



## Nematicidal Activity of Various Aqueous Extracts against Root-Knot Nematodes (*Meloidogyne chitwoodi*)

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### ABSTRACT

In this study, the effects of twelve aqueous extracts on the hatching and mortality of second-stage juveniles (J2s) of *Meloidogyne chitwoodi* were evaluated under in vitro and growth chamber conditions in 2020-2021. The mortality of the J2s increased with the increasing exposure time and concentration for all extracts. Maximum mortality was observed in 50% of all aqueous extracts. Up to 100% maximum mortality was observed in 10% of extracts of *Anethum graveolens* (100%), *Eruca sativa* (100%), *Ficus carica* (100%), *Juglans regia* (100%), *Melia azedarach* (100%), *Mentha piperita* (100%), *Vitex agnus-castus* (98%), *Asphodelus aestivus* (96.4%), *Eucalyptus camaldulensis* (95.6%) after 24 hours and minimum mortality was found in 1% of extracts of *Laurus nobilis* (6.6%) after 6 hours. Moreover, maximum suppression of hatching was found at 64.2%,

61.0%, and 59.4% with extract of *A. aestivus*, *Nerium oleander*, and *V. agnus-castus* after 7 days, respectively.

The pot experiments showed that the gall index was the lowest in soils treated with *Ficus carica*, *Nerium oleander*, *Laurus nobilis*, *Eucalyptus camaldulensis* and *Zingiber officinale* extracts. The chemical composition of five aqueous extracts was analysed using gas chromatography-mass spectrometry (GC-MS), and the main components of five aqueous extracts were identified as eucalyptol, 2-methoxy-6,10-dimethyl-dodeca-2E,6Z,10Z-trienoic acid, 12-acetoxy-, dihydroedulan II and  $\alpha$ -Zingiberene. The study confirms the potential of mainly *L. nobilis*, *E. camaldulensis*, *F. carica*, *Z. officinale* and *N. oleander* extracts for the formulation of new products for controlling *M. chitwoodi*

Keywords: *Eucalyptus camaldulensis*, *Nerium oleander*, *Zingiber officinale*, Mortality, Management, GC-MS

## 1. Introduction

Plant-parasitic nematodes cause 12.6% of global crop yield loss which is equivalent to an estimated 157 billion dollars per year (Nicol et al. 2011; Das et al. 2021; Marin-Bruzos et al. 2021). In particular, root-knot nematodes (*Meloidogyne* spp.) have a large host plant spectrum and cause low crop yields. They rapidly reproduce under favourable conditions, and the increment of the population causes low crop yields (Perry et al. 2009). Over the years, researchers have developed a variety of cultural, mechanical, and chemical applications to control root-knot nematodes (Sharma et al. 2020). Nematicides can be effectively used to prevent crop losses from plant-parasitic nematodes. Chemical nematicides are still the most effective means of plant-parasitic nematode control. However, intensive nematicide use destroys beneficial microorganisms in the soil; therefore, nematicide usage is neither a healthy nor an economical solution for growers. In order to overcome these problems, it is crucial to adopt effective and environmentally friendly methods to control nematodes (Nolling & Becker 1994). One such practice would be the use of plant extracts that have nematicidal properties (Aydınli & Mennan 2014). The flowers, shoots, and leaves of *Nerium oleander* L. have been shown to have nematicidal effects on *Meloidogyne javanica* (Treub 1885; Chitwood 1949; Moosavi 2012). Plant crude extracts have a potential nematicidal effect against *M. incognita* (Sithole et al. 2021).

Columbia root-knot nematode, *Meloidogyne chitwoodi* Golden et al. 1980 is one of the most widespread root-knot nematodes that infect potatoes, vegetables, wheat, corn, tomatoes, alfalfa, and numerous weeds (Evlice & Bayram 2019). For this reason, a few nematicides should be applied at each cropping season. Currently, research on alternative methods to nematicide is primarily focused on plant extracts and their components. The effect of plant extracts and essential oils in controlling plant-parasitic nematodes is well documented (D'Addabbo et al. 2020). However, few studies have been published on the control of *M. chitwoodi* using plant extracts (Golec 2019). The study focused on the effects of aqueous extracts on the control of *Meloidogyne chitwoodi*, one of the most widespread root-knot nematodes that infects numerous crops worldwide. The study evaluated the efficacy of the extracts in vitro and pot experiments and analysed five of the extracts using gas chromatography-mass spectrometry (GC-MS). The results showed that certain plant extracts had significant nematicidal effects on *M. chitwoodi*, and these extracts could be potentially used as alternatives to synthetic nematicides.

## 2. Material and Methods

### 2.1. Nematode cultures

*Meloidogyne chitwoodi* was initially isolated from infested potato tubers collected from Niğde province (Devran et al. 2009). To obtain the pure culture, 20 egg masses were handpicked using forceps and then surface-sterilized. The egg masses were then inoculated onto four-leaf stage tomato seedlings (Cüsseli F1) and the plants were grown for 8 weeks in a growth chamber at 23±1 °C (Hussey & Barker 1973). After 8 weeks, egg masses were collected from the infested tomato roots.

### 2.2. Preparation of aqueous extracts of plant materials

The plant species were harvested from (March-June) 2019-2020 in the Adana and Hatay provinces. Rhizome (*Zingiber officinale*), whole plants (*Anethum graveolens*), and leaves (other plants) were cut, washed with tap water, and then dried in an oven at 60 °C for 72 h (Abbas et al. 2009). The dried plants were ground with a blender and the plants powder stored in a glass bottle at 4 °C. For the preparation of aqueous extracts, 100 g powder was added to 900 mL distilled water, and thoroughly mixed on a shaker for 4 h. The suspension was then filtered with muslin cloth and then centrifuged at 5,000 rpm for 15 min. The resulting supernatant was collected and stored at 4 °C in opaque bottles until used as stock solutions (Elbadri et al. 2008; Abbas et al. 2009; Oka et al. 2012). With these stock solutions, dilutions of 1%, 2.5%, 5%, and 10% were prepared by adding the necessary amount of sterile distilled water.

### 2.3. Effect of aqueous extracts on juveniles mortality

To test for juvenile mortality, J2s were exposed for 6, 12, and 24 h to the 12 extracts at 1%, 2.5%, 5%, and 10% concentrations. The effect of plant extracts was evaluated in 96-well plate using suspensions of 100 J2/mL in distilled water at 22 °C. Distilled water was used as a control. The experiments included five replicates of each treatment. The mortality of the J2s was evaluated based on the number of dead nematodes observed under a microscope. The viability of the nematodes was determined using a needle.

### 2.4. Effect of aqueous extracts on egg hatching

Hatching was assessed after 24 h, 72 h, and 7 d in 10% concentration of plant extracts using five egg masses of the same age (Moosavi 2012). This method was used because better results were obtained at a concentration of 10%. The experiments included four replicates of each treatment, including control with distilled water, and were incubated at 21-22 °C in a growth chamber.

### 2.5. Effect of aqueous extracts on *Meloidogyne chitwoodi* in pot experiments

Experiments were set up in the growth chamber. Tomato (Cüsseli F1) seedlings at the four-leaf stage were grown in a one-kg capacity with autoclaved soil and 2,000 newly hatched J2s of *M. chitwoodi* were inoculated (Elbadri et al. 2009). Then, 100 mL of each plant extract was added to the soil. The negative control was distilled water, the positive control included nematodes only. Each treatment was replicated four times, and pots were arranged in a completely randomized design. The inoculated plants were placed in a growth room at 23 °C±1, 60±10% RH, and 16:8 h L:D photoperiod and watered regularly for 8 weeks. The plant roots were evaluated according to the 0-5 gall index (Hartman & Sasser 1985). Additionally, the fresh and dry weights of roots and plant height were measured according to Aydınlı & Mennan (2014).

### 2.6. Data analysis

All experiments in the study were repeated twice. The data were analysed using one-way ANOVA (SPSS version 25) and the means were separated using Duncan's multiple range test. The pooled data from the in vitro and nematode data from the in-pot experiments were Arcsin and Ln (x+1) transformed for the homogenize error variances. Additionally, repeated measures ANOVA was used to determine the relation with time, plant species and concentration. The relative suppression rate was calculated as illustrated in Equation 1 (Yang et al. 2016).

$$= \frac{\text{Relative suppression rate (\%)} \text{ number of J2 in sterilized water} - \text{number of J2 in root exudate}}{\text{number of J2 in sterilized water}} \times 100 \quad (1)$$

### 2.7. Gas chromatography-mass spectrometry (GC-MS)

The composition of *Laurus nobilis* L. (Lurales: Lauraceae), *Eucalyptus camaldulensis* Dehnh (Rosids: Myrtaceae), *Ficus carica* L. (Rosales: Moraceae), *Nerium oleander* (Gentianales: Apocynaceae), *Zingiber officinale* (Zingiberales: Zingiberaceae) was analysed by GC-MS. The Agilent Brand 7890B, GC 7010B MS system was used for the GC-MS analysis. Using the solid phase micro extraction (SPME) method, 3 mL of plant extract was placed in a 20 mL vial, and kept at 50 °C for 10 min. Then, samples were adsorbed for 30 min using the SPME apparatus 50/30 µm fiber coated with divinylbenzene/carboxene/polydimethylsiloxane

(DVB/CAR/PDMS) Agilent. DB-Wax (60 m x 0.25 mm i.d. x 0.25  $\mu$ m; J&W Scientific-Folsom, USA) was then injected into the capillary column by desorbing for 5 min. At first, the injection temperature was kept at 250 °C, column oven temperature was kept at 40 °C for 4 min, after which the temperature was increased at a rate of 3 °C/min to 90 °C. It was then increased at a rate of 4 °C/min up to 130 °C for 4 min.

### 3. Results and Discussion

Plants produce phytochemicals that provide significant defense against pathogenic organisms and pests. The present study investigated the effects of twelve plants on *Meloidogyne chitwoodi*. Few plant extract studies have been written on the subject of *M. chitwoodi* and, for this reason, this study seeks to provide new analyses and data. Plant extracts were tested for suppression of egg hatching, mortality of juveniles and growth of tomato plants inoculated with *M. chitwoodi*. The plants' chemical components which have the most suppressive effect were determined by GC-MS. The results indicated that aqueous extracts of *F. carica* resulted in 100% mortality of J2s at 1% concentration after 12 h under laboratory conditions. The best concentration was found in 10% of all plants.

#### 3.1. Effect of aqueous extracts on the mortality of juveniles

Table 1 shows the 1% and 2.5% mortality of the J2s for the various extracts while Table 2 presents the 5% and 10% mortality of the J2s for the various extracts. In all treatments, the mortality of the J2 increased with increasing exposure time. All plant extracts showed higher mortality with 24 h exposure compared to 12 h. After 24 h, more than 50% nematode mortality was induced by 2.5% concentration of all plant extracts. Complete mortality was observed after 12 h with 1% extracts of *F. carica*. Additionally, the most effective concentration and exposure time of plant extracts were those of 10% concentration and after 24 h.

**Table 1- Effects of aqueous extracts on mortality of second-stage juveniles (J2) of *Meloidogyne chitwoodi*\* (1% and 2.5%)**

Conc. %	1%			2.50%		
	6 h	12 h	24 h	6 h	12 h	24 h
Control	1.6±0.5 <sup>s</sup>	19.0±0.7 <sup>g</sup>	28.6±0.9 <sup>j</sup>	1.6±0.5 <sup>h</sup>	19.0±0.7 <sup>g</sup>	28.6±0.9 <sup>i</sup>
<i>Anethum graveolens</i> L.	10.8±1.2 <sup>de</sup>	38.6±1.2 <sup>c</sup>	48.4±1.3 <sup>f</sup>	18.2±1.8 <sup>f</sup>	48.2±0.8 <sup>d</sup>	76.4±0.7 <sup>d</sup>
<i>Asphodelus aestivus</i> Brot.	7.4±1.5 <sup>ef</sup>	45.2±1.0 <sup>b</sup>	60.6±0.9 <sup>d</sup>	16.2±1.4 <sup>f</sup>	51.6±0.9 <sup>c</sup>	72.4±1.3 <sup>e</sup>
<i>Eruca sativa</i> Mill	13.2±1.0 <sup>d</sup>	35.2±1.5 <sup>d</sup>	53.4±1.4 <sup>e</sup>	22.2±1.3 <sup>de</sup>	51.6±0.7 <sup>c</sup>	75.8±0.8 <sup>d</sup>
<i>Eucalyptus camaldulensis</i> Dehnh	13.8±0.9 <sup>d</sup>	35.0±0.9 <sup>d</sup>	48.8±1.2 <sup>f</sup>	23.8±1.3 <sup>cd</sup>	41.2±1.6 <sup>e</sup>	64.0±0.8 <sup>f</sup>
<i>Ficus carica</i> L.	51.4±1.0 <sup>a</sup>	100±0.0 <sup>a</sup>	100±0.0 <sup>a</sup>	94.6±0.5 <sup>a</sup>	100±0.0 <sup>a</sup>	100±0.0 <sup>a</sup>
<i>Juglans regia</i> L.	22.2±1.3 <sup>b</sup>	40.6±1.5 <sup>c</sup>	57.8±1.2 <sup>d</sup>	25.4±1.2 <sup>bcd</sup>	75.4±1.0 <sup>b</sup>	94.8±0.4 <sup>b</sup>
<i>Laurus nobilis</i> L.	6.6±1.2 <sup>f</sup>	31.8±1.2 <sup>de</sup>	38.2±0.9 <sup>i</sup>	9.6±0.9 <sup>g</sup>	34.8±0.9 <sup>f</sup>	54.0±0.9 <sup>h</sup>
<i>Melia azedarach</i> L.	11.2±1.6 <sup>d</sup>	41.4±1.4 <sup>c</sup>	69.6±1.2 <sup>b</sup>	17.8±1.1 <sup>f</sup>	49.2±0.7 <sup>cd</sup>	81.0±1.1 <sup>c</sup>
<i>Mentha piperita</i> L.	21.0±1.1 <sup>bc</sup>	44.8±1.4 <sup>b</sup>	63.8±1.0 <sup>c</sup>	28.2±1.5 <sup>b</sup>	51.0±1.1 <sup>cd</sup>	80.8±0.7 <sup>c</sup>
<i>Nerium oleander</i> L.	10.6±0.9 <sup>de</sup>	24.0±1.0 <sup>f</sup>	44.0±1.1 <sup>gh</sup>	18.8±1.1 <sup>ef</sup>	35.0±1.2 <sup>f</sup>	60.8±0.8 <sup>g</sup>
<i>Vitex agnus-castus</i> L.	18.6±1.6 <sup>c</sup>	30.0±1.0 <sup>e</sup>	42.6±1.4 <sup>h</sup>	26.4±0.9 <sup>bc</sup>	35.6±1.0 <sup>f</sup>	65.0±1.3 <sup>f</sup>
<i>Zingiber officinale</i> Roscoe	12.2±1.0 <sup>d</sup>	32.4±1.3 <sup>de</sup>	46.4±0.9 <sup>fg</sup>	18.4±1.1 <sup>f</sup>	39.6±1.1 <sup>e</sup>	60.4±0.9 <sup>g</sup>

\*: Means followed by the same letters within columns are not significantly different according to Duncan test at P<0.05. The results are expressed as mean  $\pm$  standard error

**Table 2- Effects of aqueous extracts on mortality of second-stage juveniles (J2) of *Meloidogyne chitwoodi*\* (5% and 10%)**

Conc. % Exposure time	5%			10%		
	6 h	12 h	24 h	6 h	12 h	24 h
Control	1.6±0.5 <sup>i</sup>	19.0±0.7 <sup>h</sup>	28.6±0.9 <sup>i</sup>	1.6±0.5 <sup>h</sup>	19.0±0.7 <sup>h</sup>	28.6±0.9 <sup>f</sup>
<i>Anethum graveolens</i> L.	26.4±1.3 <sup>de</sup>	56.0±1.0 <sup>d</sup>	89.2±0.9 <sup>d</sup>	33.2±0.9 <sup>e</sup>	64.2±1.3 <sup>d</sup>	100±0.0 <sup>a</sup>
<i>Asphodelus aestivus</i> Brot.	18.6±1.6 <sup>g</sup>	57.4±0.9 <sup>d</sup>	81.4±0.8 <sup>f</sup>	58.2±1.4 <sup>b</sup>	90.0±0.8 <sup>c</sup>	96.4±0.6 <sup>c</sup>
<i>Eruca sativa</i> Mill	25.0±0.9 <sup>ef</sup>	68.0±1.0 <sup>c</sup>	87.6±0.5 <sup>d</sup>	28.2±0.7 <sup>f</sup>	89.4±0.7 <sup>c</sup>	100±0.0 <sup>a</sup>
<i>Eucalyptus camaldulensis</i> Dehnh	29.2±0.9 <sup>cd</sup>	50.6±1.0 <sup>e</sup>	84.4±0.6 <sup>e</sup>	32.8±0.7 <sup>e</sup>	55.6±1.2 <sup>f</sup>	95.6±0.4 <sup>c</sup>
<i>Ficus carica</i> L.	100±0.0 <sup>a</sup>	100±0.0 <sup>a</sup>	100±0.0 <sup>a</sup>	100±0.0 <sup>a</sup>	100±0.0 <sup>a</sup>	100±0.0 <sup>a</sup>
<i>Juglans regia</i> L.	37.2±0.9 <sup>b</sup>	88.2±1.2 <sup>b</sup>	100±0.0 <sup>a</sup>	45.0±0.8 <sup>c</sup>	95.0±0.7 <sup>b</sup>	100±0.0 <sup>a</sup>
<i>Laurus nobilis</i> L.	14.8±0.9 <sup>h</sup>	38.2±1.0 <sup>g</sup>	62.4±1.1 <sup>h</sup>	19.8±1.1 <sup>g</sup>	45.2±1.2 <sup>g</sup>	79.8±0.9 <sup>e</sup>
<i>Melia azedarach</i> L.	23.2±1.2 <sup>f</sup>	55.6±0.9 <sup>d</sup>	94.6±0.7 <sup>c</sup>	27.4±0.9 <sup>f</sup>	59.2±1.4 <sup>ef</sup>	100±0.0 <sup>a</sup>
<i>Mentha piperita</i> L.	35.0±1.2 <sup>b</sup>	55.6±0.7 <sup>d</sup>	98.2±0.6 <sup>b</sup>	38.0±0.7 <sup>d</sup>	62.8±1.2 <sup>de</sup>	100±0.0 <sup>a</sup>
<i>Nerium oleander</i> L.	25.4±0.9 <sup>ef</sup>	47.8±0.9 <sup>ef</sup>	75.6±0.7 <sup>g</sup>	29.8±1.0 <sup>f</sup>	62.6±1.5 <sup>de</sup>	86.8±0.9 <sup>d</sup>
<i>Vitex agnus-castus</i> L.	31.6±1.1 <sup>c</sup>	54.4±1.2 <sup>d</sup>	84.6±0.8 <sup>e</sup>	37.2±0.8 <sup>d</sup>	65.2±0.9 <sup>d</sup>	97.6±0.7 <sup>b</sup>
<i>Zingiber officinale</i> Roscoe	27.8±0.7 <sup>de</sup>	46.4±0.9 <sup>f</sup>	73.8±1.0 <sup>g</sup>	29.4±1.2 <sup>f</sup>	56.6±1.7 <sup>f</sup>	88.6±0.9 <sup>d</sup>

\*: Means followed by the same letters within columns are not significantly different according to Duncan test at P<0.05. The results are expressed as mean ± standard error

### 3.2. Effect of aqueous extracts on the egg hatching

All tested samples were found to increase and decrease the egg hatching rate in egg masses of *M. chitwoodi* compared to the control group (distilled water). The extract from *Asphodelus aestivus* had the highest effect on the hatching at all times and reduced egg hatching by 64.2% at the end of the seventh day. This was followed by *Nerium oleander*, *Vitex agnus-castus*, and *Anethum graveolens* with 61%, 59.4%, and 53.6% respectively. The lowest effect was found in the *M. azedarach* extract with 15.2% at the end of the seventh day. The results are presented in and Table 3.

**Table 3- Effect of aqueous extracts on egg hatching and relative suppression rate (%) to control of *Meloidogyne chitwoodi* in vitro condition\***

Plant species	24 h (Mean±SE)	Relative suppression rate (%)	72 h (Mean±SE)	Relative suppression rate (%)	7 days (Mean±SE)	Relative suppression rate (%)
Control	64±2.2 <sup>a</sup>	0.0	70±1.1 <sup>a</sup>	0.0	133±2.2 <sup>a</sup>	0.0
<i>Anethum graveolens</i>	45±3.1 <sup>bcd</sup>	29.6	55±3.5 <sup>bc</sup>	21.7	62±2.7 <sup>fg</sup>	53.6
<i>Asphodelus aestivus</i>	42±2.7 <sup>d</sup>	34.3	44±2.2 <sup>d</sup>	37.6	48±2.8 <sup>i</sup>	64.2
<i>Eruca sativa</i>	52±2.5 <sup>bc</sup>	19.0	55±2.4 <sup>c</sup>	36.2	89±2.8 <sup>d</sup>	33.1
<i>Eucalyptus camaldulensis</i>	46±1.6 <sup>bcd</sup>	28.3	67±1.3 <sup>a</sup>	4.6	78±1.8 <sup>e</sup>	41.4
<i>Ficus carica</i>	47±2.4 <sup>bcd</sup>	26.5	48±1.5 <sup>cd</sup>	31.6	103±4.8 <sup>c</sup>	22.8
<i>Juglans regia</i>	62±2.2 <sup>a</sup>	2.6	64±3.1 <sup>ab</sup>	9.6	107±2.5 <sup>bc</sup>	20.1
<i>Laurus nobilis</i>	52±2.0 <sup>b</sup>	18.2	55±2.6 <sup>bc</sup>	21.7	70±2.9 <sup>ef</sup>	47.2
<i>Melia azedarach</i>	60±3.3 <sup>a</sup>	7.0	66±4.5 <sup>a</sup>	6.8	113±2.8 <sup>b</sup>	15.2
<i>Mentha piperita</i>	62±2.3 <sup>a</sup>	3.4	64±2.6 <sup>ab</sup>	9.6	79±2.8 <sup>e</sup>	41.0
<i>Nerium oleander</i>	43±2.0 <sup>d</sup>	33.0	50±3.8 <sup>cd</sup>	28.4	52±3.4 <sup>h</sup>	61.0
<i>Vitex agnus-castus</i>	44±2.3 <sup>cd</sup>	31.1	51±2.5 <sup>cd</sup>	27.3	54±2.0 <sup>gh</sup>	59.4
<i>Zingiber officinale</i>	51±3.0 <sup>bc</sup>	19.8	55±2.2 <sup>c</sup>	22.0	62±2.5 <sup>fg</sup>	53.2

\*: Means followed by the same letters within columns are not significantly different according to Duncan test at P<0.05

The interaction of time-plants, time-concentration and time-plant-concentration were statistically significant at  $P < 0.001$  and, consequently, it was found that significant relationship exist between time, plant extracts, and concentration and that they influenced each other (Supplementary File 1).

### 3.3. Pot experiment

Pot experiment indicated that certain aqueous extracts effectively controlled *M. chitwoodi* by reducing juvenile infection. (Table 4). The root gall index was the lowest in the soil with extracts from *F. carica*, *N. oleander*, *Eucalyptus camaldulensis*, *Zingiber officinale* and *L. nobilis* compared to the other plant extracts and the positive control. Most of the aqueous extracts in the soil increased the plant height and decreased the fresh and dry root weights compared to the negative control. The highest plant height, fresh and dry root weights were observed with *N. oleander*, *E. camaldulensis*, *Juglans regia* *Anethum graveolens*, while the lowest plant height, fresh and dry root weights were determined in *Asphodelus aestivus*, *Ficus carica* respectively.

**Table 4- Effect of aqueous extracts on number of gall index, dry and fresh root weight, plant height in tomato plants for 8 weeks after inoculation with nematodes\***

<i>Plant species</i>	<i>Root gall index (0-5)</i>	<i>Root fresh weight (g) (Mean±SE)</i>	<i>Root dry weight (g) (Mean±SE)</i>	<i>Plant height (cm) (Mean±SE)</i>
<i>Anethum graveolens</i>	3.5±0.3 <sup>abc</sup>	51.8±2.9 <sup>ab</sup>	19.8±2.9 <sup>ab</sup>	65.5±2.5 <sup>cde</sup>
<i>Asphodelus aestivus</i>	3.0±0.0 <sup>bc</sup>	50.1±10.5 <sup>abc</sup>	17.1±6.3 <sup>ab</sup>	59.5±1.3 <sup>e</sup>
<i>Eruca sativa</i>	3.3±1.1 <sup>bc</sup>	44.6±14.2 <sup>abc</sup>	13.1±6.2 <sup>ab</sup>	72.0±1.4 <sup>bcd</sup>
<i>Eucalyptus camaldulensis</i>	2.8±0.5 <sup>bc</sup>	56.1±16.5 <sup>abc</sup>	16.8±6.1 <sup>ab</sup>	83.3±3.9 <sup>a</sup>
<i>Ficus carica</i>	2.5±0.3 <sup>bc</sup>	23.9±2.4 <sup>c</sup>	7.2±1.1 <sup>b</sup>	78.5±1.9 <sup>ab</sup>
<i>Juglans regia</i>	4.0±0.0 <sup>ab</sup>	61.9±12.1 <sup>ab</sup>	17.2±2.8 <sup>ab</sup>	65.5±1.2 <sup>cde</sup>
<i>Laurus nobilis</i>	2.5±0.3 <sup>bc</sup>	51.7±10.5 <sup>abc</sup>	14.5±3.3 <sup>ab</sup>	67.5±2.1 <sup>cde</sup>
<i>Melia azedarach</i>	3.0±0.0 <sup>bc</sup>	55.9±5.4 <sup>ab</sup>	17.3±3.2 <sup>ab</sup>	71.3±2.1 <sup>bcd</sup>
<i>Mentha piperita</i>	4.3±0.5 <sup>ab</sup>	57.4±4.4 <sup>ab</sup>	16.2±4.4 <sup>ab</sup>	63.0±0.7 <sup>de</sup>
<i>Nerium oleander</i>	2.0±0.0 <sup>c</sup>	41.8±8.9 <sup>abc</sup>	16.0±5.6 <sup>ab</sup>	84.5±8.8 <sup>a</sup>
<i>Vitex agnus-castus</i>	4.0±0.4 <sup>ab</sup>	34.0±8.1 <sup>bc</sup>	16.9±2.6 <sup>ab</sup>	74.0±2.1 <sup>bc</sup>
<i>Zingiber officinale</i>	2.5±0.3 <sup>bc</sup>	47.6±4.2 <sup>abc</sup>	12.9±3.9 <sup>ab</sup>	71.3±1.3 <sup>bcd</sup>
Positive Control	5.0±0.3 <sup>a</sup>	41.7±12.8 <sup>bc</sup>	15.0±6.4 <sup>ab</sup>	68.5±0.8 <sup>cde</sup>
Negative Control	0.0±0.0 <sup>d</sup>	72.4±6.8 <sup>a</sup>	24.5±3.6 <sup>a</sup>	64.0±1.4 <sup>de</sup>

\*: Means followed by the same letters within columns are not significantly different according to Duncan test at  $P < 0.05$

### 4. Gas chromatography-mass spectrometry analysis

The chemical components of the plant extracts showing the highest nematicidal effect against *M. chitwoodi* were analysed by GC-MS. Details of the chemical compositions of plants extracts are shown in Supplementary File 2, Supplementary File 3, Supplementary File 4, Supplementary File 5, and Supplementary File 6. Several plants tested in this study have previously been tested against other *Meloidogyne* species. In an earlier study, *F. carica* extracts were found to have a significant paralysis effect on J2 of *M. javanica* after 72 hours and an inhibition of egg hatching because of various alkaloids and metabolites (Alves et al. 2020). *Nerium oleander* which is known as phytotoxic caused plants and juveniles to die and suppressed the egg hatching *M. javanica* (Moosavi 2012). In the present study, *N. oleander* killed 86.8% of J2s in 24 h and inhibited 52% egg hatching of *M. chitwoodi*. *Mentha piperita* L. was used to nematicidal effect on nematodes. It has been reported that *Meloidogyne arenaria* (Neal 1889) and *M. javanica* died in *M. piperita* at all concentrations of the aqueous extracts tested (Aydinli et al. 2019). In the present study, similar results were observed *M. chitwoodi*. These results can be attributed to the presence of toxic substances in the aqueous extracts. The nematicidal activity of ginger against *M. javanica* was examined in vitro and a higher concentration (100%) suppressed egg hatching and juvenile mortality (Zareen et al. 2003). In vitro results showed that the suppression of egg hatching and mortality of the J2 increased with exposure time to aqueous extracts of *Z. officinale*. Research has shown that *Eucalyptus* spp. extracts can give high mortality of *M. javanica*, J2s whereas other studies reported no such mortality (Ahmed et al. 2010). These previous findings suggest that the plants tested in this study may have broad-spectrum nematicidal activity against different *Meloidogyne* species, which is promising for their potential use in integrated pest management programs. Further research is needed to identify the specific nematicidal compounds present in these plants and to determine their efficacy

against other plant-parasitic nematodes. The present study demonstrated a high mortality rate for *M. chitwoodi* with *E. camaldulensis*. Different plant parts could provide different nematicidal activity (Aviles-Gomez et al. 2022). Nematicidal activity against *M. incognita* has been reported for the essential oil from fruits of *V. agnus-castus* (Ntalli et al. 2010). *Anethum graveolens* fruits significantly reduced *M. incognita* infection (Kim et al. 2003). In the present study, leaf extracts were used against *M. chitwoodi* in vitro and in tomato plants. In this dose-response experiment, the mortality of J2s and inhibition egg hatching increase with increasing exposure time to plant extracts. Ntalli et al. (2011) investigated antagonistic and synergistic actions of *J. regia* components. *Juglans regia* was highly nematicidal with up to 100% in vitro mortality of *M. incognita* race 2 (Laxmikant 2019). Similar results were observed in vitro as well as in the pot experiment. *Eruca sativa* provides powerful natural nematicidal effects against *M. incognita* (Aissani et al. 2015). In the present study, the gall index for *M. chitwoodi* was lower than four in pots treated with extracts of *F. carica*, *E. camaldulensis*, *N. oleander*, *L. nobilis*, *A. aestivus*, *A. graveolens*, *M. azedarach*, *Z. officinale* and *E. sativa*. GC-MS analysis was used to identify the composition of *F. carica*, *N. oleander*, *E. camaldulensis*, *L. nobilis* and *Zingiber officinale* extracts.

Alves et al. (2020) report that *F. carica* leaf extracts had components that affected the developmental process of the nematode. In the present study, ketones and monoterpenoids were found in *F. carica*. Although it has been reported that eucalyptol, which is one of the compositions of *F. carica*, is not always highly effective on nematodes. Similar results were reported by Caboni et al. (2013) and Mava et al. (2013). However, active components of other higher plant components and natural products (fatty acids) may be more effective against nematodes (Chitwood 2002). The inhibitory effect of these plant extracts on the eggs of *Meloidogyne* and J2s could be related to chemical components, such as coumarins, alkaloids, saponins, ketones, aldehydes, flavonoids, benzamides, and amides (Liu et al. 2011; Ntalli et al. 2011). It was shown that it is worth noting that the fatty acids, dihydroedulan II, and cathinone were found in *N. oleander* extracts (Cirlini et al. 2016) which is consistent with the findings of the present study. In addition, several studies have indicated that fatty acids can have considerable nematicidal activity (Davis et al. 1997; Duschatzky et al. 2004). *L. nobilis* was also found to have a few monoterpene components with nematicidal activity, such as linalool. In a recent study, spathulenol, a tricyclic sesquiterpene with 5,10-cycloaromadendrane skeleton, eucalyptol (monoterpene) in *E. camaldulensis*, was found to have useful bioactive against nematodes (Duschatzky et al. 2004, Faria et al. 2010). *Z. officinale* is a medicinal plant and its ingredients have been shown to be effective in previous studies (Youssef et al. 2015). Overall, the present study suggests that these plant extracts, or their main or minor compositions, may serve as effective nematicides. However, further studies are needed to investigate their potential for use in controlling nematode populations in agricultural settings.

#### 4. Conclusions

This study investigated the *F. carica*, *N. oleander*, *Zingiber officinale* and *L. nobilis* as potential sources of new nematicidal products. These plant extracts, or their main or minor compositions may serve as nematicides. Many plants are known as a source of naturally occurring nematicidal compounds. Plant species, plant part, harvest time, extraction method, nematode species and treatment conditions all affect the measured nematicidal activity of such plant components.

Plant extracts can be used as repellents, stimulants or hatching inhibitors, and as nematicide depending on the properties of the target phytoparasitic compounds and nematodes. Future studies need to further assess plant extracts for their usefulness in integrated pest management programs, particularly for the control of plant parasitic nematodes.

Finally, *F. carica*, *N. oleander*, *L. nobilis*, *A. aestivus*, *A. graveolens*, *M. azedarach*, *E. camaldulensis*, *Juglans regia*, *Zingiber officinale* and *E. sativa* were the potential plants that showed the most effective results in in vitro and pot experiments. However, further research is needed to determine the efficacy, safety, and environmental impact of using these plant extracts as nematicides. Therefore, it is recommended that a combination of different control methods are used, including cultural practices, biological control agents, and chemical nematicides, to manage plant parasitic nematodes effectively and sustainably.

#### Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

#### Declarations

**Conflict of interest** The author has no competing interests to declare that are relevant to the content of this article.

**Ethics approval and consent to participate.** All ethical aspects are considered.

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