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Larvicidal Activity of Ajowan (*Trachyspermum ammi*) and Peru Balsam (*Myroxylon pereira*) Oils and Blends of Their Constituents against Mosquito, *Aedes aegypti*, Acute Toxicity on Water Flea, *Daphnia magna*, and Aqueous Residue

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ABSTRACT: This study evaluated the larvicidal activity of 20 plant essential oils and components from ajowan (*Trachyspermum ammi*) and Peru balsam (*Myroxylon pereira*) oils against the mosquito, *Aedes aegypti*. Of the 20 plant essential oils, ajowan and Peru balsam oils at 0.1 mg/mL exhibited 100 and 97.5% larval mortality, respectively. At this same concentration, the individual constituents, (+)-camphene, benzoic acid, thymol, carvacrol, benzyl benzonate, and benzyl *trans*-cinnamate, caused 100% mortality. The toxicity of blends of constituents identified in two active oils indicated that thymol and benzyl benzoate were major contributors to the larvicidal activity of the artificial blend. This study also tested the acute toxicity of these two active oils and their major constituents against the water flea, *Daphnia magna*. Peru balsam oil and benzyl *trans*-cinnamate were the most toxic to *D. magna*. Two days after the treatment, residues of ajowan and Peru balsalm oils in water were 36.2 and 85.1%, respectively. Less than 50% of benzyl *trans*-cinnamate and thymol were detected in the water at 2 days after treatment. The results show that the essential oils of ajowan and Peru balsam and some of their constituents have potential as botanical insecticides against *Ae. aegypti* mosquito larvae.

KEYWORDS: *plant essential oils, Trachyspermum ammi, Myroxylon pereira, larvicidal activity, mosquito, Daphnia magna, residue in water*

INTRODUCTION

Mosquitoes are one of the best-known vectors of several diseases such as malaria, dengue fever, and Japanese encephalitis. Synthetic pesticides such as temephos, fenithion, diflubenzuron, and methoprene have been used to control mosquitoes for several decades.[1] Although effective, their repeated use for several decades has caused side effects such as environmental and human health concerns, undesirable effects on nontarget organisms, and disruption of biological control by natural enemy. Research has continued in order to find new and safe mosquito control agents to reduce environmental and human health concerns.

Plant essential oil is a good source for mosquito control agents. Essential oils are natural volatile substances found in a variety of plant species. When isolated from plants, essential oils are not usually extracted as chemically pure substances; they consist of mixtures of many compounds such as alcohols, aldehydes, ketones, esters, aromatic phenols, and lactones, as well as monoterpenes and sesquiterpenes.[2] Moreover, some essential oils are known to be safe because they are commonly used as fragrances and flavoring agents in foods and beverages.[3,4]

Many essential oils have been reported to demonstrate larvicidal activity against mosquitoes. Peppermint (*Mentha piperita*) oil was toxic for larvae of *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*.[5] The essential oil of *Tagetes patula* was effective against the larvae of three mosquito species.[6] Essential oils of cinnamon leaves[7] and *Eupatorium betonicaeforme* (DC. Baker) roots[8] have demonstrated strong insecticidal activity against *Ae. aegypti* larvae. Furthermore, plant essential oils are highly volatile, and there is little concern for residue remaining in the field and water.[9,10]

In this study, we evaluated the larvicidal activities of 20 plant essential oils and then analyzed the constituents of 2 of the oils that proved effective against *Ae. aegypti* larvae. We also tested the larvicidal activity of individual and blends of the identified compounds to elucidate the key compounds in insecticidal activity. In addition, we evaluated the acute toxicity of the 2 oils and their major constituents against the water flea, *Daphnia magna*, and determined their residue levels in water.

MATERIALS AND METHODS

Plant Essential Oils and Chemicals. The plant essential oils used in this experiment are listed in Table 1. The essential oils were purchased from Jinarome (USA). Camphene (80%), (+)-limonene (97%), 1,8-cineole (99%), (−)-α-pinene (97%), and myrcene (95%) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Thymol (99%), *p*-cymene (95%), α-terpinene (85%), and γ-terpinene (97%) were purchased from Fluka (Buchs, Switzerland). (+)-α-Pinene (95%), (−)-limonene (95%), carvacrol (95%), vanillin (98%), cinnamic acid (98%), benzyl benzoate (99%), benzyl cinnamate (98%), and β-pinene (94%) were purchased from Tokyo Kasei (Tokyo, Japan), and benzyl alcohol (99%) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Benzoic acid (99.5%) was supplied by Junsei Chemical Co. Ltd. (Tokyo, Japan). (*E*)-Nerolidol (96%) was

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Table 1. Plant Essential Oils Tested

plant essential oil	plant species	family name	part
cananga	*Cananga odorata*	Annonaceae	blossoms
ajowan	*Trachyspermum ammi*	Apiaceae	seeds
coriander	*Coriandrum sativum*	Apiaceae	fruits
galbanum	*Ferula galbaniflua*	Apiaceae	resin
artemisia afra	*Artemisia afra*	Asteraceae	flowering plant
davana	*Artemisia pallens*	Asteraceae	leaves
fokienia wood	*Fokienia hodginsii*	Cupressaceae	wood
gurijum	*Diplerocarpus lurbinalus*	Dipterocarpaceae	resin
copaiva balm	*Copaifera reticulata*	Fabaceae	resin
Peru balsam	*Myroxylon pereira*	Fabaceae	resin
geranium	*Pelargonium graveolens*	Geraniaceae	leaves
hyssop	*Hyssopus officinalis*	Lamiaceae	flowering plant
lavandin	*Lavandula hybrida*	Lamiaceae	flowering plant
litsea	*Lisea cubeba*	Lamiaceae	fruits
patchouli	*Pogostemom patchouli*	Lamiaceae	whole plant
cajeput	*Melaleuca cajuputii*	Myrtaceae	leaves
larch	*Larix europea*	Pinaceae	resin
amyris	*Amyris balsamifera*	Rutaceae	wood
prtitgrain	*Citrus aurantium* ssp. *amara*	Rutaceae	leaves
cardamone	*Elettaria cardamomum*	Zingiberaceae	seeds

synthesized in the laboratory. Values in parentheses indicate the purity of the compounds.

Insects. *Ae. aegypti* cultures were maintained in the laboratory, without exposure to any insecticides. Adults of *Ae. aegypti* were maintained on a 10% sugar solution. A live mouse in a steel cage was supplied for blood. Larvae were reared in plastic pans (24 × 35 × 5 cm) containing sterilized diet and water. Colonies were reared at 26 ± 1 °C, with a relative humidity of 60 ± 5% under a 16:8 h light/dark cycle.

Gas Chromatography (GC). GC analysis was performed using an Agilent 6890N (Santa Clara, CA, USA) equipped with a flame ionization detector (FID). The retention times of the compounds for comparison with authenticated compounds were measured using DB-1MS and HP-Innowax columns (30 m × 0.25 mm i.d., film thickness = 0.25 μm, J&W Scientific). The oven temperature was programmed to be isothermal at 40 °C for 1 min, raised to 250 °C at the rate of 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. To determine the configurations of limonene and α-pinene, a chiral column-Beta DEX 120 (30 m × 0.25 mm i.d., film thickness = 0.25 μm, Supelco, Bellefonte, PA, USA) was used. The oven temperature for this procedure was programmed as mentioned above. The helium flow rate was maintained at 1.0 mL/min.

GC–Mass Spectrometry. Essential oils of ajowan and Peru balsam were analyzed using a gas chromatograph (Agilent 7890A)–mass spectrometer (Agilent 5975C MSD) (GC-MS) equipped with a DB-5MS column (30 m × 0.25 mm i.d., film thickness = 0.25 μm, J&W Scientific). The oven temperature was programmed the same as that used for the GC-FID analysis. The carrier gas was helium at the flow rate of 1.0 mL/min. The GC column effluent was directly introduced into the source of the MS via a transfer line (at 250 °C). Ionization was obtained by electron impact (70 eV, source temperature = 230 °C), and the scan range was 41–400 amu. Most of the essential oil components were identified by comparing the mass spectra of each peak with those of authenticated samples obtained from the NIST MS library. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_7–C_{20}), under the same operating conditions.[11] Constituents were further identified by enhancing the integrated area by co-injection with the essential oil and authentic samples. The oil components were then quantified by adding two internal standards, namely, undecane (purity = 99%; Wako) and pentadecane (purity = 99%; Wako), without the use of correction factors.

Table 2. Larvicidal Activity of Plant Essential Oils against Larvae of *Aedes aegypti*

plant essential oil	mortality[a] (%, mean ± SE, *N* = 4) 0.1 mg/mL	0.05 mg/mL	0.025 mg/mL
cananga	40.0 ± 4.1 cdef	–[b]	–
ajowan	100 a	80.0 ± 4.1 a	7.5 ± 2.5 b
coriander	70.0 ± 7.1 abc	2.5 ± 2.5 cd	–
galbanum	90.0 ± 7.1 ab	25.0 ± 2.8 bc	–
artemisia afra	2.5 ± 2.5 f	–	–
davana	50.0 ± 5.7 bcde	–	–
fokienia	57.5 ± 2.5 abcd	–	–
gurjum	17.5 ± 4.7 def	–	–
copaiva balm	10.0 ± 7.1 ef		
Peru balsam	97.5 ± 2. a5	95.0 ± 2.8 a	57.5 ± 2.5 a
geranium	82.5 ± 2.5 abc	0 d	–
hyssop	95.0 ± 2.8 a	32.5 ± 8.5 b	–
lavandin	7.5 ± 4.7 ef	–	–
litsea	50.0 ± 9.1 bcde	–	–
patchouli	97.5 ± 2.5 a	32.5 ± 8.5 b	–
cajeput	10.0 ± 7.1 ef	–	–
larch	87.5 ± 7.5 ab	17.5 ± 4.7 bcd	–
amyris	47.5 ± 7.5 bcde	–	–
prtitgrain	47.5 ± 4.7 bcde	–	–
cardamone	67.5 ± 2.5 abc	–	–
control	0 f	0 d	0 b
	$F_{20,63} = 106.7, p < 0.0001$	$F_{7,24} = 67.7, p < 0.0001$	$F_{2,9} = 16.6, p < 0.0001$

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

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Larvicidal Activity Test. Larval testing was performed according to the methods used by Park et al.[12] Briefly, essential oils and constituents were serially diluted from an initial 0.01% (weight/volume) stock solution prepared in acetone. One milliliter of each oil or constituent was suspended in 200 mL of distilled water in 270 mL paper cups. Ten early third-instar larvae of *Ae. aegypti* were separately moved into the cup using a pipet. A separate set of cups that received 1 mL of acetone only served as the controls. Treated and control larvae were maintained at the same conditions used for colony maintenance, and larvicidal activity was investigated 48 h after treatment. All treatments were repeated four times.

Acute Toxicity Test against *D. magna*. In this bioassay, we used 24-h-old *D. magna* neonates obtained from a colony maintained at the National Academy of Agriculture Science, Suwon, Republic of Korea. Acute toxicity tests were conducted according to the standard methods of the U.S. EPA.[13] The temperature was maintained at 20 ± 1 °C under a 16:8 h light/dark cycle. The stock solutions used for this assay were the same as those used in the mosquito bioassays described above. Control tanks received only acetone. Twenty *D. magna* neonates were used per test. All of the tests were repeated four times. Five concentrations were used to determine LC_{50} values (ajowan oil, 5, 7.5, 11.3, 16.9, and 25.3 mg/L; Peru balsam oil, 1.0, 1.7, 2.89, 4.91, and 8.35 mg/L; benzyl benzoate, 1.0, 1.8, 3.24, 5.8, and 10.5 mg/L; thymol, 1.0, 1.8, 3.24, 5.8, and 10.5 mg/L; benzyl cinnamate, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/L). Mortality was determined 48 h after treatment.

Residue in Water. Each essential oil and constituent was dissolved in acetone and added to a glass beaker filled with distilled water (200 mL). The concentration of each test solution was 0.1 mg/mL. One milliliter of a test solution was sampled at days 2 and 7 to determine the residue of ajowan and Peru balsam oils and their constituents that remained in the water. Solutions were stirred and mixed with a small amount of NaCl, the solution was extracted with 2 mL of hexane containing undecane, and the solution was directly analyzed using GC-FID. GC analysis was performed using an Agilent 6890N equipped with FID. Retention times for comparison with authentic compounds were measured using DB-1MS and HP-Innowax columns (30 m × 0.25 mm i.d., 0.25 mm film thickness, J&W Scientific). The oven temperature was programmed to be isothermal at 40 °C for 1 min, then raised to 250 °C 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. The carrier gas flowed at the rate of 1.0 mL/min. Undecane was used as the internal standard (IS), and the concentration was adjusted to 0.01 and 0.001 mg/mL for test solutions extracted on days 2 and 7, respectively. Oil residues were determined by comparing the total area of all constituents with that of the IS.

Statistical Analysis. The percentages of mortality for mosquito larvae and *Daphnia* were determined and transformed to arcsine square-root values prior to analysis of variance (ANOVA). Treatment mean values were compared and separated using Scheffe's test. Mean (± SE) values of untransformed data have been reported. The LC_{50} values were calculated using probit analysis.[14]

RESULTS AND DISCUSSION

Larvicidal Activities of the Plant Essential Oils. The larvicidal activities of plant essential oils against *Ae. aegypti* are listed in Table 2. Among the oils tested, ajowan, Peru balsam, hyssop, and patchouli essential oils demonstrated strong larvicidal activity (≥90% mortality) at a concentration of 0.1 mg/mL. The larvicidal activities of ajowan and Peru balsam were 80 and 95%, respectively, at a concentration of 0.05 mg/mL. The other oils showed weak larvicidal activities. Nematicidal or antitermitic activities of ajowan oil have been evaluated in previous studies,[15,16] but there have not been any reports on the insecticidal activity of Peru balsam oil.

Chemical Analysis of the Active Oils. The chemical compositions of ajowan and Peru balsam oils are given in Table 3. Totals of 13 and 7 constituents were identified from ajowan and Peru balsam essential oils, respectively. The most abundant constituent in ajowan was thymol, followed by γ-terpinene, *p*-cymene, and β-pinene. The most abundant component in Peru balsam oil was benzyl benzoate, followed by benzyl cinnamate, benzoic acid, and (*E*)-nerolidol. Chemical analysis of ajowan oil has been performed in previous studies,[15,16] but the chemical analysis of Peru balsam oil has not been conducted.

Table 3. Chemical Analysis of *Trachyspermum ammi* and *Myroxylon pereira* Essential Oils

compound	RI		amount (w/w, %)	
	DB-1	Innowax	ajowan	Peru balsalm
α-pinene	928	1021	0.87	–
(−)-α-pinene			–[a]	
(+)-α-pinene			0.87	
camphene	940	1064	0.1	–
β-pinene	967	1108	1.26	–
β-myrcene	982	1165	0.48	–
benzyl alcohol	1003	1884	–	0.62
α-terpinene	1007	1181	0.13	–
p-cymene	1010	1275	24.4	–
1,8-cineole	1018	1209	0.32	–
limonene	1020	1200	0.44	–
(−)-limonene			0.08	
(+)-limonene			0.36	
γ-terpinene	1050	1248	27.77	–
benzoic acid	1140	2448	–	8.91
terpin-4-ol	1159	1610	0.32	
thymol	1273	2207	41.77	–
carvacrol	1278	2235	0.55	–
vanillin	1348	2578	–	0.93
cinnamic acid	1387	2882	–	3.01
(*E*)-nerolidol	1548	2049	–	3.41
benzyl benzoate	1724	2528	–	66.24
benzyl cinnamate	2042	2084	–	15.92
sum			98.41	99.04

[a]Not detected.

Larvicidal Activity of the Constituents and Blends. The larvicidal activities of the constituents derived from ajowan and Peru balsam essential oils are shown in Table 4. The larvicidal activities of benzyl benzoate and benzyl cinnamate were 100% at concentrations from 0.025 to 0.1 mg/mL. Larvae in paper cups treated with thymol at concentrations of ≥0.05 mg/mL showed >95% mortality; however, when larvae were exposed to 0.025 mg/mL thymol, the resulting mortality was only 35%. Larval mortality after treatments with 0.1 mg/mL (+)-camphene, carvacrol, and benzoic acid was 100%, but mortalities were reduced to 15, 27.5, and 57.5%, respectively, when the treatment concentrations were reduced to 0.05 mg/mL. The larvicidal activities of α-pinene, β-pinene, β-myrcene, α-terpinene, *p*-cymene, 1,8-cineole, limonene, γ-terpinene, terpinene-4-ol, and (*E*)-nerolidol have been reported in our previous study.[10]

Bioassays with artificial mixtures showed that blends of ajowan and Peru balsam containing 12 and 7 known constituents of the 2 oils were the most toxic (Figures 1 and 2). Mortality caused by artificial mixtures of all the constituents present did not differ significantly from the mortalities caused by the two essential oils (Figures 1 and 2; $p < 0.0001$). Component elimination assays of ajowan oil (Figure 1) indicated that the omission of thymol, *p*-cymene, and γ-

Table 4. Larvicidal Activity Components from *Trachyspermum ammi* and *Myroxylon pereira* Essential Oils against *Aedes aegypti*

	larvicidal activity[a] (%, mean ± SE, $N = 4$)			
compound	0.1 mg/mL	0.05 mg/mL	0.025 mg/mL	0.0125 mg/mL
(−)-camphene	77.5 ± 4.7 b	35.0 ± 6.4 cd	–[b]	–
(+)-camphene	100 a	15.0 ± 5.0 de	–	–
benzyl alcohol	25.0 ± 6.4 c	–	–	–
benzoic acid	100 a	57.5 ± 4.7 b	–	–
thymol	100 a	97.5 ± 2.5 a	35.0 ± 5.0 b	
carvacrol	100 a	27.5 ± 2.5 cd	–	–
vanillin	0 d	–	–	–
cinnamic acid	92.5 ± 4.7 ab	42.5 ± 2.5 bc	–	–
benzyl benzoate	100 a	100 a	100 a	37.5 ± 4.7 b
benzyl cinnamate	100 a	100 a	100 a	87.5 ± 6.2 a
control	0 c	0 d	0 c	0 c
	$F_{11,36} = 36.8, p < 0.0001$	$F_{8,27} = 48.1, p < 0.0001$	$F_{3,12} = 25.0, p < 0.0001$	$F_{2,9} = 83.3, p < 0.0001$

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

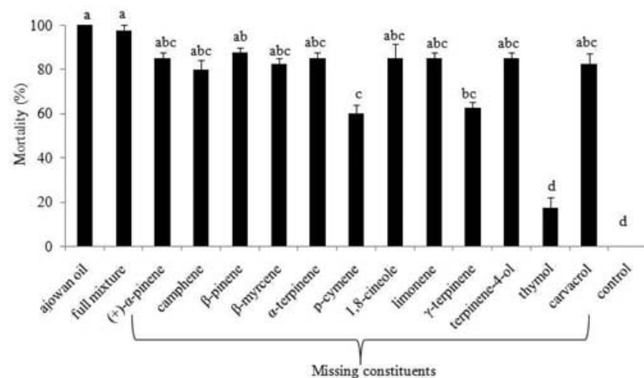

Figure 1. Mortality caused by the oil, full mixture, and selected blends of the constituents of ajowan oil in *Ae. aegypti* larvae. The concentrations of ajowan oil and full mixture were 0.1 and 0.09828 mg/mL, respectively. The concentrations of other blends were determined by removing each constituent equivalent to the ratio identified in ajowan oil. Mean values corresponding to each treatment with different letters are significantly different from each other ($F_{14,45}$ = 47.77, p < 0.0001, Scheffe's test).

terpinene from the artificial mixture caused a significant decrease in the toxicity of the blend ($F_{14,45}$ = 47.77, p < 0.0001). This result indicated that thymol, *p*-cymene, and *γ*-terpinene act synergistically in larvicidal activity against *Ae. aegypti*. Jiang et al.[17] have already reported that plant defense chemicals with more than one mode of action are especially suitable for crop protection. Thymol was the major contributor to the toxicity of the essential oil, followed by *p*-cymene and *γ*-terpinene, although the larvicidal activities of thymol, *p*-cymene, and *γ*-terpinene were similar in this and previous studies.[10] This might be due to the difference in the composition rate of these compounds in ajowan oil. Removal of most of the other constituents ((+)-*α*-pinene, camphene, *β*-pinene, *β*-myrcene, *α*-terpinene, 1,8-cineole, limonene, terpinen-4-ol, and carvacrol) from the mixture had no significant effect on the toxicity of the blend, although limone, *α*-terpinene, camphene, and carvacrol demonstrated larvicidal activities against *Ae. aegypti* in this and previous studies.[10] The quantity of these compounds that was necessary to produce larvicidal activity in an artificial mixture was low because the compounds were a minor proportion of ajowan oil (<1%).

The omission of whole constituents of Peru balsam oil from the artificial mixture did not cause a significant decrease in the toxicity of the blend at a concentration equivalent to 0.1 mg of

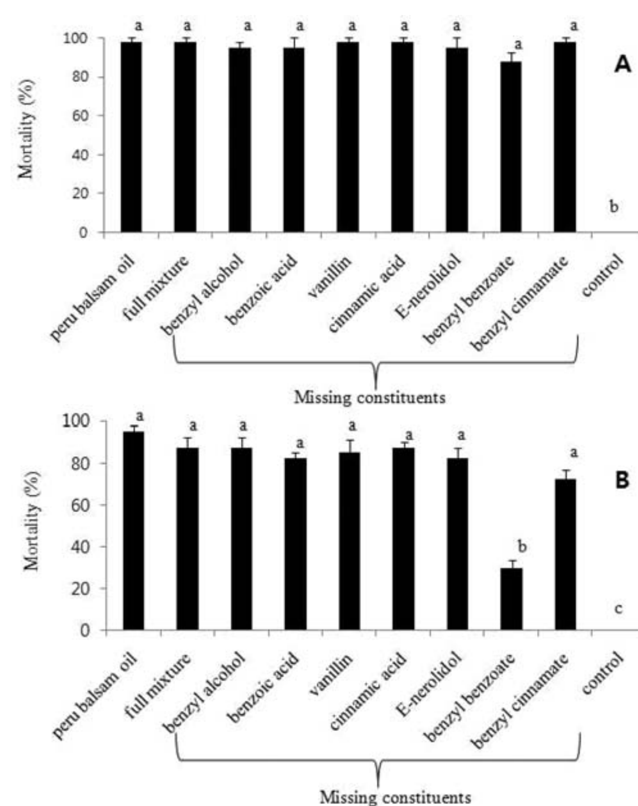

Figure 2. Mortality caused by the oil, full mixture, and selected blends of constituents of Peru balsam oil in *Ae. aegypti* larvae. (A) The concentrations of Peru balsam oil and full mixture were 0.1 and 0.09904 mg/mL, respectively. Mean values corresponding to each treatment with different letters are significantly different from each other ($F_{9,30}$ = 68.33, p < 0.0001). (B) The concentrations of Peru balsam oil and full mixture were 0.05 and 0.04952 mg/mL, respectively. The concentrations of other blends were determined by removing each constituent equivalent to the ratio identified in Peru balsam oil. Mean values corresponding to each treatment with different letters are significantly different from each other ($F_{9,30}$ = 45.0, p < 0.0001, Scheffe's test).

Peru balsam oil (Figure 2A). To find the major contributor of larvicidal activity, we tested an artificial mixture at concentrations equivalent to 0.05 mg/mL Peru balsam oil. At this concentration, omission of six constituents (benzyl alcohol, benzoic acid, vanillin, cinnamic acid, (*E*)-nerolidol, and benzyl cinnamate) from the artificial mixture did not cause a significant decrease in the toxicity of the blend; however, the omission of benzyl benzoate resulted in a significant decrease in toxicity ($F_{9,30} = 68.33$, $p < 0.0001$). This result indicated that benzyl benzoate was the major contributor to the toxicity of Peru balsam oil at a concentration equivalent to 0.05 mg/mL Peru balsam oil (Figure 2B). In addition, two compounds, benzyl benzoate and benzyl cinnamate, are major contributors to the toxicity at a concentration equivalent to 0.1 mg/mL Peru balsam oil because the omission of benzyl benzoate from the artificial mixture did not cause a significant decrease in the toxicity of the blend. At this concentration, the amount of benzyl cinnamate was 0.01576 mg/mL in the artificial mixture, and benzyl cinnamate showed 87.5% larvicidal activity against *Ae. aegypti* at 0.0125 mg/mL in the toxicity assays of individual compounds (Table 4). The effect of the other compounds was minimum because of their small contribution to the total composition of Peru balsam oil, although the larvicidal activities of benzoic acid and cinnamic acid were strong in the toxicity tests of individual compounds. The results of comparative toxicity tests of artificial blends indicated that it is important to maintain a consistent quantity of constituents identified as major contributors of insecticidal activity for the commercialization of plant essential oils as mosquito control agents.

Acute Toxicity of Plant Essential Oils and Their Components against *D. magna*. The water flea, *D. magna* Straus (Cladocera: Crustacea), is a commonly used organism in various toxicological studies in several countries.[18] In this study, Peru balsam oil was more toxic to *D. magna* than ajowan oil (Table 5). LC_{50} values of Peru balsam and ajowan oils were 3.89 and 8.53 mg/L, respectively. Among the constituents tested, benzyl cinnamate was the most toxic, with an LC_{50} value of 2.50 mg/L. The acute toxicity of some Myrtaceae plant essential oils or their components to aquatic organisms has been assessed.[10,19−22] *Melaleuca dissitiflora* and *Eucalyptus globulus* oils were classified as nontoxic (EC_{50} > 100 mg/L). Other oils, such as those of *Melaleuca quinquenervia* and *Melaleuca linariifolia*, or their constituents, such as *p*-cymene, limonene, *γ*-terpinene, *α*-terpinene, and (*E*)-nerolidol, were classified as moderately toxic (EC_{50} = 1−10 mg/L). LC_{50} values of Peru balsam and ajowan oil or their constituents were between 1 and 10 mg/L against *D. magna*, and these were classified as moderately toxic. However, toxicities of ajowan and Peru balsam oils or their major compounds were very weak, in comparison with those of synthetic pesticides such as temephos, which is the most widely used agent to control mosquito larvae. The LC_{50} value of temephos is 0.011 ppb,[23] and temephos is about 0.77 million-fold more toxic than ajowan essential oil.

Table 5. Acute Toxicity of *Trachyspermum ammi* and *Myroxylon pereira* Essential Oils and Their Major Components against *Daphnia magna*

essential oil or compound	slope (± SE)	LC_{50} (mg/L, 48 h)	95% confidence limit
ajowan	7.73 ± 1.40	8.53	7.55−9.64
Peru balsam	7.95 ± 1.68	3.89	2.85−4.91
benzyl benzoate	2.80 ± 0.50	5.09	3.99−6.86
benzyl cinnamate	3.62 ± 0.59	2.50	2.00−3.16
thymol	15.2 ± 3.31	5.94	5.45−6.53

Residue in Water. The residues of ajowan and Peru balsam essential oils and their major components in water are shown in Table 6. Two days after treatment, the residue of Peru balsam, benzyl benzoate, benzyl cinnamate, and thymol were >60%, whereas ajowan oil was present at lower concentrations. Seven days after the treatment, we detected 34.7 and 26.5% of Peru balsam and ajowan oil, respectively. Residues of benzyl benzoate, thymol, and benzyl cinnamate were 62.6, 48.9, and 42.5%, respectively. The advantage of botanical insecticides based on plant essential oils or their components is the minimal residue detected when these are applied in the field. However, few studies on the residue of plant essential oils or their components in water have been performed.[10] Park et al.[10] reported that residues of some Myrtaceae essential oils and their components, such as allyl isothiocyanate, *p*-cymene, limonene, *γ*-terpinene, *α*-terpinene, and (*E*)-nerolidol, were <40% after 7 days in water. Because many pesticides are highly toxic to aquatic organisms such as fish or aquatic invertebrates, the monitoring of pesticide residues in water is very important.[24] Another problem is that pesticides sprayed in the environment undergo chemical, physical, and biological changes, and the degraded compounds may be more harmful than the parent compounds.[25] For example, the transformation products of temephos are temephos sulfoxide, temephos sulfone, temephos sulfide, and sulfone phenols, and there has been no report on the effect of these compounds on the aqueous ecosystem. In this study, the residues were <50% of the test amounts at 7 days after the treatment for all oil and their components, except benzyl benzoate. Our results indicated that the oils and their components could easily volatilize in water within a few days after application, thus minimizing their effect on the aqueous ecosystem.

Table 6. Residue of Essential Oils and Their Active Components in Water

essential oil or compound	residue[a] (%, mean ± SE)	
	2 days	7 days
ajowan	36.2 ± 1.0 d	26.5 ± 0.7 d
Peru balsam	85.1 ± 2.5 a	34.7 ± 1.8 bc
benzyl benzoate	78.8 ± 2.9 a	62.6 ± 7.2 a
benzyl cinnamate	65.0 ± 2.2 b	42.5 ± 3.4 bc
thymol	63.5 ± 2.4 b	48.9 ± 1.6 ab
	$F_{4,15} = 21.7$, $p < 0.0001$	$F_{4,15} = 57.3$, $p < 0.0001$

[a]Means within a column followed by the same letters are not significantly different (Scheffe's test,).

In conclusion, ajowan and Peru balsam plant essential oils and their constituents appear to be useful as natural larvicides against *Ae. aegypti* because they have many advantages such as a minimal effect on the water ecosystem and low residue in water. For the practical use of these essential oils and their constituents as novel larvicides, further studies are required to improve larvicidal potency and stability and to reduce costs.

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Notes

The authors declare no competing financial interest.

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