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Environment

Control of *Meloidogyne javanica* with aqueous extract of *Trichilia claussenii*

Controle de *Meloidogyne javanica* com extrato aquoso de *Trichilia claussenii*

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ABSTRACT

Root-knot nematodes (*Meloidogyne javanica*) cause severe damage to numerous crops, highlighting the need for sustainable management strategies. This study aimed to evaluate the *in vitro* effect of the aqueous extract of *Trichilia claussenii* on the hatching of *M. javanica* eggs and to assess its influence on the initial development of lettuce (*Lactuca sativa* L.). The extract was tested at different concentrations (0–10%) and egg hatching was quantified five days after incubation by counting second-stage juveniles (J2) in microtiter plates. The same extract concentrations were applied to lettuce seedlings to evaluate potential phytotoxic effects on plant length and green mass after 14 days of growth. The results revealed a linear increase in nematicidal efficiency with rising extract concentration, reaching more than 60% inhibition of egg hatching at 4% and around 80% at higher doses. However, increasing concentrations negatively affected lettuce growth parameters. The 4% concentration presented an optimal balance between nematode control and minimal phytotoxicity. These findings demonstrate the potential of *T. claussenii* extract as a natural alternative for integrated nematode management, reducing reliance on synthetic nematicides and contributing to more sustainable agricultural systems.

Keywords: Nematodes; Plant extract; Egg hatching; *Lactuca sativa* L.

RESUMO

O nematoide-das-galhas (*Meloidogyne javanica)* é responsável por causar grandes prejuízos em diversas culturas, exigindo assim, estratégias de manejo específicas. Com isso, desenvolver alternativas para compor o manejo integrado é importante, como o uso de extratos de plantas bioativas. O objetivo do estudo foi avaliar o efeito in vitro do extrato vegetal de *Trichilia claussenii* na eclosão de ovos do nematoide das galhas, M. javanica e a influência do extrato vegetal em plantas de alface (*Lactuca* *sativa* L.). O extrato de T. claussenii foi testado nas concentrações de 0%, 2%, 4%, 6%, 8% e 10%. O efeito do extrato sobre a eclosão dos ovos foi avaliado em laboratório contabilizando os juvenis em segundo estágio (J2), eclodidos após cinco dias da incubação dos ovos, em placa de microtitulação. As mesmas concentrações de extrato foram aplicadas na cultura da alface para observar a influência no desenvolvimento inicial da cultura. As mudas de alface foram transplantas para recipiente plástico,e passados 14 dias, avaliado o comprimento e massa verde das plantas. Conclui-se que o extrato é uma alternativa no manejo integrado de nematoides, doses crescentes do extrato vegetal de *T. claussenii* apresentam eficiência linear sobre a eclosão de juvenis de *M. javanica*, entretanto, apresenta efeito negativo no desenvolvimento inicial de alface. A concentração de 4% de extrato apresenta controle superior a 60% na eclosão de *M. javanica* e baixa interferência negativa na cultura da alface.

Palavras-chave: Nematoides; Extrato vegetal; Eclosão de ovos; *Lactuca sativa* L.

1 INTRODUCTION

Phytonematoids are essential pests in most agricultural crops and are responsible for significant production losses and generating high costs for their control (Inomoto, 2016). Among the range of nematode species, the genus *Meloidogyne* stands out as one of the most damaging pathogens of cultivated plants. Due to the wide variety of hosts and adaptability to diverse environmental conditions, sustainable nematode management is complicated (Tarini et al., 2020). Nematode infection in host roots begins with second-stage juveniles and can lead to the formation of galls due to hyperplasia (Lemes et al., 2022). The symptoms of phytonematode-induced diseases are often mistaken for nutritional deficiency; however, they cause alterations and damage to plant tissues, resulting in profound physiological changes, consequently hindering the absorption and transportation of water and nutrients (Inomoto, 2016; Silva; Inomoto, 2022). As a result, plants can show size inequality, symptoms such as chlorosis and wiltingand a reduction in productivity.

Gall nematodes (*Meloidogyne spp.*) are an essential group of phytopathogensand their control is fundamental to the profitability of agricultural production (Azlay et al., 2023). They are obligate parasitesand prolonged periods without host plants can lead to their disappearance in the area. However, they often survive on infesting hosts in the absence of susceptible crops. The most commonly occurring species in Brazil are *M. incognita* and *M. javanica*. Although these species have a sedentary life habit, their multiplication and permanence in the soil make management very difficult, as there are different susceptibility reactions between lettuce cultivars, which can reach 9604 eggs per gram of root in susceptible cultivars (Pinheiro et al., 2020). Most species of the *Meloidogyne* genus survive best in regions with soil temperatures above 28°C, but *M. incognita* and *M. javanica* are cosmopolitan and well-adapted to Brazil's different climatic conditions (Oliveira et al., 2024).

The occurrence of nematodes in productive areas represents a challenge for production, especially in horticultural crops (Lopes et al., 2021). Lettuce (*Lactuca sativa* L.), considered Brazil's most consumed leafy vegetable, has a short cycle, allowing the area to be used for several crop cycles. Due to the current agricultural model, based on the monoculture of susceptible crops, phytosanitary problems have intensified, often making areas unviable. As demonstrated by Rabello et al. (2021), the successive cultivation of lettuce in regions infested by *M. javanica* becomes unviable from the second cycle of the crop onwards. Likewise, pest control methods must not be harmful to the crop.

There is currently no way of eradicating nematodes, only management methods, which face limitations. Chemical control of nematodes has been the most widely used method over time. Still, concerns about the environment and human health have stimulated the development of alternative control strategies, which include cultural practices, stimulation or introduction of antagonistic organisms, their metabolites, or bioactive compounds (Azlay et al., 2023)and using plant extracts to control pathogens that cause plant diseases, including nematodes (Neves et al., 2021).

In the search for more sustainable and efficient extraction techniques, ultrasound-assisted extraction (UAE) has emerged as a green technology capable of improving the recovery of bioactive compounds from plant matrices. This method uses acoustic cavitation to rupture cell walls, enhancing solvent penetration and compound release, while significantly reducing solvent consumption, energy demandand extraction time (Chemat et al., 2017). Compared with conventional techniques such as maceration and Soxhlet extraction, UAE is recognized for its high efficiency, reproducibilityand environmental safety, aligning with the principles of green chemistry and sustainable production of bioinputs.

Antagonistic plants can affect the nematode population by preventing penetration and interfering with the development of these parasites, as they contain nematicidal/nematostatic compounds in their tissues and exudates (Balah et al., 2019). They are used as plant extracts or essential oils, applied to the leaf area of plants or specific parts and incorporated into the soil, among other ways. Recent studies with extracts of *Vassobia breviflora* (Solanaceae) have demonstrated the presence of bioactive compounds with antioxidant, antibacterialand cytotoxic activities, reinforcing the potential of plant-derived metabolites as alternative agents for pathogen control (Viana et al., 2022; Viana et al., 2025).

In recent decades, plants from the Meliaceae family have constantly attracted the scientific community's attention, as they have substances with insecticidal properties, justifying the phytochemical interest of their natural constituents (Braga et al., 2020). The species with the greatest visibility within this family is Azadirachta indica (neem), known for being an excellent option in the biological control of pests and diseases (Muhammad et al., 2020). Based on the outstanding results seen with neem, other meliaceae have also attracted research interest, intending to find new species and releases with insecticidal activity (Sarkar et al., 2021).

Many studies have reported the biological and pharmacological activities of crude extracts and pure chemical constituents isolated from different parts of species of the Trichilia genus (Fatema et al., 2024). Among the activities of substances and/or extracts obtained from plants of the Trichilia genus are anti-food and insecticidal properties (Bogorni et al., 2005).

Trichilia claussenii, popularly known as Catiguá, belongs to the Meliaceae family and can be found in the Atlantic Forest and some regions of Brazil, such as the Pampa Biome (Muellner-Riehl et al., 2022). Its use as a bioinsecticide has already been tested and proven on agriculturally important pest species such as Spodoptera frugiperda (Baldin et al., 2020). The main bioactive compounds found in Trichilia species are limonoids, alkaloidsand flavonoids, which have insecticidal properties and can cause feeding inhibition, direct toxicityand repellency of agricultural pests (Passos et al., 2021). These bioactive compounds can be an alternative to synthetic insecticides, offering a more sustainable alternative in the agricultural bio-input market (Fan et al., 2022). However, using Trichilia extract in nematode control is a promising approach for sustainable agricultureand scientific information on this subject is scarce.

For the above, this study aimed to evaluate the in vitro effect of the aqueous extract of *Trichilia claussenii* on the hatching of *Meloidogyne javanica* eggs and the influence of the plant extract on the development of healthy, uninoculated lettuce plants. The relevance of this work lies in providing innovative and sustainable alternatives for nematode management, particularly in horticultural crops such as lettuce, which are highly vulnerable to infestations and suffer significant productivity losses. By exploring the potential of *T. claussenii* as a source of bioactive compounds with nematicidal activity, this research contributes to expanding the portfolio of plant-based bioinputs and supports the transition toward more sustainable agricultural systems, reducing dependence on synthetic nematicides and aligning with the global demand for safer and environmentally friendly practices.

2 METHODOLOGY

2.1 Obtaining the aqueous extract of *Trichilia claussenii*

Samples of *T. claussenii* were collected in the central region of Rio Grande do Sul, Brazil. The samples were then dried in an oven at 50ºC for 48 hours. The fruits and leaves of *Trichilia claussenii* were then separated and crushed. The aqueous extract was obtained through ultrasound-assisted extraction (UAE), using a 400 W high-intensity ultrasound processor with a frequency of 24 kHz (Hielscher, model UP 400S, Germany) equipped with a probe (Model H22, Tip 22). Extractions were carried out using approximately 50 g of biomass (leaves and fruit) and 500 mL of solvent (H2O distilled water). The extraction time was set at 10 min. After the extractions, the samples were subjected to vacuum filtration. The crude extract was diluted in distilled and sterilized water to obtain different concentrations: 0% (control), 2%, 4%, 6%, 8% and 10%, giving six treatments.

2.2 Composition analysis of aqueous extract of *Trichilia claussenii*

Gas chromatography analyses were conducted using a GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) coupled to a GCMS-QP2010 Ultra mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an automatic AOC-20is series injector (Shimadzu, Kyoto, Japan). The chromatographic separation was achieved using an Rtx®-5ms GC column (30m x 0.25mm, 0.25μm film thickness) composed of 5% diphenyl and 95% dimethyl polysiloxane (Restek Corporation, Bellefonte, PA, USA). Helium was used as the carrier gas at a flow rate of 1.20 $mL.min^{-1}$. The injector temperature was consistently maintained at 250°C. Volatile compounds were injected in splitless mode. The initial oven temperature was set at 40°C for 1 minute, then increased to 90°C at a rate of 1°$C.min^{-1}$, followed by an increase to 140°C at a rate of 2°$C.min^{-1}$and finally to 250°C at a rate of 40°$C.min^{-1}$, where it was held for 17.25 minutes. Both the interface and ion source temperatures were maintained at 250°C. Mass spectra were acquired over the m/z range of 50–550 amu with a scan rate of 0.30 scans. s^{-1}. Volatiles were identified using a single quadrupole mass spectrometer in electron impact (EI) mode at 70 eV in scan acquisition mode. Individual components were identified by matching their mass fragmentation patterns with those in the

Wiley Registry of Mass Spectral Data (Palisade Corporation, Newfield, NY). Only compounds with a mass spectral matching score greater than 80% were considered for further analysis.

2.3 Obtaining and multiplying *Meloidogyne javanica* inoculum

Due to the high susceptibility of some tomato cultivars (*Solanum lycopersicum* L.) to gall nematodes, research institutions use these plants in greenhouse conditions to maintain and/or multiply populations or collections of gall nematodes for studies (Pinheiro et al., 2014). On this basis, the gall nematode inoculum was maintained on tomato plants (*Solunum lycopersicon* cv. Gaúcho). After this period, the eggs were extracted according to the methodology described by Bonetti & Ferraz (1981). To do this, the tomato roots were washed in running water to remove the adhered soil, then chopped into 1-2 cm pieces and ground in a blender with a 0.5% sodium hypochlorite (NaCl) solution for approximately 30 seconds. The material was then passed through a set of sieves. The material collected on the 500-mesh sieve was added to tubes and centrifuged for 5 minutes at 1750 rpm with caulin. The supernatant was discardedand sucrose solution was added to the tubes, which were centrifuged again for 1 minute at 1750 rpm. At the end of the centrifugation process, the egg suspension was passed through a 500-mesh sieve, where the eggs were retained and collected with the aid of a pissette in a beaker.

2.4 Effect of aqueous extract of *Trichilia claussenii* on the hatching of J2 of *Meloidogyne javanica*

To conduct the hatching test, suspensions containing 10 µl of *M. javanica* eggs were placed in microtiter plate wells (Elisa type), along with the respective extract concentration of each treatment, 0, 2µl, 4µl, 6µl, 8µl and 10µland distilled water to complete 100 µl in each well. After adding the components to the wells, the eggs and larvae present in each well were counted. The plates were sealed with aluminum foil and placed in a BOD at 28ºC. After 5 days of incubation, evaluations were carried out under a stereoscopic microscope, where the J2 stage (hatched larvae) was counted to determine the percentage of hatched juveniles.

From the average data, the formula proposed by Abbott (1925) was used to calculate agronomic efficiency, where AE = (T - t) x 100 / T, where "T" is the number of live juveniles in the controland "t" the number of live juveniles in the treatment.

2.5 Evaluation of the influence of the extract on lettuce

Lettuce seeds, *Lactuca sativa*, cv. Rainha de Maio, were sown in Styrofoam trays containing commercial Carolina Soil® substrate and kept in a greenhouse. The seedlings were transplanted into plastic containers with a capacity of 50ml containing the same substrate used for sowing and were relocated to a growing room with 12h of light and 22ºC. Then 5mL of the extract diluted in distilled water was applied at concentrations of 0% (control), 2%, 4%, 6%, 8% and 10%. Irrigation was carried out every two days with 5 mL of distilled water per glass. After 14 days, the variables green mass and plant length were assessed, where the roots of the plants were washed to remove any residue of the substrate, after which they were weighed on a precision scale to determine the green mass and measured to obtain the plant length.

2.6 Statistical analysis

For both tests, J2 hatching and lettuce growth, six treatments and six replications were used in a completely randomized design (DIC). Two outliers were removed from each treatment for the variables under study from the data measured in the tests. The average value was taken as the basis, excluding the highest and lowest values, leaving four repetitions per treatment. This was followed by the F test and subsequent linear regression for the variables ($P<0.05$). All the data was submitted to Pearson's correlation test to identify interactions between variables.

3 RESULTS

The compounds identified in the extracts are presented in Tables 1 and 2. A total of 41 and 29 compounds were extracted from the leaves and fruits of *Trichilia claussenii*, which are predominantly divided into the classes of phenols, terpenes, sterols, estersand carboxylic acids (Figure 1). Water can dissolve polar compounds, so phenols were mainly extracted from these botanical parts (leaves and fruits) using UAE.

Table 1 – Compounds identified in the leaf extract of *Trichilia claussenii*

(To be continued...)

Compound	Area
Catechol	40.25
γ-Sitosterol	7.48
Kaur-16-ene	5.06
N-Hexadecanoic acid	3.24
Trans-Sinapyl alcohol	2.86
Squalene	2.76
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2.63
(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	2.42
Octadecanoic acid, 2,3-dihydroxypropyl ester	2.39
Stigmasterol	2.39
Neophytadiene	2.09
13-Docosenamide, (Z)-	1.96
Phenol, 2,6-dimethoxy-	1.88
Trans-(2-Ethylcyclopentyl)methyl acetate	1.85
Δ-Cadinene (CAS)	1.81
2-Methoxy-4-vinylphenol	1.76
Ergost-5-en-3-ol, (3.beta.,24R)- (CAS)	1.39
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	1.28
3′,5′-Dimethoxyacetophenone	1.17
α-Copaene	1.17
trans-Caryophyllene	1.03
1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	1.03
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.95
α-Tocopherol-.beta.-D-mannoside	0.94
Undec-10-ynoic acid, tridec-2-yn-1-yl ester	0.88
Hexadecanal	0.64
Aromandendrene	0.62
Tetrapentacontane	0.62
Ethyl linoleate	0.61
γ-Tocopherol	0.60
Trans-Isoeugenol	0.53

Table 1 – Compounds identified in the leaf extract of *Trichilia claussenii*

(Conclusion)

Compound	Area
9,12-Octadecadienoic acid (Z,Z)-	0.52
N-Nonadecanol-1	0.46
α-Muurolene	0.45
Phenol, 2,4-Bis(1,1-Dimethylethyl)-	0.44
α-Cubebene	0.40
Ethanone, 1-(2,3-dihydro-1,1-dimethyl-1H-inden-4-yl)-	0.35
α-Cadinol	0.33
Tetrapentacontane	0.31
Cis-9-Hexadecenal	0.23
Neophytadiene	0.21

Source: Authors (2026)

Table 2 – Compounds identified in the fruit extract of *Trichilia claussenii*

(To be continued...)

Compound	Area
Catechol	19.92
Quinic acid	19.83
N-Hexadecanoic acid	8.02
13-Docosenamide, (Z)-	7.67
Phenol, 2,6-dimethoxy-	7.20
4-vinylphenol	4.56
Oleic Acid	3.76
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2.96
9,12-Octadecadienoic acid (Z,Z)-	2.62
Trans,trans-9,12-Octadecadienoic acid, propyl ester	2.30
2-Methoxy-4-vinylphenol	2.22
N-Butyl cyanoacetate	1.77
Cyclopentadecanone	1.54
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-	1.45
Ethyl linoleate	1.43
Caryophyllene	1.42
9,12-Octadecadienoic acid (Z,Z)-	1.39
Octadecanoic acid, 2,3-dihydroxypropyl ester	1.38
Z,E-2,13-Octadecadien-1-ol	1.33
Undec-10-ynoic acid, undec-2-en-1-yl ester	1.26
9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	1.08
N-Nonadecanol-1	0.83
(-)-Caryophyllene oxide	0.78
9,12-Octadecadienoic acid (Z,Z)-	0.77
α-Copaene	0.69
Hexadecane	0.65

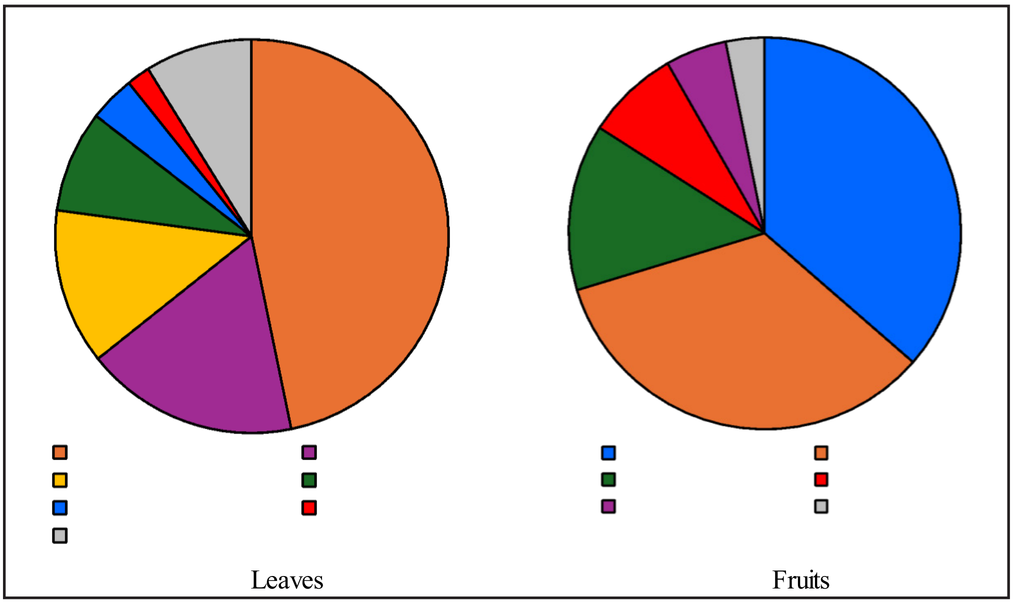
Table 2 – Compounds identified in the fruit extract of *Trichilia claussenii*

(Conclusion)

Compound	Area
γ-Muurolene	0.56
Bifenthrin	0.47
1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)-	0.18

Source: Author's (2026)

Figure 1 – Classes of compounds identified in the extracts of *Trichilia claussenii*



Source: Author's (2026)

Based on the data obtained using Pearson's correlation test (Table 3), the *Trichilia claussenii* extract concentration (CONC) variable shows significant positive interaction with agronomic efficiency on juvenile hatching (EC). On the other hand, the correlation is negative with the variables green plant mass (MV) and plant length (COMP).

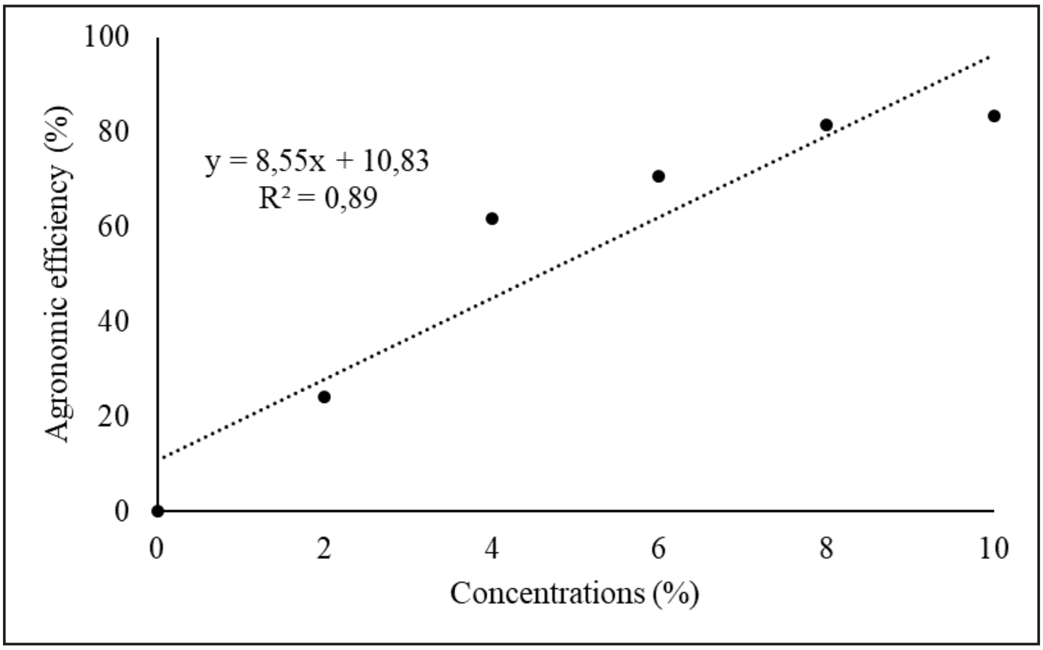
Using the average data of hatched juveniles, a regression was generated according to Figure 2. It shows a linear trend in agronomic efficiency as the doses increase, reaching an efficacy of more than 60% with a concentration of 4% extract. Figure 2 shows that the 8% and 10% concentrations of *T. claussenii* were 80% effective, with no significant difference between the doses in terms of effectiveness in hatching juveniles of *Meloidogyne javanica*.

Table 3 – Pearson's correlation for the variables concentration of *Trichilia claussenii* extract (CONC), green mass of lettuce plants (MV), length of lettuce plants (COMP)and agronomic efficiency on egg hatching (EC)

	CONC	MV	COMP	EC
CONC	1			
MV	-0,8700*	1		
COMP	-0,9583**	0,9225**	1	
EC	0,9415**	-0,8468*	-0,9052*	1

**: Significant at 1% probability by t-test; * Significant at 5% probability by t-test.
Source: Author's 2026

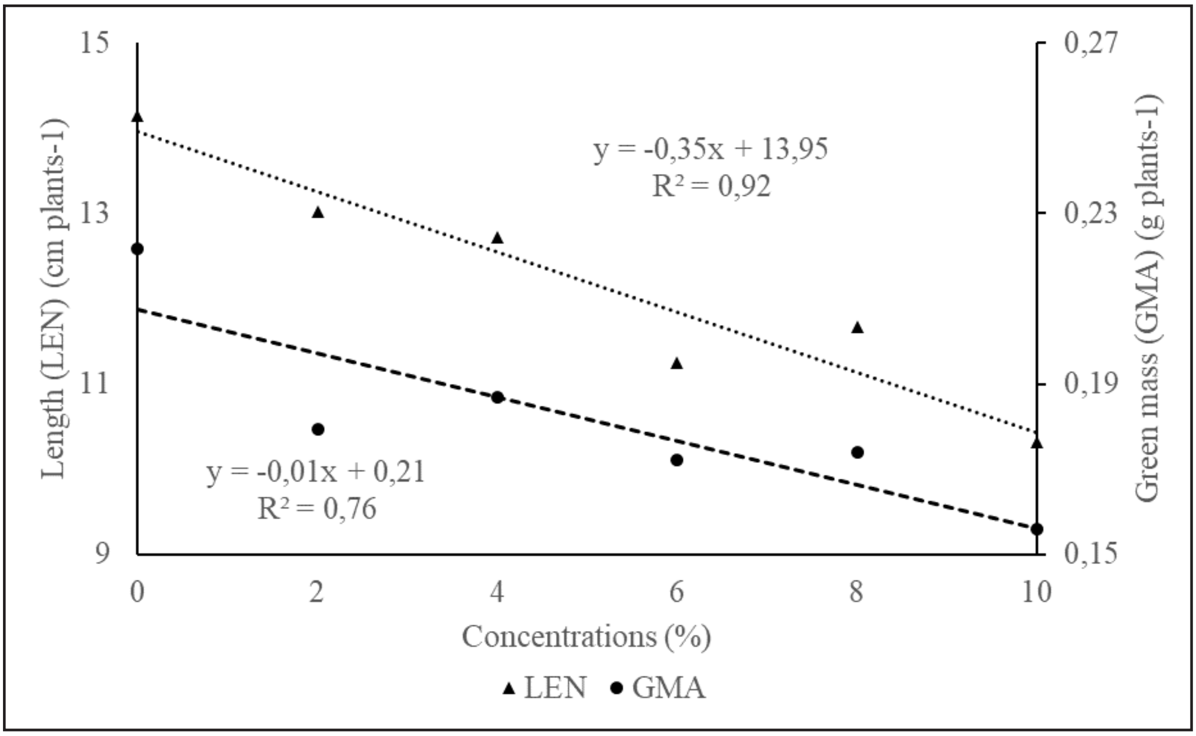
Figure 2 – Agronomic efficiency of *Trichilia claussenii* extract at concentrations of 0, 2, 4, 6, 8and 10% on the hatching of *Meloidogyne javanica* juveniles after five days of incubation



*Significant to the F test (P < 0.05; standard error = 12.8).
**Agronomic efficiency values as proposed by Abbott (1925), where AE = (T - t) x 100 / T, where "T" is the number of live juveniles in the controland "t" is the number of live juveniles in the treatment
Source: Author's 2026

Figure 3 shows a negative influence on the initial development of lettuce seedlings with increasing concentrations of *Trichilia claussenii* extract. Although the increase in extract concentration has a negative effect on the lettuce, the damage is compensated for by the reduction in the nematode population.

Figure 3 – Influence of *Trichilia claussenii* extract at concentrations of 0, 2, 4, 6, 8and 10% on the variables of length and green mass in lettuce plants (*Lactuca sativa*), cv. Rainha de Maio grown in pots



*Significant to F test ($P < 0.05$; standard error = 0.44 and 0.01 for plant length and green mass, respectively)

4 DISCUSSION

Biosynthesis processes in plants produce secondary metabolites, such as the ones above in the extracts obtained by UAE. In a review study, species of the genus *Trichilia* with a high relative frequency indicate the presence of secondary metabolites of the terpenoid metabolic pathway and mevalonic-acid and malonic-acid pathways. Among the metabolites in *Trichilia*, highly oxygenated triterpenes were observed with a remarkable frequency. Phytochemical studies of the genus *Trichilia* have allowed the isolation and identification of 334 compounds belonging to the following classes: monoterpenes, sesquiterpenes, diterpenes, triterpenes, steroids, limonoids, flavonoids, coumarinsand glycosylated lignans. Overall, *Trichilia* displays antimicrobial, anti-inflammatory, insecticidaland cytotoxic activities (Fatema et al*.,* 2024).

Only a few studies have been developed elsewhere with *Trichilia* for nematode control. For example, when comparing the extracts of the species from the Meliaceae family, *Trichilia claussenii* showed higher anti-parasite potential in vitro than *Melia azedarach*. *Trichilia claussenii* extract presented an LC_{50} of 263.8 μg/mL and a LC_{99} of 522.5 μg/mL in the egg hatch testand LC_{50} of 1.1 μg/mL and LC_{99} of 26.4 μg/mL in the larval development test (Cala et al. 2012). Aqueous extracts with concentration of 100 μg/mL of leaves and fruits from *Trichilia* sp. were tested on eggs and in the larval development test on *Haemonchus contortus*. The number of eggs reduced by a half when using the extracts if compared to negative control (only water) (Cunha et al. 2014).

However, no study was found that reported the control of *Meloidogyne javanica* with aqueous extract of *Trichilia claussenii*. Elsewhere, some compounds identified in the extracts have important bioactivities for other examples of biological control. For example, quinic acid was also identified in the extracts obtained from fruits of *Solanum viarum*. The extracts presented 62.02 ± 0.11 and 77.05 ± 0.15% inhibition of mycelial growth of *Fusarium graminearum* and *Sclerotinia sclerotiorum*, respectively (Confortin et al. 2024). The major compound found in extracts from leaves of *Lupinus albescens* was stigmasterol. Aqueous extracts at a concentration of 5 g/L presented 60.5 ± 0.2 and 57.2 ± 0.1% inhibition of *Fusarium oxysporum* and *Fusarium verticillioides* (Confortin et al. 2019).

Monoterpenes exhibited potent acaricidal activity against two-spotted spider mite (*Tetranychus urticae* Koch). The use of natural products may be considered an important alternative acaricide to control *Tetranychus urticae*, since they constitute a rich source of bioactive compounds that are biodegradable, non-toxicand potentially suitable for use as pesticide (Cavalcanti et al. 2010). Screening of 55 plant extracts against second stage juveniles of *Meloidogyne incognita* was conducted. The extracts were obtained from leaves, stemsand roots of 20 native Yucatecan plants, of which 13 species were characterized as endemic. Results showed that *Eugenia winzerlingii* leaf extract induced at 300 ppm mortalities of 77% and 84% after 48 and 72 h, respectively. Also, extracts from leaves of *Trichilia arborea* C. DC. induced at 250 and 500 ppm mortalities of 13 and 85% after 72 h, respectively (Cristóbal-Alejo et al. 2006).

The average data of hatched juveniles indicates a linear trend in agronomic efficiency as the doses increase. This corroborates Baldin et al. (2020) in a test with Trichilia pallida to control the insect Bemisia tabaci, who observed that the 3% concentration showed 100% mortality one day after application.

The bioactives present in the Trichilia plant extract may have interfered directly with the nematodes through direct toxicity, causing disturbances in biological functions (Atolani et al. 2020). In addition, the extract used in the experiment may have reduced hatching by causing hormonal changes during the ecdysis process in larval development, as observed by Bogorni et al. (2005) in Spodoptera frugiperda caterpillars. Cruz-Estrada et al. (2013), using an aqueous extract of Trichilia arborea, observed high mortality in Bemisia tabaci eggs at a concentration of 3%, which supports that species belonging to the Meliaceae family have high insecticidal activity. In addition, Trichilia arborea has also been shown to be active against plant pathogens such as the nematode *Meloidogyne incognita*, with 100% mortality of J2 at a concentration of 500ppm (Cristóbal-Alejo, 2006).

Despite the negative effect of increasing the concentration of the extract on lettuce, the reduction in the nematode population compensates for the damage. Peixoto et al. (2011) showed that initial inoculums of 2000, 4000 and 6000 nematode eggs and J2 reduced the commercial production of lettuce by 3.04%, 12.13% and 11.61%, respectively. Belan et al. (2011) state that the leaf expansion of young cherry tomato plants is negatively affected by an increase in the initial inoculum of *M. javanica*.

Extracts from other plants can also compromise the initial development of lettuce. For example, Vargas et al. (2019) observed the phytotoxic effect of Tocoyena formosa and Rudgea viburnoides extracts on lettuce roots in initial growth. Similarly, allelopathy is a naturally occurring phenomenon in which allelopathic plants suppress the growth or fitness of neighboring plants. Soln et al. (2021) state that allelopathic compounds interfere with cell division, membrane permeability and enzyme activation in plant rootlets.

Subedi et al. (2020) state that chemical and biological agents have moderate control over *Meloidogyne incognita*. It is therefore necessary to carry out integrated management by looking for methods and products that maintain or reduce the population of soil nematodes. Control is challenging, with El-Marzoky et al. (2022) demonstrating the need to use 500 ppm of abamectin in the treatment of cucumber seeds to control *Meloidogyne incognita*and the best results in terms of control and development of tomato plants grown in the presence of M. incognita were observed when combining chemical and biological treatment (El-Ashry et al. 2021). Therefore, this work contributes to the knowledge gap on the use of Trichilia species in the control of the nematode *Meloidogyne javanica*.

The ultrasound-assisted extraction (UAE) protocol adopted in this study proved to be efficient in obtaining bioactive fractions from *Trichilia claussenii*. Compared with conventional extraction methods such as maceration or Soxhlet, UAE offers significant advantages, including shorter extraction time, lower solvent consumptionand enhanced recovery of thermolabile compounds (Chemat et al., 2017). These characteristics make UAE an environmentally friendly and reproducible technique suitable for the development of plant-based bioinputs. Moreover, the reproducibility and scalability of this protocol facilitate its potential use in future formulation and field trials, reducing the time and energy required for extract preparation without compromising biological activity.

5 CONCLUSIONS

Aqueous extracts of *Trichilia claussenii* contain phenolic- and terpenoid-rich fractions capable of suppressing *Meloidogyne javanica* egg hatching in vitro in a dose-dependent manner. Concentrations ≥4% achieved >60% inhibition of J2 hatchingand 8–10% reached ≈80% efficacy.

There is a linear increase in the agronomic efficiency of the aqueous extract of *Trichilia claussenii* on nematode hatching as the concentration increases;

Field deployment should target the lowest effective dose (≈4%) to balance nematode suppression with minimal phytotoxicity; higher doses may be reserved for pre-plant or non-crop windows.

The aqueous extract of *Trichilia claussenii* can complement integrated nematode management (resistant cultivars, rotations, organic amendmentsand biocontrol agents), reducing reliance on synthetic nematicides.;

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