Essential Oils of *Echinophora lamondiana* (Apiales: Umbelliferae): A Relationship Between Chemical Profile and Biting Deterrence and Larvicidal Activity Against Mosquitoes (Diptera: Culicidae)

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ABSTRACT The essential oils from the flower, leaf, and stem of *Echinophora lamondiana* B.Yildiz et Z.Bahcecioglu were analyzed by gas chromatography–flame ionization detection and gas chromatography–mass spectrometry. In total, 41, 37, and 44 compounds were identified, which accounted for 98.0, 99.1, and 97.0% of flower, leaf, and stem essential oils, respectively. The monoterpenic hydrocarbons were found to be high in all samples of the essential oils. The major components of essential oils from flower, leaf, and stem of *E. lamondiana* were δ-3-carene (61.9, 75.0, and 65.9%, respectively), α-phellandrene (20.3, 14.1, and 12.8%, respectively), and terpinolene (2.7, 3.3, and 2.9%, respectively). Flower and leaf essential oils and terpinolene produced biting deterrence similar to 25 nmol/cm^2 *N*, *N*-diethyl-*meta*-toluamide (DEET; 97%) against *Aedes aegypti* (L.) and *Anopheles quadrimaculatus* Say. Compounds (+)-δ-3-carene, (*R*)-(-)-α-phellandrene, and water-distilled essential oils were significantly less repellent than DEET. Among essential oils, leaf oil was the least toxic of the oils, with an LC_{50} value of 138.3 ppm, whereas flower essential oil killed only 32% larvae, and no mortality of stem oil at highest tested dosages against *Ae aegypti* was observed. Terpinolene and α-phellandrene showed higher toxicity than δ-3-carene in both the species. In contrast to *Ae. aegypti*, all the essential oils showed toxicity in *An. quadrimaculatus*, and toxicity was higher in leaf oil than the other two oils. These results could be useful in finding new, safe, and more effective natural biopesticides and biting deterrent or repellents against *Ae. aegypti*.

KEY WORDS *Echinophora lamondiana*, biting deterrent, repellent, larvicide, *mosquito*

Introduction

Mosquitoes are vectors for many pathogens that cause human diseases including dengue fever, yellow fever, and malaria. These illnesses can result in high rates of human morbidity and mortality in environments where appropriate medical resources are not available. The primary method of mosquito control relies on the use of biological and synthetic insecticides. Due to long-term repeated chemical use, mosquito species have acquired resistance against commonly used insecticides, and especially against commercial pyrethroids (Chandre et al. 1999). There is an urgent need to develop alternative insecticides and insect repellents to manage these disease vectors. Plant-derived products including essential oils may offer an alternative and effective means of managing populations of these mosquitoes. Such products may be attractive as botanical pesticide candidates, as they are environmentally nonpersistent and can generally possess low mammalian toxicities, unlike many of the available synthetic alternatives (Isman 2006). One potential source of natural compounds for use as insecticides and repellents is from plants. In addition to compounds from these sources having the potential to be insecticides and insect repellents, natural compounds are often more environmentally friendly than synthetic chemicals (Cantrell et al. 2011; Ali et al. 2013a,b).

The genus *Echinophora* L. (Apiaceae) is represented in the flora of Turkey by seven species, including four endemic species (Davis 1982, Özhatay et al. 2008-2009). Similarly, four species of the genus *Echinophora* are reported from Iran including two endemic species *Echinophora platyloba* DC and *Echinophora cinerea*

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14. ABSTRACT

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(Boiss) Hedge et Lamond (Heywood 1985, Mozaffarian 1996). In Iran, fresh and dried aerial parts of *E. platyloba* are used in flavoring cheese and yogurt, and for preventing fungal growth on foods like tomato paste and pickled cucumber. *E. platyloba*, an endemic species, is reported to be from central and western provinces of Iran (Sajjadi and Ghannadi 2002, Asghari et al. 2003). Ethanolic extract of *E. platyloba* is reported to have antimicrobial (Asghari et al. 2007) and antifungal (Avijgan et al. 2006) properties. In Turkey, *Echinophora tenuifolia* subsp. *sibthorpiana* known as "tarhana otu or çörtük" and *Echinophora tournefortii* known as "dikenli çörtük" are believed to have wound-healing properties, and its 5% infusions are used for gastric ulcers (Baytop 1984, 1994; Kivanc 1988; Tuzlaci 2006). *E. t. sibthorpiana* (Guss.) Tutin is also used in preparations of tarhana (fermented wheat–yogurt mixture) and added to meaty meals and pickles for flavoring (Ibanoglu 1995). *Echinophora orientalis* has been reported to be added to "helva," a Turkish sweet, to impart fragrance and tenderness (Tuzlaci 2006). The essential oil of *E. t. sibthorpiana* was reported to have high mosquito larvicidal activity, exhibiting LC_{50} values near 60 mg liter^{-1} against *Culex pipiens* L. (Evergetis et al. 2013). No information is available on the insecticidal or repellent properties of the essential oils of *Echinophora lamondiana* B.Yildiz et Z.Bahcecioglu. This study reports the larvicidal activity and biting deterrency of essential oils from the flower, leaf, and stem of *E. lamondiana* and major compounds against yellow fever mosquito, *Aedes aegypti* (L.), and common malaria mosquito, *Anopheles quadrimaculatus* Say.

Materials and Methods

General. (+)-δ-3-Carene (CAS number 498-15-7), (*R*)-(−)-α-phellandrene (CAS number 4221-98-1), and terpinolene (CAS number 586-62-9) were purchased from Sigma-Aldrich Co., St. Louis, MO.

Plant Material. The aerial parts of *E. lamondiana* were collected at flowering stage from Malatya between Hekimhan and Hasancelebi at 1140 m. Voucher specimen was kept at GAZI, Gazi University, Ankara, Turkey (Aytac 9199).

Isolation of the Essential Oils. Flowers, leaves, and stems were separately subjected to water distillation for 3 h using a Clevenger-type apparatus to obtain essential oils. *Echinophora* oils were then calculated on a moisture-free basis for flowers, leaves, and stems as 1.26, 2.09, and 0.54%.

Gas Chromatography–Mass Spectrometry (GC-MS). The oils were analyzed by capillary gas chromatography–flame ionization detection (GC-FID) and GC-MS techniques using an Agilent 5975 GC-MSD system (Agilent, Palo Alto, CA; SEM A. S., Istanbul, Turkey). The same column and analytical conditions were used for both GC-MS and GC-FID. HP-Innowax FSC column (60 m by 0.25 mm, 0.25-μm film thickness, Agilent, Walt & Jennings Scientific, Wilmington, DE) was used with helium as a carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The split ratio was adjusted at 40:1. Flame ionization detection and injector temperature were performed at 250°C. Mass spectra were taken at 70 eV. Mass range was from m/z 35 to 450. It was injected with 1 μl of sample solution (10% in hexane).

Gas Chromatography–Flame Ionization Detection. The GC-FID analysis was carried out using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). FID detector temperature was set at 300°C. To obtain the same elution order with GC-MS, simultaneous injection was done by using the same column and appropriate operational conditions.

Identification and Quantification of Compounds. Identification of the volatile constituents was achieved by parallel comparison of their retention indices (RI) and mass spectra with data published in the WILEY GC/MS Library (Wiley, New York, NY), MASSFINDER *software 4.0* (Dr. Hochmuth Scientific Consulting, Hamburg, Germany), ADAMS Library and NIST Library (Adams 2007), and the in-house "Başer Library of Essential Oil Constituents" database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions. A C8-C40 *n*-alkane standard solution (Fluka, Buchs, Switzerland) was used to spike the samples in the calculation of RI. Quantification of volatile components was performed on the basis of their GC-FID peak areas using integration data. The enantiomeric distribution of chrial compounds in the *E. lamondiana* essential oil was not performed.

Insects. *Ae. aegypti* and *An. quadrimaculatus* larvae and adults used in these studies were from the laboratory colonies maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, U.S. Department of Agriculture–Agricultural Research Service (USDA-ARS), Gainesville, FL. For biting deterrence bioassays, eggs were hatched and the insects were reared to the adult stage in the laboratory and maintained at 27 ± 2°C, 60 ± 10% relative humidity (RH), and a photoperiod of 12:12 (L: D) h. In this study, 8- to 13-d-old adult females were used. For larval bioassays, the eggs were hatched and the larvae were maintained at the above temperature.

Mosquito Biting Bioassays. Experiments were conducted by using a six-celled in vitro Klun and Debboun (K&D) module bioassay system developed by Klun et al. (2005) for quantitative evaluation of biting deterrence of candidate compounds. Briefly, the assay system consists of a six-well reservoir with each of the 3- by 4-cm wells containing 6 ml of blood. As described by Ali et al. (2012), a feeding solution consisting of CPDA-1 (citrate-phosphate-dextrose-adenine) and ATP was used instead of blood. Green fluorescent tracer dye (www.blacklightworld.com) was used to determine the feeding by the females. Essential oils from the leaf, flower, and stem of *E. lamondiana* and its major compounds were tested in this study. Treatments of essential oils from flower, leaf, and stem of *E. lamondiana* were applied at 10 μg/cm^2; pure compounds were

tested at 25 nmol/cm^2, and DEET (97%, *N*, *N*-diethyl-*meta*-toluamide; Sigma Aldrich, St. Louis, MO) at 25 nmol/cm^2 was used as positive control. All the treatments were freshly prepared in molecular biology-grade 100% ethanol (Fisher Scientific Chemical Co. Fairlawn, NJ) at the time of the bioassay.

The temperature of the solution in the reservoirs was maintained at 37°C by continuously passing the warm water through the reservoir using a circulatory bath. The reservoirs were covered with a layer of collagen membrane (Devro, Sandy Run, SC). The test compounds were randomly applied to six 4- by 5-cm areas of organdy cloth and positioned over the membrane-covered CPDA-1 + ATP solution with a Teflon separator placed between the treated cloth and the six-celled module to prevent contamination of the module. A six-celled K&D module containing five female mosquitoes per cell was positioned over cloth treatments covering the six CPDA-1 + ATP solution membrane wells, and trap doors were opened to expose the treatments to these females. The number of mosquitoes biting through cloth treatments in each cell was recorded after a 3-min exposure, and mosquitoes were prodded back into the cells to check the actual feeding. Mosquitoes were squashed and the presence of green fluorescent tracer dye (or not) in the gut was used as an indicator of feeding. A replicate consisted of six treatments: four test compounds, DEET (a standard biting deterrent), and ethanol-treated organdy as solvent control applied randomly. Two sets of five replications each with five females per treatment were conducted on two different days using a newly treated organdy and a new batch of females in each replication. Treatments were replicated 10 times.

In-Cage Mosquito Repellent Assay. Screening studies used approximately 500 colony-reared female *Ae. aegypti* (Orlando strain, 1952), aged 5–10 d old and maintained on 10% sugar solution. Mosquitoes used for testing were maintained at a temperature of 27 ± 2°C, 60 ± 10% RH, and a photoperiod of 12:12 (L:D) h. Because stock cages of mosquitoes contained both males and females, a drawbox was used to preselect females that responded to human odors for the appropriate host-seeking behavior (Posey and Schreck 1981). Because the experimental compounds screened in these studies have unknown toxicology, they should not be applied directly to the skin. In light of this, candidate compounds were treated on muslin cloth as a means to test the compounds without topical application (U.S. Department of Agriculture [USDA] 1977). Compounds are measured in separate vials and dissolved into a solvent that evaporates rapidly, e.g., acetone. A 5- by 10-cm segment of muslin cloth is then added to the vial containing the compound in the solution. The cloth is removed and dried until the solvent evaporates. When dry, a volunteer can affix the treated cloth to cover a 32-cm^2 opening on a specially designed vinyl sleeve. The hand of the volunteer is gloved to protect from bites, and the only accessible area for mosquitoes to bite is through the opening in the sleeve. The volunteer places their arm in a testing cage (approximately 46 cm by 36 cm by 36 cm ≈ 59,000 cm^3) containing approximately 500 of the preselected female *Ae. aegypti* mosquitoes for 1 min. The cloth does not come into direct contact with the skin because of a stocking worn underneath the sleeve to provide a small barrier between the cloth and skin. The use of skin emanations is needed to attract mosquitoes to the opening in the vinyl sleeve. However, just as with other laboratory-based screening methods, the performance of a compound on cloth only partially reflects what the performance would be like if applied directly on skin.

The threshold amount of a repellent needed to prevent bites is estimated by measuring the minimum effective dosage (MED) of the repellent (USDA 1977, Katritzky et al. 2010). A range of concentrations on cloth was used in these experiments, starting with a high concentration of 1.5 mg/cm^2, provided sufficient material was available to produce a solution with this initial concentration. Serial dilutions were made from 1.5 to 0.075 mg/cm^2 and from there to 0.0375 mg/cm^2, and so on, as needed until a compound failed to prevent bites. If less than five bites are received (1% of 500), then the compound is considered to be repellent at the tested concentration. Because these studies involved human volunteers, all participants were required to provide written informed consent to participate. The MED data are reported as the mean (±SE) of all subjects for each compound (three volunteers per compound tested). All data are collected in accordance with the approved University of Florida Institutional Review Board 01 (UF IRB-01) project titled, "Laboratory Evaluation of Repellents for Personal Protection from Mosquitoes and Biting Flies" (Project number 636-2005).

Larval Bioassays. Bioassays were conducted to test *E. lamondiana* essential oils for their larvicidal activity against *Ae. aegypti* and *An. quadrimaculatus* by using the bioassay system described by Pridgeon et al. (2009). Eggs were hatched and larvae were held overnight in the hatching cup in a temperature-controlled room maintained at a temperature of 27 ± 2°C, 60 ± 10% RH, and a photoperiod of 12:12 (L:D) h. Five 1-d-old *Ae. aegypti* or *An. quadrimaculatus* larvae were added in a droplet of water to each well of 24-well plates (BD Labware, Franklin Lakes, NJ) by use of a disposable 22.5-cm Pasteur pipette. Fifty microliters of larval diet (2% slurry of 3:2 Beef Liver powder [Now Foods, Bloomingdale, IL]) and Brewer's yeast (Lewis Laboratories Ltd., Westport, CT) were added to each well by using a Finnpipette stepper (Thermo Fisher, Vantaa, Finland). All tested chemicals were diluted in dimethyl sulfoxide (DMSO). Eleven microliters of the test chemical was added to the labeled wells, whereas 11 µl of DMSO was added to control treatments. After the treatment application, the plates were swirled in clockwise and counter-clockwise motions, front and back, and side-to-side five times to ensure even mixing of the chemicals. Larval mortality was recorded 24-h posttreatment. Larvae that showed no movement in the well after manual disturbance of water were recorded as dead. A series of five concentrations ranging between 125 and 6.25 ppm were used in each treatment to obtain a range of mortality.

Treatments were replicated 10 times for each essential oil and pure compound.

Statistical Analyses. As the K&D module bioassay system can handle only four treatments along with negative and positive controls, to make direct comparisons among more than four test compounds and to compensate for variation in overall response among replicates, biting deterrent activity was quantified as Biting Deterrence Index (BDI; Ali et al. 2012). The BDIs were calculated using the following formula:

$$[BDI_{i,j,k}] = \left[\frac{PNB_{i,j,k} - PNB_{c,j,k}}{PNB_{d,j,k} - PNB_{c,j,k}}\right]$$

Where $PNB_{i,j,k}$ denotes the proportion of females not biting when exposed to test compound i for replication j and day k ($i = 1$–4, $j = 1$–5, $k = 1$–2), $PNB_{c,j,k}$ denotes the proportion of females not biting the solvent control "c" for replication j and day k ($j = 1$–5, $k = 1$–2), and $PNB_{d,j,k}$ denotes the proportion of females not biting in response to DEET "d"(positive control) for replication j and day k ($j = 1$–5, $k = 1$–2). This formula makes an adjustment for interday variation in response and incorporates information from the solvent control as well as the positive control.

A BDI value of 0 indicates an effect similar to ethanol, while a value significantly greater than 0 indicates biting deterrent effect relative to ethanol. BDI values that are not significantly different from 1 are statistically similar to DEET. BDI values were analyzed using SAS Proc ANOVA (single factor: test compound [fixed]; SAS Institute 2007), and means were separated using the Ryan–Einot–Gabriel–Welsch Multiple Range Test. To determine whether confidence intervals include the values of 0 or 1 for treatments, Scheffe's multiple comparison procedure with the option of CLM was used in SAS. LC_{50} values for larvicidal data were calculated by using SAS, Proc Probit. Control mortality was corrected by using Abbott's formula (Abbott 1925). Toxicity was compared among treatments based on nonoverlapping 95% CI (Savin et al. 1977).

Results and Discussion

Essential oils of flower, leaf, and stem from *E. lamondiana* were separately subjected to GC-FID and GC-MS analyses. The compounds characterized and reported with their relative percentages are listed in Table 1. In total, 41, 37, and 44 compounds were identified, which accounted for 98, 99.1, and 97.0% of flower, leaf, and stem essential oils, respectively. The monoterpenic hydrocarbons were found to be high in all samples of essential oils. The major components of essential oils from flower, leaf, and stem of *E. lamondiana* were δ-3-carene (61.9, 75, 65.9%, respectively), α-phellandrene (20.3, 14.1, 12.8%, respectively), and terpinolene (2.7, 3.3, 2.9%, respectively; Fig. 1). Limonene (1.6, 1.7, 1.8%, respectively), β-phellandrene (1.8, 1.2, 1.4%, respectively), and *p*-cymene (1.9, 1.3, 3.8%, respectively) were followed as second majority of compounds. Essential oil from herbal parts of *E. lamondiana* is reported to be rich in δ-3-carene (48%), α-phellandrene (28%), and *p*-cymene (7%; Baser et al. 2000), and results from this study show a similar trend. Glamoclija et al. (2011) reported high

Table 1. Chemical composition of the essential oils of *E. lamondiana*

RRI	Compound	Flower %	Leaf %	Stem %
1032	α-Pinene	0.9	0.6	1.0
1035	α-Thujene	0.2	0.1	0.1
1118	β-Pinene	2.0	0.1	–
1132	Sabinene	0.1	0.1	0.1
1159	δ-3-carene	61.9	75.0	65.9
1176	α-Phellandrene	20.3	14.1	12.8
1187	*o*-Cymene	0.1	<0.1	0.4
1188	α-Terpinene	0.1	0.1	0.1
1195	Sylvestrene	–	0.1	0.1
1203	Limonene	1.6	1.7	1.8
1218	β-Phellandrene	1.8	1.2	1.4
1246	(*Z*)-β-Ocimene	<0.1	<0.1	0.1
1255	γ-Terpinene	0.2	0.2	0.2
1266	(*E*)-β-Ocimene	0.1	0.2	0.2
1280	*p*-Cymene	1.9	1.3	3.8
1286	Isoterpinolene	0.4	0.5	0.4
1290	Terpinolene	2.7	3.3	2.9
1296	Octanal	–	–	<0.1
1400	Nonanal	0.5	<0.1	0.1
1408	Hexyl 2-methyl butyrate	–	<0.1	–
1412	3,5-Nonadiyne	–	<0.1	0.1
1419	2,5-Dimethylstyrene	–	<0.1	0.1
1477	4,8-Epoxyterpinolene	–	<0.1	0.1
1553	Linalool	–	0.1	0.2
1579	*trans*-p-Menth-2-en-1-ol	–	<0.1	<0.1
1655	(*E*)-2-Decenal	0.1	–	<0.1
1662	Pulegone	0.1	–	0.1
1674	*p*-Mentha-1,5-dien-8-ol	–	–	0.5
1685	*p*-Mentha-1(7),2-dien-8-ol[a]	0.2	–	1.3
1700	*p*-Mentha-1,8-dien-4-ol (= *Limonen-4-ol*)	–	–	0.1
1706	α-Terpineol	–	–	0.2
1726	Germacrene D	<0.1	–	<0.1
1742	β-Selinene	0.1	–	0.2
1755	Bicyclogermacrene	0.1	–	–
1764	(*E*)-2-Undecenal	0.1	–	–
1805	Germacrene B	0.4	–	0.3
1811	*m*-Cymene-8-ol	0.1	–	0.3
1814	Liguloxide	0.1	–	0.3
1823	*p*-Mentha-1(7),5-dien-2-ol	0.3	–	0.5
1896	Nona-3,5-diyn-2-yl acetate	0.3	0.1	0.3
1921	α-Phellandrene epoxide	–	<0.1	0.1
1949	Piperitenone	–	–	<0.1
2017	3,5-Nonadiyn-2-ol	0.1	<0.1	–
2045	Carotol	0.2	<0.1	–
2084	Octanoic acid	0.1	–	–
2120	Nonanoic acid	0.4	0.1	–
2121	Isothymol	–	<0.1	0.1
2144	Spathulenol	0.2	<0.1	0.3
2198	Thymol	0.1	0.1	–
2239	Carvacrol	–	–	0.2
2296	Myristicin	0.2	<0.1	0.2
2298	Decanoic acid	0.1	<0.1	–
2503	Dodecanoic acid	0.1	<0.1	–
2622	Phytol	–	<0.1	<0.1
2655	Benzyl benzoate	0.1	–	–
2670	Tetradecanoic acid (= *Myristic acid*)	0.2	–	0.1
2931	Hexadecanoic acid	0.2	0.1	–
	Total	**98.7**	**99.1**	**97.0**

RRI, relative retention indices calculated against *n*-alkanes.
% calculated from FID data.
[a] Tentatively identified using WILEY GC/MS Library.

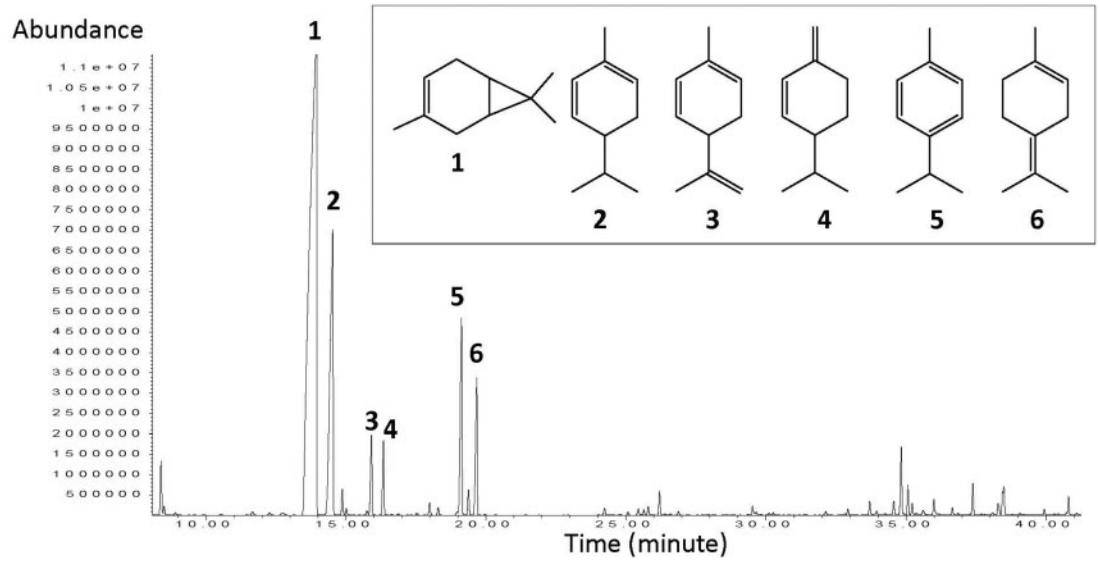

Fig. 1. Total ion chromatogram of water-distilled *E. lamondiana* stem essential oil and its representative compounds (**1**: δ-3-carene, **2**: α-phellandrene, **3**: limonene, **4**: β-phellandrene, **5**: *p*-cymene, **6**: terpinolene).

percentage of δ-3-carene (61%) in *E. spinosa* essential oil. Published data reflect chemical diversity among *Echinophora* essential oils from Turkey. *Echinophora* essential oils are found to be rich in α-phellandrene (Kubeczka 1982; Baser et al. 1996, 1998). The essential oil of *E. t. sibthorpiana* contained α-phellandrene (15.5%), *p*-cymene (1 1.0%), and methyl eugenol (58.7%) and the essential oil of *Echinophora chrysantha* contained α-phellandrene (47.7–61.0%) and β-phellandrene (8.2%) as major compounds. Other monoterpene-rich oils are the oil of *E. tournefortii* with α-pinene (9.6%) and caryophyllene oxide (12.8%), the oil of *E. trichophylla* with sabinene (27.3%) and 2,6-dimethyl-l,3(*E*),5(*E*),7-octatetraene (13.7%), and the oil of *E. orientalis* with myrcene (34.2%) and *p*-cymene (18.9%). α-Phellandrene (36.8%), *p*-cymene (27.3%), and α-pinene (15%) were reported as major constituents of the oil of *E. spinosa*, which is not a native plant of Turkey (Kubeczka 1982; Baser et al. 1996, 1998). A monoterpene α-phellandrene and a phenylpropanoid compound methyl eugenol were reported as major constituents of *E. t. sibthorpiana* (Akgül and Chialva 1989, Baser et al. 1994, Ahmad 1999, Özcan et al. 2002, Georgiou et al. 2010). Among sesquiterpene-rich oils, *E. carvifolia* essential oil had germacrene D (31.1%) and β-caryophyllene (5.3%) as major compounds (Ibanoglu et al. 1995).

Mean BDI values of essential oils of various parts of *E. lamondiana* and major compounds against *Ae. aegypti* are presented in Figure 2. Based on 95% CI, biting deterrent activity of essential oils of *E. lamondiana* flower and leaf at 10 μg/cm^2 were statistically similar to DEET at 25 nmol/cm^2. Biting deterrent activity of terpinolene was similar to DEET, whereas activity of (*R*)-(−)-α-phellandrene and (+)-δ-3-carene was significantly lower than DEET at 25 nmol/cm^2. Mean BDI values of essential oils of various parts of *E. lamondiana* and major compounds against *An. quadrimaculatus* are presented in Figure 3. Based on 95% CI, biting deterrent activity of essential oils of *E. lamondiana* flower and leaf at 10 μg/cm^2 were statistically similar to DEET at 25 nmol/cm^2. Biting deterrent activity of terpinolene and (+)-δ-3-carene was also similar to DEET, whereas activity of (*R*)-(−)-α-phellandrene was lower than DEET. In in vivo cloth patch bioassays, terpinolene (MED: 0.023 ± 0.000 mg/cm^2) was found to be as repellent as DEET (0.023 ± 0.000 mg/cm^2), whereas (+)-δ-3-carene, with a MED value of 0.078 ±0.016 mg/cm^2, was less repellent than DEET (0.018 ± 0.003 mg/cm^2) in a separate series of repellent bioassays. This is the first report of biting deterrent activity of *E. lamondiana* essential oil against *Ae. aegypti* and *An. quadrimaculatus*.

Data on the toxicity of essential oils of various parts of *E. lamondiana* and their chemical constituents against 1-d-old *Ae. aegypti* and *An. quadrimaculatus* larvae are given in Table 2. In *Ae. aegypti*, leaf essential oil was the most toxic of all oils, with an LC_{50} value of 138.3 ppm, whereas flower essential oil killed only 32% larvae, and there was no mortality in stem oil at highest dose of 125 ppm against *Ae aegypti.* Terpinolene and α-phellandrene showed high toxicity, whereas (+)-δ-3-carene was not active at the highest dose of 100 ppm in *Ae. aegypti*.

In *An. quadrimaculatus*, essential oils of leaf, flower, and stem showed LC_{50} values of 26.5, 4.9, and 65.6 ppm, respectively. Based on 95% CI, leaf essential oil was significantly more toxic than the other two oils. Among pure compounds, toxicity was similar in (*R*)(−)-α-phellandrene (LC_{50} = 15.6 ppm) and terpinolene (LC_{50} = 15.6 ppm) which was more toxic than (+)-δ-3-carene (LC_{50} = 42.2 ppm). This is the first report of larvicidal activity of *E. lamondiana* essential oil against *Ae. aegypti* and *An. quadrimaculatus*.

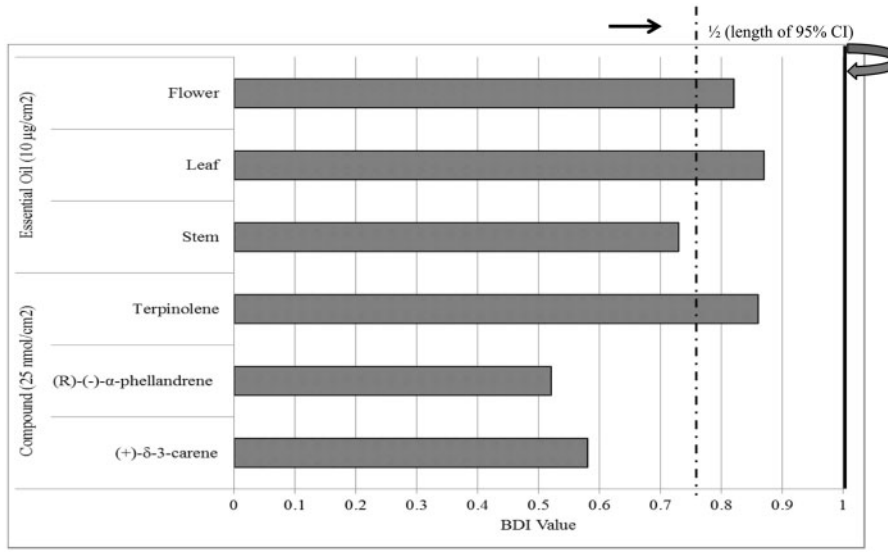

Fig. 2. Mean BDI values of the essential oils from flower, leaf, and stem of *E. lamondiana* and major compounds against female *Ae. aegypti*. Ethanol was the solvent control and DEET at 25 nmol/cm^2 was used as positive control. BDI values that fall between one-half of the length of 95% CI and value of 1 are similar to DEET.

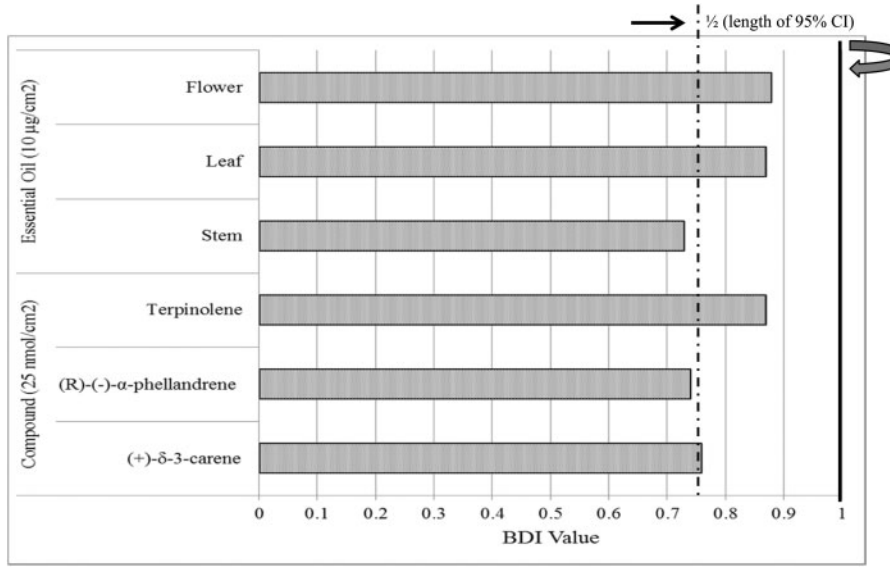

Fig. 3. Mean BDI values of the essential oils from flower, leaf, and stem of *E. lamondiana* and major compounds against female *An. quadrimaculatus*. Ethanol was the solvent control and DEET at 25 nmol/cm^2 was used as positive control. BDI values that fall between one-half of the length of 95% CI and value of 1 are similar to DEET.

The essential oil of *E. t. sibthorpiana* was reported to have high mosquito larvicidal activity, exhibiting LC_{50} values near 60 mg liter^{-1} against third- and early fourth-instar larvae of *Cx. pipiens* (Evergetis et al. 2013). (*R*)-(−)-α-Phellandrene is reported to have larvicidal activity (LC_{50} value of 38.20 mg/liter) against *Cx. pipiens* (Evergetis et al. 2013). α-Phellandrene and terpinolene are reported to show larvicidal activity against *Ae. aegypti* larvae, with LC_{50} values of 23.08 and 15.3 ppm, respectively (Perumalsamy et al. 2009). These data corroborate the results of this study for *Ae. aegypti*.

In conclusion, essential oils of various parts of *E. lamondiana* are rich sources of (+)-δ-3-carene, (*R*)-(−)-α-phellandrene, and terpinolene. Essential oils from the flower and leaf of *E. lamondiana*, and the pure compound terpinolene, showed biting deterrent activity similar to DEET. This is the first report on

Table 2. Toxicity of *E. lamondiana* essential oils and its major constituents against 1-d-old larvae of *Ae. aegypti* and *An. quadrimaculatus* 24-h post treatment

Oil or compound	*Ae. aegypti*				*An. quadrimaculatus*			
	LC_{50} ppm (95% CI)[a]	LC_{90} ppm (95% CI)	χ^2	df[b]	LC_{50} ppm (95% CI)	LC_{90} ppm (95% CI)	χ^2	df
Leaf	138.3 (131.2–145.9)	169.1 (158.3–188.4)	40.9	28	26.2 (23.3–29.5)	46.0 (39.4–57.8)	64.9	38
Flower	–[c]				46.9 (41.4–53.3)	90.4 (73.3–115.4)	73.1	38
Stem	–[d]				65.6 (58.1–74.3)	119.1 (100.9–151.9)	65.1	38
(*R*)-(−)-α-phellandrene	11.4 (10.2–12.8)	19.3 (16.7–24.0)	60.4	38	15.6 (13.5–18.1)	36.4 (29.7–48.8)	73.2	38
Terpinolene	14.0 (11.9–16.6)	21.4 (17.7–31.00)	25.7	28	20.9 (18.7–23.6)	36.8 (31.5–46.2)	64.8	38
(+)-δ-3-carene	–[e]				42.9 (38.0–48.6)	80.0 (67.7–102.4)	68.1	38

[a] 95% CI is the confidence interval.
[b] df refers to degree of freedom.
[c] Killed only 32% of the larvae at the highest dose of 125 ppm.
[d] No larvicidal mortality at the maximum dose of 125 ppm.
[e] No larvicidal mortality at the maximum dose of 100 ppm.

biting deterrent activity of these oils against mosquitoes. Terpinolene showed good repellency in human-based bioassay. Although (+)-δ-3-carene was not as repellent as DEET, both of these compounds are good candidates for use in repellent blends, provided that their use on skin is toxicologically favorable. Essential oil derived from the leaf gave better larval toxicity than the flower and stem essential oils. Among the pure compounds, terpinolene and (*R*)-(−)-α-phellandrene gave higher larval mortality than the essential oils in both species. Findings of this study suggest that the essential oil from leaf, flower and effective constituents of *E. lamondiana* may be explored as potential biting deterrents. Further investigations for the mode of action of (*R*)-(−)-α-phellandrene and terpinolene effects on nontarget organisms and field evaluation should be carried out to explore their utility as environmentally benign biopesticides.

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