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Efficacy of Mosquito Coils: Cross-resistance to Pyrethroids in *Aedes aegypti* (Diptera: Culicidae) From Indonesia

Zheng Hua Amelia-Yap,[1] Chee Dhang Chen,[2] Mohd Sofian-Azirun,[2] Koon Weng Lau,[2] I. Wayan Suana,[3] Harmonis -,[4] Edy Syahputra,[5] Abdul Razak,[6] and Van Lun Low[1,7]

[1]Tropical Infectious Diseases Research and Education Centre (TIDREC), University of Malaya, 50603 Kuala Lumpur, Malaysia, [2]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia, [3]Faculty of Mathematics and Natural Science, University of Mataram, Jl. Majapahit No. 62, Mataram 83125, Nusa Tenggara Barat, Indonesia, [4]Faculty of Forestry, Mulawarman University, Jl. Ki Hajar Dewantara, Kampus Gunung Kelua, Samarinda 75119, Kalimantan Timur, Indonesia, [5]Faculty of Agriculture, Tanjungpura University, Jl. Prof. Dr. Hadari Nawawi, Pontianak 78124, Kalimantan Barat, Indonesia, [6]Faculty of Mathematics and Natural Science, Padang State University, Jl. Prof. Dr. Hamka, Kampus Air Tawar, Padang 25131, Sumatera Barat, Indonesia, and [7]Corresponding author, e-mail: lucaslow24@gmail.com

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Abstract

Aedes aegypti (L.) (Diptera: Culicidae) is the primary vector of several arthropod-borne viral infectious diseases globally. Relentless vector control efforts are performed to curtail disease transmissions, insecticides remain as the first line of defense in Indonesia. With a dearth of publication on the efficacy of mosquito coil in Indonesia, this is the first report related to mosquito coil despite its common use in households. *Ae. aegypti* mosquitoes were sampled from nine regencies in Indonesia and tested using the glass-chamber method against three commercially available local pyrethroid-based mosquito coils containing d-allethrin, transfluthrin, and metofluthrin. The 50% knockdown time of female *Ae. aegypti* tested with d-allethrin, transfluthrin, and metofluthrin containing coils ranged from 0.65 to 14.32; 0.8 to 16.4; and 0.78 to 20.57 min, respectively. Mortality rates in accordance with WHO resistance indicators showed that strains from Denpasar, Mataram, Kuningan, Padang, Samarinda, and Sumba Timur were resistant (<80% mortality rate), whereas strains from Manggarai Barat, Dompu, and Pontianak were susceptible (>98% mortality rate) to the active ingredients assayed. Moreover, the knockdown rates between d-allethrin and transfluthrin, d-allethrin and metofluthrin, as well as transfluthrin and metofluthrin displayed significant associations, portraying the presence of cross-resistance within pyrethroid insecticides. The minimal insecticidal effect of mosquito coils against some Indonesian *Ae. aegypti* also pointed out the development of pyrethroid resistance, prompting a revamping of the vector control system.

Key words: mosquito coil, insecticide resistance, d-allethrin, transfluthrin, metofluthrin

Many countries are at risks of dengue fever (DF) infections. With an estimation of 50–100 million reported cases annually (World Health Organization [WHO] 2017), dengue virus is the most widespread mosquito-borne human virus worldwide. In Indonesia, dengue was first described in 1779 (Hanley and Weaver 2010), and the disease has since then dramatically expanded in distribution over the years. DF remains to be a major public health concern in Indonesia with the presence of recurrent epidemic cycles. In 2015, there was a total of 126,675 reported dengue cases with 1,229 deaths (MOH 2015), signifying an appalling fact that the disease burden in the country has never ceased. Contributory factor of this issue may very much be ascribed to the high availability of man-made or natural containers as breeding sites for *Aedes aegypti* (L.) (Diptera: Culicidae), the anthropophilic primary dengue vector in Indonesia that lives in close proximity with humans to obtain blood meals. Effective control of *Ae. aegypti* is utterly challenging when both vector and virus have deep rooted and spread rapidly in disease-endemic regions. Due to the absence of safe and effective treatment or vaccine for dengue to date, vector control strategies against *Ae. aegypti* continue to be the cornerstone in preventing dengue transmission and outbreak control.

The bottom line in inhibiting dengue transmission is to decrease human-vector contact with the use of synthetic chemicals. The use of multiple classes of synthetic insecticides has been largely practiced in vector control strategies, with the massive majority dominating the insecticide market is pyrethroid-based formulations. In Indonesia, pyrethroids are widely incorporated into household insecticide products to control mosquitoes, and mosquito coils are most commonly used personal protection products especially in residential areas to shield users against mosquito bites. The choice of main active ingredients of mosquito coils is pyrethroid as suggested by WHO due to the quick knockdown effect on mosquitoes using low doses with low mammalian toxicity. The mosquito coil is extensively introduced because of the low cost, ease of use without the need of equipment or electricity, and wide cultural acceptance, as smoke is widely used in many cultures in eradicating mosquitoes (Biran et al. 2007). Despite the presence of other delivery formats such as vaporizer mats, aerosols, and liquid vaporizers, mosquito coils are still the most popular choice globally (Yap et al. 2003). These continuing efforts in fighting dengue in Indonesia may not be sustainable when control method of the vector is dealing with many challenges that insecticides are deemed to be decreasing in susceptibility due to the issue insecticide resistance development. When insects are exposed to certain insecticides for a period of time, selection pressure occurs among them throughout the generations, producing insecticide-resistant offspring (Lee et al. 2003).

Despite coils provide a means of disease control in preventing mosquito bites and the strong dependence on this tool for vector control, the insecticide resistance status of *Ae. aegypti* in Indonesia to mosquito coils has never been monitored. After several decades of use of pyrethroids, the susceptibility level of these insecticides against *Ae. aegypti* faces an issue due to the development of insecticide resistance that is commonly underestimated and easily overlooked. To carry out effective and sustainable vector control measures, there possesses a crucial need in determining the susceptibility of the major vector of dengue to pyrethroid-based mosquito coils regularly used in mosquito control because this will aid in delaying the development of resistance in mosquito vectors. In Indonesia, information on the resistance of the primary dengue vector *Ae. aegypti* to mosquito coils remains unknown. Therefore, this research was initiated to examine the efficacy of pyrethroid-based mosquito coils containing either d-allethrin, transfluthrin, or metofluthrin against adult *Ae. aegypti* populations from nine regencies in Indonesia.

Materials and Methods

Study Sites

Ae. aegypti was sampled from a total of nine regencies (nine study sites) in Indonesia using mosquito-oviposition trap (ovitrap). The details of each of the sampling site are tabulated in Table 1.

Ovitrap Preparation and Sample Collection

Ovitraps were set up based on protocol developed by Lee (1992). An ovitrap is consisted of a black-painted 300-ml plastic cup with dimensions of 9.0 cm height, 6.5 cm base diameter, and 7.8 cm opening diameter. A 2.5 cm × 10.0 cm × 0.3 cm paddle made of hardboard was positioned diagonally into each of the ovitrap. The ovitrap was then filled with chlorine-free tap water up to a height of 5.5 cm. In total, 40 ovitraps were placed randomly within the study sites at each of the nine regencies, with the best conditions of close proximity to other potential breeding sites protected from direct sunlight and rainfalls. Five days later, the ovitraps were collected and transported back to the laboratory for further identification at adult stage (Fig. 1).

Mosquito Colonization

Adult *Ae. aegypti* mosquitoes were identified and colonized in respective wooden cages measuring 30 cm × 30 cm × 30 cm covered with netting according to locations. Mosquitoes from the same study sites were pooled at the laboratory. Ten percent of sucrose solution was provided for the adult mosquitoes as food source. Four- to five-day-old female adults were supplied with bloodmeal to produce F_1 generation that would be used for subsequent susceptibility test. An oviposition site that was made of plastic cup containing 200 ml of chlorine-free water lined with filter paper was placed into the cage. The collected eggs were allowed to hatch in plastic containers filled with chlorine-free water measuring 25 cm × 30 cm × 5 cm. Larvae were fed with beef liver powder. Larvae that pupated were then transferred into a small plastic cup and introduced into the rearing cage for the adults to emerge. *Ae. aegypti* Bora-Bora strain was used as the reference susceptible group.

Table 1. Geographical description of *Aedes aegypti* collection sites in Indonesia

Sunda Islands	Islands	Provinces	Regencies	Study sites	GPS coordinates
Greater Sunda Islands	Java	West Java	Kuningan	Kuningan	6° 13′ 5.260″ S 106° 50′ 15.936″ E
	Sumatra	West Sumatra	Padang	Air Tawar Barat	0° 53′ 48.260″ S 100° 20′ 45.265″ E
	Borneo	East Kalimantan	Samarinda	Sidodadi	0° 28′ 41.646″ S 117° 08′ 46.441″ E
		West Kalimantan	Pontianak	Bangka Belitung Laut	0° 3′ 31.967″ S 109° 21′ 19.322″ E
Lesser Sunda Islands	Bali	Bali	Denpasar	Sanur	8° 41′ 10.254″ S 115° 15′ 23.634″ E
	Lombok	West Nusa Tenggara	Mataram	Ampenan	8° 34′ 13.911″ S 116° 05′ 08.575″ E
				Pagesangan	8° 36′ 2.666″ S 116° 06′ 07.080″ E
	Sumbawa		Dompu	Bada	8° 32′ 20.878″ S 118° 27′ 28.799″ E
	Flores	East Nusa Tenggara	Manggarai Barat	Labuan Bajo	8° 29′ 34.269″ S 119° 52′ 40.889″ E
			Sumba Timur	Waingapu	9° 39′ 49.331″ S 120° 16′ 17.321″ E

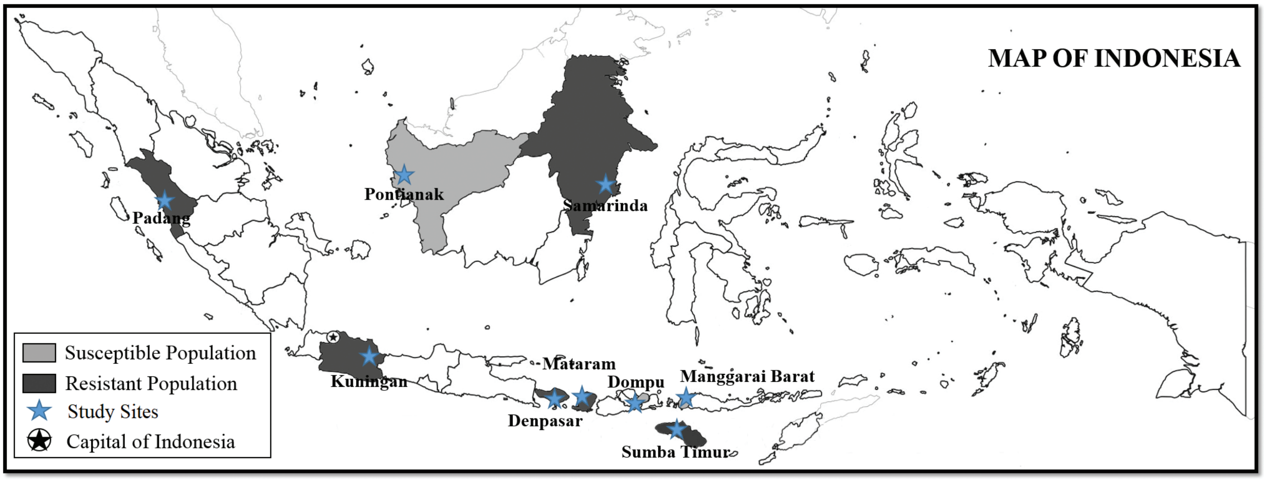

Fig. 1. Collection sites of *Aedes aegypti* in Indonesia.

Mosquito Coil Bioassay

Three easily available commercial mosquito coils from Indonesian market with different content of active ingredients were used in mosquito coil bioassays, specifically d-allethrin 0.25% w/w, transfluthrin 0.03% w/w, and metofluthrin 0.0097% w/w. The bioassay was accomplished in accordance with the standardized protocol described by Standards and Industrial Research Institute of Malaysia (SIRIM 1986), and a resistance indicator from WHO (2016) was adopted. The test was performed in a 70 cm × 70 cm × 70 cm see-through glass chamber, with a sliding window measuring 18 cm × 20 cm located at the bottom middle of the chamber door to ease the placement of coil and the release of mosquitoes. The conditions of the laboratory were controlled at a temperature of 27 ± 2°C and relative humidity of 80 ± 10%. Mosquito coil weighted 0.50 g was fixed on a coil stand, with both ends ignited inside the chamber. Both smoldered remains of coil and coil stand were eliminated after the coil was completely burnt. To ensure the coil smoke distributed evenly throughout the chamber, a small electric fan was switched on in it. In total, twenty, 2- to 5-d-old sugar-fed adult *Ae. aegypti* females were released into the chamber to expose to the coils separately in each replicate. The number of knocked-down mosquitoes was counted and recorded for every minute up to 20 min. Mosquitoes that were failed to fly or no longer maintained in normal posture would be considered as knockdown. Electric aspirator was used to collect all tested mosquitoes after 20 min of exposure time and subsequently transferred into a clean plastic container for 24-h post-treatment effect. The containers were covered up tightly with netting to prevent mosquitoes from escaping. The mosquitoes were provided with 10% sucrose solution soaked in cotton wool as food source. All the mosquitoes were kept at 27 ± 2°C and 80 ± 10% relative humidity during the 24-h recovery period. Mortality was recorded 24 h after the initial exposure period. Dead and alive mosquitoes after recovery were relocated to individual microfuge tubes and stored at −20°C for future use. Subsequently, used chambers were wiped thoroughly using detergent and water before the next test would be carried out. To ensure the cage is not contaminated by insecticide after cleaning, control experiments were carried out by releasing the adult females into the chamber without exposing to any coils for 20 min. An acceptable mortality rate for the control mosquitoes should strictly be 0%. Toxicological tests were repeated three times for each study site and active ingredient.

Data Analyses

Mosquito coil bioassay data were pooled and analyzed from at least three replicates of tests. The data within the range from 5 to 95% knockdown were subjected to probit analysis (Finney 1971) using the computer software SPSS (version 21) to obtain 50% knockdown time (KT_{50}). The resistance ratios (RR) of all field-collected *Ae. aegypti* mosquitoes were calculated using the formula as follows:

$$RR = \frac{KT_{50} \text{ of field strain}}{KT_{50} \text{ of reference strain}}$$

RR values of <5 indicate low resistance, 5–10 indicate medium resistance, whereas >10 indicate high resistance (Mazzarri and Georghiou 1995). Comparative measure of knockdown and mortality between the study sites was performed by one-way analysis of variance (ANOVA) using SPSS version 20. Tukey's test was used to separate means in significant ANOVAs, $P < 0.05$. Spearman's rank-order correlation analysis between a pair of knockdown rates was performed to determine the presence of cross-resistance (Bisset et al. 1997). Spearman's rank-order correlations between each coil for *Ae. aegypti* were performed to determine the presence of cross-resistance (Bisset et al. 1997). The mortality rate after a 24 h post-treatment was used to evaluate the susceptibility status of the mosquitoes, with criteria as stated by WHO (2016):

- Mortality rate of ≥98%: susceptible to insecticide;
- Mortality rate of <98%: possible development of resistance to insecticide;
- Mortality rate of <90%: resistance to insecticide.

Results

The susceptibility status of adult *Ae. aegypti* mosquitoes to the pyrethroid-based mosquito coils is presented in Tables 2 and 3. In each active ingredient tested, the adult mosquitoes revealed different trends of susceptibility across all study sites. Exposure of Bora-Bora laboratory reference colony to the mosquito coils caused 100% mortality rate in all replicates, with KT_{50} of 0.54, 0.76, and 0.84 to d-allethrin, transfluthrin, and metofluthrin, respectively. For field samples, the KT_{50} of female adult *Ae. aegypti* tested with d-allethrin, transfluthrin, and metofluthrin containing coils ranged from 0.65 to 14.32 min (the longest KT_{50} population: Sumba Timur); 0.8 to

Table 2. KT_{50} and RR of Indonesian *Aedes aegypti* adults against d-allethrin (0.25%), transfluthrin (0.03%), and metofluthrin (0.0097%)

Locations	Active ingredients					
	d-Allethrin		Transfluthrin		Metofluthrin	
	KT_{50} (min) (95% CL)	RR	KT_{50} (min) (95% CL)	RR	KT_{50} (min) (95% CL)	RR
Reference	0.54 (0.38–0.65)		0.76 (0.65–0.87)		0.84 (0.73–0.95)	
Denpasar	8.91 (7.71–10.40)	16.62	11.74 (10.02–14.17)	15.40	10.32 (9.15–11.80)	12.26
Mataram	4.52 (4.00–5.07)	8.44	5.54 (4.77–6.37)	7.27	11.19 (9.69–13.20)	13.29
Kuningan	10.39 (9.44–11.51)	19.38	15.05 (13.15–17.77)	19.75	14.36 (12.48–17.07)	17.06
Manggarai Barat	1.00 (0.85–1.14)	1.87	1.13 (0.98–1.27)	1.48	1.36 (1.21–1.51)	1.61
Dompu	0.66 (0.53–0.76)	1.22	0.78 (0.62–0.91)	1.02	0.78 (0.66–0.90)	0.93
Padang	4.87 (3.99–5.78)	9.09	4.33 (3.92–4.74)	5.68	4.96 (4.48–5.45)	5.89
Pontianak	0.99 (0.83–1.14)	1.84	0.80 (0.70–0.91)	1.06	0.94 (0.82–1.06)	1.12
Samarinda	8.64 (7.78–9.65)	16.13	16.40 (14.14–19.78)	21.52	20.57 (17.30–26.05)	24.43
Sumba Timur	14.32 (12.63–16.66)	26.72	6.67 (5.67–7.75)	8.76	9.82 (8.92–10.85)	11.66

CL (confidence limit).

Table 3. Percentages of knockdown and mortality of Indonesian *Aedes aegypti* adults against d-allethrin (0.25%), transfluthrin (0.03%), and metofluthrin (0.0097%)

Regencies	Knockdown			Mortality		
	d-Allethrin 0.25%	Transfluthrin 0.03%	Metofluthrin 0.01%	d-Allethrin 0.25%	Transfluthrin 0.03%	Metofluthrin 0.01%
Reference	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100.00 ± 0.00	100 ± 0
Denpasar	63.33 ± 1.67^{a}	55 ± 0^{a}	61.67 ± 0.33^{a}	$^{R}11.67 \pm 0.33^{a}$	$^{R}8.33 \pm 0.33^{a}$	$^{R}21.67 \pm 0.33^{a}$
Mataram	78.3 ± 0.33^{abce}	78.3 ± 0.33^{abce}	66.67 ± 0.33^{abce}	$^{R}50 \pm 0.58^{abce}$	$^{R}56.67 \pm 0.67abc^{abce}$	$^{R}41.67 \pm 0.67^{abce}$
Kuningan	61.67 ± 0.33^{c}	55 ± 0^{bc}	55 ± 0.58^{abc}	$^{R}15 \pm 1.53^{bc}$	$^{R}13.3 \pm 0.33^{bc}$	$^{R}5 \pm 0.577^{abc}$
Manggarai Barat	100 ± 0^{abcd}	100 ± 0^{abcd}	100 ± 0^{abcd}	$^{S}100 \pm 0^{abcd}$	$^{S}100 \pm 0^{abcd}$	$^{S}100 \pm 0^{abcd}$
Dompu	100 ± 0^{ab}	100 ± 0^{ab}	100 ± 0^{ab}	$^{S}98.3 \pm 0.33^{ab}$	$^{S}100 \pm 0^{ab}$	$^{S}100 \pm 0^{ab}$
Padang	71.67 ± 0.33^{abdef}	91.67 ± 0.33^{acdef}	86.67 ± 0.33^{bcdef}	$^{R}30 \pm 0.58^{abdef}$	$^{R}50 \pm 1.53^{acdef}$	$^{R}26.67 \pm 0.67^{bcdef}$
Pontianak	100 ± 0^{abcfg}	100 ± 0^{abcfg}	100 ± 0^{abcfg}	$^{S}100 \pm 0^{abcfg}$	$^{S}100 \pm 0^{abcfg}$	$^{S}100 \pm 0^{abcfg}$
Samarinda	70 ± 0^{acdeg}	58.3 ± 0.33^{bdefgh}	51.67 ± 0.67^{bdefgh}	$^{R}38.3 \pm 0.88^{acdeg}$	$^{R}15 \pm 0.58^{bdefgh}$	$^{R}11.67 \pm 0.33^{bdefgh}$
Sumba Timur	55 ± 0^{acdeg}	63.3 ± 0.67^{acdegh}	68.3 ± 0.67^{acdegh}	$^{R}45 \pm 0.58^{acdeg}$	$^{R}41.67 \pm 0.88^{acdegh}$	$^{R}36.67 \pm 1.33^{acdegh}$
One-way ANOVA	$P < 0.0001$ $F = 126.27$ df = (9, 20)	$P < 0.0001$ $F = 148.08$ df = (9, 20)	$P < 0.0001$ $F = 196.69$ df = (9, 20)	$P < 0.0001$ $F = 126.27$ df = (9, 20)	$P < 0.0001$ $F = 148.08$ df = (9, 20)	$P < 0.0001$ $F = 196.69$ df = (9, 20)

Means followed by a different letter were significantly different, $P < 0.05$, Tukey's test.
R = resistant (mortality < 80%) and S = susceptible (mortality > 98%) as determined by WHO (2016).
Knockdown rate was determined after 20-min exposure; mortality was calculated 24 h post-exposure.

16.4 min (the longest KT_{50} population: Samarinda); and 0.78 to 20.57 min (the longest KT_{50} population: Samarinda), respectively.

Mortality percentage for all the populations tested against d-allethrin, transfluthrin, and metofluthrin ranged from 11.67 to 100%, 8.33 to 100%, and 5 to 100%, respectively. Populations from Manggarai Barat and Pontianak exhibited full mortality to all three active ingredients tested, revealing high susceptibility. Mortality rates in accordance with WHO resistance indicators showed that mosquito strains from Denpasar, Mataram, Kuningan, Padang, Samarinda, and Sumba Timur were resistant (<80% mortality rate), whereas mosquito strains from Manggarai Barat, Dompu, and Pontianak were susceptible (>98% mortality rate) to the all the active ingredients assayed.

Spearman's rank-order correlation showed significant associations between the knockdown rates of d-allethrin and transfluthrin ($r = 0.833$; $P = 0.005$), d-allethrin and metofluthrin ($r = 0.700$;

P = 0.036), as well as transfluthrin and metofluthrin (r = 0.950; P = 0.000).

Discussion

Pioneer studies in 2005 and 2007 revealed *Ae. aegypti* populations in Indonesia recorded the occurrence of insecticide resistance to permethrin, deltamethrin, cypermethrin, and d-allethrin (Astari and Ahmad 2005, Ahmad et al. 2007). More recently, *Ae. aegypti* populations from several main cities of Indonesia continued to show the unrelenting development of insecticide resistance to a wide range of pyrethroids despite a 10-yr gap in between these studies (Hamid et al. 2017a,b), reflecting this particular class of insecticide remains the mainstay in mosquito control program. In accordance with the vast majority of reports from past studies (Astari and Ahmad 2005; Ahmad et al. 2007; Hamid et al. 2017a,b), detection of resistance in a few Indonesian *Ae. aegypti* populations to pyrethroid-based mosquito coils in this study is therefore conjecturable. The high reliance and extended use of pyrethroids for *Ae. aegypti* have unquestionably given rise to the prevalence of pyrethroid resistance. In particular, the use of fast-acting chemical insecticides for mosquito control exerts strong selection pressures that may favor the survival of resistant mosquitoes. For instance, the low mortality percentage of *Ae. aegypti* from Denpasar appeared to disclose high resistance status to all the active ingredients tested. This can be attributed to the high degree of mosquito control in the island to stop the outbreak when there was a record showing 6,898 DF cases with 38 deaths from 2014 to 2016. Furthermore, the use of pyrethroid-based spatial repellents other than mosquito coils such as mosquito-repelling lotion or patch is also suspected to be a large pyrethroid resistance contributory factor due to the rocketing growth of tourism in the island over the years. The potential of wild mosquito populations conferring resistance to these pyrethroid-based spatial repellents has been reported in Belize (Wagman et al. 2015) and Puerto Rico (Agramonte et al. 2016).

In the present study, adult *Ae. aegypti* collected from several study sites in Indonesia exhibited various trends in resistance against the three pyrethroid active ingredients assayed. High resistance level of *Ae. aegypti* exerted by the pyrethroid-based interventions in certain provinces is not particularly remarkable because pyrethroid resistance in this species of mosquito has long been logged in some countries, e.g., Malaysia, Thailand, and Singapore (Wan-Norafikah et al. 2010, Chuaycharoensuk et al. 2011, Koou et al. 2014). More recently, a nationwide study has been conducted in Malaysian *Ae. aegypti* and revealed that all strains were resistant to metofluthrin, d-allethrin, d-trans-allethrin, and prallethrin (Chin et al. 2017). This suggests pyrethroids only cause minimal insecticidal effect on pyrethroid-resistant *Ae. aegypti* as their efficacies have been compromised. It is also noteworthy to mention that KT_{50} of this study may not display direct representativeness to natural settings when the tested mosquitoes are restrained in a glass chamber where they may be exposed to higher concentrations of insecticides relative to when they are not strictly confined. Despite the limitations, glass-chamber method allows measurement of the responses of *Ae. aegypti* against a wide range of chemical accurately. This may not be probable in field settings with the presence of many environmental factors that can affect the efficacy of the products.

Recovery was observed in resistant strains after a 24-h post-treatment period in an insecticide-free setting, indicating the knockdown effect may be a temporary phenomenon for some populations. The discrepancies between the percentage of knockdown and mortality of adult Indonesian *Ae. aegypti* stress that they have been subjected to strong selection pressure after continuous exposure to pyrethroids for years, not forgetting the effect from the application of insecticides for crop protection and control of other medically important pests (Sekiyama et al. 2007, Thorburn 2015). It can also be ascribed to the role of mosquito coil as a spatial repellent that aids in declining mosquito behavior within the insecticide-exposed distance rather than killing (Chin et al. 2017).

Previous studies on insecticide resistance in Indonesia mainly focused on merely several main cities in Central Java Province or touristic islands, neglecting other cities although Indonesia has more than 900 inhabited islands stretched from east to west over 5,000 km. This geographical bias exposed the aforementioned cities to be high dengue-prone areas. On the contrary, the findings of this study showed that pyrethroid resistance is present in more than half of the tested populations, suggesting pyrethroid has long been used in the study areas. This also suggests the presence of geographical expansion in this species when resistant mosquitoes from small cities may have migrated from the historical dengue hotspots. The distribution of pyrethroid resistance *Ae. aegypti* populations is applicable when their eggs are capable in undergoing desiccation for a prolonged interval that allow them to put human travel in good use through dispersal across the country.

In Indonesia, remarkably alike as most of Southeast Asian countries, many insecticides have been used for mosquito control. It comes as no surprise when the case of resistance to dichlorodiphenyltrichloroethane (DDT) by *Ae. aegypti* is very common in Indonesia (Brengues et al. 2003) because DDT was the first chemical in widespread use to control *Ae. aegypti* and was introduced to the country since 1950s (Bang et al. 1982). Later, the government has banned the use in 1970s and subsequently substituted by pyrethroids. Resistance to both organochlorine and pyrethroid is conferred by point mutations on the voltage-gated sodium channel of insects, causing the insecticide resistance in *Ae. aegypti* arises through knockdown resistance (*kdr*; Ranson et al. 2000, Hemingway et al. 2004, Amelia-Yap et al. 2018). Based on the Spearman's rank-order correlation, the significant correlations between the knockdown rates of all active ingredients revealed the presence of cross-resistance within the pyrethroid group of insecticides. Cross-resistance occurs when there is overlapping of certain mechanisms due to insecticide pressure. It is believed the tolerance to single pyrethroid insecticide might possibly result in cross-resistance to other insecticides resided in this particular class (Lee et al. 2003, Kawada et al. 2009). The detection of cross-resistance is not unique to Indonesia because it has been reported in several countries among *Ae. aegypti*, i.e., Singapore (Koou et al. 2014), Mexico (Flores et al. 2013), and Malaysia (Chin et al. 2017). Constant presence of cross-resistance within the insecticides tested therefore suggested that they are no longer effective to control the mosquitoes in the studied populations.

Household insecticide formulations other than mosquito coils, e.g., liquid vaporizer, aerosol, and mats have been broadly introduced and easily obtained in Indonesian markets. The formulations of these products usually contained active ingredients from the pyrethroid group of insecticides, namely, d-allethrin, tetramethrin, d-trans-allethrin, prallethrin, transfluthrin, *s*-bioallethrin, permethrin, deltamethrin, and d-phenothrin (Yap et al. 2000). In response to the excessive reliance on these pyrethroid-based household insecticide products in indoor setting, high insecticide resistance was therefore detected in this study. This is attributable to the endophilic behavior of *Ae. aegypti*, which mean there are higher chances for them to expose or get in contact with the chemical released by these products and hence, the development of resistance via selection pressure (Chen et al. 2005). As the first coil study assessing the susceptibility status of *Ae. aegypti* against pyrethroid-based mosquito coils in Indonesia, the result provides an insight that varying insecticidal effects were exerted to Indonesian *Ae. aegypti*. This suggests the necessity of rotating different chemicals in different localities and the use of pyrethroids should be abandoned in locations with pyrethroid-resistant *Ae. aegypti*. The findings provided may contribute to the local authorities on the susceptibility data to be referred to in opting for an appropriate vector control program. Moreover, with the fact that the bioassays were conducted under laboratory conditions, there exists the probability that these products may not elicit ideal mortality responses to all strains for end user. Thus, it is suggested to employ semifield test system to ensure accuracy of the findings.

In conclusion, most of the adult *Ae. aegypti* populations tested have developed resistance to pyrethroid-based mosquito coils. The result of this study is contrasted to the results of some pioneer studies (Astuti et al. 2014, Putra et al. 2016) in Indonesia that demonstrated either susceptible or tolerant *Ae. aegypti*. This indicates that pyrethroid resistance has successfully developed in the country due to high reliance of routine application over the past years. Although the resistance ratios from some study sites remained low, the resistance may have built up gradually that provinces with susceptible strains may result in resistant in the future if the same control approach is still in use in a long run. This strongly suggests the abandoning of pyrethroid in vector control program and the reconstruction of well-structured national control measures urgently. However, due to its less hazardous characteristic to humans than other classes of insecticides, there possesses a possibility for the reintroduction of pyrethroid into the national vector control program in Indonesia later. Thus, the monitoring of the efficacy of pyrethroid against *Ae. aegypti* still has to be adhered closely, with the intention of turning back to its use at a proper time. With a critical need of effective mosquito control tools to combat resting mosquitoes, this study has accomplished in good time in reviewing the efficacies of Indonesian mosquito coils because this aids in gaining valuable knowledge to develop highly effective spatial repellents that can be complementing other control strategies to eliminate dengue. Last, the detection of insecticide resistance in some populations in this study infers the necessity of carrying out biochemical and molecular studies to characterize the resistance mechanisms in *Ae. aegypti*.

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