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RESEARCH ARTICLE

Canola seed cake extracts for the control of *Pratylenchus brachyurus*

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ABSTRACT

The lesion nematode *Pratylenchus brachyurus* is a major pest of several crops. Given its wide geographic distribution and broad host range, the search for innovative strategies to manage the rising populations of these parasites has become increasingly necessary. This study aimed to evaluate the effect of different dilutions of canola (*Brassica napus* L.) seed cake extracts on *P. brachyurus* mortality, reproduction, and penetration. Nematode mortality was assessed in vitro assay using a 3 × 7 (Extraction method × Dilution factor) factorial design with four replicates and two trials. For evaluation of nematode reproduction, a greenhouse experiment was carried out using a 2 × 4 (Application method × Dilution factor) factorial design with eight replicates and two trials. Nematode penetration was evaluated in a greenhouse experiment with a 2 × 5 (Seed treatment × Evaluation period) factorial design. Canola seed cake extracts were analyzed by high-performance liquid chromatography. The maximum nematode mortality values estimated from in vitro results were 78% and 100%, achieved with 10.88% and 10.15% extract, respectively. In the reproduction experiment, both seed treatment and in-furrow application resulted in effective nematode control, reaching 76% in the first trial and over 90% in the second trial compared with the control. In the penetration test, extract treatment reduced nematode number by 53% and 56% at 20 and 25 d after inoculation, respectively, compared with the control. The major compounds identified in canola seed cake extracts were ferulic, *trans*-cinnamic, caffeic, coumaric, chlorogenic, and gallic acids, along with the flavonoids quercetin and kaempferol.

Key words: *Brassica*, lesion nematode, nematicidal compounds, organic waste, plant extracts.

INTRODUCTION

Plant-parasitic nematodes are among the main phytosanitary problems affecting Brazilian agriculture. Soybean crops are the most severely affected, given their widespread cultivation across the country and the predominance of soybean-maize cropping systems (Nomura et al., 2024). The main nematodes that cause damage to soybean crops are *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, *Meloidogyne javanica* (Treub) Chitwood, *M. incognita* (Kofoid & White) Chitwood, *Heterodera glycines* Ichinohe, and the emerging nematodes *Helicotylenchus dihystera* (Cobb) Sher and *Scutellonema brachyurus* Andrassy (Machado et al., 2019). Among these, *P. brachyurus*, commonly known as the lesion nematode, stands out as the most widely distributed. The nematode is found in 75.73% of samples collected nationwide, with detection rates ranging from 86.85% in the Cerrado/Northern region to 57.63% in the Southern region (Syngenta, 2024).

Pratylenchus brachyurus is a migratory endoparasitic nematode that does not establish specific feeding sites. Owing to its migratory habits and feeding behavior, *P. brachyurus* causes lesions throughout the entire root system. All mobile stages (second-, third-, and fourth-stage juveniles and adults) are infective, and females

can lay their eggs either inside plant roots or in the surrounding soil (Nomura et al., 2024). The difficulty in controlling this nematode is aggravated by its wide range of hosts and the lack of resistant cultivars (Debiasi et al., 2016; Cruz et al., 2020). Additionally, the fact that the nematode can complete its life cycle inside the host reduces the chances of exposure to chemical or biological nematicides.

In the search for alternative methods to control *P. brachyurus* and other plant-parasitic nematodes, studies have focused on identifying new nematicidal compounds. Notably, phytochemicals, usually in the form of extracts, which may come from plants or even algae and agricultural wastes have shown great promise in nematode management (Ntalli and Caboni, 2012; Rinaldi et al., 2023). Phytochemicals can also enhance the efficiency and half-life of active ingredients, expanding the biochemical market for the major crops produced in the country (Lengai et al., 2020). Numerous phytochemicals were found to be environmentally friendly and effective for controlling nematodes (Khan et al., 2020). For instance, a product based on garlic (*Allium sativum* L.) extract is registered with the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA, Ministério da Agricultura, Pecuária e Abastecimento) for the control of *M. incognita, P. brachyurus*, and *Pratylenchus zeae* Graham in soybean, tomato, and sugarcane (AGROFIT, 2024).

Phytochemicals found in *Brassica* species have been the focus of numerous studies on nematode control. Extracts and compounds obtained from these species exert nematicidal effects (Tavares-Silva et al., 2017), commonly attributed to the presence of glucosinolate (Ntalli and Caboni, 2012). Upon decomposition of *Brassica* waste in soil, glucosinolate undergoes chemical and enzymatic reactions that result in the formation of allyl isothiocyanate, organic cyanides, and other compounds that are toxic to nematode eggs and juveniles (Oliveira et al., 2011; D'Addabbo et al., 2020). Glucosinolates from wild mustard (*Brassica juncea* (L.) Czern.) are nematicidal to eggs and second-stage juveniles of *M. incognita* (Oliveira et al., 2011). Another study showed that isothiocyanates from papaya (*Carica papaya* L.) seeds possess nematicidal activity, causing significant mortality to *M. incognita* juveniles (Gomes et al., 2020).

Extracts from plant wastes represent an efficient and cost-effective source of bioactive compounds. Seed cakes are known to contain high concentrations of nematicidal substances. Research has demonstrated the nematicidal potential of castor bean (*Ricinus communis* L.) seed cake extract and crambe (*Crambe abyssinica* Hochst ex R.E.Fr.) seed cake for controlling *P. brachyurus* (Tavares-Silva et al., 2015; Tarini et al., 2020; Izidoro et al., 2021).

In view of the low cost and great nematicidal potential of *Brassica* seed cakes, this study aimed to evaluate the nematicidal effect of canola seed cake extract on *P. brachyurus* in an in vitro assay, assess the potential of the extract in controlling nematode penetration and reproduction in soybean roots, and characterize its phenolic profile.

MATERIALS AND METHODS

General experimental procedures

The experiments were carried out at the Phytopathology Laboratory and in a greenhouse (23°47′55″ S 53°18′48″ W, 430 m a.s.l.) at the Universidade Estadual de Maringá (UEM), Umuarama Regional Campus, Paraná, Brazil. All experiments followed a completely randomized design. An in vitro mortality experiment and an in vivo reproduction experiment were conducted at two different times to verify the results. The same batch of canola (*Brassica napus* L.) seed cake was used in all experiments, thereby minimizing experimental errors. The seed cake was kindly provided by the Biodiesel Laboratory at the Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brazil.

Experiment 1: *Pratylenchus brachyurus* toxicity after exposure to canola seed cake extracts

An in vitro bioassay was carried out according to a 3 × 7 (Extraction method × Dilution factor) factorial design with four replicates and two independent trials. Aqueous extracts were prepared by mixing canola seed cake and distilled water in a 1:10 ratio. Three extraction methods were used: Maceration at room temperature, infusion at 100 °C, and autoclaving at 120 °C for 20 min. The mixtures were left to stand for 24 h and then filtered through gauze. The resulting crude extracts were diluted in distilled water to 0 (control), 2.5%, 5.0%, 7.5%, 10.0%, 12.5%, and 15.0%.

Nematodes were obtained from a pure population maintained on soybean (*Glycine max* (L.) Merr.) in a greenhouse and extracted from plant roots according to the method proposed by Hussey and Barker (1973) and adapted by Boneti and Ferraz (1981). Following extraction, the suspension was deposited in a Baermann

funnel for 48 h. After this period, active individuals were recovered in a beaker, and the resulting suspension was calibrated in a Peters chamber under a light microscope at 100X magnification.

For the assessment of nematicidal effects, 9 mL each dilution and 1 mL suspension containing approximately 100 individuals of *P. brachyurus* were added to Falcon tubes. The tubes were maintained at 25 °C in a biochemical oxygen demand incubator. Nematode mortality was assessed after 48 h by counting live and dead individuals. Nematodes were considered dead when they remained immobile after exposure to 10% sodium hydroxide (0.1 N) (Chen and Dickson, 2000), added at the exact moment of evaluation of each sample. The results are expressed as mortality percentage (%).

Experiment 2: *Pratylenchus brachyurus* reproduction in soybean treated with canola seed cake extract

Nematode reproduction was assessed in two independent greenhouse trials. The first trial lasted from January to March 2023, with minimum, average, and maximum temperatures of 21.7, 25.1, and 29.4 °C. The second trial lasted from October to December 2023, with minimum, average, and maximum temperatures of 20.5, 24.4, and 29.1 °C. The soybean cultivar was M6210 IPRO.

Both trials were organized in a 2 × 4 factorial arrangement, with eight replicates. The first factor was application method (seed treatment and in-furrow application). The second factor was extract dilution (0%, 5%, 10%, and 15%). Each experimental unit consisted of a Styrofoam cup containing 950 mL of a 2:1 mixture of soil and sand, previously autoclaved at 120 °C for 2 h. The canola seed cake extract was obtained by maceration in water at room temperature, as described above.

Seed treatment was performed using 5 mL extract per kilogram of seed. The extracts at the respective dilutions were added to a plastic bag containing soybean seeds. The mixture was gently shaken, and seeds were left to dry naturally. Treated seeds were sown in their respective experimental units. In-furrow treatments were applied at a rate of 200 L ha^{-1} (approximately 0.8 mL per plant). An automatic pipette was used to deposit the dilutions in the sowing furrow. The furrows were opened manually in the soil. Then, one soybean seed was planted where the extract was pipetted.

Five days after sowing, the plants were inoculated with a 2 mL suspension containing approximately 500 individuals of *P. brachyurus*. The inoculum was obtained from a pure population of the nematode maintained on soybean in a greenhouse for 2 mo. Nematodes were extracted from plant roots by the method of Hussey and Barker (1973) as adapted by Boneti and Ferraz (1981).

The plants were kept in pots for 70 d after inoculation (DAI) and then collected for evaluation. First, roots and shoots were separated. Then, roots were washed, placed on absorbent paper to remove excess water, and weighed. Nematodes were extracted by the aforementioned method and counted in a Peters chamber under a light microscope at 100X magnification. The total nematode number was divided by the root fresh weight to obtain the population density (number of nematodes per gram of root). Shoots were used to determine plant height, shoot fresh weight, and shoot dry weight. Shoot dry weight was measured after drying the samples for 72 h in a forced air circulation oven at 65 °C.

Experiment 3: *Pratylenchus brachyurus* penetration in soybean treated with canola seed cake extract

Nematode penetration in soybean roots was assessed in a greenhouse experiment conducted between October and November 2023. The minimum, average, and maximum temperatures were 20, 24, and 29 °C. Soybean 'M6210 IPRO' was used. The experiment followed a 2 × 5 factorial design. The first factor comprised seed treatment with an aqueous extract of canola seed cake at 15% (treated seeds and untreated control). The second factor comprised five evaluation periods (5, 10, 15, 20, and 25 DAI). Experimental units consisted of Styrofoam cups containing 280 mL 2:1 soil:sand mixture (autoclaved at 120 °C for 2 h). Extract preparation and the experimental setup were as described in the reproduction test.

At each evaluation period, five plants per treatment were collected, cut into 1 cm pieces, and subjected to the staining method described by Bybd et al. (1983). After cooling, roots were placed in 30 mL glycerol acidified with two drops 5 M hydrochloric acid (HCl). This procedure aimed to preserve samples until analysis. All root fragments were analyzed. Root fragments were placed between two microscope slides, and the number of individuals inside the root system was counted under a light microscope at 100X magnification.

Experiment 4: Phenolic characterization of canola seed cake extract by high-performance liquid chromatography

The aqueous extract of canola seed cake was purified with 1 M barium hydroxide and 5% zinc sulfate solutions, filtered through a hydrophobic polyvinylidene difluoride membrane (0.45 µm pore size, 25 mm diameter), and analyzed by using a HPLC 20A high-performance liquid chromatograph (Prominence, Shimadzu, Kyoto, Japan) coupled to a UV detector (SPD-20A, Shimadzu) operating at 280 and 320 nm. A column oven (CTO-20A, Shimadzu) was used to maintain a C-18 column (Shim-pack CLC-ODS(H), 25 cm × 4.6 mm × 5 mm, Shimadzu) at 25 °C. The sample (20 µL) was injected using a manual injector (SIL-10A, Shimadzu) and a quaternary pump (LC-20AT, Shimadzu) operated at a flow rate of 0.8 mL min^{-1}.

For chromatographic separation, ultrapure water acidified with 0.05% formic acid (A) and methanol acidified with 0.1% formic acid (B) were used as mobile phases. Gradient elution was performed as follows: 0.01 to 5 min, 20% B; 5 to 25 min, 50% B; and 25 to 30 min, 80% B. For quantification, solutions of gallic, coumaric, ferulic, caffeic, and *trans*-cinnamic acids and solutions of the flavonoids quercetin and kaempferol (1 to 10 mg mL^{-1}) were used to construct calibration curves ($R^2 > 0.99$). Results are expressed in mg 100 g^{-1} sample (Donadone et al., 2020).

Statistical analysis

Experimental data were subjected to Shapiro-Wilk and Levene's tests to assess normality and homoscedasticity, respectively. Then, the data were analyzed by two-way ANOVA. When the interaction between factors was significant, factors were analyzed within each level. When the interaction was nonsignificant, factors were analyzed separately.

In the in vitro assay, when significant differences were identified, dilution means were subjected to quadratic and linear regression analysis, and extraction methods were compared using Tukey's test at the 5% significance level. For in vivo experiments, dilution effects were studied by regression analysis, and application methods and treatments were analyzed by Tukey's test at the 5% significance level. All analyses were carried out using SISVAR software (Ferreira, 2019).

RESULTS

Experiment 1: *Pratylenchus brachyurus* toxicity after exposure to canola seed cake extracts

In the toxicity bioassays, there were nonsignificant interaction effects between factors, but the main effects were significant. Regarding extraction method, the highest *P. brachyurus* mortalities in Trial 1 were achieved using extracts obtained by infusion (63.73%) and maceration (66.63%), as compared with autoclaving (52.16%). In Trial 2, nematode mortality rates did not differ according to extraction method, with 81.53%, 78.40%, and 75.96% mortality achieved using infused, macerated, and autoclaved extracts, respectively. In both trials, the dilution factor was explained by a quadratic regression model, with maximum mortalities of 78.04% and 100.00% predicted to occur using dilutions of 10.88% (Figure 1A) and 10.15% (Figure 1B), respectively. And also, nematodes exposed to canola cake extract exhibited internal degradations and cuticle deformations (Figure 2).

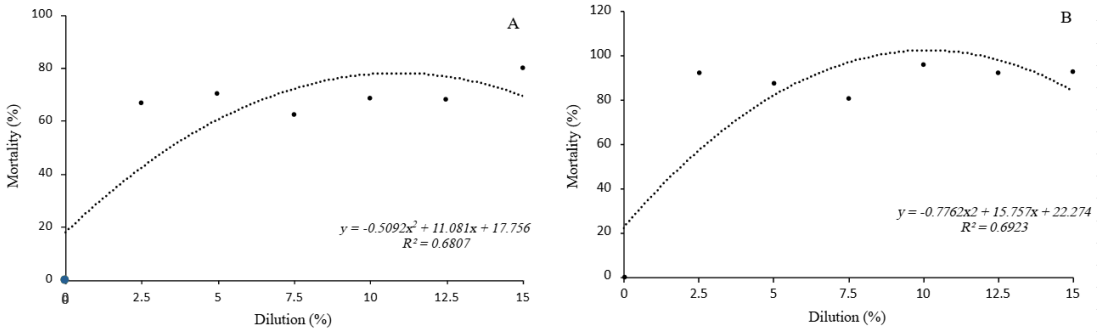

Figure 1. In vitro mortality of *Pratylenchus brachyurus* after 48 h exposure to different dilutions of the aqueous extract of canola seed cake in (A) Trial 1 and (B) Trial 2. Trial 1 was conducted in the laboratory, and Trial 2 was conducted under the same conditions, but performed 2 wk later. Trial 1 (A) *p*-value: 0.00; Trial 2 (B) *p*-value: 0.00.

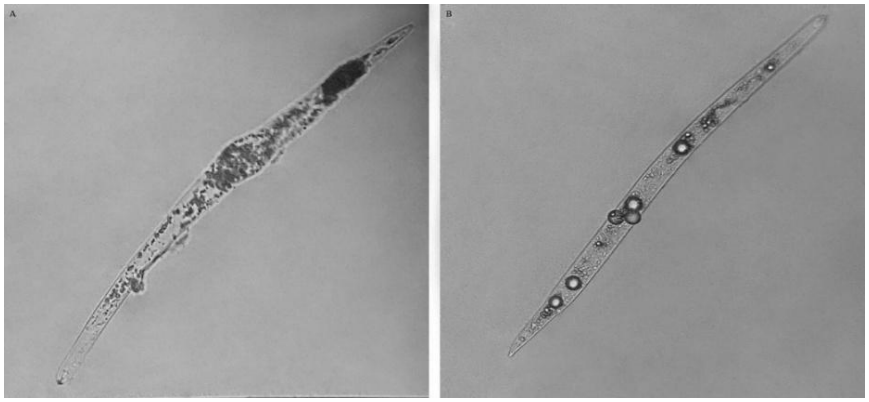

Figure 2. (A) Deformation and (B) degradation of *Pratylenchus brachyurus* exposed to canola seed cake extract in in vitro assays.

Experiment 2: *Pratylenchus brachyurus* reproduction in soybean treated with canola seed cake extract

Nematode population density was not influenced by the interaction effects of factors in Trial 1. The mean effects of application method were also nonsignificant. In furrow application resulted in a mean nematode population density of 199 nematodes g^{-1} root, and seed treatment of 210 nematodes g^{-1} root. The main effect of extract dilution was significant, and data were explained by a quadratic model (Figure 3A). The lowest nematode population density, 82 nematodes g^{-1} root, was estimated to be achieved using 10.98% extract, being much lower than the population density observed in the control, namely of 442 nematodes g^{-1} root. In Trial 2, interaction effects were significant: The highest efficiencies were obtained using dilutions of 13.48% and 14.69% for in-furrow application and seed treatment, respectively, resulting in nematode population densities of 49 and 238 nematodes g^{-1} root, respectively. Significant differences between application methods were only observed in assays using extracts at 10% dilution (Figure 3B), with in-furrow application being more efficient in controlling nematodes.

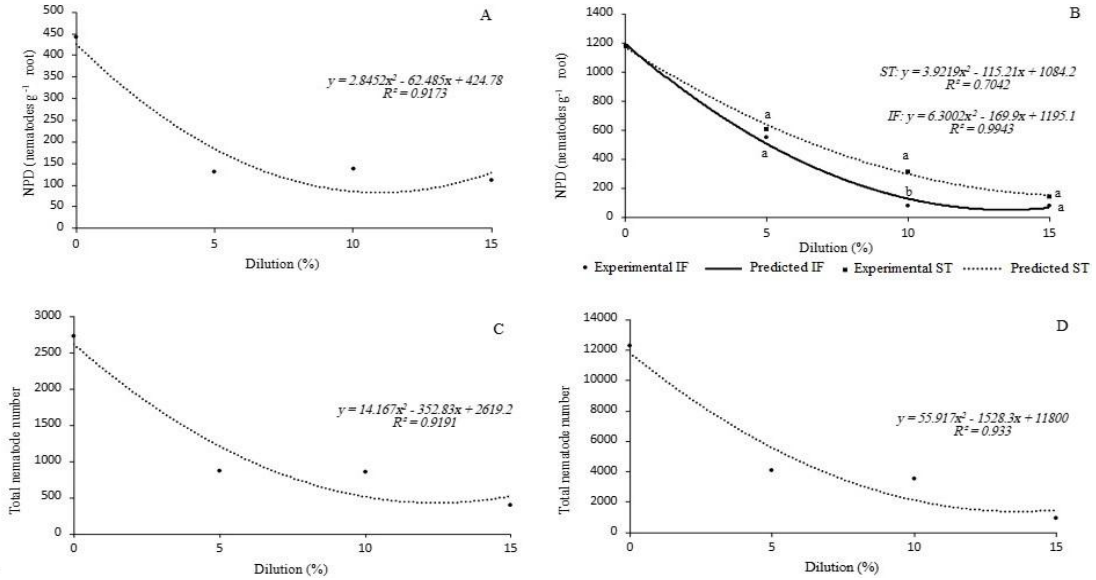

Figure 3. Nematode population density (A and B) and total nematode number (C and D) in roots of soybean treated with canola seed cake extract via seed treatment (ST) or in-furrow (IF) application and evaluated at 70 d after inoculation with 500 individuals of *Pratylenchus brachyurus* in Trial 1 (A and C) and Trial 2 (B and D). (A) *p*-value: 0.00; (B) *p*-value: 0.0045 (C) *p*-value: 0.00; (D) *p*-value: 0.00.

Total nematode number was not influenced by the interaction effects of factors in either trial, but the main effects were significant. In-furrow application was more effective than seed treatment in controlling nematodes in both trials. The mean total nematode numbers were 1025 for in-furrow application and 1400 for seed treatment in Trial 1, respectively, and 4323 for in-furrow application and 6140 for seed treatment in Trial 2, respectively. In both trials, extract dilution was explained by a quadratic model. The lowest nematode numbers (422 and 1357 nematodes) were predicted to be achieved using 12.45% extract in Trial 1 (Figure 3C) and 13.67% extract in Trial 2 (Figure 3D), respectively.

The main and interaction effects of factors did not significantly influence plant height, shoot fresh weight, or shoot dry weight in Trial 1 (data not shown). However, in Trial 2, plant height was influenced by interaction effects. The regression model provided a good fit to in-furrow application data only. Differences between application methods were significant only for extracts used at 5% dilution (Figure 4A). Under these conditions, seed treatment promoted higher plant height (68.02 cm) than in-furrow application (49.67 cm). The lowest plant height (51.21 cm) was estimated to be achieved by using 7.05% extract applied in furrow. There were nonsignificant differences in shoot fresh weight, with means ranging from 48.81 to 58.01 g. Similarly, interaction effects were not significant for shoot dry weight, but the main effects of dilution were significant, being explained by a linear model (Figure 4B).

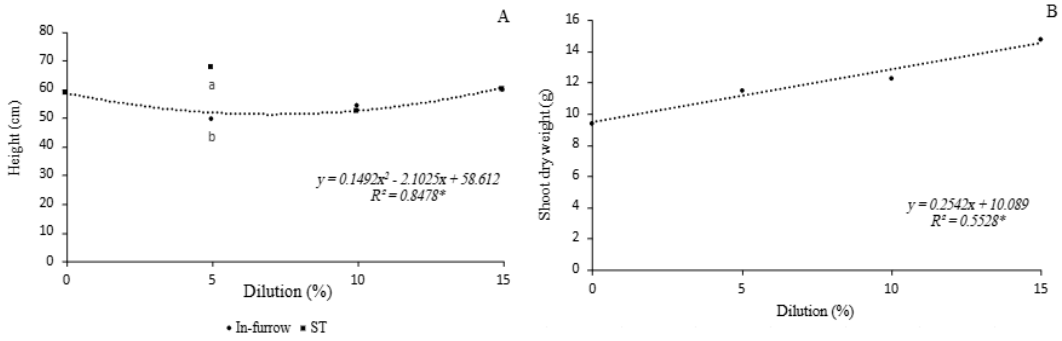

Figure 4. Plant height (A) and shoot dry weight (B) of soybean plants inoculated with *Pratylenchus brachyurus* and treated with different dilutions of canola seed cake extract by seed treatment or in-furrow application (Trial 2).

Factors exerted significant main effects on root weight in Trial 1. The highest mean was obtained in the seed treatment group (6.94 g), as compared with the in-furrow application group (5.07 g). The effect of extract dilution on root weight was explained by a linear model (Figure 5A). In Trial 2, the interaction effects were significant, but regression models did not provide a good fit to the experimental data. Extracts at 5% dilution provided an increase in root weight when used as seed treatment (14.50 g), as compared with in-furrow application (6.68 g) (Figure 5B).

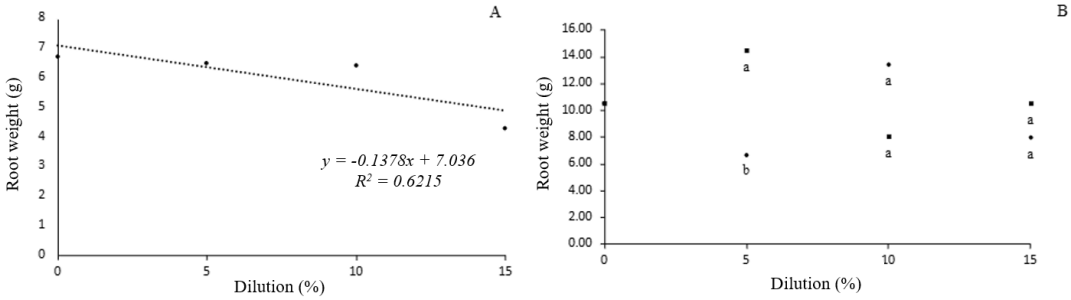

Figure 5. Root weight of soybean plants inoculated with *Pratylenchus brachyurus* and treated with different dilutions of canola seed cake extract by seed treatment, Trial 1 (A), Trial 2 (B).

Experiment 3: *Pratylenchus brachyurus* penetration in soybean treated with canola seed cake extract

Aqueous extracts of canola seed cake reduced *P. brachyurus* penetration into soybean roots when applied as seed treatment at 15% dilution. Nematode population density increased linearly with time in the untreated control. However, regression models did not fit well to data on treated plants. At 20 and 25 DAI, the nematode population density of treated plants (151 and 161 nematodes g^{-1} root, respectively) was lower than that of the control (324 and 349 nematodes g^{-1} root, respectively) (Figure 6A).

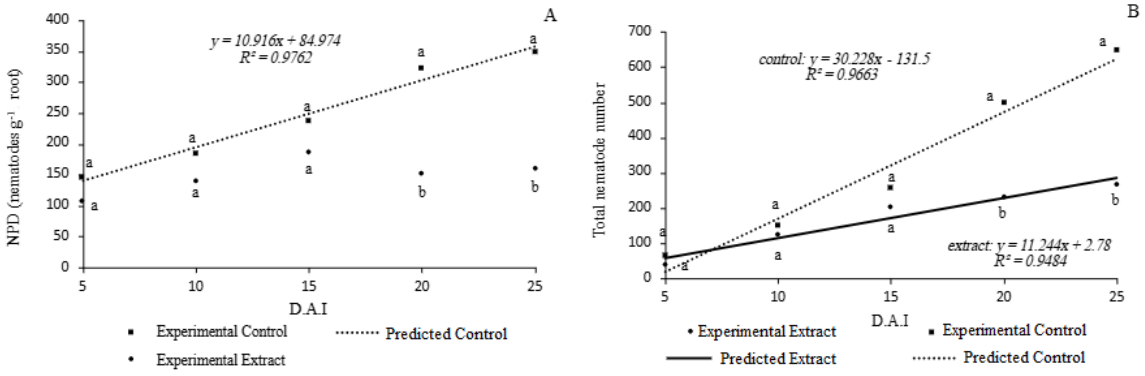

Figure 6. Nematode population density (A) and total nematode number (B) in roots of soybean treated or not (control) with 15% canola seed cake extract by seed treatment and evaluated at 5, 10, 15, 20, and 25 d after inoculation (DAI).

Similar findings were observed for total nematode number, but the regression fitted a linear model for both treatments (Figure 6B). Differences were observed at 20 and 25 DAI, with respective means of 232 and 264 nematodes in treated plants, compared with 495 and 646 nematodes in the control. Therefore, treatments afforded reductions of 46.87% and 40.87% in the number of nematodes that penetrated plants.

Root weight increased linearly with time (Figure 7), regardless of seed treatment. In other words, the interaction effect and the main effect of treatment were nonsignificant.

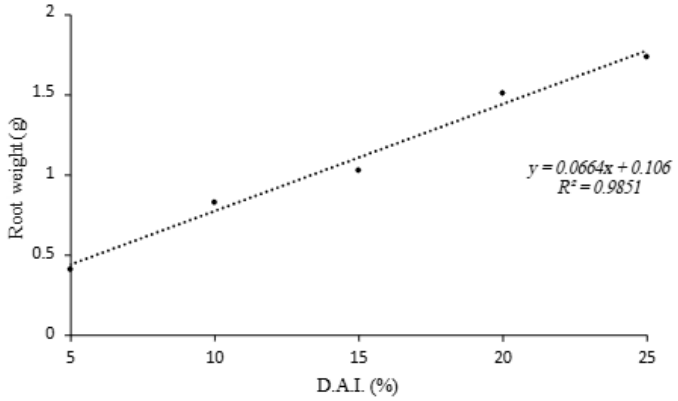

Figure 7. Root weight of soybean plants treated or not (control) with 15% canola seed cake extract by seed treatment and evaluated at 5, 10, 15, 20, and 25 d after inoculation (DAI) with *Pratylenchus brachyurus*.

Experiment 4: Phenolic characterization of canola seed cake extract by high-performance liquid chromatography

Phenolic compounds were among the major components of canola seed cake extract, in particular ferulic, *trans*-cinnamic, caffeic, coumaric, chlorogenic, and gallic acids, as well as the flavonoids kaempferol and quercetin (Table 1).

Table 1. Composition of canola seed cake extract, as determined by high-performance liquid chromatography. Values represent the mean ± standard deviation.

Compound	Concentration (mg 100 g^{-1} sample)
Ferulic acid	168.77 ± 2.60
trans-Cinnamic acid	95.51 ± 0.03
Caffeic acid	52.25 ± 0.25
Coumaric acid	28.91 ± 0.03
Chlorogenic acid	20.23 ± 0.31
Gallic acid	11.12 ± 0.19
Kaempferol	3.85 ± 0.08
Quercetin	1.93 ± 0.02

DISCUSSION

Brassica species have been widely studied for nematode management, with several reports highlighting the nematicidal and ovicidal effects of their extracts and derivatives (Tavares-Silva et al., 2017; Tarini et al., 2020). Canola extract, for instance, exhibited nematicidal and ovicidal activity against *Meloidogyne incognita* (Kuhn et al., 2015). These effects are commonly attributed to the presence of glucosinolates, compounds known for their nematicidal properties (Ntalli and Caboni, 2012). In vitro assays demonstrated that glucosinolates from *B. juncea* extract induced over 70% mortality in potato cyst nematode (*Globodera pallida*) (Ngala et al., 2015).

Here, canola seed cake treatments induced nematode mortality, regardless of the extraction method. Similar findings were reported for crambe cake extract, where the extraction method did not significantly influence nematicidal activity against *M. javanica* eggs or juveniles (Tarini et al., 2020). In vivo experiments confirmed the efficacy of canola extracts in nematode control. In a previous study, canola extract was found to successfully reduce *M. incognita* reproduction (Kuhn et al., 2015). Residues from other *Brassica* spp. have shown nematicidal potential, including sonicated mustard (*B. campestris* L.) seed cake extract, which caused the mortality, paralysis, and low infectivity of J2 in tomato plants (Nadeem et al., 2021). Crambe cake (Tavares-Silva et al., 2015) and its aqueous extract (Tarini et al., 2020) promoted the control of *M. javanica* and *P. brachyurus*.

In addition to the application of waste, such as seed cakes, *Brassica* cultivation has the potential to reduce nematode populations (Tavares-Silva et al., 2015). Glucosinolates in the soil, stemming from plant decomposition, are transformed into allyl isothiocyanate, organic cyanides, and other compounds via chemical and enzymatic reactions. These compounds are toxic toward nematode eggs and juveniles (Oliveira et al., 2011; D'Addabbo et al., 2020). *Brassica napus* cake was found to contain several glucosinolates after high-performance liquid chromatographic purification (Višnjevec et al., 2021). Biofumigation with *B. juncea* 'Terrafit' combined with allyl isothiocyanate reduced *Meloidogyne hapla* populations (Dahlin and Hallmann, 2020).

Secondary metabolites produced by *Brassica* spp. may exert phytotonic effects on soybean. A study revealed that the growth of some cultivars can be enhanced by canola extract, increasing shoot dry weight, root weight, and root length (Borella et al., 2017). Nematode-infected soybean plants treated with castor bean cake extract (Izidoro et al., 2021) and crambe cake extract (Tavares-Silva et al., 2015; Tarini et al., 2020) were found to have improved vegetative growth, possibly due to the nutrients present in extracts. Nevertheless, it is important to consider the potential phytotoxic effects of extracts derived from *Brassica* (Ferreira et al., 2018).

Canola extract was found to exert allelopathic effects on soybean, inhibiting germination and increasing the number of abnormal plants (Borella et al., 2017).

The effects observed in the current study may be attributed to the numerous secondary metabolites found in extracts, such as flavonoids, alkaloids, and saponins, which are commonly observed in Brassicaceae species, including canola. These compounds have been identified by chemical characterization studies and widely reported in the literature for their effectiveness in controlling plant-parasitic nematodes (Khan et al., 2020; Tarini et al., 2020; Hussain et al., 2022).

Phenolic acids, including those identified in the canola seed cake extract, are known to promote nematode control, inhibiting egg hatching and killing juveniles of *Meloidogyne* spp. (Ohri and Pannu, 2010; Aoudia et al., 2012). A study analyzing the nematicidal activity of gallic acid showed that it inhibits egg hatching in *M. incognita* by deforming and destroying the eggshell, thereby resulting in juvenile deformity and death (Nguyen et al., 2013). Another possible mechanism of action is via 4-coumaric acid-CoA, which plays a role in the activation of coumaric acid and other phenolic compounds. The enzyme is essential in the synthesis of lignin, which strengthens plants against nematodes, forming physical barriers that prevent their penetration (Wang et al., 2021). Variations in the phenolic composition of plant extracts may explain their different mechanisms of action against nematodes, which reduces the chance of resistance development by these parasites (Aoudia et al., 2012).

Flavonoids also contribute to parasite control by repelling nematodes and affecting their fertility and respiration (Chin et al., 2018). Studies have shown that flavonoids reduced the number of *M. javanica* galls and egg masses (Aoudia et al., 2012). Quercetin and kaempferol were associated with mobility reduction in *M. incognita* juveniles via the oxidation of individuals (Wuyts et al., 2006).

The results of this study are consistent with numerous reports in the literature highlighting the nematicidal potential of the compounds identified here, as well as other bioactive substances found in *Brassica* species. These findings underscore the potential of organic residues as sustainable, eco-friendly, and cost-effective alternatives for nematode management. Future research should aim to elucidate the mechanisms of action of the identified compounds and screen other plant species for bioactive molecules with nematicidal potential, supporting the development of phytochemical-based nematicides.

CONCLUSIONS

The results of this study indicate that canola seed cake extract is a promising alternative for the management of *Pratylenchus brachyurus*. In addition to its nematicidal efficiency and the presence of phenolic acids and flavonoids as major bioactive compounds, the seed cake, as an agricultural by-product, offers a sustainable and cost-effective solution. By reducing reliance on synthetic pesticides, this approach supports integrated nematode management strategies and promotes the development of safer and ecologically balanced agricultural practices.

Author contributions

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