Insecticidal Activity of Monoterpenoids to Western Corn Rootworm (Coleoptera: Chrysomelidae), Twospotted Spider Mite (Acari: Tetranychidae), and House Fly (Diptera: Muscidae)

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ABSTRACT Acute toxicities of 34 naturally occurring monoterpenoids were evaluated against 3 important arthropod pest species; the larva of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte; the adult of the twospotted spider mite, *Tetranychus urticae* Koch; and the adult house fly, *Musca domestica* L. Potential larvicidal or acaricidal activities of each monoterpenoid were determined by topical application, leaf-dip method, soil bioassay, and greenhouse pot tests. Phytotoxicity was also tested on a corn plant. Citronellic acid and thymol were the most topically toxic against the house fly, and citronellol and thujone were the most effective on the western corn rootworm. Most of the monoterpenoids were lethal to the twospotted spider mite at high concentrations; carvomenthenol and terpinen-4-ol were especially effective. A wide range of monoterpenoids showed some larvicidal activity against the western corn rootworm in the soil bioassay. Perillaldehyde, the most toxic (LC_{50} = 3 μg/g) in soil, was only 1/3 as toxic as carbofuran, a commercial soil insecticide (LC_{50} = 1 μg/g). Selected monoterpenoids also effectively protected corn roots from attack by the western corn rootworm larvae under greenhouse conditions. α-Terpineol was the best monoterpenoid in the greenhouse pot test. The acute toxicity of monoterpenoids was low relative to conventional insecticides. Some monoterpenoids were phytotoxic to corn roots and leaves. *l*-Carvone was the most phytotoxic, whereas pulegone was the safest. The results with thymyl ethyl ether, one of the synthetic derivatives of thymol, showed a potential of derivatization to reduce monoterpenoid phytotoxicity.

KEY WORDS *Diabrotica virgifera virgifera*, *Tetranychus urticae*, *Musca domestica*, monoterpenoids, insecticidal activity

NATURAL PRODUCTS HAVE been used for centuries to protect crops from pest invasions, and they are well known to have a range of useful biological properties against insect pests, fungal, bacterial and viral diseases, and weeds. They may be more rapidly degraded in the environment than synthetic compounds and some have increased specificity that favors beneficial insects (Plimmer 1993, Pillmoor et al. 1993). Plants can produce a diverse range of secondary metabolites such as terpenoids (mono-, sesqui-, and di-), alkaloids, polyacetylenes, flavonoids, and sugars (Benner 1993). As pesticides, terpenoids are some of the most successful (Duke 1991).

Monoterpenoids, 10-carbon compounds based on 2 isoprene (C_5H_8) units, are widely distributed in the essential oils (steam volatile and odorous constituents) of plants (Dev et al. 1982, Banthorpe 1991), and >1,000 naturally occurring monoterpenoids have been isolated from higher plants (e.g., mint, pine, cedar, citrus, and eucalyptus) (Charlwood and Charlwood 1991). They may be safe because they were used originally as food flavors, perfumes, decongestants, external analgesics, and antiseptics (Templeton 1969). Some of them have shown promise as natural insect pest control agents because they naturally provide plants with chemical defenses against phytophagous insects and plant pathogens. Monoterpenoids are typically rather lipophilic compounds and thus may interfere with basic metabolic biochemical, physiological, and behavioral functions of insects (Brattsten 1983). Some exhibit acute toxicity, whereas others are repellents (Watanabe et al. 1993), antifeedants (Hough-Goldstein 1990), or affect on growth and development (Karr and Coats 1992) or reproduction (Sharma and Saxena 1974). Earlier evaluations of monoterpenoids on various insects in our laboratory have established their activity as ovicides, fumigants, and contact toxicants (Karr and Coats 1988, Rice and Coats 1994, Tsao et al. 1995). Mechanisms of toxic action have not been elucidated; however, the onset of symptoms is usually rapid and is manifested as agitation, hyperactivity, and quick knockdown. Karr et al. (1990) and Coats et al. (1991) reported neurotoxic effects of mono-

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terpenoids in earthworms as indicated by adverse electrophysiological effects. In some insects, cytochrome P450-dependent monooxygenase enzyme systems metabolize many terpenes to polar products that can be excreted (Brattsten 1983).

Interactions between monoterpenoids and pests have been studied for many years, but better understanding of these complex relationships could provide a basis for using natural products in biorational approaches for better management of pest organisms. The objective of this study was to examine the spectrum of insecticidal activities of naturally occurring monoterpenoids against some important target pests through systematic bioassays.

Materials and Methods

Chemical Compounds. The natural monoterpenoids were purchased from Aldrich (Milwaukee, WI), Sigma (St. Louis, MO), and Pfaltz and Bauer (Waterbury, CT). Technical grade chlorpyrifos (DowElanco, Indianapolis, IN), carbofuran (U.S. EPA, Washington, DC), and 20% (AI) pyrethrins (Pet Chemical, Miami Springs, FL) were obtained and served as commercial standards for comparisons. The synthesis of thymyl ethyl ether was achieved using thymol, a monoterpenoid phenol, and an alkyl halide in the presence of the phase transfer catalyst, benzyltributylammonium bromide (BTAB) and NaOH, with a mixture of methylene chloride and water (50:50 by volume). Details on the synthesis and identification of thymyl ethyl ether derivative have been reported previously (Rice and Coats 1994, Tsao et al. 1995).

Insects. All the test insects were supplied from laboratory colonies that were being reared and maintained at 25 ± 1°C, 40–60% RH, and a photoperiod of 14:10 (L:D) h in the Pesticide Toxicology Laboratory at the Department of Entomology, Iowa State University. The nondiapausing western corn rootworm eggs were obtained from French Agricultural Research (Lamberton, MN). A special cheesecloth wick method was designed to prepare healthy corn roots (5 cm long) and maintain proper humidity for the western corn rootworm larvae, which lives in soil and need special rearing techniques (Jackson 1986). The twospotted spider mites were maintained in laboratory culture on kidney bean, *Phaseolus vulgaris* var. *humilis* Alefeld, seedlings (3 wk after germination in 250-ml plastic cups under greenhouse conditions) in white enamel pans (250 by 350 by 70 mm) with a water barrier (Ahn et al. 1993). The house flies (Orlando Regular; a susceptible strain) were reared on fly media (Purina Labchow, Ralston Purina, St. Louis, MO) for larvae and a diet of powdered milk and sugar for adults by the method of Saito et al. (1992).

Topical Application Bioassay. For topical application, monoterpenoid solutions were prepared with certified acetone. Monoterpenoid solutions were applied with an electric microapplicator, which delivered 1-μl aliquots of certified acetone alone as the control or serial dilution of each solution to the thoracic dorsum of adult house flies (5 d after eclosion; anesthetized with CO_2 and ice) and the abdominal dorsum of 3rd-instar western corn rootworms (picked up with insect forceps without anesthesia). These methods were basically modified from those described by several researchers (Bull and Pryor 1991, De Souza et al. 1992, Saito et al. 1992, Elzen et al. 1994, Rice and Coats 1994). The house flies were transferred to paper cups (100 by 60 mm) and provided with an aqueous 5% sugar solution in a cotton roll, and the western corn rootworm larvae were transferred to petri dishes (100 by 15 mm) and provided with 5 corn seedlings (3 cm long), on wet filter papers, for food under standard conditions (25 ± 1°C, 40–60% RH). Pyrethrins (20% [AI]) were used as a standard in comparison. At least 60 insects per dosage (3 replications) were used at a minimum of 5 concentrations based on preliminary tests to determine the appropriate range of concentrations. Toxicity was assessed 24 h after treatment. Insects were considered dead when tactile stimuli elicited no visible normal reaction.

Acaricidal Bioassay. The leaf-dip method was used to determine acaricidal activity of monoterpenoids against the twospotted spider mite. Monoterpenoids were dissolved in certified acetone (a maximum volume of 5% in the solution) on the basis of weight and diluted with distilled water containing Triton X-100 (200 ppm; J. T. Baker Chemical, Phillipsburg, NJ) as a wetting agent in glass bottles (40 by 80 mm). An aliquot of certified acetone in water was prepared as the control. Kidney bean leaves (40 by 40 mm) were cut from the plant just before treatment, and dipped and shaken for 30 s with forceps in a wide-mouth bottle of solution. The treated leaves were held at 25 ± 1°C until water on the leaf surface had dried and then placed into a petri dish (100 by 15 mm) with wet filter papers (Whatman No. 1, Springfield Mill, Kent, U.K.) to provide water and prevent mites from escaping. Ten adult mites per replicate were transferred onto each treated kidney bean leaf with a fine brush under the stereomicroscope. Each treatment was replicated 2 or 3 times at a minimum of 5 concentrations based on preliminary range-finding results. Acaricidal activity was assessed under the stereomicroscope 24, 48, and 72 h after treatment. Twospotted spider mites were considered dead if prodding with a fine pin elicited no visible normal reaction of appendages under the stereomicroscope.

Soil Bioassay. A soil bioassay for the determination of larvicidal activity of monoterpenoids in soil against 3rd-instar western corn rootworm larvae was performed as described below according to the methods of Coats (1986) and Rice and Coats (1994), with only minor variations. Appropriate amounts of monoterpenoid stock solutions mixed with certified acetone (a maximum of 10% in total

Table 1. Mortality of adult *M. domestica* and *D. virgifera virgifera* 24 h after topical application of monoterpenoids

Monoterpenoid	*M. domestica*					*D. virgifera virgifera*				
	n^a	Slope ± SE	LD_{50}^b	(95% CL)[c]	χ^2	n	Slope ± SE	LD_{50}^b	(95% CL)[c]	χ^2
Alcohols										
Borneol	240	—	>500	—	—	—	—	—	—	—
Carveol	260	4.76 ± 0.74	157	(118–222)	41	80	3.29 ± 0.35	90	(71–113)	88
Carvomenthenol	235	8.10 ± 1.23	152	(126–182)	43	—	—	—	—	—
Citronellol	240	6.39 ± 1.11	64	(53–80)	33	80	2.55 ± 0.22	15	(12–20)	130
Geraniol	235	6.08 ± 1.13	73	(59–93)	29	90	44.5 ±[d]	112	[d]	[d]
Isopulegol	240	7.16 ± 1.44	91	(75–117)	25	100	2.72 ± 0.25	47	(36–61)	119
Linalool	245	5.33 ± 0.95	116	(89–166)	32	80	42.0 ±[d]	108	[d]	[d]
l-Menthol	261	3.23 ± 0.46	147	(112–202)	50	—	—	—	—	—
Perillyl alcohol	261	6.75 ± 1.22	72	(60–89)	31	80	29.8 ±[d]	11	[d]	[d]
α-Terpineol	218	4.79 ± 0.89	173	(132–223)	29	80	44.5 ±[d]	112	[d]	[d]
Terpinen-4-ol	240	3.50 ± 0.51	79	(61–109)	47	90	3.29 ± 0.35	90	(71–113)	88
Verbenol	240	3.78 ± 0.59	202	(147–301)	41	—	—	—	—	—
Phenols										
Carvacrol	224	4.28 ± 0.74	92	(68–125)	33	—	—	—	—	—
Eugenol	200	8.02 ± 1.53	77	(65–93)	28	120	30.5 ±[d]	12	[d]	[d]
Thymol	225	8.99 ± 1.69	29	(24–34)	28	—	—	—	—	—
Ketones										
d-Carvone	220	2.30 ± 0.34	143	(102–213)	46	90	3.28 ± 0.35	90	(71–113)	88
l-Carvone	240	7.54 ± 1.72	102	(85–131)	19	80	106 ±[d]	224	[d]	[d]
l-Fenchone	240	3.31 ± 0.55	222	(164–319)	36	79	1.36 ± 0.13	79	(53–122)	115
Menthone	205	3.31 ± 0.51	98	(74–140)	42	—	—	—	—	—
Pulegone	240	6.66 ± 1.14	39	(32–48)	34	90	2.92 ± 0.27	38	(29–50)	121
Thujone	240	4.95 ± 0.84	62	(50–81)	35	100	1.84 ± 0.45	12	(9–17)	151
Verbenone	240	3.45 ± 0.54	247	(188–348)	41	—	—	—	—	—
Aldehydes										
Citral	260	4.68 ± 0.74	54	(43–69)	40	—	—	—	—	—
Citronellal	253	7.84 ± 1.41	66	(56–80)	31	—	—	—	—	—
Perillaldehyde	217	5.15 ± 0.73	43	(36–52)	50	100	74.4 ±[d]	32	[d]	[d]
Acid										
Citronellic acid	322	3.85 ± 0.65	32	(24–41)	35	80	30.4 ±[d]	11	(19–26)	[d]
Ether										
1,8-Cineole	240	3.56 ± 1.59	281	(219–383)	36	85	106 ±[d]	224	[d]	[d]
Hydrocarbons										
Limonene (R)	240	2.89 ± 0.45	68	(50–95)	42	80	106 ±[d]	224	[d]	[d]
Limonene (S)	223	3.57 ± 0.51	50	(46–79)	49	81	1.99 ± 0.20	88	(68–121)	104
Myrcene	233	6.29 ± 1.22	167	(125–251)	27	80	—	>500	—	—
α-Pinene	240	4.03 ± 0.68	112	(85–163)	35	90	43.5 ±[d]	445	[d]	[d]
α-Terpinene	238	3.45 ± 0.56	117	(85–175)	38	100	—	>500	—	—
γ-Terpinene	223	3.85 ± 0.65	214	(154–305)	35	80	—	>500	—	—
Standards										
Pyrethrins[e]	355	4.46 ± 0.68	8	(6–10)	43	—	—	—	—	—

[a] Number of insects tested.
[b] LD_{50} data were determined by probit analysis (SAS Institute 1991): dosage in microgram per insect.
[c] 95% CL. Insecticidal activity is considered significantly different when the 95% CL fail to overlap.
[d] Numbers are not reliable.
[e] Concentration adjusted for 20% (AI).

water solution) were added to distilled water. The different concentrations of monoterpenoid solutions (12 ml) were added to petri dishes (100 by 15 mm) containing 50 g of untreated sandy clay loam soil (52% sand, 26% silt, 22% clay; 2.7% organic matter; pH 5.7) that had been sieved and autoclaved. The treated soil was tumbled and mixed thoroughly, and then kept uncovered for 20 min to allow acetone evaporation at room temperature. An aliquot of certified acetone in distilled water (10%, 12 ml) was added to the soil in the petri dish as the control, and technical grade chlorpyrifos and carbofuran were used as standards. Five germinated corn kernels (3 cm long) were added to each petri dish, and 10 third instars were transferred from rearing boxes to the center of the soil. The petri dishes were covered with lids and stacked from lesser to greater concentrations to prevent an accumulated effect of greater concentrations in the upper petri dishes caused by the volatility of the monoterpenoids. In the preliminary test, a few insects escaped from the petri dishes through the gap made by growing corn shoots, so the stack of petri dishes was secured with sticky tape and kept in an incubator at 25 ± 1°C, 40–60% RH, and a photoperiod of 12:12 (L:D) h for

Table 2. Acaricidal activity of monoterpenoids from the primary screening against *T. urticae* at 24 and 72 h by leaf-dip application

Monoterpenoid	Mortality (%) ± SEM			
	10,000 ppm[a]		1,000 ppm	
	24 h[b]	72 h	24 h	72 h
Alcohols				
Carveol	100 ± 0	—	0 ± 0	40 ± 10
Carvomenthenol	100 ± 0	—	20 ± 6	67 ± 9
Citronellol	100 ± 0	—	70 ± 12	100 ± 0
Geraniol	100 ± 0	—	40 ± 0	50 ± 10
10-Hydroxygeraniol	0 ± 0	30 ± 12	0 ± 0	40 ± 10
Isopulegol	100 ± 0	—	0 ± 0	30 ± 0
Linalool	100 ± 0	—	10 ± 10	10 ± 0
l-Menthol	100 ± 0	—	0 ± 0	30 ± 0
Perillyl alcohol	100 ± 0	—	20 ± 0	40 ± 10
α-Terpineol	100 ± 0	—	10 ± 10	35 ± 5
Terpinen-4-ol	100 ± 0	—	0 ± 0	30 ± 0
Verbenol	30 ± 6	60 ± 0	10 ± 0	10 ± 0
Phenols				
Carvacrol	100 ± 0	—	90 ± 6	100 ± 0
Eugenol	100 ± 0	—	10 ± 10	60 ± 0
Thymol	100 ± 0	—	30 ± 10	100 ± 0
Ketones				
d-Carvone	100 ± 0	—	0 ± 0	30 ± 0
l-Carvone	100 ± 0	—	25 ± 5	90 ± 10
l-Fenchone	100 ± 0	—	10 ± 10	10 ± 0
Menthone	100 ± 0	—	10 ± 10	40 ± 0
Pulegone	100 ± 0	—	0 ± 0	10 ± 0
Thujone	100 ± 0	—	0 ± 0	0 ± 0
Verbenone	NA	NA	60 ± 40	80 ± 10
Aldehydes				
Citral	100 ± 0	—	0 ± 0	50 ± 0
Citronellal	100 ± 0	—	0 ± 0	0 ± 0
Acid				
Citronellic acid	100 ± 0	—	10 ± 0	10 ± 0
Ether				
1,8-Cineole	90 ± 10	90 ± 0	10 ± 10	40 ± 30
Hydrocarbons				
Limonene (S)	100 ± 0	—	20 ± 0	20 ± 10
α-Terpinene	25 ± 15	41 ± 19	10 ± 10	30 ± 0
γ-Terpinene	100 ± 0	—	0 ± 0	10 ± 0

All values are the mean ± SEM of 3 replicates. NA, not available.
[a] Concentrations of monoterpenoids in distilled water with 200 ppm Triton X-100.
[b] Time after exposure in hours unless otherwise stated.

48 h. At least 7 concentrations per compound based on the primary range-finding test were replicated. Larvicidal activity was determined as mortality at 48 h. Larvae were considered dead if they could not crawl normally when they were stimulated with a pin after the soil was dumped into a metal pan.

Greenhouse Pot Test. The greenhouse pot test, which has the advantage of simulating a field exposure, was conducted to determine the corn root protection effect of monoterpenoids against the western corn rootworm as a longer-term soil bioassay under greenhouse conditions (Coats 1986). Clay pots (5,800 ml) were used with untreated soil for this test. When 1 corn plant per pot reached the 9-leaf stage, 500 nondiapausing western corn rootworm eggs were placed 5 cm deep in the soil around the corn root zone in each pot and covered with the same soil. Under these conditions, the egg and larval stages lasted ≈2 and 4 wk, respectively. Pots were also set up for monitoring larval growth and timing the chemical application. The 1-liter monoterpenoid solutions were prepared with certified acetone (1% volume in total solution) in water and applied to the soil surface when the majority of corn rootworm eggs had hatched. Acetone only in water was added as the control. The concentrations (500, 300, and 0 ppm) were calculated considering the concentration of the monoterpenoids (micrograms) per gram of soil in a pot (2,900 g soil). At 3 wk after treatment, roots were harvested from pots and washed with high-pressure water. The results were evaluated as the protection value using a rating system: 0 was used for no damage and 100 was used for total damage (no roots). Root weight was also measured in treated and control plants.

Table 3. Acaricidal activity of some selected monoterpenoids against *T. urticae* at 48 h by leaf-dip application

Monoterpenoid	n^a	Slope ± SE	LC_{50}^b	95% CL[c]	χ^2
Alcohols					
Carvomenthenol	120	2.35 ± 0.26	59	42–76	81
Chlorothymol	120	4.16 ± 0.75	584	470–687	31
Citronellol	120	5.42 ± 0.99	548	455–625	30
Geraniol	120	1.47 ± 0.20	235	136–363	57
Perillyl alcohol	120	2.79 ± 0.34	614	504–752	68
Terpinen-4-ol	120	2.37 ± 0.24	96	74–122	97
Phenols					
Carvacrol	120	4.61 ± 0.81	629	526–729	32
Eugenol	120	2.05 ± 0.24	219	164–278	74
Thymol	120	3.62 ± 0.35	555	478–653	105
Ketones					
Carvone	120	1.86 ± 0.22	273	204–350	75

[a] Number of insects tested.
[b] LC_{50} data were determined by probit analysis (SAS Institute 1991); concentration (ppm) in solution.
[c] 95% CL. Insecticidal activity is considered significantly different when the 95% CL fail to overlap.

Phytotoxicity Test. A Styrofoam cup soil bioassay was used to evaluate phytotoxicity of monoterpenoids on corn plants and roots. Two plants per cup (8 oz) were grown to the 4-leaf stage under greenhouse conditions. Appropriate amounts of monoterpenoid solutions mixed with certified acetone (a maximum of 10% in the water solution) were added to distilled water and dropped with a pasteur pipette onto the surface of soil around the corn plants. Acetone alone in water was added as the control. Four concentrations (500, 100, 50, and 0 ppm) were replicated 6 times (replicate = 1 cup) in 2 groups for evaluation 3 and 10 d after treatment. Phytotoxicity was determined by using a rating system on both leaf and root parts: 0 was used for no damage and 100 was used for total damage (no roots and no plants). The dry weight of the roots also measured.

Statistical Analysis. All means of data obtained from the various toxicity bioassays were corrected with Abbott's formula (Abbott 1925). Data were subjected to probit analysis to estimate LD_{50}s and LC_{50}s (Finney 1971, SAS Institute 1991). Treatment means were compared and separated by using the least significant differences (LSD) at $p < 0.05$ (SAS Institute 1991).

Results and Discussion

Topical Application Bioassay. The structural characteristics of monoterpenoids such as shape, degree of saturation, and types of functional groups influenced the insecticidal activity and species-specific insecticidal susceptibilities (Table 1). Alcohol and phenol forms of monoterpenoids were more toxic than ketones, aldehydes, ether, or hydrocarbons in the corn rootworm test, whereas the most effective monoterpenoids in the house fly test were spread among the alcohol and phenol, ketone, acid, and aldehyde groups (Table 1). Citronellic acid, limonene, perillaldehyde, pulegone, and thymol were most effective against the house fly, whereas citronellic acid, citronellol, eugenol, perillyl alcohol, and thujone were most effective against the western corn rootworm. *l*-Carvone was more toxic than *d*-carvone to the house fly, whereas the reverse was true for the corn rootworm. Limonene (S) was more effective than limonene (R) against both insects; however, the differences were not so big in the house fly tests.

The acute topical toxicity of monoterpenoids was much less than that of commercial insecticides; the most effective monoterpenoid, thymol (LD_{50} = 29 μg per fly), was at least 3 times less toxic than natural pyrethrins (LD_{50} = 8 μg per fly). Other monoterpenoids were 4–30 times less toxic. The toxic trend showed that the monoterpenoid groups active in the house fly test were quite different from the trend in the western corn rootworm test. Similar trends were reported previously with regard to the acute topical activity of the house fly (Karr and Coats 1988, Rice and Coats 1994). Compared with Rice and Coats (1994), some of the topical LD_{50} values were slightly different, but only 2 of them exceeded a 2-fold difference (thujone and perillaldehyde). These variations can be explained by differences in rearing procedures and bioassay techniques.

Insects responded to the topical administration of some monoterpenoids such as eugenol, carvacrol, and thymol with hyperactivity, followed by quiescence; surviving insects at lower concentrations recovered after a few hours. These effects suggest that immediate neurotoxicities are induced, and then monoterpenoids are quickly metabolized or eliminated, although the mode of action is not known (Harwood et al. 1990). Harwood et al. also suggested that hydrophobicity of compounds influenced the penetration through the cuticle and piperonyl butoxide synergized the toxic effects. Gunderson et al. (1985) reported that pulegone was an effective defense against *Spodoptera eridania* (Cramer) because of its interference with feeding behavior, development, and reproduction, not because of its acute toxicity.

Acaricidal Bioassay. The 30 monoterpenoids tested against the twospotted spider mite showed mild acaricidal activity from the primary screening (Table 2). Toxicity of monoterpenoids differed depending on concentrations and exposure times. All the monoterpenoids tested except 1,8-cineol, 10-hydroxygeraniol, α-terpineol, verbenol, and verbenone caused 100% mortality at the highest concentration (10,000 ppm) 24 h after treatment. Carvacrol was most effective at lower concentrations, followed by citronellol. Geraniol produced 100% mortality at 10,000 ppm, whereas 10-hydroxygeraniol, which was similar in chemical structure, showed 0% mortality. Longer exposure time (72 h) increased acaricidal effects. Some monoterpenoids showed similar acaricidal activity at lower concen-

Table 4. Mortality of 3rd-instar *D. virgifera virgifera* at 48 h by soil application of monoterpenoids

Monoterpenoid	n^a	Slope ± SE	$LC_{50}{}^b$	95% CL[c]	χ^2
Alcohols					
Borneol	180	4.33 ± 1.37	30	17–48	10
Carveol	180	2.68 ± 0.19	10	8–11	193
Carvomenthenol	180	16.1 ± 5.63	259	210–317	8
Citronellol	200	10.4 ± 3.43	21	16–27	9
Geraniol	200	8.70 ± 1.88	43	35–51	21
10-Hydroxygeraniol	180	—	>1,000	—	—
Isopulegol	190	6.53 ± 1.31	74	61–90	25
Linalool	220	3.49 ± 0.82	241	151–407	18
l-Menthol	180	5.37 ± 0.45	70	64–78	141
Perillyl alcohol	180	5.61 ± 1.68	77	47–111	11
α-Terpineol	140	5.58 ± 1.94	95	76–200	8
Terpinen-4-ol	180	7.84 ± 2.34	66	49–87	11
Verbenol	180	5.94 ± 1.72	89	60–127	12
Phenols					
Carvacrol	160	5.51 ± 1.65	42	26–62	11
Eugenol	180	17.2 ± 5.98	87	72–107	8
Thymol	190	4.71 ± 1.55	20	10–29	9
Ketones					
d-Carvone	180	3.37 ± 0.89	72	31–113	14
l-Carvone	180	9.33 ± 2.34	56	45–67	16
l-Fenchone	180	3.68 ± 0.97	37	19–59	14
Menthone	200	5.11 ± 1.35	312	207–421	14
Pulegone	180	5.00 ± 1.52	63	43–93	11
Thujone	180	2.89 ± 0.86	146	56–238	11
Verbenone	200	3.60 ± 1.08	131	75–211	11
Aldehydes					
Citral	200	10.5 ± 2.86	42	33–52	14
Citronellal	180	7.23 ± 2.15	146	96–193	11
Perillaldehyde	190	2.31 ± 0.06	3	3–4	[d]
Acid					
Citronellic acid	180	—	>1,000	—	—
Ether					
1,8-Cineole	180	—	>1,000	—	—
Hydrocarbons					
Limonene (R)	170	—	>1,000	—	—
Limonene (S)	180	—	>1,000	—	—
Myrcene	180	3.99 ± 1.04	684	436–1,282	15
α-Pinene	180	—	>1,000	—	—
α-Terpinene	190	—	>1,000	—	—
γ-Terpinene	170	—	>1,000	—	—
Standards					
Carbofuran	260	5.23 ± 1.34	1	0.8–1.8	15
Chlorpyrifos	240	4.66 ± 0.90	1	0.7–1.4	27

[a] Number of insects tested.
[b] LC_{50} data were determined by probit analysis (SAS Institute 1991): dosage in μg/g in soil.
[c] 95% CL. Insecticidal activity is considered significantly different when the 95% CL fail to overlap.
[d] Numbers are not reliable.

trations, 1,000 and 100 ppm (data were not shown here on 100 ppm). As Delaplane (1992) reported on the efficacy of menthol to control infestations of the tracheal mites *Acarapis woodi* (Rennie) in honey bees, menthol had some acaricidal activity against the spider mite. The 10 most effective monoterpenoids (carvacrol, carvomenthenol, carvone, chlorothymol, citronellol, eugenol, geraniol, perillyl alcohol, terpinen-4-ol, and thymol) were evaluated in more detailed acaricidal activity tests (Table 3). Of these, carvomenthenol and terpinen-4-ol showed greater acaricidal activity (LC_{50} = 59 and 96 ppm, respectively) than others. Because monoterpenoids have no quick contact activity on the mites, a long exposure time is needed for physiological actions such as an antifeedant effect. Most monoterpenoids were phytotoxic to kidney bean leaves determined by visual inspection at high concentrations during this test, except for verbenone, carvomenthenol, and 10-hydroxygeraniol (data were not shown).

Soil Bioassay. A wide range of monoterpenoids showed some degree of larvicidal activity against the western corn rootworm in the laboratory soil bioassay (Table 4). The LC_{50} values differed with respect to monoterpenoid structures. Some of the

Table 5. Corn root protection effect of selected monoterpenoids against *D. virgifera virgifera* in greenhouse tests when 500 eggs were added per pot

Monoterpenoid	Root damage[a]				Root dry wt, g[b]			
	300 ppm	50 ppm	0 ppm	No larvae	300 ppm	50 ppm	0 ppm	No larvae
Alcohols								
Carveol	3.3 ± 3.3b	56.7 ± 3.3a	50 ± 0a	0 ± 0b	5.53 ± 0.85b	4.39 ± 0.46bc	3.09 ± 0.52c	8.75 ± 0.39a
Citronellol	3.3 ± 3.3b	6.7 ± 3.3b	50 ± 0a	0 ± 0b	6.79 ± 1.50a	6.41 ± 0.69a	3.09 ± 0.52b	8.75 ± 0.39a
α-Terpineol	0 ± 0b	0 ± 0b	50 ± 0a	0 ± 0b	6.42 ± 0.14b	8.37 ± 1.19ab	3.09 ± 0.52c	8.75 ± 0.39a
Aldehyde								
Perillaldehyde	0 ± 0b	3.3 ± 3.3b	50 ± 0a	0 ± 0b	6.59 ± 0.63b	5.66 ± 0.86b	3.09 ± 0.52c	8.75 ± 0.39a

Mean ± SEM of 3 replicates. Means within a row followed by the same letter are not significantly different (LSD, $P = 0.05$ [SAS Institute 1991]).

[a] Rating: 0 means no damage to roots and 100 means roots totally destroyed (by visual inspection).

[b] Biomass of corn root.

alcoholic and phenolic monoterpenoids such as carveol, citronellol, and thymol were significantly more toxic (LC_{50} = 10–21 μg/g in soil) than ketones (LC_{50} = 37–312 μg/g) and others (LC_{50} = 684–>1,000 μg/g). Perillaldehyde (LC_{50} = 3 μg/g in soil) was the most effective of the 34 monoterpenoids tested. Khoshkhoo et al. (1993) noted that some plant growth bioregulators could increase the terpenoid aldehyde associated with defense mechanisms of cotton plant against the root-knot nematode *Meloidogyne incognita* (Kofoid & White). The standards for comparison as soil insecticides, carbofuran (LC_{50} = 1 μg/g) and chlorpyrifos (LC_{50} = 1 μg/g), were 3 times more toxic than the most effective monoterpenoid, perillaldehyde. Limonene, which is used commercially as an ectoparasite killing agent in flea shampoos, and 1,8-cineole, which has some herbicidal activity, were much less toxic than others.

Karr and Coats (1988) reported that *d*-limonene exhibited slight toxicity, and the appearance of dead western corn rootworm larvae was quite unusual: the cuticle of the larva was very soft and darkened and the body seemed to be partly liq-

Table 6. Phytotoxicity of monoterpenoids to corn roots (expressed as dry weight)

Monoterpenoid	DAT	Root biomass, g			
		0 ppm	50 ppm	100 ppm	500 ppm
Alcohols					
Citronellol	3	0.14 ± 0.01ab	0.13 ± 0.00a	0.15 ± 0.01a	0.15 ± 0.01a
	10	0.25 ± 0.01a	0.17 ± 0.00b	0.24 ± 0.01a	0.19 ± 0.01b
Geraniol	3	0.14 ± 0.01a	0.15 ± 0.01a	0.11 ± 0.01b	0.14 ± 0.01b
	10	0.25 ± 0.01a	0.22 ± 0.00b	0.23 ± 0.01ab	0.12 ± 0.01c
Menthol	3	0.14 ± 0.01ab	0.15 ± 0.01a	0.09 ± 0.01c	0.12 ± 0.00b
	10	0.25 ± 0.01a	0.19 ± 0.03b	0.21 ± 0.01ab	0.13 ± 0.01c
Phenols					
Eugenol	3	0.14 ± 0.01ab	0.13 ± 0.00ab	0.14 ± 0.00a	0.12 ± 0.00b
	10	0.25 ± 0.01a	0.24 ± 0.02a	0.22 ± 0.01a	0.11 ± 0.00b
Thymol	3	0.14 ± 0.01a	0.13 ± 0.01a	0.14 ± 0.01a	0.13 ± 0.01a
	10	0.25 ± 0.01ab	0.22 ± 0.02b	0.26 ± 0.02a	0.06 ± 0.01c
Ketones					
l-Carvone	3	0.14 ± 0.01a	0.14 ± 0.01a	0.14 ± 0.00a	0.10 ± 0.01b
	10	0.25 ± 0.01a	0.25 ± 0.02a	0.20 ± 0.01b	0.11 ± 0.01c
Pulegone	3	0.14 ± 0.01ab	0.16 ± 0.00a	0.14 ± 0.01b	0.14 ± 0.01b
	10	0.25 ± 0.01a	0.19 ± 0.01a	0.22 ± 0.00a	0.25 ± 0.01a
Aldehyde					
Citral	3	0.14 ± 0.01ab	0.13 ± 0.00ab	0.13 ± 0.01a	0.12 ± 0.01b
	10	0.25 ± 0.01a	0.23 ± 0.00ab	0.21 ± 0.01b	0.10 ± 0.01c
Hydrocarbon					
α-Pinene	3	0.14 ± 0.01a	0.14 ± 0.00a	0.15 ± 0.01a	0.13 ± 0.01a
	10	0.25 ± 0.01a	0.24 ± 0.02a	0.22 ± 0.01a	0.22 ± 0.01a
Synthetic ether					
Thymyl ethyl ether[a]	3	0 14 ± 0.01a	0.11 ± 0.01a	0.14 ± 0.01a	0.13 ± 0.02a
	10	0.25 ± 0.01ab	0.22 ± 0.01c	0.21 ± 0.02b	0.26 ± 0.01a

All values are the mean ± SEM of 6 replicates. Means within a row and treatment time followed by the same letter are not significantly different (LSD, $P = 0.05$ [SAS Institute 1991]). DAT, days after treatment.

[a] Thymyl ethyl ether is a thymol derivative synthesized in our laboratory.

uefied. Nonspecific nematicidal activity was exhibited by several monoterpenoid constituents of some essential plant oils against several nematode species having a similar living habitat with the western corn rootworm (Sangwan et al. 1990). Several monoterpenoids are being evaluated in field plots and could be considered potential alternatives for natural control of corn rootworms, a key pest in the corn belt.

Greenhouse Pot Test. Some representative monoterpenoids such as carveol, citronellol, perillaldehyde, and α-terpineol, which showed good larvicidal activity, were selected for evaluation under greenhouse conditions. Table 5 shows the corn root protection effect of selected monoterpenoids against the western corn rootworm with our 0–100 rating system when 500 eggs were applied per pot. The monoterpenoids tested protected corn roots from attack by corn rootworm larvae. With no monoterpenoid, corn roots were damaged to a rating of 50, and the mean dry weight of corn roots was 3.09 g (Table 5). α-Terpineol, the most effective monoterpenoid, prevented any damage to the corn roots, and resulted in a weight of 8.37 g with the 50-ppm treatment. Carveol was the weakest rootworm insecticide among the 4 monoterpenoids tested.

Phytotoxicity Test. Some monoterpenoids were phytotoxic to both corn roots and corn leaves 3 and 10 d after treatment, as indicated by changes in corn root weight (Table 6) and by visual inspection (Table 7). *l*-Carvone was the most phytotoxic (0.10 and 0.11 g in the 500-ppm treatment at 3 and 10 d after treatment, respectively) in comparison with controls (0.14 and 0.25 g, respectively) (Table 6). Pulegone was a safer monoterpenoid in this experiment. Thymyl ethyl ether, a synthetic derivative of thymol, also was safer (root weight 0.26 g) than the parent compound, thymol (0.06 g), at 500 ppm 10 d after treatment. Longer exposure increased phytotoxic effects. *l*-Carvone, eugenol, and thymol were the most phytotoxic to both parts of the plant on the basis of visual inspection (Table 7). Eugenol, for instance, destroyed 100% of the corn leaves at 500 ppm and 80% of the corn roots. α-Pinene and pulegone did not show phytotoxicity. Derivatization of monoterpenoid parent compounds might reduce monoterpenoid phytotoxicity on the basis of our results with thymyl ethyl ether, one of the thymol derivatives, which showed 0% plant damage at 500 ppm, whereas thymol showed 100% phytotoxicity.

Duke (1991) noted that all plants produce secondary compounds that are phytotoxic to some degree, and camphor, 1,8-cineole, and pulegone are among the more phytotoxic compounds of the hundreds of known plant-derived monoterpenes. Terpenoids are more potent as growth inhibitors than as germination inhibitors. Cinmethylin, a synthetic herbicide for grassy weeds, is structurally related to 1,8-cineole (Grayson et al. 1987), and it has low mammalian toxicity and shows no tendency to accumulate in the environment. Some monoterpenoids can be used as allelochemicals and as herbicide lead compounds; however, monoterpenoids can also damage crop plants, an important consideration if they were to be used in the field. Other biological activities with insects, mites, nematodes, and phytopathogenic bacteria and fungi as well as allelopathic interactions in higher plants are well reviewed in recent literature in chemical ecology (Salama and Saleh 1984, Sheppard 1984, Moleyar and Narasimham 1987, Sangwan et al. 1990, Watanabe et al. 1990, Charlwood and Charlwood 1991, Duke 1991, Delaplane 1992). Consequently, several monoterpenoids have been considered as alternatives to conventional pesticides for a more natural means of control. *d*-Limonene is an active ingredient of some commercially available flea shampoos (Karr and Coats 1988), certain monoterpenoids such as pulegone and citronellal are used as mosquito repellents, and 1,8-cineole is the structural base of cinmethylin, a herbicide (Duke 1987). Characteristics of these simple compounds

Table 7. Phytotoxicity of selected monoterpenoids on corn leaves and roots by visual inspection 10 d after treatment

Monoterpenoid	Site	Damage rating[a] 0 ppm[b]	50 ppm	100 ppm	500 ppm
Alcohols					
Citronellol	L	0 ± 0b	0 ± 0b	0 ± 0b	30 ± 0a
	R	0 ± 0d	20 ± 0b	10 ± 0c	30 ± 0a
Geraniol	L	0 ± 0c	0 ± 0c	20 ± 0b	40 ± 0a
	R	0 ± 0d	10 ± 0c	30 ± 10b	60 ± 20a
l-Menthol	L	0 ± 0b	10 ± 10b	20 ± 10b	60 ± 20a
	R	0 ± 0d	10 ± 0c	60 ± 20b	80 ± 20a
Phenols					
Eugenol	L	0 ± 0d	20 ± 0c	40 ± 10b	100 ± 0a
	R	0 ± 0c	0 ± 0c	30 ± 10b	80 ± 10a
Thymol	L	0 ± 0b	0 ± 0b	0 ± 0b	100 ± 0a
	R	0 ± 0d	40 ± 10b	30 ± 0c	90 ± 20a
Ketones					
l-Carvone	L	0 ± 0b	0 ± 0b	0 ± 0b	90 ± 10a
	R	0 ± 0c	30 ± 0b	70 ± 10a	70 ± 20a
Pulegone	L	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
	R	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
Aldehyde					
Citral	L	0 ± 0c	0 ± 0c	30 ± 0b	50 ± 10a
	R	0 ± 0c	0 ± 0c	10 ± 0b	90 ± 10a
Hydrocarbon					
α-Pinene	L	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
	R	0 ± 0b	0 ± 0b	10 ± 0a	10 ± 0a
Synthetic ether					
Thymyl ethyl ether[c]	L	0 ± 0b	0 ± 0b	20 ± 0a	0 ± 0b
	R	0 ± 0c	70 ± 0a	70 ± 10a	30 ± 10b

All values are the mean ± SEM of 6 replicates. Means within a row and treatment time followed by the same letter are not significantly different (LSD, $P = 0.05$ [SAS Institute 1991]). L, leaves; R, roots.

[a] Rating: 0 means no damage to roots (or leaves) and 100 means roots (or leaves) totally destroyed (by visual inspection).

[b] Concentration (ppm) in soil.

[c] Thymyl ethyl ether is a thymol derivative synthesized in our laboratory.

that make them favorable for development as environmentally safe insect control agents include their insect toxicity and repellency, low mammalian toxicity, and biodegradability.

In summary, the toxicity of the tested monoterpenoids to the western corn rootworm, the twospotted spider mite, and the house fly reflects their wide spectrum of insecticidal properties. Monoterpenoid potency varied considerably, and chemical properties and structural diversities can elicit different toxic effects. Some monoterpenoids were phytotoxic to corn and kidney bean. Our data showed that bioactivity of monoterpenoids was significantly less than that of conventional organic insecticides; however, they can be effective under conditions that allow high-concentration uses of these generally safe chemicals, because monoterpenoids are considered to be safe to mammals and biodegradable in the environment. More specific studies on structure-activity relationships and their mode of action should provide a better understanding of the bioactivity of monoterpenoids.

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