Effect of Plant Essential Oils on Mortality of the Stem Nematode (*Ditylenchus dipsaci*)

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Abstract

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The aim of this study was to assess the nematicidal activity of different essential oils from medicinal and aromatic plants for use in nematode management. Essential oils of *Eugenia caryophyllata*, *Origanum compactum*, *Origanum vulgare, Thymus matschiana* and *Thymus vulgaris* showed nematicidal activity against *Ditylenchus dipsaci*.

Keywords: *Ditylenchus dipsaci*; essential oils; control

The use of pesticides on agricultural crops has recently been revised and restricted by European Legislations (Reg. CE 396/2005 and 1095/2007) and more attention is now focused on environmental safety and human and animal health. Therefore, it is necessary to find new alternative control strategies for protecting plants against attacks by phytoparasitic nematodes and soilborne pathogens; these strategies have to be environmentally sound and economically feasible for a sustainable agriculture. During the last decade, emphasis has been put on a wide range of control options, such as green manures, soil amendments, crop rotations, biofumigations, soil solarisation, steam, resistant varieties, grafting, mycorrhizae, ozone treatments, biocidal plants or their derived products and biological control agents (Gamliel *et al*. 2000; Tamietti & Valentino 2000; Tjamos *et al*. 2000; Vannacci & Gullino 2000; Ciccarese *et al*. 2008; Sasanelli *et al*. 2008). Studies have also focused on the nematicidal activity of plant extracts or essential oils (Oka *et al*. 2000; Rodriguez-Kabana & Simmons 2005). In this field, attention has so far been concentrated on root-knot nematodes of the genus *Meloidogyne* as these are the most widespread nematode pests on a global scale. The range of substances tested in recent years includes aqueous and ethanol extracts of *Carum carvi*, *Mentha rotundifolia*, *Origanum vulgare* (Oka *et al*. 2000), *Calotropis procera* (Reina *et al*. 2002), *Artemisia vulgaris* (Costa *et al*. 2003), *Tamarindus indica*, *Cassia siamea*, *Isoberlinia doka*, *Dolnix regia*, *Cassia sieberiana* (Bello *et al*. 2006), *Tagetes erecta* (Natarajan *et al*. 2006), *Avicennia marina* (Tariq *et al*. 2007), *Chromolaena odorata* (Thoden *et al*. 2007), *Ruta*

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graveolens (Sasanelli et al. 2007), *Azadirachta indica* (Javed *et al*. 2007, 2008), and *Phyllanthus niruri* (Shakil *et al*. 2008). An extensive list of active compounds (alkaloids, diterpenes, phenols, polyacetylenes, sesquiterpenes, thyenil derivatives) which can be used in plant parasitic nematode management was compiled by Chitwood (2002). Several studies were also carried out on the effect of plant compounds on free living nematodes (Langat *et al*. 2008). Insunza and Valenzuela (1995) evaluated the nematicidal activity of several Chilean medicinal plants on the stem nematode *Ditylenchus dipsaci*. During the last years, this nematode has become an important pest of onion in the Czech Republic. In the European Community, in addition to methyl bromide, with the exception of critical uses, also 1,3-dichloropropene, Aldicarb, Thionazin and Cadusafos have been banned and the management of this quarantine pest is very problematic. For the control of this nematode, Czech growers are entirely dependent on soil treatment before transplanting. Therefore, different essential oils were extracted from 35 medicinal plant species and screened *in vitro* for their effect on the mortality of *Ditylenchus dipsaci*.

MATERIAL AND METHODS

Aerial parts of 35 medicinal plant species (Table 1) were dried, crushed and their essential oils were extracted by distillation of 30 g of plant material, for 1.5 h and with 700 ml distilled water, in a classical steam distillation apparatus. Each essential oil was separated in a separation funnel. Subsequently, essential oil solutions (10% ethanol, v/v) were prepared and diluted in water containing 0.3% Tween 20 (v/v) to obtain the concentrations used in the experiment. A population of the nematode *Ditylenchus dipsaci* (Kuehn) Filipjev was reared on salad chicory. The nematodes were extracted from infested tissues by a modified Baermann's funnel method (Southey 1986). Consequently the suspensions used for screening contained all development stages of the nematodes. The nematode suspensions were diluted to contain roughly 50 individuals in 25 µl of water. This volume of diluted suspension was pipetted into wells of cultivation plates. Every time, after filling 10 wells, the nematodes in a test droplet were counted to confirm the homogeneity of the suspension. Appropriate essential oil dilutions were then added into the wells to obtain 1500 ppm; 3000 ppm; 5000 ppm and 7500 ppm concentrations. Untreated nematode suspensions were used as controls, where the concentrations of ethanol and Tween 20 were equivalent to those in treatments with essential oils. The mortality of the nematodes was observed after 3 and 6 h under a stereomicroscope. Nematodes stimulated on head or vulva that did not display mobility, after transfer to distilled water for 1 hours. All tests were conducted under laboratory conditions, at 21 ± 3°C, using an entirely randomised design. Each treatment was replicated three times and percentage nematode mortality was calculated for each treatment according to the expression: % Mortality = [100 – (100 × SEO)]/SC, where SEO is the number of nematodes that survived in essential oil and SC is the number of nematodes that survived in the control. Data from the experiment were statistically analysed after transformation in arcsine root square values, by analysis of variance (ANOVA) and means compared by Tukey's test.

RESULTS AND DISCUSSION

A significantly increased mortality of *D. dipsaci* was obtained by exposure to essential oils of *Eugenia caryophyllata*, *Origanum compactum*, *Origanum vulgare, Thymus vulgaris* and *T. matschiana,* with which only the concentrations of 5000 and 7500 ppm were effective (Table 1). Generally, length of exposure did not affect nematode mortality remarkably, although 6 h exposure to essential oil from *Thymus matschiana* at 3000 ppm revealed a significant mortality in comparison to 3 h exposure time. The effect of exposure of the nematode for 3 and 6 h to essential oils from other plant species did not significantly differ from the untreated control. No effect on nematode mortality was observed in essential oils from a mix of different *Tagetes* spp. and from *Tagetes bipinata*, despite that plants from the genus *Tagetes* (*T. erecta*, *T. minuta* and *T. patula*) are regularly used for nematode control. This is done especially in the form of mixed cultures of *Tagetes* spp. as interculture vegetables (cover crop grown before planting cash crop) and in commercial formulations, and their nematicidal effect has been known for a long time (Suatmadji 1969). However, our results are similar to findings by Sasanelli and

Table 1. Effect of different concentrations of essential oils from different plant species on per cent mortality of *Ditylenchus dipsaci,* after exposure for 3 and 6 hours

Plant species	Concentration (ppm)	Mortality after 3h treatment (%)	Mortality after 6h treatment (%)
Abies sibirica Ledeb.	1500	0.0 ± 0*	1.3 ± 2.3
	3000	0.7 ± 1.2	4.0 ± 2.0
	5000	1.3 ± 1.2	4.0 ± 2.0
	7500	1.3 ± 2.3	1.3 ± 2.3
Acorus calamus L.	1500	6.7 ± 8.3	8.7 ± 7.0
	3000	7.3 ± 5.8	14.0 ± 7.2
	5000	4.7 ± 5.0	6.0 ± 3.5
	7500	3.3 ± 5.8	5.3 ± 5.8
Amyris balsamifera	1500	0.7 ± 1.2	4.7 ± 5.0
	3000	2.7 ± 3.1	6.0 ± 0.0
	5000	0.7 ± 1.2	2.0 ± 2.0
	7500	5.3 ± 5.0	6.7 ± 6.1
Artemisia absinthium L.	1500	3.3 ± 1.2	5.3 ± 1.2
	3000	3.3 ± 3.1	10.0 ± 10.0
	5000	0.7 ± 1.2	6.0 ± 5.3
	7500	3.3 ± 5.8	8.7 ± 5.8
Citrus aurantifolia	1500	2.7 ± 3.1	1.3 ± 1.2
	3000	0.7 ± 1.2	8.0 ± 3.5
	5000	4.7 ± 2.0	12.7 ± 7.0
	7500	2.7 ± 2.3	9.3 ± 3.1
Citrus limonum Osbeck	1500	1.3 ± 1.2	4.0 ± 5.3
	3000	1.3 ± 1.2	8.0 ± 6.0
	5000	4.0 ± 4.0	4.7 ± 4.2
	7500	2.7 ± 3.1	7.3 ± 5.0
Eugenia caryophyllata	1500	34.7 ± 48,2	65.3 ± 36.3
	3000	64.7 ± 27.3	88.0 ± 8.0
	5000	**78.0** ± 26.2**	**93.3** ± 4.2**
	7500	**80.7** ± 17.9**	92.0 ± 2.0
Juniperus communis L.	1500	0.0 ± 0.0	2.7 ± 1.2
	3000	10.0 ± 12.2	16.7 ± 11.7
	5000	16 ± 9.0	17.3 ± 11.0
	7500	15.3 ± 21.4	16.7 ± 21.9
Juniperus virginiana L.	1500	18.0 ± 27.8	20.0 ± 27.8
	3000	19.3 ± 28.4	20.0 ± 28.0
	5000	18.0 ± 29.5	23.3 ± 28.3
	7500	22.0 ± 32.9	28.0 ± 27.7
Lavandula angustifolia P. Mill.	1500	6.7 ± 11.5	7.3 ± 12.7
	3000	11.3 ± 12.1	12.7 ± 14.2
	5000	11.3 ± 16.2	17.3 ± 12.7
	7500	18.0 ± 19.3	22.0 ± 17.3
Lavandula latifolia Medik.	1500	0.7 ± 1.2	1.3 ± 1.2
	3000	2.0 ± 2.0	3.3 ± 3.1
	5000	2.7 ± 3.1	7.3 ± 5.0
	7500	6.7 ± 4.6	7.3 ± 4.2

Table 1 to be continued

Plant species	Concentration (ppm)	Mortality after 3h treatment (%)	Mortality after 6h treatment (%)
Melaleuca quinquenervia (Cav.) Blake	1500	5.3 ± 6.1	13.3 ± 19.7
	3000	4.0 ± 6.9	26.7 ± 23.1
	5000	6.7 ± 8.3	30.0 ± 22.5
	7500	3.3 ± 3.1	31.3 ± 24.2
Melissa officinalis L.	1500	2.7 ± 2.3	5.3 ± 3.1
	3000	0.0 ± 0.0	2.0 ± 2.0
	5000	20.0 ± 34.6	28.0 ± 45.1
	7500	24.0 ± 31.7	44.0 ± 46.1
Mentha arvensis L.	1500	38.0 ± 45.0	65.3 ± 46.4
	3000	39.3 ± 42.4	70.0 ± 41.8
	5000	44.0 ± 41.6	66.0 ± 39.9
	7500	59.3 ± 34.9	80.7 ± 26.6
Mentha citrata L.	1500	5.3 ± 6.1	17.3 ± 23.4
	3000	10.0 ± 10.6	23.3 ± 14.2
	5000	21.3 ± 25.3	50.0 ± 17.8
	7500	47.3 ± 38.3	66.0 ± 22.7
Mentha pulegium L.	1500	10.0 ± 14.0	34.0 ± 52.0
	3000	18.7 ± 18.9	38.0 ± 50.3
	5000	31.3 ± 50.8	33.3 ± 49.3
	7500	38.0 ± 45.7	43.3 ± 44.7
Mentha spicata L.	1500	16.0 ± 22.7	32.7 ± 49.8
	3000	20.7 ± 20.4	38.0 ± 46.9
	5000	24.0 ± 15.1	41.3 ± 44.3
	7500	33.3 ± 47.7	43.0 ± 44.1
Nepeta cataria L.	1500	3.3 ± 3.1	5.3 ± 3.1
	3000	11.3 ± 11.0	13.3 ± 14.5
	5000	10.7 ± 16.8	32.0 ± 41.8
	7500	15.3 ± 21.6	33.3 ± 49.3
Ocimum basilicum L.	1500	20.0 ± 16.0	28.0 ± 20.9
	3000	16.0 ± 15.9	42.7 ± 36.1
	5000	44.0 ± 48.7	70.7 ± 32.3
	7500	59.3 ± 38.1	75.3 ± 29.1
Origanum compactum L.	1500	**82.7** ± 18.1**	**92.7** ± 5.0**
	3000	**100.0** ± 0.0**	**100.0** ± 0.0**
	5000	**99.3** ± 1.2**	**100.0** ± 0.0**
	7500	**98.7** ± 1.2**	**100.0** ± 0.0**
Origanum majorana L.	1500	18.7 ± 18.9	28.7 ± 21.4
	3000	31.3 ± 25.3	55.3 ± 36.5
	5000	22.7 ± 12.1	42.0 ± 33.0
	7500	33.0 ± 47.7	74.7 ± 28.3
Origanum vulgare L.	1500	**98.7** ± 1.2**	**99.3** ± 1.2**
	3000	**99.3** ± 1.2**	**100.0** ± 0.0**
	5000	**100.0** ± 0.0**	**100.0** ± 0.0**
	7500	**99.7** ± 1.6**	**99.3** ± 1.2**

Table 1 to be continued

Plant species	Concentration (ppm)	Mortality after 3h treatment (%)	Mortality after 6h treatment (%)
Pelargonium graveolens L'Heritier	1500	28.0 ± 27.7	37.3 ± 24.0
	3000	28.7 ± 24.2	67.7 ± 45.6
	5000	30.7 ± 16.8	64.7 ± 37.4
	7500	29.3 ± 19.7	65.3 ± 42.7
Pelargonium roseum Willd..	1500	23,3 ± 15.3	58.0 ± 40.9
	3000	38.7 ± 49.7	64.7 ± 47.5
	5000	46.7 ± 45.1	58 ± 50.5
	7500	53.3 ± 46.2	58.7 ± 50.8
Pogostemon cablin (Blanco) Benth	1500	3.3 ± 5.8	4.0 ± 6.9
	3000	4.0 ± 6.9	4.7 ± 8.1
	5000	4.0 ± 5.3	17.3 ± 20.5
	7500	5.3 ± 6.1	20.7 ± 19.4
Pongamia sp.	1500	1.3 ± 1.2	10.0 ± 10.0
	3000	1.3 ± 2.3	9.3 ± 9.5
	5000	1.3 ± 1.2	8.0 ± 12.2
	7500	10.0 ± 6.0	18.7 ± 13.3
Rosmarinus officinalis L.	1500	13.3 ± 11.5	20.7 ± 19.0
	3000	6.0 ± 10.4	14.0 ± 19.1
	5000	8.7 ± 13.3	22.0 ± 32.9
	7500	4.0 ± 0.0	19.3 ± 18.1
Salvia officinalis L.	1500	0.0 ± 0.0	4.7 ± 8.1
	3000	0.0 ± 0.0	1.3 ± 1.2
	5000	3.3 ± 1.2	18.7 ± 18.9
	7500	4.0 ± 2.0	19.3 ± 14.5
Salvia sclarea L.	1500	4.0 ± 5.3	5.3 ± 6.1
	3000	5.3 ± 4.2	18.0 ± 19.3
	5000	5.3 ± 9.2	19.3 ± 18.1
	7500	2.7 ± 2.3	22.0 ± 19.1
Tagetes bipinata L.	1500	15.3 ± 9.9	30.0 ± 12.2
	3000	12.7 ± 13.0	25.3 ± 12.2
	5000	16.7 ± 20.8	23.3 ± 15.3
	7500	23.3 ± 21.4	26.0 ± 20.9
Tagetes sp.	1500	5.0 ± 4.7	4.0 ± 6.9
	3000	0.7 ± 1.2	2.0 ± 2.0
	5000	0.7 ± 1.2	1.3 ± 2.3
	7500	0.7 ± 1.2	1.3 ± 1.2
Thuja occidentalis L.	1500	9.3 ± 9.0	17.3 ± 21.9
	3000	8.7 ± 7.0	24.7 ± 22.1
	5000	6.7 ± 9.9	25.3 ± 24.0
	7500	6.0 ± 5.3	22.7 ± 25.7
Thymus matschiana L.	1500	31.3 ± 9.9	56.7 ± 27.2
	3000	63.3 ± 44.5	90.7 ± 8.1
	5000	**82.0** ** **± 19.3**	**94.7** ** **± 4.2**
	7500	**98.7** ** **± 1.2**	**100.0** ** **± 0.0**

Table 1 to be continued

Plant species	Concentration (ppm)	Mortality after 3h treatment (%)	Mortality after 6h treatment (%)
Thymus vulgaris L.	1500	**81.3** ± 18.6**	**93.3** ± 4.2**
	3000	**83.3** ± 18.6**	**94.0** ± 5.3**
	5000	**86.7** ± 10.1**	**95.3** ± 1.2**
	7500	**98.3** ± 2.0**	**98.7** ± 1.2**
Tsuga canadensis (L.) Carriére	1500	2.7 ± 2.3	16.0 ± 20.8
	3000	2.0 ± 2.0	17.3 ± 16.7
	5000	3.3 ± 5.8	20.7 ± 26.1
	7500	4.7 ± 6.4	26.7 ± 18.9
Zingiber officinale Rosc.	1500	3.3 ± 3.1	4.7 ± 5.0
	3000	8.7 ± 2.3	20.7 ± 16.8
	5000	6.7 ± 5.8	22.0 ± 14.0
	7500	12.0 ± 8.0	26.7 ± 8.1
Control		1.3 ± 1.2	4.0 ± 2.0

Each value is an average of three replications ± SD (standard deviation); **averages marked in bold are statistically different from the untreated control ($P = 0.05$)

D'Addabbo (1993). These authors did not observe nematicidal activity of *T. erecta* against the root-knot nematode *Meloidogyne incognita* in an *in vitro* experiment. It is possible that the main plant compound responsible for the nematicidal effect of *Tagetes* species, α-therthienyl, was destroyed during the distillation process (Wang *et al.* 2007). An extract from *Eugenia winzerlingii* leaves showed nematicidal activity against the root-knot nematode *M. incognita* (Cristóbal-Alejo *et al.* 2006), but no comparison is possible because a different target organism and plant compound were used. Similar results were obtained by Elbadri *et al.* (2008), who tested the effect of extracts from *Ocimum basilicum* leaves and seeds on *M. incognita* mortality. The suppressive effect of plant extracts on the ability *of Ditylenchus dipsaci* to infest garlic is well known only for *Plantago major*, *Ruta graveolens*, *Aristotelia chilensis, Chenopodium ambrosioides* and *Ovidia pillopillo* (Insunza & Valenzuela 1995). None of the concentrations of *Mentha spicata* essential oil, at both 3 h and 6 h exposure time, showed nematicidal activity against *D. dipsaci*, although it has been effective against the root-knot nematode *M. javanica* (Oka *et al.* 2000). The essential oil of *Origanum vulgare* greatly suppressed the *D. dipsaci* population in comparison to the untreated control. In an earlier study, the essential oil from the same plant, with a high content of carvacrol and thymol, was effective against *M. javanica* (Oka *et al.* 2000). The two compounds involved in the nematicidal activity on *M. javanica* might also be effective on *D. dipsaci*. The essential oils from *Rosmarinus officinalis* and *Salvia officinalis* did not show nematicidal activity on *D. dipsaci*, whereas these oils were effective in the control of the second stage juveniles and egg hatch of *M. javanica* (Oka *et al.* 2000). Similar to the essential oil from *Origanum vulgare*, that from *Thymus vulgaris* suppressed *D. dipsaci*. This nematicidal activity might also be due to thymol, the main component (40%) in the essential oil of *T. vulgaris*. In addition to thymol, this essential oil contains carvacrol (at 7%), another strong nematicidal compound (Oka *et al.* 2000). Many components of the essential oils that revealed nematicidal activity, such as carvacrol, thymol, carvone, limonene, Artemisia ketone, and t-anathole, have also been reported for their biocidal effect on insects, fungi, bacteria and weeds (Kurita *et al.* 1981; Janssen *et al.* 1987; Muller-Riebau *et al.* 1995; Landolt *et al.* 1999; Isman 2000; Rodriguez-Kabana & Simmons 2005). Therefore, soil treatment with essential oils could have a potential for soil disinfestation as an alternative to chemicals. However, while it is clear that several plants contain compounds which can be used to protect crops against plant parasitic nematodes (Grainge & Ahmed 1988; Chitwood 2002), the mechanisms involved in the nematicidal activity are known only for few groups of plants. In conclusion, our results have

demonstrated the nematicidal properties of essential oils of *Eugenia caryophyllata*, *Origanum compactum*, *O. vulgare, Thymus matschiana* and *T. vulgaris* against *Ditylenchus dipsaci*. Yet further investigations are necessary to evaluate economic aspects and to determine the most appropriate rates and concentrations of the different essential oils and their feasibility for use in field or enclosed conditions.

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