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Control of *Meloidogyne javanica* by Formulations of *Inula viscosa* Leaf Extracts

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Abstract: *Inula viscosa* is a perennial plant that is widely distributed in Mediterranean countries. Formulations of *I. viscosa* extracts were tested for their effectiveness in control of *Meloidogyne javanica* in laboratory, growth chamber, microplot, and field experiments. Oily pastes were obtained by extraction of dry leaves with a mixture of acetone and *n*-hexane or *n*-hexane alone, followed by evaporation of the solvents. Emulsifiable concentrate formulations of the pastes killed *M. javanica* juveniles in sand at a concentration of 0.01% (paste, w/w) or greater and reduced the galling index of cucumber seedlings as well as the galling index and numbers of nematode eggs on tomato plants in growth chamber experiments. In microplot experiments, the hexane-extract formulation at 26 g paste/m^2 reduced nematode infection on tomato plants in one of two experiments. In a field experiment, a reduction of 40% in root galling index by one of two formulations was observed on lettuce plants. The plant extracts have potential as a natural nematicide, although the formulations need improvement.

Key words: botanical nematicide, *Inula viscosa,* management, *Meloidogyne javanica*, nematode, phytochemical, root-knot nematode, sesquiterpenic acid.

The root-knot nematode *Meloidogyne javanica* causes serious damage in vegetable crops in Israel, especially in organic vegetable production systems. Incorporation of organic amendments into the soil or soil solarization does not always produce sufficient nematode control. While the use of resistant cultivars or rootstocks is an effective nematode management tool, they are not available in all vegetable crops. Further, nematode resistance, such as that in tomato, is often undermined by high soil temperatures such as those that prevail in Israel. Consequently, nematode management strategies that can be used in organic farming systems are in demand and one such practice may be use of natural "nematicides." Such products also may be used in conventional farming systems due to concerns for environmental and food safety.

Chemicals produced by plants are a potential source of new chemistry for development of new pesticidal compounds. Nematicidal phytochemicals are generally safe for the environment and humans (Chitwood, 2002). Chinese herbal remedies may be a source of new nematicidal compounds (Ferris and Zheng, 1999; Zasada et al., 2002). Many nematicidal phytochemicals from a great variety of chemical structures have been isolated from numerous plant families (Gommers and Bakker, 1988; Chitwood, 2002). A majority of these nematicidal phytochemicals isolated have been from the plant family Asteraceae (Gommers and Bakker, 1988). α-Terthienyl and related compounds were isolated from *Tagetes* spp. and have been shown to be nematicidal at low concentrations in vitro (Uhlenbroek and Bijloo, 1958, 1959). These phytochemicals, however, were not effective in nematode control in soil (Gommers and Bakker, 1988).

Polyacetylenes are another chemical group from the Asteraceae with nematicidal activity. For example, nematicidal polyacetylenes have been isolated from flowers of *Carthamus tinctorius* and roots of *Cirsium japonicum* (Kogiso et al., 1976; Kawazu et al., 1980), and dithioacetylenes have been isolated from *Milleria quinqueflora*, *Iva xanthiifolia*, *Ambrosia artemisiifolia*, *Ambrosia trifida*, *Schkuhria pinnata*, and *Eriophyllym caespitosum* (Gommers and voor in 't Holt, 1976). Thiarubrine C isolated from the roots of *Rudbeckia hirta* has been shown to have nematicidal activity against *M. incognita* and *Pratylenchus penetrans* (Sánchez de Viala et al., 1998).

Unfortunately, none of these compounds or their derivatives has been developed into commercial nematicides. Plant essential oils, mainly monoterpenes, have been evaluated for their nematicidal activity, and some were highly effective in nematode suppression (Oka et al., 2000; Oka, 2001). However, use of natural essential oils as nematicides is not cost effective. Various neem tree (*Azadirachta indica* A. Juss.) preparations are well known, commercially available nematode control products derived from plants (Mojumdar, 1995).

Recently, elecampane (*Inula viscosa*, syn. *Cupularia viscosa*, *Dittrichia viscosa*) (Asteraceae), a widespread plant in Mediterranean countries, has been found to have nematicidal activity in the shoot (Oka et al., 2001). This plant has antifungal activity as well, and several foliar fungal diseases have been controlled by the plant extracts (Cohen, 1998; Wang et al., 2004). Another species, *I. helenium*, has been known to have anthelmintic activity, probably due to sesquiterpenoid lactones such as alantolactone (Mahajan et al., 1986; Bourrel et al., 1993). Sesquiterpenic acids (costic acid and isocostic acid) from *I. viscosa* leaf extracts were found to be the nematicidal phytochemicals (Oka et al., 2001). A mixture of these compounds was toxic to *M. javanica* at concentrations as low as 50 mg/kg in soil. However, formulating the plant extract is essential for commercial use of the nematicidal extracts. In the present study, the toxicity of formulated plant extracts of *I. viscosa* against *M. javanica* was evaluated in laboratory, growth chamber, microplot, and field experiments.

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Materials and Methods

Nematode: Eggs of *M. javanica* were extracted from infected tomato (*Lycopersicon esculentum* cv. Daniela) roots with NaOCl (Hussey and Barker, 1973). Second-stage juveniles emerging from eggs spread on a 30-µm-pore sieve were collected daily, stored at 15°C for no longer than 5 d, and used in experiments.

Plant extracts: Fully expanded air-dried leaves of *I. viscosa* grown at Kramim, Israel, were immersed and stirred overnight in a mixture of acetone and *n*-hexane (9:1, v/v) or *n*-hexane at 100 g/liter solvent at room temperature. The extracts were filtered through Whatman No. 1 paper, and vacuum dried at 45°C to 50°C. Acetone-*n*-hexane extract (AHE) paste and *n*-hexane extract (HE) paste were obtained without water residues. Typical yields of AHE and HE paste were 9% to 15% and 0.5% to 0.7% (w/w) of dry leaves, respectively, depending on harvest period.

Formulations: Three emulsifiable concentrate (EC) formulations were prepared by adding emulsifiers (Oka and Ben-Daniel, 1999): (i) AHE-EC, 37.5% (w/w) AHE paste, pH 3.5; (ii) AHEC-EC, 34.4% (w/w) AHE paste, pH 2.5; and (iii) HE-EC, 56.6% (w/w) HE paste. The plant leaf powder prepared by pulverizing air-dried (40°C for 8 hr) leaves was also used as a control treatment in some experiments. For comparative purposes, blind formulations of AHE-EC, AHEC-EC, and HE-EC were prepared by replacing AHE or HE pastes with water.

Laboratory experiment: AHE-EC, AHEC-EC, or HE-EC diluted with 1 ml of water was added to 10 g dry untreated dune sand (pH 8.5) in 25-cm^3 glass bottles at concentrations of 0.01%, 0.02%, and 0.04% (paste, w/w). The leaf powder was also mixed into the sand at concentrations of 0.1%, 0.2%, and 0.4% (w/w), followed by addition of 1 ml water. Two hundred *M. javanica* J2 in 200 µl of water were introduced into the sand. Nontreated sand receiving 1 ml water served as a control. The bottles were capped and held at a room temperature (24-27°C). Seven d after treatment, live J2 were recovered by spreading the sand on a sieve (60-µm-pore size, 3.0-cm-diam.) in a petri dish with water for 5 hr, and J2 that passed the sieve were counted. Each treatment had four replicates, and the experiment was performed twice. The blank formulations of AHE-EC, AHEC-EC, and HE-EC were also tested by the same method at the same time.

Growth chamber experiments: AHE-EC, AHEC-EC, or HE-EC diluted in 25 ml water was incorporated into 250 g dry sand in 180-cm^3 plastic pots at concentrations of 0.01%, 0.02%, and 0.04% (paste, w/w). Two thousand *M. javanica* J2 in 2 ml water were introduced into the sand via five 2-cm-deep holes and were incubated for 7 d at 27°C. Germinating cucumber (Zeraim Gedera cv. Dlila) seeds (with about 1-cm-long roots) were planted after the incubation period. Seedlings were uprooted 10 d later, and gall indices (GI) were assessed according to a 0-to-5 scale (0 = no infection; 1 = 1%–20% of roots galled; 2 = 21%–40%; 3 = 41%–60%; 4 = 61%–80%; and 5 = 81%–100%). Nontreated sand and the blank formulations were used as controls. Each treatment had eight replicates, and the experiment was performed twice.

In the second set of growth chamber experiments, AHE-EC, AHEC-EC, or HE-EC diluted in 100 ml water was mixed into 1.0 kg dry sand in 750-cm^3 pots at concentrations of 0.01%, 0.02%, and 0.04% (paste, w/w). The soil was infested with 2,000 *M. javanica* J2/pot. A 1-mon-old tomato (Hazera Genetics cv. Daniela) seedling was planted into each pot 7 d later, maintained at 27°C ± 2°C in a growth chamber with 13-hr days, and received 50 ml 0.1% of fertilizer (20-20-20, N-P-K,) weekly. Fresh shoot weight, root GI, and number of nematode eggs per plant on root system were determined 40 d after planting. Each treatment had five replicates, and the experiment was performed twice.

Bucket experiment: Ten-liter buckets filled with 10 kg of dry sand were each infested with 20,000 *M. javanica* J2 by mixing a nematode suspension into the soil. The soils were then mixed with AHEC-EC or HE-EC diluted in 1 liter water at concentrations of 0.01% and 0.02% (paste, w/w). A 1-mon-old tomato (cv. Daniela) seedling was planted in each pot 10 d after treatment, kept in a growth chamber at 27°C ± 2°C, and fertilized weekly with 200 ml 0.1% solution of 20-20-20 (N-P-K). Fresh shoot weight, root GI, and number of nematode eggs per plant on the root system were determined 60 d after planting. Each treatment had five replicates, and the experiment was performed twice.

Field microplot experiment: Plastic containers (1.0-m diam., 1.0-m depth) filled with 650 liters of sandy soil (pH 7.8, organic matter < 0.01%) and buried in a field were initially infested with approximately 40,000 *M. javanica* eggs each by mixing a nematode egg suspension into the soil. Four 1-mon-old tomato (Hazera Genetics cv. Bernadine) seedlings were planted in each container and grown for 3 mon (June through August), and the root systems of the tomato plants were removed. One mon later, the soil in the containers was manually tilled, and then 26 g or 52 g paste of AHEC-EC, or 26 g or 52 g paste of HE-EC, diluted in 40 liters water, were applied to the soil manually. *Meloidogyne javanica* pre-treatment density was 2.6 ± 2.2 J2/50 g soil (dry base) in the top 30 cm of the soil. Four tomato (Hazera Genetics cv. Abigail) seedlings were planted (10 October 2003) in each container 10 d after treatment. Each container received 2 liters water every 2 d by drip irrigation and 250 ml of 0.1% solution of 20-20-20 (N-P-K) weekly. Fresh shoot weight, root GI, and number of nematode eggs on tomato root systems were determined 60 d after planting. Each treatment had five replicates.

Before starting the second microplot experiment, to-

TABLE 1. Number of *Meloidogyne javanica* second-stage juveniles (J2) recovered from sand treated with leaf powder or emulsifiable concentrates of *Inula viscosa* leaf extract pastes.[a]

	Control	Leaf powder (%)			AHE-EC[b] (% paste)			AHEC-EC[c] (% paste)			HE-EC[d] (% paste)		
		0.1	0.2	0.4	0.01	0.02	0.04	0.01	0.02	0.04	0.01	0.02	0.04
Experiment 1	77.7 a	—	—	—	25.0 b	13.5 bc	1.3 cd	11.0 cd	2.8 cd	0 d	14.3 cd	6.2 cd	0 d
Experiment 2	104.3 a	24.2 b	0.6 c	0 c	27.0 b	12.5 bc	2.5 c	9.5 bc	2.3 c	0 c	13.1 c	2.6 c	0 c
Blank formulation[e]	111.2 a	—	—	—	71.1 bc	55.5 cd	21.7 d	52.8 b	29.5 bc	8.1 d	98.8 a	89.3 b	56.0 bc

[a] Data are means of four replicates. Means within a row followed by a common letter are not different according to the Tukey-Kramer HSD test ($\alpha = 0.05$).
[b] AHE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaves extracted with acetone-hexane.
[c] AHEC-EC = pH-modified AHE-EC.
[d] HE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaves extracted with *n*-hexane.
[e] Leaf extract pastes of the formulations were replaced with water.

mato (cv. Daniela) plants were grown in the microplots for 2 mon to increase nematode population densities. The initial *M. javanica* population density in the microplot soil was 25.3 ± 13.7 J2/50 g soil in the top 30 cm 3 wk after uprooting the plants. The application of AHEC-EC or HE-EC was similar to that of the first microplot experiment. Ten days after treatment, 50 g soil was collected from the microplots at a depth of 15 to 30 cm, and J2 were extracted via Baermann funnels. Four tomato (cv. Daniela) seedlings were planted (25 July 2004) in each container and irrigated and fertilized similarly to the method described above. Fresh shoot weight, root GI, and number of nematode eggs per plant on root system of the plants were determined 45 d after planting. Each treatment had five replicates.

Field experiment: A field experiment was conducted at the Zohar Experiment Station, Arava R&D, Ein-Tamar, Israel, in a field with sandy soil (pH 7.8, organic matter < 0.1 %) naturally infested by *M. javanica* (approx. 0.4 J2/g). The field was divided into 32 experimental units (1.2-m wide × 5-m long) of eight treatments. The following treatments were evaluated: soil drenching of lettuce planting holes 10 d before transplanting with 0.5, 1, or 2 g paste of AHE-EC or AHEC-EC formulation in 50 ml water, and paste formulation then leached into the soil with drip irrigation (2 liters/planting hole) immediately after drenching; and metham sodium (185 kg/ha) applied with drip irrigation under a plastic sheet 3 wk before transplanting. Nontreated units served as a control. Twenty-two 4-wk-old lettuce seedlings (Hazera Genetics cv. Noga) were planted per plot on 15 April 2003 and were irrigated daily with about 40 liters of water containing a fertilizer (8-3-5, N-P-K at a concentration of 0.125%). Root GI of six plants per plot was recorded 20 d after planting. The crop was harvested 30 d after planting. Fresh head weights (yields) and root gall indices at harvest were recorded. Each treatment had four replicates.

Data analysis: Data were statistically analyzed by analysis of variance, and means were separated according to Tukey-Kramer HSD test ($\alpha = 0.05$). All calculations were performed with JMP (SAS Institute, Cary, NC).

RESULTS

Laboratory experiment: The formulations AHE-EC, AHEC-EC, and HE-EC at concentrations as low as 0.01% (paste, w/w) reduced ($P < 0.001$) the number of recovered J2 from the sand. No J2 were recovered at 0.04%, except from the soil treated with AHE-EC (Table 1). The blank formations of AHE-EC and AHEC-EC showed moderate nematicidal activity, whereas that of HE-EC was far weaker than the original formulations. The leaf powder also showed nematicidal activity.

Growth chamber experiments: In the bioassay with cucumber seedlings, the cucumber seedlings grown in the sand treated with the three formulations or the leaf powder showed little or no galling on the root, whereas the control plant roots were heavily galled (Table 2).

TABLE 2. Root galling index (GI: 0–5) caused by *Meloidogyne javanica* of cucumber seedlings grown in sand treated with formulations of *Inula viscosa* extract pastes in 180-cm^3 pots.[a]

	Control	Leaf powder (% paste)			AHE-EC[b] (% paste)			AHEC-EC[c] (% paste)			HE-EC[d] (% paste)		
		0.1	0.2	0.4	0.01	0.02	0.04	0.01	0.02	0.04	0.01	0.02	0.04
Experiment 1	4.2 a	0.4 b	0 b	0 b	0.3 b	0 b	0 b	0.5 b	0 b	0 b	0.2 b	0 b	0 b
Experiment 2	3.6 a	—	—	—	0.3 b	0 b	n.t.	0.3 b	0 b	n.t.	0 b	0 b	n.t.
Blank formulation[e]	3.1 a	—	—	—	2.1 ab	1.6 b	1.4 b	2.8 ab	1.9 ab	1.6 b	2.4 ab	2.6 ab	2.1 ab

[a] Data are means of eight replicates. Means within a row followed by a common letter are not different according to Tukey-Kramer HSD test ($\alpha = 0.05$).
[b] AHE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaves extracted with acetone-hexane.
[c] AHEC-EC = pH-modified AHE-EC.
[d] HE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaves extracted with *n*-hexane.
[e] Leaf extract pastes of the formulations were replaced with water.

The blank formulations of AHE-EC and AHEC-EC reduced the galling index at high concentrations, whereas that of HE-EC did not. Treatment of sand with the formulations at high concentrations caused a reduction in tomato fresh shoot weight compared to the control (Table 3). Galling index and numbers of nematode eggs per plant were lower ($P < 0.001$) at all the concentrations than those of control plants (Table 3).

Bucket experiments: The GI and numbers of nematode eggs per plant in AHEC-EC- and HE-EC-treated soils were lower ($P < 0.001$) than those of control tomato plants in the two experiments (Table 4). There were no differences in fresh shoot weight ($P > 0.05$) among the treatments.

Microplot experiment: In the first experiment, GI of tomato plants grown in the soil treated with both concentrations of HE-EC was lower ($P < 0.05$) than that of control plants (Table 5). No difference ($P > 0.05$) in fresh shoot weight of tomato plants was found among the treatments. In the second experiment, the number of J2 in the control soil at 10 d after treatment was higher ($P < 0.001$) than in soils treated with AHEC-EC or HE-EC. No differences ($P > 0.05$) in fresh shoot weight or GI were found among the treatments. There was no treatment effect on eggs per plant in either experiment.

Field experiment: The lettuce plants grown in the metham sodium-treated soil had almost no root galls (Table 6). The treatments with AHEC-EC at three doses reduced the root GI, except for the treatment at a concentration of 2.0 g paste/planting hole 20 d after planting. There was no difference ($P > 0.05$) in fresh shoot weight of the plants among the treatments.

TABLE 4. Root galling index (GI), fresh shoot weights (FSW), and number of *Meloidogyne javanica* eggs of tomato plants grown in nematode-infested soil treated with emulsifiable concentrates of *Inula viscosa* extract pastes in 10-liter buckets.[a]

	Control	AHEC-EC[b] (% paste)		HE-EC[c] (% paste)	
		0.01	0.02	0.01	0.02
Experiment 1					
FSW (g)	90.2 a	69.2 a	83.7 a	75.0 a	80.1 a
GI (0–5)	3.9 a	1.8 b	1.3 bc	0.2 c	0.1 c
Eggs/plant[d]	332,750 a	32,267 b	14,667 b	1,173 bc	293 c
Experiment 2					
FSW (g)	36.9 a	43.5 a	53.3 a	38.1 a	5.9 a
GI (0–5)	5.0 a	2.8 b	0.8 c	1.9 b	0.1 c
Eggs/plant[d]	656,700 a	42,720 b	27,950 b	25,360 b	3,227 b

[a] Data are means of five replicates. Means within a row followed by a common letter are not different according to Tukey-Kramer HSD test ($\alpha = 0.05$).
[b] AHE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaf extract with acetone-hexane.
[c] HE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaf extract with *n*-hexane.
[d] Statistical analysis was done after transformation to $\log_{10}(x + 1)$.

Discussion

The present study used formulations of *I. viscosa* leaf extracts based upon two extraction methodologies. The main difference between these extracts was that HE contains a higher percentage of costic and isocostic acid in the paste than the paste of AHE (unpubl. data). However, only a slight difference in nematode control efficacy was seen in the 10-liter bucket experiment, probably due to other nematicidal compounds present in the acetone-hexane extract (Oka et al., 2001). A mixture of these sesquiterpenic acids was found to be one of the nematicidal components in the plant extract (Oka et al., 2001). These sesquiterpenic acids and their derivatives have been found in *I. viscosa* and other plants in the genus, as well as in related plants (Shtacher and Kashman, 1970; Ulubelen et al., 1987; Zdero et al., 1988; Öksüz and Topçu, 1991). Although the leaf powder and leaf extracts with solvents showed nematicidal activity against *M. javanica* in our previous study (Oka et al., 2001), one of the most important factors for commercial agrochemical development is the influence of formulations on the stability of active ingredients, dispersal in the soil, and convenience of handling and field application. The most common formulation of nematicides used in Israel is a liquid that

TABLE 3. Root galling index (GI), fresh shoot weights (FSW), and number of *Meloidogyne javanica* eggs per root system of tomato plants grown in nematode-infested soil treated with emulsifiable concentrates of *Inula viscosa* extract pastes in 750-cm^3 pots.[a]

	Control	AHE-EC[b] (% paste)			AHEC-EC[c] (% paste)			HE-EC[d] (% paste)		
		0.01	0.02	0.04	0.01	0.02	0.04	0.01	0.02	0.04
Experiment 1										
FSW (g)	16.1 a	11.2 ab	14.9 abc	7.9 bc	15.4 abc	10.3 abc	8.6 c	16.8 a	15.3 abc	11.6 abc
GI (0–5)	2.9 a	0.5 b	0.3 b	0 b	0.5 b	0.1 b	0 b	0.1 b	0 b	0 b
Eggs/plant[e]	54,560 a	5,573 b	2,933 b	0 c	2,787 b	0 c	0 c	5,573 b	0	0 c
Experiment 2										
FSW (g)	11.8 a	9.3 ab	8.6 bc	8.5 bc	9.1 ab	9.3 ab	9.4 ab	8.9 ab	7.9 bc	7.2 c
GI (0–5)	3.9 a	0.9 b	0.4 bc	0 c	0.9 b	0.6 bc	0 c	0.3 bc	0.3 bc	0 c
Eggs/plant[e]	32,550 a	8,863 ab	3,764 abc	0 d	4,500 ab	1,200 abc	0 d	2,250 abc	300 cd	0 d

[a] Data are means of five replicates. Means within a row followed by a common letter are not different according to Tukey-Kramer HSD test ($\alpha = 0.05$).
[b] AHE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaves extracted with acetone-hexane.
[c] AHEC-EC = pH-modified AHE-EC.
[d] HE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaves extracted with *n*-hexane.
[e] Statistical analysis was done after transformation to $\log_{10}(x + 1)$.

TABLE 5. Root galling index (GI), fresh shoot weights (FSW), and number of *Meloidogyne javanica* eggs of tomato plants grown in nematode-infested soil treated with emulsifiable concentrates of *Inula viscosa* extract pastes in field microplots.[a]

	Control	AHEC-EC[b] (g paste/m^2)		HE-EC[c] (g paste/m^2)	
		26	52	26	52
Experiment 1					
FSW (g)	38.5 a	37.3 a	41.8 a	49.8 a	41.2 a
GI (1–10)	7.7 a	5.9 ab	5.4 ab	4.5 b	3.3 b
Eggs/10 g root[d]	805,162 a	723,724 a	513,689 a	365,462 a	277,626 a
Experiment 2					
J2/50 g soil[e]	31.0 a	0.8 b	0.5 b	2.5 b	0 b
FSW (g)	31.5 ba	53.9 a	64.5 a	45.1 a	44.8 a
GI (1–10)	9.6 a	7.4 a	8.3 a	9.2 a	8.2 a
Eggs/10 g root[d]	706,500 a	603,000 a	439,312 a	714,375 a	338,625 a

[a] Data are means and SD of 20 plants. Means within a row followed by a common letter are not different according to Tukey-Kramer HSD test ($\alpha = 0.05$).
[b] AHE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaf extract with acetone-hexane.
[c] HE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaf extract with *n*-hexane.
[d] Statistical analysis was done after transformation to $\log_{10}(x + 1)$.
[e] Number of juveniles 10 d after treatment.

can be applied via drip irrigation systems (i.e., chemigation). The emulsifier used for the oily pastes of the *I. viscosa* extracts affected the nematode when it was applied at higher concentrations. It inhibited oxidation of the active ingredients, maintained longer shelf life, and reduced the pH (unpubl. data). Lowering pH may make the active ingredient more stable in the formulation and in soil and change percentages of dissociated acids, as pH of most agricultural soils in Israel is neutral to weak basic.

In our laboratory, growth chamber, and bucket experiments where only the J2 were tested, the formulations effectively controlled *M. javanica*. The results may be attributable to the fact that the formulations have higher nematicidal activity against J2 than against eggs (unpubl. data) or to rapid product degradation after application in soil. In most pot experiments, tomato growth was inhibited by the formulations, resulting in smaller fresh shoot weights than in the control plants, which were more severely infected with the nematode. This growth inhibition could be overcome by delaying planting after soil treatment (Oka et al., 2001).

In the microplot experiments, a large volume of water containing the formulations was applied in order to disperse the active ingredient uniformly and deeply in the soil. In the first experiment, HE-EC reduced the galling index and number of nematode eggs. High initial populations of the nematode in the second experiment resulted in no significant differences in fresh shoot weight or galling index among the treatments. Nematode juveniles located deeper in the soil that had not come in contact with the treatments may have infected the plants in the treated soils. Application methods that drive the formulations to deeper soil need to be developed.

In the field experiment, a smaller volume of diluted formulations than used in the microplot experiment was drenched in planting holes, and the soil was then irrigated via a drip system. Only a slight nematicidal effect was observed with the AHEC-EC formulation, probably due to non-uniform incorporation of the active ingredient into the soil. The active ingredient might adhere to the upper soil layer and not reach deeper levels. Thus, mechanical delivery of the formulation into the soil or improvement of the formulation may result in better performance. In pot experiments, the EC formulations used in the present study also showed antifungal activity against soil-borne fungi *Rhizoctonia solani* and *Sclerotium rolfsii* (Oka et al., 2003) and foliar fungal diseases (Wang et al., 2004).

TABLE 6. Root galling index (GI) at 20 and 30 d after planting and fresh shoot weights (FSW: kg/10 plants) at 30 d of lettuce plants grown in a *Meloidogyne javanica*-infested field treated with metham sodium (185 kg/ha) or three doses of emulsifiable concentrates of *Inula viscosa* extract pastes.

	Control	Metham sodium	AHE-EC[a] (g paste/planting hole)			AHEC-EC[b] (g paste/planting hole)		
			0.75	1.5	2.0	0.75	1.5	2.0
GI at 20 d[c]	2.6 a	0.1 d	2.7 a	2.1 ab	2.8 a	1.3 bc	0.9 cd	2.7 a
GI at 30 d[d]	4.2 a	0 d	3.5 ab	3.4 ab	4.1 a	1.9 c	2.8 b	3.0 b
FSW (kg/10 plants)	1.5 a	2.1 a	1.8 a	1.8 a	1.7 a	2.2 a	1.6 a	1.7 a

[a] AHE-EC = emulsifiable concentrate prepared from *Inula viscosa* leaves extracted with acetone-hexane.
[b] AHEC-EC = pH-modified AHE-EC.
[c] Data are means of six plants.
[d] Data are means of 30 plants.
Means within a row followed by a common letter are not different according to Tukey-Kramer HSD test ($\alpha = 0.05$).

In conclusion, these results suggest that the EC formulations of *I. viscosa* extract are potential nematicides that can be used in organic and conventional agricultural systems if the formulations are improved for soil drenching or chemigation. A disadvantage of this formulation and the active ingredient is the phytotocixity that does not allow use of the product during the growing season. Non-phytotoxic nematicidal compounds from plants must be found and developed into a commercial product.

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