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ORIGINAL ARTICLE

Control of citrus nematode *Tylenchulus semipenetrans* (Tylenchida: Tylenchulidae) using plant-based products under *in vitro* and *in vivo* conditions

Seyedeh Zohreh Asadi[1], Salar Jamali[1]*, Mohammad Ghadamyari[1], Vahid Mottaghitalab[2]

[1] Plant Protection Department, Faculty of Agricultural Sciences, University of Guilan, Rasht 41635-1314, Iran
[2] Textile Engineering Department, University of Guilan, Rasht 41635-1314, Iran

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*Corresponding address:
jamali_s2002@yahoo.com

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Abstract

The citrus nematode (*Tylenchulus semipentrans*) is one of the most important parasitic nematodes affecting citrus trees, causing gradual decline and reduced yield. Potential risks, high costs and environmental consequences of chemical compounds have led researchers to explore non-chemical methods such as using plant-based products for nematode management. The present study was conducted to control citrus nematodes using essential oil and water extract of *Artemisia annua* and methanolic extract of *Melia azederach* under laboratory and greenhouse conditions. *In vitro* bioassays were carried out, and the effects on toxicity, mortality, and egg hatching were assessed. The highest *in vitro* nematistatic activity was recorded for 250 ppm of *A. annua* essential oil and 500 ppm of *M. azederach* methanol extract by 100% paralysis of nematodes after 48 h. Furthermore, the highest nematicidal activity of *A. annua* essential oil, aqueous extract and methanolic extract of *M. azederach* was recorded to be about 60–100%, 40–87% and 38–100%, respectively. Among all concentrations of *M. azederach* methanolic extract and high concentrations of *A. annua* essential oil and aqueous extract, the repellents and motility inhibitors for nematodes were found. The results of egg hatching showed that essential oil of *A. annua* at a concentration of 250 ppm had the greatest reduction of egg hatching. In a greenhouse experiment, all the treatments were found to be significantly effective against the citrus nematode population in soil and roots compared to the control. Maximum reduction was observed in 500 ppm of methanolic extract of *Melia azederach*. Growth parameters (plant height, fresh and dry shoot and root weight) increased compared to the control when treatments were applied. Based on the results, plants such as *A. annua* and *M. azedarach* are considered to be promising control agents for citrus nematodes. The results indicate that products derived from these plants may be potential candidates for formulating new nematicides suitable for sustainable nematode management, although field trials are still needed to demonstrate their effectiveness for commercial use.

Keywords: *Artemisia annua*, citrus, essential oil, *Melia azederach*, *Tylenchulus semipenetrans*

Introduction

Citrus orchards are infested with a wide range of plant-parasitic nematodes (PPN), but *Tylenchulus semipenetrans* (Cobb, 1913) is the most damaging citrus nematode, causing the gradual decline and reduced yield of citrus worldwide. It is a semi-endoparasitic nematode that feeds on deeper cortical cells. Maximum yield loss due to *T. semipenetrans* is estimated to be between 10 to 30% depending on the level of infestation, nematode population, soil characteristics, rootstock susceptibility, and management methods (Duncan and Cohn 1990). In recent years, nematode control in citrus has been achieved using fumigant and non-fumigant nematicides. Chemical nematicides, however, require better alternatives for nematode management due to their harmful effects on the environment, non-target organisms, and plants. A wide spectrum of plant

materials with nematicidal activity has been reported for the development of environmentally friendly alternatives (Atolani and Fabiyi 2020). Phytochemicals have been widely reported as probable sources of bioactive elements for the development of natural nematicides (Oka 2001).

The use of plant materials or extracts in nematode-infested soils either directly affects soil microbes or stimulates them to reduce nematode populations (Ahmad *et al*. 2004). *Artemisia* spp. (Mugwort), belonging to the Asteraceae family, is known for its nematicidal properties in citrus orchards. More than 1000 biodynamic compounds and various types of secondary metabolites such as polyacetylenes, phenolic hydrocarbons, oxygenated aliphatic hydrocarbons, furans, flavonoids, alkamides, and coumarins have been reported from *Artemisia* spp. (Saadali *et al*. 2001). Essential oils can be used as biocontrol agents with antibacterial, antifungal, repellent, insecticidal, nematicidal, and phytotoxic effects. In this regard, several researchers have identified potential new biopesticides from *Artemisia* genus. Most *Artemisia* compounds have insecticidal, antiulcer, antidiabetic, antispasmodic, antifungal, anticancer, antimalarial, antibacterial, antioxidant, antihistaminic, anthelmintic, and anti-allergic properties from a pharmacological point of view (Bora and Sharma 2011).

Researchers have reported nematicidal activity of *Artemisia annua* against the mortality of second-stage larvae and the prevention of egg hatching. For example, *A. annua* extracts have been found to be effective against various nematodes, including *Meloidogyne* spp. and *Pratylenchus* spp. (Dias *et al*. 2000; Shakil *et al*. 2004; Zahabi Asl 2011; D'Addabbo *et al*. 2013; Jalali Sendi and Khosravi 2014; D'Addabbo *et al*. 2017), *A. vulgaris* (Costa *et al*. 2003), *A. judaica*, *A. arborescens* and *A. dracunculus* (Oka *et al*. 2000), *A. herba-alba* (Avato *et al*. 2017), *A. absinthium* (García-Rodríguez *et al*. 2015, Kundu *et al*. 2021), *A. elegantissimia* and *A. incisa* (Khan *et al*. 2019), against root knot nematode, *Meloidogyne* spp.

Another medicinal plant with nematicidal properties is Cinnaberry (*Melia azedarach* L.) from the Meliaceae family. It mainly grows in northern Iran and warm and tropical regions of the country. In the mid-twentieth century, scientists began using the Meliaceae family to control nematodes. Nematicidal activity of *Azadirachta indica* L. from this family was discovered in India (Singh and Sitaramaiah 1970). *Melia azedarach* from this family also has insecticidal properties that have been reported in many studies (Abou-Fakhr Hammad *et al*. 2001; Banchio *et al*. 2003; Carpinella *et al*. 2003; Sengottayan Senthil and Sehoon 2006; Nathan 2006) and does not have negative effects on beneficial insects (Charleston *et al*. 2005), indicating its potential use in biological control programs. The mortality of nematodes was improved by increasing the exposure time to *A. indica* extract. Under laboratory conditions, the cherrylaurel treatment had the highest larval mortality. The 'S' standard extract (50 g of plants were mixed in 100 ml of water and the extract was prepared) was more lethal to *T. semipenetrans* larvae than other concentrations (Ahmad *et al*. 2004). Extracts from *A. indica* leaves, *Allium sativum* Woodville (Garlic) and *Tagetes erecta* L. (African marigold) were tested against *Meloidogyne incognita* on tomato under *in vitro* conditions, in pots and under field conditions. The highest effect of neem extract was observed after 24 and 48 h. In soil, all treatments significantly reduced root galling, nematode population, and increased plant growth and yield (Abo-Elyousr *et al*. 2010). Increasing the concentration of plant extracts and exposure time to *A. sativum* L., *Brassica campestris* L., *Capsicum frutescents* L., *Glycyrrhiza glabra* L., *Datura innoxia* L., *Chenopodium botrys* (L.) Mosyakin & Clemants and *Foeniculum vulgare* Mill. increased the mortality of *T. semipenetrans* in laboratory experiments. In pot trials, the nematode population in the seedlings treated with *A. sativum*, *C. frutescents* and *F. vulgare*, decreased compared to the control (Ayazpour *et al*. 2010). Aqueous extracts of marigold leaves and flowers, castor beans and garlic controlled the root-knot nematode on tomato plants (Tibugari *et al*. 2012).

Because of studies and obtained results, it is to be expected that the essential oil and aqueous extract of *A. annua* and methanolic extract of *M. azedarach* extract will have a favorable nematicidal effect on citrus nematodes. Accordingly, the aim of this study was to investigate the nematicidal potential of the essential oil (AEO) and aqueous extract of *A. annua* (AWE) and the methanolic extract of *M. azedarach* (MME) under *in vitro* and *in vivo* conditions, and to determine their efficacy against the second-stage larval mortality, egg hatching of *T. semipenetrans*, as well as their chemotaxis behavior and growth parameters of *Citrus aurantifolia*.

Materials and Methods

Isolation of nematodes

In the spring of 2022, root samples of *C. aurantifolia* (Mexican lime) and soil surrounding the roots were collected from citrus orchards in northern Iran. Soil samples were collected approximately 1.5 m from the trunk at a depth of 20–30 cm using a handheld auger (approximately 6 cm in diameter and 20–30 cm in depth) from three sides of each tree (El-Nagdi *et al*. 2010; Bakr *et al*. 2011). Three samples from each tree were thoroughly mixed, and a reference sample of about 500 g was prepared. The tray method was used to extract live nematode larvae from the soil (Whitehead and Heming 1965). The samples were kept at 4°C.

Nematode eggs were extracted from the roots (Hussey and Barker 1973). The number of individuals was counted using a light microscope (Amen and Hasabo 1995). Citrus nematode, *T. semipenetrans,* was identified based on the morphology of the adult and larval stages, according to established classification works (Crozzoli *et al.* 1998).

Preparation of essential oil and extracts

Artemissia annua was collected from Rasht and its essential oil was extracted from dried leaves and stems. Essential oil of *A. annua* extraction was carried out using a Clevenger type apparatus, using 50 g of dried plant powder mixed with 750 ml of distilled water. Extraction lasted for about 2 h, and the collected essences were separated by sodium sulfate and transferred to brown glass vials and stored at 4°C in a refrigerator. To prepare the water extract of *A. annua*, 150 g of aerial parts of *A. annua* were chopped and boiled in 750 ml of water for 15 min. The extract was then filtered through filter paper (Whatman).

The seeds of *M. azedarach* were collected from Ramsar province and dried after washing with water. The method of Ardakani *et al.* (2011) was used to prepare the methanolic extract of *M. azedarach*. Spectrophotometric and gas chromatography-mass spectrometry (GC-MS) analyses were performed using an Agilent GC-7890A with helium gas as the carrier (Oftadeh *et al.* 2016).

In vitro experiments

Toxicity assay

The nematicidal potential of *A. annua* essential oil and aqueous extract and *M. azedarach* methanol extract were evaluated against citrus nematode at concentrations of 62.5, 125, 187.5, and 250 ppm (essential oil of *A. annua*) and 125, 250, 375, and 500 ppm (water extract of *A. annua* and methanolic extract of *M. azedarach*) for 24 and 48 h of exposure.

A suspension of 100 *T. semipenetrans* J2 in 0.5 ml of distilled water was poured into each well of a 24-well cell culture plate. The nematodes were allowed to settle for 10 min, and excess water was removed with a micropipette. Concentrations of the essential oil of *A. annua* and methanolic extract of *M. azedarach* were prepared in dimethyl sulfoxide (DMSO). One milliliter of each concentration was added to each well in four replicates. Fosthiazate was used as a positive control, and DMSO and distilled water were used as negative controls. The plates were incubated in the dark at 22 ± 2°C. At the end of each exposure period, nematodes were observed under a light microscope, and the number of immobile nematodes was counted. Nematodes in each treatment were washed twice with distilled water and kept for an additional 24 h to evaluate the potential nematicidal activity of the plant extracts. The absence of movement after immersion in water was considered evidence of nematode mortality.

Hatching assay

To evaluate egg hatching inhibition, approximately 20–25 nematode eggs were exposed to different concentrations of the extracts, and incubated at 24 ± 2°C for 7 days and the percentage of hatched eggs was recorded after this time. The experiments were conducted with four replicates and DMSO (AEO, MME) and DW (AWE) were used for controls.

Chemotaxis assay

In this experiment, repellent/attractant activity was measured using the Wuyts *et al.* (2006) method. Petri dishes were divided into 16 sections and 0.5% agar solution was poured into Petri dishes (5 cm in diameter). Then, 100 µl of test compounds at four concentrations (AEO: 62.5, 125, 187.5, and 250 ppm; AWE, MME: 125, 250, 375, and 500 ppm) were added. Distilled water (DW) was used as a control, 1% acetic acid as a repellent, and 1 M calcium chloride ($CaCl_2$) as an attractant. Twenty nematodes were added to the inner circle, and the nematode movement was stopped by spraying 70% ethanol on the Petri dishes. The results were recorded after 2 h using the scoring system proposed by Wuyts *et al.* (2006) based on the nematode location (Cf: chemotaxis factor).

In vivo tests

In the experiment to test the nematicidal activity of plant essential oils and extracts against *T. semipenetrans*, 6-month-old *C. aurantifolia* seedlings were planted in plastic pots containing approximately 2 kg of sterilized sandy loam soil (2 : 1). Each pot was inoculated with 4000 J2 nematodes in four holes around the citrus seedling stem. The pots were incubated at 25°C (day) and 24°C (night). Five days after inoculation, the pots were irrigated with 100 ml of concentrations (62.5, 125, 187.5, and 250 ppm (AEO) and 125, 250, 375, and 500 ppm (AWE and MME)) per pot. Each treatment had four replicates. Fosthiazate was used as a chemical nematicide, and a non-infected control and an infected control treated with the extract were included. After 70 days, the seedlings were carefully removed from the pots and the roots were washed with water. Data on plant height, fresh and dry shoot and root weight, final nematode population in 100 g of soil, and the number of females per gram of root were recorded. A completely randomized experimental design was used in both cases.

Statistical analysis

The data were analyzed using the SAS software package and subjected to analysis of variance (ANOVA). Mean comparisons were performed using the Tukey multiple comparison test ($p < 0.05$). The LD values were determined using the Polo-Plus software (2002). The laboratory experiments had four replicates, and 100 J2 were used in each replicate. The greenhouse experiment consisted of four replicates with an initial inoculation of 4000 *T. semipenetrans* J2 population.

Results

Chemical composition of plant extracts

Artemissia annua essential oil contains approximately 31 chemical compounds, including α-pinene (21.17%), camphor (15.02%), beta-selinene (9.3%), 1,8-cineole (6.46%), pinocarvone (3.6%), and germacrene-d (5.61%). Arteannuin B (30.2%), 4-vinylguaiacol (16.27%), scopoletin (8.64%), camphor (8.8%), trans--pinocarveol (4.43%), and 4'-methylxanthotoxin (4.09%) are present in *A. annua* aqueous extract. The main coumarins present in *M. azederach* methanolic extract are phenol, 4-ethyl-2-methoxy-phenol (55.18%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (14.08%), 5-methyl-2-furfural (7.14%), methyl succinyl anhydride (3.85%), and guaiacol (3.74%).

In vitro tests

The nematicidal activity of AEO at concentrations of 62.5, 125, 187.5, and 250 ppm, AWE and MME at concentrations of 125, 250, 375, and 500 ppm against citrus nematode was evaluated, and the results are presented in Table 1. Mortality rates increased with increasing concentrations of essential oils and extracts and exposure time. In all treatments, the highest percentage of mortality was observed at the highest concentration and 48 h after treatment. As shown in Table 1, the maximum larval mortality (100%) was calculated at the highest concentration by MME and AEO.

Table 1. Nematicidal effects of the essential oil and aqueous extract of *Artemisia annua* and the methanolic extract of *Melia azedarach* on infective juveniles of *Tylenchulus semipentrans*

Treatment	Concentration [ppm]	Immobile J2 after 24 h	Immobile J2 after 48 h	Dead J2 after 24 h water treatment
Essential oil of *A. annua*	62.5	43 ± 1.77 d	60 ± 1.87 d	60 ± 1.87 d
	125.0	48 ± 1.47 c	78 ± 1.22 c	78 ± 1.22 c
	187.5	58 ± 1.22 b	90 ± 1.08 b	90 ± 1.08 b
	250.0	73 ± 3.02 a	100 ± 0.00 a	100 ± 0.00 a
DMSO	–	2 ± 0.4 e	7 ± 0.4 e	10 ± 0.7 e
Control	–	2 ± 0.8 e	5 ± 0.9 e	10 ± 1.3 e
Aqueous extract of *A. annua*	125	29 ± 0.70 d	45 ± 1.08 c	40 ± 1.22 d
	250	36 ± 1.4 c	64 ± 0.70 b	52 ± 0.70 c
	375	43 ± 0.81 b	85 ± 1.63 b	78 ± 1.58 b
	500	58 ± 0.91 a	90 ± 1.08 a	87 ± 0.40 a
Control	–	2 ± 0.8 e	5 ± 0.9 d	10 ± 1.3 e
Methanolic extract of *M. azedarach*	125	40 ± 1.29 c	54 ± 2.67 c	38 ± 1.48 c
	250	63 ± 1.08 b	76 ± 3.16 b	70 ± 1.77 b
	375	78 ± 1.47 a	96 ± 2.12 a	93 ± 0.19 a
	500	83 ± 1.08 a	100 ± 0.00 a	100 ± 0.00 a
DMSO	–	2 ± 0.4 d	7 ± 0.4 d	10 ± 0.7 d
Control	–	2 ± 0.8 d	5 ± 0.9 d	10 ± 1.3 d
Fosthiazate	1	32 ± 1.1 d	51 ± 2.27 d	51 ± 1.27 d
	2	48 ± 1.01 c	71 ± 2.12 c	71 ± 1.12 c
	4	56 ± 1.95 c	94 ± 0.81 b	94 ± 0.81 b
	8	70 ± 1.4 b	100 ± 0.00 a	100 ± 0.00 a
	15	87 ± 1.2 a	100 ± 0.00 a	100 ± 0.00 a
DMSO	–	2 ± 0.4 e	7 ± 0.4 e	10 ± 0.7 e

In each separate column, means followed by different letters designate significant differences at $p < 0.05$ according to Tukey's test
DMSO – dimethyl sulfoxide

After 24 h of exposure to DW, no revival was found for nematodes previously exposed to AEO concentration, while some revival was observed for nematodes previously exposed to AWE and MME concentrations. LD_{50} values for AEO, AWE, and MME were found to be 256, 703, and 464 ppm, respectively.

The analysis of variance showed a significant decrease in egg hatching in all treatments. AEO at a concentration of 250 ppm reduced hatching to 36%, while hatching in the control group was 80% 7 days after treatment (Fig. 1). MME at a concentration of 500 ppm showed the highest inhibition of hatching. The results of the chemotaxis assay showed that MME and AEO had repellent effects at all concentrations (Table 2). Lower concentrations of AWE showed a neutral effect on nematode movement.

Table 2. Response of infected juveniles of *Tylenchulus semipentrans* to essential oil and aqueous extract of *Artemisia annua* and the methanolic extract of *Melia azedarach*

Concentration [ppm]	*T. semipentrans*	$CaCl_2$	Acetic acid	DW
Essential oil of *A. annua*				
62.5	R	A	R	N
125.0	R	A	R	N
187.5	R	A	R	N
250.0	R	A	R	N
Aqueous extract of *A. annua*				
125.0	N	A	R	N
250.0	N	A	R	N
375.0	R	A	R	N
500.0	R	A	R	N
Methanolic extract of *M. azedarach*				
125.0	R	A	R	N
250.0	R	A	R	N
375.0	R	A	R	N
500.0	R	A	R	N

1 M calcium chloride ($CaCl_2$) (attractant), 1% acetic acid (repellent); DW – distilled water; N – neutral; R – repellent; A – attractant

In vivo test

According to our results, the highest amount of growth parameters was related to the chemical nematicides and the highest concentration of essential oils and extracts after the positive control (Table 3). The lowest amount of growth parameters was related to the lowest concentration used after the negative control. The use of essential oils and extracts was more effective in terms of the number of compounds per gram of root and the number of larvae per 100 g of soil. The results showed that, in general, there was no statistically significant difference between the three initial concentrations of essential oils and extracts in growth parameters and nematode population in soil and roots, but there was a significant difference compared to the negative control. The highest reduction in the population of second-stage larvae was recorded for MME at a concentration of 500 ppm. However, the efficacy of all treatments of AEO, AWE, and MME in reducing nematode population and improving seedling growth parameters was lower than that of the chemical nematicide, but in all treatments, the improvement of growth parameters and the reduction of the nematode population compared to the nematode-infested and untreated control were evident.

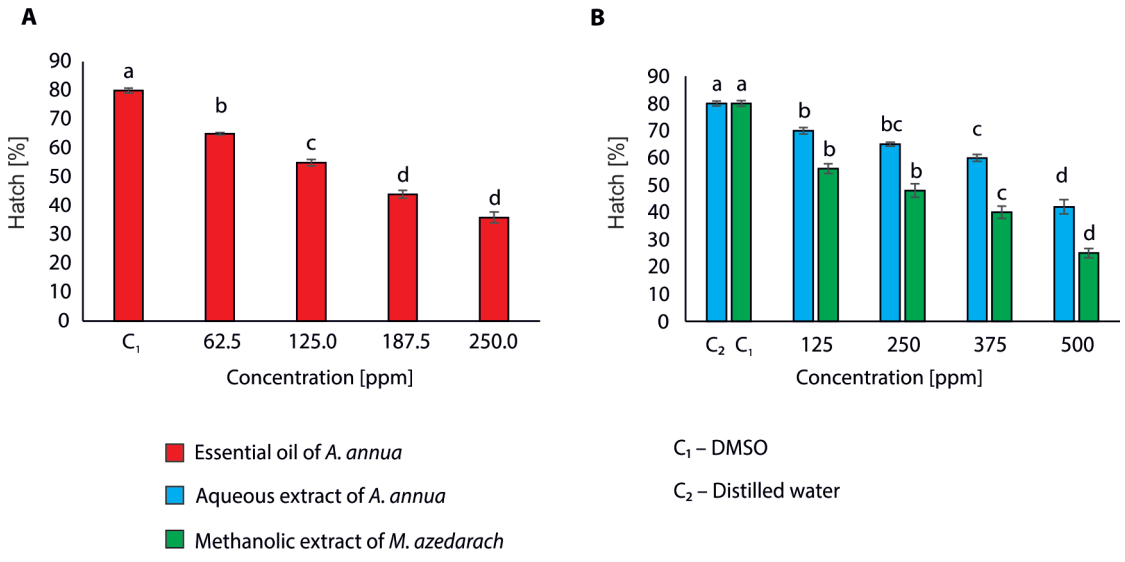

Fig. 1. Hatching percentage of second-stage juveniles (J2) of *Tylenchulus semipenetrans* after 7 days of exposure to essential oil of *Artemisia annua* (A) and aqueous extract of *Artemisia annua* and methanolic extract of *Melia azedarach* (B). Means followed by different letters are significantly different according to Tukey's test ($p < 0.05$)

Table 3. Effect of essential oil of *Artemisia annua* (A), aqueous extract of *A. annua* (B) and methanolic extract of *Melia azedarach* (C) on different growth parameters of *Citrus aurantifolia* and indicators of nematode infection in the presence of *Tylenchulus semipentrans*

A

Concentration [ppm]	Plant length	Foliage wet weight	Root wet weight	Foliage dry weight	Root dry weight	Final population of larvae in 100 gram soil	Final population of females per gram of roots
T1	107 ± 1.2 b	50 ± 1.9 bc	33 ± 1.2 c	20 ± 0.9 c	17 ± 0.5 c	170 ± 1.9 b	70 ± 0.9 b
T2	110 ± 1.2 b	53 ± 1.1 b	35 ± 0.4 c	20 ± 1.08 c	19 ± 1.08 c	168 ± 1.7 b	66 ± 1.9 b
T3	112 ± 1.4 ab	55 ± 1.08 ab	36 ± 0.7 c	23 ± 0.7 bc	20 ± 1.9 c	165 ± 1.2 b	65 ± 1.4 b
T4	112 ± 1.6 ab	56 ± 1.01 ab	35 ± 0.7 c	24 ± 1.4 bc	20 ± 1.09 c	127 ± 1.09 c	55 ± 0.7 c
Chemical nematicide	115 ± 1.8 ab	66 ± 0.7 ab	40 ± 0.4 b	26 ± 0.7 ab	25 ± 1.8 b	100 ± 1.09 d	45 ± 1.6 d
C^+	120 ± 0.4 a	69 ± 1.01 a	50 ± 0.7 a	30 ± 1.07 a	30 ± 0.4 a	0	0
C^-	100 ± 1.7 c	40 ± 1.2 c	22 ± 1.2 d	12 ± 0.8 d	10 ± 0.7 d	390 ± 2.04 a	130 ± 2.3 a

In each separate column, means followed by different letters designate significant differences at $p < 0.05$ according to Tukey's test. Concentration: T1: 62.5, T2: 125.0, T3: 187.5, T4: 250.0 ppm; C^-: the treatment infected with nematodes and not treated with the essential oil of *A. annua*, C^+: the treatment not infected with nematodes and not treated with the essential oil of *A. annua*

B

Concentration [ppm]	Plant length	Foliage wet weight	Root wet weight	Foliage dry weight	Root dry weight	Final population of larvae in 100 gram soil	Final population of females per gram of roots
T1	106 ± 1.4 c	45 ± 0.7 d	29 ± 0.9 c	18 ± 0.9 d	15 ± 1.1 c	200 ± 2.04 b	93 ± 0.7 b
T2	106 ± 1.7 c	47 ± 1.1 cd	31 ± 0.7 c	20 ± 0.4 d	17 ± 1.2 c	199 ± 1.4 b	91 ± 1.08 b
T3	108 ± 0.4 c	50 ± 0.4 c	33 ± 0.7 c	22 ± 0.5 cd	20 ± 0.4 bc	190 ± 0.7 b	88 ± 1.4 b
T4	110 ± 0.8 bc	54 ± 1.01 b	33 ± 1.6 c	24 ± 0.7 bc	21 ± 0.9 bc	135 ± 1.6 c	68 ± 2.3 c
Chemical nematicide	115 ± 1.8 ab	66 ± 0.7 b	40 ± 0.4 b	26 ± 0.7 b	25 ± 0.8 ab	100 ± 1.09 d	45 ± 1.6 d
C^+	120 ± 0.4 a	69 ± 1.01 a	50 ± 0.7 a	30 ± 1.07 a	30 ± 0.4 a	0	0
C^-	100 ± 1.7 d	40 ± 1.2 e	22 ± 1.2 d	12 ± 08 e	10 ± 0.7 d	390 ± 2.04 a	130 ± 2.3 a

In each separate column, means followed by different letters designate significant differences at $p < 0.05$ according to Tukey's test. Concentration: T1: 125.0, T2: 250.0, T3: 375.0, T4: 500.0 ppm; C^-: the treatment infected with nematodes and not treated with the aqueous extract of *A. annua*; C^+: the treatment not infected with nematodes and not treated with the aqueous extract of *A. annua*

C

Concentration [ppm]	Plant length	Foliage wet weight	Root wet weight	Foliage dry weight	Root dry weight	Final population of larvae in 100 gram soil	Final population of females per gram of roots
T1	104 ± 2.2 cd	53 ± 1.5 c	30 ± 1.08 c	19 ± 0.8 d	17 ± 0.5 d	143 ± 1.9 b	63 ± 0.9 b
T2	105 ± 2.1 cd	53 ± 0.9 c	30 ± 1.2 c	19 ± 0.5 d	17 ± 0.9 d	142 ± 1.4b c	61 ± 1.08 b
T3	110 ± 1.4 bc	55 ± 1 bc	36 ± 1.02 b	21 ± 0.5 cd	20 ± 0.9 cd	133 ± 1.4 c	58 ± 0.8 b
T4	113 ± 1.3 b	59 ± 0.7 b	38 ± 1.08 b	25 ± 1.5 bc	21 ± 0.7 c	120 ± 0.9 d	50 ± 0.7 c
Chemical nematicide	115 ± 1.8 ab	66 ± 0.7 a	40 ± 0.4 b	26 ± 0.7 b	25 ± 0.8 b	100 ± 1.09 e	45 ± 1.6 c
C+	120 ± 0.4 a	69 ± 1.01 a	50 ± 0.7 a	30 ± 1.07 a	30 ± 0.4 a	0	0
C^-	100 ± 1.7 d	40 ± 1.2 e	22 ± 1.2 d	12 ± 08 e	10 ± 0.7 e	390 ± 2.04 a	130 ± 2.3 a

In each separate column, means followed by different letters designate significant differences at $p < 0.05$ according to Tukey's test. Concentration: T1: 125.0, T2: 250.0, T3: 375.0, T4: 500 ppm; C^-: the treatment infected with nematodes and not treated with the methanolic extract of *M. azedarach*; C^+: the treatment not infected with nematodes and not treated with the methanolic extract of *M. azedarach*

Discussion

Plant parasitic nematodes are one of the major causes of annual losses in global agricultural production. Chemical nematicides can control nematodes, but due to their persistence in soil and water, indirect effects on non-target organisms and potential hazards to the environment, suitable methods for controlling nematodes that are both effective and environmentally friendly need to be found (Pathak *et al.* 2022). Plant essential oils may be a widely available green source, and their breakdown into non-toxic products does not have any adverse effects on non-target organisms or

the environment (Isman 2000). This study is the first report on the effects of nematicidal activity in vitro and in vivo with the water extract and essential oil of *A. annua* and the methanol extract of *M. azedarach* on *T. semipentrans*. Several nematicidal activities have been reported for *A. annua* against other PPNs. The nematicidal properties of *A. annua* on AWE and its major components have been identified for different nematode species (Shakil *et al.* 2004; D'Addabbo *et al.* 2013). In agreement with our results, D'Addabbo *et al.* (2013) reported high mortality of second-stage larvae of *M. incognita* and significant inhibition of *M. incognita* egg hatching when exposed to concentrations similar to those used in our experiments. AEO was also investigated for its activity against soil-borne pathogens and pests such as root-knot nematodes. Mohammad *et al.* (2022) investigated the nematicidal activity of aqueous extracts of *A. annua* on *M. incognita* under laboratory and greenhouse conditions. The results showed that the mortality of nematodes at 250 and 500 ppm after 48 h was 82 and 80%, respectively, and no return was seen after retaking the nematodes in distilled water. Similar to our results, the results of another study showed that the essential oil of *A. annua* (α-pinene and 1, 8 cineole) acts as a repellent for nematodes (Mwamba *et al.* 2021). Shakil *et al.* (2004) reported complete mortality of *M. incognita* larvae at concentrations of 500 and 250 ppm of MME. As the concentration of MME increased, nematode infestation decreased. These results are consistent with those obtained by El Nagdi and Mansour (2003), Hosseini-Nejad (2004), Cristobal Aljaro *et al.* (2006), Ardakani *et al.* (2009), Ntalli *et al.* (2010), Katooli *et al.* (2010), Ardakani (2011), Aoudia *et al.* (2012) and Kavousi *et al.* (2012).

The topic under investigation was the effect of AEO, AWE, and MME on nematode behavior. MME was found to be a potent nematicide for citrus nematodes, while AEO and AWE were able to act as repellents at high concentrations. The extract can prevent nematodes from being attracted to the root area and reduces their feeding on roots. In the pot experiment, the water extract and essential oil of *A. annua* and the methanol extract of *M. azedarach* were tested against a chemical nematicide, Fostiazate, on citrus nematodes. Each treatment was compared with a nematode-infected control (C^+) and a nematode-infected and untreated control (C^-). The results showed that Fostiazate had a better efficacy in reducing nematode populations and increasing growth parameters (shoot weight as the most suitable performance indicator under greenhouse conditions). On the other hand, among the plants with nematicidal properties, *M. azedarach* was more effective than *A. annua*, although AEO and AWE were also effective against citrus nematodes. Improved growth of tomatoes has been previously reported with *A. annua* food or water extract. The results showed that the growth of tomato and potato plants increased compared to the control and untreated treatment with Phytophthora (D'Addabbo *et al.* 2017). According to our results, Fostiazate has a better efficacy in reducing citrus nematode populations than the tested plant materials, but due to its cost-effectiveness, high environmental hazards, persistence in soil, it has lower priority than the plant species used in this study. Plant-based pesticides are not harmful to humans as they have less residual effect than synthetic chemicals and easily decompose. They are effective and have lower risks at low levels. Using biological logic for managing nematodes is an effective and environmentally friendly action.

Plant-based products, as a significant source of nematicidal compounds, can be widely used and replace chemical nematicides. Therefore, the evaluation of plant materials for controlling citrus nematodes as one of the major destructive factors in citrus is very valuable. According to the results, plants such as *A. annua* and *M. azedarach* are considered as promising control agents for citrus nematodes. The results suggest that *A. annua* and *M. azederach* products may be potential candidates for formulating new nematicides suitable for sustainable nematode management, although field trials are still needed to demonstrate effective commercial application.

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