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Insecticidal and Acetylcholine Esterase Inhibition Activity of Apiaceae Plant Essential Oils and Their Constituents against Adults of German Cockroach (*Blattella germanica*)

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ABSTRACT: We evaluated the insecticidal and acetylcholine esterase (AChE) inhibition activity of 11 Apiaceae plant essential oils and their constituents in adult male and female *Blattella germanica*. Of the 11 Apiaceae plant essential oils tested, dill (*Anethum graveolens*), carvi (*Carum carvi*), and cumin (*Cuminum cyminum*) demonstrated >90% fumigant toxicity against adult male German cockroaches at a concentration of 5 mg/filter paper. In a contact toxicity test, dill (*Anethum graveolens*), carvi (*Carum carvi*), cumin (*Cuminum cyminum*), and ajowan (*Trachyspermum ammi*) produced strong insecticidal activity against adult male and female German cockroaches. Among the test compounds, (*S*)-(+)-carvone, 1,8-cineole, *trans*-dihydrocarvone, cuminaldehyde, *trans*-anethole, *p*-cymene, and γ-terpinene demonstrated strong fumigant toxicity against adult male and female *B. germanica*. In a contact toxicity test, carveol, cuminaldehyde, (*S*)-(+)-carvone, *trans*-anethole, thymol, and *p*-cymene showed strong contact toxicity against adult male and female *B. germanica*. IC_{50} values of α-pinene, carvacrol, and dihydrocarvone against female AChE were 0.28, 0.17, and 0.78 mg/mL, respectively. The toxicity of the blends of constituents identified in 4 active oils indicated that carvone, cuminaldehyde, and thymol were major contributors to the fumigant activity or contact toxicity of the artificial blend.

KEYWORDS: *Apiaceae plant essential oils, fumigant toxicity, contact toxicity, German cockroach, acetylcholine esterase inhibition*

INTRODUCTION

The German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae), is a small cockroach about 1.3 cm (0.51 in) to 1.6 cm (0.63 in) in length, and is commonly found in houses, schools, hospitals, and other large buildings. They are considered important indicators of hygiene, because they can cause allergic reactions in sensitive people,[1] and transmit several human pathogens such as viruses, bacteria, protozoa, and helminthes.[2] Control of German cockroaches is primarily dependent on continued applications of residual insecticides, such as chlorpyrifos, DDVP, pyrethroids, and bendiocarb.[3] However, their repeated use has resulted in the development of resistance and has caused serious human health concerns.[1,4] Because of the many side effects of these conventional pesticides, the development of new and safe German cockroach control agents is essential.[5,6]

Plant essential oils are good candidates as German cockroach control agents. They can be easily extracted by steam-distillation, and they consist of mixtures of many bioactive compounds, such as alcohols, aldehydes, ketones, esters, aromatic phenols, and lactones as well as monoterpenes and sesquiterpenes.[7] Many essential oils and their constituents demonstrate insecticidal or repellent activity against the German cockroach.[8−12] Furthermore, plant essential oils are highly volatile and there is little concern regarding their residue in the field and water.[13,14] In this study, we investigated the insecticidal and acetylcholine esterase (AChE) inhibition activities of plant essential oils and their components against the German cockroach in order to find potential alternatives to current insecticides and their mode of action.

MATERIALS AND METHODS

Plant Essential Oils and Chemicals. Essential oils of ammi visnaga (*Ammi visnaga*), celery (*Apium graveolens*), pastinak (*Pastinaca sativa*), and parsely (*Petroselinum sativum*) were purchased from Oshadhi (Weinstrasse, Bühl/Baden, Germany). Dill (*Anethum graveolens*), carvi (*Carum carvi*), coriander (*Coriandrum sativum*), carrotseed (*Daucus carota*), cumin (*Cuminum cyminum*), galbanum (*Ferula galbaniflua*), and ajowan (*Trachryspermum ammi*) were purchased from Jinarome (USA). These plant essential oils are listed in Table 1. Carveol (purity, 97%), (*S*)-(+)-carvone (96%), 1,8-cineole (99%), (+)-limonene (97%), myrcene (95%), cuminaldehyde (98%), *trans*-

Table 1. List of Apiaceae Plant Essential Oils Tested

oil	scientific name	region	part
ammi visnaga	*Ammi visnaga*	Morocco	flowering plant
dill	*Anethum graveolens*	Bulgaria	seeds
celery	*Apium graveolens*	France	seeds
carvi	*Carum carvi*	Egypt	seeds
coriander	*Coriandrum sativum*	Russia	herb
cumin	*Cuminum cyminum*	Egypt	seeds
carrotseed	*Daucus carota*	France	seeds
galbanum	*Ferula galbaniflua*	Iran	resin
pastinak	*Pastinaca sativa*	Croatia	whole plant
parsely	*Petroselinum sativum*	Hungary	herb
ajowan	*Trachyspermum ammi*	India	seeds

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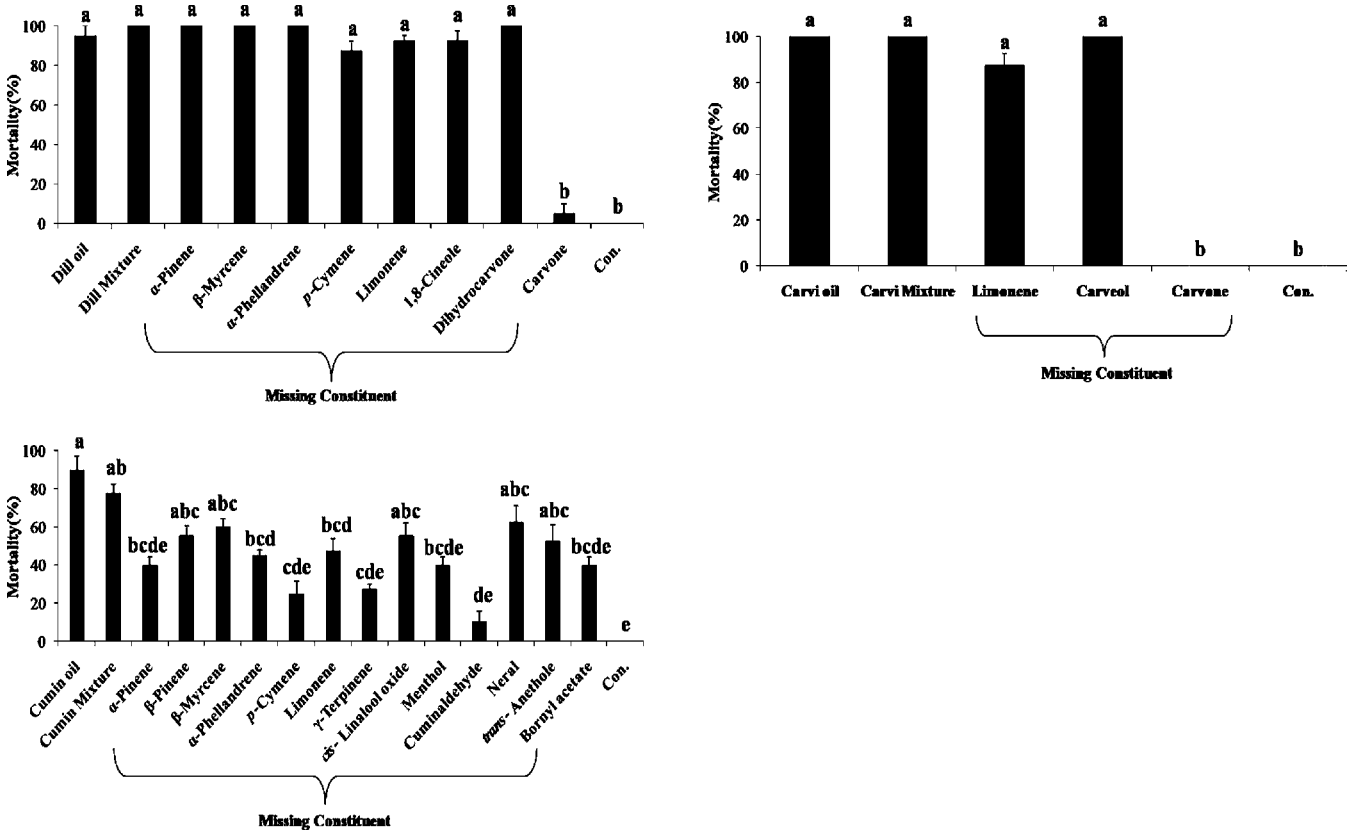

Figure 1. Fumigant toxicity of the oil, full mixture, and selected blends of the constituents of dill, carvi, and cumin oils in German cockroach male adults. The concentration of dill and carvi oil was 20 mg/filter paper. The concentrations of the full mixture of dill and carvi oils were 15.82 mg/filter paper and 16.78 mg/filter paper, respectively. The concentrations of cumin oil and the full mixture were 5 mg/filter paper and 3.82 mg/filter paper, respectively. The concentrations of other blends were determined by removing each constituent equivalent to the ratio identified in dill, carvi, and cumin oils. Mean values corresponding to each treatment with different letters are significantly different from each other (dill oil, $F_{10,33} = 37.12$, $p < 0.0001$; carvi oil, $F_{5,18} = 15.27$, $p < 0.0001$; cumin oil, $F_{15,48} = 120.31$, $p < 0.0001$, Scheffe's test).

anethole, and dihydrocarvone (98%) were obtained from Sigma-Aldrich (Milwaukee, WI). *p*-Cymene (95%), *γ*-terpinene (97%), *α*-terpinene (85%), terpinen-4-ol (99%), menthol (99%), linalool oxide (97%), and thymol (99%) were purchased from Fluka (Buchs, Switzerland). (+)-*α*-Pinene (95%), bornyl acetate (70%), *β*-pinene (94%), carvacrol (95%), and *α*-phellandrene (65%) were obtained from Tokyo Kasei (Tokyo, Japan). Acetone was purchased from Merck (99.8%), and neral (98%) was synthesized in the laboratory.

Insect. *B. germanica* was cultured in the laboratory without exposure to any insecticide. The cockroaches were provided water from a glass flask fitted with a cotton stopper and dried mouse food. The cockroaches were maintained at 27 ± 1 °C and 60% RH under a 16:8 h light:dark cycle.

Gas Chromatography. Gas chromatography (GC) analysis was performed using Agilent 6890N (Santa Clara, CA, USA) equipped with a flame ionization detector (FID). Retention times, for comparison with those of authentic compounds, were measured using DB-1MS and HP-INNOWAX columns (internal diameter [i.d.], 30 m × 0.25 mm; film thickness, 0.25 μm; J&W Scientific, Folsom, CA, USA). The oven temperature was programmed as isothermal at 40 °C for 1 min, then raised to 250 at 6 °C/min, and held at this temperature for 4 min. Helium was used as the carrier gas at the rate of 1.5 mL/min. For chiral GC separation of carvone, a Beta DEX 225 (i.d., 30 m × 0.25 mm; film thickness, 0.25 μm; Supelco) was used. The temperature program was as follows: 130 °C for 10 min and then increased to 200 °C at a rate of 10 °C/min. The carrier gas had a flow rate of 1.0 mL/min. Retention indices were determined in relation to a homologous series of *n*-alkanes (C_7−C_{20}), (C_8−C_{22}) under the same operating conditions. Further identification was made by enhancing the integrated area by coinjection with oil and authentic samples.

Gas Chromatography−Mass Spectrometry. Essential oils were analyzed using a gas chromatograph (Agilent 7890A)−mass spectrometer (Agilent 5975C MSD) (GC−MS) (Santa Clara, CA, USA) equipped with a DB-5MS column (i.d., 30 m × 0.25 mm; film thickness, 0.25 μm; J&W Scientific, Folsom, CA, USA). The oven temperature was programmed as in GC-FID analysis. Helium was used as the carrier gas at the rate of 1.0 mL/min. An effluent of the GC column was introduced directly into the source of the MS via a transfer line (250 °C). Ionization was achieved using an electron impacter (70 eV; source temperature, 230 °C). The scan range was 41−400 amu. Most components of the oil were tentatively identified by comparing the mass spectra of each peak with those of authentic samples in the NIST MS library.

Fumigant Toxicity Test. A paper disk (8 mm, Advantec) treated with the essential oil or compound being tested was placed in the bottom lid of a glass cylinder (diameter, 9.5 cm; height, 19 cm) with a wire sieve fitted 9.5 cm above the bottom; thereafter, the lid was sealed with Parafilm (Pechiney Plastic Packaging Company, Chicago, USA). Ten adult male and female German cockroaches were placed on the sieve. This prevented direct contact of the cockroach with the test plant oils and compounds. The insects were maintained at 25 ± 1 °C and 60% RH. The adult cockroaches were considered dead if their appendages did not move when prodded with a brush. Cumulative mortalities were determined 48 h after treatment. All treatments were replicated 4 times.

Contact Toxicity Test. Appropriate doses of the test compounds dissolved in acetone were topically applied to the thorax of the adult male and female German cockroaches (anesthetized using CO_2) with a microapplicator (Burkard, Hertfordshire, U.K.). The controls received acetone (1 μL). Batches of 10 treated adults were put into a Petri dish

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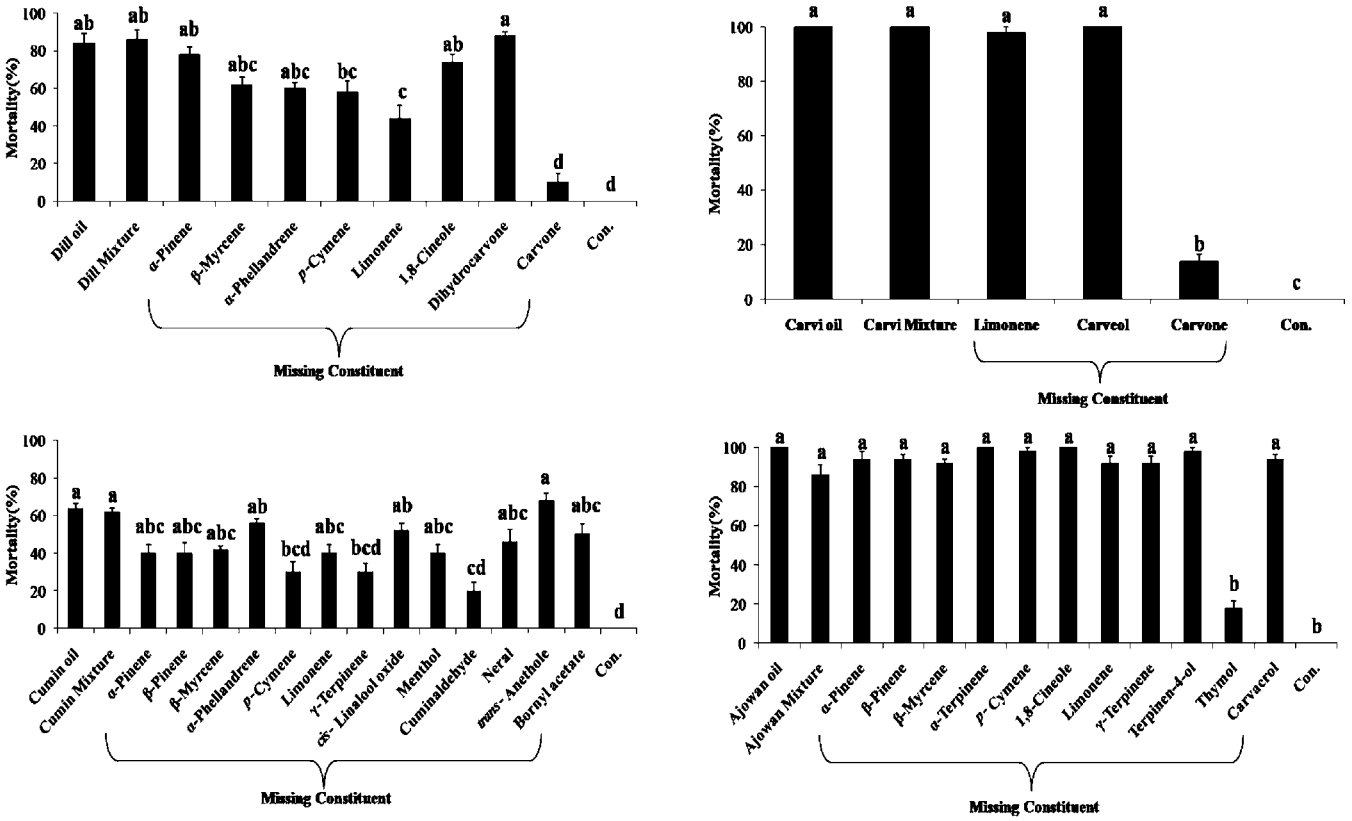

Figure 2. Contact toxicity of the oil, full mixture, and selected blends of the constituents of dill, carvi, cumin, and ajowan oils in female adult German cockroaches. The concentration of dill, carvi, and ajowan oils was 2 mg/♀. The concentration of cumin oils was 1 mg/♀. The concentrations of artificial mixtures of dill, carvi, and ajowan oils were 1.58, 1.66, and 1.96 mg/♀, respectively. The concentration of artificial mixtures of cumin oil was 0.76 mg/♀. The concentrations of other blends were determined by removing each constituent equivalent to the ratio identified in dill, carvi, cumin, and ajowan oils. Mean values corresponding to each treatment with different letters are significantly different from each other (dill oil, $F_{10,44} = 95.45$, $p < 0.0001$; carvi oil, $F_{5,248} = 8.33$, $p < 0.0001$; cumin oil, $F_{15,64} = 88.75$; ajowan oil, $F_{13,564} = 47.85$ $p < 0.0001$, Scheffe's test).

(diameter, 9.5 cm; height, 2 cm). Mortality was determined 48 h after treatment. Each assay was performed 5 times.

Comparative Toxicities. To determine the contribution of each constituent to fumigant or contact toxicity against German cockroaches, we prepared blends of all constituents for 4 active oils that mimicked the natural oils. We also prepared a number of blends, each lacking 1 constituent (Figures 1 and 2). Blends were based on the natural composition of the 4 active oils, as indicated by GC-FID (Table 4). In a fumigant toxicity test, the concentration of dill and carvi oils was 20 mg/filter paper. The concentrations of the full mixture of dill and carvi oils were 15.82 mg/filter paper and 16.78 mg/filter paper, respectively. The concentrations of cumin oil and the full mixture were 5 mg/filter paper and 3.82 mg/filter paper, respectively. The concentrations of other blends were determined by removing each constituent equivalent to the ratio identified in dill, carvi, and cumin oils. In the contact toxicity test, the concentration of dill, carvi, and ajowan oil was 2 mg/♀. The concentration of cumin oils was 1 mg/♀. The concentrations of artificial mixtures of dill, carvi, and ajowan oils were 1.58, 1.66, and 1.96 mg/♀, respectively. The concentration of artificial mixtures of cumin oil was 0.76 mg/♀. The concentrations of other blends were determined by removing each constituent equivalent to the ratio identified in dill, carvi, cumin, and ajowan oils. We also compared the toxicities of the complete and incomplete blends with those of the pure 4 active oils.

Primary AChE Inhibition Assay and IC_{50} Estimation. To extract crude protein, an adult cockroach was ground using a glass tissue-grinder (Wheaton Industries Inc., Millville, NJ) in protein extraction buffer (0.1 M Tris-HCl containing 0.02 M NaCl and 0.5% Triton X-100, pH 7.8) and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). The ground cockroach was centrifuged at 15,000 rpm for 15 min, and the crude protein was collected from the ground mixture. All procedures to extract the crude protein were performed under 4 °C. The AChE activity was measured using the modified Ellman method.[15] All chemicals tested were diluted to 100 mg/mL in acetone (carvacrol and thymol were diluted to 50 mg/mL). Then, 2 μL of the chemicals (final concentration was 0.5 mg/mL or 1 mg/mL) and 30 μL of crude protein were mixed in a 96-well microplate containing 148 μL of protein extraction buffer. Next, the mixture was incubated for 10 min at room temperature and then acetylthiocholine iodide (final concentration, 1 mM) and 5,5′-dithiobis(2-nitrobenzoic acid) (final concentration, 0.4 mM) were added. The control was treated by adding acetone without other chemicals. The enzyme activity was measured for 30 min at 1 min intervals at 405 nm and rt. The inhibition rate of the treatment against control was calculated in percentage by using the following formula:

$$\%\ \text{inhibition rate} = 100 - (\text{enzyme activity of treatment} / \text{enzyme activity of control} \times 100)$$

The primary AChE inhibition assay was replicated at least 3 times. Three chemicals—α-pinene, carvacrol, and dihydrocarvone—were selected to determine IC_{50} values because of their higher inhibition rate. The following concentrations of α-pinene, carvacrol, and dihydrocarvone were used: 2, 1, 0.5, 0.1, and 0.05 mg/mL. All treatments were replicated 3 times at each concentration. The enzyme activity was measured as described above, and the IC_{50} was estimated using probit analysis.[16]

Statistical Analysis. The percentage of mortality and primary AChE inhibition rate was determined and transformed to arcsine square-root values for analysis of variance. Treatment mean values were compared and analyzed using Scheffe's test.[16] Mean (±SE) values of untransformed data have been reported.

Table 2. Fumigant Toxicity of 11 Apiaceae Plant Essential Oils against Male and Female Adults of German Cockroach

	mortality (%, mean ± SE, N = 40)						
	male					female	
plant essential oils	20[a]	10	5	2.5	1.25	20	10
ammi visnaga	17.5 ± 4.8 b[b]	0 c	–[c]	–	–	2.5 ± 2.5 d	–
dill	100 a	100 a	100 a	27.5 ± 7.5 b	2.5 ± 2.5 a	95.0 ± 5.0 a	7.5 ± 4.8 bc
celery	12.5 ± 4.8 bc	–	–	–	–	2.5 ± 2.5 d	–
carvi	100 a	100 a	100 a	60.0 ± 4.0 a	2.5 ± 2.5 a	97.5 ± 2.5 a	20 ab
coriander	95.0 ± 2.9 a	12.5 ± 4.8 bc	–	–	–	37.5 ± 4.8 c	5.5 ± 5.0 bc
cumin	100 a	100 a	90.0 ± 7.0 a	2.5 ± 2.5 c	–	87.5 ± 6.3 ab	0 c
carrotseed	10 bc	–	–	–	–	0 d	–
galbanum	15.0 ± 2.9 bc	–	–	–	–	10.0 ± 4.1 d	–
pastinak	2.5 ± 2.5 bc	–	–	–	–	0 d	–
parsely	97.5 ± 2.5 a	7.5 ± 4.8 bc	–	–	–	15.0 ± 2.9 cd	–
ajowan	100 a	17.5 ± 2.5 b	–	–	–	65.0 ± 2.9 b	32.5 ± 4.8 a
control	0 c	0 c	0 b	0 c	0 a	0 d	0 c
	$F_{11,36}$ = 25.0	$F_{7,24}$ = 26.04	$F_{3,12}$ = 50.0	$F_{3,12}$ = 79.17	$F_{2,9}$ = 16.67	$F_{11,36}$ = 46.5	$F_{5,18}$ = 47.2
	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p = 0.6224	p < 0.0001	p < 0.0001

[a]mg/filter paper. [b]Means within a column followed by the same letters are not significantly different (Scheffe's test). [c]Not tested.

Table 3. Contact Toxicity of 11 Apiaceae Plant Essential Oils against Male and Female Adults of German Cockroach

	mortality (%, mean ± SE, N = 50)					
	male				female	
plant essential oils	2[a]	1	0.5	0.25	2	1
ammi visnaga	98.0 ± 2.0 a[b]	10.0 ± 3.1 cd	–[c]	–	12.0 ± 4.9 d	–
dill	100 a	100 a	62.0 ± 3.7 ab	6.0 ± 4.0 b	84.0 ± 5.0 ab	20.0 ± 3.2 cd
celery	100 a	74.0 ± 5.1 b	4.0 ± 2.4 d	–	96.0 ± 4.0 a	22.0 ± 5.8 cd
carvi	100 a	100 a	20.0 ± 5.5 cd	–	96.0 ± 4.0 a	50.0 ± 5.5 ab
coriander	86.0 ± 2.4 b	26.0 ± 4.0 c	–	–	72.0 ± 2.0 b	34.0 ± 2.4 bc
cumin	100 a	100 a	42.0 ± 5.8 bc	0 b	100 a	64.0 ± 2.4 a
carrotseed	100 a	100 a	18.0 ± 8.0 cd	–	74.0 ± 2.4 b	34.0 ± 2.4 bc
galbanum	82.0 ± 2.0 b	12.0 ± 3.7 cd	–	–	40.0 ± 3.1 c	–
pastinak	100 a	92.0 ± 4.9 ab	8.0 ± 3.7 d	–	64.0 ± 2.4 b	10.0 ± 3.2 d
parsely	100 a	24.0 ± 6.0 c	–	–	66.0 ± 2.4 b	8.0 ± 2.0 d
ajowan	100 a	100 a	76.0 ± 5.0 a	24.0 ± 2.4 a	100 a	42.0 ± 5.8 abc
control	0 c	0 d	0 d	0 b	0 d	0 d
	$F_{11,48}$ = 5.8	$F_{11,48}$ = 52.5	$F_{7,32}$ = 117.5	$F_{3,16}$ = 27.5	$F_{11,48}$ = 47.5	$F_{9,40}$ = 70.0
	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001

[a]mg/adult. [b]Means within a column followed by the same letters are not significantly different (Scheffe's test). [c]Not tested.

RESULTS AND DISCUSSION

Fumigant and Contact Toxicities of the Plant Essential Oils. When 11 plant essential oils were subjected to bioassays, mortalities varied according to the oil type and dose (Tables 2 and 3). In a test with adult male German cockroaches, dill (*Anethum graveolens*), carvi (*Carum carvi*), and cumin (*Cuminum cyminum*) essential oils showed 100% fumigant toxicity at 20 mg/filter paper and 10 mg/filter paper concentrations. At 5 mg/filter paper concentration, these 3 oils caused ≥90% mortality. Coriander and parsley oils caused 95% and 97.5% mortality at 20 mg/mL concentration, but the mortality reduced to 12.5% and 7.5% at 10 mg/mL concentration, respectively. The other oils showed weak fumigant toxicity at 20 mg/filter paper concentration. In a contact toxicity test, dill, carvi, cumin, carrotseed, and ajowan oils demonstrated 100% insecticidal activity against male German cockroaches at 1 mg/♂ concentration. Cumin, ajowan, celery, carvi, and dill oils demonstrated >80% contact toxicity against female adults at 2 mg/♀ concentration. The other oils showed moderate or weak activity. The insecticidal or nematicidal activity of dill, carvi, cumin, and ajowan has been reported in previous studies,[17−19] but there have not been any reports about their fumigant and contact toxicities toward the German cockroach.

Chemical Components of the Plant Essential Oils. The chemical compositions of dill, carvi, cumin, and ajowan essential oils are shown in Table 4. The chemical compositions of ajowan, carvi, dill, and cumin oils have been reported in our previous study.[18,19] There was only a little difference in the composition rate of the constituents of ajowan, carvi, and dill oils. However, 5 compounds—limonene, *cis*-linalool oxide, menthol, neral, and bornyl acetate—were newly identified in cumin oil, as compared to the results of our previous study.[18] The main components of dill oil were α-pinene (0.37%), β-myrcene (0.17%), α-phellandrene (3.90%), *p*-cymene (3.05%), 1,8-cineole (1.20%), limonene (22.83%), dill ether (5.04%), *trans*-dihydrocarvone (0.9%), *cis*-dihydrocarvone (0.91%), and carvone (40.77%). Limonene (27.01%), *cis*-carveol (0.52%), *trans*-carveol (0.39%), and carvone (40.77%) were detected as the main components in carvi oil. α-Pinene (0.69%), β-pinene

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Table 4. Chemical Analysis of Dill, Carvi, Cumin, and Ajowan Essential Oils

		retention index		amount (w/w, %)			
no.	compound	DB-1MS	HP-Innowax	dill	carvi	cumin	ajowan
1	α-pinene	929	1020	0.37	–[c]	0.69	0.87
2	β-pinene	969	1107	–	–	9.14	1.26
3	β-myrcene	982	1165	0.17	–	0.53	0.48
4	α-phellandrene	995	1165	3.9	–	0.39	–
5	α-terpinene	1007	1181	–	–	–	0.13
6	*p*-cymene	1011	1273	3.05	–	26.88	24.4
7	1,8-cineole	1018	1209	1.2	–	–	0.32
8	limonene	1020	1200	22.83	27.01	0.62	0.44
9	γ-terpinene	1049	1247	–	–	16.31	27.77
10	*cis*-linalool oxide	1072	1450	–	–	0.69	–
11	menthol	1158	1643	–	–	0.58	–
12	terpinen-4-ol	1160	1611	–	–	–	0.32
13	dill ether[a]	1165	1484	5.04	–	–	–
14	*trans*-dihydrocarvone[b]	1169	1611	0.9	–	–	–
15	*cis*-dihydrocarvone[b]	1175	1631	0.91	–	–	–
16	*cis*-carveol[b]	1196	1848	–	0.52	–	–
17	*trans*-carveol[b]	1207	1879	–	0.39	–	–
18	cuminaldehyde	1210	1789	–	–	17.26	–
19	(*S*)-(+)-carvone	1213	1738	40.77	55.98	–	–
20	neral	1218	1690	–	–	0.41	–
21	*trans*-anethole	1259	1833	–	–	2.52	–
22	bornyl acetate	1267	1582	–	–	0.56	–
23	thymol	1273	2207	–	–	–	41.77
24	carvacrol	1278	2236	–	–	–	0.55
	sum			79.14	83.9	76.58	98.31

[a]Structure was tentatively identified by comparison of mass spectrum in the library. [b]Mixture of *cis*- and *trans*-isomers was determined from the literature report.[33] [c]Not detected.

(9.14%), β-myrcene (0.53%), α-phellandrene (0.39%), *p*-cymene (26.88%), limonene (0.62%), γ-terpinene (16.31%), *cis*-linalool oxide (0.69%), menthol (0.58%), cuminaldehyde (17.26%), neral (0.41%), *trans*-anethole (2.52%), and bornyl acetate (0.56%) were identified as the major components of cumin oil. The main components of ajowan oil were α-pinene (0.87%), β-pinene (1.26%), β-myrcene (0.48%), α-terpinene (0.13%), *p*-cymene (24.4%), 1,8-cineole (0.32%), limonene (0.44%), γ-terpinene (27.77%), terpinen-4-ol (0.32%), thymol (41.77%), and carvacrol (0.55%).

Fumigant and Contact Toxicities of the Individual Compounds. The fumigant and contact toxicities of the individual compounds from dill, carvi, cumin, and ajowan oils are shown in Tables 5 and 6. In a fumigant toxicity test, carvone demonstrated the strongest activity against male adults, followed by *trans*-anethole, dihydrocarvone, 1,8-cineole, γ-terpinene, and *p*-cymene. In a test with female adults, 1,8-cineole showed the strongest insecticidal activity followed by dihydrocarvone, carvone, *trans*-anethole, and cuminaldehyde. In a contact toxicity test with male adults, *trans*-anethole and thymol demonstrated the most potent activity. Insecticidal activity of carvacrol was 84% at 0.25 mg/♂, but it reduced to 4% at 0.125 mg/♂. In a contact test with female adults, thymol was the most toxic followed by *trans*-anethole, cuminaldehyde, and carvacrol. However, toxicities of plant essential oils and their components were weaker than those of conventional insecticides such as chlorpyrifos (LD_{50} = 0.015 μg/female) or deltamethrin (LD_{50} = 0.0054 μg/female).[20] Philips et al.[11] and Phillips and Appel[12] have already reported the fumigant and contact toxicities of 12 essential oil components against German cockroaches: carvacrol, 1,8-cineole, *trans*-cinnamaldehyde, citornellic acid, eugenol, geraniol, limonene, linalool, menthone, α-pinene, β-pinene, and thymol. They reported that 1,8-cineole was the most toxic to male and female adult German cockroaches in a fumigant test. The fumigant toxicity of carvacrol and thymol was less than that of α-pinene, β-pinene, and limonene. However, thymol and carvacrol showed strong contact toxicity against adult male and female German cockroaches. This observation and our results confirm that thymol and carvacrol showed insecticidal activity against German cockroaches by direct contact, and not via fumigation. In our fumigant toxicity test, mortality with α-pinene, β-pinene, and limonene was not higher than that with the other compounds. This result did not agree with the results of Phillips and Appel.[12] The difference in the insecticidal activity of these compounds might be because we used different strain of German cockroach.

Comparative Toxicities of the Blends. A fumigant toxicity test with artificial mixtures showed that blends of dill, carvi, and cumin containing 9, 4, and 13 known constituents of the 3 oils were the most toxic (Figure 1). Insecticidal activity of artificial mixtures containing all the constituents did not differ significantly from that of the 3 essential oils (Figure 1; $p < 0.0001$). Component elimination assays of dill and carvi oils indicated that the omission of carvone from the artificial mixture caused a significant decrease in the fumigant toxicity of the blend (dill oil: $F_{10,33} = 37.12$, $p < 0.0001$, carvi oil: $F_{5,18} = 15.27$, $p < 0.0001$). These results indicated that carvone is a major contributor to the fumigant toxicity of dill and carvi oils. Omission of other compounds from the artificial mixture did not cause a significant difference in the fumigant toxicity of the blend. For cumin oil, component elimination assays (Figure 1)

Table 5. Fumigant Toxicity of Components from Dill, Carvi, and Cumin Essential Oils against Male and Female Adults of German Cockroach

	mortality(%, mean ± SE, N = 40)										
	male						female				
compounds	20[a]	10	5	2.5	1.25	0.625	20	10	5	2.5	1.25
α-pinene	65.0 ± 2.9 bc[b]	12.5 ± 2.5 c	–[c]	–	–	–	52.5 ± 4.8 c[b]	12.5 ± 2.5 c	–	–	–
β-pinene	57.5 ± 4.8 c	12.5 ± 6.3 c	–	–	–	–	57.5 ± 4.8 bc	10.0 ± 4.1 c	–	–	–
β-myrcene	5.0 ± 2.9 d	–	–	–	–	–	2.5 ± 2.5 d	–	–	–	–
α-phellandrene	100 a	57.5 ± 2.5 b	2.5 ± 2.5 cd	–	–	–	100 a	70.0 ± 4.1 b	0 d	–	–
p-cymene	100 a	100 a	12.5 ± 4.8 cd	–	–	–	100 a	97.5 ± 2.5 a	10.0 ± 4.1 d	–	–
1,8-cineole	100 a	100 a	100 a	0 d	–	–	100 a	100 a	100 a	100 a	0
limonene	85.0 ± 6.5 ab	17.5 ± 7.5 c	–	–	–	–	75.0 ± 5.0 b	10.0 ± 4.1 c	–	–	–
γ-terpinene	100 a	100 a	17.5 ± 4.8 c	–	–	–	100 a	92.5 ± 4.8 a	15.0 ± 2.9 d	–	–
linalool oxide	15.0 ± 5.0 d	–	–	–	–	–	2.5 ± 2.5 d	–	–	–	–
menthol	2.5 ± 2.5 d	–	–	–	–	–	2.5 ± 2.5 d	–	–	–	–
dihydrocarvone	100 a	100 a	100 a	42.5 ± 4.8 c	2.5 ± 2.5 c	–	100 a	100 a	100 a	5.0 ± 2.9 cd	–
carveol	2.5 ± 2.5 d	–	–	–	–	–	2.5 ± 2.5 d	–	–	–	–
cuminaldehyde	100 a	97.5 ± 2.5 c	67.5 ± 2.5 b	62.5 ± 2.5 b	40 ± 7.1 b	7.5 ± 2.5	100 a	100 a	55.0 ± 2.9 c	17.5 ± 4.8 c	–
(S)-(+)-carvone	100 a	100 a	100 a	100 a	100 a	2.5 ± 2.5	100 a	100 a	97.5 ± 2.5 ab	77.5 ± 4.8 b	0
neral	17.5 ± 8.5 d	–	–	–	–	–	17.5 ± 2.5 d	–	–	–	–
trans-anethole	100 a	100 a	100 a	77.5 ± 4.8 b	7.5 ± 2.5 c	–	100 a	100 a	82.5 ± 4.8 b	15.0 ± 2.9 cd	–
bornyl acetate	80 abc	65 ± 2.8 b	10 ± 4.0 cd				7.5 ± 4.7 d				
control	0 d	0 c	0 d	0 d	0 c	0	0 d	0 c	0 d	0 d	0
	$F_{17,54}$ = 42.5	$F_{12,39}$ = 37.8	$F_{9,30}$ = 30.0	$F_{5,18}$ = 34.7	$F_{4,15}$ = 50.0	$F_{2,9}$ = 16.7	$F_{17,54}$ = 28.2	$F_{11,36}$ = 28.5	$F_{8,27}$ = 27.8	$F_{5,18}$ = 41.7	
	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p = 0.0751	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	

[a]mg/filter paper. [b]Means within a column followed by the same letters are not significantly different (Scheffe's test). [c]Not tested.

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Table 6. Contact Toxicity of Components from Dill, Carvi, Cumin, and Ajowan Essential Oils against Male and Female Adults of German Cockroach

	mortality (%, mean ± SE, N = 50)						
	male				female		
compounds	1[a]	0.5	0.25	0.125	1	0.5	0.25
α-pinene	54.0 ± 2.4 cdef[b]	38.0 ± 5.8 defgh	–[c]	–	46.0 ± 2.4 cdefg	10.0 ± 4.5 d	–
β-pinene	48.0 ± 2.0 def	6.0 ± 4.0 hi	–	–	12.0 ± 3.7 hi	–	–
β-myrcene	42.0 ± 2.0 ef	18.0 ± 3.7 fghi	–	–	34.0 ± 4.0 efgh	–	–
α-phellandrene	80.0 ± 4.5 abc	34.0 ± 2.4 defgh	0 c	–	76.0 ± 2.4 abc	2.0 ± 2.0 d	–
α-terpinene	68 ± 5.8 bcde	26 ± 5.0 efghi	–	–	42 ± 3.7 defgh	26 ± 5.0 cd	
p-cymene	98.0 ± 2.0 a	76.0 ± 2.4 abc	0 c	–	68.0 ± 2.0 bcd	4.0 ± 2.4 d	–
1,8-cineole	98.0 ± 2.0 a	56.0 ± 2.4 bcde	12.0 ± 3.7 c	–	28.0 ± 4.9 fghi	24.0 ± 4.0 cd	–
limonene	40.0 ± 5.5 f	20.0 ± 3.2 fghi	–	–	26.0 ± 5.1 fghi	–	–
γ-terpinene	90.0 ± 0.0 ab	14.0 ± 6.0 ghi	–	–	42.0 ± 3.7 defgh	14.0 ± 6.0 d	–
linalool oxide	96.0 ± 2.4 a	60.0 ± 4.5 bcd	4.0 ± 2.4 c	–	20.0 ± 6.3 fghij	–	–
menthol	52.0 ± 3.7 def	16.0 ± 4.0 fghi	–	–	18.0 ± 3.7 ghi	–	–
dihydrocarvone	98.0 ± 2.0 a	42.0 ± 4.9 defg	2.0 ± 2.0 c	–	50.0 ± 3.2 cdef	12.0 ± 2.0 d	–
carveol	96.0 ± 4.0 a	80.0 ± 3.2 ab	2.0 ± 2.0 c	–	60.0 ± 5.5 cde	20.0 ± 4.5 cd	–
cuminaldehyde	100 a	96.0 ± 2.4 a	70.0 ± 3.2 b	0 b	98.0 ± 2.0 ab	90 a	6.0 ± 2.4 b
carvone	100 a	80.0 ± 5.5 ab	16.0 ± 2.4 c	–	98.0 ± 2.0 ab	42.0 ± 3.7 bc	2.0 ± 2.0 b
neral	80.0 ± 4.5 abc	48.0 ± 5.8 bcdef	6.0 ± 4.0 c	–	22.0 ± 3.7 fghi	–	–
trans-anethole	100 a	100 a	96.0 ± 2.4 a	6.0 ± 2.4 b	100 a	96.0 ± 2.4 a	14.0 ± 4.0 b
thymol	100 a	98.0 ± 2.0 a	98.0 ± 2.0 a	62.0 ± 2.0 a	100 a	94.0 ± 4.0 a	68.0 ± 3.7 a
carvacrol	100 a	100 a	84.0 ± 2.4 ab	4.0 ± 4.0 b	94.0 ± 2.4 ab	62.0 ± 5.8 b	8.0 ± 3.7 b
bornyl acetate	74 ± 6.7 abcd	46 ± 5.0 cdefg	2.0 ± 2.0 c		46 ± 6.7 cdefg	0 d	
control	0 g	0 i	0 c	0 b	0 i	0 d	0 b
	$F_{20,84}$ = 50.4	$F_{20,84}$ = 77.1	$F_{13,56}$ = 28.5	$F_{4,20}$ = 26.0	$F_{20,84}$ = 69.5	$F_{14,60}$ = 67.3	$F_{5,24}$ = 45.0
	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001

[a]mg/adult. [b]Means within a column followed by the same letters are not significantly different (Scheffe's test). [c]Not tested.

showed that the omission of cuminaldehyde, *p*-cymene, or γ-terpinene from the artificial mixture caused a significant decrease in the toxicity of the blend ($F_{15,48}$ = 120.31, p < 0.0001). Cuminaldehyde was the major contributor to the fumigant toxicity of cumin oil, followed by *p*-cymene and γ-terpinene. This result indicated that cuminaldehyde, *p*-cymene, and γ-terpinene act synergistically in terms of insecticidal activity against German cockroaches. Jiang et al.[21] have already insisted that plant defense chemicals with more than one mode of action are especially suitable for plant protection. Omission of α-pinene, α-phellandrene, limonene, menthol, and bornyl acetate did not cause a significant difference in the fumigant toxicity of the blends, but there was a significant difference between the toxicity of the blends and cumin oil. This result indicated that unidentified compounds (23.42%) must be responsible for the total toxicity of the oil, and α-pinene, α-phellandrene, limonene, menthol, and bornyl acetate might act more synergistically with the unidentified compounds than β-pinene, β-myrcene, *cis*-linalool oixide, neral, and *trans*-anethole.

There was no significant difference in contact toxicity between dill, carvi, cumin, and ajowan oils and complete artificial mixtures of these oils (Figure 2). For dill oil, components elimination assays (Figure 2) demonstrated that omission of carvone and limonene from the mixture caused a significant difference in the contact toxicity of the blends. Carvone was also the major contributor for the contact toxicity of carvi oil (Figure 2). Omission of other compounds did not cause a significant difference in the contact toxicity of the blends. For cumin oil, several compounds were involved in the contact toxicity of the oil. Significant difference in the contact toxicity of the blends was observed when cuminaldehyde, *p*-cymene, and γ-terpinene were removed from the complete mixture. Omission of other compounds did not cause a significant difference in the contact toxicity, but toxicity of the blends was reduced, in comparison with that of cumin oil or the complete artificial mixture. This result indicated that unidentified compounds (23.42%) were responsible for the total contact toxicity of the oil, as mentioned above. Thymol was the major contributor to the contact toxicity of ajowan oil, but other compounds did not cause a significant difference in the contact toxicity of the blends (Figure 2).

Primary AChE Inhibition Assay and IC_{50} Estimation. The primary inhibition rates of the chemicals identified in the 4 active oils against the German cockroach are summarized in Figure 3. In males, carvacrol showed the highest inhibition rate (78%), followed by α-pinene (71.6%) (Figure 3). The β-pinene also exhibited >50% inhibition rate (53.4%). However, in females, α-pinene showed the highest inhibition rate (86.3%), followed by carvacrol and dihydrocarvone (55.1% and 50.6%, respectively). In the primary inhibition assay, 3 chemicals (α-pinene, carvacrol, and dihydrocarvone) that showed >50% inhibition rate were selected and their IC_{50} values against AChE were estimated. The IC_{50} of α-pinene, carvacrol, and dihydrocarvone was 0.18, 0.18, and 1.60 mg/mL, respectively, in males and 0.28, 0.17, and 0.78 mg/mL, respectively, in females (Table 7). AChE inhibition activity of phytochemicals has been investigated in several studies.[22−25] Abdelgaleil et al.[26] reported that cuminaldehyde, 1,8-cineole, (−)-limonene, and (L)-fenchone showed strong *Sitophilus oryzae* AChE inhibition activity. However, cuminaldehyde, 1,8-cineole, and (+)-limonene showed weak AChE inhibition activity in this study. Instead, carvacrol, α-pinene, and dihydrocarvone demonstrated

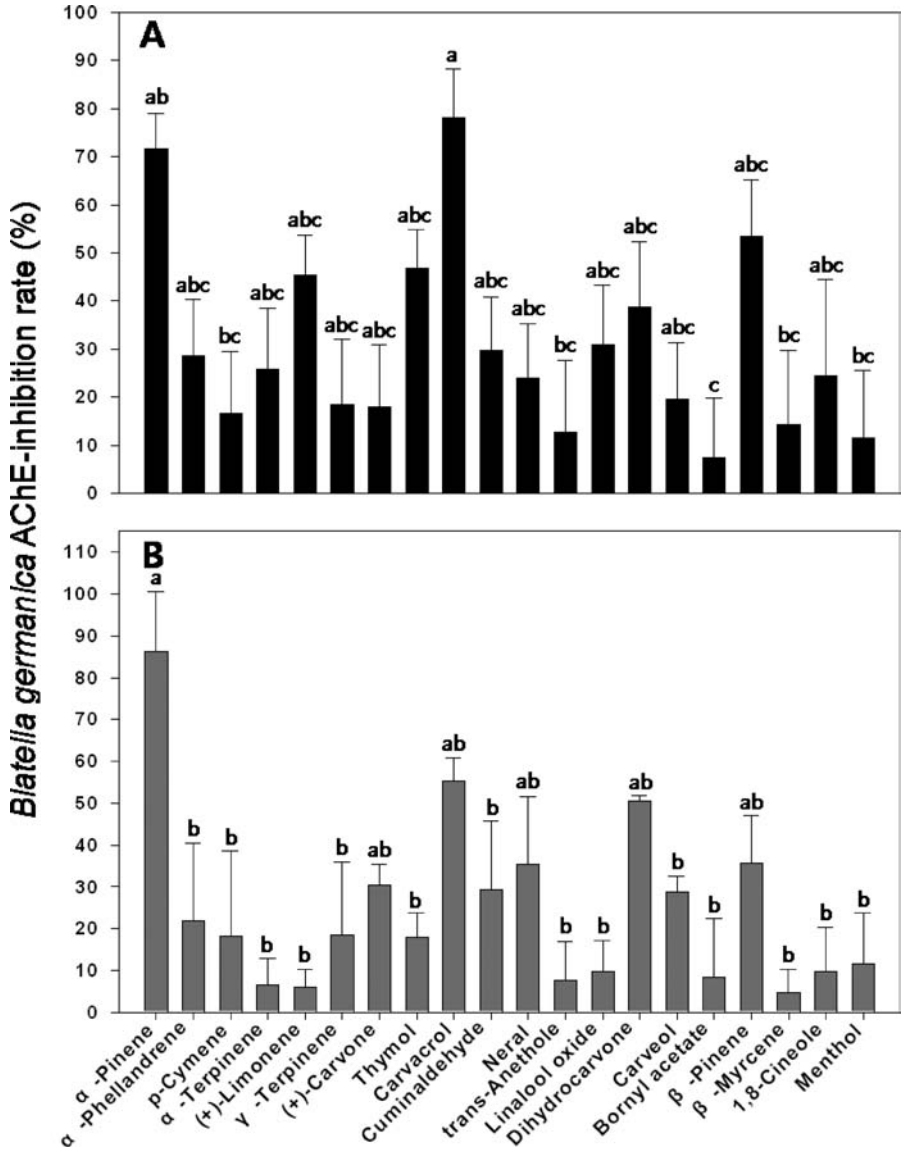

Figure 3. *Blatella germanica* acetylcholine esterase inhibition rates of constituents identified in four active oils: (A) males; (B) females. Mean values corresponding to each treatment with different letters are significantly different from each other (male, $F_{19,40} = 160.57$, $p < 0.0001$; female, $F_{19,40} = 136.28$, $p < 0.0001$, Scheffe's test).

Table 7. Acetylcholine Esterase Inhibition Activity of α-Pinene, Carvacrol, and Dihydrocarvone

	male				female			
compounds	slope	IC_{50} (mg/mL)	95% cl[a]	χ^2	slope	IC_{50} (mg/mL)	95% cl	χ^2
α-pinene	1.01 ± 0.10	0.18	0.13−0.24	1.22	0.86 ± 9.90	0.28	0.20−0.38	0.58
carvacrol	1.09 ± 0.10	0.18	0.13−0.23	6.39	1.08 ± 0.10	0.17	0.12−0.22	7.61
dihydrocarvone	0.61 ± 0.10	1.60	0.96−3.65	1.94	0.76 ± 0.10	0.78	0.54−1.23	2.74

[a]Confidence limit.

strong AChE inhibition activity. This might be attributed to the use of different insect species. Carvacrol and dihydrocarvone demonstrated strong fumigant or contact toxicity against German cockroaches in an individual compound test. Our results indicated that the toxicity of carvacrol and dihydrocarvone correlated with the ability to inhibit AChE activity. Anderson and Coats[27] also reported that carvacrol showed strong reaction for American cockroach AChE inhibition activity. However, Lei et al.[28] insisted that the nematicidal activity of carvacrol against 2 nematodes, *Caenorhabditis elegans* and *Ascaris suum*, might be mediated through a tyramine receptor. α-Pinene showed strong AChE inhibition activity, but their fumigant or contact toxicity was weak in comparison with the toxicity of the other compounds. One possibility for the weak contact or fumigant toxicity of α-pinene could be low penetration rate to the target site. However, carvacrol, α-pinene, and dihydrocarvone were not major contributors to the toxicity of the essential oils in the artificial blend test. This finding suggested that the mode of action for the essential oil is not AChE inhibition. Although little is known regarding the

mode of action of the essential oils in insects, many oils or their constituents cause symptoms that indicate a neurotoxic mode of action.[29,30] Eugenol exerts its insecticidal activity by binding to octopamine receptors.[31,32] However, the exact mode of action of essential oils and constituents tested in this study remains unclear.

Our results indicate that carvi, dill, cumin, and ajowan oils and their components could be developed as control agents against German cockroaches. For the practical use of these oils and their constituents as novel cockroach-control agents, the safety of the oils and their components in humans and nontarget organisms and their mode of action should be investigated further.

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Notes

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