

Evaluation of a formulation based on essential oils of *Allium sativum* (L.) and *Thymus vulgaris* (L.) combined with *Yucca schidigera* (Roezl) extract for control of *Meloidogyne* spp. in greenhouse tomatoes

Evaluación de un formulado a base de aceites esenciales de *Allium sativum* y *Thymus vulgaris* combinado con extracto de *Yucca schidigera* en el control de *Meloidogyne* spp. en tomate bajo invernadero

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Juvenile stage (J2) of plant parasitic nematode *Meloidogyne* spp. in optical microscopy at 40X magnification.

Photo: M.A.Tucuch-Pérez

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ABSTRACT

Tomato crop is among the more important worldwide due to its high production and consumption. However, tomatoes are affected by plant-parasitic nematode *Meloidogyne* spp., which reduce their yield and quality. To manage this issue, synthetic nematicides are commonly used. However, the use of synthetic nematicides impacts the soil microbiome and alters its structure affecting soil porosity and having effects on organic matter. In contrast, plant oils and extracts have emerged as alternative methods for controlling *Meloidogyne* spp. The objective of this research was characterize the oils of *Thymus vulgaris* and *Allium sativum*, along with an ethanolic extract of *Yucca schidigera*, and to evaluate their nematicidal activity when incorporated into a formulation against *Meloidogyne* spp. under greenhouse conditions. Phytochemical characterization was performed using qualitative techniques and measurements of antioxidant capacity (AC) and total polyphenol content (TPC). The experiment was conducted on tomato plants of the Rio Grande variety, with the following treatments: ATY formulation (*A. sativum* + *T. vulgaris* + *Y. schidigera*) at 8.0 L ha⁻¹, ATY formulation at 12.0 L ha⁻¹, chemical control (a.i. cadusafos, C₁₀H₂₃O₂PS₂) at 10.0 L ha⁻¹, positive control (inoculated), and negative control (non-inoculated). Phytochemicals with nematicidal properties, such as alkaloids, flavonoids, terpenes, saponins, and tannins, were detected. The ATY formulation (*A. sativum* + *T. vulgaris* + *Y. schidigera*) at doses of 8.0 and 12.0 L ha⁻¹ reduced the number of galls up to 97.29% and juvenile populations (J2) of *Meloidogyne* spp. up to 98.5%, and improved the plants' physiological development.

Additional key words: sustainable agriculture; biological nematicides; root-knot nematodes; plant-derived oils; plant extracts.

RESUMEN

El cultivo de tomate es uno de los más importantes a nivel mundial debido a su alta producción en diversos países. Sin embargo, el tomate es afectado por el nematodo fitopatógeno *Meloidogyne* spp., lo que reduce su rendimiento y calidad. Para manejar este problema, comúnmente se utilizan nematicidas sintéticos. No obstante, el uso de estos nematicidas impacta el microbioma del suelo y altera su estructura, afectando la porosidad del suelo y teniendo efectos sobre la materia orgánica. En contraste, los aceites y extractos vegetales han surgido como métodos alternativos para el control de *Meloidogyne* spp. El objetivo de esta investigación fue caracterizar los aceites de *Thymus vulgaris* y *Allium sativum*, junto con un extracto etanólico de *Yucca schidigera*, y evaluar su actividad nematicida cuando se incorporan en una formulación contra *Meloidogyne* spp. en condiciones de invernadero. La caracterización fitoquímica se llevó a cabo mediante técnicas cualitativas y mediciones de capacidad antioxidante (CA) y contenido total de polifenoles (CTP). El experimento se realizó en plantas de tomate de la variedad Río Grande, con los siguientes tratamientos: formulación ATY (*A. sativum* + *T. vulgaris* + *Y. schidigera*) a 8.0 L ha⁻¹, formulación ATY a 12.0 L ha⁻¹, control químico (i.a. cadusafos, C₁₀H₂₃O₂PS₂) a 10.0 L ha⁻¹, control positivo (inoculado) y control negativo (no inoculado). Se detectaron fitoquímicos con propiedades nematicidas, como alcaloides, flavonoides, terpenos, saponinas y taninos. La formulación ATY (*A. sativum* + *T. vulgaris* + *Y. schidigera*), en dosis de 8.0 y 12.0 L ha⁻¹, redujo el número de agallas hasta en un 97.29% y la población de juveniles (J2) de *Meloidogyne* spp. hasta en un 98.5%, además de mejorar el desarrollo fisiológico de las plantas.

Palabras clave adicionales: agricultura sostenible; nematicidas biológicos; nematodo agallador; aceites vegetales; extractos vegetales.

INTRODUCTION

Tomato crop is one of the most widely produced vegetables worldwide; however, it is affected by phytosanitary problems caused by phytopathogenic microorganisms such as fungi, bacteria, viruses, and plant-parasitic nematodes (Tucuch-Pérez *et al.*, 2021). The plant-parasitic nematode *Meloidogyne* spp. are considered the more destructive worldwide in tomato, which has a global production nearing 186,107,972.48 t (Sithole *et al.*, 2021; FAO, 2024). In this regard, it is estimated that *Meloidogyne* spp. can cause losses of 70 % in tomato crops where it is detected (Padilla-Hurtado *et al.*, 2022). Consequently, farmers are compelled to control these plant-

parasitic nematodes with the application of synthetic nematicides due to their effectiveness and rapid action, such as organophosphates, carbamates, metham sodium, methyl bromide, aldicarb, oxamyl, etoprop, fenamifos, and 1,3-dichloropropene (Chen *et al.*, 2020).

However, it has been documented that the continuous and indiscriminate use of chemical nematicides causes environmental problems such as soil degradation, greenhouse gas pollution, and human health impacts, as they exhibit carcinogenic, cytotoxic, and mutagenic effects (Sithole *et al.*, 2021). In contrast, plant oils and extracts represent an attractive option owing to the high number of phytochemicals present in their metabolism, such as terpenoids, alkaloids, flavonoids, tannins, among others, which have been associated with nematostatic or nematicidal activity, acting as repellents, deterrents, respiratory poisons, or contact toxins (Al-Saleem *et al.*, 2018; Tucuch-Pérez *et al.*, 2023).

In this regard, several studies have demonstrated the capacity of plant extracts to control *Meloidogyne* spp., such as the species *Argemone mexicana* (L.) and *Citrullus colocynthis* (L.) Schrad. which contain phytochemicals as flavonoids, tannis and saponins with nematicidal activity against *Meloidogyne* spp. (Khan *et al.*, 2017). Additionally, studies have shown that phytochemicals compounds from *Larrea divaricate* (Cav.) and *Phoenix dactylifera* (L.) species exhibit nematicidal effects against *Meloidogyne* spp. (Alam and El-Nuby, 2019; Gómez *et al.*, 2021).

The current trend in agriculture has focused on shifting production systems towards organic and sustainable farming by using biorational and biological products such as plant extracts and beneficial microorganisms. Therefore, the objective of this research was phytochemically characterize oils from the plant species *Allium sativum* and *Thymus vulgaris*, as well as an ethanolic extract of *Yucca schidigera*, and determined their biological efficacy when combined in a formulation (Formulation ATY) against the root-knot nematode *Meloidogyne* spp. in tomato crop under greenhouse conditions.

MATERIALS AND METHODS

Obtaining plant extracts

The oils of *A. sativum* and *T. vulgaris*, the extract of *Y. schidigera*, and the ATY formulation were provided by the company GreenCorp Biorganiks de México S.A. de C.V.

Phytochemical analysis of oil from *Allium sativum* and *Thymus vulgaris*, and ethanolic extract from *Yucca schidigera*

The presence or absence of phytochemicals was determined by colorimetric methods, observing color changes when a phytochemical was present in the extracts. The extracts were used at 100% concentration. The tests performed included: alkaloids (Dragendorff and Sonheschain reagents), carbohydrates (Molisch's reagent), carotenoids (H₂SO₄ and FeCl₃ reagents), coumarins (Ehrlich's reagent), flavonoids (Shinoda and 1% NaOH reagents), reducing sugars (Fehling and Benedict's reagents), cyanogenic glycosides (Grignard's reagent), purines (HCl test), quinones (NH₄OH and H₂SO₄ reagents for anthraquinones and Börntrager's test for benzoquinones), saponins (foam test, Bouchard's reagent for steroidal saponins, and Rosenthaler's reagent), terpenoids (Ac₂O reagent), and tannins (FeCl₃ and ferrocyanide reagents) (Arredondo-Valdés *et al.*, 2021).

DPPH antioxidant capacity in oil extracts of *Allium sativum* and *Thymus vulgaris*, and ethanolic extract of *Yucca schidigera*

The antioxidant capacity (AC) of DPPH (2,2-difenil-1-picrilhidrazilo) was determined using the methodology proposed by Ramírez-Méndez *et al.* (2024), with some modifications. A 10 µL aliquot of each oil and extract was placed in a 96-well polystyrene microplate, followed by the addition of 290 µL of DPPH reagent prepared in absolute methanol (CH₃OH) at a concentration of 100 µM. The reaction was carried out by incubating the samples in the dark for 30 min. After the reaction time, the plates were read at 515 nm. The DPPH inhibition percentage of the extracts was calculated using the absorbance of 80 % methanol as the control. The AC of DPPH was determined using the following Eq. 1:

$$\text{DPPH Inhibition (\%)} = (\text{Absorbance of the control} / \text{Absorbance of extract}) \times 100 \quad (1)$$

Total polyphenol content in extracts of *Allium sativum* and *Thymus vulgaris*, and ethanolic extract of *Yucca schidigera*

Total polyphenol content (TPC) was determined using the Folin-Ciocalteu method. A 10 µL aliquot of each oil and extract was diluted with 80% methanol. A total of 790 µL of sterile distilled water, 50 µL of Folin-Ciocalteu reagent, and 150 µL of 20% sodium carbonate (Na₂CO₃) were added. The samples were kept in the dark for 1 h, and absorbance was measured at 765 nm.

The TPC (mg g⁻¹) was determined from a gallic acid a calibration curve prepared using gallic acid (C₇H₆O₅) and expressed as gallic acid equivalents (GAE) (Ramírez-Méndez *et al.*, 2024).

Biological effectiveness study under greenhouse conditions

Inoculation of *Meloidogyne* spp. in pots. The Research and Agronomic Development Department of GreenCorp Biorganiks de México S.A. de C.V. provided the inoculum, identified with the code MGP001. Saladette tomato plants (Rio Grande variety) were used (with 30 days of development), which were transplanted into 4 kg pots. The substrate consisted of a 1:1 mixture of soil and sterilized peat moss. The inoculation with juveniles in J2 stage of *Meloidogyne* spp. was performed 1 week after transplantation, using two pots as the experimental unit. Pure cultures of *Meloidogyne* spp. were maintained on tomato plants. Intact roots were extracted, and egg masses were subsequently collected. The eggs were kept at 27°C for 96 h to obtain second-stage juveniles (J2). The inoculation was conducted using approximately 1,500 J2 juveniles and 500 eggs per pot (Kankam and Adomako, 2014).

Application of treatments. The treatments were applied 3 d after inoculation using a backpack sprayer. The application method was via drench, with 50 mL per plant. Three applications were performed, with a 15-d interval between each application. The experimental design was a completely randomized design with six replicates per treatment. The treatments used in the study were five: Formulation ATY (*A. sativum* + *T. vulgaris* + *Y. schidigera*) at 8.0 L ha⁻¹, Formulation ATY (*A. sativum* + *T. vulgaris* + *Y. schidigera*) at 12.0 L ha⁻¹, chemical control (i.a cadusafos) at 10.0 L h⁻¹, positive control (inoculated), and negative control (Non-inoculated, only water).

Evaluated variables. The evaluation of variables was conducted two months after inoculation. The severity of infection was assessed by counting the number of galls per 100 g of root and the juvenile population (J2)/100 g of substrate. For the number of galls, 100 g of root were weighed, and the number of galls present on the roots was counted. On the other hand, juveniles in the soil were extracted using the centrifugal flotation technique with sugar, taking 100 g of soil from each pot, and they were observed and counted under the microscope. Additionally, the dry weight of roots and foliage was determined.

Statistical analysis

The biological efficacy test was established under a completely randomized design with six replicates per treatment. The data from the evaluated variables were subjected to ANOVA and a mean comparison test was performed with Tukey ($P \leq 0.05$), using SAS software version 9.0.

RESULTS AND DISCUSSION

Phytochemical analysis of *Allium sativum* and *Thymus vulgaris* oils, and ethanol extract of *Yucca schidigera*

The analysis showed variations in the phytochemical profiles of the three materials examined, in the case of *A. sativum* oil, alkaloids, carbohydrates, coumarins, flavonoids, reduced sugars, saponins, terpenes, and tannins were observed; for *T. vulgaris* oil, carbohydrates, coumarins, flavonoids, quinones, terpenoids, saponins, and tannins were detected. Finally, the analysis of the ethanol extract of *Y. schidigera* exhibited alkaloids, carbohydrates, flavonoids, saponins, terpenoids, and tannins.

Table 1. Phytochemical compounds identified in *A. sativum* oil, *T. vulgaris* oil, and *Y. schidigera* ethanolic extract.

Plant species	Phytochemical compounds											
	A	C	Ca	Cu	F	AR	GC	P	Q	S	TE	TA
<i>Allium sativum</i>	+	+	-	+	+	+	-	-	-	+	+	+
<i>Thymus vulgaris</i>	-	+	-	+	+	-	-	-	+	+	+	+
<i>Yucca schidigera</i>	+	+	-	-	+	-	-	-	-	+	+	+

+ = phytochemical present; - = phytochemical not present; A = alkaloids; C = carbohydrates; Ca = carotenoids; Cu = coumarins; F = flavonoids; AR = reducing sugars; GC = cyanogenic glycosides; P = purines; Q = quinones; S = saponins; TE = terpenoids; TA = tannins.

The phytochemical compounds in plant oils and extracts can vary due to various factors such as the environment, harvest time, species, etc. As a result, several authors have identified botanical compounds present in *A. sativum*, such as alkaloids, saponins, flavonoids, and tannins, which provide various interesting properties, including antimicrobial activity (Matsuura, 2001; Baek *et al.*, 2019; El-Saber *et al.*, 2020; Akullo *et al.*, 2023). In this context, the phytochemical characterization of compounds present in *T. vulgaris* has also been conducted, and compounds such as flavonoids, alkaloids, saponins, terpenoids, and glycosides have been described (Saleem *et al.*, 2022). Finally, *Y. schidigera* has also been studied, as plants from the Agavaceae family

are rich in phytochemical compounds. Botanical compounds such as saponins, flavonoids, and terpenoids have been reported in this species (Góngora-Chi *et al.*, 2024; Fan *et al.*, 2024). All these compounds present in oils and extracts confer various biological activities, enabling them to be used by plants to resist diseases and infections due to their antimicrobial and antioxidant properties (Saleem *et al.*, 2022).

Antioxidant capacity of DPPH and total polyphenol content in *Allium sativum* oil, *Thymus vulgaris* oil, and *Yucca schidigera* ethanol extract

Regarding the AC, statistical differences were observed between *T. vulgaris* oil and *A. sativum* oil, and *Y. schidigera* extract, with *T. vulgaris* oil showing the highest AC at 92.1% ($P=0.003$). On the other hand, for TPC, statistical differences were observed among all treatments, with *T. vulgaris* oil again exhibiting the highest TPC at 132.4 GAE mg⁻¹ ($P=0.021$) (Tab. 2). These results suggest that *T. vulgaris* oil has a higher capacity to neutralize free radicals than the other two plant species, which may have a lower capacity to act on free radicals.

Several authors have reported AC and TPC in extracts that control phytopathogenic microorganisms, for example, Nhung and Quoc (2024) observed an AC of 59.67 to 92.13% in a *Chromolaena odorata* (L.) R.M.King & H.Rob. extract when used as a nematicide against the nematode *Radopholus similis* (Cobb) Thorne. Regarding TPC, Ramírez-Méndez *et al.* (2024) described a TPC of 61 mg per g and 112 mg per g in extracts of *Fouquieria splendens* (Engelm.) and *Agave striata* (Zucc.), respectively, for the control to 100% of *Pythium aphanidermatum* (Edson.) Fitzp. and *Rhizoctonia solani* (Kühn.).

Table 2. Antioxidant capacity and total polyphenol content in *A. sativum* and *T. vulgaris* oils, and *Y. schidigera* ethanolic extract.

Plant species	Antioxidant capacity (%)	Total polyphenol content (GAE per mg)
<i>Allium sativum</i>	84.2±1.7 b	78.9±2.0 b
<i>Thymus vulgaris</i>	92.1±1.2 a	132.4±1.8 a
<i>Yucca schidigera</i>	80.1±2.2 b	57.2±1.4 c

Literals with the same letter are statistically equal (Tukey, $P\leq 0.05$).

Biological effectiveness study under greenhouse conditions

The phytochemical compounds in plant oils and extracts confer nematocidal activity, as evidenced by reduced root damage caused by *Meloidogyne* spp. nematodes. This research

observed statistical differences in the number of galls/100 g of root across all treatments compared to the positive control. A significant reduction in galls was observed in the treatments with the ATY formulation at doses of 12.0 and 8.0 L ha⁻¹, which presented 3.2 and 15 galls/100 g of root ($P=0.0015$) (Fig. 1), showing a similar effect to the chemical control with the a.i. Cadusafos. Similarly, the juvenile population (J2) of *Meloidogyne* spp. per 100 g of substrate was also affected by the applied treatments, with statistical differences observed between all treatments compared to the positive control. The ATY formulation at a dose of 12.0 L ha⁻¹ induced the lowest population with 2.5 J2 *Meloidogyne* spp./100 g of substrate, followed by the 8.0 L ha⁻¹ dose with 4.7 J2 *Meloidogyne* spp./100 g of substrate ($P=0.047$). Once again, a similar effect to the chemical control was observed with 1.75 J2 *Meloidogyne* spp./100 g of substrate (Fig. 2).

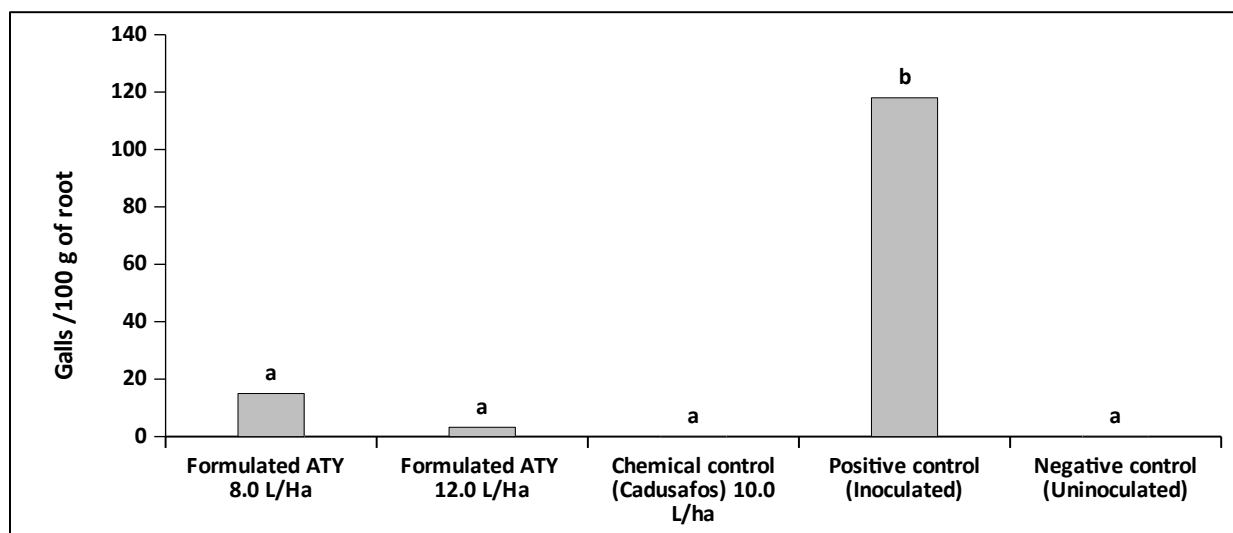


Figure 1. Effect of the formulation based on *A. sativum* and *T. vulgaris* oils mixed with an ethanolic extract of *Y. schidigera* on the number of galls of *Meloidogyne* spp. Literals with the same letter are statistically equal (Tukey, $P\leq 0.05$).

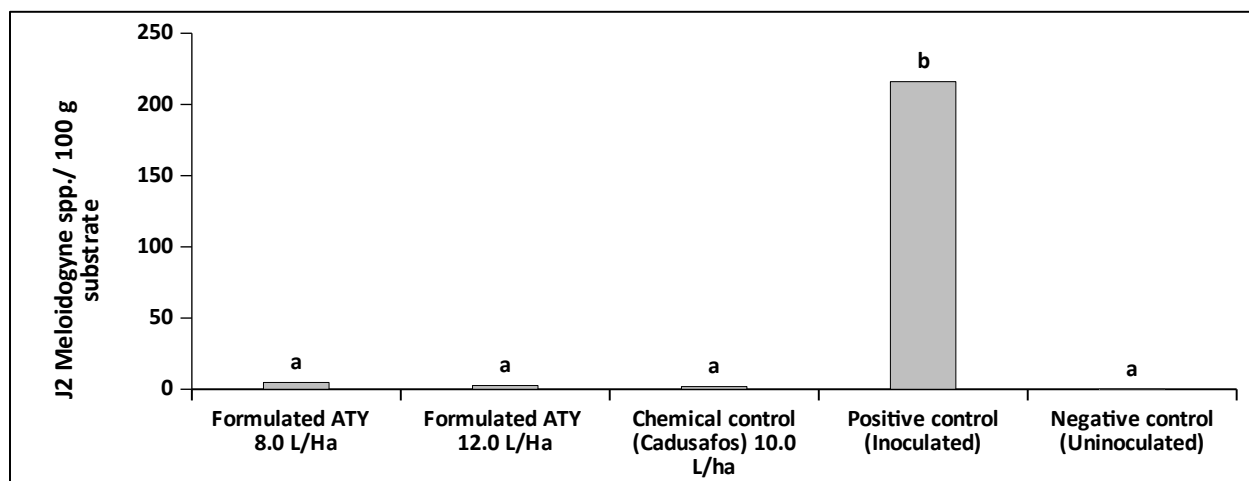


Figure 2. Effect of the formulation based on *A. sativum* and *T. vulgaris* oils mixed with an ethanolic extract of *Y. schidigera* on the juvenile (J2) population of *Meloidogyne* spp. literals with the same letter are statistically equal (Tukey, $P \leq 0.05$).

Reducing the population of phytopathogenic nematodes helps improve the physiological development of plants. Statistical differences were once again observed between treatments compared to the positive control for the variable corresponding to root dry weight. Thus, plants treated with the positive control produced the least root dry weight, with 10.2 g, while the treatments with the formulated ATY induced the highest root dry weight in the tomato plants, with 23.27 g for the 8.0 L ha⁻¹ dose and 20.17 g for the 12.0 L ha⁻¹ dose ($P=0.008$) (Fig. 3). Similarly, the dry weight of the foliage also increased as the population of *Meloidogyne* spp. decreased. Statistical differences were observed between all treatments compared to the positive control, except for the chemical control treatment. Once again, the formulated ATY treatments induced the highest production of dry foliage weight, with 48.4 g for the 8.0 L ha⁻¹ dose and 44.77 g for the 12.0 L ha⁻¹ dose ($P=0.032$) (Fig. 4).

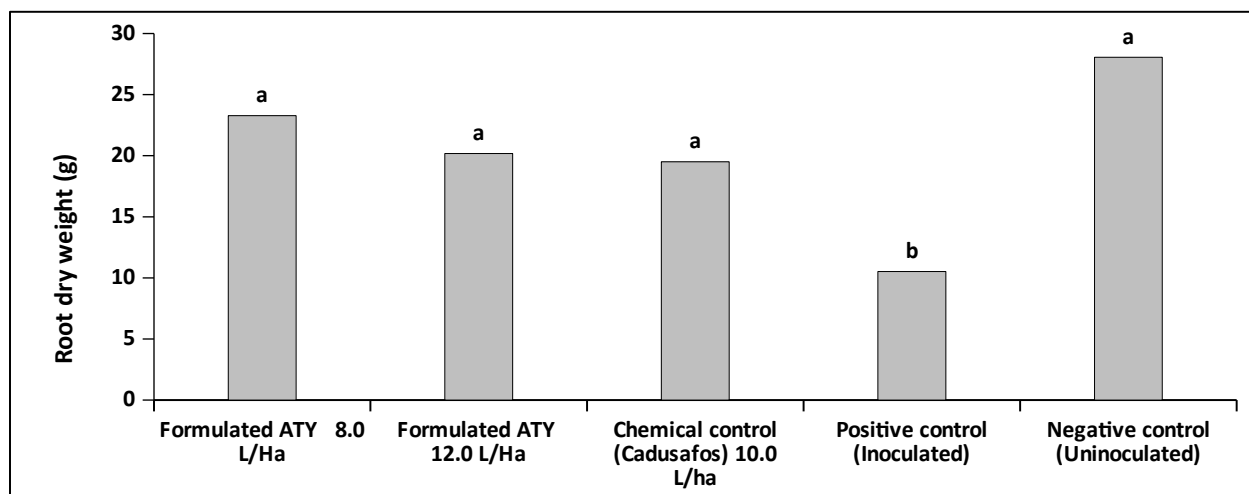


Figure 3. Effect of the formulation based on *A. sativum* and *T. vulgaris* oil mixed with an ethanolic extract of *Y. schidigera* on the root dry weight in tomato plants. Literals with the same letter are statistically equal (Tukey, $P \leq 0.05$).

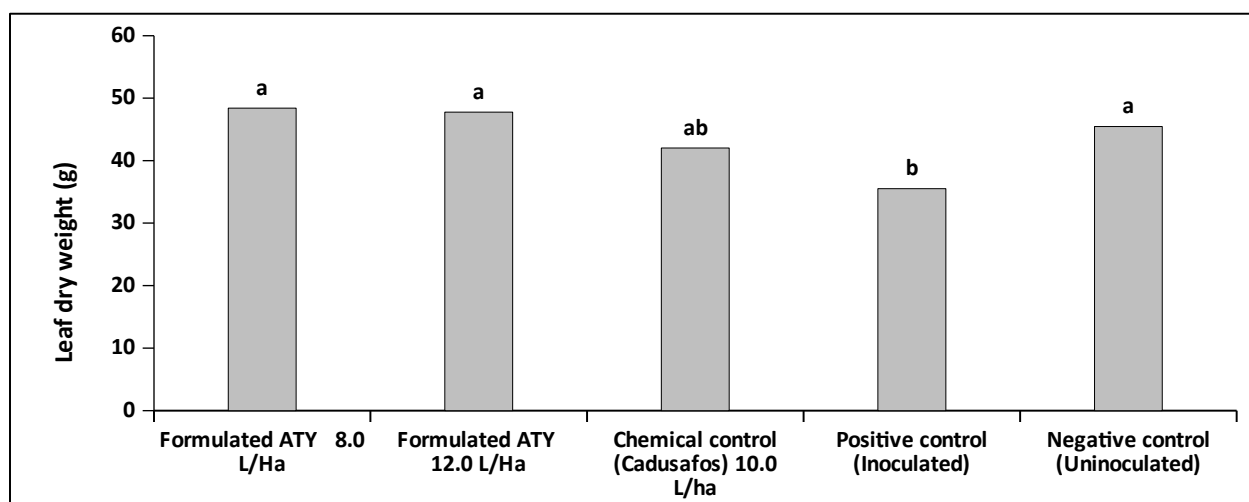


Figure 4. Effect of the formulation based on *A. sativum* and *T. vulgaris* oil mixed with an ethanolic extract of *Y. schidigera* on the foliage dry weight in tomato plants. Literals with the same letter are statistically equal (Tukey, $P \leq 0.05$).

Plants produce phytochemicals as part of their metabolism. Some of the biochemical processes involved in the production of bioactive compounds are the phenylpropanoid pathway, which, through shikimic acid and the production of phenylalanine, induces the synthesis of phenolic compounds such as flavonoids, tannins, and coumarins (Lin *et al.*, 2016), and mevalonate pathway, which synthesizes terpenes that serve as precursors for saponins (Couillaud *et al.*, 2021). Due to their nematicidal activity, all of these compounds are present in plant oils and

extracts, making them a sustainable alternative for the control of phytopathogenic microorganisms such as nematodes of the genus *Meloidogyne* (Tucuch-Pérez *et al.*, 2023).

Thus, the effect observed in the treatments on the variables evaluated in this study may be attributed to the presence of all phytochemicals in the oils of *A. sativum* and *T. vulgaris* and the extract of *Y. schidigera*. These compounds, at higher doses, present higher concentrations, which provide protection against nematodes, allowing for improved physiological development of the plants. It has been described that alkaloids inhibit acetylcholinesterase activity, causing an instability between the neurotransmitters acetylcholine and γ -aminobutyric acid, affecting the nervous system of nematodes (Jang *et al.*, 2015). Flavonoids inhibit egg hatching, possess repellent effects, and activate systemic resistance in plants (Chin *et al.*, 2018). Meanwhile, saponins alter the cellular membranes of nematode cuticles and may decrease cholesterol in the eggs, affecting their development and hatching (D'Addabbo *et al.*, 2020).

Terpenes and tannins also exhibit nematicidal activity through various mechanisms of action. Some studies indicate that terpenes affect the nervous system by inhibiting the cell membrane's tyramine receptors and ion channels (Tucuch-Pérez *et al.*, 2023). Meanwhile, the nematicidal activity of tannins has been correlated with their ability to induce morphological changes in the nematode cuticle, affecting their mobility and their ability to molt in larval stages, in addition to acting on the nervous and digestive systems at the enzymatic level (Guo *et al.*, 2017; Greiffer *et al.*, 2022).

Several authors have reported nematicidal activity from plant oils and extracts. Plants, through their metabolism produce phytochemical compounds with nematicidal activity. It has been documented that species such as *T. vulgaris* and *A. sativum* generate a wide variety of botanical compounds through their metabolism, which, when extracted as oils, have demonstrated efficacy in controlling various nematode genera, including *Meloidogyne* spp., *Pratylenchus* spp., and *Ditylenchus* spp. (Faria *et al.*, 2016; Jardim *et al.*, 2020; Galisteo *et al.*, 2022; Catani *et al.*, 2023). Additionally, plant extracts have also proven to be a viable alternative for the control of plant-parasitic nematodes, for example: Velasco-Azorsa *et al.* (2021) documented that extracts from *Adenophyllum aurantium* (L.) R.M.King & H.Rob., *Alloispermum integrifolium* (DC.) H. Rob. and *Tournefortia densiflora* (Kunth.) showed nematicidal activity against the false root-knot nematode *Nacobbus aberrans* (Thorne). Our results suggest that the combination of *T. vulgaris* and *A. sativum* oils with *Y. schidigera* extract can develop a highly nematicidal formulation,

which could serve as an effective alternative for the control and management of *Meloidogyne* spp.

CONCLUSION

Oils from *A. sativum* and *T. vulgaris* and the extract from *Y. schidigera* contain various phytochemical compounds that confer them nematicidal properties, including alkaloids, flavonoids, saponins, terpenes, and tannins. These bioactive compounds also provide CA and a high CPT. Thus, a formulation was developed to control *Meloidogyne* spp., comprising oil from *A. sativum* and *T. vulgaris* and extract from *Y. schidigera*. This formulation, applied at doses of 8.0 and 12.0 L ha⁻¹, enhanced the physiological development of tomato plants by reducing the population of juvenile *Meloidogyne* spp. (J2), decreasing root gall formation and improving the physiological processes of the plants. Therefore, a formulation based on oil from *A. sativum* and *T. vulgaris* and extract from *Y. schidigera* represents a promising alternative for managing and controlling *Meloidogyne* spp. in tomato crops.

Conflict of interests: The authors declare no conflict of interest.

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