Phytopathol. Mediterr. (2003) 42, 123–128

Effect of plant extracts and essential oils on root-knot nematode

Luma Al-Banna[1], Rula M. Darwish[2] and Talal Aburjai[2]

[1] Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan
[2] Department of Pharmaceutics and Pharmaceutical Technology and Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan

Summary. The nematicidal activity of methanolic extracts (20 μg ml^{-1}) from twenty Jordanian plant species against two species of root-knot nematodes *in vitro* was evaluated. Whole-plant extract of *Hypericum androsaemum* showed the highest activity (11% mortality) against *Meloidogyne javanica* after 24 h of incubation. However, leaf extract of *Origanum syriacum* also increased *M. javanica* mortality markedly a day later, reaching 59 and 82% after 48 and 72 h of exposure respectively. Against *M. incognita* the response of leaf extracts was somewhat different, with leaf extract of *Artemisia herba alba* the most effective causing 22, 51, 54% mortality after 24, 48 and 72 h of exposure respectively. With a tenfold concentration (200 μg ml^{-1}) of those plant extracts thought to contain volatile oils, the second-stage juveniles (J2) mortality of both nematodes increased after 24 and 72 h of incubation. Nematicidal tests of some volatile oils that are active ingredients of the plants tested revealed that geraniol, thymol, and camphor were the most effective against *M. javanica* J2s, with 91, 60, 56% mortality respectively after 72 h of exposure. Cineole, menthol, and pinene were not effective against this nematode. Against *M. incognita* J2s, the most effective oil components were carvacol, thymol, and geraniol, with mortalities of 100, 90, and 74% respectively after 72 h of exposure. Cineole was the least effective against *M. incognita*.

Key words: *Meloidogyne javanica*, *Meloidogyne incognita*, mortality.

Introduction

In Jordan, the two nematode species, *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid *et* White) Chitwood attack several crops, producing an average annual losses of about 10% of irrigated vegetable crops in the Jordan Valley (Abu Gharbieh, 1994). These losses have led to attempts at control by various methods, mainly chemical (Abu-Gharbieh, 1994; Badawi and Abu Gharbieh, 2000), but also with solarization, an environmentally friendly means of nematode suppression (Abu Gharbieh, 1994; Abu El-Asal, 1998). A further method of control, alternative to chemicals, is the use of potential nematicides, to be integrated with other control methods. In this contest the effects of extracts or extract components on egg hatching, mortality, the immobility of second-stage juveniles (J2s), and root galling has been studied in many laboratories (Sangwan *et al.*, 1985; Saxena *et al.*, 1987; Abid *et al.*, 1997; Nath and Mukherjee, 2000; Oka *et al.*, 2000) with varying results. The objective of the present study was to evaluate twenty Jordanian plant extracts and their main components for their nematicidal activity against two Jordanian nematode populations; *M. javanica* and *M. incognita*.

Corresponding author: L. Al-Banna
Fax: +962 6 5355577
E-mail: lalbanna@agr.ju.edu.jo

Materials and methods

Plant material

Plants were either collected from the field or purchased from the local market (Table 1). Talal Aburjai identified the plants and voucher specimens were deposited in his research laboratory at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan.

Extraction and sample preparation

Dried, finely powdered plant materials were extracted in a Soxhlet with 2 l of methanol for 4 h except for *Capparis spinosa*, which was extracted for 10 h. Solvents were then evaporated under reduced pressure and the different extracts were conserved in tightly sealed glass vials and weighed (Table 1). The completely dried plant extracts were dissolved in absolute methanol to give a stock solution of 20 mg ml^{-1}. *Euphorbia macroclada* latex was obtained by cutting and squeezing the stem of the plant and was examined directly.

Analytical grade camphor, carvacrol, cineole, eugenol, geraniol, menthol, menthone, pinene, terpenine, thujon, and thymol were purchased from Acros (Acros organics, Morris Plains, NJ, USA) and used as standards in biological assays.

Nematode inoculum

Populations of *M. javanica* and *M. incognita* were collected from naturally infested fields in Jordan. Pure cultures were maintained on tomato roots in pots in the greenhouse at the University campus. Second-stage juveniles were obtained from hatched eggs by incubating hand picked egg masses in a water suspension at 24°C for 24 h.

Biological assays

Approximately 50 J2s were incubated in an aqueous suspension (15 ml) of each plant extract (20 μg ml^{-1} water) for 72 h at 24°C in a Petri dish. J2s in water with methanol served as controls. Eight plants extracts that contain volatile oils were also assayed at 200 μg ml^{-1} water. Moreover, approximately 100 J2s were incubated in 5 ml sus-

Table 1. Ethnobotanic data of plants studied.

Botanical name	Voucher specimen[a]	Family	Parts[b] used
Achillea santolina L.	Abbadi 2000-7	Lamiaceae	L, F
Anagyris foetida L.	Al-abd. 1999-2	Leguminosae	L, T
Artemisia herba-alba Asso.	Abbadi 2000-8	Compositae	L
Capparis spinosa L.	Abbadi 1999-20	Capparidaceae	R
Echinops polyceras Boiss.	Al-abd. 1999-3	Compositae	W
Eruca sativa Mill.	M	Cruciferae	T
Euphorbia macroclada L.	Al-abd. 1998-11	Euphorbiaceae	LX
Ferula harmonis L.	M	Umbelliferae	R
Gundelia tournefortii L.	Abbadi 2000-24	Compositae	W
Hibiscus sabdariffa L.	Abbadi 2000-18	Malvaceae	C
Hypericum androsaemum L.	Abbadi 99-23	Gittiferae	W
Lepidium sativum L.	M	Umbelliferae	T
Mentha piperita L.	M	Lamiaceae	L
Origanum syriacum L.	Abbadi 00-19	Lamiaceae	L
Phlomis brachydon (Boiss.) Zohary	Al-abd. 99-4	Lamiaceae	W
Pimpinella anisum L.	M	Umbelliferae	T
Teucrium polium L.	Abbadi 1999-5	Lamiaceae	L
Thea sinensis L.	M	Theaceae	L
Trigonella foenum-graecum L.	M	Leguminosae	T
Varthemia iphionoides Boiss. & Blanche	Abbadi 1999-10	Compositae	L

[a] Voucher specimens: purchased from the local market (M), or collected from different growing areas in Jordan by Mouna Al-abd (Al-abd) or Amal Abbadi (Abbadi) in 1998, 1999, and 2000 and given a number.
[b] L, leaves; T, fruits; F, flowers; R, roots; C, calyx; W, whole plant; LX, latex.

pensions of each of eleven volatile oils purchased from Acros (0.5 µg ml^{-1} water) at 24°C for 72 hours. The number of dead J2s was recorded every 24 hours for three days. Percent mortality was determined daily for each extract and volatile oil. The solvents used showed no nematode activity at the test concentration.

All data obtained were statistically analyzed using the Chi square test.

Results

Nematicidal activity of tested plants

Extracts of *H. androsaemum* were most active (11% mortality) against J2s of *M. javanica* after 24 h of incubation (Table 2). Of the other tested plants, none showed interesting results against this nematode at this time. However, when the exposure time was extended to 48 and 72 h, J2 mortality increased significantly in almost all the extracts assayed. For example, mortality caused by leaf extract of *O. syriacum* reached 59% after 48 h and 82% after 72 h. Extracts from *Anagyris foetida*, *H. androsaemum, Thea sinensis* and *Varthemia iphionoides* also showed increased mortality after 48 h (18– to 54%). Exposure to *Euphorbia macroclada, Ferula harmonis, Gundelia tournifortii, Hibiscus sabdariffa*, *Lepidium sativum* and *Pimpinella anisum* for 72 h produced a relatively modest mortality of J2, but higher than that at 24 h. Extracts from *A. herba alba*, *Achillea santolina, Capparis spinosa, Echinops polycerus, Eruca sativa, Mentha piperita, Phlomis brachydon*, *Trigonella graecum* and *Teucrium polium* were practically ineffective even after 72 h.

Meloidogyne incognita responded differently to plant extracts than did *M. javanica* (Table 2). Leaf extract of *A. herba alba*, ineffective against *M. javanica*, was the most effective here, producing 22, 51, and 54% of J2 mortality after 24, 48, and 72 h respectively. Extracts of *E. macroclada* and *P. anisum* were also effective (P=0.001) with substantial

Table 2. Effect of twenty plant extracts (20 µg ml^{-1}) on mortality of second-stage juveniles (J2s) of *Meloidogyne javanica* and *M. incognita*.

Botanical name	*M. javanica* J2 mortality (%) after			*M. incognita* J2 mortality (%) after		
	24 h	48 h	72 h	24 h	48 h	72 h
Achillea santolina	0	2	2	8	17	21
Anagyris foetida	4	22***	25***	1	1	5
Artemisia herba-alba	1	2	10	22*	51***	54***
Capparis spinosa	2	2	9	1	5	15
Echinops polycerus	4	5	8	5	5	8
Eruca sativa	5	5	5	5	14	16
Euphorbia macroclada	2	3	16*	6	35***	46**
Ferula harmonis	2	3	17*	2	9	16
Gundelia tournefortii	5	7	19*	11	13	13
Hibiscus sabdariffa	1	7	14	16	18	18
Hypericum androsaemum	11	54***	54***	14	14	14
Lepidium sativum	0	2	27***	15	18	20
Mentha piperita	3	3	11	4	10	10
Origanum syriacum	3	59***	82***	4	6	29
Phlomis brachydon	4	4	4	2	2	12
Pimpinella anisum	5	6	16*	17	41***	48**
Teucrium polium	0	0	4	2	6	8
Thea sinensis	3	19***	19*	4	13	20
Trigonella graecum	0	5	10	4	4	5
Varthemia iphionoides	0	18**	27***	0	14	23
Control	3	3	6	9	10	26

* P<0.05, ** P<0.01, ***P<0.001 compared to the control.

J2 mortality after 48 h of incubation. Extracts of all other plants, except *O. syriacum* showed a percent mortality lower than that of untreated J2s.

Percent mortality of *M. javanica* caused by extracts assayed at 200 μg ml^{-1} was generally higher than that caused by the same extracts at 20 μg ml^{-1} after 24 and 72 h (Table 3). *Origanum syriacum* was already effective against *M. javanica* at the lower concentration, and its activity against that nematode was not improved by increasing the concentration. Fruit extract of *P. anisum* at the higher concentration was as effective as was extract of *O. syriacum*.

With *M. incognita*, the tenfold concentration of these plant extracts caused an increase in J2 mortality after 24 and 72 h. *O. syriacum* was the most effective here too, with a remarkable 53% J2 mortality after only 24 h, increasing to 80% after 72 h. Extracts of *A. herba alba*, *M. piperita* and *E. polycerus* also showed increased J2 mortality, though to a less extent.

Volatile oils known to be major components of

Table 3. Effect of eight plant extracts (200 μg ml^{-1}) on mortality of second stage juveniles (J2s) of *Meloidogyne javanica* and *M. incognita*.

Botanical name	*M. javanica* J2 mortality (%) after		*M. incognita* J2 mortality (%) after	
	24 h	72 h	24 h	72 h
Achillea santolina	21	34	9	18
Artemisia herba-alba	25*	33	22	56***
Echinops polycerus	28**	41*	25	63***
Mentha piperita	21	45**	20	60***
Origanum syriacum	35***	70***	53***	80***
Phlomis brachydon	21	54***	19	31
Pimpinella anisum	30**	74***	28	44*
Teucrium polium	27*	45**	20	40
Control	12	24	17	28

* $P<0.05$, ** $P<0.01$, ***$P<0.001$ compared to the control.

Table 4. Effect of essential oil main component (0.5 μg ml^{-1}) on mortality of second stage juveniles (J2s) of *Meloidogyne javanica* and *M. incognita*.

Volatile oil	*M. javanica* J2 mortality (%) after			*M. incognita* J2 mortality (%) after		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Camphor	30***	56***	56***	31***	31***	31***
Carvacrol	6	32***	39***	91***	98***	100***
Cineole	4	6	10	9	12	12
Eugenol	13*	27***	30***	10	21*	25**
Geraniol	16**	45***	91***	60***	60***	74***
Menthol	4	4	4	15	36***	36***
Menthone	9	15**	15	45***	45***	45***
Pinene	5	5	13	53***	53***	53***
Terperine	8	25***	25***	11	25**	26**
Thijon	0	14*	17*	25**	51***	51***
Thymol	40***	60***	60***	76***	86**	90***
Control	3	3	6	8	8	8

* $P<0.05$, ** $P<0.01$, ***$P<0.001$ compared to the control.

some of these plants were tested in their pure form for nematicidal activity. Geraniol, thymol, and camphor were the most effective in causing *M. javanica* J2 mortality: thymol and camphor after 24 and 48 h (40–30% and 60–56% mortality respectively), and geraniol after 48 and 72 h (45 and 91% mortality respectively).

Cineole, menthol, and pinene were not effective against *M. javanica*. While carvacrol, eugenol, menthone, terpenine and thymol caused a low to moderate mortality at 48 and 72 h.

Although thymol and geraniol caused high J2 mortality of *M. incognita*, carvacrol was the most effective, causing 91, 98, and 100% mortality after 24, 48, and 72 h of incubation respectively. All the other essential oils except cineole caused significan mortality of *M. javanica* and *M. incognita*.

Discussion

This is the first report on the nematicidal effects of plant methanolic extracts against local populations of these two common root-knot nematodes. Some of the promising extracts (*A. herba alba, E. polycerus, E. macroclada, P. brachydon,* and *P. anisum*) have never been tested at 200 µg ml^{-1} against either species. Extract of *O. siriacum* was tested by Oka *et al.* (2000) who found that it caused a high percent mortality of *M. javanica* J2s.

The response of the nematodes varied with the type of plant, the extract concentration, and the exposure time (Table 2 and 3). For example, *H. androsaemum* and *O. syriacum* were most active against J2s of *M. javanica*, whereas *A. herba alba, E. macroclada*, and *P. anisum* were most effective against J2s of *M. incognita* when used at the lower concentration (20 µg ml^{-1}).

Time of exposure to the extracts affected the mortality of J2s of both nematodes. *O. syriacum* was most lethal against *M. javanica* J2s after 48 and 72 h of incubation, while *H. androsaemum* was most effective already after 24 h.

Increasing the concentration of some promising plant extracts to 200 µg ml^{-1} increased the mortality of both nematodes. Oka *et al.* (2000) also reported that a higher concentration (1000 µl l^{-1}) of certain plant extracts led to an increase in the mortality of *M. javanica* J2s. However, in our study leaf extract of *O. syriacum* was as effective at the lower as at the higher concentration.

Many of the plants tested are known to contain volatile oils as active ingredients. Several studies have reported that the volatile oils have a role in the nematicidal effect of these plants (Sangwan *et al.*, 1985; Malik *et al.*, 1987; Saxena *et al.*, 1987; Oka *et al.*, 2000). The present *in vitro* study found that some of these oils were very effective against one or both nematodes at relatively low concentrations. Further investigations are needed to determine the proportion of volatile oil in each extract, the most promising extracts, their mode of action, and the effect of combinations of volatile oils. In addition, field applications of promising extracts should be conducted to verify their nematicidal effectiveness.

Acknowledgements

We thank Toujan Abu Shwaimah for technical assistance. This study was supported by the Deanship of Scientific Research, University of Jordan.

Literature cited

Abid M., M.I. Choudhary, M.A. Maqbool and A. Ur-Rahman, 1997. Preliminary screening of some plants for their nematicidal activity against *Meloidogyne javanica*. *Nematologia Mediterranea* 25, 155–157.

Abu-Gharbieh W.I., 1994. *Root Knot Nematodes, Meloidogyne* spp. *in Jordan, Biology and Control*. 2nd Edition, Publications of the University of Jordan, Amman, Jordan, 97 pp.

Abu El-Asal M.I., 1998. *Treatment of Transparent Polyethylene Sheets after Soil Solarization for Direct Transplanting, and their Effect on Root-knot Nematode*. M.Sc. Thesis. Faculty of Agriculture, University of Jordan, Amman, Jordan, 70 pp.

Badawi S.M. and W.I. Abu-Gharbieh, 2000. Efficacy of certain non fumigant nematicides for the control of *Meloidogyne javanica* on tomato. *Pakistan Journal of Nematology* 18, 59–68.

Barker K.R., R.S. Hussey, L.R. Krusberg, G.W. Bird, R.A. Dunn, H. Ferris, V.R. Ferris, D.W. Freckmann, C.J. Gabriel, P.S Grewal, A.E. MacGuidwin, D.L. Riddle, P.A. Roberts and D.P. Schmitt, 1994. Plant and soil nematodes: Societal impact and focus for the future. *Journal of Nematology* 26, 127–137.

Malik M.S., N.K. Sangwan, K.S. Dhindsa, K.K. Verman and D.S. Bhatti, 1987. Nematocidal efficacy of some monoterpenes and related derivatives. *Pesticides* 21, 30–32.

Nath R.C. and B. Mukherjee, 2000. *Dioscorea floribunda*, a potential source of nematicides of plant origin. *Nematologia Mediterranea* 28, 145–149.

Oka Y., S. Necar, 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., K.K. Verma, B.S. Verma, M.S. Malik and K.S. Dhindsa, 1985. Nematicidal activity of essential oils of Cymbopogon grasses. *Nematologica* 31, 93–99.

Saxena D.B., B.K. Goswami and S.S. Tomar, 1987. Nematicidal activity of some essential oils against *Meloidogyne incognita*. *Indian Perfume* 31, 150–154.

Accepted for publication: May 22, 2003