Journal of the American Mosquito Control Association, 36(4):227–232, 2020

LARVICIDIDAL ACTIVITY OF NATURAL REPELLENTS AGAINST THE DENGUE VECTOR, *AEDES AEGYPTI*

SHOUGANG ZHANG,[1,2] KAI BLORE,[1] RUI-DE XUE,[1,3] WHITNEY A. QUALLS,[1] STEVE CERMAK[4] AND JUN-WEI ZHU[5]

ABSTRACT. The present research aimed to evaluate the larvicidal activity of several recently discovered natural repellents formulated in lotions against larvae of *Aedes aegypti*. We used a modified larval bioassay method by the World Health Organization standards in evaluating larval mortality at 24-, 48-, and 72-h exposure. Among the test repellents, 2-undecanone showed 100% mortality of *Ae. aegypti* larvae, followed by catnip oil, capric acid, coconut oil fatty acids, methyl caprate, methyl laurate, and coconut oil methyl esters. The repellent, 2-undecanone showed median lethal concentration (LC_{50}) values of 73.07, 26.45, and 15.68 ppm at 24-, 48-, and 72-h exposure, respectively. Larvicidal activity varied among the other repellents tested.

KEY WORDS *Aedes aegypti*, dengue vector, larvicide, mosquito control, natural repellent product

INTRODUCTION

Aedes aegypti (L.), an important public health pest, is widely distributed around tropical and subtropical zones of the world and is currently spreading worldwide (Al-Abri et al. 2019). *Aedes aegypti*, a known vector of chikungunya, dengue, Zika, and yellow fever viruses, is highly adapted to the urban environment, often found within and around households (Liu et al. 2019, Martin et al. 2019). Control of this mosquito species primarily relies on source reduction and massive use of insecticides, which has led to the development of resistance in this container-inhabiting species to organochlorine, organophosphate, carbamate, and pyrethroid insecticides (Amelia-Yap et al. 2018, Al-Abri et al. 2019, Dusfour et al. 2019). Therefore, it is necessary to investigate and develop new mosquito control tools that are environmentally safe and effective to mitigate the insecticide resistance. Botanical products with insecticidal properties can be used in the control of mosquitoes with the goal of interrupting disease transmission and resistance development.

There is an increasing interest in research using botanical products as potential insecticides for the control of pests (Ghosh et al. 2012). Recently, several botanical products have been discovered with strong repellency against adult mosquitoes coupled with larvicidal activity against mosquitoes (Zhu et al. 2006, 2018; Witting-Bissinger et al. 2008; Roh et al. 2020). In the present study, we compared the larvicidal activity of some selected botanical product-based mosquito repellent compounds against *Ae. aegypti* larvae.

MATERIALS AND METHODS

Aedes aegypti (Orlando 1952 susceptible strain) mosquitoes were originally acquired as eggs from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, and have been maintained as colonies at Anastasia Mosquito Control District insectary, St. Augustine, FL. They are reared at 26.6 ± 1°C, 70.0 ± 10% relative humidity, and a 14:10 light:dark (LD) photoperiod. The eggs of mosquitoes were hatched, and larvae reared to late 3rd instar in large plastic trays (12 × 35 × 50 cm), containing distilled water. The larvae were fed a mixture of yeast and powdered fish food.

Botanical repellent compounds (15% 2-undecanone, 2-U; 7% capric acid, C10 FA; 7% coconut fatty acids, FA; 7% methyl caprate, C10 ME; 7% methyl laurate, C12 ME; 7% methyl esters of coconut fatty acids, and 10% catnip oil) were prepared in 2 carriers (Aroma Land® hand and body lotion, unscented (AL) and Coppertone lotion (CL)). The 15 treatments (including 2 controls) are listed in Table 1. All tested samples were prepared before the experiments.

The test products were purchased from the following sources: Aroma Land (AL) hand and body lotion (unscented) from Aroma Land Inc. (Santa Fe, NM); Coppertone® tanning sunscreen lotion, SPF 8, water resistant (80 min) from Bayer HealthCare LLC (Whippany, NJ); boron trifluoride diethyl etherate and 2-undecanone (99%) from Sigma-Aldrich Co. (St. Louis, MO); capric acid (96%) from Acros Organics (Morris Plains, NJ); coconut fatty acid 745 food grade kosher from Acme Hardesty (Blue Bell, PA); lauric acid (97%) from Pfaltz & Bauer (Waterbury, CT); catnip essential oil from Bramble Berry

[1] Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092.
[2] Nanjing Center for Disease Control and Prevention, Nanjing, 210003, China.
[3] To whom correspondence should be addressed.
[4] U.S. Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL 61604.
[5] USDA/ARS, Agroecosystem Management Research Unit, University of Nebraska, Lincoln, NE 68583.

Table 1. Larvicidal activity of natural product repellent compounds against *Ae. aegypti* larvae at a concentration of 1,000 ppm under laboratory conditions.

No. and plant extract	Mortality (%)[1]		
	24 h	48 h	72 h
1			
Control (AL lotion, 0% Ingredient)	0 fg	0.0 def	0.0 de
2-Undecanone in AL	100 a	100 a	100 a
Methyl caprate in AL	100 a	100 a	100 a
Methyl laurate in AL	40 cdef	50 cde	63.3 cd
Methyl esters of coconut FA in AL	10 cfg	20 df	33.3 e
Catnip oil in AL	0 g	36.7 cde	76.7 bc
Control (Coppertone lotion, 0% Ingredient)	6.7 g	30.0 f	66.7 f
2			
2-Undecanone in CL	100 a	100 a	100 a
Capric acid in CL	86.7 ab	100 a	100 a
Coconut FA in CL	50 bcde	80 abc	93.3 ab
Capric acid/coconut FA (1:1) in CL	66.7 abcd	83.3 abc	96.7 a
Methyl caprate in CL	83.3 abc	100 a	100 a
Methyl laurate in CL	33.3 cdfg	73.3 abce	96.7 a
Methyl esters of coconut FA in CL	6.7 fg	23.3 df	50.0 de
10% catnip oil in CL	96.7 a	100 a	100 a

[1] Means with the same letter in a column are not significantly different ($P = 0.05$).

(Bellingham, WA); hexanes, ethyl acetate, sodium sulfate, sodium chloride, sodium phosphate monobasic monohydrate from Fisher Scientific Co. (Fairlawn, NJ); and methanol from EMD Millipore Co. (Billerica, MA), Filter paper Watman No. 54 was purchased from Whatman (Clifton, NJ).

Preparation of synthetic methyl esters

Acid-catalyzed esterification reactions were conducted with solvent in a 1-liter round-bottom flask. A solution of boron trifluoride diethyl etherate (0.4 M and 9.45 ml) in methanol (190.55 ml) was added to 100 g of the starting fatty acid (e.g., capric acid, lauric acid, or coconut fatty acid). The reaction was heated to reflux with a cold condenser. After 24 h, the flask contents were allowed to cool at room temperature and transferred to a separator funnel followed by the addition of 50 ml of a 1:1 ethyl acetate:hexane solution. The pH of the solution was then adjusted to 5.0 to 6.0, using distilled H_2O and a final wash with pH 5 buffer (NaH_2PO_4, 519 g in 4 liter H_2O). The organic layer was then washed with a saturated sodium chloride solution, dried over sodium sulfate, and filtered with Whatman No. 54 filter paper. All reactions were concentrated in vacuo and then Kugelrohr distilled under vacuum (0.013–0.067 kPa) up to 100–110°C to yield a purified and colorless distillate of methyl esters. The final product was then filtered with Whatman No. 54 paper.

Preparation of natural product repellents in carrier lotions

Samples were prepared by weighing each natural product repellent into a tared 118 or 236 ml Qorpak® glass jar depending on the final sample size desired. The corresponding amount and type of carrier lotion was then added to the jar. The samples were then vigorously mixed using a Cat Scientific X120 Handheld Homogenizer Drive with a T10 dispersing tool fitted with a V type generator. Mixing time varied depending on sample size. The samples were mixed from 60 to 70 sec, while larger 148 ml samples were mixed from 120 to 135 sec. The compounds that were solid at room temperature, i.e., capric acid, coconut fatty acid, and the capric/coco fatty acid mixture, were gently heated on a steam bath before weighing and then again immediately before mixing. The homogenizer was wiped clean between each sample and then rinsed with acetone. Additionally, the homogenizer was submerged in a clean jar of acetone and turned on to remove any residual products from the dispersing tool. The remaining acetone was then blown off with the use of an air hose.

The larval bioassay was performed in the laboratory following the WHO Bioassay Guidelines with some modifications (WHO 2005). Ten 3rd instar *Ae. eagypti* were released into a 266 ml clear plastic cup (Dart, No. Tp9r Solo Ultra Clear) containing 99 ml of distilled water and 1 ml of the test material. Three replicates were carried out simultaneously for each treatment. Controls were exposed to the same amount of distilled water only. Treatment and control cups were kept in an incubator (Precision, Low Temperature Illuminated Incubator 818) set at 26.6°C ± 1°C with a 12:12 LD photoperiod. Ambient relatively humidity in the incubators was 50 ± 10%. During the test period, the larvae were not provided with any food. Dead larvae were recorded after 24, 48, and 72 h.

Data were analyzed using SPSS 20.0. The mortality data were subjected to chi-square test and probit analysis for the median lethal concentration

Table 2. LC_{50} and LC_{90} of natural product repellent compounds against 3rd instars of *Aedes aegypti* under laboratory conditions.

Plant extracts	Time (h)	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	95% confidence level of LC_{50}	95% confidence level of LC_{90}	χ^2
15% 2-U in AL	24	219.09	1,514.66	$Y = -3.57 + 1.53x$	—	—	202.50
	48	130.82	959.06	$Y = -3.14 + 1.48x$	—	—	43.54
	72	84.45	760.46	$Y = -2.59 + 1.34x$	11.13–1,031.79	137.736–9,459,542,645	9.83
7% C10 ME in AL	24	558.71	1,265.59	$Y = -9.91 + 3.61x$	—	—	23.13
	48	471.33	1,383.35	$Y = -7.33 + 2.74x$	—	—	17.81
	72	408.32	1,189.28	$Y = -7.21 + 2.76x$	157.14–3,768.00	560.62–72,733,192.41	11.39
15% 2-U in CL	24	73.07	629.74	$Y = -2.55 + 1.37x$	10.8–918.06	135.37–2,910,670.02	7.85
	48	26.45	647.02	$Y = -1.29 + 0.87x$	1.88–1,925.15	81.89–2,268,471,740	10.51
	72[1]	15.68	432.89	$Y = -1.06 + 0.89x$	2.07–175.46	60.16–576,416.23	7.21
7% C10 ME in CL	24	656.67	1,153.17	$Y = -14.76 + 5.24x$	572.00–768.82	944.73–1,637.94	0.10
	48	471.81	721.31	$Y = -18.59 + 6.95x$	419.66–533.26	620.72–939.64	0.86
	72	418.64	572.47	$Y = -24.72 + 9.43x$	378.19–463.15	509.09–705.54	0.02
7% C12 ME in CL	24	962.18	2,424.55	$Y = -9.53 + 3.19x$	474.34–13,759.88	1,255.01–737,850,207.0	17.82
	48	623.36	1,329.75	$Y = -10.89 + 3.90x$	530.50–724.03	1,094.39–1,783.58	3.17
	72	451.17	945.36	$Y = -10.59 + 3.99x$	381.27–525.87	785.60–1,227.65	4.28
10% catnip oil in CL	24	609.77	844.12	$Y = -25.27 + 9.07x$	548.85–682.92	741.53–1,070.25	0.39
	48	411.58	596.18	$Y = -20.82 + 7.96x$	367.76–459.58	522.16–756.68	0.13
	72	390.76	546.86	$Y = -22.76 + 8.78x$	350.58–433.62	483.72–682.73	0.07

[1] The significant difference on the nonoverlapping of 95% fiducial limits.

(LC_{50}) values with 95% confidence level and the 90% lethal concentration (LC_{90}). Analysis of variance (ANOVA) was used in linear regression. The significant difference was calculated at the 0.05 level. The significant differences in LC_{50} and LC_{90} values were based on the nonoverlapping of 95% confidence levels.

RESULTS

The results of mortality from the botanical repellents at 1,000 ppm against larvae of *Ae. aegypti* are presented in Table 1. No mortality was found from the AL lotions. However, larval mortality from 6.7% to 66% was observed for the CL at 24- to 72-h exposure. Highest mortality (100%) was observed with 2-undecanone formulated in both lotions. A significantly higher mortality was also demonstrated by methyl caprate in AL and CL, and capric acid and catnip oil in CL. Mortality of catnip oil in AL lotion was lower than that in CL. Among the test repellent compounds, 2-undecanone showed 100% mortality of *Ae. aegypti* larvae, followed by catnip oil, capric acid, coconut oil fatty acids, methyl caprate, methyl laurate, and coconut oil methyl esters.

The LC_{50} and LC_{90} values of the selected repellent compounds including 2-undecanone, methyl caprate, methyl laurate, and catnip oil in 2 lotions are shown in Table 2. Capric acid, mixture of capric acid/coconut fatty acids, and coconut fatty acid in CL lotion resulted in 50%, 80%, and 90% mortality at 24, 48, and 72 h, respectively; however, no mortality was observed at 500 or 800 ppm of these products, hindering the LC_{50} calculation. The highest larvicidal activity was recorded for 2-undecanone in CL lotion with LC_{50} value of 15.68 ppm at 72-h exposure, followed by its AL lotion with an LC_{50} of 84.45 ppm 72 h posttreament. The repellent, 2-undecanone and methyl caprate in AL lotion showed large deviations in mortality at lower concentrations, which also resulted in no LC_{50} values at 24- and 48-h exposure. At 72 h, larvicidal efficacy of 2-undecanone was found to be significantly different, compared with other products ($P < 0.05$). All LC_{90} of these repellent compounds in lotions showed no significant difference.

The dose response of the 6 repellent products against *Ae. aegypti* larvae is shown in Fig. 1. The log dose-probit mortality responses to the lotions were correlated ($0.695 \leq R^2 \leq 0.934$, $P < 0.05$). Out of all the tested repellents, 2-undecanone was found to perform with the best larvicidal activity, followed by catnip oil, methyl caprate, and methyl laurate.

DISCUSSION

Plant extracts including natural product repellents have been reported to exhibit insecticidal activity including larvicidal, adulticidal, ovicidal. These could be used to develop promising new insecticide formulations that are biodegradable and nontoxic to nontarget organisms, thus presenting a significant potential for future integrated vector management (IVM) programs (Pavela et al. 2019). The results of this study employing different botanical product repellents in 2 lotions against larvae of *Ae. aegypti* indicate their unique use, in addition to adult repellency (Zhu et al. 2018).

Our study has demonstrated strong larvicidal activity against *Ae. aegypti* with LC_{50} values of 73.07, 26.45, and 15.68 ppm after 24-, 48-, and 72-h exposure, respectively. The repellent 2-undecanone, a well-known natural product repellent identified from tomato skin, is considered a broad spectrum

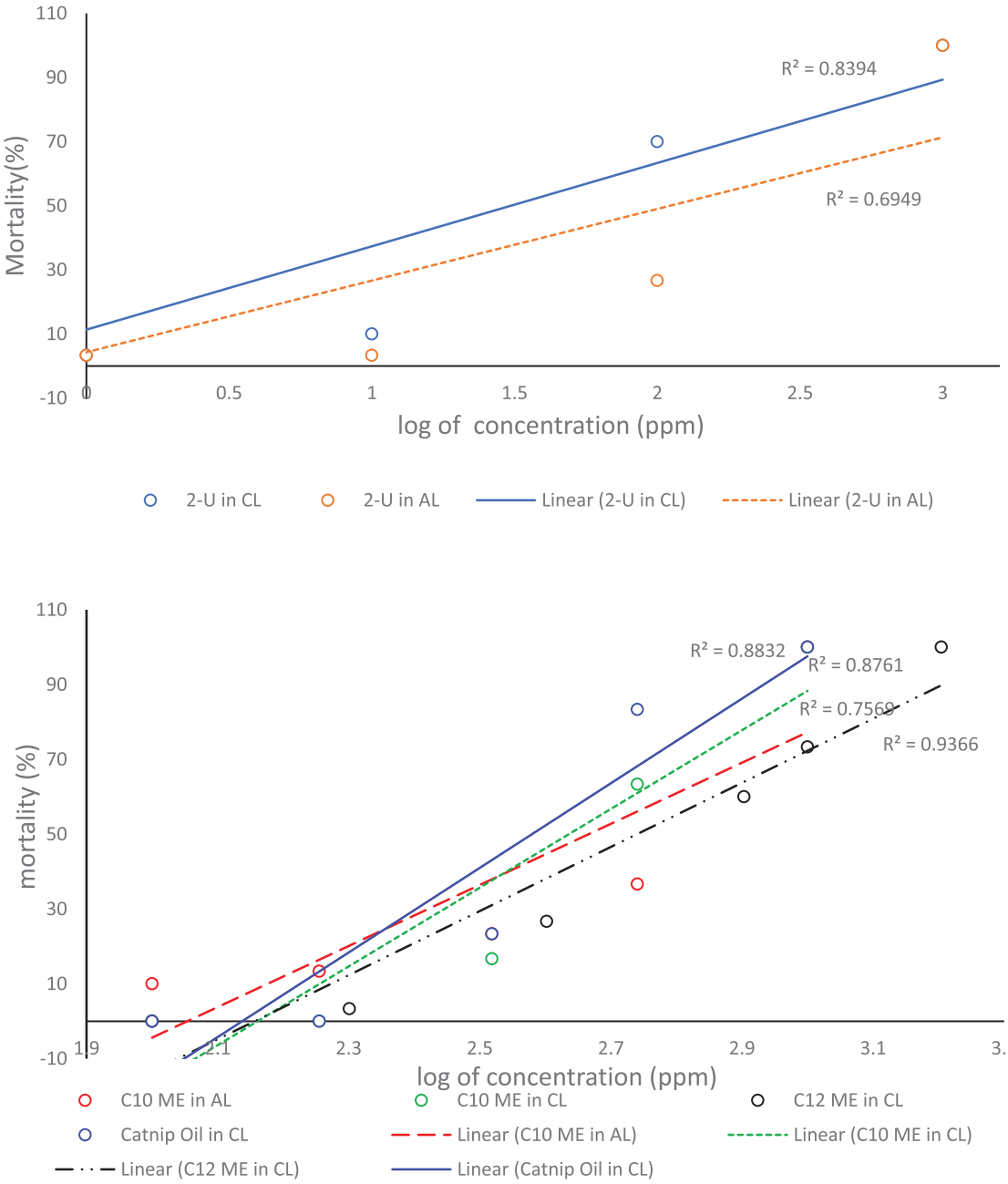

Fig. 1. Regression graphs of repellent lotions against larval *Aedes aegypti* exposure at 48 h. Methyl caprate (C10 ME), methyl laurate (C12 ME).

arthropod repellent (Witting-Bissinger et al. 2008) when used in large amounts with commercial formulations at concentrations ranging from 5% to 20%. The compound 2-undecanone, used as one of the major components at 43.7% in *Ruta chalepensis* L., repels mosquitoes, ticks, and other insects effectively (Pérez López et al. 2015). The compound 2-undecanone has been reported to elicit responses by the octenol receptor (C Neuron) located on the maxillary palps of *Ae. aegypti* (Grant and Dickens 2011).

Previous studies have demonstrated catnip oil to possess strong spatial repellency against various adult mosquitoes in addition to its larvicidal impacts (Bernier et al. 2005, Zhu et al. 2006). Bernier et al. (2005) showed 100% knockdown and 76–100%

mortality of adult *Ae*. *aegypti* when exposed to 2–3% catnip formulation. A subsequent study by Sathantriphop et al. (2015) used catnip concentrations ranging between 5% and 10% to achieve 100% knockdown and mortality as well. Similarly, EZ-nepetalactone and ZE-nepetalactone, 2 primary compositional compounds of catnip oil, showed effective repellency and larvicidal activity against *Aedes* mosquitoes (Zhu et al. 2006, Polsomboon et al. 2008). Crude catnip oil (0.01–1.00%) at lower concentrations provided up to 97.2% repellency against adult mosquitoes (Reichert et al. 2019).

Zhu et al. (2018) reported capric and lauric acids derived from coconut oil as novel, inexpensive, and highly efficacious repellent compounds that are active against a broad array of blood-sucking arthropods, including biting flies, ticks, bed bugs, and mosquitoes. Although the coconut fatty acids exhibited strong repellency against biting flies, bed bugs, and ticks, a relatively high concentration of the coconut fatty acids was required at the minimum effective dosage in comparison to *N*,*N*-diethyl-3-methylbenzamide (Deet) in order to prevent biting by the yellow fever mosquitoes. Recently, Roh et al. (2020) has reported that methyl caprate and laurate act as strong repellents against biting flies, *Stomoxy calcitrans* (L.). In the present study, we have demonstrated that these 2 methyl esters in AL and CL possess a weak larvicidal activity. However, capric acid in lotions was effective at 1,000 ppm.

Foley and Frances (2005) reported methylated coconut oil as toxic to both *Anopheles farauti* (Laveran) and *Culex annulirostris* (Skuse). In the present study, methyl esters of coconut fatty acids showed lower larvicidal activity in AL or no effectiveness in CL (lotions). This could be explained by the fact that different formulations have different insecticidal effect on various mosquito species.

In summary, natural product repellents formulated in lotions against adult mosquitoes can also be applied to control mosquito larvae. However, additional factors, such as chemistries of different carriers, may result in significant differences in larvicidal activity. More studies are needed to fully understand the larvicidal mechanisms. Further spatial and contact repellency tests from the test repellent lotions are underway to be evaluated under outdoor conditions against *Ae. aegypti*.

ACKNOWLEDGMENTS

The authors thank N. Acevedo, M. Pearson, and L.M. Bangonan for their technical assistance during the course of this study.

REFERENCES CITED

Al-Abri SS, Kurup PJ, Al Manji A, Al Kindi H, Al Wahaibi A, Al Jardani A, Mahmoud OA, Al Balushi L, Al Rawahi B, Al Fahdi F, Al Siyabi H, Al Balushi Z, Al Mahrooqi S, Al Manji A, Al Sharji A, Al Harthi K, Al Abri B, Al-Raidan A, Al Bahri Z, Al-Mukhaini S, Amin M, Prasanna AR, Petersen E, Al Ajmi F. 2019. Control of the 2018–2019 dengue fever outbreak in Oman: a country previously without local transmission. *Int J Infect Dis* 90:97–103.

Amelia-Yap ZH, Chen CD, Sofian-Azirun M, Low VL. 2018. Pyrethroid resistance in the dengue vector *Aedes aegypti* in Southeast Asia: present situation and prospects for management. *Parasit Vectors* 11:332–348.

Bernier UR, Furman KD, Kline DL, Allan SA, Barnard DR. 2005. Comparison of contact and spatial repellency of catnip oil and N, N-diethyl-3-methylbenzamide (deet) against mosquitoes. *J Med Entomol* 42:306–311.

Dusfour I, Vontas J, David JP, Weetman D, Fonseca DM, Corbel V, Raghavendra K, Coulibaly MB, Martins AJ, Kasai S, Chandre F. 2019. Management of insecticide resistance in the major *Aedes* vectors of arboviruses: advances and challenges. *PLoS Negl Trop Dis* 13:e0007615.

Foley DH, Frances SP. 2005. Laboratory evaluation of methylated coconut oil as a larvicide for *Anopheles farauti* and *Culex annulirostris*. *J Am Mosq Control Assoc* 21:477–479.

Ghosh A, Chowdhury N, Chandra G. 2012. Plant extracts as potential mosquito larvicides. *Indian J Med Res* 135:581–598.

Grant AJ, Dickens JC. 2011. Functional characterization of the octenol receptor neuron on the maxillary palps of the yellow fever mosquito, *Aedes aegypti*. *PLoS One* 6:e21785.

Liu P, Lu L, Jiang J, Guo Y, Yang M, Liu Q. 2019. The expanding pattern of *Aedes aegypti* in southern Yunnan, China: insights from microsatellite and mitochondrial DNA markers. *Parasit Vectors* 12:561–572.

Martin E, Borucki MK, Thissen J, Garcia-Luna S, Hwang M, Wise de Valdez M. 2019. Mosquito-borne viruses and insect-specific viruses revealed in field-collected mosquitoes by a monitoring tool adapted from a microbial detection array. *Appl Environ Microbiol* 85:e01202–01219

Pavela R, Maggi F, Iannarelli R, Benelli G. 2019. Plant extracts for developing mosquito larvicides: from laboratory to the field, with insights on the modes of action. *Acta Trop* 193:236–271.

Pérez López LA, de la Torre YC, Cirio AT, de Torres NW, Flores Suárez AE, Aranda RS. 2015. Essential oils from *Zanthoxylum fagara* Wild Lime, *Ruta chalepensis* L. and *Thymus vulgaris* L.: composition and activity against *Aedes aegypti* larvae. *Pak J Pharm Sci* 28:1911–1915.

Polsomboon S, Grieco JP, Achee NL, Chauhan KR, Tanasinchayakul S, Pothikasikorn J, Chareonviriyaphap T. 2008. Behavioral responses of catnip (*Nepeta cataria*) by two species of mosquitoes, *Aedes aegypti* and *Anopheles harrisoni*, in Thailand. *J Am Mosq Control Assoc* 24:513–519.

Reichert W, Ejercito J, Guda T, Dong X, Wu Q, Ray A, Simon JE. 2019. Repellency assessment of *Nepeta cataria* essential oils and isolated nepetalactones on *Aedes aegypti*. *Sci Rep* 9:1524–1532.

Roh GH, Zhou XJ, Wang YS, Cermak SC, Kenar JA, Lehmann A, Han BY, Taylor DB, Zeng XP, Park CG, Brewer GJ, Zhu JJ. 2020. Spatial repellency, antifeedant activity and toxicity of three medium chain fatty acids and their methyl esters of coconut fatty acid against stable flies. *Pest Manage Sci* 76:405–414.

Sathantriphop S, Achee NL, Sanguanpong U, Chareonviriyaphap T. 2015. The effects of plant essential oils on escape response and mortality rate of *Aedes aegypti* and *Anopheles minimus*. *J Vector Ecol* 40:318–326.

WHO [World Health Organization]. 2005. Guidelines for laboratory and field testing of mosquito larvicides. Geneva, Switzerland: World Health Organization.

Witting-Bissinger BE, Stumpf CF, Donohue KV, Apperson CS, Roe RM. 2008. Novel arthropod repellent, BioUD, is an efficacious alternative to deet. *J Med Entomol* 45:891–898.

Zhu JW, Cermak SC, Kenar JA, Brewer G, Haynes KF, Boxler D, Baker PD, Wang D, Wang C, Li AY, Xue RD, Shen Y, Wang F, Agramonte NM, Bernier UR, de Oliveira Filho JG, Borges LMF, Friesen K, Taylor DB. 2018. Better than DEET repellent compounds derived from coconut oil. *Sci Rep* 8:14053.

Zhu JW, Zeng X, Ma Y, Liu T, Qian K, Han Y, Xue S, Tucker B, Schultz G, Coats J, Rowley W, Zhang A. 2006. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *J Am Mosq Control Assoc* 22:515–522.