

Research Report

Present status of the insecticide susceptibility of *Aedes* mosquitoes in Thailand

Rawewan Srisawat^{1*}, Narumon Komalamisra^{1*}, Theerawit Phanphoowong¹, Tomohiko Takasaki², Lucky Ronald Runtuwene³, Ichiro Kurane², Hironari Narita³, Yuki Eshita^{4*}

Abstract

The current method for interrupting dengue fever transmission is spraying insecticide in the area affected by the epidemic. In such cases, information about the insecticide susceptibility of the vector would allow an appropriate insecticide to be applied. It is also a key factor for control programs. Therefore, the insecticide susceptibility of *Aedes aegypti* and *Ae. albopictus* were investigated using the WHO susceptibility test kit. Adult mosquitoes or larvae collected from Bangkok, Phra Nakhon Si Ayutthaya, Chanthaburi, and Prachin Buri from 2008–2010 and reared in a laboratory were exposed to insecticide for 1 hour at discriminating diagnostic doses recommended by the WHO, and mortality was examined after 24 hour. In general, *Ae. aegypti* were tolerant or resistant to all insecticides except malathion. Their degrees of insecticide resistance ranged from high to low in Bangkok, Phra Nakhon Si Ayutthaya, Prachin Buri, and Chanthaburi, respectively. In the Bang Khae and Thung Khru districts of Bangkok, insecticide resistance increased each year. *knock down* resistance, which is related to pyrethroid resistance, was observed in *Ae. aegypti*. On the other hand, *Ae. albopictus* from Prachin Buri, Bangkok, and Phang-nga were able to tolerate DDT. However no *Ae. albopictus* that were tolerant/resistant to pyrethroid or malathion were found in this study.

Key words: Insecticide susceptibility, *knock down* resistance, *Aedes* mosquito, Thailand

Introduction

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are vector borne diseases that are transmitted to humans through the bites of *Aedes* mosquitoes. Several tropical countries have suffered dengue epidemics that

caused serious levels of illness and death among children over the last 20 years¹⁾. In Thailand, the first outbreak of DF took place in 1958, and it still cyclically recrudesces today. In 2009, a total 52,919 cases including 46 lethal cases were reported by the Division of Epidemiology, Ministry of Public Health²⁾.

Among the numerous strategies applied to control dengue fever outbreaks, insecticide spraying is used to disrupt dengue fever outbreaks after cases have been reported. Once mosquitoes have been frequently exposed to insecticides, they develop insecticide resistance, which is now a major problem for vector control.

After the first dengue fever outbreak in 1958, DDT (organochlorine) was widely used to control *Ae. aegypti*. Many mosquito species were reported to be DDT

¹ Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

² Virology 1, National Institute of Infectious Diseases, Tokyo, Japan

³ Ohkagakuen University, Aichi, Japan

⁴ Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, Japan

* E-mail address for corresponding authors: tmrsw@mahidol.ac.th (RS), tmnkm2004@yahoo.com (NK) and yeshita@oita-u.ac.jp (YE)

resistant in 1992³⁾. However, *Ae. aegypti* was reported to be resistant to DDT in 1964 in Bangkok and Nakhon Ratchasima, Thailand and in Bangkok alone in 1969⁴⁾. Organophosphate and carbamate compounds, which were used in agriculture, were subsequently replaced with pyrethroid compounds⁵⁾. In the early 1990s, pyrethroids (e.g., permethrin, deltamethrin, lambda-cyhalothrin, and etofenprox) were introduced for controlling malaria (for impregnated bed nets and as indoor residual sprays) and dengue (space sprays)⁶⁾. At the moment, pyrethroids are the major insecticide group used for vector control in Thailand. In particular, deltamethrin has been used as the major adulticide in the national *Aedes* control program (until the present day), as reported by the Division of General Communicable Disease, CDC Department, Ministry of Public Health⁷⁾. The long term use of pyrethroid in Thailand leads mosquitoes to be resistant. One of the major mechanisms causing pyrethroid resistance is point mutations in the domain II of sodium channel gene. It is known as knockdown resistance (*kdr*) and linked to change in sensitivity of sodium channel to pyrethroid. At least four mutations (923, 982, 1011 and 1016 amino acid residues) were reported in permethrin-resistant *Ae. aegypti*^{8), 9), 10)}.

The insecticide susceptibility of *Aedes* can be characterized as susceptible, tolerant, or resistant. Moreover, the mechanistic level of resistance provides a powerful tool for improving resistance detection. Data about the insecticide susceptibility and resistance mechanism of vectors would greatly aid insecticide management for disease control. Basically, the development of resistance is increased with selection pressure, and the constant use of the same group of insecticides will produce a strong selection pressure that will lead to faster development of resistance. Therefore, understanding the current status of insecticide susceptibility is important for successful vector control management and vector surveillance.

Many factors need to be considered when attempting to develop a vector control program. One important factor is the insecticide resistance of the vector, which would allow appropriate dosages and insecticides to be used.

We report the current of insecticide susceptibility of *Aedes* from different localities and outline insecticides that are effective and ineffective for reducing this population. The pyrethroid resistance mechanism caused by point mutations in the domain II of sodium channel gene is also discussed.

Materials and Methods

Mosquitoes

Adults or larvae of *Ae. aegypti* and *Ae. albopictus* were collected from Bangkok, Phra Nakhon Si Ayutthaya, Chanthaburi, Phang-nga and Prachin Buri from 2008–2010. They were reared under laboratory conditions at 26–28°C under natural light-dark cycles (12: 12h) and 70–80% relative humidity. At least 100 female mosquitoes of each strain were used in each susceptibility test. *Aedes aegypti* that survived from exposure with insecticide were assigned as field insecticide resistant mosquito.

Insecticide

Paper impregnated with 0.05% deltamethrin, 0.75% permethrin, 5% malathion, 4% DDT, 0.05% lambda-cyhalothrin, 0.15% cyfluthrin, 0.1% propoxur, or 0.1% bendiocarb was obtained from the Vector Control Research Unit, School of Biological Sciences, University Sain, Malaysia.

Insecticide susceptibility test

Twenty-five 2 to 5-day old non-blooded female mosquitoes had their insecticide susceptibility tested according to the standard WHO procedure¹¹⁾. The discriminating doses of insecticides used in this study were recommended by WHO and were as follows: 0.75% permethrin, 0.05% deltamethrin, 5% malathion, 4% DDT, 0.1% propoxur, 0.15% cyfluthrin, 0.05% lambda-cyhalothrin, and 0.1% bendiocarb. The mosquitoes were kept in a holding tube for 1 hour before being exposed to the insecticide. Subsequently, the mosquitoes were transferred gently from the holding tube to the exposure tube containing insecticide-impregnated paper. The mosquitoes were exposed to the insecticide for 1 hour

and then transferred back to the holding tube. Ten percent sugar solution was provided to the mosquito, and mortality was observed after 24 hour. The control mosquitoes were exposed to paper containing silicone oil without insecticide for pyrethroids, olive oil without insecticide for organophosphates, and Risella oil without insecticide for organochlorines, respectively. When the mortality of the control mosquitoes was over 20%, the experiment was discarded. When the mortality was over 5% but less than 20%, mortality was adjusted using Abbott's formula:

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Knockdown resistance (*kdr*) gene detection

To detect *kdr* mutations, total RNA was isolated from individual mosquitoes according to the method of Srisawat et al⁹. Subsequently, first-strand cDNA was synthesized at 50 °C for 30 min using SuperScript™ One-step RT-PCR and the PLATINUM Taq reaction mixture (Invitrogen, USA). The specific primers used were NaF (forward primer) 5' CGT GGC GCT GTC GTT GCT C 3' and NaR (reverse primer) 5' CTT GTT CGT TTC GTT GTC GGC 3'. Thirty-five PCR cycles of 94°C for 30 s, 65°C for 30 s, and 68°C for 1 min were followed by a final extension step at 68°C for 2 min. The PCR products were purified and sequenced by Macrogen Inc using the ABI BigDye Terminator Cycle Sequencing kit with a 3730xl Automated DNA sequencer (PE Applied Biosystems, USA).

Results

Insecticide susceptibility test

Insecticide susceptibility status was defined according to percentage mortality as follows: 100–98% was designated as susceptible; <98% and >80% was designated as tolerant, which needed to be confirmed; and <80% was designated as resistant¹¹. According to these criteria, the *Ae. aegypti* collected at four sites showed resistance to DDT, bendiocarb, lambda-

cyhalothrin, and propoxur but they were all sensitive to malathion (Table 1). Tolerance to permethrin was only detected in the mosquitoes collected from Chanthaburi (94% mortality) whereas the remaining *Ae. aegypti* were resistant. Differences in deltamethrin susceptibility were detected among the four sites. The *Ae. aegypti* collected in Chanthaburi in 2009 were susceptible. However the opposite result was detected in 2010. The *Ae. aegypti* collected in Prachin Buri developed resistance to deltamethrin that needed to be confirmed, as did those found in Bangkok (Thung Khru and Taling Chan) and Phra Nakhon Si Ayutthaya (Bang Sai). On the other hand, the *Ae. albopictus* from Prachin Buri, Bangkok, and Phang-nga were only tolerant of DDT (Table 2), and all *Ae. albopictus* were susceptible to deltamethrin, permethrin, and malathion.

Knockdown resistance (*kdr*) gene detection

The field permethrin-resistant *Ae. aegypti*, which survived from 0.75% permethrin exposure, had their *kdr* gene sequences analyzed (especially the domain IIS6), and two point mutations were found in these populations. Both the S989P and V1016G substitutions were found in the same sample. Of the three genotypes (SS, SR, and RR), the SS genotype was found in the majority of 36 individuals derived from 42 permethrin-resistant *Ae. aegypti* at both mutation sites, followed by SR (4 individuals) and RR (2 individuals).

Discussion

Based on our results, *Ae. aegypti* were resistant to pyrethroids (permethrin, deltamethrin, lambda-cyhalothrin, and cyfluthrin), carbamates (propoxur and bendiocarb), and DDT. However, they were susceptible to organophosphates (malathion). This result was similar to the results of previous reports about the insecticide susceptibility of *Ae. aegypti* from many parts of Thailand^{7,12,13,14,15}. In the case of the resistance of *Ae. aegypti* to lambda-cyhalothrin and cyfluthrin, our results were different from those found in *Ae. aegypti* collected in northern Thailand, which were still susceptible to these

Table 1. Mortality (%) of *Aedes aegypti* females at 24 hours after exposure to 0.05% deltamethrin, 0.75% permethrin, 5% malathion, 4% DDT, 0.1% propoxur, 0.15% cyfluthrin, 0.1% bendiocarb, or 0.05% lambda-cyhalothrin

Province	District	Year	0.05% Deltamethrin	0.75% Permethrin	5% Malathion	4% DDT	0.1% Propoxur	0.15% Cyfluthrin	0.1% Bendiocarb	0.05% Lambda-cyhalothrin
Bangkok	Bang Khae	2008	66.27	16.96	99.88	1.08	-	-	-	-
		2009	47.15	3.65	99.00	0.25	6.35	42.10	27.35	19.45
	Thung Khru	2005	96.40	57.60	100.00	0.80	-	92.00	-	-
		2008	46.00	10.00	99.00	0.00	-	-	-	-
	Taling Chan	2005	82.00	57.60	99.00	0.00	-	9.00	-	-
		2010	56.00	19.00	100.00	0.00	8.00	5.00	5.00	38.00
Phra Nakhon	Sena	2010	32.00	7.00	-	1.00	-	-	-	-
Si Ayutthaya	Phra Nakhon	2010	51.00	2.00	9.00	2.00	-	-	-	-
	Bang Sai	2010	86.00	1.00	100.00	0.00	-	-	-	-
Prachin Buri	-	2008	96.00	51.00	100.00	2.00	-	-	-	-
	-	2010	86.00	19.00	98.50	0.00	22.50	40.00	56.50	1.00
Chanthaburi	-	2009	100.00	94.00	100.00	2.00	-	-	-	-
	Pawa	2010	27.00	1.00	100.00	0.00	14.00	35.00	59.00	0.00

- not detected

Table 2. Mortality (%) of *Aedes albopictus* females at 24 hours after exposure to 0.05% deltamethrin, 0.75% permethrin, 5% malathion, or 4% DDT

Province	Year	0.05% Deltamethrin	0.75% Permethrin	5% Malathion	4% DDT
Phang-nga	2009	99.00	100.00	100.00	85.00
Chanthaburi	2008	100.00	99.50	99.50	-
Prachin Buri	2009	100.00	100.00	100.00	80.00
Bangkok (Taling Chan)	2009	100.00	100.00	100.00	95.00

- not detected

compounds¹²⁾.

In general, the mortality rates of these insecticides gradually decreased each year, for example, in the Bang Khae districts, the mortality rate of permethrin decreased from 66.27 (year 2008) to 47.15 (year 2009), and similar results were found in Chanthaburi and Prachin Buri.

In Chanthaburi, the large increase in pyrethroid resistance (permethrin and deltamethrin) observed in 2010 was the result of the distribution of insecticide impregnated nets and indoor residual spray for malaria control in 2009 in this area. In contrast, the *Ae. aegypti* in Bangkok were resistant to permethrin and deltamethrin due to an ongoing dengue vector control program involving fogging the epidemic area with insecticide and the frequent use of insecticide household products (i.e., mats, liquid products, mosquito coils, and aerosol sprays). The most popular insecticide was pyrethroid because of its fast efficiency (knock down effect) and low toxicity.

The chance of exposure to insecticide in Bangkok (metropolitan) was therefore higher than in rural areas (Prachin Buri and Chanthaburi), which led to enhanced resistance.

The first report of DDT resistance in *Ae. aegypti* in Thailand was published in 1966 by Neely¹⁶⁾. A recent study found that *Ae. aegypti* were still resistant to DDT, even though it has not been imported into Thailand since 1995¹²⁾. It was suspected that DDT resistance confers cross-resistance to pyrethroids, as DDT and pyrethroids have the same resistance mechanism. They affect the insect's nervous system and paralyze it. In addition, DDT persists in the environment, making it dangerous to humans and animals.

At present, malathion was effective at controlling *Ae. aegypti* in the adult stage, and temephos was useful for controlling the larval stage. Both are organophosphate insecticides. Malathion was unpopular

because it is an acetylcholinesterase inhibitor that causes mosquito paralysis due to over stimulation at cholinergic nerve terminals. And also, it inhibits human acetylcholinesterase, which is a particular problem in spraymen who are frequently exposed to organophosphate insecticides¹⁷. However, it has now been accepted that temephos is safe when used at the dosage recommended for killing larval stages.

This study showed that *Ae. aegypti* were more resistant to most insecticides than *Ae. albopictus*. One possible reason for this is the different habitats of the two *Aedes* species; i.e., *Ae. aegypti* breed and rest in houses; hence, they are more likely to encounter insecticides than *Ae. albopictus*, which breed and rest outdoor.

One resistance mechanism to pyrethroids is an amino acid substitution in domain II of the sodium channel gene, which is a known target site. Only 989 and 1016 amino acid substitutions among five-reported substitutions^{8,9,10,13} were found in this study. The percentage of heterozygous and homozygous resistant *Ae. aegypti* was very low (14.28%). However, the high permethrin-resistant phenotype was observed. Thus, the frequency of this resistance allele should be investigated further in natural populations.

These results implied that the use of pyrethroid compounds for control should be cautioned against due to their reduced effectiveness. The continuous use of these compounds would be a selection pressure for the development of greater insecticide resistance in the surrounding area. Other groups of insecticides would therefore be more appropriate for vector control management.

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タイの *Aedes* (ヤブカ) 属蚊の殺虫剤感受性の現状

Rawewan Srisawat^{1*}, Narumon Komalamisra^{1*}, Theerawit Phanphoo Wong¹, 高崎 智彦²,
Lucky Ronald Runtuwene³, 倉根 一郎², 成田 弘成³, 江下 優樹^{4*}

抄 録

Dengue熱の伝播を止めるのに現在使われている方法は、流行地域に殺虫剤を噴霧することである。そのような場合、媒介昆虫の殺虫剤感受性についての情報があれば、適切な殺虫剤を散布できるであろう。このことは対策プログラムの重要な要素でもある。そこで、WHO 感受性評価キットを使って、ネッタイシマカ *Aedes aegypti* とヒトスジシマカ *Ae. albopictus* の殺虫剤に対する感受性を調べた。2008年から2010年にかけて、バンコク、Phra Nakhon Si Ayutthaya (プラ・ナコーン・シー・アユタヤー)、Chantha Buri (チャンタブリー)、Prachin Buri (プラチンブリー) で採集し、実験室で飼育した蚊の成虫または幼虫に、WHO が推奨している識別判定が可能な投与量の殺虫剤に1時間暴露させ、24時間後の死亡率を調べた。おおむね、ネッタイシマカはマラチオンを除く全ての殺虫剤に対して耐性あるいは抵抗性を示した。殺虫剤抵抗性の程度は、バンコク、プラ・ナコーン・シー・アユタヤー、プラチンブリー、チャンタブリーで、それぞれ高いレベルから低いレベルまで分布していた。バンコクの Bang Khae (バンケー) 区および Thung Khru (トゥンクル) 区では、殺虫剤に対する抵抗性が毎年増してきていた。ピレスロイド抵抗性と関係があるノックダウン型抵抗性 (*kdr*) 型抵抗性が、ネッタイシマカで観察された。一方、プラチンブリー、バンコク、Phang-nga (パンガー) から採集したヒトスジシマカは、DDT に対して耐性を示した。しかし、ピレスロイドあるいはマラチオンに対して耐性/抵抗性を示したヒトスジシマカはいなかった。

キーワード：殺虫剤感受性、ノックダウン型抵抗性、*Aedes* (ヤブカ) 属蚊、タイ

¹ Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

² 国立感染症研究所ウイルス1部

³ 桜花学園大学、日本赤十字豊田看護大学非常勤講師

⁴ 大分大学医学部感染予防医学講座

* 連絡先 E-mail: tmrsw@mahidol.ac.th (RS)、tmnkm2004@yahoo.com (NK)、yeshita@oita-u.ac.jp (YE)