Journal of Medical Entomology, XX(X), 2018, 1–5
doi: 10.1093/jme/tjy208
Research

Monitoring Insecticide Resistance Profiles of *Aedes aegypti* (Diptera: Culicidae) in the Sunda Islands of Indonesia Based on Diagnostic Doses of Larvicides

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Subject Editor: Douglas Norris

Received 5 September 2018; Editorial decision 23 October 2018

Abstract

This study was conducted to monitor the susceptibility status of *Aedes aegypti* (Linnaeus) larvae in the Sunda Islands of Indonesia against various organophosphates and organochlorines. Larval bioassay was performed in accordance with the World Health Organization standard protocol. Field-collected and reference strains of *Ae. aegypti* larvae were tested against diagnostic doses of eight larvicides belonging to organophosphates and organochlorines, namely bromophos (0.050 mg/liter), chlopyrifos (0.002 mg/liter), fenitrothion (0.020 mg/liter), fenthion (0.025 mg/liter), malathion (0.125 mg/liter), temephos (0.012 mg/liter), DDT (0.012 mg/liter), and dieldrin (0.025 mg/liter). Mortality rates of larvae were recorded at 24-h posttreatment. This study showed that *Ae. aegypti* larvae from Padang, Samarinda, Manggarai Barat, and South Central Timor were susceptible to both fenitrothion and dieldrin (mortality rates ≥ 98%). About 6 out of 10 field strains of *Ae. aegypti* larvae were resistant (<80% mortality rates) against fenthion, whereas *Ae. aegypti* larvae from Kuningan, Samarinda, Sumba, and South Central Timor exhibited some degrees of resistance (mortality rates 80–98%). All field-collected *Ae. aegypti* larvae were resistant against diagnostic doses of chlorpyrifos, malathion, temephos, and DDT with mortality rates ranging from 0 to 74.67%. Continued insecticide susceptibility studies are essential to identify the efficacy of insecticides for an improved dengue vector control and to delay the development of insecticide resistance.

Key words: *Aedes aegypti*, larvicide, diagnostic dose, insecticide resistance, Indonesia

Dengue is a mosquito-borne viral disease which is currently considered an important public health problem due to its rapid spread in all regions of the world in recent years. In 1968, the first dengue outbreak was reported in Surabaya and Jakarta, Indonesia, with a total of 24 deaths (Sumarmo 1987). Since then, dengue cases have increased annually. In 2015, a total of 126,675 dengue cases with 1,229 deaths were reported, an increase in dengue cases over the previous year (Ministry of Health Republic of Indonesia 2016). Although an experimental dengue vaccine has been distributed to the Asian countries including Indonesia in 2016, there are certain limitations that need further investigation (Wichmann et al. 2017). Hence, the most effective approach in preventing the transmission of dengue virus is through interventions targeting the primary vector, *Aedes aegypti* (Linnaeus).

Since *Ae. aegypti* prefers artificial containers, this provides the opportunity to target the mosquitoes at the immature stage (Koou et al. 2014). Larviciding is also practical because larvicides can be easily obtained and applied directly by the community. Organophosphates have been used since the 1970s in Indonesia to control this vector (Ahmad et al. 2009). The neighboring countries of Malaysia, Singapore, and Thailand also utilize this same approach to control mosquitoes (Chen et al. 2005, Koou et al. 2014, Thongwat and Bunchu 2015). Organochlorines had been used in Indonesia for malaria eradication programs since the 1950s and were banned in the 1970s (Asih et al. 2012).

Excessive use and improper concentrations of larvicides can lead to insecticide resistance. Several studies have evaluated the status of

organophosphate resistance in *Ae. aegypti* in Indonesia (Widiarti et al. 2011, Mulyatno et al. 2012, Dwi et al. 2015, Putra et al. 2016). However, these previous studies focused only on certain parts of Indonesia (i.e., Surabaya, Bandung, and Yogyakarta on Java Island) and did not specify the diagnostic doses of the larvicides. Hence, the effectiveness of temephos, which is the most widely used, and other larvicides (i.e., organochlorines) remains unknown in many parts of Indonesia. Accordingly, this study aimed to monitor the resistance profile of the country based on diagnostic doses of eight organophosphate and organochlorine larvicides against *Ae. aegypti* larvae from 10 regencies of Indonesia.

Materials and Methods

Study Sites

Aedes aegypti eggs were collected from 10 residential areas across 10 regencies located in the Greater Sunda Islands and Lesser Sunda Islands of Indonesia. All collections were conducted in suburban areas. The geographical descriptions of study sites are shown in Table 1.

Collection Method

Ovitraps were prepared according to Lee (1992). Each ovitrap used was a 300-ml plastic container with a 6.5-cm diameter base, 9.0 cm in height, and a 7.8-cm diameter. A layer of black paint was sprayed on the outer wall of the container, whereas the inner surface of the container remains unsprayed. An oviposition paddle made from hardboard (10 × 2.5 × 0.3 cm) was placed diagonally into each ovitrap. Tap water that was aired overnight to allow chlorine evaporation was filled into each ovitrap to a level of 5.5 cm and the ovitraps were placed randomly in both indoor and outdoor of selected houses. Ovitraps were placed on the ground 25 m apart from each other.

Colonization of Mosquitoes

Ovitraps were collected after 5 d and brought back to the laboratory. The contents were poured into individual plastic containers, together with the paddle. Each container was added with overnight tap water and the larvae were allowed to grow in the laboratory. A small amount (0.01 g) of dried, powdered beef liver was added into each container as larval food. The hatched larvae were reared to adults in cages for species identification. Three days after emergence, *Ae. aegypti* female mosquitoes were identified and blood-fed by using a BALB/c mouse (albino, laboratory-bred strain). Two days after blood feeding, female mosquitoes were allowed to lay eggs. An oviposition site was prepared using a plastic cup (4.7-cm base diameter, 7.4-cm opening diameter, and 7.7-cm height) containing 200-ml overnight tap water and lined with filter paper. The eggs were removed, air dried, and the filter paper was immersed into overnight tap water to hatch the eggs. The hatched larvae were designated as first generation (F1). Late third-instar or early fourth-instar larvae of the F1 were used for bioassay. A laboratory reference strain of *Ae. aegypti* larvae (Bora-bora strain), which has been colonized under insecticide free conditions for 60 generations, was used for comparison.

Larvicides

The larvae were tested against diagnostic dosages of bromophos (0.050 mg/liter), chlorpyrifos (0.002 mg/liter), fenitrothion (0.020 mg/liter), fenthion (0.025 mg/liter), malathion (0.125 mg/liter), temephos (0.012 mg/liter), dieldrin (0.025 mg/liter), and DDT (0.012 mg/liter). All stock solutions of larvicides were supplied from the WHOPES Collaborating Centre in University Science of Malaysia, Penang. The concentration for each larvicide was prepared by dilution to 31.25 mg/liter for bromophos, fenitrothion, fenthion; 312.5 mg/liter for temephos; 6.25 mg/L for chlorpyrifos; 8% for malathion; 4% for DDT, and 1% for dieldrin.

Larval Bioassay

The larvicidal activity of each insecticide was assessed according to the World Health Organization (WHO) standard procedure for larval bioassay (WHO 2005). Twenty-five late third-instar larvae were introduced into 250 ml of test solution containing larvicides and ethanol in a 300-ml paper cup for 24 h. The concentrations were obtained by diluting commercial grade larvicide stock solutions with absolute ethanol. For the control, 1 ml of ethanol was added to 249-ml overnight tap water. The experiment was run in triplicate, and the

Table 1. Geographical description of study sites in Indonesia

Sunda Island	Island	Province	Regency	Study site	Coordinate
Greater Sunda Islands	Java	West Java	Kuningan	Kuningan	S 6°13′5.260″ E 106°50′15.936″
	Sumatra	West Sumatra	Padang	Air Tawar Barat	S 0°53′48.260″ E 100°20′45.265″
	Borneo	East Kalimantan	Samarinda	Sidodadi	S 0°28′41°.646″ E 117°08′46°.441″
		West Kalimantan	Pontianak	Bangka Belitung Laut	S 0°3′31.967″ E 109°21′19.322″
Lesser Sunda Islands	Bali	Bali	Denpasar	Sanur	S 8°41′10°.254″ E 115°15′23.634″
	Lombok	West Nusa Tenggara	Mataram	Pagesangan	S 8°36′2.666″ E 116°06′07.080″
	Sumbawa		Dompu	Bada	S 8°32′20.878″ E 118°27′28.799″
	Flores	East Nusa Tenggara	Manggarai Barat	Labuan Bajo	S 8°29 ′ 34.269″ E 119°52′40.889″
	Sumba		East Sumba	Waingapu	S 9°39′49.331″ E 120°16′17.321″
	Timor		South Central Timor	Soe	S 9°51′33.538″ E 124°15′44.345″

mortality of the larvae was assessed after 24 h. Larvae were considered dead if they sank to the bottom of the paper cups and failed to move or float after being probed (Othman et al. 2010).

Data Analysis

The percentage mortality was determined by dividing the number of dead larvae by the total number of larvae tested. The observed mortality was corrected using Abbot's formula, if the control mortality was between 5 and 20%. The larvae were considered 'susceptible' if the mortality was >98%, 'possibly resistant' if the mortality was between 90 and 97%, and 'resistant' if the mortality was <98% (WHO 2016). Mortality rates were recorded at 24-h after exposure.

$$\text{Percentage of mortality } (\%) = \frac{\text{Number of dead larvae}}{\text{Total number of larvae tested}} \times 100$$

All the data obtained were analyzed using SPSS Version 25.

Results

Susceptibility Status of Organophosphate Larvicides Against Field-Collected *Ae. aegypti* Larvae

Aedes aegypti from Kuningan, Padang, Samarinda, Pontianak, Dompu, Manggarai Barat, East Sumba, and Timor showed 98.67–100% mortality rates against fenitrothion, whereas *Ae. aegypti* from Denpasar was resistant (85% mortality). Mosquitoes from Mataram showed the possibility of resistance with mortality rates ranging from 90 to 97% (Table 2).

A wide range of mortalities were also observed in the field strain against the diagnostic doses of malathion and temephos, with zero mortality up to the 76% mortality, demonstrating the resistance status of both larvicides across all study sites.

On the other hand, all field-collected *Ae. aegypti* showed resistance against fenthion, chlorpyrifos, and bromophos with mortality rates ranging from 0 to 88.00%, indicating that these insecticides were no longer effective against the field populations of *Ae. aegypti* larvae (Table 2).

Susceptibility Status of Organochlorine Larvicides Against Field-Collected *Ae. aegypti* Larvae

All 10 field populations were resistant to DDT; eight of which (i.e., Kuningan, Samarinda, Pontianak, Denpasar, Mataram, Dompu, East Sumba, and South Central Timor) demonstrated zero mortality after 24 h of exposure. Five populations (Padang, Samarinda, Denpasar, Mataram, and Manggarai Barat) were fully susceptible against a diagnostic dose of dieldrin with 100% mortality rates. Pontianak and Dompu showed low mortality rates of 53.33 and 37.33%, respectively, against dieldrin, whereas Kuningan, East Sumba, and South Central Timor showed low to moderate resistance with mortality rates ranging from 81.33 to 97.33% (Table 2).

Discussion

The *Ae. aegypti* larvae in this study showed different degrees of susceptibility toward the organophosphate and organochloride larvicides. Resistance of larvae toward a few organophosphates has been reported in Indonesia (Mulyatno et al. 2012, Putra et al. 2016) and other South-East Asian countries, such as in Thailand, Malaysia, and Singapore (Wesson 1990, Chen et al. 2005, Ponlawat et al. 2005, Chareonviriyaphap et al. 2013, Chen et al. 2013, Koou et al. 2014, Thongwat and Bunchu 2015). Mass larviciding using temephos has been implemented in Indonesia to reduce *Ae. aegypti* populations since the early 1970s (Putra et al. 2016). However, due to its prolonged use, *Ae. aegypti* larval populations have developed resistance against the diagnostic dose of temephos. In this study, field strains of *Ae. aegypti* larvae exhibited resistance with mortality rates ranging from 4 to 76%. Similar findings have been reported in *Ae. aegypti* from Surabaya, Indonesia, and Selangor, Malaysia against the diagnostic dosage of temephos (0.012 mg/liter) with mortality rates

Table 2. Percentage mortalities of *Ae. aegypti* larvae from 10 study sites in Indonesia against diagnostic dosages of organophosphate and organochlorine larvicides after 24 h of exposure

Location	Insecticides							
	Organophosphate						Organochlorine	
	Fenitrothion 0.02 mg/liter	Fenthion 0.025 mg/liter	Chlorpyrifos 0.002 mg/liter	Bromophos 0.05 mg/liter	Malathion 0.125 mg/liter	Temephos 0.012 mg/liter	Dieldrin 0.025 mg/liter	DDT 0.012 mg/liter
Reference	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	92.00 ± 0.58^{P}	89.33 ± 1.67^{R}	0.00 ± 0.00^{R}	9.33 ± 0.33^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}
Kuningan	98.67 ± 0.33^{S}	88.00 ± 2.00^{R}	4.00 ± 0.00^{R}	81.33 ± 1.67^{R}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	97.33 ± 0.67^{P}	0.00 ± 0.00^{R}
Padang	100.00 ± 0.00^{S}	33.33 ± 0.88^{R}	46.67 ± 0.88^{R}	40.00 ± 0.58^{R}	0.00 ± 0.00^{R}	4.00 ± 0.00^{R}	100.00 ± 0.00^{S}	4.00 ± 0.00^{R}
Samarinda	100.00 ± 0.00^{S}	85.33 ± 0.88^{R}	1.33 ± 0.33^{R}	82.67 ± 0.33^{R}	1.33 ± 0.33^{R}	8.00 ± 0.00^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}
Pontianak	100.00 ± 0.00^{S}	61.33 ± 1.45^{R}	74.67 ± 1.86^{R}	38.67 ± 0.33^{R}	0.00 ± 0.00^{R}	4.00 ± 0.58^{R}	53.33 ± 1.76^{R}	0.00 ± 0.00^{R}
Denpasar	85.00 ± 0.33^{R}	11.00 ± 0.33^{R}	1.33 ± 0.33^{R}	20.00 ± 2.31^{R}	0.00 ± 0.00^{R}	25.00 ± 2.33^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}
Mataram	97.00 ± 0.33^{P}	39.00 ± 1.33^{R}	4.00 ± 0.00^{R}	9.30 ± 0.33^{R}	0.00 ± 0.00^{R}	9.30 ± 0.33^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}
Dompu	100.00 ± 0.00^{S}	49.33 ± 1.67^{R}	0.00 ± 0.00^{R}	54.67 ± 0.33^{R}	1.33 ± 0.33^{R}	49.33 ± 0.88^{R}	37.33 ± 0.67^{R}	0.00 ± 0.00^{R}
Manggarai Barat	100.00 ± 0.00^{S}	35.00 ± 1.33^{R}	2.67 ± 0.33^{R}	0.00 ± 0.00^{R}	1.33 ± 0.33^{R}	76.00 ± 0.00^{R}	100.00 ± 0.00^{S}	2.67 ± 0.33^{R}
East Sumba	98.67 ± 0.33^{S}	88.00 ± 1.00^{R}	25.33 ± 0.88^{R}	81.33 ± 2.33^{R}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	93.33 ± 0.67^{P}	0.00 ± 0.00^{R}
South Central Timor	100.00 ± 0.00^{S}	97.33 ± 0.67^{P}	9.33 ± 0.33^{R}	44.00 ± 1.00^{R}	33.33 ± 0.33^{R}	10.67 ± 0.33^{R}	81.33 ± 2.40^{R}	0.00 ± 0.00^{R}
	$P = 0.00$ $F = 18.57$ df = (10,22)	$P = 0.00$ $F = 96.81$ df = (10,22)	$P = 0.00$ $F = 375.28$ df = (10,22)	$P = 0.00$ $F = 54.75$ df = (10,22)	$P = 0.00$ $F = 153.10$ df = (10,22)	$P = 0.00$ $F = 458.00$ df = (10,22)	$P = 0.00$ $F = 2231.76$ df = (10,22)	$P = 0.00$ $F = 11.80$ df = (10,22)

R, resistant (mortality <90%); S, susceptible (mortality ≥ 98%), P, possible resistance (mortality 90–97%) as determined by WHO (2016).

ranging from 16 to 60% (Chen et al. 2005, Mulyatno et al. 2012). Low mortality rates (0–1.33%) were also observed in field strain larvae exposed to malathion, possibly due to the use of malathion in fogging to control adult mosquitoes over the past 36 yr (Putra et al. 2016).

Chlorpyrifos is not directly used to control the *Ae. aegypti* population in most countries including Indonesia. However, medium to high resistance to chlorpyrifos was observed in all field populations in this study. This is most probably due to cross-resistance to temephos or other organophosphates. Likewise, Rodriguez et al. (2002) reported that *Ae. aegypti* larvae from Venezuela and Cuba showed a similar trend of cross-reactivity to chlorpyrifos. This study also showed that all field strains of *Ae. aegypti* larvae were resistant to bromophos. In addition to its use as a larvicide to control mosquitoes, bromophos is used for fly control and in poultry farms (Rozendaal 1997). The pesticide residues of bromophos may indirectly affect mosquitoes as an environmental contaminate (Thongwat and Bunchu 2015).

Aedes aegypti larvae from Mataram and Denpasar had the lowest mortality rates against both fenthion and fenitrothion. However, fenthion and fenitrothion are not commonly used to control *Aedes* mosquitoes in Indonesia. In contrast, fenitrothion has been commonly used in other countries such as Thailand and Cuba to control *Aedes* mosquitoes (Bisset et al. 2013, Thongwat and Bunchu 2015). It is possible that the insecticide-resistant mosquitoes may have been accidentally transported from other countries by planes or ships (WHO 2017).

DDT was used for malarial control programs in Indonesia from 1950 until it was banned in 1970 (Asih et al. 2012). Although it has not been used for insecticide residual spray since the 20th century, larval populations in Indonesia are still resistant toward this insecticide class. Prior to the present study, the susceptibility of DDT against Indonesian *Ae. aegypti* larvae has not been reported. All tested field strain larvae were found resistant against DDT, probably due the excessive use of DDT in Indonesia over those past two decades (Dia et al. 2012). Nevertheless, DDT resistance has been reported in *Ae. aegypti* in Asia including Malaysia and India (Nazni et al. 2009, Mohsin et al. 2016). An increased level of enzyme glutathione S-transferase (GST) has been found to be involved in DDT resistance in insects (Tang and Tu 1994). DDT dehydrochlorination is the major route to detoxify DDT, the most common resistance mechanism in mosquitoes (Brown 1986, Hemingway et al. 2000). In addition, DDT resistance is also associated with a mutation in the target site of voltage-gated sodium channel (kdr) (Hemingway et al. 2000, Amelia-Yap et al. 2018). *Aedes aegypti* from Semarang, Indonesia, has previously been found resistant to DDT with an elevated level of GST and two kdr mutations. (Brengues et al. 2003).

Dieldrin was only used in Indonesia for a decade, from 1955 to 1965, before it was banned (Asih et al. 2012). In the present study, half of the populations were susceptible to this insecticide. However, three populations were resistant against the diagnostic dose. Resistance to dieldrin has been linked with mutations occurring in the gamma amino-butyric acid receptor in various insects, including *Aedes albopictus* (Ffrench-Constant et al. 2000, Low et al. 2015). Du et al. (2005) also reported that a substitution of alenine296 to glycine has been associated with dieldrin resistance in a laboratory strain of *Anopheles gambiae*. Thus far, there is no evidence of this mutation in *Ae. aegypti*.

In this study, the laboratory-reared Bora-bora reference strain of *Ae. aegypti* showed some level of resistance against bromophos, temephos, malathion, and DDT. Pasteur et al. (1995) documented resistance of *Culex quinquefasciatus* in Bora-bora against temephos. Resistance in Bora-bora is expected because malathion, fenitrothion, and temephos have been widely used in the vector control of *Ae. aegypti* (Failloux et al. 1994). Although this reference strain has been colonized in an insecticide free condition for >60 generations, resistance to these insecticides is still present. Similar observations (i.e., DDT resistance) have also been found in Malaysian laboratory strains of *Aedes aegypti* (Nazni et al. 2009) and *Cx. quinquefasciatus* (Low et al. 2013).

In conclusion, this study indicates that the commonly used insecticides (temephos and malathion) to control *Ae. aegypti* are no longer effective at the diagnostic doses. Alternative control strategies are recommended for vector control programs. Further, continued insecticide susceptibility studies are essential to identify the efficacy of insecticides for improved dengue vector control and to delay the development of insecticide resistance.

Acknowledgments

We are grateful for the financial support from the University of Malaya (RP021B/16SUS and RP021D/16SUS). We thank Mr. Muhammad Rasul Abdullah Halim for field assistance.

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