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Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*

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Summary. The nematicidal activity of the essential oil/pure components and plant extracts of naturally grown aromatic plant species against hatching, migration and mortality of the root knot nematode *Meloidogyne incognita* was investigated. The pure components carvacrol, thymol, and linalool at 1, 2 and 4 mg liter^{-1} concentrations were the most toxic against *M. incognita* second-stage juveniles (J2s) followed by terpineol and menthone. Hatching was completely inhibited at low concentrations (2, 4 mg liter^{-1}) of carvacrol, thymol, and linalool. Clove extracts (1 mg liter^{-1}) of *Allium sativum* significantly reduced hatching activity to below 8%, followed by flower extracts of *Foeniculum vulgare* which reduced hatching to below 25%. These extracts were also toxic against J2s of *M. incognita* (LC_{50} 43) followed by leaf extracts of *Pinus pinea, Origanum syriacum*, *Mentha microcorphylla*, *Eucalyptus* spp. and *Citrus sinensis* with an estimated LC_{50} of 44, 50, 65, 66 and 121 ppm respectively. Flower extracts of *F. vulgare* had the highest effect on J2 mortality in sand (86%). The highest concentration of essential oils (6%) was detected in leaf extracts of *Origanium syriacum*. Over 30 major components were identified in all the plant extracts tested.

Key words: aromatic plants, root-knot nematodes, carvacrol, thymol, linalool.

Introduction

Agrochemicals have had a major role in improving yields in food production. However, concern has arisen about the negative impact that such chemicals have on human health and the environment. Some pesticides have active ingredients that act as hormone disruptors, and may cause loss of fertility, carcinogenesis and mutagenesis. The widespread application of agrochemicals to most cash crops has meant that pesticides are present in the ecosystems, aquifers and water systems of most agricultural areas. In the long term, this could have repercussions on both the environment and human health (Dinham, 1993). There is therefore an urgent need to replace pesticides with alternative means of control that are less toxic and more environmentally friendly. Plant extracts containing volatile compounds, especially essential oils, have been found to possess antimicrobial, insecticidal and nematicidal activity (Marban-Mendoza *et al*., 1987; Thackray *et al*., 1990; Digrak, 1999; Okoko, *et al*., 1999). Certain plants kill or repel pests, disrupt their life cycle, or discourage them from feeding. Plant extracts may contain volatile and non-volatile components (Brown and Morra, 1997). Some of these components may be detected at a distance by olfaction and act as attractants or repellents (Pickett and Stephenson, 1980).

Nematodes occur in virtually all soil types. Worldwide annual crop losses caused by nematodes, mainly the root-knot nematodes (RKN) *Me-*

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loidogyne spp. and the cyst nematodes *Globodera* spp. and *Heterodera* spp., are estimated at approximately US$ 100 billion (Sasser and Freckman, 1987). They attack a wide range of economically important crops in horticultural, agricultural and forest systems. Nematode control is essentially preventive; because once a plant is parasitized it is impossible to kill the nematode without also destroying the plant. Controlling nematodes is very difficult and relies heavily on the use of soil fumigants with toxic and expensive nematicides. Methyl bromide is effective against nematodes, but it is harmful to human beings and to the environment including beneficial organisms. There is an urgent need to look for natural compounds with less toxicity and a low environmental impact. Lebanon is committed to phasing out methyl bromide and to find alternatives to this biocide. Little information has been published on the status of nematodes and their control in Lebanon. One way to search for environmentally benign nematicidal control agents is to screen naturally occurring compounds in plants. Lebanon is a country rich in medicinal and aromatic plants (Nehmeh, 1978). Volatile compounds from such plants, especially their essential oils, have been found to possess antimicrobial and insecticidal activity (Oka *et al.,* 2001), but so far only a few essential oils and their components have been evaluated for their nematicidal properties.

This paper reports the identification of the chemical components of essential oils isolated from eighteen plants, and the effect that plant extracts and pure essential oil components, from various medicinal and aromatic plants have on the hatching, migration and mortality of *M. incognita* second-stage juveniles (J2s).

Materials and methods

Nematode inocula

Meloidogyne incognita isolates were maintained on tomato *(Lycopersicon esculentum L.*) in pot cultures. Inocula of freshly hatched J2s were obtained from egg masses in distilled water. Only J2s that hatched within a 24-h period were used for experiments.

Tomato-root diffusate and soil leachate

Tomato-root diffusate from 4-week-old plants was collected using a technique similar to that of Fenwick (1949). Soil leachate was collected from pots containing the same soil but without plants.

Isolation of essential oils

Essential oils were extracted from plant material collected from different regions of Lebanon (Table 1) as described by Traboulsi *et al*., 2002. Between one hundred and 200 g of fresh plant material per species was used. Ethyl alcohol (99%) was used as a solvent in the extraction process and 100 ml was used for each extraction run. Every run consisted of four extraction cycles, 20 min each, at a heating temperature of 100ºC and a cooling temperature of 50ºC. The plant extracts were reduced to a volume of 50 ml using a rotary evaporator (90ºC) and stored at 4ºC until required. To determine the concentration of an essential oil in each plant extract, 10 g of each plant part was weighed and evaporated in an oven at 100ºC. The remaining material was then weighed and the percentage of essential oil determined.

Gas chromatography/mass spectrometry analysis

The plant material, air-dried at room temperature for about one week, was subjected to hydrodistillation for 4 h according to the standard method using a Clevenger-type distillation apparatus (Traboulsi *et al.,* 2002). Plant components were determined by gas chromatography (GC) (Hewlett-Packard) coupled to an HP 5871A mass spectrometer detector and equipped with an on-column DBI (30 m$\times$0.20$\times$0.05 μm). The temperature programme consisted of an initial temperature of 50ºC; hold 3 min^{-1}; ramp rate 3ºC min^{-1}, final temperature 220ºC; hold 65 min^{-1}; column flow rate 0.6 ml d'He/mi constant. The injection temperature was 200ºC with an injection volume of 2 μl/min. The mass spectrometer settings were: electron impact ionization mode with 70 eV electron energy, scan mass range m/z 50–400. Detection temperature was 270ºC using the retention time and peak area as a mean of measure (Table 2). Components were identified by comparing the GC retention and mass spectra with those reported in the literature. Pure essential oils of commercial origin were kindly supplied by Jean-Marie Bessiere (Ecole Nationale Supérieure de Chimie de Montpellier, France). Each oil was separated from water with a Pasteur pipette, dried by fil-

Table 1. Location, plant parts, harvest dates, and percentage of essential oils of the plants.

Plant species	Common name	Location	Plant part	Harvest date	Essential oil (%)
Andropogon nardus	Lemon grass	Mount Lebanon	Leaves	April 2005	2.1
Allium sativum	Garlic	Market	Cloves	April 2005	0.3
Chrysanthemum coronarium	Chrysanthemum	Mount Lebanon	Whole plant	April 2005	n.a.
Cinnamomum zeylanicum	Cinnamom	Mount Lebanon	Bark	April 2005	0.5
Citrus sinensis	Sweet orange	Bourghoulieh	Leaves	June 2000	0.25
Eucalyptus spp.	Eucalyptus	Hazmieh (Mount)	Leaves	April 2000	0.4
Foeniculum vulgare	Fennel	Tabaria (South)	Flowers	May 2000	1.0
Lavandula stoechas	Lavander	Pchemon (Mount)	Flowers	June 1998	0.8
Laurus nobilis	Laurel	Nabay	Leaves	April 2000	0.5
Matricara discoidea	Chamomile	Mount Lebanon	Whole plant	April 2005	0.9
Mentha microphylla	Mint	Mashgara (Bekaa)	Leaves, flowers	April 1998	1.1
Myrtus communis	Myrtle	Akkar	Leaves	September 1998	1.2
Origanum syriacum	Oregano	Mashgara (Bekaa)	Leaves	September 1998	6.0
Pelargonium graveolens	Pelargonium	Mount Lebanon	Leaves	April 2005	0.8
Pimpinella anisum	Anise	Mount Lebanon	Seeds	April 2005	2.6
Pinus pinea	Pine	Mashgara (Bekaa)	Leaves	April 2000	0.5
Pistacia lentiscus	Mastic tree	Mashgara (Bekaa)	Leaves	September 1998	0.2
Salvia officinalis	Sage	Mount Lebanon	leaves, stem, flowers	April 2005	1.3
Tagetes patula	Marigold	Mount Lebanon	Leaves, stem, flowers	April 2005	1.3

n.a., Data not available.

tration over anhydrous sodium sulphate and stored at -20°C in a sealed dark bottle until analysis. The oil yields (Table 1) were calculated relative to the mass of dry plant material.

Effect of plant extracts with tomato-root diffusate on J2 hatching

Bioassays were carried out as described by Ibrahim *et al*. (1993), using four batches of 25 egg masses in 2 ml of each test solution in cavity watch glasses at 25–27°C. Distilled water (DW), soil leachate (SL) and tomato-root diffusate (TRD), diluted 1:3, and were used as control. TRD were diluted with various concentrations (0.125, 0.250, 0.5, 1, 2, 4 mg l^{-1}) of plant extracts or pure components as treatments.

Hatched J2s were counted weekly for 6 weeks. Every week, a fresh solution with or without TRD plus plant extracts or pure components was added. At the end of each experiment, the egg masses were disrupted and the number of eggs containing unhatched J2s was counted to determine the percentage of hatching. All treatments were replicated four times and each experiment was repeated twice.

Effect of plant extracts on J2 migration in sand

One hundred freshly hatched J2s were placed in cavity watch glasses containing 5, 10, 25, 50, 100 mg l^{-1} of each plant extract in 2 ml DW. After 2, 4, 8 and 24 hours the J2s were removed and placed on a sand column (height 1.5 cm, sand particle size 250–500 μm) in plastic tubes (3.0×4.0 cm) sealed at the bottom with a nylon mesh (53-μm aperture). The column was placed upright in a cavity watch glass containing DW and kept at room temperature (25–27°C). After 24 hours the number of J2s that had migrated through the mesh was recorded. The controls for all experiments without plant extracts were treated identically. This experiment was conducted twice.

Effect of plant extracts on mortality of J2s

Fifty newly hatched J2s were placed in cavity watch glasses containing 5, 10, 25, 50 and 100 mg l^{-1} of each plant extract in 2 ml of DW. After 24 hours, plant extracts were removed and the J2s placed in DW and left to recover. The J2s were examined after 24 hours and only J2s that were unable to move were considered dead. This experi-

Table 2. Percentage of major components of the essential oil in each plant species tested.

Plant species (plant part)	Major components of essential oil (%)
Allium sativum (cloves)	Allicin[a], allyl-methyltrisulphide[a], diallyldisulphide[a], diallytrisulphide[a], diallytetrasulphide[a], allypropyldisulphide[a]
Cinnamomum zeylanicum (bark)	Methyl chavicol (74%), 1,8 cineole (2.1%), linalool (2.6%), caryophyllene (3.1%), eugenol (2.5%)
Andropogon nardus (leaves)	Citral (75.2%), myrcene (18.5%), geraniol (16.9%), limonene (7.7%)
Citrus sinensis (leaves)	Limonene (40%), linalyl acetate (37%), minolool (16%), bergaptene (4%)
Eucalyptus spp. (leaves)	1,8 cineol (30%), camphor (18%), α-pinene (19%), borneol (17%), borneol acetate (5%)
Foeniculum vulgare (flowers)	Limonene (63.6%), anethole (25.5%), fenchyl acetate (2.6%), α-pinene (0.97%), myrcene (0.98%), estragole (1.1%)
Laurus nobilis (leaves)	1,8 cineole (44%), α-pinene (4.5%), β-pinene (2.2%), sabinene (8.9%), methyl eugenol (1.9%)
Lavandula stoechas (flowers)	Fenchone (40.1%), 1,8 cineole (11.7%), bornyl acetate (5.8%), myrtenyl acetate (2.8%), myrtenol (2.1%), α-pinene (2%), viridiflorol (1.9%)
Matricaria discoidea (whole plant)	Chamazulene (14%), farnesene[a], farnesol[a]
Mentha microphylla (leaves, flowers)	Piperitenone (54.2%), pulegone (10.7%), piperitenone oxide (11.3%), menthone (3.3%), 1,8 cineole (2.8%)
Myrtus communis (leaves)	1,8 cineole (40%), α-pinene (17%), linalool (9.9%), α-terpineol (7.9%), geranyl acetate (4.5%), myrtenyl acetate (7%), α-terpinyl acetate (2%)
Origanum syriacum (leaves)	Carvacrol (61%), thymol (21.8%), δ-terpinene (4%), p-cymene (5.5%), myrcene (1.2%), α-terpinene (1.3%)
Pelargonium graveolens (leaves)	Citronellol (21.7%), linalool (17.3%), geraniol (14%), menthone (1.1%)
Pimpinella anisum (seeds)	Transanethole (85%), estragole (0.57%), linalool (1.5%), α-terpineol (1.5%)
Pinus pinea (leaves)	Limonene (74.6%) 1,8 cineole (4.3%), α-pinene (3.8%), myrcene (2.7%), β-caryophyllene (3.4%)
Salvia officinalis (leaves, stem, flowers)	α-thujene (26.8%), limonene (22.9%), linalyl acetate (17.4), ocimene (10.7%), linalool (5.7%), myrcene (3.6%) α-pinene (2.5%)
Tagetes patula (leaves, stem, flowers)	Linalool (26.8), limonene (22, 9%), linalyl acetate (17.4%), ocimene (10.7%)

[a], Data not available.

ment was repeated twice. Data were subjected to Sigma Stat 2.0 analysis of variance (ANOVA), and the LC was calculated using probit analysis.

Results

Plant extracts

The highest concentration (6%) of essential oil was detected in leaf extracts of *Origanum syriacum* L. (Table 1).

In the plant extracts more than 30 major components were detected. 1,8- cineole and linalool were common in a number of plants: *Cinnamomum zeylanicum*, *Laurus nobilis*, *Lavandula stoechas*, *Mentha microphylla*, *Myrtus communis*, *Pelargonium graveolens*, *Pimpinella anisum, Pinus pinea, Salvia officinalis* and *Tagetes patula*. Myrtenyl acetate was detected in both *L. stoechas* and *M. communis*. The highest percentage of essential oils occurred in *P. anisum* (transanethole, 85%), followed by *Cymbopogon citrates* L. (citral, 75.2%), *Cinnamomum zeylanicum* (eugenol, 71.3%), and *Origanum syriacum* (carvacrol, 61%) (Table 1). The lowest percentage was detected in *P. anisum* (estragole, 0.57 %) (Table 1).

Effect of pure components with or without tomato-root diffusate on J2 hatching

The effect of pure components on hatching of J2s was related to component concentration (Tables 3 and 4). More than 95% of the J2 hatched when the egg masses were incubated with TRD and DW alone, but when the egg masses were exposed to the pure components with TRD at 1 mg l^{-1} for 6 weeks, the response varied according to the component. Thymol, carvacrol, and linalool almost completely inhibited hatching, while menthone and *(1S)-(-)-α*-pinen reduced hatching to 27 and 39% respectively. 1,8 cineol or cineole and *(1R)-(+)-α*-pinen were less effective against hatching (Table 3). Hatching did not exceed 6% with carvacrol, thymol and linalool at 2 mg l^{-1}, but at 4 mg l^{-1} hatching was completely inhibited from the first week with carvacrol, thymol and linalool and was reduced significantly to 15% with menthone (Table 4). At concentrations of 0.125, 0.250, 0.5 mg l^{-1}, however, carvacrol, linalool, thymol and menthone did not significantly affect hatching (data not shown). Hatching in SL did not exceed 46% (Tables 3 and 4).

Effect of plant extracts with or without tomato-root diffusate on hatching of J2s

The effect of 11 of the essential oils on the hatching of J2s is shown in Table 5. Essential oil of *A. sativum* had the greatest inhibitory effect, reducing hatching to less than 8% followed by *F. vulgare*, which reduced hatching to 25.3%. The effect of *A. sativum* was detected from the first week, when hatching was only 3.3% and increased very slowly during the remaining 5 weeks. *F. vulgare* also had an inhibitory effect, but it was not fully expressed until the second week. *C. coronarium, P. palestina*, *M. discoidea* and *T. patula* did not significantly inhibit hatching as compared with the control. In the majority of the treatments, the greatest reduction in hatching occurred in the first two weeks.

Effect of essential oils on the migration of J2s

Toxicity lines were established for each of the six plant extracts and the LC_{50} and LC_{90}, slope values were calculated (Table 6). Extracts from *F. vulgare* were the most toxic (LC_{50} 43 ppm) followed by extracts of *P. pinea, O. syriacum*, *M. microcorphylla*, *Eucalyptus* spp. and *C. sinensis* with an LC_{50} of 44, 50, 65, 66 and 121 ppm respectively. There were no significant differences between *P. pinea, O. syriacum*, *M. microcorphylla* and *Eucalyptus* spp. However, *C. sinensis* was significantly different from all the other essential oils.

Effect of plant extracts on mortality of J2s

Even at the lower concentrations all the six plant extracts began to affect J2s after minutes of exposure, with slower body movements becoming apparent. As concentrations increased from 5 to 100 mg l^{-1} there was a corresponding increase in J2 mortality (Table 7). At a concentration of 100 mg l^{-1}, *F. vulgare* had the greatest effect on J2 mortality, followed by *P. pinea*, *O. syriacum* and *Eucalyptus*. *C. sinensis* had the lowest effect on mortality.

Discussion

Allelochemicals are plant-produced compounds that affect the activity of other organisms and are thought to be toxins and secondary metabolites that act as attractants or deterrents (Dodds, 1996; Brown and Morra, 1997). Sudan grass, for example, contains a chemical called d'hurrin that degrades into hydrogen cyanide, which is a powerful nematicide (Wider and Abawi, 2000). In our study all the essential oil/plant extracts tested had a nematicidal effect and affected the hatching of *M. incognita* J2s even at the lowest concentration (1 mg l^{-1}). Inhibition of hatching persisted as long as the eggs were exposed to the essential oil/plant extracts. When the eggs were transferred to TRD alone, hatching resumed, but the degree of recovery was affected by the essential oil/ plant extracts concentrations to which the eggs had previously been exposed.

Essential oils from various plants have shown promise as sources for nematicides. Most of these plants are aromatic and culinary herbs that contain nematicidal compounds such as carvacrol and thymol (Oka *et al.,* 2000). Over 20 major compounds of the essential oils were identified, but the most toxic against *M. incognita* J2s were carvacrol, linalool, thymol and menthone. At very low concentrations (1 mg l^{-1}) several oils immobilized the juveniles and some also reduced hatching. Essential oils from: caraway, fennel, applemint, spearmint, Syrian oregano, and oregano showed a very high nematicidal activity (Oka *et al.,* 2000).

Table 3. Effect of pure components concentrations on hatching of second stage juveniles of *Meloidogyne incognita*.

Exposure time (week)	DW[a]	SL[a]	TRD[a]	Pure component concentration (1.0 mg l^{-1} a.i.)[b] + TRD[a]							
				Carvacrol	Thymol	Linalool	Terpineol	Menthone	1,8 Cineole	Pinen (1S) -(-)-α	Pinen (1 R) -(+)-α
1	244 a	739 b	504 a	40 c	7 c	22 c	117 d	427 d	835 ade	232 de	424 de
2	856	811	1287	224	8	234	582	765	1210	681	860
3	1029	1394	2991	54	4	2	1684	170	405	494	903
4	4459	728	1762	7	5	17	364	7	125	569	1009
5	2043	190	1325	3	4	4	256	5	250	753	1609
6	114	45	712	0	0	0	62	1	125	675	325
Total	8745	3907	7869	328	28	301	3065	1374	2950	3404	5130
% hatch	95.4	46.0	98.1	3.8	0.5	5	35.0	27.0	56.0	39.0	59.0

[a] DW, distilled water; SL, soil leachate, TRD, tomato root diffusate.
[b] Data are the means of four replications. Data followed by the same letter are not significantly different at $P<0.05$.

Table 4. Effect of pure component concentrations on hatching of second stage juveniles of *Meloidogyne incognita*.

Exposure time (week)	DW[a]	SL[a]	TRD[a]	Pure component concentration +TRD[b]							
				2 mg l^{-1}				4 mg l^{-1}			
				Carvacrol	Thymol	Linalool	Menthone	Carvacrol	Thymol	Linalool	Menthone
1	244 a	739 b	504 a	404	129	71	342	17	4	4	124
2	856	811	1287	82	1	52	176	3	0	0	274
3	1029	1394	2991	13	1	22	508	1	0	0	327
4	4459	728	1762	4	0	109	529	0	0	0	996
5	2043	190	1325	1	0	20	333	0	0	0.7	89
6	114	45	712	0	11	8	246	0	0	0	383
Total	8745	3907	7869	504	140	282	2134	21	4	4.7	2193
% hatch	95.4	46	98.1	5.3	1.5	6	20	0.2	0.04	0.04	15

[a] DW, distilled water; SL, soil leachate, TRD, tomato root diffusate.
[b] Data are the means of two replications. Data followed by the same letter are not significantly different at $P<0.05$.

Table 5**.** The effect of plant extracts at 1.0 mg l^{-1} on the hatching of second stage juveniles of *Meloidogyne incognita* (accumulative percentage).

Plant species (plant part)	Hatching time [a]					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Allium sativum (cloves)	3.32	4.1	6.2	6.8	7.3	7.6 a
Andropogon nardus (leaves)	17.35	33.3	37.2	38.3	38.5	38.6 b
Chrysanthemum coronarium (whole plant)	28.4	82.2	95.5	96.8	97.3	97.4 d
Cinnamomum zeylanicum (bark)	43.4	67.3	70.5	71.4	72.1	72.6 c
Foeniculum vulgare (flowers)	20.61	22.5	24.4	25.1	25.2	25.3 b
Matricaria discoidea (whole plant)	38.5	82.3	92.7	93.6	93.9	94.1 d
Pelargonium graveolens (leaves)	15.65	42.1	48.5	49.9	50.2	50.5 bc
Pimpinella anisum (seeds)	33.94	43.3	44.7	45.7	46.1	46.3 bc
Pistacia palestina (leaves)	46.7	82.1	94.4	96.5	96.8	96.9 d
Salvia officinalis (leaves, stem, flowers)	22.54	53.5	64.2	65.4	65.6	65.8 c
Tagetes patula (leaves, stem, flowers)	36.1	70.6	86.4	89.9	91.4	92.0 d
Control (TRD)	42.1	82.8	96.3	97.0	97.3	97.31 d

[a] Data are the means of two replications. Data followed by a common letter are not significantly different at $P<0.05$.

Table 6. The effect of plant extracts on the migration of second stage juveniles of *Meloidogyne incognita*.

Plant species	LC_{50} (ppm)	LC_{90} (ppm)	LC_{50} ppm (95% confidence limits)	Slope	Slope function
Citrus sinensis	121	260	146.168 - 100.165	1.208	3.324
Eucalyptus sp.	66	132	80.058 - 54.410	1.213	3.015
Foeniculum vulgare	43	105.5	50.697 - 36.471	1.179	2.523
Mentha microphylla	65	160	82.615 - 51.140	1.271	3.888
Origanum syriacum	50	124	61.900 - 40.387	1.238	3.342
Pinus pinea	44	129	54.824 - 35.313	1.246	4.178

Data are the means of two replications.

Table 7. Mortality of second stage juveniles (J2s) of *Meloidogyne incognita* after 24 h exposure to plant extracts.

Plant species	J2s tested (No.)	Mortality (%) at different concentrations (mg l^{-1})[a]				
		5	10	25	50	100
Foeniculum vulgare	79	1	8	24	63	86
Pinus pinea	81	10	19	37	63	69
Origanum syriacum	82	5	16	29	52	65
Mentha microphylla	85	6	21	31	45	56
Eucalyptus sp.	79	3	10	28	46	63
Citrus sinensis	84	4	12	14	25	29

[a] Data are means of two replications.

Concentration of 1,8-cineole (40%) is high in many plants (Kojima *et al.,* 1998; Obeng-Ofori and Reichmuth, 1999). In our study 1,8 cineole, carvacrol, linalool, and thymol were detected in several plants but at different concentrations. The highest concentration of carvacrol (61%) was detected in *O. syriacum* plant extracts. Some pure essential oils (carvacrol, linalool, thymol and menthone) exhibited nematode-suppressive characteristics equivalent to that of cadusafos, a synthetic pesticide (Ibrahim and Haydock, 1999). The EC value for essential oils against hatching *M. incognita* J2s was very low (0.125-4.0 mg l^{-1}) compared with 0.49 mg l^{-1} for oxamyl and 4.63 mg l^{-1} for aldicarb against *Globodera rostochiensis* on agar plates. A concentration of 0.1 and 0.5 mg l^{-1} of oxamyl inhibited the orientation of *M. incognita* toward the host roots (Wright *et al.,* 1980) and affected the orientation of *G. rostochiensis* J2s toward potato roots (Evans and Wright, 1982). The mode of action of essential oils against nematodes is still not fully understood. In insects, several essential oils inhibit acetylcholinesterase activity (Ryan and Byrne, 1988). Some common components of essential oils such as carvacrol, *t*-anethole, and thymol have been found to be insecticidal (Isman, 1999, Traboulsi *et al*., 2002) and some, to be nematicidal (Oka *et al*., 2000). Zuckerman and Esnard (1994) reported that some plant extracts with nematicidal activity may also affect behaviour, such as the nematode's ability to recognise the host.

As a result of enzyme degradation, *Brassica* spp. like rapeseed and mustard release bio-products that have a nematode-suppressive effect by interfering with the nematode reproductive cycle (Brown and Morra, 1997). Rapeseed and Sudan grass green manures grown prior to potatoes in a field reduced RKN in potatoes by 86% (Stark, 1995). The LC_{90} for thymol against *M. arenaria* in the soil was 161 ppm and the effectiveness of thymol was enhanced synergistically when it was combined with a synthetic benzaldehyde (Soler-Serratosa *et al.,* 1995). In our experiments thymol gave almost total inhibition at a very low concentration (0.5 mg l^{-1}), while *F. vulgare*, which contains thymol, had the highest effect on J2 mortality at LC_{90} of 100 mg l^{-1}.

Nematodes are attracted to *T. patula* (French marigold) roots but when they invade them, the roots release ozone, killing the nematodes (Ogden, 1997). Moreover, *T. patula* was the most effective plant in lowering RKN populations (Ogden, 1997). In our study marigold crude extracts stimulated the hatching of *M. incognita* J2s, however, it may have affected the life cycle after invasion. Belcher and Hussey (1977) reported that *T. patula* acted as a trap crop to *M. incognita*, preventing giant cell initiation. On the other hand, a minimum concentration of 1 mg l^{-1} of *A. sativum* and *F. vulgare* significantly ($P<0.05$) decreased *M. incognita* J2s to 7.6 and 25% respectively. Leaf extracts of *Crotalaria virgulata* subsp. *grantina* had a nemostatic effect on the J2s of *M. incognita* at the same low concentration (Jourand *et al.,* 2003). Pérez *et al.* (2003) reported that *C. coronarium* extracts also reduced hatching, J2 survival and the reproduction rate of *M. artiellia in vitro*, but in our research *C. coronarium* stimulated the hatching of *M. incognita* J2s. Leaf powder of rock fleabane (*Inula viscose*) at a concentration of 0.1% in sand reduced hatching of J2s of *M. javanica* and the citrus nematode (*Tylenchulus semipenetrans*), though the stem-bulb nematode *Ditylenchus dipsaci* was unaffected (Oka *et al*., 2001). Clove extract and Nimbecidine did not show any potential to control *Aphelenchoides fragariae* (Jagdale and Grewal, 2002). Further research is required to explore the natural biocidal activity of plant extracts against nematodes.

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