JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

Nematicidal Activity of Mint Aqueous Extracts against the Root-Knot Nematode *Meloidogyne incognita*

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ABSTRACT: The nematicidal activity and chemical characterization of aqueous extracts and essential oils of three mint species, namely, *Mentha* × *piperita*, *Mentha spicata*, and *Mentha pulegium*, were investigated. The phytochemical analysis of the essential oils was performed by means of GC-MS, whereas the aqueous extracts were analyzed by LC-MS. The most abundant terpenes were isomenthone, menthone, menthol, pulegone, and carvone, and the water extracts yielded mainly chlorogenic acid, salvianolic acid B, luteolin-7-*O*-rutinoside, and rosmarinic acid. The water extracts exhibited significant nematicidal activity against *Meloidogyne incognita*, and the $EC_{50/72h}$ values were calculated at 1005, 745, and 300 mg/L for *M.* × *piperita*, *M. pulegium*, and *M. spicata*, respectively. Only the essential oil from *M. spicata* showed a nematicidal activity with an $EC_{50/72h}$ of 358 mg/L. Interestingly, menthofuran and carvone showed $EC_{50/48h}$ values of 127 and 730 mg/L, respectively. On the other hand, salicylic acid, isolated in the aqueous extracts, exhibited EC_{50} values at 24 and 48 h of 298 ± 92 and 288 ± 79 mg/L, respectively.

KEYWORDS: *essential oil, nematicide, botanical pesticide, allelochemical, reactive carbonyl species, intercropping practices*

INTRODUCTION

Nematodes of the genus *Meloidogyne* are tiny worms that attack the roots of agricultural crops and play an important role in the predisposition of the host plant to invasion by secondary parasites,[1] resulting in detrimental effects on the harvest in terms of quality and quantity.[2]

In past decades, synthetic nematicides have been the only weapon used against these parasites, but their use is nowadays somewhat reduced, and many products have actually been phased-out from the market because their indiscriminate use affected nontarget organisms with consequential problems for the environment.[3,4] The development of an alternative eco-friendly tool to control crop-damaging nematodes represents an important challenge. Soil solarization is the most diffused nonchemical method to control nematodes, but this is effective only up to a depth of 20 cm in the soil,[5] and when the temperature increases, those nematodes still surviving in the deeper soil layers can migrate upward and reinfest the previously disinfested area.[6] The practice of intercropping could be another way to delay or inhibit nematodes' reinfestation, although it is not always suitable when intensive cropping is involved.[7]

To date, the research on alternative methods for nematode control is focused on the variety of botanical species growing in the Mediterranean basin used as rich sources of nematicidal agents.[8,9] Recently, our research group discovered that the volatile allelopathic metabolite furfural present in *Melia azedarach* L. and the allylisothiocyanate extracted from horseradish (*Armoracia rusticana*) roots were active against root-knot nematodes.[10−12] Similarly, we reported the potent nematicidal activity of phthalaldehyde, salicylaldehyde, and cinnamic aldehyde against *Meloidogyne incognita*, which exhibited EC_{50} values at 24 h of 11 ± 6, 11 ± 1, and 12 ± 5 mg/L, respectively.[13]

Consequently, the possible application of plants' water extracts directly on the field without the use of toxic organic solvents or coformulants could be of interest in relation to crop protection.

In the present study, we focused our attention first into finding the exact composition of aqueous extracts of dry leaves of *Mentha* × *piperita*, *Mentha spicata*, and *Mentha pulegium* using liquid chromatography−mass spectrometry (LC-MS) and of the essential oils by means of gas chromatography−mass spectrometry (GC-MS). Subsequently, we tested the nematicidal activity of these extracts and some selected compounds.

MATERIALS AND METHODS

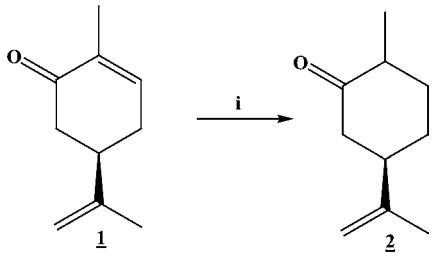
Chemicals. Analytical standards of α-pinene (99.0% w/w, d = 0.858 g/mL at 20 °C), D-limonene (97.0% w/w, d = 0.842 g/mL at 20 °C), eucalyptol (99% w/w, d = 0.921 g/mL at 25 °C), menthone (97% w/w), menthofuran (≥99.0% w/w, d = 0.97 g/mL at 20 °C), menthol (99% w/w, d = 0.89 g/mL at 25 °C), menthyl acetate (97% w/w, d = 0.922 g/mL at 25 °C), *E*-caryophyllene (98.5% w/w), carvone (98% w/v, d = 0.959 g/mL at 25 °C), and salvianolic acid B were purchased from Sigma-Aldrich, Italy. All solvents and reagents were of pesticide grade.

General Procedure for the Preparation of Dihydrocarvone.[14] A mixture of fine zinc powder (18.65 mmol), KOH (8.66 mmmol), MeOH (5 mL), and water (2 mL) was refluxed under vigorous stirring for a few minutes (Scheme 1). Then a solution of product **1** in 5 mL of MeOH was slowly added drop by drop and the mixture refluxed for 24 h; the reaction was monitored by TLC (hexane/ethyl acetate 3:1). The cooled mixture was then filtered and the filtrate concentrated to give a crude purple oil that was dispersed in water and extracted with ethyl acetate (10 mL × 3). The organic layer, previously dried on $MgSO_4$, was concentrated in vacuum to give **2** as a light yellow oil (yield % = 92). ^{1}H NMR (500 MHz, $CDCl_3$)

Received: May 28, 2013
Accepted: September 19, 2013
Published: September 19, 2013

dx.doi.org/10.1021/jf403684h | J. Agric. Food Chem. 2013, 61, 9784−9788

Scheme 1. Reagent and Conditions



i Zn, KOH, MeOH 95%, H_2O, reflux, 24 h

δ 4.70 (br s, 1H), 4.68 (br s, 1H), 2.40−2.23 (m, 5H), 2.07−1.99 (m, 2H), 1.87−1.79 (m, 2H), 1.69 (m, 2H), 0.980 (d, J = 6.5 Hz, 3H).

Plant Materials. Dried aerial parts of *M.* × *piperita*, *M. spicata*, and *M. pulegium* were bought from a local supplier (Laboratorio d'erbe Sauro, Verona, Italy).

Isolation and Chemical Characterization of the Essential Oils. The dried leaves were subjected to water distillation using a Clevenger apparatus (Winzer) for 3 h according to the fifth edition of the *European Pharmacopoeia*. One hundred grams of aromatic material was added to 1 L of distilled water in a 2 L glass flask. The essential oils (EOs) obtained were dried over anhydrous Na_2SO_4 and stored in dark glass vials with Teflon-sealed caps at −20 °C until used. The chromatographic separation of the EOs for component identification purposes was performed on an Agilent Technologies 6850 gas chromatograph coupled with a 5973 mass selective detector and a 7683B series injector autosampler, and the injection was performed in splitless mode. The resulting data were elaborated using MSD ChemStation. The column was 5% phenylmethylpolysiloxane (30 m × 0.25 mm; film thickness = 0.25 μm). Injector temperature was kept at 250 °C. The oven temperature was programmed as follows: from 50 to 230 °C (5 °C/min) in 36 min and kept at this temperature for 2 min. The carrier gas was helium with a flow of 1 mL/min, and 1 μL of the sample was injected. The MS setting were as follows: ionization voltage, 70 eV; scan rate, 2.91 scan/s; mass range, 50−550; transfer line, 230 °C. The components of the EOs were identified by (a) comparison of their relative retention times and mass fragmentation with those of authentic standards and (b) computer matching against NIST98, as well as retention indices as calculated according to Kovats, for alkanes C9−C24 compared with those reported by Adams.[15] Quantitative analysis of each component was carried out with an external standard method.

Chemical Characterization of the Aqueous Extracts. A Varian tandem mass spectrometer (Palo Alto, CA, USA) consisting of a ProStar 410 autosampler, two ProStar 210 pumps, and a 1200 L triple-quadrupole mass spectrometer equipped with an electrospray ionization source was used. Varian MS workstation version 6.7 software was used for data acquisition and processing. Ten grams of dried sample was infused in 100 mL of water or methanol at room temperature and sonicated for 15 min. One milliliter of the aqueous extract was filtered through a PTFE filter of 0.45 μm. The chromatographic separation was performed on a Waters XTerra RP-18 column (4.6 mm × 250 mm i.d., 5 μm). The mobile phase consisted of (A) methanol and (B) bidistilled water. The solvent gradient started at 50% A and 50% B, reached 100% A in 20 min, and was kept in this condition for 30 min. The mobile phase, previously degassed with high-purity helium, was pumped at a flow rate of 0.3 mL/min, and the injection volume was 20 μL. The electrospray ionization−mass spectrometer was operated in the ion switching mode.[16] The electrospray capillary potential was set to 48 V, whereas the shield was at 600 V. Nitrogen at 49 mTorr was used as a drying gas for solvent evaporation. The temperatures of the atmospheric pressure ionization (API) housing and of the drying gas were kept at 65 and 250 °C, respectively. The scan time was 45 min. Quantitative analysis of each component was carried out with an external standard method. Due to the lack of biological activity of the methanolic extracts, they were not subjected to chemical composition analysis.

Nematode Population. A population of *M. incognita* originally obtained from tomato (*Solanum lycopersicum* L.) roots collected from a greenhouse in Vassilika, Thessaloniki, northern Greece, was reared on plants of tomato cv. Belladonna, a cultivar that is very susceptible to root-knot nematodes. All plants were maintained in a growth chamber at 25−28 °C, 60% relative humidity, and 16 h photoperiod, in plastic pots (18 cm diameter) containing a 10:1 (v/v) mixture of peat and perlite. Plants used for inoculations were 7 weeks old, at the five-leaf stage. After 40 days, the plants were uprooted, and the roots were washed free of soil and cut into 2 cm pieces. Eggs were extracted according to the sodium hypochlorite procedure,[17] and second-stage juveniles (J2s) were allowed to hatch in modified Baermann funnels at 28 °C. All J2s hatching in the first 3 days were discarded. After 24 h, J2s were collected and used in the experiments.

Nematicidal Assay. The nematicidal activity of the EOs and pure compounds, in terms of nematode juveniles' motility suppression, was tested, and the EC_{50} values were calculated. Stock solutions of pure compounds were prepared by dilution with methanol or water, whereas working solutions were obtained by dilution with distilled water containing the polysorbate surfactant 20 (Tween-20). Final concentrations of methanol and Tween-20 in each well never exceeded 1 and 0.3% v/v, respectively, because preliminary trials showed that the motility of nematodes exposed at those concentration levels was similar to the motility of nematodes maintained in distilled water.[18] Distilled water, as well as a mixture of water with methanol and Tween-20 at concentrations equivalent to those in the treatment wells, were used as controls. Water extracts were diluted with water. Twenty juveniles were used per each treatment well in Cellstar 96-well plates (Greiner bio-one). The plates were covered to prevent evaporation and kept in dark conditions at 28 °C. Border wells containing plain water with nematodes were placed around the wells of each treatment to check the vapor drift among wells that could possibly interfere with the efficacy results. Juveniles were observed with the aid of an inverted microscope (Euromex, The Netherlands) at 40× after 72 h and were ranked into two distinct categories: motile or immotile.

Statistical Analysis. The motility experiments were replicated six times, and each experiment was performed twice. The percentages of immotile J2s in the microwell assays were corrected by elimination of the natural death/immotility in the water control according to the formula corrected % = [(mortality % in treatment − mortality % in control)/(100 − mortality % in control)] × 100. Data were analyzed by ANOVA and combined over time. Because ANOVA indicated no significant treatment by time interaction, the means were averaged over all experiments. Corrected percentages of immotile J2s treated with test compounds were subjected to nonlinear regression analysis using the log−logistic equation proposed by Seefeldt et al.:[19] $Y = C + (D - C)/\{1 + \exp[b\,(\log(x) - \log(EC_{50}))]\}$, where C = the lower limit, D = the upper limit, b = the slope at the EC_{50}, and EC_{50} = the test compound concentration required for 50% death/immotility of nematodes after elimination of the control (natural death/immotility). In the regression equation, the test compound concentration (% w/v) was the independent variable (x) and the immotile J2 (percentage increase over water control) was the dependent variable (y). The mean value of the six replicates per essential oil and compound concentration and immersion period was used to calculate the EC_{50} value.

Table 1. EC_{50} Values of Different Mint Extracts against *M. incognita* Calculated at 72 h of Immersion in Test Solutions[a]

	EC_{50} (±SD) (mg/L)		
extract	*M. piperita*	*M. spicata*	*M. pulegium*
water	1005 ± 33	300 ± 106	745 ± 74
methanolic	>1000	>1000	>1000
essential oil	>1500	358 ± 111	>1500[b]

[a]If SD values are not presented and the EC_{50} values are reported >1000 or 1500, they are outside the test concentration range and are estimated higher than the upper concentration level (1000 or 1500 mg/L). [b]Data reported in Ntalli et al.[24]

Table 2. LC-MS (ESI) Characteristics of Three Mint Species Components

				concn (μg/g ± SD; n = 3)		
RT (min)	MW (m/z)	parent ion (m/z)	compd	*M. pulegium*	*M. spicata*	*M. piperita*
10.47	354.0	353.0 $[M - H]^-$	chlorogenic acid	453 ± 41	209 ± 34	92.8 ± 20.5
11.39	718.0	717.0 $[M - H]^-$	salvianolic acid E[a]	NM[b]	NM	NM
12.20	180.0	179.0 $[M - H]^-$	caffeic acid	33.5 ± 10.2	45.9 ± 9.4	53.3 ± 10.7
12.69	594.1	595.0 $[M + H]^+$	luteolin-7-*O*-rutinoside	31.0 ± 9.9	386 ± 89	1196 ± 151
13.10	718.0	717.0 $[M - H]^-$	salvianolic acid B	216 ± 23	164 ± 41	321 ± 35
13.21	610.0	609.0 $[M - H]^-$	rutin	28.6 ± 8.4	72.6 ± 12.3	57.9 ± 5.5
13.30	360.1	359.1 $[M - H]^-$	rosmarinic acid	374 ± 81	1600 ± 253	496 ± 50
13.61	448.0	449.0 $[M + H]^+$	luteolin-7-*O*-glucoside	ND[c]	22.3 ± 5.0	184 ± 35
14.00	432.1	433.1 $[M + H]^+$	apigenin-7-*O*-glucoside	ND	223 ± 20	174 ± 21
16.72	138.0	137.1 $[M - H]^-$	salicylic acid	52.9 ± 10.0	52.5 ± 5.9	57.4 ± 9.7
17.80	286.2	285.1 $[M - H]^-$	luteolin	14.8 ± 5.0	ND	32.5 ± 8.0
19.14	270.0	269 $[M - H]^-$	apigenin	ND	ND	24.7 ± 4.9

[a]Tentatively identified as salvianolic acid E (Dong et al.[27]). [b]NM, not measured (lack of a commercially available authentic standard). [c]ND, not detected (limit of detection for luteolin and apigenin of 0.5 mg/L and 1 mg/L for luteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside).

Table 3. Chemical Composition and Percent Content[a] of the Essential Oil Components of the Three Mint Species

compound (in order of elution)[b]	RI[c]	*M. piperita*	*M. spicata*	*M. pulegium*
α-pinene	939	0.58 ± 0.20		0.69 ± 0.31
β-pinene	980	0.97 ± 0.15		0.66 ± 0.12
3-octanol	993			3.56 ± 0.90
D-limonene	1031	3.04 ± 0.66	2.97 ± 0.59	0.63 ± 0.24
eucalyptol	1033	2.33 ± 1.11		0.71 ± 0.31
isopulegon	1146	0.69 ± 0.28		3.98 ± 0.88
menthofuran	1164	2.80 ± 1.00		
(−)-lavandulol	1166		0.72 ± 0.10	
isomenthone	1173	9.76 ± 2.10		11.27 ± 2.12
menthone	1173	20.46 ± 3.78	1.9 ± 0.4	18.77 ± 3.78
menthol	1182	47.04 ± 5.55	1.97 ± 0.38	
neo-isomenthol	1189	0.63 ± 0.26		
dihydrocarvone	1193		8.35 ± 3.16	
neodihydrocarveol			3.28 ± 1.11	
cis-carveol	1202	0.55 ± 0.20		
(*Z*)-carveol, 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol, *cis*-mentha-1,8-dien-6-ol			1.59 ± 0.32	
paramenth-4-(8)-en-3-one	1237	0.99 ± 0.22		1.56 ± 0.32
pulegone				34.17 ± 5.12
carvone	1242		60.43 ± 8.79	
piperitone	1252	0.49 ± 0.34		
3-cyclohexen-1-one				1.67 ± 0.66
4-deoxypyridoxine				1.22 ± 0.45
menthyl acetate	1292	1.67 ± 0.58		
cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1*R*,2*S*,5*R*)			4.08 ± 1.17	
piperitenone oxide	1362			4.70 ± 0.97
1-methoxy-3,5-dimethylcyclohexene				0.51 ± 0.26
(−)-β-bourbonene	1382	1.05 ± 0.34	2.04 ± 0.56	
β-elemene	1391	0.55 ± 0.12	1.15 ± 0.50	
caryophyllene	1404	1.42 ± 0.98	2.26 ± 0.42	
1-(3-methoxypyrazinyl)ethanone				0.62 ± 0.12
caryophyllene oxide	1582		1.11 ± 0.33	
total identified compounds (%)		95.01 ± 20.66	91.85 ± 11.56	84.72 ± 12.50

[a]Mean value of three determinations (three replicates ± SD) calculated from GC-MS areas. [b]Compounds are listed in order of elution from a DB5 (30 m, 0.25 mm, 0.25 μm) capillary column. Identification by comparison of mass spectra with the respective data of NIST library in total ion current (TIC) and the literature, as well as retention indices. [c]Calculated according to Kovats.

RESULTS AND DISCUSSION

Recently, we reported the strong nematicidal activity of some plant water extracts and their potential use under field conditions if compared with organic solvent extracts.[20] The attractiveness of the use of plant water extracts is the cost effectiveness of their production, their potential use in developing countries, the environmental friendliness, and the fact that they can be easily prepared. Interestingly, according to our results, the aqueous

extracts of dried aerial parts of mint are more active than the methanolic extract and the essential oils. In particular, the water extracts exhibited significant nematicidal activity against *M. incognita*, and the $EC_{50/72h}$ values are calculated at 1005, 745, and 300 mg/L for *M.* × *piperita*, *M. pulegium*, and *M. spicata*, respectively (Table 1). Conversely, the only active essential oil was obtained from *M. spicata* (EC_{50} = 358 mg/L). According to the literature, the essential oils of *M.* × *piperita* and *M. pulegium* cause root-knot nematode J2 immobilization and egg-hatching inhibition at 1000 mg/L, whereas *M. spicata*, in accordance with our findings, exhibits a higher activity (EC_{50} = 293 mg/L),[21−23] probably related to the presence of the nematicidal carvone (EC_{50} = 115 mg/kg).[24] It is interesting to note that mint species are nonhosts or at cases susceptible to infestation by *Meloidogyne* spp.,[25] whereas *M.* × *piperita* is able to control *M. javanica* when used as soil amendant[26] through its water-soluble and volatile compounds.

The chemical composition analysis reveals that the water extracts are rich in flavonoids and carboxylic acids, whereas in the essential oils the terpene derivatives are predominant (Tables 2 and 3). On the other hand, methanolic extracts are not biologically active, so their chemical composition was no futher investigated. According to the LC-MS analysis, all three mint water extracts yielded luteolin-7-*O*-rutinoside (Table 2), rosmarinic acid, salvianolic acid B, salicylic acid, chlorogenic acid, caffeic acid, and rutin. Interestingly, only salicylic acid is active against *M. incognita*, showing EC_{50} values at 24 and 48 h of 298 ± 92 and 288 ± 79 mg/L (Table 4). As reported by Aoudia et al., caffeic acid does not have nematicidal activity at a concentration of 1000 mg/L;[20] we observed a similar behavior with chlorogenic acid at the same concentration. Similarly, the aglycone luteolin and luteolin-7-*O*-rutinoside do not exhibit any activity against *M. incognita* when tested at 1000 mg/L (Table 4).

Table 4. Nematicidal Activity of Selected Compounds

	$EC_{50/24h}$ (mg/L)	$EC_{50/48h}$(mg/L)
compounds found in the EO		
α-pinene	>1000	>1000
β-pinene	>1000	>1000
D-limonene	>1000	>1000
eucalyptol	>1000	>1000
isomenthone	>1000	>1000
menthone	>1000	>1000
cis-(−)-carveol	>1000	>1000
menthofuran	152 ± 21	127 ± 39
carvone	754 ± 37	730 ± 60
menthol	>1000	>1000
menthyl acetate	>1000	>1000
caryophyllene	>1000	>1000
pulegone[a]	150 (0.99)	<117
dihydrocarvone	>1000	>1000
compounds found in the aqueous extract		
salvianolic acid B	>1000	>1000
salicylic acid[b]	298 ± 92	288 ± 79
chlorogenic acid	>1000	>1000
luteolin	>1000	>1000
rosmarinic acid	>1000	>1000
caffeic acid[b]	>1000	NT[c]
ferulic acid[b]	>1000	NT
rutin	>1000	>1000
luteolin-7-*O*-rutinoside	>1000	>1000
apigenin-7-*O*-rutinoside	>1000	>1000

[a]Data reported in Ntalli et al.[10,24] and presented as EC_{50} (*μ*g/mL) and R^2. [b]Data reported in Aoudia et al.[20] [c]NT, not tested.

The main constituents identified in the essential oils are the terpenes isomenthone (9.76 and 11.27% in *M.* × *piperita* and *M. pulegium*, respectively), menthone (20.46 and 18.77% in *M.* × *piperita* and *M. pulegium*, respectively), menthol (47.04% in *M.* × *piperita*), pulegone (34.17% in *M. pulegium*), and carvone (60.43% in *M. spicata*) (Table 3). In our previous work we reported on the strong nematicidal activity of *M. pulegium* essential oil, which exhibits an EC_{50} value of 3.15 *μ*L/mL after 96 h for *M. incognita*. In that case the activity was attributed to the presence, in the essential oil, of high concentrations of pulegone (51.5% w/w), a terpene considerably active against *M. incognita* ($EC_{50/24h}$ = 150 mg/L) but not present in *M.* × *piperita*.[24]

In the present work we show that menthofuran and carvone (see Figure 1 for structures) exhibit EC_{50} values of 127 and 730 mg/L, respectively, after 48 h for *M. incognita* (Table 4). On the other hand, when we tested dihydrocarvone we did not measure any nematicidal activity; this may indicate the carbonyl functionality as a key factor. This is in agreement with our previous work in which the unsatured aldehydes (*E*,*E*)-2,4-decadienal and (*E*)-2-decenal from *Ailanthus altissima* showed strong nematicidal activity.[19]

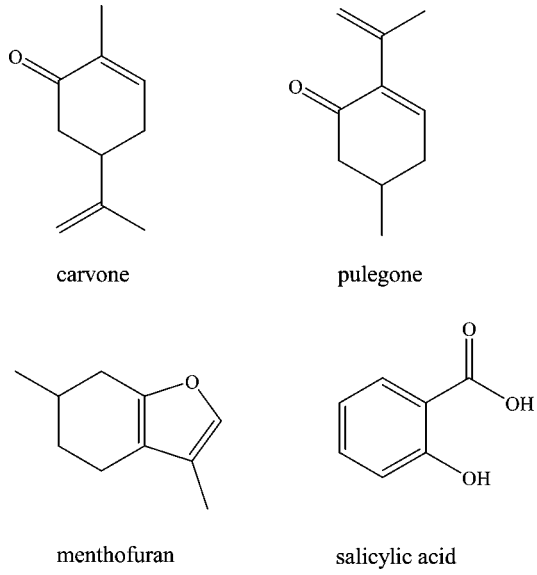


Figure 1. Chemical structures of the nematicidal agents in mint.

Although numerous studies on the subject have been carried out, the exact mode of action of reactive carbonyl species has not been exactly determined yet, but it is likely that these compounds work on more than one level, probably glycating the nematode's external cuticle and inhibiting the V-ATPase enzymes.[14]

In conclusion, reactive carbonyl species have a strong and fast-acting effect on the root-knot nematode *M. incognita*, causing the nematode to become paralyzed and eventually die. The authors believe that mint species containing reactive carbonyl compounds have potential use as bionematicides. In addition, the fact that the highest nematicidal activity was exhibited by the water extract rather than the essential oils of the mint species strengthens the scenario of incorporating the use of mint species in integrated crop management programs and in particular by using it as a green manure or in intercropping

dx.doi.org/10.1021/jf403684h | J. Agric. Food Chem. 2013, 61, 9784−9788

practices. However, before definitive conclusions can be reached, trials under field conditions are needed to verify the applicability of mint water extracts in the control of nematodes.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Jayasinghe, U. L.; Kumarihamy, B. M.; Bandara, A. G.; Vasquez, E. A.; Kraus, W. Nematicidal activity of some Sri Lankan plants. *Nat. Prod. Res.* **2003**, *17*, 259−262.

(2) Kerry, B. R. Exploitation of the nematophagous fungus, *Verticillium chlamydosporium* Goddard for the biological control of root-knot nematodes (*Meloidogyne* spp.). In *Fungi as Biocontrol Agents*; Butt, T. M., Jackson, C., Magan, N., Eds.; CAB International: Wallingford, UK, 2001; pp 155−167.

(3) Qui, S.-J.; Can, J.-Y.; Liu, W.-P.; Becker, J. O. Degradation and absorption of fosthiazate in soil. *J. Agric. Food Chem.* **2004**, *52*, 6239−6242.

(4) Karpouzas, D. G.; Karanasios, E.; Menkissoglu-Spiroudi, U. Enhanced microbial degradation of cadusafos in soils from potato monoculture: demonstration and characterization. *Chemosphere* **2004**, *56*, 549−559.

(5) Madulu, J. D.; Trudgill, D. L. Influence of temperature on the development and survival of *Meloidogyne javanica*. *Nematologica* **1994**, *40*, 230−243.

(6) Ogbuji, R. O. Soil depth distribution of the root-knot nematode (*Meloidogyne incognita*) from two farmlands in a humid tropical environment. *GeoJournal* **1981**, *5*, 79−80.

(7) Pyrowolakis, A.; Westphal, A.; Sikora, R. A.; Becker, J. O. Identification of root knot nematode suppressive soils. *Appl. Soil Ecol.* **2002**, *19*, 51−56.

(8) Ntalli, N. G.; Caboni, P. Botanical nematicides in the mediterranean basin. *Phytochem. Rev.* **2012**, 1−9.

(9) Ntalli, N. G.; Caboni, P. Botanical nematicides: a review. *J. Agric. Food Chem.* **2012**, *60*, 9929−9940.

(10) Ntalli, N. G.; Vargiu, S.; Menkissoglu-Spiroudi, U.; Caboni, P. Nematicidal carboxylic acids and aldehydes from *Melia azedarach* fruits. *J. Agric. Food Chem.* **2010**, *58*, 11390−11394.

(11) Cavoski, I.; Chami, Z. Al.; Bouzebboudja, F.; Sasanelli, N.; Simeone, V.; Mondelli, D.; Miano, T.; Sarais, G.; Ntalli, N. G.; Caboni, P. *Melia azadarach* controls *Meloidogyne incognita* and triggers plant defence mechanisms on cucumber. *Crop Prot.* **2012**, 85−90.

(12) Aissani, N.; Tedeschi, P.; Maietti, A.; Brandolini, V.; Garau, V. L.; Caboni, P. Nematicidal activity of allylisothiocyanate from horseradish (*Armoracia rusticana*) roots against *Meloidogyne incognita*. *J. Agric. Food Chem.* **2013**, *61*, 4723−4727.

(13) Caboni, P.; Aissani, N.; Cabras, T.; Falqui, A.; Marotta, R.; Liori, B.; Ntalli, N.; Sarais, G.; Sasanelli, N.; Tocco, G. Potent nematicidal activity of phthalaldehyde, salicylaldehyde, and cinnamic aldehyde against *Meloidogyne incognita*. *J. Agric. Food Chem.* **2013**, *6*, 1794−1803.

(14) de Faria, M. L.; de A. Magalhães, R.; Silva, F. C.; de O. Matias, L. G.; Ceschi, M. A.; Brocksom, U.; Brocksom, T. J. Enantiodivergent syntheses of cycloheptenone intermediates for guaiane sesquiterpenes. *Tetrahedron: Asymmetry* **2000**, *11*, 4093−4103.

(15) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured: Carol Stream, IL, 2007.

(16) Caboni, P.; Sarais, G.; Angioni, A.; Vargiu, S.; Pagnozzi, D.; Cabras, P.; Casida, J. E. Liquid chromatography-tandem mass spectrometric ion-switching determination of chlorantraniliprole and flubendiamide in fruits and vegetables. *J. Agric. Food Chem.* **2008**, *56*, 7696−7699.

(17) Hussey, R. S.; Barker, K. R. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Report.* **1973**, *57*, 1025−1028.

(18) Caboni, P.; Ntalli, N. G.; Aissani, N.; Cavoski, I.; Angioni, A. Nematicidal activity of (*E*,*E*)-2,4-decadienal and (*E*)-2-decenal from *Ailanthus altissima* against *Meloidogyne javanica*. *J. Agric. Food Chem.* **2012**, *60*, 1146−1151.

(19) Seefeldt, S. S.; Jensen, J. E.; Fuerst, E. P. Log-logistic analysis of herbicide rate response relationship. *Weed Technol.* **1995**, *9*, 218−227.

(20) Aoudia, H.; Ntalli, N.; Aissani, N.; Yahiaoui-Zaidi, R.; Caboni, P. Nematotoxic phenolic compounds from *Melia azedarach* against *Meloidogyne incognita*. *J. Agric. Food Chem.* **2012**, *60*, 11675−11680.

(21) Andrés, M. F.; González-Coloma, A.; Sanz, J.; Burillo, J.; Sainz, P. Nematicidal activity of essential oils: a review. *Phytochem. Rev.* **2012**, 1−20.

(22) Pandey, R.; Kalra, A.; Tandon, S.; Mehrotra, N.; Singh, H. N.; Kumar, S. Essential oils as potent sources of nematicidal compounds. *J. Phytopathol.* **2000**, *148*, 501−502.

(23) Oka, Y.; Nacar, S.; Putievsky, E.; Ravid, U.; Yaniv, Z.; Spiegel, Y. Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology* **2000**, *90*, 710−715.

(24) Ntalli, N. G.; Ferrari, F.; Giannakou, I.; Menkissoglu-Spiroudi, U. Phytochemistry and nematicidal activity of the essential oils from 8 greek lamiaceae aromatic plants and 13 terpene components. *J. Agric. Food Chem.* **2010**, *58*, 7856−7863.

(25) Walker, J. T.; Melin, J. B. *Mentha piperita*, *Mentha spicata* and effects of their essential oils on *Meloidogyne* in soils. *J. Nematol.* **1996**, *28*, 629−635.

(26) Klein, E.; Katan, J.; Gamliel, A. Soil suppressiveness to *Meloidogyne javanica* as induced by organic amendments and solarization in greenhouse crops. *Crop Prot.* **2012**, *39*, 26−32.

(27) Dong, J.; Zhu, Y.; Gao, X.; Chang, Y.; Wang, M.; Zhang, P. Qualitative and quantitative analysis of the major constituents in Chinese medicinal preparation Dan-Lou tablet by ultra high performance liquid chromatography/diode-array detector/quadrupole time-of-flight tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2013**, *80*, 50−62.