EFFECT OF ESSENTIAL OILS ON THE ROOT-KNOT NEMATODE MANAGEMENT

Deisy Xavier Amora[1*], Guilherme Silva de Podestá[2], Pedro Henrique França Grupioni[2], Érica das Graças Carvalho Nasu[3], Leonardo Domingues de Figueiredo[4], Fernanda Carla Ferreira[5], Leandro Grassi de Freitas[6], Everaldo Antônio Lopes[7], Silamar Ferraz[6]

ABSTRACT:

In an attempt to find alternative compounds for the management of root-knot nematodes, whether essential oils would be absorbed by plants and affect the development of *Meloidogyne javanica* inside tomato roots was evaluated. First, the viability, *in vitro*, of the essential oils of *Artemisia absinthium* (wormwood), *Mentha* × *piperita* (peppermint), *Origanum vulgare* (oregano) and *Thymus vulgaris* (thyme) was assessed. Then, the same essential oils were tested in pot experiments, at concentrations of 0.25% (v / v) and 0.5% (v / v). In *in vitro* tests, all essential oils were effective in killing the juveniles, resulting in mortality rates of 99.0% at both concentrations. The essential oils of *Mentha* × *piperita*, *O. vulgare* and *T. vulgaris* were effective in reducing nematode hatching to about 2.0%. In tests conducted under greenhouse conditions, however, tomato plants treated with the *essential oils of A. absinthium, Mentha* × *piperita and O. vulgare had increased the number of M. javanica eggs and galls.* Therefore, despite being highly toxic to nematodes *in vitro*, some of the essential oils tested in this study increased nematode development.

KEYWORDS: Alternative control, *Artemisia absinthium*, *Meloidogyne javanica, Mentha* × *piperita*, *Origanum vulgare, Thymus vulgaris*

EFEITO DE ÓLEOS ESSENCIAIS NO CONTROLE DOS NEMATOIDES DAS GALHAS

RESUMO:

Na tentativa de encontrar compostos alternativos para o manejo dos nematoides das galhas, avaliou-se se óleos essenciais de plantas medicinais podem ser absorvidos pelas plantas e afetar o desenvolvimento de *Meloidogyne javanica* dentro de raízes de plantas de tomate. Primeiro, foi avaliado, *in vitro*, a viabilidade dos óleos essenciais de *Artemisia absinthium* (artemísia), *Mentha* × *piperita* (hortelã), *Origanum vulgare* (orégano) e *Thymus vulgaris* (tomilho). Em seguida, foram testados os mesmos óleos essenciais em experimentos em vasos nas concentrações de 0,25% (v / v) e 0,5% (v / v). Nos testes *in vitro*, todos os óleos essenciais foram eficazes em matar os juvenis do nematoide, resultando em taxas de mortalidade de 99,0%, em ambas as concentrações. Os óleos essenciais de *Mentha* × *piperita*, *O. vulgare* e *T. vulgaris* foram eficazes na redução da eclosão do nematoide, com taxas de cerca 2,0% de eclosão. Nos experimentos realizados em casa de vegetação, no entanto, as plantas de tomate tratadas com os óleos essenciais de *A. absinthium*, *Mentha* × *piperita* e *O. vulgare* tiveram aumento no número de ovos e galhas de *M. javanica*. Assim, apesar de serem altamente tóxicos para os nematoides *in vitro*, alguns dos óleos essenciais testados neste estudo estimularam o desenvolvimento de *M. javanica*.

PALAVRAS-CHAVE: Controle alternativo, *Artemisia absinthium*, *Meloidogyne javanica, Mentha* × *piperita*, *Origanum vulgare, Thymus vulgaris*

1- Engenheira Agrônoma, DSc., Universidade Federal de Viçosa, Av. P.H. Rolfs, Campus Universitário, CEP 36570-900, Viçosa (MG), Brasil. deisyamora@yahoo.com.br. *Corresponding author
2 - Engenheiro Agrônomo, DSc., Professor do Curso de Engenharia Agronômica, Universidade Federal da Paraíba – Campus Areia, Rodovia PB-079, CEP: 58397-000, Areia (PB) Brasil. oguipodesta@yahoo.com.br
3 - Bióloga, DSc., Universidade Federal de Viçosa, Av. P.H. Rolfs, Campus Universitário, CEP 36570-900, Viçosa (MG), Brasil. ericanasu@gmail.com
4 - Engenheiro Agrônomo, MSc., Universidade Federal de Viçosa, Av. P.H. Rolfs, Campus Universitário, CEP 36570-900, Viçosa (MG), Brasil. leonardoagronomiaufv@bol.com.br
5 - Engenheira Agrônoma, MSc., Doutoranda em Fitopatologia. Universidade Federal de Viçosa, Av. P.H. Rolfs, Campus Universitário, CEP 36570-900, Viçosa (MG), Brasil. fe.carla1@gmail.com
6 - Engenheiros Agrônomos, DSc., Professores do Curso de Engenharia Agronômica, Universidade Federal de Viçosa, Av. P.H. Rolfs, Campus Universitário, CEP 36570-900, Viçosa (MG), Brasil. leandro@ufv.br; silamar@ufv.br
7 - Engenheiro Agrônomo, DSc., Professor do Curso de Engenharia Agronômica, Universidade Federal de Viçosa - Campus Rio Paranaíba, Instituto de Ciências Agrárias, 38810-000 – Rio Paranaíba/MG – Brasil. everaldolopes@ufv.br

Received 18 Nov, 2016 • Accepted 28 Jun, 2017

INTRODUCTION

Nematodes of the genus *Meloidogyne* Goeldi are harmful to most economically important crops worldwide and it is a pathogen of hard control. The use of synthetic nematicides is becoming limited due to their elevated costs and to the demand for food without pesticide residues and for safer working conditions for the farming community. Besides this, the growing market for organic products opens a new path for alternative forms of pathogen control. Thus, researchers have been searching for sustainable ways of managing nematodes (Ferraz et al., 2010).

The study of nematicidal compounds in plants is well established. The nematicidal properties of several medicinal plants have been revealed by experiments using tinctures, extracts, incorporation of organic matter, among others (Pandey and Kalra, 2010; Chaudhary et al., 2013; Kokalis-Burelle et al., 2013). Plants have metabolic pathways that may produce secondary compounds that provide protection, attract pollinators and promote environmental adaptation. Thus, many higher plants contain compounds with nematicidal properties that can kill, inhibit hatching or motility of nematodes (Chitwood, 2002).

The essential oils extracted from plants are substances rich in secondary compounds and are complex mixtures that may contain 20 to 60 components in different concentrations. Thus, a good way to explore the nematicidal potential of plants is through the use of these essential oils (Andrés et al., 2012). Besides the *in vitro* tests, essential oils have been tested in different ways to decrease nematode population. The essential oils of cinnamon (*Cinnamomum zeylanicum* Blume), verbena (*Verbena officinalis* L.) and mustard (*Brassica nigra* (L.) Koch) reduced the population of *Aphelenchoides besseyi* Cristie. from seeds of *Brachiaria brizantha* (A. Rich.) Stapf 'Marandu' (Monteiro et al., 2014). Additionally, the soil fumigation with essential oils of *Eucalyptus citriodora* Hook*., E. globulus* Labill*., Pelargonium asperum* Ehrh. ex. Spreng. and *Ruta graveolens* L. reduced *Meloidogyne incognita* (Kofoid & White) Chitwood multiplication and gall formation on tomato roots (Laquale et al., 2015).

Since *Meloidogyne* sp. is an endoparasite, a translocable compound that could affect the nematode inside root plants is desirable. This compound could be found in the essential oils, once it contains several antimicrobial ingredients that work through various modes of action. Eugenol, a constituent of *Ocimum sanctum* L. and others plants, has shown effect on the viability of nematodes, in addition to a systemic effect (Bala and Sukul, 1987; Li et al., 2013; Moreira et al., 2013). In line with these findings, for screening a systemic compound with nematicidal activity, we chose to work with essential oils because they are generally more complex and have more concentrated active compounds than tinctures or extracts. Thereby, the aim of this study was to determine whether essential oils extracted from wormwood (*Artemisia absinthium* L.), peppermint (*Mentha* × *piperita* L.), oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) would affect the development of *Meloidogyne javanica* (Treub) Chitwood when applied on the leaves and on the root system of infected tomato plants.

MATERIAL AND METHODS

The inoculum of *Meloidogyne javanica* was obtained from tomato roots (*Solanum lycopersicum* L.) cultivar 'Santa Clara' grown in a greenhouse at the Federal University of Viçosa, Minas Gerais, Brazil. Nematode eggs were extracted according to the method developed by Hussey and Barker (1973) and modified by Boneti and Ferraz (1981).

The essential oils of *Artemisia absinthium*, *Mentha* × *piperita*, *Origanum vulgare* and *Thymus vulgaris*, used in the experiments, were acquired at Laszlo Aromaterapia (Belo Horizonte, Minas Gerais, Brazil). The essential oils, obtained from leaves of the medicinal plants by steam distillation, were analyzed by high-resolution gas chromatography. Oil constitutions, provided by the manufacturer, are listed in Table 1.

Because of the insolubility of essential oils in water, all experiments were carried out using solutions of essential oils in 2% dimethylsulfoxide (DMSO). Essential oils were evaluated at concentrations of 0.25% and 0.5% (v/v). A screening test was made to check the phytotoxicity of essential oils on tomato leaves and roots, being 0.5% the highest dose that did not kill the plants (data not shown). Half of this dose was chosen as the second dose to be tested. Purified water and 2% DMSO solution (v / v) were used as controls.

Table 1. Chemical composition of essential oils extracted from leaves of *Artemisia absinthium, Mentha × piperita*, *Origanum vulgare* and *Thymus vulgaris*.

Chemical composition of essential oils (%)							
Artemisia absinthium		*Mentha × piperita*		*Origanum vulgare*		*Thymus vulgaris*	
β-thuione	51.1	Menthol	47.0	Carvacrol	77	Thymol	56.0
Linalyl acetate	24.3	Menthone	26.0	Thymol	4.3	p-cymene	31.0
Linalool	2.6	Neomenthol	11.0	p-cymene	4.0	γ-terpinene	5.4
α-thujone	2.6	Menthyl acetate	5.7	γ-terpinene	3.2	Myrcene	1.6
Sabinene	2.5	Isomenthol	2.9	Caryophyllene	1.5	α-pinene	1.4
Camphor	1.9	β-caryophyllene	1.0	Limonene	1.4	1,8-cineole	1.2
Caryophyllene	1.5	1,8-cineole	0.9	Phellandrene	1.2	Camphene	0.9
Myrcene	1.4	Isomenthone	0.6	Camphene	1.1	Linalool	0.7
Cineole	1.4	Limonene	0.5	Terpinen-4-ol	0.8	α-thujene	0.6
Spathulenol	1.4	α-terpinene	0.2	1,8-cineole	0.7	β- caryophyllene	0.3
Germacrene d	0.7	α-pinene	0.1	Camphor	0.7	Limonene	0.2
α-pinene	0.5	β-pinene	0.1	α-pinene	0.5	β-pinene	0.1
		p-cymene	0.1	α-terpineol	0.2		

Essential oils were obtained from leaves of medicinal plants by steam distillation. The constitutional analysis, provided by the manufacturer, was conducted through high-resolution gas chromatography.

In vitro experiments

Before assaying the effect of the essential oils under greenhouse conditions, the viability of the essential oils was checked *in vitro* on second stage juveniles (J_2) of *M. javanica* by measuring hatching and mortality. For the hatching test, 1 mL of a suspension containing approximately 300 eggs was placed in a Petri dish (5 cm diameter) containing 4.0 mL of essential oils diluted at concentrations of 0.25% and 0.5% (v/v). The plates were incubated at 26° C in the dark for 10 days. The hatched juveniles were counted every 48 h, totaling five counts. At each evaluation, the average number of hatched J_2 on three plates was recorded for each treatment. The hatching analysis was performed by measuring the area under the curve of hatching progress (AUCHP):

$$AACPE = \sum_{i=1}^{n-1} \left(\frac{Y_i + Y_i + 1}{2} \right) \times (T_i + 1 + T_i)$$

Where:

n - number of observations;

Yi - percentage of hatching to the i^{th} observation;

Ti - time in days to the i^{th} observation.

For the mortality test, juveniles were obtained from eggs of *M. javanica* 48 hours after incubation in a Baermann funnel at 26ºC. In each Petri dish, 1 mL of suspension containing approximately 300 juveniles + 4.0 mL of essential oil diluted at concentrations of 0.25% and 0.5% (v/v) were added. Afterwards, the plates were incubated at 26°C in the dark for 24 h. To recover nematodes from potential immobility, the contents of each plate were rinsed under running tap water on a 500-mesh sieve and subsequently placed back on the plates with pure water and incubated for 24 h. Next, the percentage of dead J_2 was evaluated.

Both experiments were performed in a completely randomized 4 x 2 + 2 factorial design (4 essential oils x 2 concentrations + 2 additional controls - water and 2% DMSO) with three replications. The experimental unit consisted of one Petri dish.

In vivo experiments

Experiments were set up under greenhouse conditions in order to test two different ways to applicate the essential oils on plants: application on the leaves and on the soil. In the *in vivo* experiments, the essential oils' ability to be absorbed by roots or by leaves and to inhibit the development of *M. javanica* inside tomato roots was assessed. Two concentrations of essential oils, 0.25% and 0.5%, prepared as previously described, were ad-

ded to the soil or sprayed on the leaves of tomato plants. Plastic pots with 1 L capacity were filled with a mixture of soil and sand (1:1) pretreated with methyl bromide (80 cm^3/m^3 soil). Each pot was infested with 5,000 eggs of *M. javanica* before a tomato seedling approximately three weeks old was planted. To verify a possible systemic effect of the treatments, the essential oils applications started seven days after inoculation, when juveniles had already entered the roots. The application of essential oils was done as follows: when via soil, 10 mL of essential oil were pipetted directly around the stem of the plant; when via shoots, the essential oils were sprayed using a hand sprayer until the liquid covered all the leaves and was about to run off. When essential oils were sprayed on leaves, the pots were wrapped in plastic bags at each spraying, avoiding roots absorption, according to the methodology described by Bala and Sukul (1987). Applications were made weekly until the end of the experiment. The numbers of galls and eggs of *M. javanica* in the roots of the tomato plants were evaluated 60 days after soil infestation.

The experiment was conducted twice and performed in a completely randomized design using a 4 x 2 + 2 factorial scheme (4 essential oils x 2 concentrations + 2 controls - water and 2% DMSO) with seven replicates. The experimental unit consisted of one pot containing one plant. Data were subjected to analysis of variance and treatment means were compared by Tukey test (the effect of essential oils) and F (effect of dose) at 5% probability. Treatments were compared to control conditions by the Dunnet test and significance was set at 5%.

RESULTS AND DISCUSSION

The essential oils of *A. absinthium*, *Mentha × piperita, O. vulgare* and *T. vulgaris* caused mortality of *M. javanica* juveniles at a rate higher than 99.0%, regardless of the concentration evaluated (Table 2). All the essential oils reduced the hatching of nematode compared to the control condition, regardless of the dose (Table 2). Although the essential oil of *A. absinthium* did not inhibit hatching in the same proportion as of the other plants, it was still able to maintain hatching levels lower than those of the control, with the concentration of 0.5% being the most effective (Table 2).

Table 2. Rate of mortality and hatching of second stage juveniles of *Meloidogyne javanica* in distilled water, DMSO (2%) and essential oils of *Artemisia absinthium*, *Mentha × piperita*, *Origanum vulgare* and *Thymus vulgaris* at concentrations of 0.25% and 0.5%.

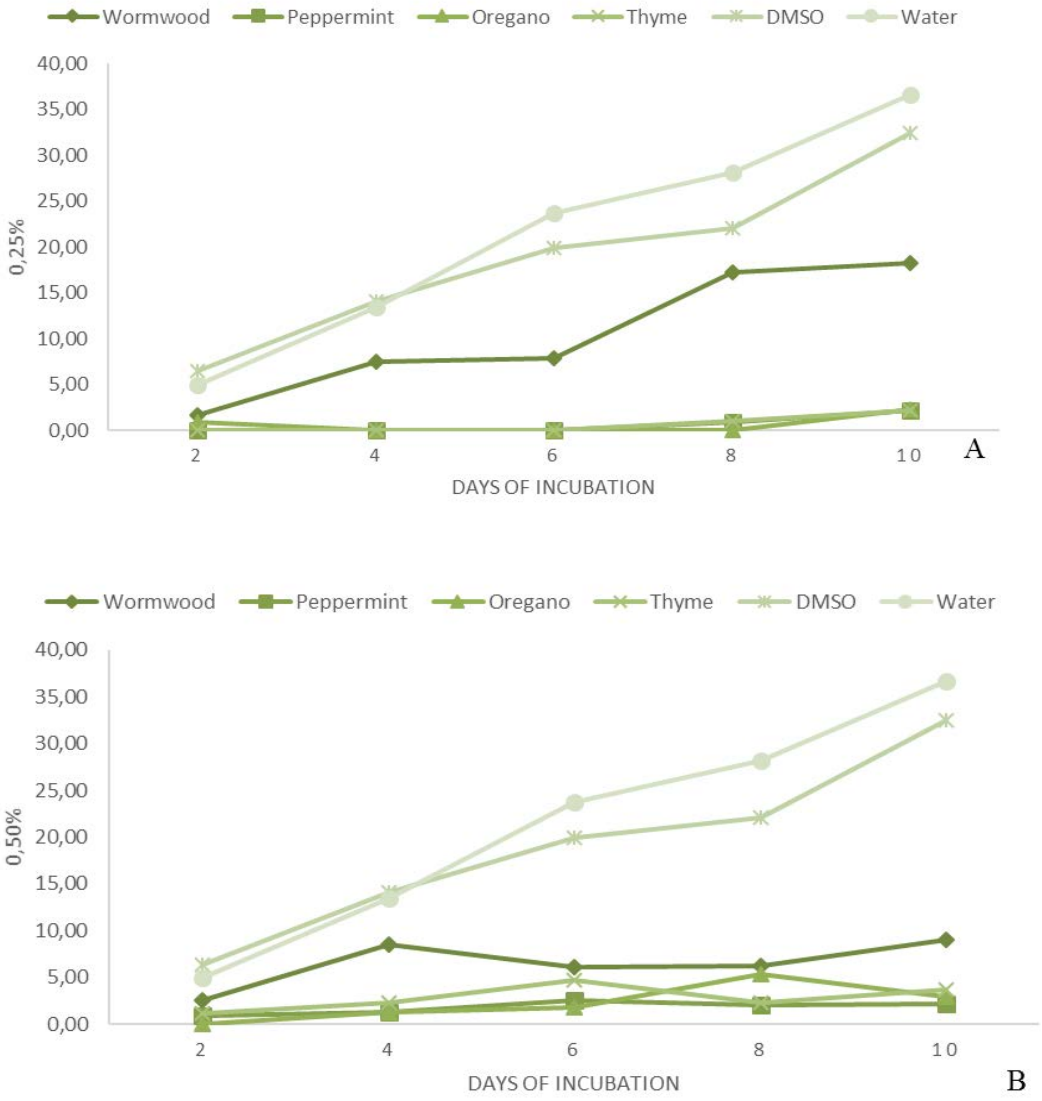
Treatments	Mortality rate [a]			Hatching rate [a]		
	0.25%	0.50%	Means	0.25%	0.50%	Means
A. absinthium	99.4	99.5	99.3[ns] *	18.2 Aa	9.0 Ba	13.6*
Mentha × piperita	99.1	99.2	99.6 *	2.1 Ab	2.2 Ab	2.1*
O. vulgare	99.4	99.8	99.3 *	2.4 Ab	3.0 Ab	2.7*
T. vulgaris	99.4	99.6	99.8 *	3.2 Ab	3.7 Ab	3.5*
Mean	99.3 [ns]	99.5		6.5	4.5	
DMSO		17.5			82.4	
Water		15.9			86.6	

[a]Data were transformed to Log 10 (x +1) to conform to the assumptions of analysis of variance. Original means are presented. Means followed by the same lowercase letters within columns and uppercase letters within rows did not differ significantly by the Tukey test at 5% probability; ns= not significant by the F test at 5% probability. Means followed by * differ statistically from Water by Dunnett's test at 5% probability.

The essential oils mentioned above reduced the hatching of nematode throughout the incubation period of the eggs at both concentrations tested, but 0.25% was the most efficient. On the other hand, the essential oil of *A. absinthium* resulted in greater amounts of hatching, but these numbers were still lower than in controls. At 0.25% concentration, there was a delay in nematode hatching until the sixth day of incubation. After this period, the hatching rate increased differently at the 0.5% dose, which in this case was the best (Figure 1).

The efficacy of nematicidal compounds present in the essential oils was confirmed in the *in vitro* tests. The success of this assay may be due to the fact that the lipophilic molecules of essential oils pass freely through the cell wall and cytoplasmic membrane. They disrupt the lipopolysaccharide layers, phospholipid, and fatty acids, making them permeable (Nazzaro et al., 2013). Little is known about the mechanism of action of essential oils on nematodes, but this issue is being explored in tests with several microorganisms (Sengul et al., 2011). And, in an experiment performed *in vitro* with essential

oils of *O. vulgare* and *T. vulgaris*, which are rich in thymol and carvacrol, these compounds (especially when working together) promoted the disintegration of the *Escherichia coli* outer membrane (Pei et al., 2009).



	AUCHP		
Essential Oil	0.25%	0.50%	Mean
A. absinthium	85.3 Aa	53.4 Ba	69.4 a *
Mentha × piperita	3.9 Bb	14.9 Ab	9.4 b *
O. vulgare	3.3 Bb	19.8 Ab	11.6 b *
T. vulgaris	4.3 Bb	23.5 Ab	13.9 b *
Means	24.4 ^{ns}	27.9	
DMSO		151.1	
Water		172.2	

AUCHP values followed by the same lowercase letters within columns and uppercase letters within rows did not differ significantly by the Tukey test at 5% probability; ns = not significant by the F test at 5% probability. Means followed by * differ statistically from the Water by Dunnet's test at 5% probability

Figure 1. Temporal dynamics and values of the area under the curve of hatching progress (AUCHP) of second stage juveniles of *Meloidogyne javanica* incubated for 10 days at 26°C in distilled water, DMSO (2%) and essential oils of wormwood (*Artemisia absinthium*), peppermint (*Mentha × piperita*), oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) at concentrations of 0.25% (A) and 0.5% (B).

There are several studies showing the nematicidal activity of the components of essential oils from different plants *in vitro* (Bai et al., 2011; Ntalli et al., 2011) and under greenhouse conditions. The addition of essential oils of *Allium sativum* L. and *T. vulgaris* in the soil, at a volume of 50 uL per plant, caused a reduction in the number of galls and in the egg mass of *M. incognita* race 2 in tomato plants (Cetintas and Yarba, 2010). In another study, the researchers achieved the control of *M. javanica* control combining *Steinernema carpocapsae* (Weiser, 1955) and diallyl disulfide, a volatile component of garlic essential oil (Anastasiadis et al., 2011). On the other hand, the essential oil of *Rosmarinus officinalis* L. did not induce resistance to *M. javanica* and *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev e Sch. Stekhoven, (1941) in soybean (Mattei et al., 2013). In the current study, we were interested in testing the indirect action of the oils on the nematode, by applying the essential oils after the entrance of pathogens.

Following the positive laboratory test results, we evaluated all essential oils under greenhouse conditions. In the present work, applying essential oils to tomato plant shoots did not reduce the number of galls or eggs of *M. javanica*, regardless of the applied dose (Table 2). On the other hand, spraying oils of *A. absinthium* and *Mentha* × *piperita* (experiment II) actually increased the number of galls, and there was also an increase in the number of nematode eggs in plants treated with oils of *A. absinthium* (experiment II) and *O. vulgare* (experiment I) (Table 3). Finally, applying essential oils directly to the soil at 0.25% and 0.5% concentrations did not reduce the number of galls or eggs of *M. javanica* in tomato plants (Table 4).

Table 3. Number of galls and eggs of *Meloidogyne javanica* in roots of tomato plants treated by spraying distilled water, DMSO (2%) and essential oils of *Artemisia absinthium*, *Mentha* × *piperita*, *Origanum vulgare* and *Thymus vulgaris* at concentrations of 0.25% and 0.5%.

Essential oils	Galls per gram of root						Eggs per gram of root					
	Experiment I			Experiment II			Experiment I			Experiment II		
	0.25%	0.50%	Mean	0.25%	0.50%	Mean	0.25%	0.50%	Mean	0.25%	0.50%	Mean
A. absinthium	92	72	82 ^{ns}	89*	82*	86*	7343	8135	7739	27300*	27409*	27355*
T. vulgaris	89	105	97	65	65	65	9912	7960	8936	15923	18656	17290
Mentha × *piperita*	93	91	92	98*	93*	96*	8437	8329	8383	16714	18753	17734
O. vulgare	104	87	96	48	52	50	12800*	13970*	13385*	15830	16589	16214
Mean	95	89		75	73		9623	9599		20192	22339	
Water		111			52			8458			22467	
DMSO		105			52			7428			20564	

ns = means not significant by the F test at 5% probability. Means followed by * are statistically different from the control water by Dunnett's test at 5% probability.

Table 4. Number of galls and eggs of *Meloidogyne javanica* in tomato plant roots treated by the addition to the soil of distilled water, DMSO (2%) and essential oils of *Artemisia absinthium*, *Mentha* × *piperita*, *Origanum vulgare* and *Thymus vulgaris* at concentrations of 0.25% and 0.5%.

Essential oils	Galls per gram root						Eggs per gram root					
	Experiment I			Experiment II			Experiment I			Experiment II		
	0.25%	0.50%	Mean	0.25%	0.50%	Mean	0.25%	0.50%	Mean	0.25%	0.50%	Mean
A. absinthium	101	103	102 ^{ns}	52	34	43 ^{ns}	5893	4984	5434 ^{ns}	13123	13902	13513 ^{ns}
T. vulgaris	108	104	106	47	53	50	4990	6575	5782	11395	13667	12531
Mentha × *piperita*	87	96	92	44	97	70	7436	6262	6849	13982	15095	14539
O. vulgare	106	109	108	52	40	46	6777	9998*	6888	15580	18385	16982
Mean	101 ^{ns}	103		49 ^{ns}	56		6274 ^{ns}	6205		13520 ^{ns}	15262	
Water		99			53			6871			14836	
DMSO		94			50			6645			15095	

ns = Means not significant by the F test at 5% probability. Means followed by * differ statistically from the control water, by Dunnett's test at 5% probability.

Interestingly, although rich in nematicide compounds, essential oils of *Mentha* × *piperita* and *O. vulgare* stimulated the nematode proliferation. The compounds thymol and carvacrol are commonly found in essential oils of *O. vulgare* from different parts of the world (Silva et al., 2010). Several researchers have tested the essential oil of *O. vulgare* and its components, confirming their efficacy in the controlling of different nematodes. In *in vitro* and greenhouse tests, they caused a reduction in the population of *M. javanica*, *M. arenaria* and *Heterodera glycines* (Oka et al., 2000; Soler-Serratosa et al., 1996). The essential oil of *A. absinthium* also increased the nematode population. There is little information in the literature on the effect of beta-thujone, the major component of the essential oil of *A. absinthium*, on nematodes and other microorganisms. But, essential oils thujone-free were not effective on killing nematode juveniles (García-Rodríguez et al., 2015). It is known that the plant has bactericidal effects and is widely used in some regions of the world for their antihelminthic properties (Abad et al., 2012). Although compounds found in greater concentrations are the most active in the essential oil formulation, it is known that compounds which are present in lower concentrations can be critical to essential oils' mode of action, by acting synergistically (Bassolé and Juliani, 2012).

Several factors may have interfered in the action of the nematicide components present in the essentials tested in this work. Possible volatilization of the active substances present in the oils may lead them to lose their activity when available for plant uptake. Compounds that are toxic to nematodes may have been metabolized by the plant or by soil microorganisms into simpler organic compounds with no nematicidal effect (Moreira and Siqueira, 2002). These factors may have stimulated the development of the nematodes in the plant roots. Moreover, chemical and physical factors, which include moisture and soil aeration, as well as the pH of the soil solution and the chemicals present in it, may influence the hatching process. Many organic and inorganic compounds that are excreted by the roots in the soil can stimulate the hatching of juveniles of some species of *Meloidogyne* (Perry et al., 2009). The compounds present in essential oils absorbed by plants may have altered the composition of root exudates, stimulating hatching and making the roots of tomato plants more attractive to the juveniles.

In the current study, treating plants with essential oils was not effective at controlling *M. javanica*. In fact, some of the essential oil compounds increased nematode development within tomato roots. The use of these compounds should be further studied, especially their mode of action, formulation and other factors that may affect the viability of the nematicidal components and their effect on the environment.

CONCLUSION

Although essential oils are a rich source of toxic compounds to nematodes in vitro, the essential oils applied on the leaves and on the roots of tomato plants did not control M. javanica. Essential oils derived from A. absinthium, Mentha × piperita and O. vulgare favored nematode development and should not be used as a systemic tool for controlling root-knot nematodes

REFERENCES

Abad, M.J.; Bedoya, L.M.; Apaza, L. & Bermejo, P. (2012). The Artemisia L. Genus: A Review of Bioactive Essential Oils. **Molecules** 17(12): 2542–2566. http://doi.org/10.3390/molecules17032542

Anastasiadis, I.; Kimbaris, A.C.; Kormpi, M.; Polissiou, M.G. & Karanastasi, E. (2011). The effect of a garlic essential oil component and entomopathogenic nematodes on the suppression of *Meloidogyne javanica* on tomato. **Hellenic Plant Protection Journal** 4(1): 21–24. Retrieved from http://www.bpi.gr/files/journal/2011/january/Volume 4 - Issue 1 (January 2011).pdf

Andrés, M.F.; González-Coloma, A.; Sanz, J.; Burillo, J. & Sainz, P. (2012). Nematicidal activity of essential oils: a review. **Phytochemistry Reviews** 11(4): 371–390. http://doi.org/10.1007/s11101-012-9263-3

Bai, C.Q.; Liu, Z.L. & Liu, Q.Z. (2011). Nematicidal constituents from the essential oil of *Chenopodium ambrosioides* aerial parts. **E-Journal of Chemistry** 8(S1): S143–S148. Retrieved from http://dx.doi.org/10.1155/2011/470862

Bala, S.K. & Sukul, N.C. (1987). Systemic nematicidal effect of eugenol. **Nematropica** 17(2): 219–222.

Bassolé, I.H.N. & Juliani, H.R. (2012). Essential oils in combination and their antimicrobial properties. **Molecules**, 17(12), 3989–4006. http://doi.org/10.3390/molecules17043989

Boneti, J.I. da S. & Ferraz, S. (1981). Modificações do mét-

odo de Hussey & Barker para extração de ovos de *Meloidogyne exigua* em raizes de cafeeiro. **Fitopatologia Brasileira** 6: 553.

Cetintas, R. & Yarba, M.M. (2010). Nematicidal effects of five plant essential oils on the southern root-knot nematode, *Meloidogyne incognita* race 2. **Journal of Animal and Veterinary Advances** 9(2): 222–225. http://doi.org/10.3923/javaa.2010.222.225

Chaudhary, K.K.; Haile, A.; Ayresea, Z.G.; Semereab, G. & Weldegergish, T. (2013). Nematicidal activity of eritrean weed plants against the root-knot nematode *Meloidogyne incognita*. **Nematropica** 43(2): 9784–9788. Retrieved from http://journals.fcla.edu/nematropica/article/view/82709/79653

Chitwood, D.J. (2002). Phytochemical based strategies for nematode control. **Annual Review of Phytopathology** 40(1): 221–249. http://doi.org/10.1146/annurev.phyto.40.032602.130045

Ferraz, S.; Freitas, L.G. de, Lopes, E.A. & Dias-Arieira, C.R. (2010). **Manejo sustentável de fitonematoides.** Viçosa-MG: Editora UFV.

García-Rodríguez, J.J.; Andrés, M.-F.; Ibañez-Escribano, A.; Julio, L.F.; Burillo, J.; Bolás-Fernández, F. & González-Coloma, A. (2015). Selective nematicidal effects of essential oils from two cultivated *Artemisia absinthium* populations. **Zeitschrift Für Naturforschung C** 70(9–10): 275–280. http://doi.org/10.1515/znc-2015-0109

Hussey, R.S. & Barker, K.. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. **Plant Disease Reporter** 57: 1025–1028.

Kokalis-Burelle, N.; Butler, D. M.; Rosskopf, E.N. & Society, T. (2013). Evaluation of cover crops with potential for use in anaerobic soil disinfestation (ASD) for susceptibility to three species of *Meloidogyne*. **Journal of Nematology** 45(4): 272–278. Retrieved from http://journals.fcla.edu/jon/article/view/82688

Laquale, S.; Candido, V.; Avato, P.; Argentieri, M. P. & D'Addabbo, T. (2015). Essential oils as soil biofumigants for the control of the root-knot nematode *Meloidogyne incognita* on tomato. **Annals of Applied Biology** 167(2): 217–224. http://doi.org/10.1111/aab.12221

Li, H.Q.; Liu, Q.Z.; Liu, Z.L.; Du, S.S. & Deng, Z.W. (2013). Chemical composition and nematicidal activity of essential oil of agastache rugosa against *Meloidogyne incognita*. **Molecules** 18(4): 4170–4180. http://doi.org/10.3390/molecules18044170

Mattei, D.; Dias-Arieira, C.R.; Higashi Puerari, H.; Santo Dadazio, T.; Roldi, M. & da Silva, T.R.B. (2013). Evaluation of *Rosmarinus officinalis* essential oil in inducing resistance to *Meloidogyne javanica* and *Pratylenchus brachyurus* in soybean. **Journal of Food, Agriculture and Environment** 11(3–4): 1171–1175. Retrieved from http://world-food.net/download/journals/2013-issue_3&4/2013-issue_3&4-agriculture/a106.pdf

Monteiro, T.S.A.; Nasu, É. das G.C.; Guimarães, C.P.; Neves, W. dos S.; Mizobutsi, E.H. & Freitas, L.G. de. (2014). Redução de inóculo de *Aphelenchoides besseyi* em sementes de *Brachiaria brizantha* tratadas com óleos essenciais. **Ciência Rural** 44(7): 1149–1154. http://doi.org/10.1590/0103-8478cr20120383

Moreira, F. de S. & Siqueira, J.O. (2002). Xenobióticos no solo. In F.M. de S. Moreira & J.O. Siqueira (Eds.), **Microbiologia e bioquímica do solo**. Lavras-MG: Editora UFLA. p.626.

Moreira, L.C.B.; Vieira, B.S.; Mota Júnior, C.V. da, Lopes, E.A. & Canedo, E.J. (2013). Ação nematicida do eugenol em tomateiro. **Pesquisa Agropecuária Tropical** 43(3): 286–291. http://doi.org/10.1590/S1983-40632013000300011

Nazzaro, F.; Fratianni, F.; De Martino, L.; Coppola, R. & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. **Pharmaceuticals** 6(12): 1451–1474. http://doi.org/10.3390/ph6121451

Ntalli, N.G.; Ferrari, F.; Giannakou, I. & Menkissoglu-Spiroudi, U. (2011). Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematicidal activity of essential oils from seven plants indigenous to Greece. **Pest Management Science** 67(3): 341–351. http://doi.org/10.1002/ps.2070

Oka, Y.; Nacar, S.; Putievsky, E.; Ravid, U.; Yaniv, Z. & Spiegel, Y. (2000). Nematicidal activity of essential oils and their components against the root-knot nematode. **Phytopathology** 90(7): 710–715. http://doi.org/10.1094/PHYTO.2000.90.7.710

Pandey, R. & Kalra, A. (2010). Inhibitory effects of vermicompost produced from agro-waste of medicinal and aromatic plants on egg hatching in *Meloidogyne incognita* (Kofoid and White) Chitwood. **Current Science** 98(6):

833–835. Retrieved from http://admin.indiaenvironmentportal.org.in/files/vermicompost produced from agro waste.pdf

Pei, R.-S.; Zhou, F.; Ji, B.-P. & Xu, J. (2009). Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against *E. coli* with an improved method. **Journal of Food Science** 74(7): M379-83. http://doi.org/10.1111/j.1750-3841.2009.01287.x

Perry, R.N.; Moens, M. & Starr, J.L. (Eds.). (2009). **Root-knot nematodes**. Wallingford: CABI. http://doi.org/10.1079/9781845934927.0000

Sengul, M.; Ercisli, S.; Yildiz, H.; Gungor, N.; Kavaz, A. & Çetina, B. (2011). Antioxidant, antimicrobial activity and total phenolic content within the aerial parts of *Artemisia absinthum*, *Artemisia santonicum* and *Saponaria officinalis*. **Iranian Journal of Pharmaceutical Research** 10(1): 49–56. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24363680

Silva, J.P.L.; Duarte-Almeida, J.M.; Perez, D.V. & Franco, B.D.G. de M. (2010). Óleo essencial de orégano: interferência da composição química na atividade frente a *Salmonella enteritidis*. **Ciência E Tecnologia de Alimentos** 30(1): 136–141. http://doi.org/10.1590/S0101-20612010000500021

Soler-Serratosa, A.; Kokalis-Burelle, N.; Rodríguez-Kábana, R.; Weaver, C.F. & King, P.S. (1996). Allelochemicals for control of plant-parasitic nematodes.1.in vivo nematicidal efficacy of thymol and thymol/benzaldehyde combinations. **Nematropica** 26(1): 57–71. Retrieved from http://journals.fcla.edu/nematropica/article/view/64146/61814