al., 2018; Pestana, Gouveia, & Abrantes, 2009). However, extracts of several species of the genus Solanaceae have nematicidal effect (Babaali et al., 2017; de Mendonça et al., 2010; Gousset et al., 2005; Nandakumar et al., 2017).

The nematotoxic activity observed in aqueous extract of *S. stramonifolium*, and the ID/ED that derive from it, possesses a hydrophilic nature and therefore, most likely, include molecules such as alkaloids (Babaali et al., 2021), saponins and hydrophylic proteins. These proteins might have an influence on these parasitic phytonematodes. The result obtained corroborate with previous studies that also indicated the nematotoxic or nematicidal activity of aqueous seed extracts of antagonistic plants such as *Canavalia ensiformis* and *Crotalaria spectabilis* against *M. incognita* (Almeida et al., 2008; Rocha et al., 2017). Aqueous extracts obtained from both *Tagetes erecta* and *T. patula* seeds caused a higher mortality to *Heterodera schachtii*, *M. hapla* and *Pratylenchus penetrans* than the control extracts from radish, tomato and corn seeds (Riga, Hooper, & Potter, 2005). A similar result is observed with the *Azadirachta indica* (A. Juss) aqueous seed extract, which killed more than 98% of the soybean cyst nematode *H. glycines* (Silva, Oliveira, Jham, & Aguiar, 2008). Additionally, previous literature reports identified that extracts obtained from diverse plant organs show nematicidal activity. Leaf aqueous extract from *Mentha spp.* exhibited a 50% nematicidal effect against *M. incognita* at several concentrations (*M. piperita*—300 µg mL⁻¹; *M. pulegium*—745 µg mL⁻¹; and *M. spicata*—1005 µg mL⁻¹) (Caboni et al., 2013). Compounds associated with this nematicidal effect were identified as carvone, pulegone, salicylic acid and

menthofuran (Caboni et al., 2013). In other studies, several seed extracts have shown to exhibit activity against *M. incognita*, such as *A. indica* and *Melia azedarach* (Khurma & Singh, 1997).

The compounds associated with nematocidal effect may vary from large proteins to small molecules. The dialysis provides an effective process that is able to separate compounds by molecular size or mass. The ID compounds are mostly larger than 3.5 kDa, suggesting that nematotoxic activity might be related to proteinaceous compounds (e.g., seeds storage proteins). These proteins are classified in different families (e.g., albumin, globulin, prolamin and glutelin) and they represent up to 40% of the seed's dry weight (Erbaş, Tonguç, & Şanlı, 2016; Shewry, Napier, & Tatham, 1995). Moreover, they function as nutrient reserve for embryo development and may also be involved in plant defense against other organisms.

The ED fraction contains compounds smaller than 3.5 kDa, like secondary metabolites (e.g., glycoalkaloid, phenolic compound) and peptides already identified in the Solanaceae family, as described by Tania et al. (2003). Several studies underlined the biological potential of secondary metabolites extracted from Solanaceae plants, such as indole alkaloids obtained from *Tabernaemontana* genus (Marinho, Simões, Barcellos, & Moura, 2016), and aporphine alkaloids obtained from *Annona crassiflora* (Justino et al., 2020).

Any potential nematotoxic compound must display thermostability in the prevailing environment, as it will be subject to temperature variations, which could otherwise compromise its properties and characteristics and, consequently, its nematotoxic activity. Results obtained in this study from ID and ED, showed that ID is slightly more thermostable than ED. Proteins maintain their three-dimensional structures due to diverse interactions between the amino acids, cofactors, the medium in which they are inserted, as well as the interaction with other protein compounds. This three-dimensional arrangement is often correlated with biological activity (Lehninger & Cox, 2014). Nevertheless, some of these interactions, such as hydrogen bonds and Van Der Waals, are particularly less stable than others, like covalent bonds, and are consequently more affected by physical factors, such as temperatures higher than that of the original biological medium (Lehninger & Cox, 2014). Based on the results obtained regarding thermostability, it was expected that the ID, which contains larger molecules, mainly proteins, would lose its nematocidal activity, due to protein denaturation. On the other hand, it was expected that secondary metabolites would maintain their nematocidal activity, due to greater stability of its compounds, solely composed of covalent bonds (Hall, 2006; Newman, Cragg, & Snader, 2000; Newman, Cragg, & Snader, 2003). Contrary to what was initially expected, the results obtained here show a loss of activity for ED when tested at a $100 \mu\text{g mL}^{-1}$ concentration in nematotoxic assay. This phenomenon possibly occurs due to the smaller amount of nematotoxic compounds effective toward *M. incognita* J₂ present in this fraction. Conversely, ED maintained its nematocidal potential when tested at a concentration of $300 \mu\text{g mL}^{-1}$. On the other hand, ID maintained its activity, which might be correlated to the thermoresistant plant proteins existent in this fraction (Silva et al., 2003). To date, based on a literature review, there are no reports of studies that corroborate the data obtained in the present study.

Our results indicate that molecules present in ED, ID and ACE are possibly not toxic to non-targeted organisms. Information regarding the cytotoxicity evaluation represents a very important characterization of various biological materials that could be used, for instance, in the development of products for agriculture (Clardy & Walsh, 2004; Oksman-Caldentey & Inze, 2004). Enterolobin, a hemolytic protein isolated from *Enterolobium contortisiliquum*, demonstrated an antitryptic activity (trypsin and chymotrypsin) (Batista et al., 1996; de Sousa & Morhy, 1989). Other tests using plant compounds isolated from *Crataegus pinnatifida* (Chinese hawthorn) seeds identified cytotoxic activity in 5 metabolites (7*S*,8*S*)-4-[2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1(hydroxymethyl) ethoxy]-3, 5-dimethoxybenzaldehyde, (+)-balanophonin, erythro-guaiacylglycerol- β -coniferyl aldehyde ether and buddlenol A, inhibiting the proliferation of OPM2 and RPMI-8226 cells (Li et al., 2013). On the other hand, studies using extract, fractions, and isolated metabolites from *C. ensiformis* indicated their activity against *M. incognita* but did not present hemolytic activity (Rocha et al., 2017). Studies using protein extract of *Caryocar brasiliense* seeds demonstrated no hemolytic activity, despite its insecticidal potential against pest insects in *in vitro* tests (Costa, Franco, Migliolo, & Dias, 2015). *Solanum capsicoides*, a plant from the Solanaceae family, also showed no hemolytic effect (Petreanu et al., 2016).

The results of bacteria and free-living nematodes assays suggest that ID, ED and ACE did not affect non-targeted organisms in the soil. Additionally, our results diverge from previous studies regarding the activity of proteinaceous extracts from *S. stramonifolium* seeds against *E. coli* and *S. aureus*, in which the ED, ID and ACE showed no activity against those bacteria, probably due the extraction used in our study. Herein, we focused on small metabolites, while the study performed by (Sarnthima & Khammuang, 2012) employed proteinaceous compounds.

Finally, the greenhouse evaluation showed that ED can be as effective as a positive control (Aldicarbe®) against J₂, as it also demonstrates a reproduction factor (RF) lower than other Solanaceae plant extracts (Ferreira, 2018) and other synthetic nematicides, such as Rugby ® (RF 0.70) (Carvalho, 2017) and, possibly, without the side-effects to the environment and human health (Taniwiryo et al., 2009).

5. Concluding Remarks

Our study suggests that dialysates and extracts obtained from *S. stramonifolium* have biotechnological potential to be explored as a natural alternative to the nematicides currently used. For they associate not only the nematocidal activity obtained from low concentrations, but also other proprieties, such as thermostability and specificity.

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