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Extrato de nim como estratégia para o controle de *Meloidogyne javanica* em plantas de tomate

Neem extracts as a strategy for the control of Meloidogyne javanica on tomato plants

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RESUMO: Os extratos de nim (*Azadirachta indica*) são comumente usados na agricultura por suas propriedades pesticidas e potenciais benefícios no manejo de pragas e proteção de culturas. Para avaliar o uso de dois extratos à base de nim no controle de *Meloidogyne javanica* em tomateiro, foram realizados experimentos com ovos e juvenis de segundo estádio (J_2) de *M. javanica* para estimar a concentração efetiva de 50% (CE_{50}) nas concentrações de 0,0. % a 0,75% (v v^{-1}) e 0,0% a 20,0% (v v^{-1}) de extrato de nim, respectivamente. As plantas de tomateiro foram tratadas com concentrações de 0,0% a 3,00% dos extratos de nim para avaliar a penetração dos J_2 de *M. javanica*. Os extratos foram aplicados em tomateiros infestados com ovos de *M. javanica* para comparar com os nematicidas químicos carbofuran e abamectina. Os valores estimados de CE_{50} para o Extrato 1 e Extrato 2 foram de 0,44% e 0,40% para a eclosão de J_2 e 13,9% e 19,3% para a mortalidade de J_2, respectivamente. As aplicações dos extratos em tomateiros infestados com J_2 de *M. javanica* não tiveram efeitos significativos na altura e na matéria seca dos tomateiros, mas resultaram em redução significativa no fator de reprodução de *M. javanica*. O tratamento com concentração de 1,00% de Extrato de nim reduziu o fator de reprodução de *M. javanica*, assim como o tratamento com abamectina. Os extratos de nim têm efeito nematicida sobre ovos e J_2 e podem suprimir a população de *M. javanica* em tomateiros. Extratos de nim têm potencial para controlar *M. javanica*.

Palavras-chave: *Azadirachta indica*; Concentração efetiva; Dose-resposta; Nematoide das galhas.

ABSTRACT: Neem (*Azadirachta indica*) extracts are commonly used in agriculture for their pesticidal properties and potential benefits in pest management and crop protection. To evaluate the use of two neem-based extracts to control *M. Meloidogyne javanica* in tomato plants, experiments were conducted with eggs and second-stage juveniles (J_2) of *M. javanica* to estimate the 50% effective concentration (EC_{50}) at concentrations of 0.0% to 0.75% (v v^{-1}) and 0.0% to 20.0% (v v^{-1}) of neem extracts, respectively. Tomato plants were treated with concentrations from 0.0% to 3.00% of the neem extracts to assess the penetration of *M. javanica* J_2. The extracts were applied in tomato plants infested with *M. javanica* eggs for comparison to carbofuran and abamectin. The estimated EC_{50} values for Extract 1 and Extract 2 were 0.44% and 0.40% for the hatching of J_2 and 13.9% and 19.3% for the mortality of J_2, respectively. The applications of the extracts on tomato plants infested with *M. javanica* J_2 had no significant effects on the height and dry matter of tomato plants but resulted in a significant reduction on the reproduction factor of *M. javanica*. The treatment with a concentration of 1.00% of neem extracts reduced the reproduction factor of *M. javanica*, as did the treatment with abamectin. Neem extracts have a nematicidal effect on eggs and second-stage juveniles of *M. javanica* and can suppress the population of *M. javanica* in tomato plants. Neem extracts have potential for controlling *M. javanica*.

Keywords: *Azadirachta indica*; Dose-response; Effective concentration; Root-knot nematode.

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1 INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a very popular fruit worldwide. In 2022, its world production was 186.1 million tonnes, cultivated on 4.9 million hectares (FAO, 2024). In Brazil, the 2022 annual production was estimated at 3.8 million tonnes, distributed on 54.5 thousand hectares (Food and Agriculture Organization, 2024). Therefore, it is essential to avoid tomato productivity losses caused by pests and diseases, especially considering the high economic value of this crop.

Nematodes are one of the causes of the major losses in susceptible tomato plants, decreasing up to 77% of the crop's yield due to *Meloidogyne* spp. Goeldi (Fullana *et al.*, 2024) parasitism. This genus, popularly known as root-knot nematode, is possibly the most important plant parasitic nematode worldwide (Mesa-Valle *et al.*, 2020). In Brazil, 18 *Meloidogyne* species have been detected, but the species *Meloidogyne javanica* (Treub) Chitwood and *Meloidogyne incognita* (Kofoid and White) Chitwood were found to be the prevalent parasitize tomato plants (Gabriel *et al.*, 2020).

Nematode management is difficult and requires the integration of several control methods. Chemical control is often employed, however, there is a limited availability of nematicides in the Brazilian market due to the prohibition of several previously utilized products. For instance, methyl bromide, banned under the Montreal Protocol, and carbofuran are no longer permitted. Currently, for the control of *Meloidogyne* spp. on tomato plants, the use of fluensulfone, metam-sodium, abamectin, and garlic extract is allowed (Brazil, 2024). The restrictions on the use of some chemicals and the need to reduce the use of pesticides due to their toxicity have favored the market of biological and alternative products for nematode control.

Alternative nematicides obtained from plants and microorganisms are less aggressive to the environment and to human health (Oka, 2010). Toxins produced by several plant species influence *Meloidogyne* spp. neem (*Azadirachta indica* A. Juss) produces secondary metabolites used for pest control and stands out for the presence of azadirachtin (Mwamula; Kabir; Lee, 2022). This substance is abundantly present in the oil extracted from neem seeds and presents an insecticidal effect (D' Errico *et al.*, 2023).

The popularization of neem oil has favored the emergence of commercial neem crops for oil production. The cold pressing of the seeds generates by-products such as the neem cake, whose application to soil has the potential to control nematodes (Javed; Abdullah; Fayyaz, 2007, Javed *et al.*, 2007; 2008; Oka, 2010). Furthermore, commercial tree management produces another by-product that results from pruning: leaves and branches. In this by-product can find nimbin, 22,23-Dihydronimocinol and quercitin on leaf, and, nimbinin, salannin and gallic acid on bark (Adusei; Azupio, 2022).

Further studies are warranted to elucidate the potential of compounds inherent in the by-products derived from neem oil production, thus paving the way for novel market opportunities and alternative strategies in nematode management. Therefore, this study aimed to evaluate the use of two extracts neem-based for controlling *M. javanica* in tomato plants.

2 MATERIAL AND METHODS

2.2 NEEM EXTRACTS

The neem plants used in the extract production were grown at the OpeNeem For Life S.A. farm (OpeNeem For Life S.A. (São Paulo, SP, Brazil), located in the town of São João de Pirabas, State of Pará, Brazil. Extract 1 was produced using the hydroalcoholic extract of neem leaves and branches. Extract 2

consists of 90% of hydro-alcoholic extract of neem leaves and branches, 5% of orange oil (d-limonene) and 5% of adjuvants.

2.3 OBTAINING THE INOCULUM OF NEMATODES

The population of *M. javanica* was obtained from tomato roots grown at Canguiri Farm, a property owned by the Federal University of Paraná and located in the city of Pinhais, State of Paraná, Brazil. Species identification was performed using the perineal pattern of adult females and the isoenzyme electrophoresis pattern (Carneiro; Almeida; Quénéhervé, 2000). The nematodes were multiplied in tomato plants of the cultivar 'Santa Clara', which were maintained in a greenhouse (room temperature ranging from 8 °C to 36 °C) and in a growth chamber with a temperature of 28 ± 3 °C and relative humidity of 80% ± 20% (InstalaFrio, Curitiba). The nematodes used as inoculum were extracted by the centrifugal flotation in sucrose with kaolin technique (Hahn *et al*., 2019).

Infected roots were mixed with water and crushed in a blender for 15 seconds. The suspension of roots and nematodes was separated by a set of 60 Mesh and 500 Mesh sieves, in that order. The suspension retained by the 500 Mesh sieve was centrifuged with kaolin at 530 g (Eppendorff, Hamburg, Germany) for 4 minutes.

The supernatant was discarded, and the compact mass of roots, kaolin, and nematodes was resuspended with sucrose solution (453.6 g of refined sugar and water to complete 1 L) and centrifuged again at 530 x g for 1 minute. The sucrose suspension containing the nematodes was poured over a set of 60 Mesh, 200 Mesh and 500 Mesh sieves and washed in running water. The suspension retained by the 500 Mesh sieve was composed mainly of eggs with occasional juveniles (J_2) and was used in the hatching tests. Simultaneously, it was subjected to an aerated hatching chamber to obtain J_2 of 24 h to 48 h lifetime (Hahn *et al*., 2019). The J_2 obtained were used in the mortality and root penetration tests on tomato plants.

2.3 HATCHING AND MORTALITY IN VITRO EXPERIMENTS OF MELOIDOGYNE JAVANICA J_2

To identify concentrations with nematicidal effect, Extract 1 and 2 were analysed in *in vitro* tests to estimate the 50% effective concentration (EC_{50}) of hatching (hatching tests) and mortality (mortality tests) of *M. javanica* J_2. Four experiments were conducted: two for the hatching tests (one for each extract), following a randomized block design with seven concentrations and three replications; and two for the mortality tests (one for each extract), following a randomized block design with eight concentrations and three replications. For the hatching tests, the concentrations of Extracts 1 and 2 used were 0.0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.75% (v v^{-1}). As for the mortality tests, the concentrations used were 0.0%, 0.5%, 1.0%, 2.5%, 5.0%, 10.0% 15.0%, and 20.0% (v v^{-1}). The experiments were conducted in 96-well flat-bottom ELISA plates, with each replication being performed in one well. In each plate well, 20 μL of nematode suspension (approximately 200 eggs for the hatching tests and 50 second-stage juveniles for the mortality tests) was deposited. In addition, 80 μL of streptomycin sulfate (0.5 mL L^{-1}, Dinâmica, Indaiatuba) with polysorbate 20 (0.5 mL L^{-1}, Tween 20®, Vetec, Rio de Janeiro) and 100 μL of the neem extracts were deposited in each well. The ELISA plates were sealed with adhesive plastic film (Politac, Polifix, Terra Preta) and incubated in the dark at 28 ± 2 °C.

The hatching of *M. javanica* J_2 was evaluated under a light microscope at 40 × magnification (Zeiss) after 16 days of incubation. Hatched J_2 and remaining eggs were quantified, and the hatching rate was calculated using Equation 1:

$$Hatching\ (\%) = \left(\frac{J_2}{J_2 + \ eggs}\right) \times 100 \quad (1)$$

The mortality experiments were evaluated after 48 hours of incubation. In each sample of the mortality test, 20 μL of NaOH solution (1 mol L^{-1}) was added. After 1 minute, the dead J_2 count was performed under a light microscope at 40 × magnification (Zeiss). The J_2 that remained straight after the addition of NaOH were considered dead (Hahn *et al.*, 2019). The mortality rates were calculated using Equation 2:

$$Mortality\ (\%) = \left(\frac{J_2\ dead}{alive\ J_2\ +\ dead\ J_2}\right) \times 100 \quad (2)$$

The experiments were repeated once.

2.4 EXPERIMENTS ON PENETRATION AND DEVELOPMENT OF MELOIDOGYNE JAVANICA J2 IN TOMATO ROOTS

To identify effective concentrations against the penetration of *M. javanica* J_2 in tomato roots that did not cause severe damage to the plants, two experiments were conducted, one using the Extract 1 and the other using Extract 2. Each experiment followed a randomized block design with five concentrations and three replications. The penetration experiments were carried out in seedling cultivation tubes of 150 mL capacity. The concentrations used for the penetration tests were 0.00%, 0.25%, 0.75%, 1.50%, and 3.00% (v v^{-1} of substrate). A previous experiment in which bare-root tomato seedlings were immersed in different concentrations of water-diluted extracts and incubated for 24 hours indicated the occurrence of plant lesions at concentrations higher than 3.00%.

The substrate used for the experiments consisted of red latosol (Oxisol) (Santos *et al*., 2018) and sand (1:1 v v^{-1}). Soil fertility was adjusted by adding 5.21 g dm^{-3} of dolomitic limestone, 0.17 g dm-3 of urea, 0.35 g dm^{-3} of KCl, and 2.78 g dm^{-3} of single superphosphate. The substrate was autoclaved at 120 °C for one hour. The concentrations were obtained by diluting the extracts (0 μL, 375 μL, 1125 μL, 2250 μL, and 4500 μL of product) in water to reach a total of 25 mL of liquid, which was applied on the substrate surface of each tube using a beaker. Tomato seedlings of the cultivar 'Santa Clara' with 30 days post-emergence and grown in a 128-cell polyethylene tray (Nutriplan, Cascavel) containing commercial substrate (MecPlant, Telêmaco Borba) were transplanted to the tubes. Subsequently, a 3 cm deep cavity was opened beside the root crown using a 2 mL conical microtube. In the cavity, 100 μL of suspension containing approximately 100 J_2 of *M. javanica* (Initial Population, IP) was deposited. The cultivation tubes were placed inside polystyrene cups (sundae model, Copaza, Içara), which were, in turn, positioned into support grids. The seedlings were maintained in a growth chamber at 25 ± 3 °C, with relative humidity of 80% ± 20%, and under a 12-hour photoperiod. Watering was done using the polystyrene cups and according to the need.

Plants were evaluated 30 days after inoculation. Initially, plant height and aerial parts' dry matter were determined using a forced-air oven at 60 °C for 72 h. The nematodes were extracted from the root system using the centrifugal flotation in sucrose with kaolin technique (Hahn *et al.,* 2019). After nematode extraction, the crushed roots retained by the 60 Mesh sieve were collected and dried to determine the plant's total dry matter. The final population (FP) was estimated based on the number of nematodes present in the roots and the reproduction factor (RF) was calculated by Equation 3:

$$RF = \frac{FP}{IP} \quad (3)$$

Due to plant death at a 3.00% concentration of the extracts, the experiments were repeated with the following intermediate concentrations: 0.0%, 0.5%, 1.0%, 2.0%, and 2.5% (v v^{-1} of substrate).

2.5 EXPERIMENT ON THE REPRODUCTION DEVELOPMENT OF *MELOIDOGYNE JAVANICA* IN TOMATO PLANTS

Since the 1.0% concentration showed satisfactory results for both extracts in penetration tests, it was used in a randomized block experiment with five treatments and four replications for comparison with the antiparasitics abamectin and carbofuran. The experiments were carried out in aluminum pots of 2 L capacity and evaluated the following treatments: Extract 1 and 2 (1.0% v v^{-1} or 20 mL per pot), carbofuran (10 μL per pot, Furadan 350 SC, FMC), abamectin (2 μL per pot, Vertimec 18 EC, Syngenta), and control (water).

The substrate was prepared according to the methodology described for the J_2 penetration and development tests and watered 24 hours before the application of the treatments. On its surface, 20 mL per pot of Extracts 1 and 2 was sprayed, as well as 20 mL of water-diluted carbofuran (10 μL per pot), and 20 mL of water-diluted abamectin (2 μL per pot). As for the control treatment, each pot received 20 mL of water. Afterward, a 3 cm deep hole was opened using a 50 mL Falcon tube.

At the bottom of the hole, 1.0 mL of suspension containing approximately 5,000 eggs of *M. javanica* was deposited. On top of the nematode suspension, a 'Santa Clara' tomato seedling with 30 days post-emergence and grown in a 128-cell polyethylene tray (Nutriplan, Cascavel) with commercial substrate (MecPlant, Telêmaco Borba) was transplanted. The plants were evaluated 90 days after inoculation and according to the methodology described for the J_2 penetration and development tests. The experiment was repeated once.

2.6 STATISTICAL ANALYSIS

Statistical analyses were performed using R software (R Core Team, 2024). Dose-response curves for hatching and mortality of J_2 were fitted and the statistical model was determined by the lowest Akaike weight (Portet, 2020) obtained by the mselect( ) function of the 'drc' package (Ritz; Strebig, 2022). Non-significant experimental terms were removed by Fisher's F test ($p<0.05$), except for blocks. Regression model parameters and EC_{50} were estimated using the functions drm( ) and ED( ) from the 'drc'. Data from J_2 penetration tests were transformed to $\log_{10}(y)$ to satisfy the assumptions of the linear regression model. Non-significant experimental terms were removed by Fisher's F test ($p<0.05$), except blocks. The effect of the concentration on the response was tested by Student's t-test on the regression model parameters using the 'agricolae' package (Mendiburu, 2023). Data from the *M. javanica* reproduction experiments were transformed to $\log_{10}(y)$ to satisfy the assumptions of the linear regression model. Studies on the significant experimental terms identified by ANOVA ($p<0.05$) were conducted through multiple comparisons of means using Tukey's test and the 'agricolae' package.

3 RESULTS AND DISCUSSION

3.1 HATCHING AND MORTALITY IN VITRO EXPERIMENTS OF *MELOIDOGYNE JAVANICA* J_2

The *in vitro* experiments presented a dose-response effect for hatching and mortality of *M. javanica*. The dose-response curves from the hatching of *M. javanica* eggs being immersed in the Extracts 1 and 2

are presented as sigmoid curves (Figure 1). The f-test indicated no significance between the experiment repetitions and, therefore, both experiments were analysed together (n = 6). The statistical model which presented the lowest Akaike's Information Criterion was the three-parameter Weibull (W2.3) for hatching and four-parameter Weibull (W2.4) for mortality of *M. javanica* J_2. According to the Akaike's Information Criterion, despite presenting low coefficient of determination values, the data fitted well to the statistical model.

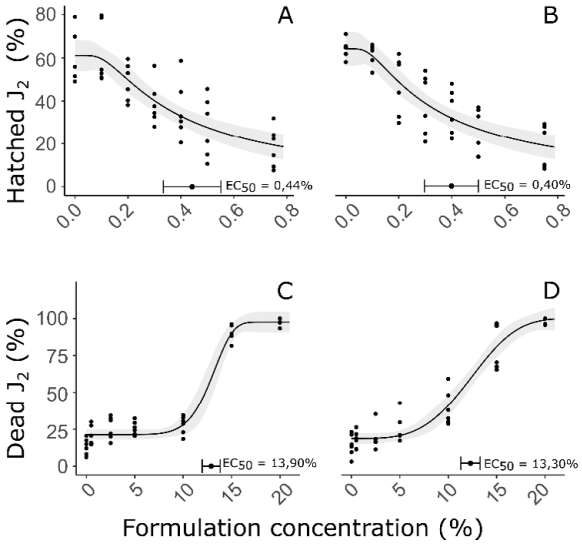

Figure 1. Percentage of hatched and dead juveniles of *Meloidogyne javanica* (J_2) after 48 hours of exposure to different concentrations of the extracts of neem (*Azadirachta indica*). Three-parameter Weibull curve for egg incubation exposure to Extract 1 with estimated EC_{50} of 0.44% (A); three-parameter Weibull curve for egg incubation exposure to Extract 2 with estimated EC_{50} of 0.40% (B); four-parameter Weibull curve for J_2 mortality exposure to Extract 1 with estimated EC_{50} of 13.90% (C); four-parameter Weibull curve for J_2 mortality exposure to Extract 2 with estimated EC_{50} of 13.30% (D). Gray bands represent the confidence interval (0.95%) of the estimated curve. The bars represent the estimated value of EC_{50} with a confidence interval (0.95%). The points represent the data (n = 6) from two experiments, each conducted in three blocks

The estimated EC_{50} values for *M. javanica* hatching correspond to a concentration of 0.44% for extract 1 and 0.40% for extract 2. Regarding the mortality tests, the best fitting model was the four-parameter Weibull, which estimated the EC_{50} concentration at 12.90% for extract 1 and 12.30% for extract 2 (Figure 1). The confidence interval for the EC_{50} estimated values indicated no statistical difference between the EC_{50} values estimated for Extracts 1 and 2 for both hatching and mortality of J_2.

3.2 EXPERIMENTS ON PENETRATION AND DEVELOPMENT OF *MELOIDOGYNE JAVANICA* J_2 IN TOMATO ROOTS

The f-test indicated no significance between the experiment repetitions and, therefore, both experiments were analysed together (n = 6). The 2.5% and 3.0% concentrations were removed from the analyses due to plant death. The effect of the products and between experiments was not significant in the linear regression model. The effect of the Extracts 1 and 2 concentrations showed no significant linear regression β value for height (0.164 and 0.152) and plant total dry matter (0.0190 and 0.0422), respectively (Figure 2).

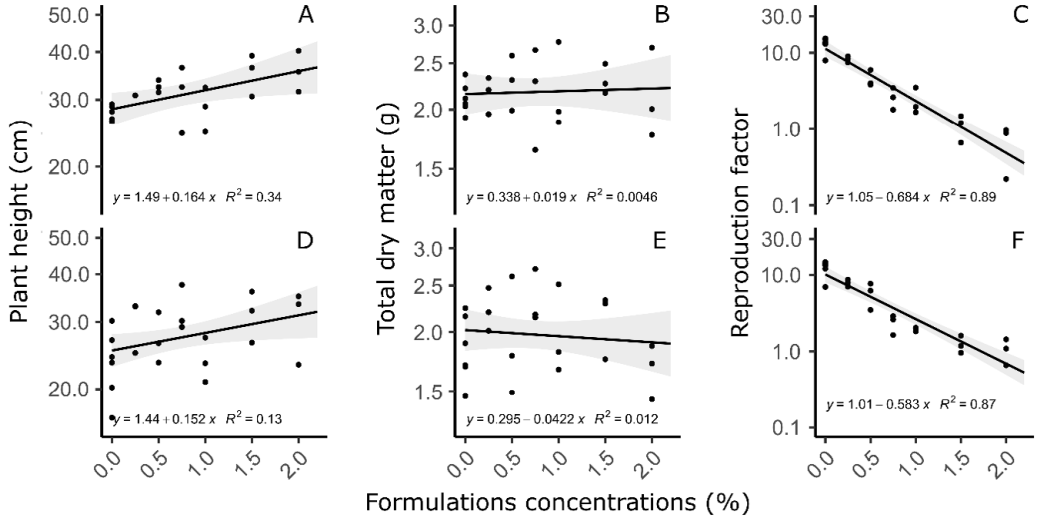

Figure 2. Effect of Extracts 1 and 2 on height, plant dry matter, and reproduction factor of *M. javanica* in tomato plants. Height of tomato plants treated with Extract 1 (A) and Extract 2 (D); total dry matter of tomato plants treated with Extract 1 (B) and Extract 2 (E); reproduction factor of *M. javanica* in tomato plants treated with Extract 1 (C) and Extract 2 (F). Gray bands represent the confidence interval (0.95%) of the estimated curve. The y-axis is represented on the log10(y) scale. The points represent the data (n = 3) from two experiments, each conducted in three blocks

The reproduction factor of *M. javanica* showed a significant reduction with the increasing Extracts 1 and 2 concentrations. In the case of Extract 1, it corresponded to 81.71% and 94.73% for the 1.0% and 2.0% concentrations, respectively. As for Extract 2, the reduction was 84.28% and 91.47% for the same concentrations, respectively. The linear regression β value for the reproduction factor was -0.684 for Extract 1 and -0.583 for Extract 2.

3.3 EXPERIMENT ON THE REPRODUCTION DEVELOPMENT OF MELOIDOGYNE JAVANICA IN TOMATO PLANTS

The f-test indicated no significance between the experiment repetitions and, therefore, both experiments were analysed together (n = 8). Data on height and total dry matter of tomato plants infested by *M. javanica* presented no statistical difference between the concentrations of Extracts 1 and 2 evaluated ($p > 0.05$). As for the reproduction factor, there was a significant difference between both extracts ($p < 0.05$). The application of Extracts 1 and 2, as well as of abamectin and carbofuran, reduced the RF of *M. javanica* in tomato plants (Figure 3). The 1.0% concentration of neem extracts reduced the RF of *M. javanica* by 48.22% for Extract 1 and 59.42% for Extract 2.

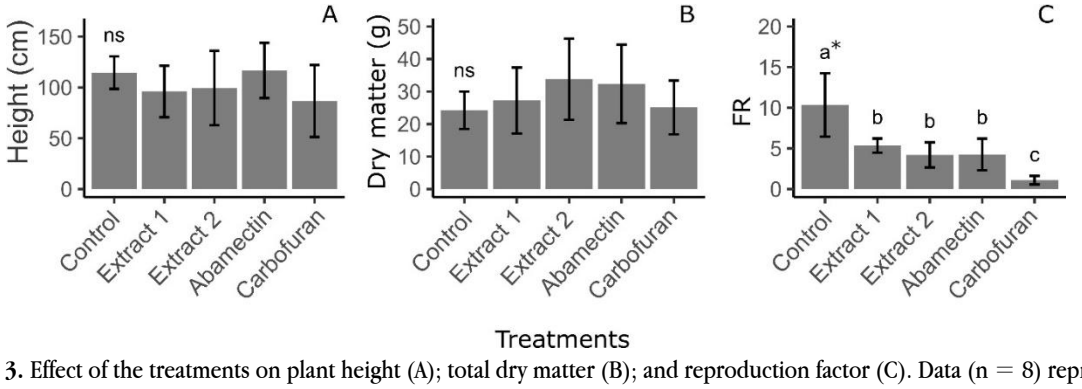

Figure 3. Effect of the treatments on plant height (A); total dry matter (B); and reproduction factor (C). Data (n = 8) represent the mean ± standard deviation of two experiments, each conducted in four blocks. Means are not statistically (ns) different by ANOVA test ($p \geq 0.05$). * Means followed by different letters are statistically different by Tukey's test ($p \leq 0.05$).

Nematodes are a group of plant pathogens of worldwide importance, and the use of plant extracts is potentially applicable for the management of these parasites. Among nematicides obtained from plant extracts, neem extracts offer potential for exploration. The products obtained from the alcoholic extract of the aerial parts of neem plants (leaves and branches), such as Extracts 1 and 2, offer potential for controlling *M. javanica* in tomato plants.

The Extracts 1 and 2 inhibited hatching and caused in vitro mortality of *M. javanica* J_2. The concentrations of Extracts 1 and 2 used in the hatching experiments were able to inhibit the hatching of *M. javanica* J_2. The aqueous and alcoholic extracts of neem leaves also reduced the hatching of *M. javanica* (Javed *et al*., 2008) and *M. incognita* (Nile *et al*., 2018) under *in vitro* conditions. The concentrations of Extracts 1 and 2 used in the experiments of mortality were able to kill *M. javanica* J_2. Similar results were observed with aqueous extract of neem cake and neem leaves, which caused the mortality of *M. javanica* J_2 (Javed *et al*., 2008). Considering the EC_{50} values for the extracts, the results indicate that, for management purposes, the application of Extracts 1 and 2 reduces both hatching and mortality of J_2. Thus, the application of lower concentrations aiming at reducing J_2 hatching possibly presents results with greater economic viability than the application of high concentrations aiming at J_2 mortality.

The penetration of J_2 in tomato roots, 30 days after inoculation and treatment with the extracts, presented a reduction in the reproduction factor of *M. javanica* with no significant effect on plants' height and total dry matter. The ethyl extract of neem cake also affects the penetration of J2 into tomato roots after 21 days of inoculation and treatment, reducing the number of nematodes in the roots without reducing plant mass (Javed *et al*., 2008). The appearance of wounds in the root crown of the plants with evolution to tumbling and death was observed after the application of concentrations equal to or greater than 2.5% of both Extracts 1 and 2. The application of the 2.0% concentration of Extracts 1 and 2 - maximum capacity supported by the plants and estimated in the penetration tests - would result in a reproduction factor reduction of 94.73% and 91.47%, respectively. The reduction in reproduction factor would be 81.71% and 84.28% for the concentration of 1.0% of Extracts 1 and 2, respectively. In other words, doubling the concentration of the product applied would only result in a reduction of 13.02%, in the case of extract 1, and 7.19%, in the case of extract 2. Thus, possibly, the application of a 2% concentration of the products in the soil is economically unfeasible, which is the reason why we decided to apply the intermediate concentration of 1% in the reproduction tests of *M. javanica*.

In the reproduction tests, the application of the extracts at a concentration of 1.0% reduced the reproduction factor of *M. javanica* in the roots of tomato plants. The reduction in reproduction factor obtained by the application of neem extracts was similar to the one obtained by the application of abamectin. However, there is a difference between the applicability of these products since, to achieve a similar control index, 20 mL per pot of Extracts 1 or 2 were required whereas 2 μL per pot of abamectin was needed. The application of high amounts of extract had already been pointed out by the need to apply more than 500 L ha^{-1} of neem extract for nematode control (Oka, 2010).

The reduction of nematodes population caused by the application of different neem extracts is reported in several studies. The application of neem leaves and powdered neem cake (Javed *et al*., 2007), crude extract (Javed *et al*., 2008), or only of neem cake (Rizvi *et al*., 2015) to soil reduces *M. javanica* infestation in tomato plants. The reproduction factor reduction in nematodes of the genus *Meloidogyne* is associated with neem compounds with nematostatic effect. One of the most well-known compounds with this effect is the azadirachtin (a triterpenoid of the limonoid class), which alteration of insect feeding, inhibits the growth and development of insects by blocking the biosynthesis of growth hormones and preventing ecdysis (D' Errico *et al.*, 2023). The aqueous extract of purified azadirachtin did not inhibit the hatching of

M. javanica J_2 (Javed *et al*., 2008) nor did it cease the development of J_2 in tomato plants (Javed; Abdullah; Fayyaz, 2007), suggesting that the compound was not absorbed or is not toxic enough to cause the death of the nematodes.

Other triterpenoids, such as nimbin and salannin, are also known to have a nematostatic effect. Similar to azadirachtin, purified nimbin and salannin do not cause the death of *M. incognita* J_2. However, they cause mobility reduction of nematodes and, consequently, reduce the penetration of J_2 into plant roots (Mojumder; Kamra; Dureja, 2002). The reduced mobility of J_2 also plays an important role in the egg hatching process (Javed *et al*., 2008).

Several factors must be considered regarding the efficiency of extracts applied to the soil. Initially, we must assume that the compounds will degrade rapidly due to the decomposition of the extracts in the field. The crude extract of neem leaves can persist in the soil and inhibit *M. javanica* for up to 16 weeks (Javed; Abdullah; Fayyaz; 2007). On the other hand, irrigation water constantly dissolves the extracts and causes their leaching at depth. Therefore, new studies proposing the application of neem extracts at different time intervals and even through fertigation need to be developed to enable the use of this technology.

4 FINAL CONSIDERATIONS

The results indicate that the concentration of 1.0% of the neem Extracts 1 and 2 can be used to control *M. javanica* in tomato plants. This concentration is higher than that required to inhibit the hatching of 50% of J_2 (0.4% for extract 1 and 0.44% for extract 2) but is not sufficient to cause the death of 50% of J_2 (13.90% for extract 1 and 13.30% for extract 2). An option to economically reduce pathogen inoculum would be to use smaller areas or hotspot areas, considering that a concentration of 1% of the extract is already high. Consequently, costs would be reduced, and the practice could be integrated into management of the disease.

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