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Nematicidal Effects of Five Plant Essential Oils on the Southern Root-Knot Nematode, *Meloidogyne incognita* Race 2

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Abstract: This research was conducted to evaluate the nematicidal activity of five plant essential oils (rosemary, thyme, mint, garlic and sesame) against Southern root-knot nematode, *Meloidogyne incognita* race 2. Trial was designed as randomized complete block design with three nematode inoculums densities (0, 1000 and 2000 J2 plant^{-1}) and three essential oil volumes (0, 50 and 150 plant μL^{-1}) replicated six times. There were no significant differences between nematode inoculums density and essential oil volumes used. However, all oil treatments suppressed nematode populations and resulted an increase in root mass tissue. Compared with control groups, among the essential oils, thyme (2.82±0.47%) and garlic (5.53±1.68%) treatments reduced root galling significantly and produced the lowest percent of galls on the plants whereas, rosemary, mint and sesame treatments were less effective in reducing root-galling. Compared with control groups thyme (2.46±0.17) and garlic (2.50±0.22) yielded also the lowest egg masses. Among five plant essential oils, application of a rate of 50 μL plant^{-1} of thyme or garlic in tomato production areas could give the best results in root nematode control and be an alternative to the current control methods. However, more studies need to be conducted in the field and greenhouse conditions to see the possible differences because nematodes and essential oils could be influenced by different experimental conditions.

Key words: *Meloidogyne incognita*, Southern root-knot nematode, plant essential oil, nematicidal activity, garlic, rosemary, mint, thyme, sesame

INTRODUCTION

It is difficult to estimate yield suppression caused by plant-pathogenic nematodes because oftentimes damage is not limited to a single nematode species. It is reported that plant-pathogenic nematodes annually cause $78 billion (Barker *et al*., 1994) to $100 billion (Sasser, 1987) in losses to agricultural production worldwide. Root-knot nematodes are major contributors of these economical losses. Yield suppression in vegetable production by root-knot nematodes is reported to range from 10% (Taylor, 1967) to 80% (Siddiqi, 1986). These nematodes have the potential to cause large economical losses on most of the important horticultural and agronomic crops grown in Turkey. However, there is little documentation on the actual amounts of these losses.

Annual production of tomato in Turkey is reported at approximately 10 million metric tons, which is 9% of the total production worldwide and this amount is increasing every year (Anonymous, 2007). Tomato is an important crop for the export market in Europe and Russia. Among all the important pests and pathogens, root-knot nematodes (*Meloidogyne* sp.) are considered as one of the greatest threats to successful tomato production in this country (Sogut and Elekcioglu, 2000a, b). Of these nematode species, *Meloidogyne incognita* race 2 is one of the most common root-knot nematode found in fields and greenhouses in Turkey. Chemical control is one of the leading management tactics that are being applied against this nematode. Within chemical control, methyl bromide was the most effective and widely used fumigant for soil borne diseases and pests including nematodes.

However, phasing out of methyl bromide since its listing as ozone depleting chemical (UNEP, 1995), triggered many scientists to put efforts to quickly develop and implement economically feasible and environmentally compatible alternatives. It is necessary to look for natural compounds with less toxicity and low environmental adverse impact. Screening naturally occurring compounds in plants is one way of searching for such nematicidal compounds. Essential oils from plants have been found to possess antimicrobial and insecticidal activity (Oka *et al*., 2000). Only a few oils or their components have been evaluated for their nematicidal effects. In present study,

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essential oils obtained from five aromatic plants were evaluated for their nematicidal effects on southern root nematode *Meloidogyne incognita* race 2.

MATERIALS AND METHODS

Source of nematode and essential oils: The culture of *Meloidogyne incognita* race 2 used in this study was obtained from West Mediterranean Agricultural Research Institute, Antalya, Turkey. Species identification was confirmed by morphological observations and photographs of perineal patterns derived from the single egg mass of the culture. Forty females were extracted from roots of tomato (*Lycopersicon esculentum var. esculentum*) grown in a growth chamber. Perineal patterns were prepared following procedures of Hartman and Sasser (1985) and morphological observations were completed within 12 h following slide preparation.

Five plant essential oils tested in this study were garlic (*Allium sativum* L.), rosemary (*Rosmarinus officinalis* L.), mint (*Mentha arvensis* L.), thyme (*Thymus vulgaris* L.) and sesame (*Sesamum indicum* L.). Source of plants, plant parts and extraction method of oils are shown in Table 1. These essential oils were purchased from ECODAB (Antalya, Turkey).

Experimental design: The experiment was designed as a 7×3×3 factorial treatment arrangement in a randomized complete block with six replications. The trial was conducted twice. Factors and the levels for each factors consisted of 7 plant essential oil treatments (5 plant essential oils (Table 1) with two control groups (Control+ with nematode only and Control-left without any applications), three nematodes (second stage juvenile (J2)) inoculum levels: control (0 J2 soil plant^{-1}), low (1000 J2 soil plant^{-1}) and high (2000 J2 soil plant^{-1}) and three essential oil application rates: control (0 μL plant^{-1}), low (50 μL plant^{-1}) and high (150 μL plant^{-1}).

Three weeks old seedlings were transplanted into 15 cm diam plastic pots containing sterilized soil. Five days after transplanting, each seedling was inoculated with the appropriate inoculum level of second stage *M. incognita* race 2 juveniles from the same inoculum suspension. Second stage nematode juveniles (J2) were obtained from hatched eggs (masses) dissected manually from tomato roots. Within the same day of nematode inoculation, plants were treated with appropriate rates of essential oils to be tested. Tests were carried out in a growth chamber for 65±2 days, with temperature averaging 28°C. Plants were watered daily and fertilized bi-weekly with Lebosol (Lebosol Dünger, GMBH) using 2 g L^{-1}.

Table 1: The source and extraction methods of plant essential oils used in the study

Source of plant	Scientific name	Plant parts	Extraction method
Garlic	*Allium sativum*	Seeds	Cold pressing
Rosemary	*Rosmarinus officinalis*	Leaves	Steam distillation
Mint	*Mentha arvensis*	Leaves	Steam distillation
Thyme	*Thymus vulgaris*	Leaves	Steam distillation
Sesame	*Sesamum indicum*	Seeds	Cold pressing

At harvest, plants were removed from pots and with root systems were washed. Root galling were assed using 0-10 scale, where 0 = no galling, 1 = 10%, …. 10 = 100% of root system galled (Zeck, 1971) and egg mass indices were assessed using a 0-5 scale, where 0 = no egg mass, 1 = 1-2 egg masses, 2 = 3-10 egg masses, 3 = 11-30 egg masses, 4 = 31-100 egg masses and 5 = >100 egg masses (Taylor and Sasser, 1978). The values of these indices were used to rate the nematode response to five plant essential oils treatments.

Statistical analysis: Data were subjected to Analysis of Variance (ANOVA) with SAS software (SAS Institute, Cary, NC) and mean separation was conducted using Duncan's Multiple Range Test ($p \leq 0.05$). The experiment was conducted twice; no differences were detected ($p > 0.05$) between the trials therefore, pooled data was analyzed and shown in Table 2-4.

RESULTS AND DISCUSSION

There was a significant effect of plant essential oils individually on root-galling, egg mass numbers, plant height and shoot fresh weight ($p \leq 0.01$) (Table 2). There was no significant interaction of plant essential oils x plant essential rates or plant essential rates x nematode inoculum levels interactions on root-galling, plant height and shoot fresh weight ($p \leq 0.05$) (Table 2). Also, there was no significant interaction of plant essential oils x plant essential oil rates x nematode inoculum levels for root-galling, plant height and shoot fresh weight ($p \leq 0.05$) (Table 2). Pooled data of two trials showed that there was a significant difference between two inoculum levels of nematodes together and its control level, however no differences were observed between two inoculum levels of nematodes individually (Table 3). Similar situation were observed also for essential oil application rates (Table 3) for all parameters measured thus, data were combined (Table 4).

The number of galls produced on relatively low for all treatments, however there were greater numbers in control pots than other treatments both inoculum levels ($p \leq 0.05$) (Table 3). *M. incognita* race 2 produced lower percentage of galls on F-191 tomato in thyme (2.82±0.47%) and garlic (5.53±1.68%) treatments than any other essential oil

Table 2: Analysis of variance for the effects seven essential oils treatments and their three application rates to three inoculum level of *Meloidogyne incognita* race 2 and their interactions on the root galling, egg mass indices, plant height and plant shoot fresh weight of tomato cv. F-191

Source of variation	df	Root galling (%)[a]	F-value	Egg mass index[b]	F-value	Plant height (cm)	F-value	Fresh weight (g)	F-value
Plant essential oils (O)	6	**	9.32	**[c]	20.19	**	3.70	**	8.50
Plant essential oils rates (R)	2	NS	2.26	*[d]	5.02	NS	0.03	NS	0.70
Nematode inoculum levels (I)	2	NS	0.05	NS[e]	1.32	*	4.94	*	4.91
OxR	12	NS	0.49	NS	1.06	NS	1.15	NS	0.61
OxI	12	*	2.95	**	7.94	NS	0.72	NS	1.06
RxI	4	NS	0.03	NS	0.03	NS	1.85	NS	1.36
OxRxI	24	NS	0.13	NS	1.75	NS	0.70	NS	1.19

[a]Gall index: 0-10 scale; where 0 = no galling, 1 = 10%, ... 10 = 100% of root system galled (Zeck, 1971). [b]Egg mass indices: 0-5 scale where, 0: No egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses and 5: >100 egg masses (Taylor and Sasser, 1978), [c]**: significant at p≤0.01 level. [d]*: significant at p≤0.05 level. [E]NS: Not Significant

Table 3: Effects of three application rates of seven plan essential oils and two inoculum level of *Meloidogyne incognita* race 2, on the root galling, egg mass indices, plant height and plant shoot fresh weight of tomato cv. F-191

	Nematode inoculum levels (J2 plant^{-1})			Essential oil application rates (µL plant^{-1})		
Parameters	0	1000	2000	0	50	150
Root galling[a]	0±0b[b]	18.70±2.60a	19.49±3.27a	29.57±7.58a	19.38±3.06b	13.75±2.53b
Egg mass indices[c]	0±0b	3.27±0.13a	3.09±0.14a	2.47±0.41b	3.13±0.14a	2.94±0.15a
Plant height (cm)	28.28±0.96a	21.41±0.86b	19.14±0.60b	23.0±1.16a	20.35±0.71a	20.18±0.91a
Shoot fresh weight (g)	10.95±0.98a	5.86±0.30b	5.04±0.23b	6.95±0.77a	5.66±0.30b	5.34±0.27b

[a]Gall index: 0-10 scale; where 0 = no galling, 1 = 10%, ... 10 = 100% of root system galled (Zeck, 1971). [b]Data were transformed with [Arcsinevx] before analysis and nontransformed data are presented. [c]Egg mass indices: 0-5 scale, where 0: No egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses and 5: >100 egg masses (Taylor and Sasser, 1978). Data are means of ten replications (pooled data of two trials). Means followed by the same letter within a row in the same inoculum level or essential oil application rate are not significantly different according to Duncan multiple range test (p≤0.05)

Table 4: Effect of seven plant essential oil treatments on root galling, egg mess indices, plant height and shoot fresh weight of *Meloidogyne incognita* race 2 infected tomato cv. F-191 (Mean±SD)

Essential oils treatments	Root galling[a]	Egg mass indices[b]	Plant height (cm)	Shoot fresh weight (g)
Garlic	5.53±1.68c[c]	2.50±0.22b	19.35±0.92bc	5.63±0.43bc
Rosemary	22.85±5.03b	3.21±0.28a	20.46±1.03bc	5.63±0.34bc
Mint	22.64±4.70b	3.64±0.21a	23.28±2.0b	5.94±0.60b
Thyme	2.82±0.47c	2.46±0.17b	17.71±0.78c	4.18±0.28c
Sesame	29.00±5.65b	3.82±0.17a	20.53±1.18bc	6.13±0.50b
Control (+)	44.35±9.07a	3.71±0.19a	20.35±1.13bc	4.95±0.47bc
Control (–)	0±0c	0±0c	28.28±0.96a	10.95±0.98a

[a]Gall index: 0-10 scale; where 0 = no galling, 1 = 10%, ... 10 = 100% of root system galled (Zeck, 1971). [b]Egg mass indices: 0-5 scale, where 0 = no egg mass, 1: 1-2 egg masses, 2: 3-10 egg masses, 3: 11-30 egg masses, 4: 31-100 egg masses and 5: >100 egg masses (Taylor and Sasser, 1978). [c]Data were transformed with [Arcsinevx] before analysis and nontransformed data are presented. Data are means of six replications (pooled data of two trials). Means followed by the same letter within a column are not significantly different according to duncan multiple range test (p≤0.05)

tested. Contradictory of these two essential oil results, sesame produced the highest root-galling (29.06±5.65%) among the treatments, coming second following control (+) group plants (p≤0.05) (Table 4).

Meloidogyne incognita race 2 produced also significantly lower number of egg mass on plants in thyme (2.46±0.17) and garlic (2.50±0.22) than those in other essential oil treatments (p≤0.05) (Table 4). Whereas, the nematode produced the highest number of egg mass in sesame (3.82±0.17) among all the treatments including control (+) group (p≤0.05) (Table 4).

The plant heights of tomato did not differ in essential oils applications rates but did differ among the essential oils individually (Table 2). Excluding nontreated control (-) group, plants treated with sesame sustained the highest plant heights as compared with the other treatments (p≤0.05). Numbers in fresh weights varied among all essential oil treatments (p≤0.05) (Table 4). Sesame (6.13±0.50) and mint (5.94±0.60) resulted the highest shoot fresh weight among all the essential oil treatments.

A few essential oils have been evaluated for their nematicidal effects (Sangwan *et al*., 1990). In a recent laboratory study, essential oils from 25 plant species and aromatic plants were evaluated for their nematicidal effect on the root-knot nematode *M. javanica*. Majority of essential oils tested immobilized nematode juveniles and/or inhibited nematode egg hatching.

Another recent study conducted by Elbadri *et al*. (2008) demonstrated that fthe of five essential oils tested against pine wood nematode, *Bursaphelenchus xylophilus*, found to be highly promising in controlling of nematodes. One of effective essential oils tested in this study was from Thyme (*Thymus vulgaris*) and results of this study corroborate the results from thyme treatment where we found a significantly lower root-galling percent and egg mass numbers caused by another plant parasitic nematode, *M. incognita* race 2.

CONCLUSION

This study evaluated nematicidal effect of five plant essential oils on the southern root-knot nematode, *Meloidogyne incognita* race 2, which is one of major pest in many vegetables plants including tomato. Present study demonstrated that garlic and thyme essential oils were more effective in reducing the nematode infestation, consequently, reducing number of egg mass and rootgalling formation caused by *M. incognita* than the other plant essential oils tested. It was evident that garlic and thyme essential oils deserves serious consideration for inclusion into nematode management tactics. Not many studies have been conducted with particular plant essential oils and *M. incognita* and the present results aid in determining how some of plant essential oils can be used also as a alternative to current chemicals.

Some measures should be put into consideration to practice for application of these essential oils. More studies need to be conducted in the field and greenhouse conditions to see the possible differences because nematodes and essential oils could be influenced by different experimental conditions. Moreover, there is also in need of research for finding possible phytotoxicity of these essential oils on the host plants before being applied.

REFERENCES

Anonymous, 2007. TURKSTAT (Turkish Statistical Institute), Prime Ministry Republic of Turkey, Agricultural Structure (Production, Price, Value). http://www.turkstat.gov.tr.

Barker, K.R., R.S. Hussey, L.R. Krusberg and G.W. Bird *et al.*, 1994. Plant and soil nematodes: Societal impact and focus for the future. J. Nematol., 26: 127-137.

Elbadri, G.A.A., D.W. Lee, J.C. Park, Y.H. Bin, H.Y. Choo, S.M. Lee and T.H. Lim, 2008. Nematicidal screening of essential oils and herbal extracts against *Bursaphelenchus xylophilus*. Plant Pathol. J., 24: 178-182.

Hartman, K.M. and J.N. Sasser, 1985. Identification of *Meloidogyne* sp. on the Basis of Differential Host Test and Perineal Pattern Morphology. In: Barker, K.R., C.C. Carter and J.N. Sasser (Eds.). An Advanced Treatise on *Meloidogyne*. Methodology, 2: 69-77. Raleigh, NC: North Carolina States Graphics.

Oka, Y., S. Nacar, E. Putievsky, U. Ravid, Z. Yaniv and Y. Spiegel, 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. Phytopathology, 90: 710-715.

Sangwan, N.K., B.S. Verma, K.K. Verma and K.S. Dhindsa, 1990. Nematicidal activity of some essential oils. Pestic. Sci., 28: 331-335. DOI: 10.1002/ps.2780280311.

Sasser, J.N., 1987. A Perspective on Nematode Problems Worldwide. In: Saxena, M.C., R.A. Sikora and J.P. Srtvastava (Eds.). Nematodes Parasitic to Cereals and Legumes in Temperate Semi-arid.

Siddiqi, M.R., 1986. Tylenchida: Parasites of plants and insects. Commonwealth Institute of Parasitology, CAB, Wallingford, Oxon, UK, pp: 645.

Sogut, M.A. and I.H. Elekcioglu, 2000a. Determination of *Meloidogyne* Goeldi, 1892 (Nemata: Heteroderidae) species and races found in vegetable growing areas of the Mediterranean region of Turkey. Turk. J. Entomol., 24 (1): 33-40.

Sogut, M.A. and I.H. Elekcioglu, 2000b. Investigation on some biological aspects of *Meloidogyne incognita* Chitwood (Nemata: Heteroderidae) race 2 on different tomato varieties. Turk. J. Entomol., 24 (2): 113-125.

Taylor, A.L., 1967. Principles of measurement of crop losses: Nematodes. Proc. FAO Symposium. Crop losses, Rome, pp: 225-232.

Taylor, A.I. and J.N. Sasser, 1978. Biology, identification and control of root-knot nematode (*Meloidogyne* sp.). North Carolina State University Graphics, Raleigh. NC 27607.

UNEP, 1995. Montreal protocol on substance that deplete the ozone layer. Methyl Bromide Technical Option Committee, Kenya, pp: 304. http://www.unep.org.

Zeck, W.M., 1971. A rating scheme for field evaluation of root-knot nematode infestation. Pflanzenschutz Nachrichten, Bayer. Published by Farbenfabriken Ag. Leverkusen, 10: 141-144.