

Resistance to Synthetic Pyrethroids in *Aedes aegypti* (Diptera: Culicidae) in Thailand

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ABSTRACT

The insecticide susceptibility level of mosquito populations is one of the major factors influencing the success of vector control. In this study, *Aedes aegypti* from seven localities with a current dengue outbreak in Thailand were subjected to synthetic pyrethroid insecticide susceptibility assays. The results revealed that *Ae. aegypti* from all localities were strongly resistant to bifenthrin, permethrin and deltamethrin. High resistance to lambda-cyhalothrin was detected from all localities with the exception of *Ae. aegypti* from Bangkok and Uttaradit which demonstrated incipient resistance. However, *Ae. aegypti* from Bangkok, Phra Nakhon Si Ayutthaya, Sakon Nakhon and Chumphon showed incipient resistance to alpha-cypermethrin whereas *Ae. aegypti* collected from Uttaradit, Mukdahan and Phatthalung were susceptible. In addition, *Ae. aegypti* from Bangkok, Phra Nakhon Si Ayutthaya and Uttaradit demonstrated incipient resistance to cypermethrin while susceptibility of *Ae. aegypti* from Mukdahan, Sakon Nakhon, Phatthalung and Chumphon to cypermethrin was observed. It was concluded that field-collected *Ae. aegypti* from all localities had developed resistance to the synthetic pyrethroids, with the majority of these being to bifenthrin, permethrin and deltamethrin.

Keywords: *Aedes aegypti*, synthetic pyrethroid, resistance, Thailand

INTRODUCTION

The incidence of dengue fever and dengue hemorrhagic fever (DF/DHF) has increased dramatically around the world in recent decades, especially in tropical and subtropical regions; about 2.5 billion people—two fifths of the world's population—are now at risk from DF/DHF and it is estimated that there are 50 million dengue infections worldwide every year (World Health Organization, 2012, 2014). The incidence of DF/DHF is still consistently high with 153,765 reported cases in Thailand (Ministry of Public Health, 2013). The disease is transmitted by *Aedes*

aegypti, a primary vector of DF and DHF (Gubler, 1997).

Aedes aegypti, a day-biting mosquito, is highly anthropophilic and often rests and feeds in or near human dwellings (Christophers, 1960). This mosquito has been found to be highly adapted to all man-made and natural environments. The key to preventing dengue transmission relies mainly on vector control, the most effective method for reducing disease transmission (Pant, 1979; Reiter and Gubler, 1997). During the past decade, several synthetic pyrethroids—namely permethrin, deltamethrin, lambda-cyhalothrin and etofenprox—were

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introduced in the malaria and dengue control program, particularly with the impregnation of bed nets (permethrin) and indoor/outdoor sprays (deltamethrin) (Chareonviriyaphap *et al.*, 1999). At present, pyrethroids are the main insecticides used in controlling vector-borne diseases throughout the country. However, the long-term continuous use of insecticides has led in some cases to high levels of chemical resistance by certain pests and disease vectors (Chareonviriyaphap *et al.*, 1999). This is considered to be a major factor in the development of resistance in mosquitoes (Chareonviriyaphap *et al.*, 1999; Sathantriphop *et al.*, 2006; Chuaycharoensuk *et al.*, 2011). Previous studies have shown the occurrence of insecticide resistance in several populations and species of mosquitoes, including *Ae. aegypti* (Chareonviriyaphap *et al.*, 1999; Ponlawat *et al.*, 2005; Jirakanjanakit *et al.*, 2007; Chuaycharoensuk *et al.*, 2011).

According to World Health Organization (WHO) guidelines for insecticide susceptible tests on mosquito populations (World Health Organization, 1998, 2006), the insecticide discriminating dose is important for determining the susceptibility status in mosquito populations. However, few diagnostic doses are currently available for insecticide susceptible assays on *Ae. aegypti* mosquitoes. In 2007, diagnostic doses of two synthetic pyrethroids (permethrin and deltamethrin) were established for *Ae. aegypti* (Jirakanjanakit *et al.*, 2007). In recent years, diagnostic doses of six synthetic pyrethroids,

consisting of alpha-cypermethrin, bifenthrin, cypermethrin, deltamethrin, lambda-cyhalothrin and permethrin, were obtained for monitoring the insecticide susceptibility of *Ae. aegypti* (Juntarajumnong *et al.*, 2012). Therefore, the aim of this study was to determine the insecticide susceptibility levels to six synthetic pyrethroids diagnostic doses recently available for *Ae. aegypti* populations collected from seven outbreak localities in Thailand.

MATERIALS AND METHODS

Study sites

Aedes aegypti larvae and pupae were collected from containers located in and around houses in seven collection sites within Thailand, namely—Bangkok (Khet Prawet), Phra Nakhon Si Ayutthaya (Ban Phraek district), Uttaradit (Tron district), Mukdahan (Mueang district), Sakon Nakhon (Mueang district), Phatthalung (Mueang district) and Chumphon (Mueang district) as shown in Figure 1. The samples were collected between 2011 and 2012 and the geographical coordinates of the locations are provided in Table 1. Larvae and pupae collected from the study sites were then reared in the Department of Entomology, Faculty of Agriculture, Kasetsart University for morphological identification and colonization. The standard insecticide susceptible laboratory strain of *Ae. aegypti* was obtained from the United States Department of Agriculture (USDA), Gainesville, Florida, USA.

Table 1 Location and global positioning system (GPS) coordinates of *Ae. aegypti* collection sites.

| Province | District | GPS coordinates | |
|--------------------------|------------|-----------------|----------------|
| Bangkok | Prawet | 13°40'9.5"N | 100°41'27.6" E |
| Phra Nakhon Si Ayutthaya | Ban Phraek | 14°38'35.9" N | 100°34'27.3" E |
| Uttaradit | Tron | 17°29'3.1" N | 100°6'23" E |
| Mukdahan | Mueang | 16°32'30.25" N | 104°43'1.1" E |
| Sakon Nakhon | Mueang | 17°15'13.3" N | 104°11'7.9" E |
| Phatthalung | Mueang | 7°36'52.7" N | 100°4'52.5" E |
| Chumphon | Mueang | 10°30'34.4" N | 99°6.5'25" E |

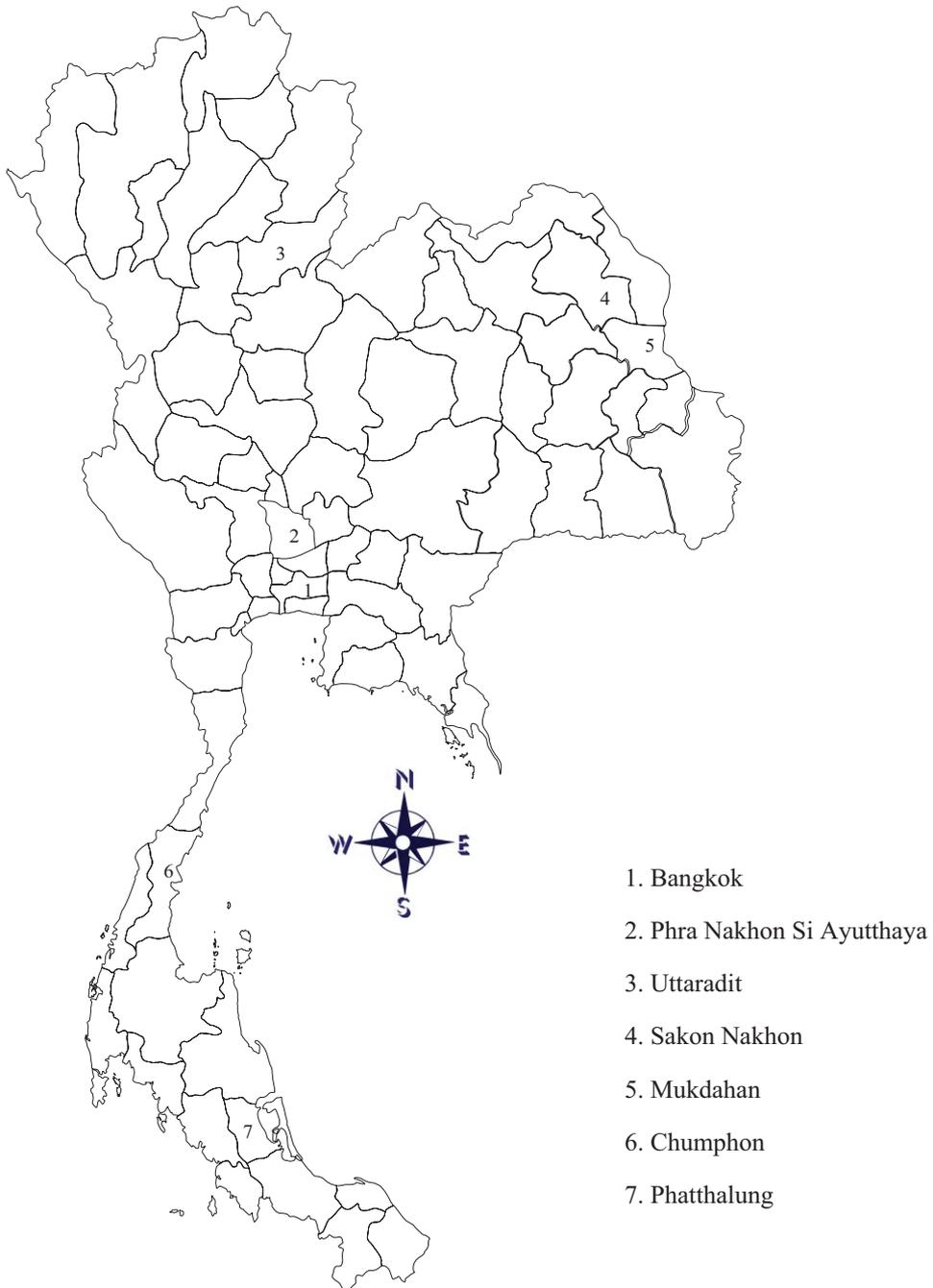


Figure 1 *Aedes aegypti* collection sites in various parts of Thailand.

Mosquito rearing

All strains of *Ae. aegypti* were reared and kept separately to ensure no accidental cross-breeding (hybridization) between populations. All developmental stages were reared in a temperature-

controlled space at $25 \pm 5^\circ\text{C}$ and $80 \pm 10\%$ relative humidity using a 12h:12h light:dark photoperiod according to the method of Kongmee *et al.* (2004). Immature stages were reared in plastic pans with identical physical and nutritional conditions

throughout the study. Pupae were transferred into cups with tap water and placed in screened cages (30 cm × 30 cm × 30 cm). Adult males and females were provided cotton pads soaked with 10% sugar solution. The resultant progeny and adults from the F₁ to F₃ generations were utilized for testing.

Insecticides

Six pyrethroid insecticides were used in this study: 1) alpha-cypermethrin (Sherwood Chemicals Public Company Limited, Bangkok, Thailand, purity 97.05%); 2) deltamethrin (Sherwood Chemicals Public Company Limited, purity 98.46%); 3) permethrin (Sherwood Chemicals Public Company Limited, purity 97.6%); 4) bifenthrin (Sherwood Chemicals Public Company Limited, purity 95.12%); 5) cypermethrin (T.J.C. Chemical Company Limited, Bangkok, Thailand, purity 92%); and 6) lambda-cyhalothrin (Syngenta Company, Bangkok, Thailand, purity 91.8%). Diagnostic concentrations for all test compounds are shown in Table 2 (Juntarajumnong *et al.*, 2012).

Insecticide-treated paper

Test papers (Whatman[®] No. 1 size 12 cm × 15 cm) were impregnated with diagnostic doses of synthetic pyrethroids, following the WHO standard protocol (World Health Organization, 1998). These synthetic pyrethroids (bifenthrin, permethrin, cypermethrin, alpha-cypermethrin, deltamethrin and lambda-cyhalothrin) were used for determining the susceptibility tests. All treated papers were treated at the rate of 2 mL of insecticide solution per paper. Control papers were impregnated with only carrier diluents (acetone and silicone oil).

Insecticide susceptibility test

Three to five day-old, non-blood fed adult females of *Ae. aegypti* were used for the susceptibility tests. Test procedures were obtained from the WHO, including analysis and

interpretation (World Health Organization, 1981a, b). Treated papers were prepared at the Department of Entomology, Faculty of Agriculture, Kasetsart University, according to WHO guidelines (World Health Organization, 1998). Each test was replicated four times. Twenty-five mosquitoes were carefully introduced into each holding tube lined with clean (untreated) paper for 1 hr to observe the health of the mosquitoes before insecticide exposure. Dead and moribund mosquitoes were removed before beginning the insecticide exposure. Mosquitoes from each holding tube were exposed for 1 hr to either insecticide-impregnated or control papers prepared in serial dilutions (determined from base-line findings). Knockdown mosquitoes were recorded after 1 hr. All mosquitoes were then carefully returned to separate clean holding tubes and provided with 10% sugar solution. Mortality was recorded at 24 hr post-exposure.

Data analysis

The mortality of *Ae. aegypti* at 24 hr was averaged for each test series. Interpretation and analysis of resistance/susceptibility status was determined according to WHO criteria (World Health Organization, 1998, 2006). If the percentage of mosquito mortality was between 98 and 100%, it was interpreted as completely susceptible. Mosquitoes were defined as incipiently resistant if the mortality rate was between 80 and 97% and were considered resistant if the mosquito mortality was less than 80%. If mortality of the control was between 5% and 20%, the test results were corrected using Abbott's formula (Abbott, 1925).

RESULTS

Diagnostic concentrations for six synthetic pyrethroids were established in previous work (Table 2). In this study, these "diagnostic doses" were used to determine the insecticide susceptibility level for eight populations of

Table 2 Diagnostic concentration of six synthetic pyrethroids based on dose/mortality relationships tested against *Ae. aegypti*, susceptible strain (US Department of Agriculture) (Juntarajumnong *et al.*, 2012).

| Insecticide | No. tested | LC ₅₀ (%) | 95% FL | LC ₉₉ (%) | 95% FL | Diagnostic concentration (%) | P > Chi square |
|----------------|------------|----------------------|---------------|----------------------|---------------|------------------------------|----------------|
| α-Cypermethrin | 302 | 0.0009 | 0.0004-0.0013 | 0.043 | 0.0220-0.1449 | 0.0863 | 0.9419 |
| Bifenthrin | 282 | 0.0185 | 0.0171-0.0202 | 0.047 | 0.0396-0.0599 | 0.0938 | 0.3082 |
| Cypermethrin | 311 | 0.0052 | 0.0031-0.0072 | 0.111 | 0.0662-0.2760 | 0.2212 | 0.1333 |
| Deltamethrin | 297 | 0.0007 | 0.0006-0.0007 | 0.002 | 0.0020-0.0034 | 0.0049 | 0.8955 |
| λ-Cyhalothrin | 299 | 0.0012 | 0.0010-0.0014 | 0.006 | 0.0043-0.0087 | 0.0116 | 0.8633 |
| Permethrin | 275 | 0.0379 | 0.0345-0.0407 | 0.073 | 0.0632-0.0922 | 0.1466 | 0.9718 |

LC = Lethal concentration; FL = Fiducial limits at 95% confidence level; LC₅₀ = Lethal concentration required to kill 50% of the tested population; LC₉₉ = Lethal concentration required to kill 99% of the tested population; Diagnostic concentration/discriminating dose calculation = $2 \times LC_{99}$.

Ae. aegypti—one laboratory and seven field populations. The results of susceptibility tests using the single diagnostic dose of 0.147% permethrin, 0.094% bifenthrin, 0.005% deltamethrin, 0.221% cypermethrin, 0.086% alpha-cypermethrin and 0.012% lambda-cyhalothrin for different populations of *Ae. aegypti* are shown in Table 3. The ability of mosquitoes to survive the diagnostic dose after 24 hr is indicative of resistance in the population as defined by the percent mortality in mosquito test populations. The results from the current study indicated that the control population (USDA) was completely susceptible to all test compounds as evidenced by 100% mortality (Table 3). Based on the diagnostic doses established by Juntarajumnong *et al.* (2012), the USDA *Ae. aegypti* control population was found to be completely susceptible to all six synthetic pyrethroids along with 100% knockdown after 60 min. All seven populations of *Ae. aegypti* were highly resistant to deltamethrin (0–37% mortality), followed by bifenthrin (4–39% mortality) and permethrin (2–55% mortality). In addition, high resistance to lambda-cyhalothrin (0–57% mortality) was detected from all localities with the exception of *Ae. aegypti* from Bangkok and Uttaradit which demonstrated incipient resistance (93–96% mortality). Moreover, the two

populations from Bangkok and Phra Nakhon Si Ayutthaya were found to have evidence of incipient resistance to alpha-cypermethrin and cypermethrin (91–97% mortality) whereas the populations from Mukdahan and Phattalung were susceptible to both insecticides indicated by 98–100% mortality. The populations from Sakon Nakhon and Chumphon demonstrated incipient resistance to alpha-cypermethrin (90–95% mortality); however, the population from Uttaradit was susceptible. The populations from Sakon Nakhon and Chumphon demonstrated susceptibility to cypermethrin (98–100% mortality) but the population from Uttaradit was incipient resistant.

In general, higher levels of physiological tolerance/resistance to bifenthrin, permethrin and deltamethrin were seen in northeastern populations (Mukdahan and Sakon Nakhon) and one southern population (Chumphon) compared to other geographical regions in Thailand. The strongest resistance to permethrin and lambda-cyhalothrin was seen in the population from Sakon Nakhon (2% mortality for permethrin and 0% mortality for lambda-cyhalothrin) whereas the highest resistance to deltamethrin was observed in the population from Mukdahan (0% mortality), followed closely by Chumphon (2% mortality) and Sakon Nakhon (6% mortality). Interestingly, the population

Table 3 Percent knockdown (KD) following 60 min exposure and mortality rate at 24 hr of *Ae. aegypti* to diagnostic concentrations of six pyrethroid insecticides.

| Area | Bifenthrin | | Permethrin | | Deltamethrin | | λ-Cyhalothrin | | α-Cypermethrin | | Cypermethrin | |
|--------------------------------------|------------|------------------|------------|------------------|--------------|------------------|---------------|------------------|----------------|------------------|--------------|------------------|
| | % KD | % Mortality ± SE | % KD | % Mortality ± SE | % KD | % Mortality ± SE | % KD | % Mortality ± SE | % KD | % Mortality ± SE | % KD | % Mortality ± SE |
| USDA (susceptible strain) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Bangkok (Prawet) | 0 | 6 | 5 | 6 ± 1.18 | 2 | 14 ± 1.02 | 16 | 93 ± 2.51 | 91 | 97 ± 2.51 | 96 | 97 ± 1.93 |
| Phra Nakhon Si Ayuthaya (Ban Phraek) | 0 | 4 | 9 | 23 ± 6.15 | 2 | 16 ± 5.01 | 1 | 26 ± 7.39 | 89 | 91 ± 3.53 | 89 | 97 ± 1.99 |
| Uttaradit (Tron) | 3 | 18 ± 6.00 | 4 | 10 ± 4.49 | 7 | 14 ± 7.75 | 17 | 96 ± 2.36 | 98 | 99 ± 1.00 | 82 | 89 ± 5.59 |
| Mukdahan (Mueang) | 1 | 8 ± 2.83 | 7 | 26 ± 2.58 | 0 | 0 | 16 | 22 ± 8.87 | 94 | 98 ± 1.15 | 97 | 99 ± 1.00 |
| Sakon Nakhon (Mueang) | 0 | 9 ± 3.15 | 0 | 2 ± 1.11 | 1 | 6 ± 2.00 | 0 | 0 | 86 | 90 ± 2.06 | 94 | 98 ± 1.85 |
| Phatthalung (Mueang) | 20 | 39 ± 3.32 | 30 | 55 ± 9.04 | 25 | 37 ± 0.91 | 24 | 57 ± 5.68 | 99 | 100 | 100 | 98 ± 1.85 |
| Chumphon (Mueang) | 1 | 5 ± 1.88 | 1 | 4 ± 1.57 | 1 | 2 ± 2.00 | 1 | 7 ± 3.28 | 85 | 95 ± 3.00 | 92 | 100 ± 1.00 |

collected from Chumphon demonstrated very high resistance to four synthetic pyrethroids (5% mortality for bifenthrin, 4% mortality for permethrin, 2% mortality for deltamethrin and 7% mortality for lambda-cyhalothrin) and showed incipient resistance to alpha-cypermethrin (95% mortality) and no resistance to cypermethrin (100% mortality). In contrast, the population from Phatthalung showed higher resistance to bifenthrin (39% mortality) and deltamethrin (37% mortality) than from permethrin (55% mortality) and lambda-cyhalothrin (57% mortality) as shown in Table 3.

In this study, the population from Bangkok, Central Thailand was strongly resistant to bifenthrin (6% mortality), permethrin (6% mortality) and deltamethrin (14% mortality). However, it showed incipient resistance to the other three synthetic pyrethroids (93% for lambda-cyhalothrin, 97% for alpha-cypermethrin and 97% for cypermethrin). In addition, the population from Phra Nakhon Si Ayutthaya in central Thailand showed high resistance to bifenthrin (4% mortality), followed by deltamethrin (16 % mortality) and permethrin (23% mortality). In brief, strong resistance to bifenthrin, permethrin and deltamethrin was observed in all field test populations whereas alpha-cypermethrin and cypermethrin demonstrated incipient or no resistance.

DISCUSSION

Aedes aegypti (L.) is the primary vector of dengue viruses in Southeast Asia, a region which represents the epicenter of disease transmission (Gubler, 1998). This vector is primarily a day-biting mosquito and is more prevalent near and inside dwellings. In addition, *Ae. aegypti* is considered a secondary vector of the chikungunya virus, a disease that has recently emerged in a more frequent epidemic form in Asia, Indian Ocean countries and southern Europe (Charrel *et al.*, 2007). *Ae. aegypti* also prefers to

rest indoors in undisturbed places, complicating control of this vector (Reiter and Gubler, 1997). Despite some research progress, an effective and commercially acceptable dengue vaccine is not yet available; thus, the prevention and control of disease transmission relies almost entirely on vector control strategies using synthetic insecticides (Roberts and Andre, 1994; Reiter *et al.*, 1995; Chareonviriyaphap *et al.*, 2004).

Synthetic compounds, including organophosphates, carbamates and pyrethroids have long been used with varying levels of success in national control programs to control dengue vectors (Reiter and Gubler, 1997). Since 1994, the Ministry of Public Health in Thailand has recommended the use of deltamethrin for emergency vector control and adulticide during dengue outbreaks and this latter chemical remains the only compound used in dengue control programs (Chareonviriyaphap *et al.*, 1999; Kongmee *et al.*, 2004). Recent studies have reported that there has been an increased deltamethrin resistance in several field populations of *Ae. aegypti* in Thailand (Jirakanjanakit *et al.*, 2007; Thanispong *et al.*, 2008; Chuaycharoensuk *et al.*, 2011). The increased incidence of resistance is raising awareness of the need for alternative insecticides or newer, more innovative methods of controlling mosquito vectors. Alpha-cypermethrin, another synthetic pyrethroid, is being used in Thai homes for protection against indoor biting mosquitoes and other arthropod pests.

Numerous synthetic pyrethroids, such as permethrin, resmethrin, cypermethrin, cyfluthrin, lambda-cyhalothrin and bifenthrin-based formulations (for example, aerosols, coils and gels), are commercially available to the general public (Paeporn *et al.*, 1996; Chareonviriyaphap *et al.*, 1999; Jirakanjanakit *et al.*, 2007). The selection for resistance to pyrethroids by mosquitoes is largely attributed to frequent exposure to sub-lethal concentrations of commonly applied chemicals and has a direct bearing on the effective management and

prevention of vector-borne diseases (Hemingway and Ranson, 2000). Many studies have reported mosquito resistance to synthetic pyrethroids in Thailand (Chareonviriyaphap *et al.*, 1999; Prapanthadara *et al.*, 2002; Somboon *et al.*, 2003; Paeporn *et al.*, 2004; Ponlawat *et al.*, 2005; Yaicharoen *et al.*, 2005; Sathantriphop *et al.*, 2006; Jirakanjanakit *et al.*, 2007; Thanispong *et al.*, 2008; Chuaycharoensuk *et al.*, 2011). However, the majority of published reports on pyrethroid resistance in *Ae. aegypti* have been restricted in their geographical scope with the susceptibility level of the insecticides needing updating frequently (Chadwick *et al.*, 1977; Chareonviriyaphap *et al.*, 1999; Paeporn *et al.*, 2004; Yaicharoen *et al.*, 2005; Sathantriphop *et al.*, 2006; Jirakanjanakit *et al.*, 2007; Thanispong *et al.*, 2008; Chuaycharoensuk *et al.*, 2011). Without a better understanding of the temporal effects and outcomes of the insecticides used to determine mosquito susceptibility, sustainable and successful vector control activities will never be positive.

As noted, WHO has established only two diagnostic doses for synthetic pyrethroids—permethrin and lambda-cyhalothrin (World Health Organization, 1998, 2006). Therefore, estimation of the resistance status of *Ae. aegypti* to pyrethroid compounds is incomplete. Recently, Juntarajumnong *et al.* (2012) developed diagnostic doses for six synthetic pyrethroids from the standard susceptible strain of *Ae. aegypti* from the USDA. These compounds currently represent the predominant chemical classes utilized for space spray applications and treated materials (for example, window curtains) in Thailand (Juntarajumnong *et al.*, 2012). In the current study, diagnostic doses were used for these compounds to investigate mosquito resistance. This study was consistent with the results from previous studies (Juntarajumnong *et al.*, 2012). It was found that there was a clear development of mosquito resistance to synthetic pyrethroids in the collected areas. In addition, incipient resistant was found to alpha-cypermethrin in some populations of *Ae.*

aegypti which were similar to those reported by Thanispong *et al.*, 2008. In the current study, five populations of *Ae. aegypti* demonstrated moderate to high resistance to lambda-cyhalothrin which had not been detected in any previous studies (Chuaycharoensuk *et al.*, 2011).

In summary, *Ae. aegypti* from several localities were resistant to bifenthrin, permethrin, deltamethrin and lambda-cyhalothrin as indicated by the low percentage mortality. Monitoring insecticide resistance should be carried out more frequently and should be increased in geographical coverage to include as many known vectors as possible. Further steps should include the identification of biochemical mechanisms responsible for resistance and should be an integral part of insecticide evaluation programs for effective integrated vector management practices.

CONCLUSION

Aedes aegypti were collected from seven localities in Thailand where there had been a dengue outbreak between 2011 and 2012. Susceptibility or resistance of mosquitoes to the synthetic pyrethroids, bifenthrin, permethrin, deltamethrin, lambda-cyhalothrin, alpha-cypermethrin and cypermethrin were determined using the WHO susceptibility test and the diagnostic doses determined by Juntarajumnong *et al.*, 2012. The results from the susceptibility tests revealed that *Ae. aegypti* from all localities were extremely resistant to bifenthrin, permethrin and deltamethrin. *Ae. aegypti* from most localities were also resistant to lambda-cyhalothrin with the exception of *Ae. aegypti* from Bangkok and Uttaradit which were incipient resistant. *Ae. aegypti* from Bangkok, Phra Nakhon Si Ayutthaya, Sakon Nakhon and Chumphon had incipient resistance to alpha-cypermethrin whereas *Ae. aegypti* collected from Uttaradit, Mukdahan and Phatthalung were still susceptible. Mosquitoes collected from all areas were found to be susceptible to cypermethrin except for Bangkok, Phra Nakhon Si Ayutthaya and Uttaradit which showed incipient

resistance.

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