Biting Deterrence, Repellency, and Larvicidal Activity of *Ruta chalepensis* (Sapindales: Rutaceae) Essential Oil and Its Major Individual Constituents Against Mosquitoes

ABBAS ALI,[1,2] BETUL DEMIRCI,[3] HULYA TUBA KIYAN,[3] ULRICH R. BERNIER,[4] MAIA TSIKOLIA,[4] DAVID E. WEDGE,[5] IKHLAS A. KHAN,[1,6,7] KEMAL HUSNU CAN BAŞER,[3,8] AND NURHAYAT TABANCA[1]

J. Med. Entomol. 50(6): 1267–1274 (2013); DOI: http://dx.doi.org/10.1603/ME12177

ABSTRACT The essential oil from aerial parts of *Ruta chalepensis* L. (Sapindales: Rutaceae) was obtained by hydrodistillation, and its chemical profile was identified using gas chromatography and gas chromatography–mass spectrometry. Compounds, 2-undecanone (43.2%), 2-nonanone (27.9%), and 2-nonyl acetate (10.6%) were the major constituents of the oil. Biting deterrent activity of *R. chalepensis* essential oil at 10 and 50 $\mu g/cm^2$, 2-undecanone at 8.5 $\mu g/cm^2$, 2-nonanone at 9 $\mu g/cm^2$, and 2-nonyl acetate at 9.3 $\mu g/cm^2$ was similar to DEET (*N*, *N*-diethyl-*meta*-toluamide) at 4.8 $\mu g/cm^2$, against *Aedes aegypti* L. Biting deterrent activity of *R. chalepensis* oil at 50 $\mu g/cm^2$ against *Anopheles quadrimaculatus* Say was statistically similar to DEET at 4.8 $\mu g/cm^2$, whereas the activity was lower in the other compounds tested. In cloth patch assay, *R. chalepensis* essential oil was effective at 187 $\mu g/cm^2$, whereas 2-undecanone was effective at 108.9 $\mu g/cm^2$ against *Ae. aegypti.* In larval bioassays, 2-undecanone showed similar toxicity whereas toxicity of *R. chalepensis* essential oil and 2-nonanone was higher at 24-h posttreatment at the LD_{50} in *An. quadrimaculatus* than *Ae. aegypti.* This study revealed that *R. chalepensis* essential oil and its major compounds were active biting deterrents against *Ae. aegypti* at higher application rates whereas only the essential oil showed activity similar to DEET against *An. quadrimaculatus*. 2-undecanone was the most active compound in in vivo repellency bioassay against *Ae. aegypti*. Chemical composition of *R. chalepensis* essential oil varies because of plant production and harvest practices, and the activity level of the essential oil may depend on the source of the sample.

KEY WORDS *Ruta chalepensis*, biting deterrent, repellent, larvicide, *Aedes aegypti*

Mosquitoes are vectors for many pathogens that cause human diseases like malaria, dengue fever, yellow fever, Rift Valley fever, and Chikungunya. When significant levels of transmission occur, epidemics can result in high rates of human morbidity and mortality. The primary method to control mosquitoes relies on use of biological and synthetic insecticides. Owing to continuous chemical use, mosquitoes have developed resistance against many currently used commercial pyrethroids (Chandre et al. 1999). Therefore, there is an urgent need to search and identify new effective alternatives to insecticides to control these vectors.

The genus *Ruta*, known as common rue, belongs to the Rutaceae family, which features many shrubby plants that are native to the Mediterranean region and are represented by 40 species in the world (Daniel 2006). Out of these 40, only two species *Ruta chalepensis* L. and *Ruta montana* L., are widespread in Turkey (Davis 1967). *R. chalepensis* is commonly called 'sedef otu' (psoriasis herb) in Turkey and is used in decoction as emmenagogue and analgesic (Baytop 1999).

R. chalepensis is used as traditional medicine in many countries to treat a variety of diseases like rheumatism, neuralgia, menstrual bleeding, fever, arthritis, hepatic diseases, antifertility, and gastrointestinal disorders (Gunaydin and Savci 2005, Gonzalez–Trujano et al. 2006). The plant also has analgesic, antipyretic, anti-inflammatory, antifungal, emmenagogue, insect repellent, molluscicidal, nematicidal, antimicrobial, antihelmintic, sedative, and antiplatelet properties (Gunaydin and Savci 2005, Shehadeh et al. 2007, Ntalli et al. 2011). *R. chalepensis* ("ruda in Mexico") is used in decoction as a medicinal remedy to treat cultural

[1] National Center for Natural Products Research, The University of Mississippi, University, MS 38677.
[2] Corresponding author, e-mail: aali@olemiss.edu.
[3] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey.
[4] Center for Medical, Agricultural and Veterinary Entomology, USDA–ARS, Gainesville, FL 32608.
[5] Natural Products Utilization Research Unit, USDA–ARS, University of Mississippi, University, MS 38677.
[6] Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677.
[7] Department of Pharmacognosy, College of Pharmacy, King Saud University, 11451 Riyadh, Saudi Arabia.
[8] Department of Botany and Microbiology, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia.

0022-2585/13/1267–1274$04.00/0 © 2013 Entomological Society of America

Report Documentation Page	*Form Approved OMB No. 0704-0188*

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE **2013**	2. REPORT TYPE	3. DATES COVERED **00-00-2013 to 00-00-2013**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Biting Deterrence, Repellency, and Larvicidal Activity of Ruta chalepensis (Sapindales: Rutaceae) Essential Oil and Its Major Individual Constituents Against Mosquitoes	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **University of Mississippi,National Center for Natural Products Research,University,MS,38677**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The essential oil from aerial parts of Ruta chalepensis L. (Sapindales: Rutaceae) was obtained by hydrodistillation, and its chemical pro?le was identi?ed using gas chromatography and gas chromatography?mass spectrometry. Compounds, 2-undecanone (43.2%), 2-nonanone (27.9%) and 2-nonyl acetate (10.6%) were the major constituents of the oil. Biting deterrent activity of R. chalepensis essential oil at 10 and 50 g/cm2, 2-undecanone at 8.5 g/cm2, 2-nonanone at 9 g/cm2 and 2-nonyl acetate at 9.3 g/cm2 was similar to DEET (N, N-diethyl-meta-toluamide) at 4.8 g/cm2 against Aedes aegypti L. Biting deterrent activity of R. chalepensis oil at 50 g/cm2 against Anopheles quadrimaculatus Say was statistically similar to DEET at 4.8 g/cm2, whereas the activity was lower in the other compounds tested. In cloth patch assay, R. chalepensis essential oil was effective at 187 g/cm2, whereas 2-undecanone was effective at 108.9 g/cm2 against Ae. aegypti. In larval bioassays 2-undecanone showed similar toxicity whereas toxicity of R. chalepensis essential oil and 2-nonanone was higher at 24-h posttreatment at the LD50 in An. quadrimaculatus than Ae. aegypti. This study revealed that R. chalepensis essential oil and its major compounds were active biting deterrents against Ae. aegypti at higher application rates whereas only the essential oil showed activity similar to DEET against An. quadrimaculatus. 2-undecanone was the most active compound in in vivo repellency bioassay against Ae. aegypti. Chemical composition of R. chalepensis essential oil varies because of plant production and harvest practices, and the activity level of the essential oil may depend on the source of the sample.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**8**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

diseases such as "empacho" (gastrointestinal illness) and is reportedly used against "mal de ojo" (evil eye) and for "spiritual cleansings" (Gonzalez–Trujano et al. 2006). Phytochemical research on *R. chalepensis* has revealed the presence of alkaloids, anthraquinones, tannins, flavonoids, cyanogenic glycosides, cardiac glycosides, triterpenes, saponins, sterols, furanocoumarins, and essential oils (Gunaydin and Savci 2005, Michael 2005, Escher et al. 2006).

An infusion of *R. chalepensis* leaves rubbed onto skin has been purported to be repellent to mosquitoes and other insects by farmers and shepherds in rural and mountainous areas of Marche and Latium, Central Italy (Guarrera 1999). In the same Italian countryside, *R. graveolens* leaves were set under the bed to repel bugs and mice (Guarrera 1999). A decoction of *Ruta* species also has been used topically against scabies, lice, and fleas, to repel insects and to treat intestinal worms in livestock (Feo et al. 2002). Because ethnobotanical studies show that *R. chalepensis* repels mosquitoes and other insects (Hadis et al. 2003); we evaluated *R. chalepensis* essential oil and its major compounds for their biting deterrent, repellent, and larvicidal activity against the yellowfever mosquito, *Ae. aegypti* and a common malaria mosquito, *An. quadrimaculatus.*

Materials and Methods

Plant Material and Chemicals. The aerial parts of *R. chalepensis* were collected in Alanya, Turkey, in May 2009, and voucher specimens were deposited at the Faculty of Pharmacy Herbarium, Anadolu University, Eskisehir, Turkey (ESSE: 14574). Standards of both 2-undecanone (purity ≥99%) and 2-nonanone (purity ≥99%) were purchased from Sigma–Aldrich (St. Louis, MO), and 2-nonyl acetate (purity ≥98%) was purchased from TCI Japan (Tokyo Kasei Kogyo Co., Ltd., Chuo-ku, Japan).

Isolation of Essential Oil. The essential oil from needles of the air-dried plant was isolated by hydrodistillation for 3-h using a Clevenger-type apparatus (European Pharmacopoeia 2005). The essential oil was dried over anhydrous sodium sulfate and stored in sealed amber vial at 4°C.

Gas Chromatography–Mass Spectrometry Analysis Conditions. The gas chromatography–mass spectrometry (GC–MS) analysis was carried out with an Agilent 5975 GC–MSD system (SEM Ltd., Istanbul, Turkey). Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness; SEM Ltd.) was used with helium as carrier gas (0.8 ml/min). The GC oven temperature was kept at 60°C for 10 min followed by an increase to 220°C at a rate of 4°C/min. The oven was kept constant at 220°C for 10 min and then the temperature increased to 240°C at a rate of 1°C/min. The split ratio was adjusted at 40:1. The oil was injected at a 20% concentration in *n*-hexane, and the analyses were carried out in triplicate. The injector temperature was set at 250°C. Mass spectra were recorded at 70 with mass range of m/z 35–450.

GC Analyses. The GC analysis was carried out using an Agilent 6890N GC system (SEM Ltd.). The flame ionization detector (FID) temperature was set at 300°C. Simultaneous auto-injection was done using an identical column under the same temperature program to obtain the same elution order with GC–MS. The relative percentage amounts of the separated compounds were calculated from peaks in the GC–FID chromatograms.

Identification of Components. Tentative identification of the essential oil components were conducted by comparison of their relative retention times (RT) with those of authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Peak matching was aided by in-house "Baser Library of Essential Oil Constituents" with commercial libraries (Wiley GC/MS Library, Adams Library, Mass Finder 3 Library; McLafferty and Stauffer 1989, Koenig et al. 2004). Components of known oils, authentic standards as well as MS literature data (Joulain and Koenig 1998; ESO 2000) were used for the characterization of components.

Insects. *Ae. aegypti* and *An. quadrimaculatus* used in these studies were from a laboratory colony maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, United States Department of Agriculture–Agricultural Research Service (USDA–ARS), Gainesville, FL (Pridgeon et al. 2007). For biting deterrence and repellent bioassays, pupae were maintained in the laboratory at 27 ± 2°C and 60 ± 10% relative humidity (RH), and 5- to 13-d-old adult females were used. For larval bioassays, the eggs were hatched, and the larvae were maintained at a temperature of 27 ± 2°C, 60 ± 10% RH, and a photoperiod of 12:12 (L:D) h.

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 bite deterrent properties 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 and ATP was used instead of blood. *R. chalepensis* essential oil, 2-undecanone, 2-nonanone, and 2-nonyl acetate were tested in this study. Treatments were applied at the following two rates (μg/cm^2): *R. chalepensis* essential oil (10, 50), 2-undecanone (4.25, 8.5), 2-nonanone (4.5, 9), and 2-nonyl acetate (4.65, 9.3), and DEET (97%, *N, N*-diethyl-*meta*-toluamide; Sigma–Aldrich) at 4.8 μg/cm^2 was used as positive control. All the treatments were freshly prepared in molecular biology grade 100% ethanol (Fisher Chemical Co., Fairlawn, NJ) at the time of bioassay.

The temperature of the solution in the reservoirs was maintained at 37.5°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 mem-

brane-covered CPDA-1 + ATP solution with a Teflon separator placed between the treated cloth and the six-celled module to prevent the 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. These mosquitoes were then squashed to determine the numbers that had actually imbibed the solution. A replicate consisted of six treatments: four test compounds, DEET (a standard biting deterrent), and ethanol-treated organdy as solvent control applied randomly. Three sets of five replications each with five females per treatment were conducted on three different days using a newly treated organdy and a new batch of females in each replication. Treatments were replicated 15 times for *Ae. aegypti* and five times for *An. quadrimaculatus.*

Mosquito Repellent Assays. *Ae. aegypti* pupae were maintained in the laboratory for adult emergence. Experiments were conducted by using 5- to 9-d-old females that were selected from the stock cages by a hand-draw box (Posey and Schreck 1981). Approximately 500 (±10%) mosquitoes, consisting primarily of females, were collected and loaded into a test cage (size of 45 by 37.5 by 35 cm) and held in the cage for 25 (±2.5) min before initiating repellency assays (Barnard et al. 2007).

An in vivo "cloth patch assay" with a series of dosages of the essential oil and its components was used to determine the minimum effective dose for repellency of *Ae. aegypti.* Minimum effective dose (MED) refers to a concentration of the chemical at which biting is <1% out of 500 females in a cage, received during 1-min exposure period. Series of the concentrations of a treatment are tested starting from the middle of the road until the desired concentration where <1% (five bites) is achieved. *R. chalepensis* essential oil, 2-undecanone, 2-nonanone, and 2-nonyl acetate were the treatments in this study. Each treatment was weighed into 2-dram vials and dissolved by addition of 2 ml molecular biology grade 100% ethanol (Fisher, Waltham, MA). The initial weight of the treatment was measured so that when one half was removed and a 50-cm^2 muslin cloth was added to the vial, the remaining 1-ml solution would produce an initial concentration of 1,500 $\mu g/cm^2$ on cloth. Serial dilutions were made analogously such that the concentrations on cloth for the remaining 1 ml solution were 750, 375, 187, 94, 47, 23, 11, and 5 $\mu g/cm^2$. Vials were sealed and stored at −4°C in a freezer until testing (normally <48 h). The conduction of a test involved removal of cloth from the vial and attaching it by staples onto two sections of card stock (5 by 2.5 cm). Approximately 2.5–5 cm of masking tape was used to secure the cloth onto the card stock. The card and cloth assembly was placed on a drying rack for 3–5 min before bioassay initiation.

A single test consisted of covering the hand of a volunteer with a soft-embossed long cuff poly glove (Atlantis Products, Mankato, MN), then by a powder-free latex glove (Diamond Grip, Microflex Corporation, Reno, NV). Thereafter, a knee-high stocking (Leggs everyday knee highs, Winston–Salem, NC) was placed over the gloved hand and arm. A plastic sleeve constructed of polyvinyl was the final layer that was then affixed over the stocking covered arm. The plastic sleeve was sealed around the arm by a Velcro strip. About half-way between the wrist and elbow a 4 by 8 cm opening was used to assess mosquito landing and biting behavior. The opening permitted attractive odors from the skin surface to emanate out and attract mosquitoes to this opening in the sleeve. During testing, this 32-cm^2 open area was covered with one of the chemical-treated muslin cloth treatments. Three volunteers (three males) provided written informed consent. The protocol was approved by the University of Florida Human Use Institutional Review Board-01 (Study #636-2005). Each volunteer had their own cage of mosquitoes.

A test started when the arm with sleeve and cloth were inserted into the mosquito cage. If 0–4 bites were received during the 1-min test period, the dosage of repellent on cloth was considered to have "passed." A failing treatment was one that received five bites out of 500 mosquitoes in the cage in 1 min. An intermediate dose (187 $\mu g/cm^2$) was tested first, and as there were four treatments and three volunteers, each volunteer received a different treatment at the start of the first set. Volunteers passed their treated cloth to the other volunteer and the next test interval was conducted. The three treated cloths were then disposed of properly, and the testing of the next set of cloths commenced. Depending on whether the concentration of a treatment passed or failed, higher or lower treatment concentrations were evaluated by the volunteers until each had pinpointed their individual concentration that produced 1% (five bites) failure point. At times, this required one or two individuals not testing during a test interval. No patch was evaluated beyond 5 min after the 3-min drying period to avoid any bias that may result from evaporative loss of the treatment from the cloth during the duration of the test. Because the mosquitoes show reduced behavioral activity on repeated exposure to repellent and attractant odors from the arm, after 10 successive exposures of treated cloths by each volunteer, the caged mosquitoes were allowed a 15-min recovery period. Based on previous assays of this nature, it was determined that a 15-min interval is sufficient for recovery from repeated chemical exposures (Katritzky et al. 2010).

Larval Bioassays. Bioassays were conducted to test *R. chalepensis* essential oil, 2-undecanone, 2-nonanone, and 2-nonyl acetate 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* 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]) was added to each well by using a Finnpipette stepper (Thermo Fisher, Vantaa, Finland) for *Ae. aegypti* and 1:1 Beef Liver powder and Brewer's yeast for *An. quadrimaculatus.* All chemicals tested were diluted in dimethyl sulfoxide (DMSO). Eleven microliters of the test chemical was added to the labeled wells, while 11 μl of DMSO was added to control treatments. After the treatment application, the plates were swirled in clock-wise and counter clockwise motions and front and back and side to side five times to ensure even mixing of the chemicals. Larval mortality was recorded 24- and 48-h posttreatment. Larvae that showed no movement in the well after manual disturbance of water by a pipette tip were recorded as dead. A series of five concentrations ranging between 300 and 6.25 ppm were used in each treatment to obtain a range of mortality between 0 and 100%. Treatments were replicated 15 times for each essential oil and chemical for *Ae. aegypti* and five times for *An. quadrimaculatus.*

Statistical Analyses. Proportion not biting (PNB) was calculated using the procedure described by Ali et al. (2012). 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 ($i = 1–4, j = 1–5, k = 1–3$), $PNB_{c,j,k}$ denotes the proportion of females not biting the solvent control c for replication j and day k ($j = 1–5, k = 1–3$), 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 ($j = 1–5, k = 1–3$). This formula makes an adjustment for inter-day 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 >0 indicates biting deterrent effect relative to ethanol. BDI values 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, Scheffé 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). Because MED are single point values, which were replicated three times, means and SEs were calculated to interpret the data.

Table 1. The composition of essential oil extracted from *R. chalepensis*

RRI[a]	Compound	Area (%) ± (SD)[b]	Identification[c]
1203	Limonene	0.1 ± 0.0	t_R, MS
1337	Geijerene	2.9 ± 0.0	t_R, MS
1372	2-octyl acetate	0.2 ± 0.0	MS
1398	2-nonanone	27.9 ± 0.8	t_R, MS
1400	Nonanal	0.5 ± 0.0	t_R, MS
1471	2-nonyl acetate	10.6 ± 0.2	t_R, MS
1496	2-decanone	2.0 ± 0.1	t_R, MS
1521	2-nonanol	0.8 ± 0.0	t_R, MS
1572	Pregeijerene B	1.8 ± 0.1	t_R, MS
1604	2-undecanone	43.2 ± 0.8	t_R, MS
1668	2-decyl acetate	2.5 ± 1.0	MS
1688	10-methyl-2-undecanone	1.0 ± 0.1	MS
1715	2-dodecanone	0.6 ± 0.1	t_R, MS
1722	2-undecanol	0.6 ± 0.1	t_R, MS
1815	2-tridecanone	1.0 ± 0.1	t_R, MS
2622	Phytol	0.7 ± 0.1	MS
	Total	96.4 ± 12.1	—

[a] RRI, relative retention indices calculated against *n*-alkanes, % calculated from FID data.

[b] Average value (area %) of three replicates. SD (±) is given with percent.

[c] Identification method: t_R, identification based on the retention times (t_R) of genuine compounds on the HP Innowax column; MS, tentatively identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

Results and Discussion

Hydrodistillation of the air-dried aerial parts of *R. chalepensis* yielded 0.84% (wt:vol) of yellowish oil with strong odor. Sixteen compounds from the essential oil were identified using GC and GC–MS which represented 96.4% of the oil of *R. chalepensis.* 2-undecanone (43.2 ± 0.8%), 2-nonanone (27.9 ± 0.8%), and 2-nonyl acetate (10.6 ± 0.2%), as main constituents, were 81.7% of the oil (Table 1).

The chemical composition of *R. chalepensis* essential oil from different parts of the world has been reported by many researchers. Baser et al. (1996), Rustaiyan et al. (2002), Dob et al. (2008), Merghache et al. (2008), and Fakhfakh et al. (2012) reported 66.49, 52.5, 28.2, 20.4–82.7, and 12.4–23.8% of 2-undecanone, respectively, in the essential oil of *R. chalepensis.* Studies by the same authors also reported 16.2, 24.1, 5.2–33.6, 20, and 14.2−41.7% of 2-nonanone, respectively, in the essential oil. Tounsi et al. (2001) reported 2-undecanone (52.5%) as the major components of *R. chalepensis* essential oil. Baser et al. (1996), Bagchi et al. (1996, 2003), Rustaiyan et al. (2002), and Gunaydin and Savci (2005) reported 2-nonyl acetate

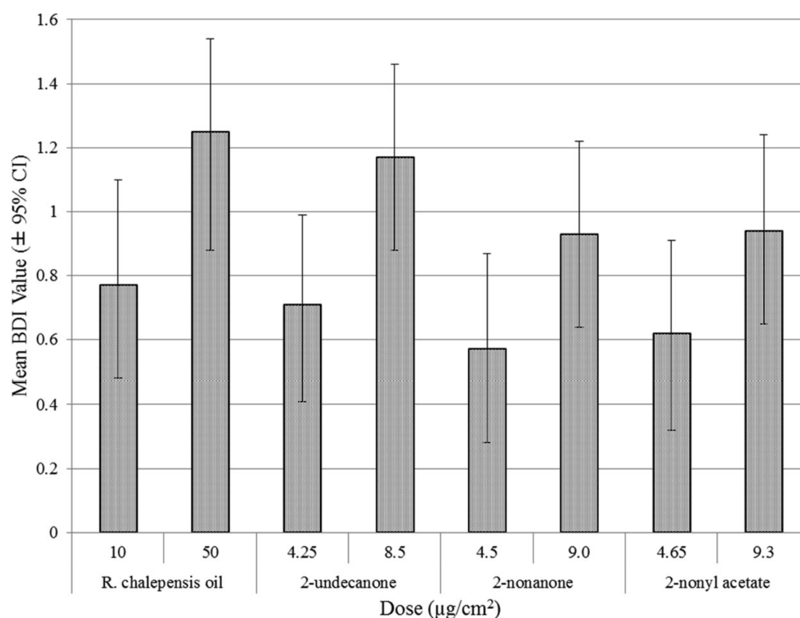

Fig. 1. Biting deterrence index (BDI ± 95% CI) values of *R. chalepensis* essential oil, 2-undecanone, 2-nonanone, and 2-nonyl acetate against *Ae. aegypti* females. A BDI value of 0 indicates an effect similar to ethanol, and BDI values not significantly different from 1 are statistically similar to DEET. Ethanol was the solvent control, and DEET at 4.8 $\mu g/cm^2$ was used as positive control.

with 2.43, 2.8–15.3, 9.1, 2.8–10.4, and 13.8% as the third major component of *R. chalepensis* essential oil, respectively. The percentage of major components found in the current study falls in the range reported by the above researchers. The variations in the composition of the essential oil are likely the result of plant parts used, harvesting time, and geographical location (Merghache et al. 2008).

The *in vitro* K&D system used in this study specifically quantified the mosquito biting-deterrent properties of *R. chalepensis* and its major constituents. The proportion of *Ae. aegypti* not biting ranged between 0.86 and 0.88 in DEET at 4.8 $\mu g/cm^2$ and 0.24 and 0.26 in the solvent control. In *in vitro* bioassay, a mosquito's decision of biting or not appeared to be dependent on properties of the compound (Ali et al. 2013). *Ae. aegypti* females that expressed a knockdown effect when exposed to *R. chalepensis* essential oil at 50 $\mu g/cm^2$ were excluded from the analyses. There were significant differences ($F = 11.7$; df = 7, 119; $P \leq 0.0001$) among the treatments, and mean BDI values of the *R. chalepensis* essential oil and its major compounds (Fig. 1). *R. chalepensis* essential oil at 10 and 50 $\mu g/cm^2$, 2-undecanone at 8.5 $\mu g/cm^2$, 2-nonanone at 9 $\mu g/cm^2$, and 2-nonyl acetate at 9.3 $\mu g/cm^2$ showed biting deterent activity similar to DEET at 4.8 $\mu g/cm^2$, whereas 2-undecanone at 4.25 $\mu g/cm^2$, 2-nonanone at 4.5 $\mu g/cm^2$, and 2-nonyl acetate at 4.65 $\mu g/cm^2$ showed significantly lower biting deterrent activity than DEET.

The biting deterrent effect of *R. chalepensis* essential oil at 50 $\mu g/cm^2$ was similar to DEET at 4.8 $\mu g/cm^2$, whereas biting deterrent activity of 2-undecanone, 2-nonanone, and 2-nonyl acetate was significantly lower than the essential oil and DEET ($F = 11.5$; df = 5, 29; $P \leq 0.0001$; Fig. 2) against *An. quadrimaculatus.* There were no differences between 2-undecanone, 2-nonanone, and 2-nonyl acetate for biting deterrent activity against *An. quadrimaculatus.* Biting deterrent effects of *R. chalepensis* essential oil at 10 $\mu g/cm^2$ and its major compounds at dosages of $\leq$4.7 $\mu g/cm^2$ showed no biting deterrent activity against *An. quadrimaculatus.*

In the cloth patch assay, *R. chalepensis* essential oil showed repellent activity at MED value of 187 ± 0 $\mu g/cm^2$ whereas the major essential oil compound, 2-undecanone, had the lowest MED value of 108.9 ± 71.1 $\mu g/cm^2$.Activity of 2-nonanone (MED = 316.8 ± 141.5 $\mu g/cm^2$) and 2-nonyl acetate (MED = 238.9 ± 141 $\mu g/cm^2$) was similar to 2-undecanone and all three were less potent than DEET (11.1 ± 8.0 $\mu g/cm^2$).

Yarnell and Abascal (2004) reported that *R. chalepensis* essential oil was more effective as repellent against *Ae. aegypti* than neem, but not as effective as lemon eucalyptus (concentration of 40–75%) or pyrethrum. Ikeshoji and Mulla (1974) reported that undecanones showed slight repellency whereas smaller ketones showed good attractancy against *Ae. aegypti and Anopheles albimanus* Wiedemann (Bernier et al. 2001). The larger ketones, such as 2-undecanone, have been shown previously to suppress the attraction of *Ae. aegypti* to L-lactic acid in a laboratory olfactometer (U.R. Bernier, unpublished results). Results from this study corroborate the findings of Barton (2003) and Roe (2004) who reported repellency of 2-undecanone against *Culex quinquefasciatus* (Say) and *Ae. aegypti.* 2-undecanone applied as 30 and 40% solution was

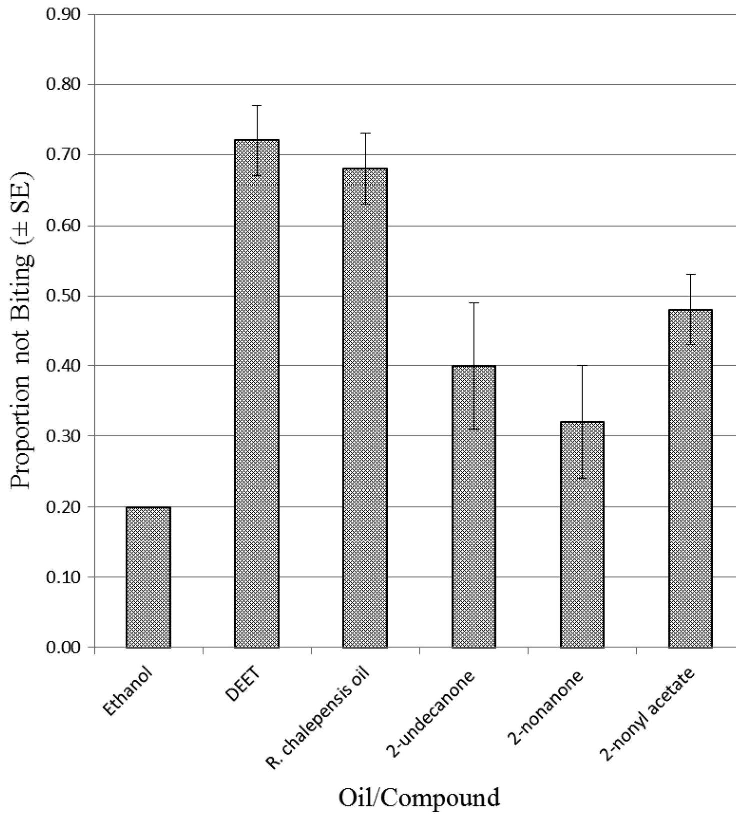

Fig. 2. Biting deterrent effects of DEET at 4.8 μg/cm², *R. chalepensis* essential oil at 50 μg/cm², 2-undecanone at 8.5 μg/cm², 2-nonanone at 9.0 μg/cm², and 2-nonyl acetate at 9.3 μg/cm² against *An. quadrimaculatus*.

100% active on human skin for 15 min against feeding *Ae. aegypti* (Barton 2003), while it showed 100% repellency for 6 h against *Cx. quinquefasciatus* both at 100 and 50% concentrations (Roe 2004). Innocent et al. (2008) also reported 100% protection at a 10% 2-undecanone concentration against *Anopheles gambiae* s.s.

R. chalepensis essential oil showed larvicidal activity against *Ae. aegypti* with LC_{50} value of 22.2 ppm, at 24-h posttreatment (Table 2). Based on nonoverlapping 95% CIs, 2-undecanone with LC_{50} value of 14.37 ppm was significantly more toxic to *Ae. aegypti* than the essential oil. Larvicidal activity of 2-nonanone was significantly lower than *R. chalepensis* essential oil and 2-undecanone, while 2-nonyl acetate which was the third major compound did not show any larvicidal activity. In *An. quadrimaculatus*, larvicidal activity of *R. chalepensis* essential oil (LC_{50} = 14.9 ppm) was statistically similar to 2-undecanone (LC_{50} = 14.2 ppm), while toxicity of 2-nonanone (LC_{50} = 65.5 ppm) was significantly lower. There was a significant difference in mortality between 24- and 48-h posttreatment in *An. quadrimaculatus* larvae.

At 24-h posttreatment, toxicity of *R. chalepensis* essential oil and 2-nonanone was significantly lower in *Ae. aegypti* than *An.quadrimaculatus*, while mortality was similar with 2-undecanone. There was significantly higher mortality in all treatments in *An. quadri-*

Table 2. Toxicity of *R. chalepensis* essential oil and its major constituents against 1-d-old larvae of *Ae. aegypti* and *An. quadrimaculatus* 24- and 48-h posttreatment

Oil/compound	Time[a]	*Ae. aegypti*				*An. quadrimaculatus*			
		LC_{50} ppm (95% CI)[a]	LC_{90} ppm (95% CI)	χ^2	df	LC_{50} ppm (95% CI)	LC_{90} ppm (95% CI)	χ^2	df
Essential oil	24	22.2 (19.4–25.4)	36.6 (31.1–47.5)	47.8	46	14.9 (11.8–18.8)	42.4 (31.2–69.7)	45.9	23
	48	21.7 (19.6–24.1)	36.6 (32.0–44.1)	78	46	>0.49[b]	>22.5[b]	4.3	23
2-undecanone	24	14.37 (12.7–16.3)	27.18 (23.1–34.3)	71.8	48	14.2 (11.3–17.6)	35.0 (26.7–54.9)	39.2	23
	48	12.6 (11.1–14.2)	23.4 (20.0–29.4)	67.9	48	>2.2[b]	>7.1[b]	3.3	23
2-nonanone	24	106.9 (95.2–119.9)	259.67 (220.9–320.3)	140.9	73	65.5 (53.0–81.1)	159.9 (122.0–245.5)	46.8	23
	48	82 3 (73.6–91.9)	185.4 (160.1–223.5)	148.7	73	10.2 (0.8–21.2)	114.2[b]	10.7	23

[a] Time refers to hours posttreatment.
[b] CIs were not estimated because of high mortality at the lower dosages in *An. quadrimaculatus* at 48-h posttreatment.

maculatus than *Ae. aegypti* at 48-h posttreatment. This is the first report on larvicidal activity of *R. chalepensis* essential oil and its major compounds against larvae of these species. Larvicidal activity of the plant essential oils against *Ae. aegypti* has been reported by previous researchers. Kim et al. (2012) reported toxicity (LC_{50}) of essential oils of cassia (58.2 ppm), rosemary (75.0 ppm), lemon eucalyptus (140.1 ppm), lemon grass (78.5 ppm), and xanthoxylum (124.1 ppm) against first instar larvae, while Cavalcanti et al. (2004) reported LC_{50} values of 63, 67, and 60 ppm for *Lippia siodise, Ocimum americanum, Ocimum gratissimum,* respectively, in third instar larvae. The essential oils from different plant sources contain many similar compounds, but in different ratio's, which are thought to be responsible for variability in larvicidal activity. Our data document that *R. chalepensis* oil contains a diverse blend of compounds, with 2-undecanone being the major compound that may be contributing to the larvicidal activity of this oil.

In conclusion, this study revealed that *R. chalepensis* essential oil and its major compounds, 2-undecanone and 2-nonanone, have larvicidal activity against *Ae. aegypti* and *An. quadrimaculatus.* At higher dosage rates, *R. chalepensis* essential oil, 2-undecanone, 2-nonanone, and 2-nonyl acetate all showed biting deterrent activity similar to DEET against *Ae. aegypti,* but the activity of 2-undecanone was significantly less than the essential oil and DEET against *An. quadrimaculatus.* In repellency bioassays, 2-undecanone was the most active compound, confirming the findings of previous repellent research on this compound with biting deterrence data being reported for the first time. At a concentration of 50 $\mu g/cm^2$, *R. chalepensis* essential oil provided knockdown of portion of *Ae. aegypti* adults. *An. quadrimaculatus* knockdown was not observed in any of the treatments. Further investigations will evaluate the toxic effect of *R. chalepensis* essential oil on mosquitoes. Because the composition of *R. chalepensis* essential oil varies depending on the plant parts used, harvesting time, or geographical location, the activity level of the essential oil may depend on the source of the sample. These results indicated that the *R. chalepensis* essential oil and 2-undecanone could be used as repellent against *Ae. aegypti.*

Acknowledgments

We thank Junaid-ur-Rehman, National Center for Natural Products Research, and Natasha M. Agramonte, USDA–ARS, Gainesville, for their technical assistance. We thank James J. Becnel and Dan Kline, USDA–ARS, Gainesville, for supplying mosquito eggs. This study was supported in part by USDA–ARS grant 56-6402-1-612 and Deployed War-Fighter Protection Research Program Grant funded by the U.S. Department of Defense through the Armed Forces Pest Management Board.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Ali, A., C. L. Cantrell, U. R. Bernier, S. O. Duke, J. C. Schneider, and I. Khan. 2012. *Aedes aegypti* (Diptera: Culicidae) biting deterrence: structure-activity relationship of saturated and unsaturated fatty acids. J. Med. Entomol. 49: 1370–1378.

Ali, A., N. Tabanca, B. Demirci, K.H.C. Baser, J. Ellis, S. Gray, B. R. Lackey, C. Murphy, I. A. Khan, and D. E. Wedge. 2013. Composition, mosquito larvicidal, biting deterrent and antifungal activity of essential oils of different plant parts of *Cupressus arizonica var. glabra* ('Carolina Sapphire'). Nat. Prod. Commun. 8: 257–260.

Bagchi, G. D., P. D. Dwivedi, A. Singh, F. Haider, and A. A. Naqvi. 1996. Variations in essential oil constituents at different growth stages of *Ruta chalepensis* L. on cultivation at North Indian Plains. J. Essent. Oil Res. 8: 413–414.

Bagchi, G. D., P. D. Dwivedi, S. Mandal, A. A. Naqvi, and S. Kumar. 2003. Essential oil constituents of *Ruta chalepensis* L. plants grown in India. Indian Perfum. 47: 39–42.

Barnard D. R., U. R. Bernier, R. D. Xue, and M. Debboun. 2007. Standard methods for testing mosquito repellents, pp. 101–108. *In* M. Debboun, S. P. Frances, and D. Strickman (eds.), Insect Repellents Principles Methods, Uses. CRC, Boca Raton, FL.

Barton, V. M. 2003. Trypsin modulating oostatic factor (TMOF) and non-peptidic analogs as novel insecticides and arthropod repellents. M.Sc. thesis, North Carolina State University, Raleigh, NC.

Başer, K.H.C., T. Özek, and S. H. Beis. 1996. Constituents of the essential oil of *Ruta chalepensis* L. from Turkey. J. Essent. Oil Res. 8: 413–414.

Baytop, T. 1999. Therapy with medicinal plants in Turkey, 2nd ed. Nobel Tip Kitapevleri, Istanbul, Turkey.

Bernier, U. R., D. L. Kline, D. R. Barnard, K. H. Posey, M. M. Booth, and R. A. Yost. 2001. Chemical composition that attracts arthropods. U.S. patent 6,267,953. U.S. Patent and Trademark Office, Washington, DC.

Cavalcanti, E.S.B., S. de Morais, M. A. Lima, and E.W.P. Santana. 2004. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti.* L. Mem. Inst. Oswalso Cruz 99: 541–544.

Chandre, F., F. Darrier, L. Manga, M. Akogbeto, O. Faye, J. Mouchet, and P. Guille. 1999. Status of pyrethroid resistance in *Anopheles gambiae* sensu lato. Bull. W.H.O. 77: 230–234.

Daniel, M. 2006. Medicinal plants: chemistry and properties. Science Publishers, Enfiled, NH.

Davis, P. H. 1967. Flora of Turkey and the East Aegean Islands, vol. 2. Edinburgh University Press, Edinburgh, Scotland.

Dob, T., D. Dahmane, B. Gauriat–Desrdy, and V. Daligault. 2008. Volatile constituents of the essential oil of *Ruta chalepensis L. subsp. Angustifolia* (Pers.) P. Cout. J. Essen. Oil Res. 20: 306–309.

Escher, S., Y. Niclass, M. Van de Waaland, and C. Starkenmann. 2006. Combinatorial synthesis by nature: volatile organic sulfur-containing constituents of *Ruta chalepensis* L. Chem. Biodivers. 3: 943–957.

ESO. 2000. The complete database of essential oils, Boelens. Aroma Chemical Information Service, Huizen, The Netherlands.

European Pharmacopoeia, Council of Europe. 2005. European Directorate for the quality of medicines & healthcare. vol. 1, 5th ed. Strasbourg, France, United Kingdom.

Fakhfakh, N., S. Zouari, M. Zouari, C. Loussayef, and N. Zouari. 2012. Chemical composition of volatile compounds and antioxidant activities of essential oil, aqueous and ethanol extracts of wild Tunisian *Ruta chalepensis* L. (Rutaceae). J. Med. Plants Res. 6: 593–600.

Feo, V. D., F. D. Simonea, and F. Senatoreb. 2002. Potential allelochemicals from the essential oil of *Ruta graveolens.* Phytochemistry 61: 573–578.

Gonzalez–Trujano, M. E., D. Carreraa, R. Ventura–Martinezd, E. Cedillo–Portugalb, and A. Navarrete. 2006. Neuropharmacological profile of an ethanol extract of *Ruta chalepensis* L. in mice. J. Ethnobot. 106: 129–135.

Guarrera, P. M. 1999. Traditional antihelmintic, antiparasitic and repellent uses of plants in Central Italy. J. Ethobot. 68: 183–192.

Gunaydin, K., and S. Savci. 2005. Phytochemical studies on *Ruta chalepensis* (Lam.) Lamarck. Nat. Prod. Res. 19: 203–210.

Hadis, M., M. Lulu, Y. Mekonnen, and T. Asfaw. 2003. Field trials on the repellent activity of four plant products against mainly *Mansonia* population in western Ethiopia. Phytother. Res. 17: 202–205.

Ikeshoji, T., and M. S. Mulla. 1974. Attractancy and repellency of alkyi carbonyl compounds for mosquito oviposition. Jap. J. Sanit. Zool. 25: 89–94.

Innocent, E., N. K. Gıkonyo, and M.H.H. Nkunya. 2008. Repellency property of long chain aliphatic methyl ketones against *Anopheles gambiae* s.s. Tanzania. J. Health Res. 10: 50–54.

Joulain, D., and W. A. Koenig. 1998. The atlas of spectra data of sesquiterpene hydrocarbons. EB-Verlag, Hamburg, Germany.

Katritzky, A. R., Z. Wang, S. Slavov, D. A. Dobchev, C. D. Hall, M. Tsikolia, U. R. Bernier, N. M. Elejalde, G. G. Clark, and K. J. Linthicum. 2010. Novel carboxamides as potential mosquito repellents. J. Med. Entomol. 47: 924–938.

Kim, S., J. Yoon, S. Baeck, S. Lee, Y. J. Ahn, and H. W. Kwon. 2012. Toxicity and synergic repellency of plant essential oil mixtures with vanillin against *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 49: 876–885.

Klun, J. A., M. Kramer, and M. Debboun. 2005. A new *in vitro* bioassay system for discovery of novel human-use mosquito repellents. J. Am. Mosq. Control Assoc. 21: 64–70.

Koenig, W. A., D. Joulain, and D. H. Hochmuth. 2004. Terpenoids and related constituents of essential oils, MassFinder 3. *In* D. H. Hochmuth (Ed), Convenient and Rapid Analysis of GC/MS, Hamburg, Germany.

McLafferty, F. W., and D. B. Stauffer. 1989. The Wiley/NBS registry of mass spectral data. J. Wiley, New York, NY.

Merghache, S., M. Hamza, M. Bendahou, and B. Tabti. 2008. Chemical composition and antimicrobial activity of *Ruta chalepensis* L. essential oil from Algeria. Asian J. Chem. 20: 2989–2996.

Michael, J. P. 2005. Quinoline, quinazoline and acridone alkaloids. Nat. Prod. Rep. 22: 627–646.

Ntalli, N. G., F. Ferrari, I. Giannakou, and U. Menkissoglu–Spiroudi. 2011. Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematicidal activity of essential oils from seven plants indigenous to Greece. Pest Manag. Sci. 67: 341–351.

Posey, K. H., and C. E. Schreck. 1981. An airflow apparatus for selecting female mosquitoes for use in repellent and attraction studies. Mosq. News 41: 566–568.

Pridgeon, J. W., K. M. Meepagala, J. J. Becnel, G. G. Clark, R. M. Pereira, and K. J. Linthicum. 2007. Structure-activity relationships of 33 piperidines as toxicants against female adults of *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 44: 263–269.

Pridgeon, J. W., J. J. Becnel, G. G. Clark, and K. J. Linthicum. 2009. A high-throughput screening method to identify potential pesticides for mosquito control. J. Med. Entomol. 46: 335–341.

Roe, R. M. 2004. Method of repelling insects. U.S. patent application 20040242703, Kind Code A 1.

Rustaiyan, A., M. Khossravi, F. S. Lotfabadi., M. Yari., S. Masoudi, and A. Monfared. 2002. Constituents of the essential oil of *Ruta chalepensis* from Iran. J. Essent. Oil Res. 14: 378–379.

SAS Institute. 2007. SAS online doc., version 9.2. SAS Institute, Cary, NC.

Savin, N. E., J. L. Robertson, L. Jacqueline, and R. M. Russell. 1977. A critical evaluation of bioassay in insecticide research: likelihood ratio tests of dose-mortality regression. Bull. Entomol. Soc. Am. 23: 257–266.

Shehadeh, M. B., F. U. Afifi, and S. M. Abu-Hamdah. 2007. Platelet aggregation inhibitors from aerial parts of *Ruta chalepensis* grown in Jordan. Integr. Med. Insights 2: 35–39.

Tounsi, M. S., W. A. Wannes, I. Ouerghemmi, K. Msaada, A. Smaouiand, and B. Marzouk. 2001. Variation in essential oil and fatty acid composition in different organs of cultivated and growing wild *Ruta chalepensis* L. Ind. Crops Prod. 33: 617–623.

Yarnel1, E., and K. Abascal. 2004. Botanical prevention and treatment of malaria: Part 1—Herbal mosquito repellants. Altern. Complement Ther. 10: 206–210.

Received 10 August 2012; accepted 15 August 2013.