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Mentha × *piperita*, *Mentha spicata* and Effects of Their Essential Oils on *Meloidogyne* in Soil

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Abstract: Six peppermint *(Mentha* × *piperita)* and six spearmint *(M. spicata)* PI accessions were inoculated with *Meloidogyne incognita* race 3 and *M. arenaria* race 2, under greenhouse conditions. No galls formed on roots of any of the plants inoculated with 1,800 eggs/pot. Fewer than two galls per root system formed on three PI accessions of peppermint inoculated with *M. incognita* at 5,400 eggs/pot. Only one peppermint accession developed galls when inoculated with *M. arenaria,* whereas none of the spearmint accessions was susceptible to this species. Plant dry weights generally were unaffected by infection with root-knot nematodes at these densities. Growing peppermint and spearmint accessions for 8 or 12 weeks in *M. arenaria*-infested soil before tomato resulted in 90% reduction of root galls compared with tomato following tomato. Cineole, eugenol, geraniol, linalool, and peppermint oils at 50 and 250 mg oil/kg soil caused no reduction in the number of galls caused by *M. arenaria* on tomato. At 1,500 mg oil/kg soil, geraniol, eugenol, linalool, and peppermint oils ($P = 0.05$) reduced the number of galls caused by *M. arenaria,* but the decrease in galling caused by *M. incognita* was not significant. Geraniol, linalool, and peppermint oil at 1,000 and 1,500 mg were phytotoxic to tomato.

Key words: cineole, eugenol, geraniol, linalool, *Meloidogyne* spp., nematode, peppermint, peppermint oil, root-knot nematode, spearmint.

In the United States, peppermint (*Mentha* × *piperita* L.) and spearmint (*M. spicata* L.) are grown commercially for oil in five states, with production around 4 million kg annually and a value of $112 million in 1993 (16). The economic value of these plants for culinary or horticultural purposes is not known, but their use could be enhanced if it could be demonstrated that growing mints was an effective means of decreasing plant-parasitic nematode population densities.

The principal nematode found on peppermint in Indiana (5) and Oregon (11) is *Pratylenchus penetrans,* whereas those species associated with damage to spearmint in Florida are *Dolichodorus heterocephalus, Pratylenchus scribneri, Belonolaimus longicaudatus,* and *Paratrichodorus christiei* (9). Though root-knot nematode species have been found on peppermint, their reproduction was not documented on this host (10,11).

Naturally occurring aromatic compounds, including the monoterpenols, have been investigated for control of certain plant diseases (4,17). These, or similar compounds, may have considerable potential for managing plant-parasitic nematodes on cotton (3) or on landscape plants. Certain essential oils, some of which are present in mints, are nematicidal to second-stage juveniles of *M. javanica* (12). Combining geraniol and thymol suppressed population densities of *M. incognita* (14), and recently, the essential oils of four medicinal plants were shown to inhibit motility of ring and lance nematode and hatching of *M. incognita* eggs (1). Neem oil extracts inhibit egg hatch of three *Meloidogyne* species (7).

The reduced availability of nematicides and concerns associated with continued pesticide usage have expanded the need for more information on plant resistance and safer methods for disease control. Priorities for lessening the societal impact of plant-parasitic nematodes include developing nematode-resistant plants and applying natural products to modify nematode behavior (2). If peppermint or spearmint are resistant to root-knot nematodes or significantly lower nematode population densities in soil, planting schemes or

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rotational systems involving these plants could be devised.

This research had the following objectives: (i) to determine the susceptibility of six peppermint and six spearmint accessions to two root-knot nematode species, *Meloidogyne arenaria* (Neal) Chitwood and *M. incognita* (Kofoid & White) Chitwood, (ii) to determine if growing mints in root-knot nematode-infested soil for a specific period would suppress the nematode population densities, (iii) to examine the effect of several essential mint-derived oils on root-knot nematode eggs in soil.

Materials and Methods

Meloidogyne incognita race 3 (Mi3) and *M. arenaria* race 2 (Ma2) populations were maintained on eggplant (cv. Black Knight) and tomato (cv. Rutgers), respectively, and their eggs collected with 1.0% NaOCl (8).

Mint susceptibility: Rooted cuttings of six peppermint and six spearmint accessions (Table 1) were obtained from the USDA National Clonal Germplasm Research Laboratory, Corvallis, Oregon. The cuttings were planted in April 1993 in pasteurized media soil-mix composed of 2 parts soil: 1 part vermiculite: 1 part Fafard Mix 3 (Conrad Fafard, Agawam, MA) in 10-cm-diam. plastic pots (360 cm^3 volume). The pots remained in a shaded, polyethylene-covered, growing house with temperatures ranging from 15 to 34 °C until the plants were well-rooted. Plants were watered daily and fertilized monthly with 50 ml from a 2.5-g/liter solution of 20-20-20 (N:P:K) (Peter's Fertilizer, Grace-Sierra Horticultural Products Co., Milpitas, CA).

Plants were moved to benches in a pad-cooled greenhouse in June 1993 and inoculated by pipetting eggs of Mi3 or Ma2 to the center of each pot at each of three inoculation levels: 0, 1,800, or 5,400 eggs/pot. There were three replications at each inoculation level of Mi3 and four replications with Ma2. The experiment, a completely randomized block design, was repeated twice with Mi3 and once with Ma2.

Seven to 8 weeks after inoculation, the number of galls per root system of each plant was counted. A week was required to harvest all experimental units. Plants were oven-dried at 90 °C, and dry weights were taken as an indication of plant growth relative to nematode inoculation levels. The length of the longest stolon of each plant infected with Ma2 and control also was measured.

Nematode population density suppression by mints: To determine if growing mints for short periods in root-knot nematode-infested soil would affect nematode population densities, single plants of three spearmint (557790, 557815, and 557818) and three peppermint (557937, 557950, and 557973) accessions were planted in 11-cm-diam. plastic pots (650 cm^3 volume) containing a 2:1 mix of pasteurized soil and Promix A (Premier Brands, Red Hill, PA) infested with 16,250 Ma2 eggs/pot. Each accession was replicated six times. In addition, a tomato (cv. Rutgers) was planted in each of six pots as an indicator of nematode infectivity. After 8 weeks' growth, three tomato and three plants of

Table 1. Mean length of longest stolon on spearmint and peppermint accessions exposed to different population densities of *Meloidogyne arenaria* race 2.

Species PI number	Length (cm) 0 Egg/pot	1,800 Eggs/pot	5,400 Eggs/pot
	Spearmint		
557790	56 a[a]	53 bc	64 ab
557793	65 a	70 a	55 bc
557814	55 ab	65 ab	73 a
557815	40 bc	46 c	53 c
557816	54 ab	55 bc	57 bc
557818	36 c	28 d	36 d
Mean	51	53	56
	Peppermint		
557937	56 ab	91 a	83 ab
557950	74 a	64 c	92 a
557968	55 ab	78 b	67 b
557972	52 b	75 bc	78 ab
557973	62 ab	71 bc	79 ab
557976	67 ab	78 b	72 b
Mean	61	76	78

[a] Mean value of four replications. Dissimilar letters within columns of each mint indicate differences by *t* test (LSD) at $P \leq 0.05$.

TABLE 2. Mean number of galls per root system on spearmint, peppermint, and Rutgers tomato after 8 and 12 weeks' growth in *Meloidogyne arenaria*-infested soil and on subsequent tomato replants.

PI number	Galls/plant				Galls/tomato replant[c]			
	8 weeks		12 weeks		8 weeks		12 weeks	
	Expt.		Expt.		Expt.		Expt.	
	I	II	I	II	I	II	I	II
				Spearmint				
557790	0[a]	0	0	0	0	0	12[b]	0
557815	0	4	2	3	1	12	4	6
557818	0	0	0	0	0	0	21	0
				Peppermint				
557937	0	0	2	0	0	0	2	0
557950	0	0	0	0	0	0	61	0
557973	0	0	0	0	0	1	1	0
				Tomato				
Rutgers	167	158	233	155	100	100	82	54

[a] Mean value of three replications in experiment I and six replications in experiment II, except five replications in last column.
[b] Contamination with nematodes suspected to have occurred during transplanting of tomato.
[c] Tomato transplants grown for 36 to 42 days after harvest of previous plants.

each accession were carefully removed, and their roots washed and examined for galls. The same day, a tomato seedling was transplanted into each pot from which a mint or tomato plant had been removed. The same procedure was repeated with the three remaining pots of each accession after 12 weeks' growth. In both instances, the transplanted tomato plants grew for 36 to 42 days before the number of galls was counted. The roots were suspended in Phloxine B solution for 15 minutes to enhance detection of egg masses (6). The experiment was repeated.

Survival of nematode eggs in oil-treated soil: The effect of five essential oils on root-knot nematode eggs was tested by mixing the oils individually with soils infested or not infested with nematode eggs and determining plant response. Cineole, eugenol, geraniol, linalool, and peppermint oils were added at 0, 50, or 250 mg oil/kg soil to 1.25 kg steam-pasteurized soil in double polyethylene bags, each infested with approximately 6,000 eggs of Ma2 or Mi3. All bags were rolled and shaken by hand and then stored, either sealed or open, in a work-room for 1 week at 10 to 21 °C. Soil from each bag was then transferred to six 240-cm^3 styrofoam cups, and one tomato (Rutgers) seedling was transplanted into each cup. The cups were randomized on a greenhouse bench and plants watered daily and fertilized as needed. The roots of each tomato were examined and the number of galls counted approximately 5 weeks after transplanting. Plant heights and dry weights of root and shoot were measured. Another experiment was conducted using 0, 250, 500, 1,000 and 1,500 mg oil/kg soil added to nematode egg-infested soil and stored in sealed bags for 1 week before planting tomato.

Data of all experiments were analyzed by General Linear Model procedure (SAS, Cary, NC) with *t* tests (LSD) used to separate the mean values.

RESULTS AND DISCUSSION

Mint susceptibility: None of the peppermint or spearmint accessions tested were hosts of Ma2 or Mi3. Although certain accessions developed a few galls at the highest inoculum density (5,400 eggs/pot), no eggs were recovered from any of the accessions producing galls and no root-galls were observed on any peppermint or

TABLE 3. Mean number of galls on root system of Rutgers tomato planted in *Meloidogyne incognita*-infested soil 1 week following soil treatments with 250 mg of oil/kg of soil in open or sealed bags.

Oil	Galls/plant	
	Open bags	Sealed bags
Cineole	48 a[a]	49 a
Eugenol	47 a	48 a
Geraniol	47 a	29 b
Linalool	55 a	47 a
Peppermint	43 a	36 a
None	52 a	41 a

[a] Mean value of six plants with separation of means across columns by Duncan's multiple-range test ($P = 0.05$).

spearmint exposed to 0 or 1,800 eggs/pot. Neither Mi3 nor Ma2 affected the dry weights of peppermint or spearmint, except that peppermint PI 557968, when infected with Mi3, had lower dry weights than the control ($P = 0.05$).

Stolon length differed between control plants and those infected with Ma2 ($P = 0.0001$). The mean lengths for all spearmint accessions inoculated with 0, 1,800, and 5,400 eggs/pot were 51, 53, and 56 cm, respectively (Table 1); the mean lengths for all peppermint were 61, 76, and 78 cm. Stolon length also varied ($P = 0.0001$) with spearmint accessions that were not inoculated; therefore, stolon length may not be as satisfactory as plant dry weights for measuring response to these population densities of root-knot nematodes. The total number of stolons produced was not recorded since the plant dry weights adequately reflected plant growth.

Nematode population suppression by mints: The suppression of root-knot population densities by mint was confirmed by growing tomato in the Ma2-infested soil previously planted to mints (Table 2). In two experiments, no galls or egg masses were observed on roots of the transplanted tomato except where spearmint PI 557815 had grown for 8 weeks previously. Tomato following tomato averaged 100 galls per root system in the 8-week set. Numerous egg masses were visible after staining with

TABLE 4. Mean number of galls per root system of Rutgers tomato growing in *Meloidogyne arenaria*- and *Meloidogyne incognita*-infested soils treated with four rates of essential oils.

Treatment	Rate (mg/kg)	No. galls/plant			
		Ma2		Mi3	
			$P > F$		$P > F$
Control	—	44[a]		43	
Cineole	250	39	NS	21	NS
	500	32		31	
	1,000	34		33	
	1,500	41		26	
Eugenol	250	28	NS	24	NS
	500	22		22	
	1,000	19		30	
	1,500	14		22	
Geraniol	250	38 a	0.0023	31	NS
	500	44 a		22	
	1,000	14 b		30	
	1,500	2 b		22	
Linalool	250	37 a	0.0235	38	NS
	500	41 a		28	
	1,000	29 ab		25	
	1,500	13 b		22	
Peppermint	250	44 a	0.0004	43	NS
	500	33 ab		29	
	1,000	21 bc		22	
	1,500	8 c		19	

[a] Mean values of six plants. Dissimilar letters within oil treatments indicate differences by t test (LSD) at $P = 0.05$.

TABLE 5. Plant height and dry weight for Rutger tomato grown in *Meloidogyne arenaria*- and *Meloidogyne incognita*-infested soil treated with essential oils.

Treatment	Rate mg/kg	Height (cm)				Dry wt. (g)			
		Ma2		Mi3		Ma2		Mi3	
		Noninf.	Infest.	Noninf.	Infest.	Noninf.	Infest.	Noninf.	Infest.
Control	—	22.3[a]	22.8	19.7	19.5	1.0	1.0	1.7	1.0
Cineole	250	20.0 a	21.8 b	20.4 b	22.5 a	0.8 a	0.5 a	1.1 b	0.7
	500	19.3 a	23.0 ab	21.0 b	24.3 a	0.6 a	0.6 a	1.5 ab	0.7
	1,000	19.5 a	25.3 a	24.3 a	23.7 a	0.7 a	0.5 a	1.3 ab	0.7
	1,500	20.7 a	24.0 ab	24.8 a	24.8 a	0.6 a	0.5 a	1.7 a	1.0
Eugenol	250	18.8 a	19.8 a	26.2 a	26.0 a	0.7 a	0.7 a	1.4 a	1.1
	500	20.7 a	22.2 a	23.8 b	25.2 a	0.7 a	0.5 ab	1.3 a	1.0
	1,000	20.8 a	22.0 a	22.3 b	24.7 a	0.6 a	0.5 b	1.2 a	1.0
	1,500	20.7 a	20.3 a	22.0 b	26.7 a	0.6 a	0.3 b	1.2 a	0.7
Geraniol	250	21.5 ab	22.3 a	20.3 a	21.0 a	0.9 a	0.8 a	0.7 a	0.9 a
	500	22.3 a	22.3 a	22.2 a	19.5 a	0.9 a	0.8 a	1.0 a	0.7 ab
	1,000	17.0 bc	14.5 b	23.2 a	19.2 a	0.3 b	0.3 b	1.0 a	0.6 b
	1,500	12.5 c	13.5 b	21.8 a	18.8 a	0.1 c	0.1 b	0.9 a	0.6 b
Linalool	250	14.5 b	17.2 a	22.3 a	27.0 a	0.8 a	0.7 a	1.4 a	1.1
	500	17.0 a	18.2 a	20.8 ab	26.8 a	0.7 ab	0.7 a	1.2 a	1.2
	1,000	18.3 a	17.8 a	21.2 a	20.8 b	0.5 b	0.5 b	1.4 a	0.8
	1,500	16.0 ab	13.0 b	18.8 b	20.2 b	0.2 c	0.2 c	1.2 a	0.8
Peppermint	250	24.0 a	21.3 a	19.5 a	17.5 a	1.2 a	0.8 a	1.2 ab	0.8
	500	24.3 a	20.2 a	21.5 a	16.8 a	0.9 b	0.5 b	1.4 a	0.7
	1,000	22.0 a	19.5 a	18.7 a	18.3 a	0.6 c	0.3 bc	0.8 b	0.6
	1,500	17.7 b	16.2 b	18.8 a	16.3 a	0.3 d	0.2 c	1.0 ab	0.6
$P > F$									
Treatments		0.0001	0.0001	0.0001	0.0001	0.0217	NS	0.0001	0.0004
Rate		0.0021	0.0001	NS	0.0549	0.0001	0.0001	NS	0.0440
Trt. × Rates		0.0002	0.0001	0.0003	0.0007	0.0003	0.0001	0.0344	NS

[a] Mean values of six replications. Dissimilar letters within oil treatments indicate differences by t test (LSD) at $P = 0.05$.

Phloxine B. Tomato planted after 12 weeks of mint growth developed a few galls in one of two experiments, indicating that contamination with nematode eggs may have occurred during transplanting. Fewer galls were noted on tomato roots after 12 weeks of prior tomato growth, probably because population densities were decreased by removing the initial heavily galled tomatoes and their accompanying egg masses.

Survival of nematode eggs in oil-treated soil: Except for geraniol, the essential oils, when added at 50 or 250 mg oil/kg to soil infested with Mi3 eggs, had little effect on the number of galls on roots of tomato assay plants (Table 3). Data for the 50-mg rate were omitted. Maintaining the treated soil in open or sealed plastic bags before planting tomato had no effect on the mean number of galls subsequently formed on roots, except with geraniol at 250 mg oil/kg soil. Oils at this concentration were not phytotoxic if bags remained open for 1 week before planting, but tomato, when planted immediately after addition of oils in a separate experiment, died within 4 days.

Geraniol, linalool, and peppermint oil reduced the number of galls caused by Ma2, depending on the rate of oil treatment (Table 4). For example, geraniol and peppermint reduced the number of galls by more than 50% at the 1,000- and 1,500-mg rate but had little effect at the 250- or 500-mg levels. Cineole had no effect on the number of galls at any of the rates tested. It was difficult to detect any differences in gall numbers due to oil treatments in the Mi3 experiment, probably because of a high standard error. The average percentage reduction in number of galls for combined rates of all oils was similar in both experiments (36% and 37%).

Certain oil treatments decreased plant heights and dry weights in both the Ma2 and Mi3 experiments (Table 5). Noninfested controls were used for each nematode species because the experiments were conducted at different times. Probability values for treatments, rates, and the treatment by rate interactions are provided. Geraniol, linalool, and peppermint oil were toxic to the tomato transplants at 1,500 mg in the Ma2 experiment, reducing dry weights an average of 78% below the 250-mg oil rate ($P = 0.05$). Heights and weights were not affected by different rates of cineole. Eugenol was not phytotoxic in the absence of nematodes.

Our results are somewhat in contrast with those reported by Sangwan et al. (12), who reported nematicidal activity as LC 50s against *M. javanica,* using these same essential oils. They found methanol and linalool to be effective at 250 and 368 μg/ml. These differences may be explained because of different evaluation systems and stage of the nematode during exposure. We exposed eggs, rather than second-stage juveniles, and added oils to soil rather than incubating nematodes in solutions. The solution system provided accurate dosage response curves, yet our soil treatments provided a closer approach to field situations. The impact of environmental factors, exposure times, and soil types on the nematicidal activity of these oils to juveniles or eggs can be easily evaluated in a soil system.

Mint oils contain 50% to 60% methanol and lesser amounts of menthone, menthyl acetate, limone, cineole, pyridines, and numerous other compounds (13,15). Although the compound(s) responsible for the observed phytotoxicity in our experiments was not identified, the similar reaction of tomato transplants in soils treated with geraniol, linalool, and peppermint suggests that the phytotoxic principle may be present in each oil. Perhaps increasing the volatization period before planting the assay plants would diminish the phytotoxicity, but any nematicidal effect on eggs would be lessened.

We conclude that the mints we evaluated are resistant or are nonhosts to Mi3 and Ma2 and, therefore, are good candidates for suppressing root-knot nematode population densities. Their effectiveness under field conditions awaits confirmation.

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