RESEARCH/INVESTIGACIÓN

AQUEOUS EXTRACT OF CASTOR BEAN SEED CAKE FOR THE CONTROL OF *PRATYLENCHUS BRACHYURUS* IN SOYBEAN

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ABSTRACT

Izidoro, Jr., A., E. J. Silva, G. Tarini, J. C. Bordin, B. A. Silva, L. Ambrosano, and C. R. Dias-Arieira. 2021. Aqueous extract of castor bean seed cake for the control of *Pratylenchus brachyurus* in soybean. Nematropica 51:1-8.

Agroindustrial wastes are sources of compounds with nematicidal potential. However, such wastes are generally only available in small quantities, which may impair commercial application. Preparing aqueous extracts of these materials may provide products with nematicidal activity. This study assessed the aqueous extract of castor bean (*Ricinus communis*) seed cake to control *Pratylenchus brachyurus* in soybean. A 10% (w/v) castor bean cake aqueous extract was prepared and diluted to 5, 10, 15, and 20% in distilled water. The negative control was 0% (without extract). First, the nematicidal activity of the extract was assessed *in vitro*. Then, the extract was applied to soil to evaluate effects on *P. brachyurus* reproduction and soybean growth. The chemical composition of the cake and the total phenolic content of the undiluted extract were also determined. Aqueous extract of castor bean seed cake at dilutions close to 20% resulted in *P. brachyurus* mortality *in vitro* of 65% and reduced *P. brachyurus* reproduction *in vivo* by approximately 60% compared to the control. A 40% increase in soybean shoot dry weight was observed with extract application. The cake proved to be rich in nutrients, and the total phenolic content of the aqueous extract was 1,078.6 mg gallic acid equivalents/100 g. Identification of nematicidal compounds in castor bean seed cake aqueous extract may guide the development of novel nematicidal products.

Key words: alternative control, bioextract, *Ricinus communis*, root lesion nematode

RESUMO

Izidoro, Jr., A., E. J. Silva, G. Tarini, J. C. Bordin, B. A. Silva, L. Ambrosano, and C. R. Dias-Arieira. 2021. Extrato aquoso de torta de mamona para o controle de *Pratylenchus brachyurus* em soja. Nematropica 51:1-8.

Resíduos da agroindústria são ricos em compostos com potencial nematicida. Porém, geralmente, estão disponíveis em quantidades reduzidas, que podem inviabilizar o uso comercial. É possível que extratos diluídos destes materiais mantenham atividade nematicida. Assim, avaliou-se o extrato aquoso de torta de mamona no controle *in vitro* e *in vivo* de *Pratylenchus brachyurus*, bem como o teor de compostos fenólicos e a composição química do resíduo. Para isso, preparou-se o extrato aquoso bruto de torta a 10% (m:v), o qual foi posteriormente diluído a 5, 10, 15 e 20% em água destilada. Tratamento sem extrato foi usado como testemunha (0%). As doses foram testadas sobre a mortalidade do nematoide *in vitro* e a reprodução em soja, em casa-de-vegetação, quando aplicado por irrigação, bem como o efeito no crescimento vegetal.

Determinou-se a composição química da torta e o teor compostos fenólicos totais do extrato bruto. O extrato aquoso na diluição próxima a 20% promoveu mortalidade de 65% dos nematoides *in vitro* e reduziu em aproximadamente 60% a reprodução de *P. brachyurus* quando comparado à testemunha. Houve incremento próximo a 40% na massa seca de parte aérea. A torta mostrou ser rica em nutrientes e o extrato apresentou 1078,56 mg equivalente de ácido gálico/100 g. A identificação de compostos nematicidas no extrato da torta de mamona, poderá auxiliar no desenvolvimento de novos nematicidas para uso comercial.

Palavras-chave: controle alternativo, bioextratos, *Ricinus communis*, nematoide das lesões radiculares

INTRODUCTION

Plant-parasitic nematodes cause substantial losses to agricultural production worldwide. In tropical regions, the root-knot nematodes *Meloidogyne incognita* and *M. javanica* and the lesion nematode *Pratylenchus brachyurus* are major yield-limiting factors in soybean production (Favoreto *et al*., 2019). These parasites have wide geographical distribution, great damage potential, and control options are limited making it difficult to maintain crop yields in nematode-infested fields.

Nematode control strategies that are effective and also environmentally friendly are needed to reduce the environmental damage caused by excessive use of pesticides. The use of agroindustrial waste has shown potential for the control of plant-parasitic nematodes in several crops (Baldin *et al*., 2012; Roldi *et al*., 2013; Dias-Arieira *et al*., 2015; Ferreira *et al*., 2018a; Brito *et al*., 2020). However, the low availability and requirements for large amounts of waste may, in some cases, limit the economic viability of this control strategy.

Castor bean seed cake, a byproduct of castor bean (*Ricinus communis*) oil extraction, has nematicidal activity (Gardiano *et al*., 2009; Devi *et al*., 2019; Pedroso *et al*., 2019;); however, availability of this byproduct is limited. Such limitation may be overcome by obtaining aqueous extracts of castor bean seed cake, with the water-soluble substances potentially maintaining nematicidal activity, even when diluted. Aqueous extracts from different parts of castor bean, including leaves, branches, and seeds were effective in reducing *M. incognita* population densities in carrot (*Daucus carota*) (Baldin *et al*., 2012).

The main nematicidal compounds in castor bean seed cake are ricin, ricinine, and agglutinin, which are released during decomposition of plant material, and are soluble in water (Annongu and Joseph, 2008; Melo and Serra, 2019). We hypothesized that an aqueous extract of castor bean seed cake has nematicidal activity against *P. brachyurus*. This research assessed the effects of castor bean seed cake aqueous extract on *P. brachyurus* mortality *in vitro* and reproduction on soybean. An additional aim was to determine the total phenolic content of the aqueous extract.

MATERIALS AND METHODS

Castor bean seed cake was obtained from the Biodiesel Laboratory of the Federal University of Lavras (Lavras, Brazil), and was stored in a refrigerator at 6-10°C until use. A crude aqueous extract was prepared by mixing 10 g of castor bean seed cake in 100 ml of distilled water at 25°C. After 24 hr of incubation, the solution was filtered through sterile gauze and diluted in distilled water to 5, 10, 15, and 20% of the extract. The negative control was 0% (without extract).

The *in vitro* test was performed under laboratory conditions in a completely randomized design. Castor bean aqueous extract dilutions were replicated five times, and the experiment was conducted twice. Each experimental unit consisted of a Falcon tube containing 4 ml of each dilution and 1 ml of a nematode suspension containing 100 *P. brachyurus*/ml. *Pratylenchus brachyurus* was maintained on soybean cv. Pintado under greenhouse conditions. To obtain nematodes for experiments, *P. brachyurus* were extracted from soybean roots according to the method of Coolen and D'Herde (1972) and incubated on a Baermann funnel for 48 hr, after which viable nematodes were collected and counted. Tubes containing nematodes and treatments were incubated in the dark in a biochemical oxygen demand incubator at 27°C for 24 hr. The number of live and dead *P. brachyurus* was determined using a Peter's chamber under a light microscope. Individuals that remained immobile after exposure to a 10% (v/v) NaOH solution (1 M) were considered dead. Results are expressed as percentage mortality.

The greenhouse experiment (23°78'91.17"S 53°25'85.12"W, 401 m elevation) was arranged in completely randomized design, with five treatments (dilutions) and five replications. The experiment was conducted twice. Trial 1 was conducted from December 2018 to February 2019, with average minimum and maximum temperatures equal to 23 and 31°C, respectively. Trial 2 was conducted from October to December 2019 with average minimum and maximum temperatures of 22 and 29°C, respectively. Polystyrene pots were filled with 500 cm^3 of a 2:1 mixture of soil and sand (autoclaved at 120°C for 2 hr). Two soybean cv. M6410 IPRO seeds were sown per pot and 5 days later seedlings were thinned to one per pot. At this time, plants were inoculated with *P. brachyurus*, obtained as described above, by applying a 1 ml suspension containing 500 nematodes into two holes in the soil near the plant. Immediately after inoculation, the holes were closed, and 10 ml of castor bean cake extract was applied to the soil by irrigation, using the same doses cited for the *in vitro* experiment. Each pot was considered an experimental unit.

At 85 days after inoculation, plants were harvested and separated into shoots and roots. The roots were thoroughly washed, blotted dry on paper towels, and weighed to obtain the root fresh weight (g). Root length (cm), shoot height (cm), and shoot fresh and dry weights (g) were also determined. For dry weight determination, shoots were dried in an air-circulating oven at 65°C for 72 hr. *Pratylenchus brachyurus* were extracted from fresh roots (Coolen and D'Herde, 1972) and counted using a Peter's chamber under a light microscope. The total nematode number was determined and divided by the root fresh weight to obtain *P. brachyurus* population density (nematodes per gram fresh root).

The total phenolic content of the aqueous extract of castor bean seed cake was assessed on three samples using the method proposed by Chen *et al*. (2015), with modifications. Aliquots (0.5 ml) of the crude extract were mixed with 2.5 ml of 10% Folin-Ciocalteau reagent and 2.0 ml of 7.5% sodium carbonate. The mixture was incubated in a water bath at 50°C for 5 min under stirring and subsequently cooled in a water bath at room temperature. Absorbance was read at 760 nm using a spectrophotometer (UV 5200s, Global Trade Technology). Total phenolic content was determined against a standard curve of gallic acid, and results are expressed as mg of gallic acid equivalents (GAE)/100 g sample. The chemical composition of castor bean seed cake (50 g) was determined by a specialized laboratory (Acqua Sollus Laboratory, Campo Mourão, Paraná, Brazil).

In vitro and *in vivo* data were subjected to analysis of variance at the 5% significance level. When significant, means were assessed by linear and quadratic polynomial regression analyses at the 5% level. Statistical analyses were performed using Sisvar version 5.3 (Ferreira, 2011).

RESULTS AND DISCUSSION

Mortality of *P. brachyurus* exposed to castor bean seed cake aqueous extract increased in a quadratic dose-dependent manner in both *in vitro* trials (Fig. 1). In Trial 1, nematode mortality ranged from 8.1 to 66.5% using extracts at dilutions ranging from 0 to 20%, respectively. Regression analysis showed that the maximum efficiency was obtained using the aqueous extract at a dilution of 22.2%, higher than that evaluated in the study (Fig. 1A). In Trial 2, the highest percentage mortality (40%) was achieved using 20% extract (Fig. 1B). Studies on the nematicidal activity of castor bean seed cake aqueous extract are scarce; however, extracts obtained from different parts of castor bean have the potential for nematode control. Extracts of shoots and leaves reduced *M. javanica* and *M. incognita* egg hatch (Bharadwai and Sharma, 2007; Gardiano *et al*., 2009; Ferreira *et al*., 2018b), whereas extracts of castor seeds increased mortality of *M. incognita* second-stage juveniles (J2) (Baldin *et al*., 2012). The nematicidal activity of castor bean has been attributed to the presence of nematicidal compounds, such as ricin, ricinine, albumin, and alkaloids (Oliveira *et al*., 2005; Carboni and Mazzonetto, 2013).

Although extracts from different parts of the castor bean plant had nematicidal activity, their chemical compositions are likely distinct. Castor bean seed cake was used in the present study because it is a byproduct of the biofuel industry, which is different from other oilseed cakes because it cannot be used for animal feed. This is due to castor bean seeds having high concentrations of

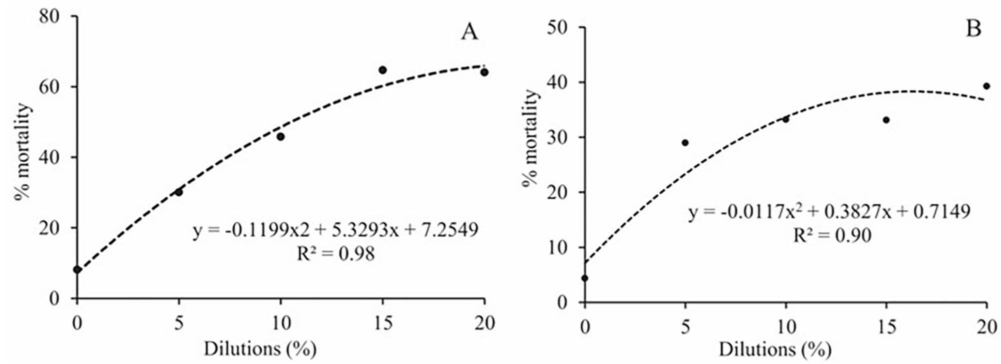

Figure 1. Mortality of *Pratylenchus brachyurus* after 24 hr of exposure to an aqueous extract of castor bean seed cake in laboratory assays. A: Trial 1; B: Trial 2

toxic substances, such as toxalbumin, a highly toxic glycoprotein (2% of seed weight) in the endosperm (Cook *et al*., 2006; Alexander, 2008). Castor bean seeds are also rich in agglutinin, proteins capable of irreversibly inactivating ribosomes, causing cell death (Olsnes, 2004; Dutra *et al*., 2006; Sousa *et al*., 2017). In fact, these compounds are probably associated with the nematicidal activity of the byproduct.

In the greenhouse experiment, total number of *P. brachyurus* in roots decreased linearly with the increasing extract dilutions in both trials (Fig. 2A, C). Soil application of 20% extract resulted in a 62 and 55% reduction in total *P. brachyurus* compared to the control in Trial 1 and 2, respectively. Similarly, in Trial 1, nematode population densities ranged from 224 to 110 nematodes/g root with application of 0 to 20% extract, respectively (Fig. 2B), while in Trial 2, reductions reached 51% (Fig. 2D).

These results corroborate other research where applications of 600, 1200, and 1800 kg/ha castor bean cake suppressed *Meloidogyne* spp. and *Pratylenchus* spp. reproduction on sugarcane (Dinardo-Miranda and Fracasso, 2010). A similar effect was observed on *Meloidogyne exigua* parasitizing coffee when densities of this nematode were significantly reduced in the roots and soil of plots treated with castor bean cake at a dose of 1000 kg/ha (Dutra et al., 2006). It is worth noting that the waste was applied directly to the soil in the referred studies, a strategy that may not be economically feasible under field conditions.

In both greenhouse trials, extracts did not influence shoot height, shoot fresh weight, root fresh weight, or root length (Table 1). However, shoot dry weight increased with higher application rates (Fig. 3A, B). In Trial 1, weights ranged from 2.54 to 3.55 g, and regression analysis showed that the largest increase (39.8%) was obtained using 19.4% extract (Fig. 3A). In Trial 2, an increase of 35.1% in shoot dry weight was achieved using 16.3% extract (Fig. 3B). Although extract application did not enhance vegetative development, no phytotoxic effects were observed on soybean. Castor bean seed cake was shown to compromise the vegetative growth of lettuce (*Lactuca sativa*) (Borges *et al*., 2007) and cowpea (*Vigna unguiculate*) (Silva *et al*., 2011), when the waste was applied directly to the soil.

Nutrient analysis of the castor bean seed cake was 49.3 g/kg N, 6.0 g/kg P, 12.6 g/kg K, 12.9 g/kg Ca, 5.0 g/kg Mg, 4.8 g/kg S, 70.0 mg/kg Zn, 63.4 mg/kg Fe, 45.6 mg/kg Mn, 14.5 mg/kg B, and 8.6 mg/kg Cu. Carbon and OM levels were 55.3% and 95.0%, respectively. The pH was 5.5, and the C:N ratio was 11:1. The macro- and micronutrient contents agreed with previous data (Silva *et al*., 2012), and were similar to those of other agricultural residues with nematicidal activity (Pasos *et al*., 2014; Ferreira *et al*., 2018a; Brito *et al*., 2020). Similarly, C and OM levels as well as pH were close to the average of other organic waste (Ferreira *et al*., 2018a; Brito *et al*., 2020). Thus, despite the dilutions of the cake, it is possible that the extract retained part of the nutrient profile, which may not have affected soybean development, but may have influenced the plant defense system, since nutrients are important regulators of metabolic pathways involved in defense responses (Oka, 2010; Marschner, 2012; Santana-Gomes *et al*., 2013; Zörb *et al*., 2013;

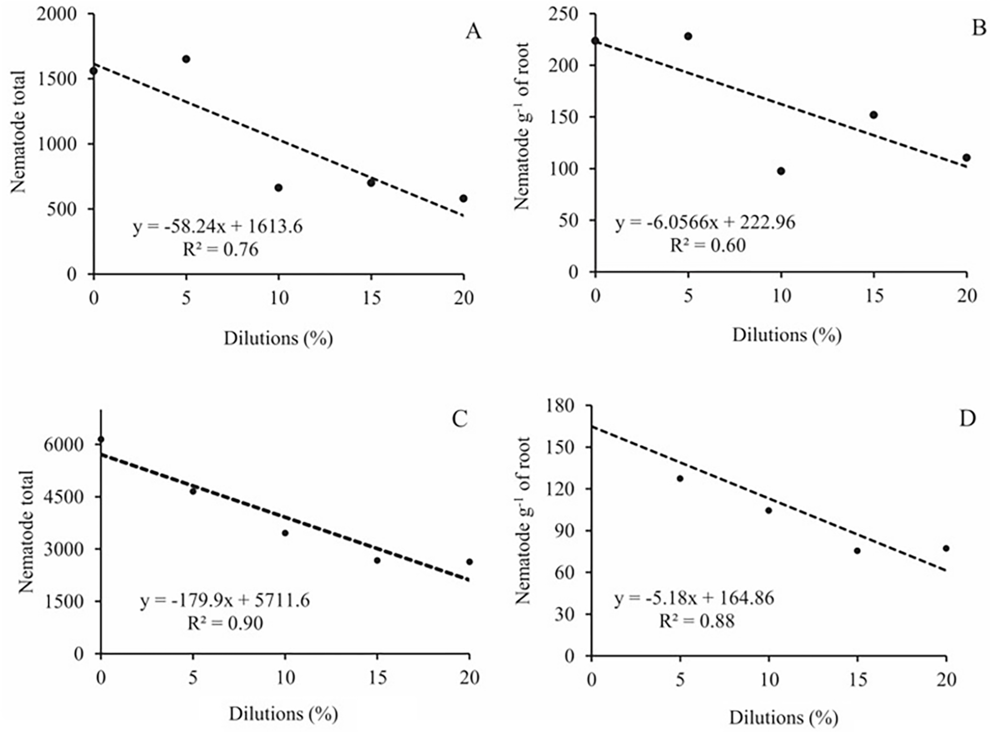

Figure 2. Total numbers (A and C) and population densities (B and D) of *Pratylenchus brachyurus* in soybean roots 85 days after nematode inoculation and soil treatment with an aqueous extract of castor bean seed cake. A and B: Trial 1; C and D: Trial 2

Hafsi *et al*., 2014; Molinari, 2016). However, this hypothesis needs to be studied in the future.

In addition to the possible nutritional effect, castor bean seed cake aqueous extract had high phenolic content, equivalent to 1078.6 mg GAE/100 g or 10.8 mg GAE/g. Phenolic composition was lower than that of methanolic extracts of another Euphorbiaceae, *Euphorbia tirucalli* which contained 65.7 to 206.2 mg GAE/g (Basma *et al*., 2011), but similar to that of wine industry residues (13.5 mg GAE/g) (Reiner *et al*., 2016). Gallic acid, the phenolic compound analyzed in this study, was shown to exert ovicidal and nematicidal effects on *M. javanica* and *M. incognita* (Nguyen *et al*., 2013; Reiner *et al*., 2016). *Meloidogyne incognita* eggs exposed to gallic acid (20.3 to 95.8 mg GAE/mL) from *Terminalia nigrovenulosa* were deformed or the egg walls were destroyed, leading to juvenile malformation and death (Nguyen *et al*., 2013).

Phenolics are a complex group of compounds with nematicidal effects, including flavonoids, isoflavones, tannins, cinnamic acid, caffeic acid, and phenylpropanoids (Ntalli *et al*., 2009; Ohri and Pannu, 2010; Nguyen *et al*., 2013). They also play a crucial role in the hypersensitivity responses of plants to pathogens, as evidenced by their accumulation in feeding sites of *Meloidogyne* spp. (Oliveira *et al*., 2019).

Although castor bean seed cake is well-known for its ability to control nematodes, this is one of the first studies to assess the activity of the aqueous extract for *P. brachyurus* control. The effectiveness was probably associated with its high phenolic content and nutrient composition. Castor bean seed cake, although derived from an oilseed, contains water-soluble substances with nematicidal effects. This finding opens the possibility for further research aimed at identifying natural compounds with nematicidal activity from this waste.

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Table 1. Plant height, fresh weight of aerial part (FWAP), fresh weight (FWR), and length (RL) of roots of soybean 85 days after inoculation with *Pratylenchus brachyurus* and application of an aqueous extract of castor bean seed cake at different dilutions.

	Trial 1				Trial 2			
Dilutions (%)	Height (cm)	FWAP (g)	FWR (g)	RL (cm)	Height (cm)	FWAP (g)	FWR (g)	RL (cm)
0	43.41^{ns}	8.22^{ns}	6.61^{ns}	18.60^{ns}	34.4^{ns}	9.00^{ns}	2.14^{ns}	2.14^{ns}
5	44.10	9.04	7.28	18.43	37.6	10.90	2.39	2.39
10	44.23	8.07	7.38	17.85	34.8	14.52	2.71	2.71
15	37.62	5.86	5.34	19.44	35.9	13.73	2.54	2.54
20	41.21	7.68	5.54	18.12	34.4	10.50	2.46	2.46
CV (%)	10.10	21.31	25.23	10.61	15.66	42.60	9.41	9.41

ns = not significant. CV = coefficient of variation.

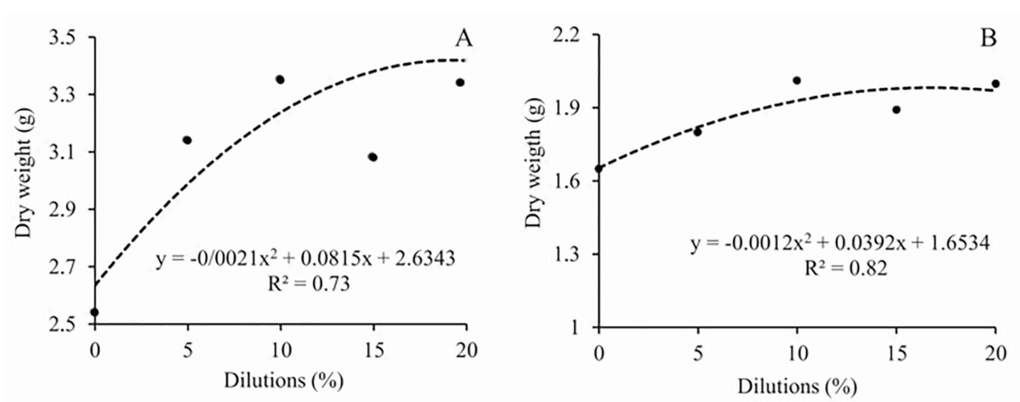

Figure 3. Shoot dry weights (g) of soybean 85 days after *Pratylenchus brachyurus* inoculation and soil treatment with an aqueous extract of castor bean seed cake. A: Trial 1; B: Trial 2

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