Journal of the American Mosquito Control Association, 34(3):210–216, 2018

RAPID IMMOBILIZATION OF ADULT *AEDES AEGYPTI* CAUSED BY PLANT ESSENTIAL OILS AT SUBLETHAL CONCENTRATIONS

EDMUND J. NORRIS,[1] MARIA ARCHEVALD-CANSOBRE,[1] AARON D. GROSS,[1,2] LYRIC C. BARTHOLOMAY[1,3] AND JOEL R. COATS[1,4]

ABSTRACT. Many synthetic insecticides cause immobilization in insect pests after they are exposed. This immobilization or knockdown is an important feature of intoxication that contributes to the abatement of pest insect populations, while preventing vectors of disease from biting and spreading pathogenic organisms to susceptible individuals. We have previously demonstrated that certain plant essential oils rapidly immobilize adult female mosquitoes that have been exposed via topical application. To further characterize this effect, adult female *Aedes aegypti* were exposed to multiple concentrations of 32 commercially available plant essential oils, and immobilization at 1 h after exposure was recorded. The dose required to produce the 1-h knockdown effect in 50% of the test population (KD_{50}) was calculated and compared with concentrations of each plant essential oil that caused mortality at 24 h. In the current study, multiple plant essential oils caused high percentage knockdown at 1 h at lower concentrations than concentrations that caused mortality at 24 h. Moreover, delayed mortality was observed in mosquitoes that were exposed to various concentrations of the 2 plant essential oils that produced significant knockdown at 1 h. These observations demonstrate an important characteristic of many plant essential oils and represent a novel means for which these oils may be incorporated into future insecticidal formulations.

KEY WORDS *Aedes aegypti*, plant essential oils, terpene, insecticides, knockdown

INTRODUCTION

Synthetic insecticides are immensely important in combatting mosquito populations throughout the world (Gramiccia and Beales 1988; Roberts et al. 2000; Lengeler 2004; Mabaso et al. 2004; WHO 2006, 2013). In particular, pyrethroid, organophosphate, and organochlorine insecticides have been the primary tools by which wild mosquito populations have been controlled (Nauen 2007, Pocquet et al. 2013). By promoting the continued influx of sodium ions across the membrane of neurons in insects, pyrethroid insecticides cause hyper-excitability of the insect nervous system, contributing to spastic paralysis and death (Narahashi 1962). Because pyrethroids readily migrate across the insect cuticle and diffuse throughout the insect, they exert toxic effects minutes after the target insect comes into contact with the compounds (Narahashi 1972). One important symptom of pyrethroid intoxication is rapid immobilization (knockdown). Unlike some slow-acting insecticides, pyrethroids are ideal for public health vector control because this knockdown effect immediately prevents mosquitoes from feeding on future hosts (Norris et al. 2015, Gross et al. 2017a). Moreover, it likely contributes to the mortality of the intoxicated insect through various scenarios. As suggested in a previous study, this knockdown may lead to increased desiccation, susceptibility to predation, and the inhibition of grooming (which mitigates the accumulation of fungal spores on the insect), all of which may lead to higher levels of mortality in field applications. As such, this knockdown effect is a crucial consideration in the development of future insecticidal formulations.

One of the obstacles facing the identification of new insecticidal formulations for the control of mosquito populations is ensuring that these new mixtures will be fast-acting and cause high mortality to the target species while simultaneously being safe for application in residential areas. Although some potent synthetic insecticides exist that cause rapid intoxication by interfering with the normal physiology of the insect nervous system, their high toxicity to other nontarget organisms and relatively long half-lives in the environment can prevent them from being viable avenues to follow in the search for new mosquito adulticides. Moreover, registration of new insecticides for the control of adult mosquitoes is prohibitively costly (Rose 2001, Ridley et al. 2017). It is paramount to identify candidate insecticides that have low mammalian toxicity, degrade rapidly, and still quickly immobilize and kill mosquitoes. Some plant essential oil compounds manifest these prerequisite characteristics due to their low cost, ready availability, rapid degradation in the environment, and safety to mammals (Isman 2011). Because of the rapid development of resistance to many of the currently available insecticides on the market (Rose 2001, Oxborough 2016), these plant essential oil components may be viable alternatives in future insecticidal formulations.

[1] Department of Entomology, Iowa State University, Ames, IA 50011.
[2] Present address: Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.
[3] Present address: Department of Pathobiological Sciences, University of Wisconsin, Madison, WI 53706.
[4] To whom correspondence should be addressed.

The bioactivity of plant essential oils is rooted in diverse molecular mechanisms of action that lead to rapid knockdown and/or mortality of exposed insects. These active sites include, but are not limited to, octopamine receptors, tyramine receptors, acetylcholinesterase, nicotinic acetylcholine receptors, and gamma-aminobutyric acid-gated chloride channels (Essam 2001, Anderson and Coats 2012, Tong et al. 2012, Gross et al. 2017b). Numerous other studies suggest that other sites of toxic action may be involved. Because some compounds making up the most common plant essential oils are neuroactive in various insect models, their utility as insecticides and their various modes of insecticidal action need to be further characterized. Due to the unique mechanisms of action of essential oils compared with other currently utilized synthetic insecticides, cross-resistance with synthetic insecticides on the market today is unlikely (Norris et al. 2015, Gross et al. 2017a). Plant essential oils also contain multiple terpenoids, which all may possess their own mechanisms of insecticidal action (Isman 2000). The development of resistance to these plant oil insecticides may be delayed due to the multiple mechanisms of action represented by different terpenoids within the formulation (Georghiou 1994). Instead, plant essential oils, and particularly the terpenoids they are made up of, may represent novel approaches in the field of vector control.

Our lab has identified numerous plant essential oils that cause rapid knockdown when applied topically to adult female *Aedes aegypti* L. alone or in combination with synthetic insecticides (Norris et al. 2015, Gross et al. 2017a). Moreover, these plant essential oils cause significant knockdown in combination with other synthetic insecticides. This effect is described as the immobilization or the inability of exposed mosquitoes to orient in the upright posture. We postulate that by more completely characterizing this effect, it will be possible to further explore the potential of natural products in the control of public health pests. For this study, we screened numerous plant essential oils with the goal of identifying which compounds cause knockdown at 1 h after exposure at low concentrations. Furthermore, our goal was to compare this knockdown effect to the 24-h mortality from the same plant oils to determine whether this knockdown effect occurred at comparable exposure thresholds as the toxicity of these oils. Finally, to evaluate whether knockdown may cause some level of delayed lethality in the laboratory, we monitored the 24-h survival of mosquitoes that were immobilized immediately postexposure.

MATERIALS AND METHODS

Mosquitoes

Adult *Ae. aegypti* mosquitoes (Liverpool strain) were reared at a constant temperature of 27°C and 70% RH, and a photoperiod of 16:8 h light:dark. This strain is susceptible to pyrethroids and other insecticides. A 10%-sucrose solution was provided to mosquitoes ad libitum via cotton pads that were replaced every other day to prevent the buildup of mold and to supply fresh water and sucrose. Adult mosquitoes in the colony cage were blood-fed once per wk to promote egg laying using defibrinated sheep blood (Hemostat Laboratories, Dixon, CA) via an artificial membrane feeder. Four days after blood feeding, eggs were collected from colony cages and were stored at 27°C and 70% RH. Eggs were hatched in an enamel pan with deionized water, and larvae were supplied different amounts of TetraMin Tropical Flakes Fish Food (Tetra, Blacksburg, VA) based on their developmental stage.

Pupae were collected, size separated using a pupal separator adjusted to allow male pupae to flow through (as they are smaller than female pupae), and placed in 1-pint (473.2 ml) cartons of separate male and female mosquitoes. Only female mosquitoes were used for bioassays.

Topical application

Similar methods as described in Norris et al. (2015) were used for the topical application of various doses of 32 plant essential oils (Table 1) to the pronotum of adult, female *Ae. aegypti* with some minor exceptions. This study differed in the doses of plant essential oils used and the duration of monitoring. Concentrations used caused between 5% and 95% knockdown at 1 h. The dose response of knockdown effect was then compared with the concentrations that caused mortality at 24 h.

Survival curves for the 2 most successful oils (clove leaf and patchouli) were compared with each other within each experiment to see if delayed mortality was caused by significant knockdown (i.e., statistically significant from the control at 0.2 μl of acetone). Experiments consisted of applying either an effective dose (ED_{05} or ED_{75}) as compared to a control treatment for the 2 most successful plant essential oils to 30 individual *Ae. aegypti* females. The ED_{05} was used as an estimation of the no adverse effects level of the plant essential oil. This dosage was compared with the effective concentration (EC_{75}) of each oil to identify whether 1-h KD would also produce delayed lethality.

Data analysis

Data were analyzed using a log-probit method described previously by Finney (1971) by using a PROBIT protocol in SAS (SAS version 9.4; Cary, NC) An OPTC correction was used to account for the normal (control) response. A minimum of 5 concentrations were used that caused between 5% and 95% 1-h KD with 3 biological replicates. This number of replications was increased until the Pearson chi-squared value was large enough to correspond to a P value of less than 0.05. Calculated KD_{50} values were compared with the fiduciary limits associated with

Table 1. The KD_{50} after topical exposure to multiple plant essential oils compared to the calculated LD_{50} (mortality recorded at 24 h).

Treatment	*n*	Slope (SE)	KD_{50}[1] (µg/g)	95% FL[1]	χ^2 (df)[2]	LD_{50}[3] (µg/g)	Less than or greater than LD_{50} (+/−)
Patchouli	1,225	1.38 (0.40)	210	6.8–610	315.5 (35)	1,500	−
Clove leaf	925	5.10 (0.91)	2,000	1,500–260	78.4 (30)	4,200	−
Cinnamon leaf	1,000	5.11 (0.77)	2,100	1,700–2,400	67.2 (31)	4,200	−
Clove bud	875	3.90 (0.96)	2,100	1,200–2,400	168.1 (23)	4,100	−
Cassia	825	2.81 (0.57)	2,300	1,400–3,200	112 (23)	3,300	
Thyme	650	5.52 (0.93)	2,600	2,200–2,900	58 (17)	3,400	−
Origanum	875	4.42 (0.63)	2,700	2,200–3,100	47 (22)	3,500	
Litsea cubeba	800	5.07 (1.24)	3,200	2,500–3,800	137.6 (23)	3,400	
Cinnamon bark	550	5.53 (0.98)	3,500	2,900–4,000	67.8 (17)	3,700	
Geranium (bourbon)	950	5.52 (0.77)	4,600	4,000–5,100	111.3 (29)	6,000	
Lemongrass	600	5.73 (0.89)	4,700	4,000–5,300	64.2 (18)	4,900	
Citronella (Java)	1,175	5.22 (0.80)	4,800	4,200–5,500	229 (39)	4,500	
Guaiacwood	775	3.40 (0.58)	5,100	3,500–6,700	117.3 (22)	10,500	−
Sandalwood	825	2.50 (0.87)	5,300	3,400–8,000	107 (26)	3,600	
Catnip	525	7.36 (2.5)	6,000	2,600–7,400	109.56 (15)	9,000	−
Amyris	825	3.63 (0.49)	6,100	5,200–7,200	79.64 (23)	9,400	
Basil (Egyptian)	975	7.39 (0.84)	9,000	8,300–9,700	69 (30)	10,900	−
Celery seed	950	5.36 (0.67)	9,000	8,000–9,900	50.4 (27)	14,600	−
Peppermint	775	3.35 (0.79)	9,400	5,000–12,000	123.1 (21)	12,700	
Cedar leaf	650	5.89 (1.4)	11,000	9,700–13,000	108.9 (18)	10,500	
Cedarwood (Texas)	600	5.44 (1.0)	11,000	9,700–1,200	31.3 (18)	10,700	
Sassafras	975	1.53 (0.45)	11,000	1,000–25,000	91.4 (26)	40,400	−
Anise seed	675	3.45 (0.57)	12,000	8,100–15,000	31.3 (20)	11,600	
Cedarwood (Moroccan)	1,050	4.43 (1.05)	18,000	16,000–23,000	154.1 (32)	12,700	+
Nutmeg (East Indies)	850	4.63 (1.2)	19,000	10,000–23,000	149.7 (25)	33,000	−
Nutmeg (West Indies)	700	7.34 (1.00)	21,000	19,000–22,000	12.03 (20)	20,200	
Rosemary	575	4.34 (1.16)	21,000	10,000–27,000	48.96 (16)	33,000	−
Wormwood	700	5.03 (1.29)	21,000	14,000–25,000	88.3 (20)	20,200	
Wintergreen	975	3.11 (0.60)	22,000	15,000–26,000	93.3 (26)	39,700	−
Orange	1,850	4.56 (1.54)	31,000	27,000–40,000	478.03 (22)	22,500	+
Sesame	725	2.89 (0.78)	32,000	24,000–70,000	67.2 (22)	15,000	+
Black pepper	875	9.05 (1.08)	34,000	32,000–36,000	72.8 (24)	31,500	

[1] All LD_{50} and KD_{50} values were calculated using an average weight of 2.54 mg/female mosquito ($n = 256$ mosquitoes).
[2] Pearson chi-square goodness-of-fit values with degrees of freedom. Degrees of freedom are used to calculate significance in the model at a threshold of ($P < 0.05$).
[3] Values reported from Norris et al. (2015).

the LD_{50} values to determine whether the 1-h KD caused by the plant essential oil occurred at statistically lower concentrations than those that correspond to lethality. The ED_{05} and ED_{75} values were calculated using the PROC PROBIT protocol in SAS described in the data analysis section of this article.

Correlation analysis of KD_{50} and LD_{50} values for each of the plant essential oils screened in this study was performed in SAS 9.4 using a PROC CORR protocol. The Pearson correlation coefficient was calculated along with the *P* value associated with the model. A P value ≤ 0.05 indicated a significant correlation between the above variables.

Kaplan-Meier survival curves were generated using GraphPad Prism (GraphPad Prism version 5.04; GraphPad, La Jolla, CA). Survival curves for the 2 most successful oils (clove leaf and patchouli) were compared within each experiment to test the null hypothesis that each curve was statistically similar to one another and the control treatment (0.2 µl of acetone) at a value of $\alpha = 0.05$.

RESULTS

The 5 most successful plant essential oils that provided knockdown (measured as KD_{50}) values of less than 3,000 ppm (µg/g mosquito) in *Ae. aegypti* were patchouli, clove leaf, cinnamon leaf, clove bud, and cassia oil, with KD_{50} values of 210, 2,000, 2,100, 2,100, and 2,300 µg/g, respectively (Table 1). Of all the oils screened, the most potent (patchouli) produced a KD_{50} value of 210 ppm, whereas the least potent (black pepper) produced a KD_{50} value of 34,000 ppm in *Ae. aegypti*. Comparing the knockdown effectiveness of both oils at this level we found that patchouli was more than 161-fold more toxic than black pepper.

Overall, plant essential oils produced lower KD_{50} values than LD_{50} values, with some exceptions.

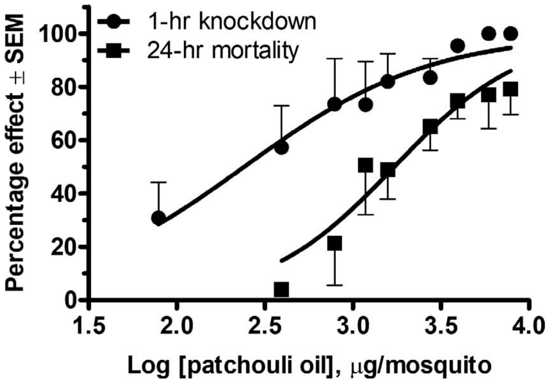

Fig. 1. Dose response of patchouli oil applied topically to adult, female *Aedes aegypti*.

Among all plant essential oils screened, 16 caused KD_{50} values at concentrations that were dissimilar to concentrations that caused mortality. An example of the differences between the 1-h KD and 24-h mortality dose response is patchouli oil (Fig. 1). It is evident that significant 1-h KD occurs at lower concentrations than those that lead to mortality at 24 h, as evidenced by the lack of 95% confidence interval overlap between the 2 oils (Table 1). Among all the plant essential oils screened, 13 caused 1-h KD at concentrations that were lower than those that cause mortality (patchouli, clove leaf, cinnamon leaf, clove bud, thyme, guaiacwood, catnip, basil [Egyptian], celery seed, sassafras, nutmeg [East Indies], rosemary, and wintergreen oil) (Table 1). Alternatively, cedarwood (Moroccan), orange, and sesame oil caused 1-h KD at concentrations that were higher than those that caused mortality.

That said, we found that a Pearson correlation coefficient of 0.723 was observed (Fig. 2), indicating that there is a strong positive, direct relationship between the KD_{50} value and the LD_{50} value of each oil. The P value of less than 0.001 suggests that the model accurately describes the correlation of these 2 datasets.

Adult mosquitoes exposed to clove leaf or patchouli oil (both being the 2 most capable oils causing sublethal knockdown at 1 h) when applied at either a KD_{05} or KD_{75} resulted in delayed mortality (Fig. 3) compared to controls. Approximately 87% (26 out of 30) of acetone (control) -exposed *Ae. aegypti* survived at the end of the 9-day experimental interval (Fig. 3A, 3B). This was in stark contrast to 30% (9 out of 30) and 40% (12 out of 30) of surviving mosquitoes that were exposed to the KD_{05} value for clove leaf oil and patchouli oil, respectively. Curves of these 2 oils were statistically significant from the control at that level but were equivalent to each other. Similar to the ED_{05} level, mosquitoes treated with an ED_{75} of patchouli oil resulted in 23% survival (7 out of 30) after 9 days. As for the ED_{75} exposure of clove leaf, 37% of mosquitoes (11 out of 30) survived until the 9th experimental day. For the KD_{05} and KD_{75} of both oils, survival significantly differed from the control at day 9. Survival curves for mosquitoes exposed to clove leaf and patchouli were significantly different compared to the control group ($P < 0.05$).

DISCUSSION

Based on our bioassay screening data, numerous plant essential oils caused significant knockdown at 1 h after individual mosquitoes were exposed to them. This knockdown is unique to mortality as it occurs at distinct concentrations from those that cause mortality. The most potent plant essential oils that cause knockdown in *Ae. aegypti* were patchouli, clove leaf, cinnamon leaf, clove bud, and cassia (Table 1). The KD_{50} values of these oils ranged from 210 to 2,300 ppm compared with the range in LD_{50} values of 1,500–4,200 ppm. The lower dosages required to cause knockdown in these oils may be an indicator for utility in mosquito control programs. Even at low doses, these plant essential oils rapidly immobilize adult mosquitoes; it is likely that this would prevent mosquitoes from blood feeding and thereby potentially transmitting pathogens. However, rapid immobilization of adult mosquitoes may allow for their use in future insecticidal formulations. By rapidly immobilizing exposed mosquitoes, plant oils may prevent insects from transmitting pathogens. Rapidly preventing mosquitoes from feeding on vertebrate hosts is an important characteristic of all successful public health pest insecticides. Furthermore, this immobilization may lead to death due to other environmental processes. These might include increased levels of predation, increased toxicity from entomopathogenic fungi due to decreased grooming, and desiccation from the inability to seek water. This phenotype is best illustrated when mosquitoes are exposed to patchouli oil (Fig. 1). Here it is evident that the concentrations required to cause this knockdown are much lower than those required to cause mortality at 24 h. Moreover, the concentrations that induce knockdown can be as low as 79 ppm, which corresponded to a 1-h KD value of approximately 30 ± 14% (for patchouli oil). These concentrations that cause bioactivity are comparable to concentrations at which synthetic insecticides are active (Pridgeon et al. 2008).

Out of the 32 oils screened in this study, 13 caused knockdown at concentrations that were significantly lower than the lethal concentrations. The mode or modes of action that underpin this knockdown is not known. It is possible that these plant essential oils and their constituents are active at various nervous system targets and are readily detoxified after this initial bioactivity. This may lead to inactivation and excretion of these components from the insect, which would necessitate higher doses in order to lead to mortality. Waliwitiya et al. (2009) illustrated that the toxicity of terpenoids, the primary constituents of a majority of plant essential oils, were more toxic after

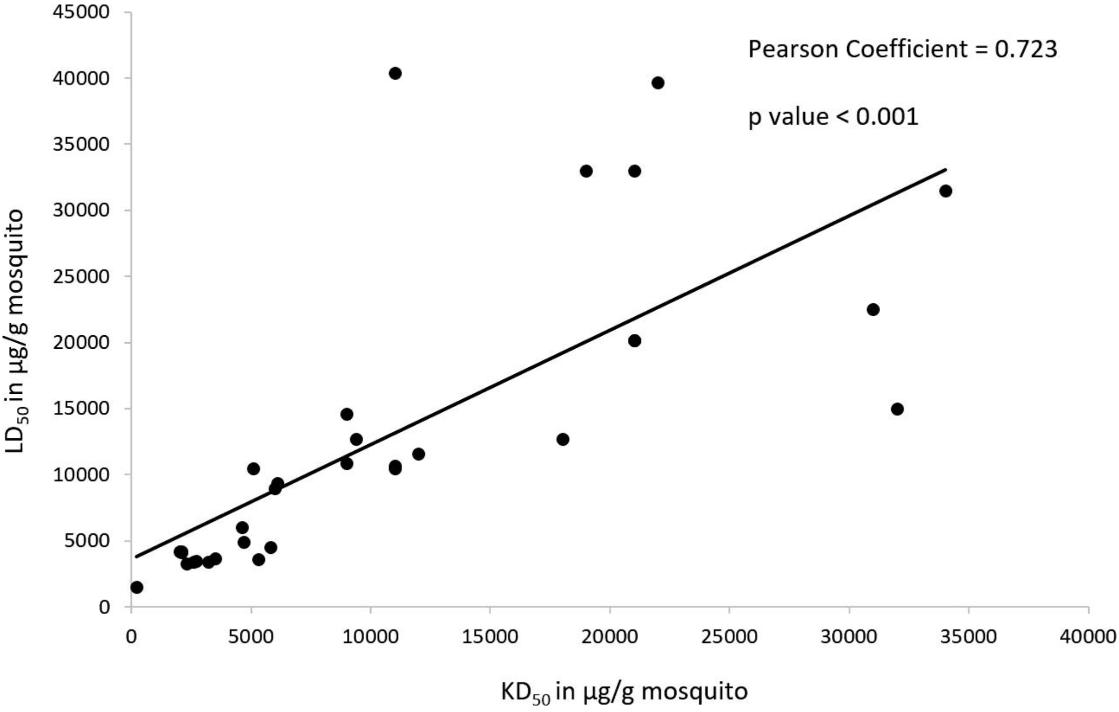

Fig. 2. Plot of KD_{50} values plotted with respect to LD_{50} values for each plant oil screened in this study. The Pearson correlation coefficient was 0.723, indicating that KD_{50} values are strongly correlated to the LD_{50} values for each plant essential oil screened. The low P value of the correlation analysis (< 0.001) demonstrates that this analysis is highly descriptive of the relationship between KD_{50} and LD_{50} values.

a preapplication of piperonyl butoxide. This may suggest that the inhibition of various detoxification enzymes, such as cytochromes P-450, may allow terpenoids within these plant oils to remain bioactive for longer periods within the exposed organism, thereby causing higher percentage toxicity.

Our current study also highlighted a correlation between plant essential oil KD_{50} and LD_{50} values. With a Pearson correlation coefficient of 0.723, we showed that the capacity of a plant essential oil to cause knockdown is strongly correlated with mortality at a later time. However, some outliers were noted in our datasets. The most significant outliers in this analysis were sassafras, nutmeg (East Indies), rosemary, and wintergreen oil. All these oils have significantly lower KD_{50} values compared with their corresponding LD_{50} values. It may be that the terpenoids that cause rapid immobilization in these

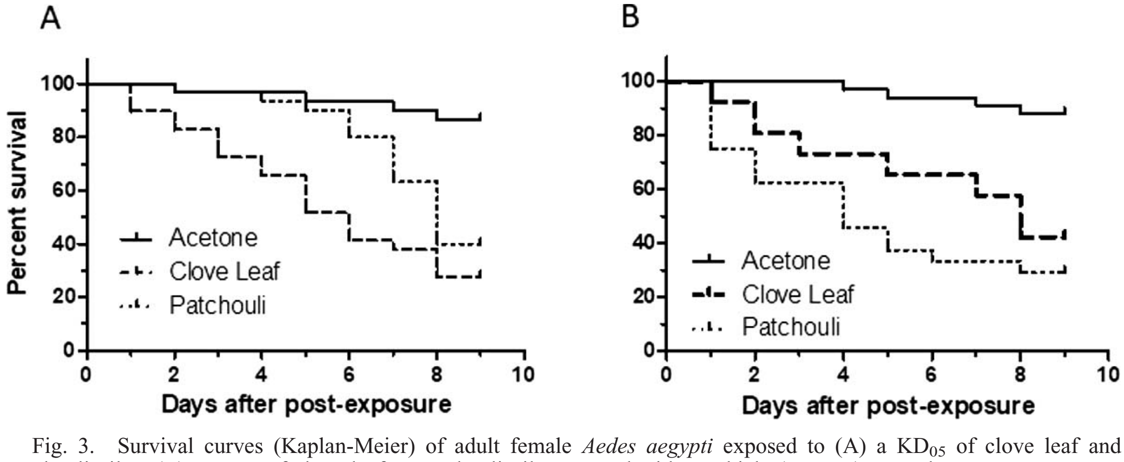

Fig. 3. Survival curves (Kaplan-Meier) of adult female *Aedes aegypti* exposed to (A) a KD_{05} of clove leaf and patchouli oil or (B) a KD_{75} of clove leaf or patchouli oil compared with a vehicle (acetone) control.

oils are more readily degraded and thus cause low levels of mortality at 24 h. These data could be valuable in the formulation of new insecticidal formulations that rely on plant essential oils or the terpenoids that are in them, as more insecticidal oils are more likely to cause rapid knockdown. This information could be utilized in the development of future blends of plant oils that cause rapid knockdown.

We also demonstrated that delayed toxicity was caused by some plant essential oils screened in this study. For this exploration, we chose to examine the latent mortality caused by the 2 oils that possessed the lowest KD_{50} values. At both an ED_{05} level (Fig. 3A) and an ED_{75} level (Fig. 3B) of plant essential oil applied, significant mortality was observed out to 9 days compared to the control treatment. This may highlight the potential of these botanical products in the control of wild mosquito populations in the future. Interestingly, in both the ED_{05} and ED_{75} exposure levels, significant delayed mortality was observed for both clove leaf and patchouli oils.

The delayed mortality caused by some of the plant essential oils screened in this study may be due to oxidative stress brought about by the detoxification of the natural insecticides at sublethal levels (at 24 h). It is well known that CYP450-dependent detoxification can lead to the increase of reactive oxygen species within cells (Hrycay and Bandiera 2015). Based on the disparity between KD and mortality according to dose, we postulate that the mortality effect is not correlated to initial immobilization of the insect by these knockdown agents in the laboratory. Very little knockdown was observed in the ED_{05}-exposure experiment compared with the ED_{75}-exposure experiment; however, delayed percentage mortality was similar in both experiments. This indicates that this delayed mortality is caused by an alternative mechanism, which warrants further investigation. Promisingly, treatments of plant essential oils at concentrations that caused little to no mortality at 24 h postexposure caused significant delayed mortality as observed out to 9 days postexposure in the Kaplan-Meier survival analysis.

In summary, this study highlights the potential of plant essential oils and natural products in the control of adult mosquitoes. The use of only 24 h mortality as a metric of efficacy (Oxborough et al. 2015) may exclude potential bioactive compounds from subsequent testing regimens and development as future insecticidal products. Here we demonstrate the utility of plant essential oils as immobilizing agents for mosquitoes, measured by the metric of percentage knockdown at 1 h. Moreover, the delayed mortality caused by a number of the most potent plant essential oils also reveals interesting new lines of research for potential novel modes of action for natural products and plant essential oils as mosquito control strategies. As insecticide resistance causes the failure of many insecticidal chemistries that have been extremely valuable in the control of mosquito populations, new agents must be explored as additives in future insecticidal formulations. Plant essential oils may represent a viable source of novel insecticidal additives.

ACKNOWLEDGMENTS

The authors thank Ryan Smith, Benjamin R. Arends, and Brendan M. Dunphy for providing *Ae. aegypti* for this study. This is a publication of the Iowa Agricultural Experiment Station. Funding for this study was provided by the Deployed Warfighter Protection Program of the Department of Defense. The award number for this project is W911QY-12-1-0003. Our industry partner for this project was EcoSMART Technologies/Kittrich Corporation.

REFERENCES CITED

Anderson JA, Coats JR. 2012. Acetylcholinesterase inhibition by nootkatone and carvacrol in arthropods. *Pestic Biochem Physiol* 102:124–128.

Essam E. 2001. Insecticidal activity of essential oils: octopaminergic sites of action. *Comp Biochem Physiol C: Toxicol Pharmacol* 130:325–337.

Finney DJ. 1971. *Probit analysis*. Cambridge, United Kingdom: Cambridge Univ. Press.

Georghiou GP. 1994. Principles of insecticide resistance management. *Phytoprotection*. 754:51–59.

Gramiccia G, Beales P. 1988. The recent history of malaria control and eradication. In Wernsdorfer WH, MacGregor I, eds. *Malaria: principles and practices of malariology*. Edinburgh, Scotland: Churchill Livingstone. p 1335–1378.

Gross AD, Norris EJ, Kimber MJ, Bartholomay LC, Coats JR. 2017a. Essential oils enhance the toxicity of permethrin against *Aedes aegypti* and *Anopheles gambiae*. *J Med Vet Entomol* 31:55–62.

Gross AD, Temeyer KB, Day TA, Pérez de León AA, Kimber MJ, Coats JR. 2017b. Interaction of plant essential oil terpenoids with the southern cattle tick tyramine receptor: a potential biopesticide target. *Chem Biol Inter* 263:1–6.

Hrycay EG, Bandiera SM. 2015. Involvement of cytochrome P450 in reactive oxygen species formation and cancer. *Adv Pharmacol* 74:35–84.

Isman M. 2000. Plant essential oils for pest and disease management. *Crop Prot* 19:603–608.

Lengeler C. 2004. Insecticide-treated bed nets and curtains for prevention of malaria. Cochrane Database Syst Rev. 2004:CD000363.

Mabaso MLH, Sharp B, Lengeler C. 2004. Historical review of malaria control in Southern Africa with emphasis on the use of indoor residual housespraying. *Trop Med Int Health* 9:846–856.

Narahashi T. 1962. Effect of the insecticide allethrin on membrane potentials of cockroach giant axons. *J Cell Compar Physiol* 59:61–65.

Narahashi T. 1972. Effects of insecticides on excitable tissues. *Adv Insect Physiol* 8:1–93.

Nauen R. 2007. Insecticide resistance in disease vectors of public health importance. *Pest Manag Sci* 63:628–633.

Norris EJ, Gross AD, Dunphy BM, Bessette S, Bartholomay LC, Coats JR. 2015. Comparison of the insecticidal characteristics of commercially available plant essential oils against *Aedes aegypti* and *Anopheles gambiae* (Diptera: Culicidae). *J Med Entomol* 52:993–1002.

Oxborough RM. 2016. Trends in US President's Malaria Initiative-funded indoor residual spray coverage and insecticide choice in sub-Saharan Africa (2008–2015): urgent need for affordable, long-lasting insecticides. *Malaria J* 15:146. doi: 10.1186/s12936-016-1201-1.

Oxborough RM, N'Guessan R, Jones R, Kitau J, Ngufor C, Malone D, Mosha FW, Rowland MW. 2015. The activity of the pyrrole insecticide chlorfenapyr in mosquito bioassay: towards a more rational testing and screening of non-neurotoxic insecticides for malaria vector control. *Malaria J* 14:124. doi: 10.1186/s12936-015-0639-x.

Pocquet N, Milesi P, Makoundou P, Unal S, Zumbo B, Atyame C, Darriet F, Dehecq JS, Thiria J, Bheecarry A, Iyaloo DP, Weill M, Chandre F, Labbe P. 2013. Multiple insecticide resistances in the disease vector *Culex p. quinquefasciatus* from western Indian Ocean. *PLOS ONE* 8:e77855.

Pridgeon JW, Pereira RM, Becnel JJ, Allan SA, Clark GG, Linthicum KJ. 2008. Susceptibility of *Aedes aegypti*, *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say to 19 pesticides with different modes of action. *J Med Entomol* 45:82–87.

Ridley DB, Moe JL, Hamon N. 2017. A voucher system to speed review could promote a new generation of insecticides to fight vector-borne diseases. *Health Aff* 36:1461–1468.

Roberts DR, Manguin S, Mouchet J. 2000. DDT house spraying and re-emerging malaria. *Lancet* 356:330–332.

Rose RI. 2001. Pesticides and public health: integrated methods of mosquito management. *Emerg Infect Dis* 7:17–23.

Tong F, Gross AD, Dolan MC, Coats JR. 2012. The phenolic monoterpenoid carvacrol inhibits the binding of nicotine to the housefly nicotinic acetylcholine receptor. *Pest Manag Sci* 69:775–780.

Waliwitiya R, Kennedy CJ, Lowenberger CA. 2009. Larvicidal and oviposition altering activity of monoterpenoids, trans-anethole and rosemary oil to the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae). *Pest Manag Sci* 65:241–248.

WHO [World Health Organization]. 2006. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. Control of neglected tropical diseases: WHO Pesticide Evaluation Scheme. Geneva, Switzerland: World Health Organization.

WHO [World Health Organization]. 2013. World malaria report 2013. Geneva, Switzerland: World Health Organization.