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Research Article
Effect of Some Plant Extracts on the Management of *Meloidogyne incognita* in Tomato (*Solanum lycopersicum*)

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Abstract

Background and Objective: Root-knot (*Meloidogyne* species) nematodes are one of the most important causes of reduced tomato (*Solanum lycopersicum)* yield in Iran. During a survey of a tomato field, *M. incognita* was identified from the rhizosphere soil samples. **Materials and Methods:** Fresh leaf extracts of neem (*Azadirachta indica*) and wild thyme (*Thymus vulgaris*), including positive control (fenamiphos 60 ppm), were investigated *in vitro* and *in vivo* for efficacy against *M. incognita* motility and reproduction. **Results:** *In vitro*, all treatments caused ($p \leq 0.05$) mortality of second-stage juveniles (J2), with the highest mortality occurring under neem leaf extracts at 48 and 72 hrs exposure. *In vivo*, with tomato inoculated at 5000 J2/seedling, all treatments reduced ($p \leq 0.05$) root galling, gall index, reproductive factor (Pi/Pf) and J2 in soil. **Conclusion:** Overall, following fenamiphos, plant extracts of neem had the highest efficacy in suppressing nematode population densities. Consequently, the neem leaf extract could be investigated as an alternative product to synthetic chemical nematicides on tomatoes under field conditions.

Key words: Management, biological control, root-knot nematode, tomato, antifungal, root galling, sodium hypochlorite

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is the main vegetable that is in high demand in Iran. According to the data released by FAO, with producing approximately 6.4 million tons, Iran became the third country of tomato production in the middle east. Several *Meloidogyne* species were associated with vegtables[1]. The genus *Meloidogyne* represents over 100 species[2], with members of the genus having over 3000 host plant species. *Meloidogyne incognita* is one of the most important species of this genus[3].

Although chemical nematicides had been successful in managing root-knot nematodes in most crops[4], the products have often adversely affected the animal, human and environmental health systems[5]. Since, plant extracts could be excellent alternatives for managing nematode population densities, most plant species have been examined for their nematicidal or nematostatic properties[6].

One of the plants well known for its pesticide properties is neem (*Azadirachta indica*). Long ago, neem has been used as a pesticide, fungicide and antifeedant by Indian farmers. The neem plants have been used against *M. javanica*[7].

The use of thyme as a soil amendment resulted in a significant decrease in *Xiphinema index* population densities in grapevine (*Vitis vinifera* L.) trials[8]. Essential oils from thyme significantly reduced the number of *M. incognita* race 2-induced galls on roots of tomato (*Solanum lycopersicum)* in pots containing 3-4 kg of sterilized soil. After 60 days, infected roots were replanted[9].

The aim of this study was to investigate the effects of a crude leaf extract from *A. indica* and *T. vulgaris* for their efficacy on the suppression of *M. incognita in vitro* and *in vivo.*

MATERIALS AND METHODS

***In vivo* rearing of *Meloidogyne* species:** A population of *Meloidogyne* species was reared on a three-week-old susceptible tomato (*S. lycopersicum*) at Plant Protection clinic, Shahrekord lab, from 2016-2017. Sixty days after rearing, infected roots were removed from pots and rinsed with running tap water to remove soil particles and debris. Roots were cut into 2-3 cm pieces and placed in a kitchen blender with 500 mL 1% sodium hypochlorite (NaOCl) solution to extract eggs and J2[10]. Eggs and J2 were then collected on a 38 µm sieve after passing the aliquot through a 125 µm sieve to remove the root debris. Eggs and J2 on a 38 µm sieve were gently washed to remove excess NaOCl solution and collected in clear water in a beaker. Eggs and J2 were then placed on the filter paper in a Petri dish and kept in a temperature-regulated incubator at 25±2°C for four days until J2 was hatched[11].

Identification of the nematode: *Meloidogyne* species isolate was identified using morphological and morphometrical characteristics as *M. incognita*[12]. Verification of the identified species was done with molecular sequencing of 28S rDNA of the monoculture females, which were previously examined with a stereo microscope (Olympus SZX10, Japan) to confirm conspecificity.

Preparation of plant extracts: Fresh leaves of *T. vulgaris* and *A. indica*, were collected from the wild in the Shahrekord and Bandar-Abbas region of Iran, respectively. The leaves were separately shade-dried at room temperature and ground using an electrical steel grinder. Fifty-gram ground material of each was poured in an Erlenmeyer flask (Thermo Fisher, USA), with 100 mL ethanol (95%) added. The flask was sealed with a Parafilm® layer, shaken for 1 hr and placed in the refrigerator for 24 hrs. The crude extracts were then filtered through a Whatman No. 2 filter paper (Maidstone, United Kingdom) and a Millipore filter (0.22 µm) to obtain a clear liquid[13], which was poured into a 100 mL glass beaker. The glass beaker was covered with aluminium foil to prevent direct light on the extract solutions, which were stored in the freezer at -18°C until being used. Three concentrations, *viz*., 2.5 and 5%, were prepared using distilled water for each extract solution.

***In vitro* experiment:** Ten mL distilled water containing 100 *M. incognita* J2 was pipetted into an 8-cm-diameter Petri dish. Two hours later, a 10 mL diluted solution for each plant species and concentration was added to the Petri dishes containing J2, with distilled water and ethanol (70%) used as untreated controls[14]. At 48 and 72 hrs post-exposure at 25°C, dead J2 per Petri dish were identified as blue-stained individuals after staining with Trypan Blue solution. Each dead nematode was probed with a needle to ensure its mortality. Treatments for *in vitro* experiments were arranged in a completely randomized design, with six replications. The experiment was repeated two times.

***In vivo* experiment:** Twenty-cm-diameter plastic pots were filled with steam-pasteurized 3 kg clay and sand mixed at 2:1 (v/v) ratio, with pH at 7 and EC = 2 dS m^{-1}. Four leaf-stage of the tomato seedling was transplanted in a newly filled plastic pot. Treatments were as for *in vitro* experiment but

due to heterogeneous conditions in the glasshouse, treatments were arranged in randomized complete block design, with five replications. Plants were maintained under greenhouse conditions at temperatures ranging between 26 and 28°C and watered as needed. Two days after inoculation, each seedling was fertilized using 5 g 2:3:2 (26) NPK fertilizer to provide essential macro-and micro-nutrients. The experiment was repeated two times.

Inoculation with *M. incognita* second-stage juveniles (J2): One hole (2-cm-diameter) was made in the rhizosphere of each seedling, with 60 mL water containing 5,000 J2 *M. incognita* added to the bottom of the hole. After 24 hrs, 2.5 and 5% suspension of crude leafy extracts from each plant species were added to each well. Fenamiphos was applied at 0.06 mg mL^{-1} tap water. Untreated control constituted tap water. Sixty days after inoculation, plants were removed from each pot and roots excised from shoots. The root system of each plant was rinsed under running tap water and blotted dry on a paper towel. Recorded plant variables included plant height (cm), root lengths (cm), dry shoot mass (g), fresh root mass (g) and dry root mass (g). The number of galls per root system and gall index, J2 per 100 mL soil sample[15] and the reproduction factor were recorded[16]:

$$\text{Reproduction factor Rf} = \frac{\text{Final population (Pf)}}{\text{Initial population (Pi)}}$$

Statistical analysis: Statistical analyses were performed using SAS (ver. 9.2) software. The significant differences among treatments were determined according to the least significant differences (LSD) at $p<0.05$ level of probability, using the SAS (ver. 9.2) software. The interaction between repeated experiments was not significant, therefore, data were pooled.

RESULTS AND DISCUSSION

***In vitro* experiment:** According to morphological (e.g., J2, females and perineal pattern) and morphometrical characters and 28S rDNA sequence of *M. incognita* used in the current study had 99% identity with the molecularly identified populations for *M. incognita*. The J2 mortality was higher for concentrations of all crude extracts after 72 hrs than those at 48 hrs (Fig. 1). During 48 hrs, lower J2 mortality (5.5%) occurred in untreated control and the 2.5% thyme (11%) treatment compared to those for other treatment concentrations. The 2.5% thyme extract treatment resulted in the lowest J2 mortality at both the 48 (11%) and 72 hrs (16.5%) intervals. The 5% neem extract resulted in the highest J2 mortality at both time points (94.9 and 97.9% at 48 and 72 hrs, respectively). The neem 2.5% treatment at 48 (95.5%) and 72 hrs (95.5%) was higher than the untreated control (Fig. 1).

***In vivo* experiment**

Nematode variables: The nematicide treatment (fenamiphos) produced the fewest J2 numbers/100 mL soil (3.62) and differed significantly from all the other treatments and the control, which was associated with the highest J2 numbers/100 mL soil (164) (Table 1).

The thyme extract (30 ppm) treatment contained the highest number of galls/root systems (70.75), however, it was lower than control, which detected 110 gall numbers per whole root system (Table 1).

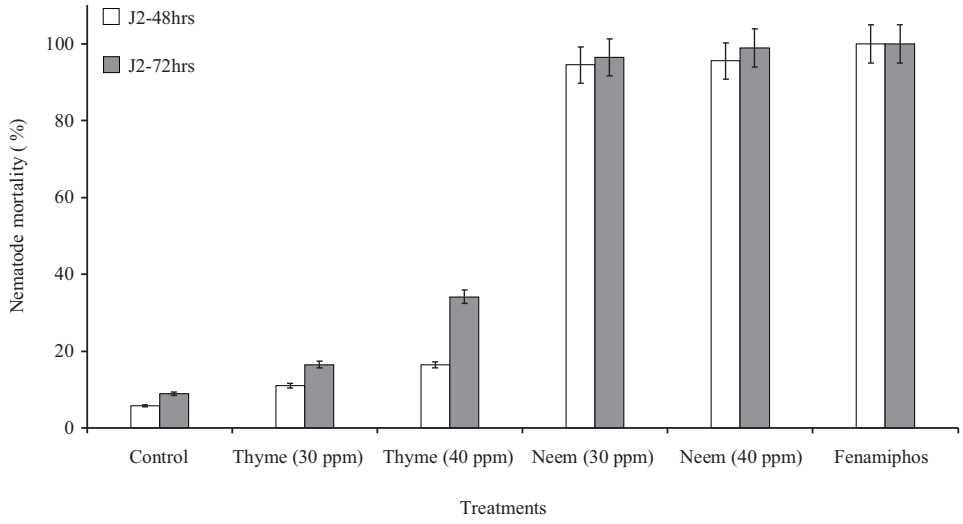

Fig. 1: *In vitro* investigation of ethanolic plant extract on the mortality of *M. incognita* after 48 and 72 hrs

Table 1: Effect of *A. indica* and *T. vulgaris* on nematode reproduction variables in tomato exposed to *M. incognita*

Treatments	J2 in soil	Gall number	Gall index	Rf
Neem extract (30 ppm)	67.25±0.5^{b}	27.12±0.5^{e}	3.71±0.04^{h}	0.27^{k}
Neem extract (40 ppm)	61.60±1.1^{b}	11.40±1^{f}	1.61±0.02^{i}	0.16^{l}
Thyme extract (30 ppm)	81.00±0.7^{c}	70.75±9^{d}	4.20±0.19^{h}	0.44^{m}
Thyme extract (40 ppm)	67.00±0.5^{b}	36.50±3^{e}	3.75±0.02^{h}	0.32^{k}
Fenamiphos (60 ppm)	3.62±0.5^{d}	7.87±0.6^{f}	1.47±0.02^{i}	0.13^{l}
Control	1.64±0.9^{a}	1.10±16 ^{g}	6.2±0.01^{j}	0.61^{n}

Values in the same column, followed by a different letter(s) are significantly different according to LSD ($p<0.05$). Each treatment had ten replications (five replications in two successive experiments). Values in parenthesis show±standard error. Rf: Initial population/final population. Similar letter(s) in a column are non-significant statistically at $p\leq0.05$

Table 2: Effect of *A. indica* and *T. vulgaris* on tomato growth parameters exposed to *M. incognita*

Treatments	Root length (cm)	Root dry weight (g)	Root fresh weight (g)	Shoot length (cm)	Shoot dry weight (g)	Shoot fresh weight (g)
Neem extract (30 ppm)	59.5±13^{b}	0.23±0.002^{f}	0.38±0.007^{i}	17.12±2^{l}	28±3 ^{o}	50±5^{r}
Neem extract (40 ppm)	50.2±5^{c}	0.17±0.001^{g}	0.34±0.005^{i}	18.40±3^{l}	41±5^{p}	85±12^{s}
Thyme extract (30 ppm)	31.47±12^{a}	0.30±0.002^{d}	0.51±0.003^{j}	24.25±3^{m}	32±4^{o}	62±9^{t}
Thyme extract (40 ppm)	41.04±12^{d}	0.25±0.002^{e}	0.49±0.003^{j}	25.25±3^{m}	28±1^{o}	48±8^{r}
Fenamiphos (60 ppm)	60.2±5^{b}	0.16±.0001^{g}	0.35±0.004^{i}	18.01±5^{l}	45±6^{p}	90±15^{s}
Control	28.87±9^{a}	0.36±0.01^{h}	0.77±0.008^{k}	10.91±6^{n}	17±2 ^{q}	25±4^{u}

Values in the same column, followed by a different letter(s) are significantly different, according to LSD ($p<0.05$). Each treatment had ten replications (five replications in two successive experiments). Similar letter(s) in a column are non-significant statistically at $p\leq0.05$

For the gall index, the nematicide treatment (fenamiphos) resulted in the lowest (1.47) and the untreated control the highest (6.2). Interestingly, the neem extract (40 ppm) treatments resulted in the lowest gall index of 1.61 that was no significant difference with fenamiphos ($p>0.5$). The Reproductive factor (Rf) was lowest in fenamiphos (0.13), followed by neem extract (40 ppm) (0.16), which was no significant difference with control ($p>0.5$) (Table 1).

Plant growth parameters: Root dry weight was the highest in fenamiphos treatment (0.16 g) followed by neem extract (40 ppm), which was 0.17 g (Table 2) that there were no significant differences ($p>0.5$). Root fresh weight in Fenamiphos (60 ppm) was 0.35 g, which showed no significant difference with neem extract (30 and 40 ppm) ($p>0.5$). The highest root length (59.5 cm) was for neem extract (30 ppm), which showed significantly different than control (28.87 cm) (Table 2). The lowest root length was 31.47 cm for thyme extract (30 ppm), which showed no significant difference from control ($p>0.5$). The highest shoot length, 25.25 cm observed for thyme extract (40 ppm), which showed significantly different from control (10.91 cm). The lowest shoot length, 17.12 cm observed for neem extract (30 ppm), which showed significantly different from the control (Table 2).

Worldwide, medicinal plants have drawn attention for their nematicidal effects for many years[17]. Our research has determined the nematicidal effects of two medicinal plants on *M. incognita*, one of the major pests in tomato orchards in southeast Iran. Our results indicated that neem and thyme extract was highly effective in killing J2 and decreasing symptoms caused by *M. incognita*. Moreover, the results were similar to those of fenamiphos.

Generally, the mortality of J2 of *Meloidogyne* spp. was higher in 48 and 72 hrs than 24 hrs after exposure to exposed plant extract[18]. Hence, in this study, 48 and 72 hrs were selected for the experiments. In the *in vitro* laboratory experiment, neem and thyme extract (40 ppm) was significantly increased the mortality of *M. incognita* J2. Similar activity has been reported for other plant products against various *Meloidogyne* spp.

In our greenhouse study, extracts of the two medicinal plants showed nematicidal effects against *M. incognita* by significantly reducing the gall index, root galling number and Rf of this nematode pest. These effects were pronounced for neem plant extracts in particular, which were highly effective against *M. incognita*. These results agree with those obtained for *M. incognita* by Hasabo and Noweer[16] with aqueous extracts of leaves of basil (*Ocimum basilicum*), marigold (*Tagetes erecta*), pyrethrum (*Chrysanthemum cinerariafolium*), Chinaberry (*Melia azedarach*) and seeds of the neem (*Azadirachta indica*) and by Sharma and Pandey[19] with *Withania somnifera* extracts. Several studies have reported between 70-100% mortality of root-knot nematodes using different aqueous extracts of neem formulations[20]. In the present study, the increase in the percentage of immobility and mortality caused by the higher concentrations of leaf extract of neem (40 ppm) was observed. This is in agreement with the previous studies[7]. It is possible that if the static (paralyzed) juveniles were not returned to the water, they

might be mistaken as dead, or the time of the observation for their revival was not long enough. Additionally, the mortality of the juveniles might increase due to the lack of oxygen in the amended dishes. However, this should have been avoided by using small volumes of extract in containers with a sizeable surface-to-air contact.

The previous work indicated that adding essential oils of *Allium sativum* and *T. vulgaris* to the soil, at a volume of 50 µL per plant, caused a reduction in the number of galls and the egg mass of *M. incognita* race 2 in tomato plants[9]. The same result was obtained in this study, in which 40 ppm of *T. vulgaris* significantly affected the reproduction factors of *M. incognita*.

Compounds such as terpenoids, particularly monoterpenoids and related phenols, are ingredients of plant essential oils and extracts and possess inhibitory effects on the hatching of J2 and nematicidal activity[21]. The nematicidal mechanisms of plant extracts are complex and still under investigation. However, numerous plant extracts have been reported to inhibit nematode biology, physiology and/or reproduction.

CONCLUSION

Nowadays, eco-friendly strategies for plant-parasitic nematode management are included in many research projects across the globe. Leaf extract of *A. indica* and *T. vulgaris* possess nematicidal characteristics and can be used for the management of root-knot nematodes. Although these plants are commonly available in most parts of Iran, however, they should be more surveyed for the control of root-knot nematodes in tomato orchards. Therefore, our findings underline thyme and neem having potential for use in an integrated pest management program.

SIGNIFICANCE STATEMENT

This study discovers the potential effect of some plant extracts on *M. incognita* that can be beneficial for the management of the plant-parasitic nematode. This study will help the researcher to uncover the critical areas of biological control of root-knot nematodes that many researchers were not able to explore. Thus a new theory on plant extracts may be arrived at.

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