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Brief communication (Original)

Comparative efficacy of spatial repellents containing *d*-allethrin and *d-trans* allethrin against the major dengue vector *Aedes aegypti* (Linnaeus)

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Background: The use of mosquito coils is a common method of protection against mosquito bites and mosquito borne diseases. These coils are widely marketed and used by households in Malaysia to prevent mosquito associated problems.
Objective: To determine the bioefficacy of commercial *d*-allethrin and *d-trans* allethrin spatial repellents against the dengue vector *Aedes aegypti*.
Method: We evaluated the knockdown and mortality effects of spatial repellents containing *d*-allethrin (0.3% w/w) and *d*-trans allethrin (0.1% and 0.15% w/w) on *Ae. aegypti* in a Peet Grady Chamber, relative to a reference product (0.2% w/w *d*-allethrin).
Results: The spatial repellent containing 0.3% *d*-allethrin had the shortest knockdown times (KT_{50} and KT_{90}) and these were significantly different from the other products including the reference coil except the 0.15% *d*-trans allethrin coil. The spatial repellent containing 0.3% *d*-allethrin elicited a mortality response of 96%, which was significantly different from the mortality response to the other coils, except for the 0.15% *d*-trans allethrin formulation.
Conclusions: Spatial repellents containing 0.3% *d*-allethrin or 0.15% *d-trans* allethrin had higher efficacies against *Ae. aegypti* than repellents containing 0.2% w/w *d*-allethrin or 0.1% *d*-trans allethrin and their use by households could offer better relief from *Ae. aegypti*.

Keywords: *Aedes aegypti*, *d*-allethrin, *d*-trans allethrin, mosquito, spatial repellent

The mosquito *Aedes aegypti* is an efficient vector of dengue and other viral diseases, such as yellow fever and chikungunya [1-4]. These diseases account for tens of thousands of human deaths worldwide annually [2-4]. *Aedes. aegypti* is a domestic vector of dengue commonly found in tropical and subtropical countries [5, 6] and is closely linked with the urban environment [1, 4, 5]. The female *Ae. aegypti* feeds preferentially on humans, even in the presence of other hosts [7] making it an effective vector of human viruses. A common method of protection against mosquito borne diseases and nuisance is vector control through the use of insecticide application or formulated devices, such as indoor residual spraying, insecticide treated nets or materials, mosquito coils, aerosols, and mats [2, 4].

The mosquito coil is a major anti-mosquito household product in Malaysia [8, 9]. They are cheaper and more easily purchased than other anti-mosquito products [8, 10] and therefore mostly favored by low-income communities [10]. Personal protection products such as mosquito coils have been in use for a very long time [11] with a reported global consumer market of about $1 billion in 2006 [12]. Commercially formulated mosquito coils mostly contain pyrethroids as active ingredients and these are released when the coils are burnt. The released gaseous active ingredients affect mosquitoes within the surroundings of the burning coil in several ways [13, 14], causing a reduction in host seeking mosquitoes [8, 10].

There are several commercially formulated coils on the Malaysian market used by households against

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dengue vectors and other mosquitoes. Mosquito coils in Malaysia are expected to meet a standard biological efficacy (bioefficacy) requirement set by the Department of Standards Malaysia [15]. This is ascertained by testing the coil products and a Malaysian Standard Reference Product (0.2% *d*-allethrin) in either a glass chamber or a Peet Grady chamber and comparing the biological efficacy of the test products with the standard reference product [15]. A test product with a knockdown time for 50% of the test mosquitoes (KT_{50}) either equal to or better than the KT_{50} for the standard reference is deemed to have achieved the standard bioefficacy requirement [15]. In view of the alarming spate of dengue cases in Malaysia over the past year [16], it is important to assess the efficacy of household products in controlling dengue vectors. A recent study evaluated the impact of pyrethroid coils on the secondary dengue vector, *Ae. albopictus* [17], but not on *Ae. aegypti*, which is the primary dengue vector in this region. This study assayed the efficacies of spatial repellents containing *d*-allethrin and *d-trans* allethrin in comparison with a standard reference against *Ae. aegypti* to assess their level of personal protection.

Materials and methods

Coil formulations

The mosquito coils were obtained from consumer shops and their formulations were 0.1% (w/w) or 0.15% (w/w) *d-trans* allethrin, or 0.3% (w/w) *d*-allethrin. The test products (0.3% *d*-allethrin or 0.15% *d-trans* allethrin) have earlier been evaluated on *Ae. albopictus* and had significant insecticidal effect under laboratory condition [17]. The standard reference coil (0.2% *d*-allethrin) for evaluating the efficacy of mosquito coils in Malaysia [15] served as the reference product.

Mosquito samples

A susceptible *Ae. aegypti* strain reared in the Vector Control Research Unit (VCRU) of the Universiti Sains Malaysia (USM) according to standard protocols was used with due respect. The mosquito larvae were reared on a powdered blend of cat biscuit (Friskies), beef liver, yeast, and milk powder in a 2:1:1:1 proportion. The adults were allowed free access to 10% sucrose solution. Sucrose-fed female adult mosquitoes, which were aged 3–5 days were used for the testing and their numbers limited to what was necessary to conduct the experiments. The mosquitoes were reared at 27 ± 2°C and 80 ± 10% relative humidity.

Efficacy test

A biological efficiency test was conducted in a Peet Grady chamber according to the World Health Organization's method [18] at 29 ± 2°C and relative humidity of 70 ± 5%. Four nylon covered cages each containing 25 test mosquitoes were hung in the four designated corners of the chamber. An upward facing fan in the center of the chamber circulated the air in the chamber. The mosquito coil to be tested was affixed to a stand, lit, and placed on a flat dish on top of the fan's guard rail. The number of mosquitoes knocked down was counted at 1 min intervals for 10 min, then 2 min interval (until the 20th min), and then 5 min interval for a total exposure period of 60 min. The remaining mosquitoes were then held in paper cups provided with 10 % sucrose solution and their mortality recorded after 24 hours. A no coil exposure of mosquitoes in the chamber was used as the control. The bioefficacy test was conducted in triplicate for all the coils and the control.

Statistical analyses

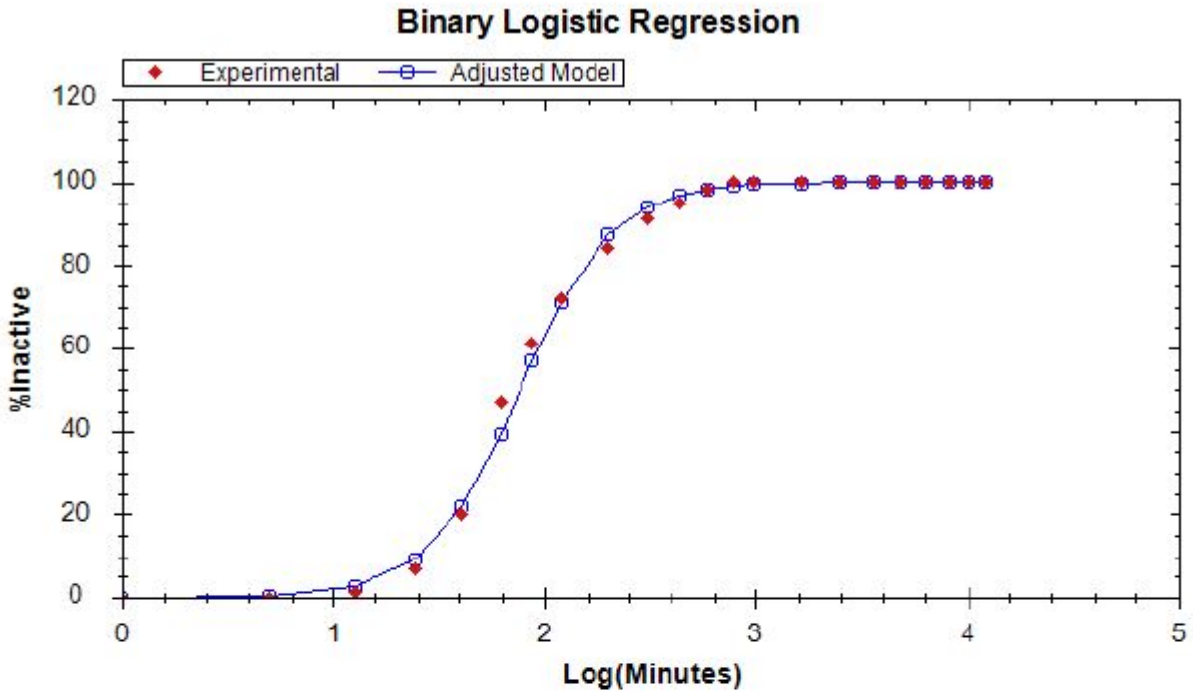
Time–response logistic regression was conducted to obtain the KT_{50} and the 90% knockdown time (KT_{90}) of the test mosquitoes, the confidence intervals (CI), slopes and standard errors (SE) using Qcal software [19]. The mortality data was arcsine transformed. The transformed data was analyzed using a one-way analysis of variance using IBM SPSS Statistics for Windows (version 20.0; IBM Corp, Armonk, NY, USA). Mean mortality was separated using the least significant difference test at $P < 0.05$.

Results

Knockdown durations

The KT_{50} of mosquitoes exposed to the reference coil was longer and significantly different ($P < 0.05$) from the 0.3% *d*-allethrin- and 0.15% *d-trans* allethrin-containing coils, but not different from the 0.1% *d-trans* allethrin-containing coil. The KT_{50} responses between coils containing the same active ingredients (*d*-allethrin or *d-trans* allethrin) group were significantly different ($P < 0.05$). However, across the active ingredient groupings, the KT_{50} of the higher concentrations (0.3% *d*-allethrin and 0.15% *d-trans* allethrin) and the lower concentrations (0.2% *d*-allethrin and 0.1% *d-trans* allethrin) were not significantly different (**Figure 1, Table 1**).

A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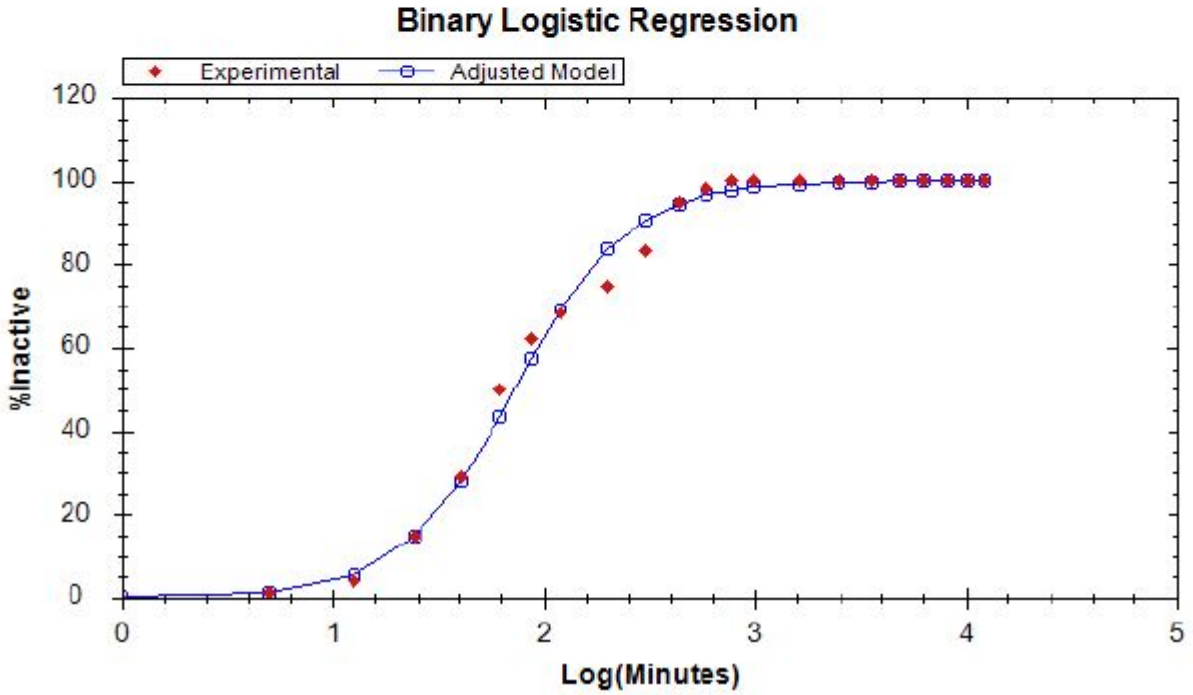
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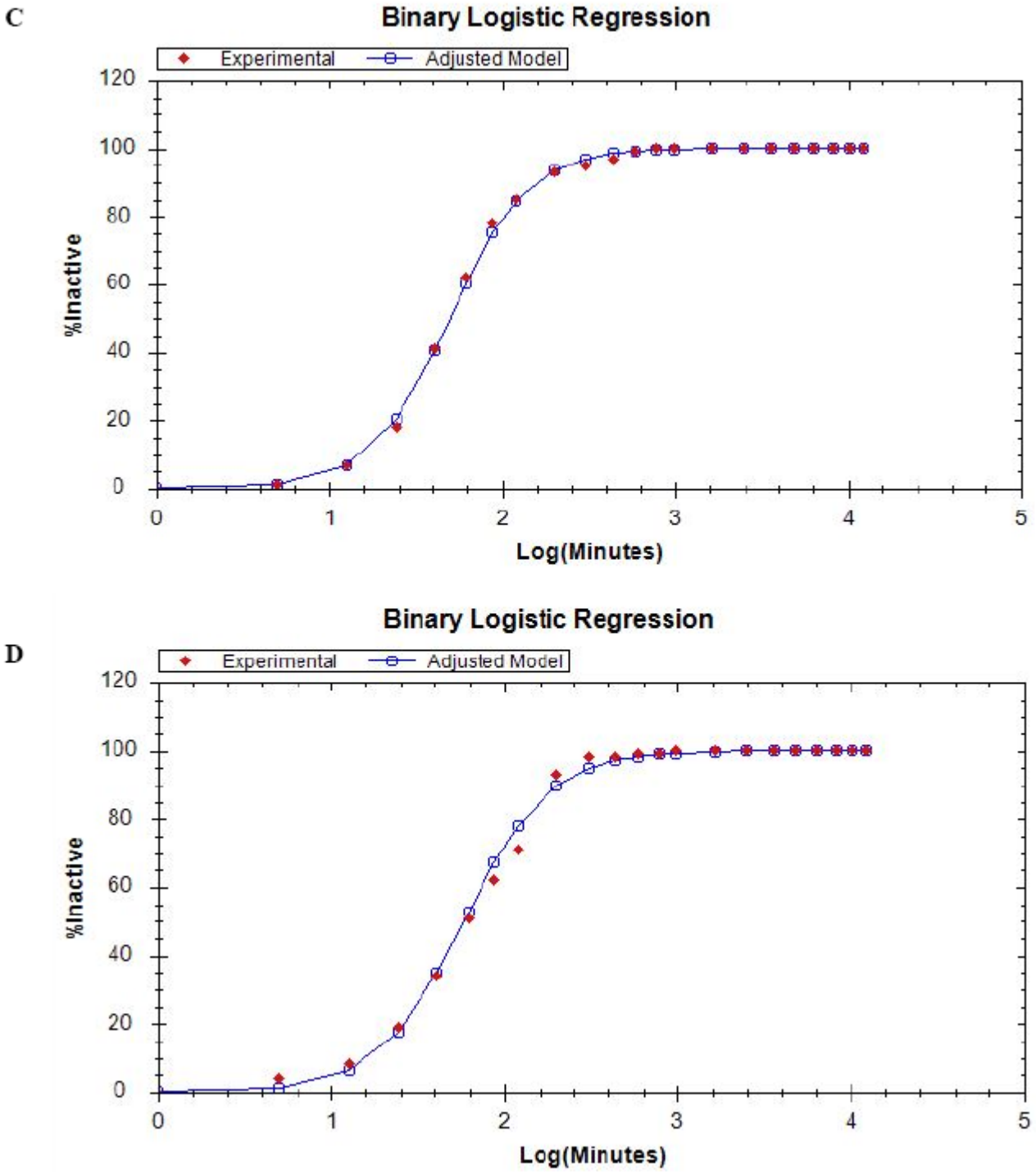

Figure 1. Binary logistic regression analysis of the knockdown effect of spatial repellents on *Aedes aegypti* generated using IRMA-Qcal software. (A) 0.2% *d*-allethrin; (B) 0.3% *d*-allethrin; (C) 0.1% *d-trans* allethrin; (D) 0.15% *d-trans* allethrin. Details of the knockdown times and slopes are in **Table 1**.

Table 1. Knockdown duration for *Aedes aegypti* exposed to smouldering *d*-allethrin or *d-trans* allethrin-containing coils

Active ingredient (a.i.)	% w/w a.i.	KT_{50} (min) (95% CI)	KT_{90} (min) (95% CI)	Slope ± SE
d-allethrin	0.3	5.45[a] (5.22–5.69)	8.98[a] (8.44–9.55)	4.40 ± 0.25
	0.2	6.45[b] (6.17–6.74)	11.67[b] (10.92–12.47)	3.71 ± 0.19
d-trans allethrin	0.15	5.84[a] (5.60–6.11)	10.08[ac] (9.45–10.74)	3.99 ± 0.22
	0.1	6.59[b] (6.34–6.85)	10.59[bc] (9.98–11.25)	4.63 ± 0.25
Control	–	–	–	–

Mean values with the same superscript letters in the same column were not significantly different ($P < 0.05$)

The reference coil had the longest KT_{90} duration, which was significantly different ($P < 0.05$) from the 0.3% *d*-allethrin and 0.15% *d-trans* allethrin-containing coils. Unlike the KT_{50}, the KT_{90} of the *d-trans* allethrin-containing coils were not significantly different, although the concentrations in the coils were different. However, the *d*-allethrin coils had KT_{90} values that were significantly different. Mosquitoes exposed to smoke of the coil containing 0.3% *d*-allethrin had the shortest KT_{50} and KT_{90} values.

Toxicity effect

The mean mortality effects for the *Ae. aegypti* as a result of exposure to the smoldering coils were between 57% and 96%. The effect of the reference coil was not different from the 0.15% *d-trans* allethrin coil; however, the effect was different from the other coils ($P < 0.05$). The 0.3% *d*-allethrin coil effected a significantly higher mortality than the reference coil ($P < 0.05$). The mosquitoes exposed to the smouldering 0.1% *d-trans* allethrin-containing coil had the lowest mortality for all the coil types tested ($P < 0.05$) (**Table 2**).

Discussion

The smoke from the smouldering mosquito coils used in this study produced median knockdown times of less than 7 min in *Ae. aegypti*, with coils containing higher concentration of their respective active ingredients knocking down the mosquitoes more quickly than those with lower concentrations of the same active ingredients. This is consistent with the observation that knockdown is positively influenced by high doses of active ingredients [20]. In an earlier study in which two mosquito species were exposed to mosquito coils using a glass chamber (70 cm × 70 cm × 70 cm), the KT_{50} of 1–5-day-old female *Ae. aegypti* tested with 0.2% *d*-allethrin- or 0.1% *d-trans* allethrin-containing coils were less than 3 min, while similarly aged *Culex quinquefasciatus* exposed to the same coils had higher KT_{50}, more than twice the *Ae. aegypti* KT_{50} [8]. Another study nearly a decade later using a *d*-allethrin-containing mat (36 mg/mat) in a glass chamber showed shorter KT_{50} of 1.38 min for *Ae. aegypti* and a KT_{50} of 8.36 min for *Cx. quinquefasciatus* [21], which is relatively similar to the KT_{50} of *Cx. quinquefasciatus* in the earlier study [8]. However, in this present study, the KT_{50} of

Table 2. Mean mortality (%) of *Aedes aegypti* exposed to smouldering *d*-allethrin and *d-trans* allethrin coils

Active ingredient (a.i)	% w/w a.i.	Mortality ± SE (%)	95 % CI
d-allethrin	0.3	96.00 ± 2.08[a]	91.91–100.09
	0.2	82.67 ± 3.76[b]	75.30–90.04
d-trans allethrin	0.15	85.67 ± 4.18[b,a]	77.49–93.85
	0.1	57.00 ± 7.81[c]	41.69–72.31
Control	–	0.33 ± 0.33[d]	–0.33–0.99

Mean values with the same letters in the same column are not significantly different ($P < 0.05$)

Ae. aegypti exposed to the smouldering *d*-allethrin- and *d-trans* allethrin-containing coils were much higher [8]. This may be a consequence of the different test chambers used, because the Peet Grady chamber is larger than the glass chamber and therefore would contain less insecticide per unit volume compared with the glass chamber. The 0.3% *d*-allethrin- and 0.15% *d-trans* allethrin-containing coils significantly knocked down 50% of the *Ae. aegypti* population more quickly than the reference coil. The KT_{50} of these two coils were lower and therefore better than the standard reference. However, the KT_{50} effect of the standard reference coil and the 0.1% *d-trans* allethrin-containing coil were not significantly different. The time to knock down 90% of the test *Ae. aegypti* was shorter when the mosquitoes were exposed to the 0.3% *d*-allethrin-containing coil compared with the other coils studied, including the reference coil, and this was significantly different with the exception of the KT_{90} of *Ae. aegypti* exposed to the 0.15% *d-trans* allethrin-containing coil. For the *d-trans* allethrin-containing coils, the KT_{90} of the mosquito populations were not significantly different, although they knocked down 90% of the test mosquitoes more quickly than the reference coil. Using these commercial *d-trans* allethrin-containing coils and the 0.3% *d*-allethrin-containing coil paralyses the mosquitoes more quickly than the reference coil, and could therefore diminish their ability to be in contact with a host to take a blood meal. An analysis of the KT_{90} of the test coils showed that they all performed better than the standard reference, although the difference between the standard reference and the 0.1% *d-trans* allethrin-containing coil was not significant. However, the knockdown times of the *Ae. aegypti* population tested with 0.3% allethrin- and 0.15% *d*-trans allethrin-containing coils shows that it is less susceptible to be knocked down relative to the closely related species *Ae. albopictus* [17].

Spatial repellents affect mosquitoes in several ways including repellency, biting inhibition, knockdown and mortality [17, 20-22], thereby reducing the number of biting mosquitoes [8, 21]. However, mortality is a less pronounced effect in the natural setting and it has been postulated that coils do not kill mosquitoes under end-user conditions [20]. The 0.3% *d*-allethrin-containing coil induced the highest mortality in the *Ae. aegypti* mosquitoes and was significantly different from all the other coils, except the 0.15% *d*-trans allethrin coil. The 0.15% *d-trans* allethrin also caused a high mortality in the mosquitoes than the standard reference coil, but this difference in mortality response was not significantly different to the response from the standard coil.

The extent of mortality induced by these coils especially the 0.3% *d*-allethrin and 0.15% *d-trans* allethrin are much higher than in an earlier study [8]. In the earlier study, mortalities of 13.67% (*Ae. aegypti*) and 7.33% (*Cx. quinquefasciatus*) as a result of the 0.2% *d*-allethrin coil and 17.50% (*Ae. aegypti*) and 8.17% (*Cx. quinquefasciatus*) as a result of 0.1% *d*-trans allethrin coil were induced [8]. Although that study [8] used a smaller testing area (glass chamber), the knockdown exposure period was shorter (10 min for *Ae. aegypti* and 20 min for *Cx. quinquefasciatus*), therefore low mortality observed. However, the *Ae. aegypti* mortality in the present study when assessed with 0.3% *d*-allethrin- and 0.15% *d-trans* allethrin-containing coils is within the mortality range of *Ae. albopictus* exposed to similar products [17].

Comparative analysis of the mortality effects of the coils showed that 0.3% *d*-allethrin- and 0.15% *d*-trans allethrin-containing coils were more effective than the standard reference. The control used for examining the natural mortality [23] in the mosquito coil bioefficacy studies is usually no-coil exposure or exposure to a blank coil or sometimes both [8, 17, 22, 24]. Smoke from non-insecticidal mosquito coils can affect mosquito behavior, therefore the use of a no-coil exposure control group in lieu of a blank coil as used in this study could over-estimate the effectiveness of the spatial repellents. The inert components of mosquito coils can also aid the efficient and effective use of the coils [15]. The specific components of the inert ingredients of these commercial spatial repellents were not available from the label information. It is plausible that differences in the inert ingredients of the test products may contribute to the observed spatial effect of the products on the mosquitos.

Conclusion

A bioefficacy test showed the varying effects of *d*-allethrin and *d*-trans allethrin spatial repellents on *Ae. aegypti* under laboratory conditions. It is important to conduct further studies on the effectiveness of these and other spatial repellents on this dengue vector in the natural end-user setting and their impact on its vectorial capability.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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