Diagnostic Doses of Two Pyrethroids Currently Used for Control of
*Aedes aegypti* L. (Diptera: Culicidae), a Vector of Dengue

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**ABSTRACT**

Determining the diagnostic dose is crucial in evaluating the susceptibility of a mosquito population to certain insecticides. Presently, the diagnostic doses of only two pyrethroids (permethrin and \(\lambda\)-cyhalothrin) have been published for monitoring insecticide susceptibility of *Aedes aegypti*. Therefore, the objective of this study was to establish the diagnostic doses for two synthetic pyrethroids—bifenthrin and \(\alpha\)-cypermethrin—currently used in dengue vector control, using the United State Department of Agriculture (USDA) standard strain of *Aedes aegypti*. These diagnostic doses were subsequently used to evaluate the susceptibility status in field collected specimens of *Ae. aegypti*. Based on the baseline susceptibility levels, all three field strains demonstrated varying levels of physiological resistance to each compound. Strong resistance to bifenthrin was seen in one strain from Nong Khai resulting in 14.14% mortality whereas complete mortality (100%) was observed in the USDA standard strain when exposed to the established diagnostic doses of these two synthetic pyrethroids. These diagnostic doses could be applied for monitoring the susceptibility status in mosquito populations across Thailand.

**Keywords:** insecticide, diagnostic dose, *Aedes aegypti*, pyrethroids, Thailand

**INTRODUCTION**

Over 100 countries around the world are at risk from a wide variety of vector-borne diseases, including dengue fever (DF) and dengue hemorrhagic fever (DHF), with an estimated 2.5 billion people at risk of contracting dengue many of whom live in Southeast Asia (WHO, 2002). Approximately 50–100 million people are infected annually with dengue viruses worldwide, primarily in crowded, impoverished urban regions of the world (WHO, 2009). In Southeast Asia, DHF cases have been increasing from an annual rate of below 10,000 in the 1960s to more than 200,000 in the 1990s (Gibbons and Vaughn, 2002; WHO, 2009). In Thailand, there were 115,434 dengue cases in 2010 with 141 reported deaths (Bureau of the Vector-borne Diseases, 2010).

The four viral serotypes (DEN-1, 2, 3, 4) are transmitted primarily by *Aedes aegypti* (L.), a highly efficient mosquito vector that is often found in and near human dwellings. In Thailand, this mosquito-borne disease causes tremendous morbidity and mortality each year. To date, no effective or commercial multivalent dengue vaccine is available. Prevention of this disease...
remains almost entirely dependent on using various methods of vector control by attacking both the adult and immature stages of the mosquito. Vector control remains the most effective means of reducing the risk of virus transmission (Reiter and Gubler, 1997; WHO, 1999). Unfortunately, *Ae. aegypti* has confounded organized control efforts due to its close association with humans and its exploitation of domestic and peridomestic environments. In Thailand, the standard vector control techniques are based on the use of chemicals and source reduction.

Many chemical compounds, including organophosphates, carbamates, synthetic pyrethroids and so-called bio-rational pesticides (bacterial toxins and insect growth regulators) have long been used in national public health vector control programs (Reiter and Gubler, 1997; WHO, 1998a). In Thailand, several synthetic pyrethroids, for example, deltamethrin, cyfluthrin, bifenthrin, α-cypermethrin, and permethrin, have been introduced into the public market for controlling *Ae. aegypti* (Chareonviriyaphap *et al.*, 1999; Somboon *et al.*, 2003; Thanispong *et al.*, 2010). However, control of this mosquito has been hampered by the development of resistance to these insecticidal compounds in *Ae. aegypti* populations throughout Thailand (Chareonviriyaphap *et al.*, 1999; Somboon *et al.*, 2003). The selection for resistance to pyrethroids has largely been attributed to the frequent use of the same chemical class of compounds and is believed to have a direct bearing on the effective management and prevention of vector-borne diseases in general (Hemingway and Ranson, 2000). Although, several published reports have described the status of pyrethroid resistance in *Ae. aegypti* populations in Thailand (Chadwick *et al.*, 1977; Charoenviriyaphap *et al.*, 1999; Paeporn *et al.*, 2004; Yaicharoen *et al.*, 2005; Sathantriphop *et al.*, 2006; Jirakanjanakit *et al.*, 2007; Thanispong *et al.*, 2008), these reports were based on the use of ‘diagnostic’ doses established by the World Health Organization (WHO, 1998b). Baseline insecticide susceptibility has not been established for the detection of insecticide resistance in any populations of *Ae. aegypti* in Thailand. The aim of this study was to establish the diagnostic dosage of two synthetic pyrethroids—bifenthrin and α-cypermethrin—using the USDA susceptible standard strain of *Ae. aegypti*. These values were then used to determine the susceptibility of field strains of *Ae. aegypti* in Thailand.

**MATERIALS AND METHODS**

**Mosquito strains**

A susceptible standard strain of *Ae. aegypti* (USDA) was used to establish the baseline susceptibility levels of two synthetic pyrethroids that were used to determine the susceptibility status of three local strains of *Ae. aegypti* as follows: 1) the USDA laboratory strain was provided by the Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida, U.S.A. This colony has been maintained in colony for over 40 y; 2) the Kanchanaburi strain was obtained in May 2010 as larvae from outdoor container habitats at Pu Teuy village, Sai Yok district (14°20'12.1″N, 98°59'19″E), Kanchanaburi province, an area west of Bangkok; 3) the Khon Kaen strain was obtained in November 2010 as larvae from outdoor container habitats in Muang district (16°24’52″N, 102°05’5″E), Khon kaen province, northeast Thailand; 4) the Nong Khai strain was obtained in November 2010 as larvae from outdoor container habitats in Tha Bor district (17°51’51.1″N, 102°34’30.5″E), Nong Khai province, northeast Thailand.

**Mosquito rearing**

Mosquito larvae and pupae were collected from each site, brought back to the insectary at the Department of Entomology, Kasetsart University, and reared to the adult stage. Adult mosquitoes identified as *Ae. aegypti* were transferred to a mosquito cage and permitted guinea-pig blood on the third/fourth day post emergence. Two
days postbloodfeeding, oviposition dishes were placed in the cages containing gravid females. All strains were colonized separately under laboratory-controlled conditions at 25 ± 3°C and 75 ± 15% RH in the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

**Chemicals**

Two insecticides—namely, α-cypermethrin (purity 97.05%; Cherwood Chemical Ltd; Bangkok, Thailand) and bifenthrin (purity 95.12%; Cherwood Chemical Ltd; Bangkok, Thailand)—were used in the susceptibility testing.

**Insecticide impregnated paper**

Test papers 12 × 15 cm² impregnated with each chemical at a single diagnostic dose (99% lethal dose (LD₉₉) × 2) established from the USDA standard susceptible strain, were prepared in the laboratory at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand (Table 2). All papers were treated at the rate of 2 mL of the insecticide solution per 180 cm².

**Susceptibility assay**

The level of susceptibility of each population to the two synthetic pyrethroids was assessed by exposing 25 sugar fed 3–5 day-old female mosquitoes to a single diagnostic dose established from the USDA standard susceptible strain. Standard testing procedures were followed according to WHO guidelines (1998b). After a 60 min exposure, tested and control mosquitoes were transferred to separate holding containers and mortalities were recorded after 24 h post-exposure. The test was repeated four times and an average susceptibility level was derived for each strain. Based on the percent mortality in the test population, mosquito survival was indicative of the degree of physiological resistance in that population.

**Analysis**

Control mortality was corrected using Abbott’s formula (Finney, 1971). The LD₉₉ value was calculated from a dosage-mortality regression line using the log-probit program from the Statistical Analysis System (SAS) software (SAS, 2002). The estimate of the 99% lethal dose (LD₉₉) was determined from four replicates conducted on the USDA susceptible strain of *Ae. aegypti*. Double the concentration for LD₉₉ was determined as the “diagnostic dose” and used for susceptibility/resistance tests on *Ae.aegypti* field strains. Determination of resistance/susceptibility status was done according to WHO criteria (WHO, 1998b).

**RESULTS**

The baseline susceptibility levels to synthetic pyrethroids currently used in dengue vector control in Thailand were established from a standard susceptible strain (USDA). A baseline was established using the insecticide doses which gave mortality ranging between 10 and 95% in the USDA susceptible strain. Small chi-square values (0.9419 for α-cypermethrin and 0.3082 for bifenthrin) demonstrated that the responses of *Ae. aegypti* mosquitoes to each compound in the susceptibility trials satisfied a linear model (*P > 0.05*) as shown in Table 2. The 50% lethal dose (LD₅₀ value) and the 99% lethal dose (LC₉₉ value) to two synthetic pyrethroids against *Ae. aegypti* (USDA) were determined by using log-probit analysis (SAS, 2002). Alphacypermethrin produced an LD₅₀ value of 0.0009% whereas bifenthrin gave an LD₅₀ value of 0.0185%. With LD₉₉ values, α-cypermethrin produced the dose at 0.043% whereas bifenthrin yielded the dose at 0.047%. A single diagnostic concentration (double concentration of LD₉₉) of α-cypermethrin (0.086%) and bifenthrin (0.094%) was obtained (Table 2). The established diagnostic dose was subsequently used to determine the susceptibility level of three field strains of *Ae. aegypti* (Kanchanaburi, Khon
Kaen and Nong Khai) (Table 1).

The results of the susceptibility tests using the established diagnostic dose for the two synthetic pyrethroids are presented in Table 3. The ability of mosquitoes to survive the diagnostic dose after 24 h is indicative of resistance in the population. Based on WHO recommendations, the interpretation of insecticide susceptibility results is divided into three categories: 1) mosquitoes are susceptible to insecticides if the percent mortality is between 98 and 100%; 2) mosquitoes demonstrate incipient insecticide resistance if the percent mortality is between 80 and 97%; or 3) mosquitoes are resistant if the percent mortality is less than 80% (WHO, 1998b).

The results of the current study demonstrated that the USDA standard strain was completely susceptible to all compounds as indicated by 100% mortality to all compounds tested. However, when the *Ae. aegypti* from the three different localities were tested, various levels of tolerance/resistance to the two synthetic pyrethroids were revealed (Table 3). Based on established diagnostic dosages, a trend of tolerance/resistance to the two pyrethroids was identified in the three field strains with mortality rates ranging between 9.68% for bifenthrin and 97.96% for α-cypermethrin. The Khon Kaen and Nong Khai strains were found greatly resistant to bifenthrin as indicated by the low percent mortality results (9.68% for Khon Kaen and 14.14% for Nong Khai). The Kanchanaburi and Nong Khai strains of *Ae. aegypti* were slightly tolerant to α-cypermethrin (97.83% and 97.96% mortality, respectively).

**DISCUSSION**

Globally, mosquito-borne diseases kill thousands of people each year (WHO, 2009). Mosquitoes are a nuisance and a public health threat and serve as potential vectors of parasitic

<table>
<thead>
<tr>
<th>Collection site/province</th>
<th>Village</th>
<th>District</th>
<th>GPS coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanchanaburi</td>
<td>Pu Teuy</td>
<td>Sai Yok</td>
<td>14°20'12.1&quot;N, 98°59'19&quot;E</td>
</tr>
<tr>
<td>Khon kaen</td>
<td>Non Ton</td>
<td>Muang</td>
<td>16°24'52&quot;N, 102°05'5&quot;E</td>
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<tr>
<td>Nong Khai</td>
<td>Pa Ngew</td>
<td>Tha Bor</td>
<td>17°51'51.1&quot;N, 102°34'30.5&quot;E</td>
</tr>
</tbody>
</table>

**Table 2** Susceptibility data of two pyrethroids based on dose/mortality relationships tested against *Aedes aegypti*, USDA strain.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>No. Tested</th>
<th>LD₅₀ (%)</th>
<th>95% CI</th>
<th>LD₉₀ (%)</th>
<th>95% CI</th>
<th>Diagnostic dose (%)</th>
<th>Chi square</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Cypermethrin</td>
<td>302</td>
<td>0.0009</td>
<td>0.0004-0.0013</td>
<td>0.043</td>
<td>0.0220-0.1449</td>
<td>0.0863</td>
<td>0.9419</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>282</td>
<td>0.0185</td>
<td>0.0171-0.0202</td>
<td>0.047</td>
<td>0.0396-0.0599</td>
<td>0.0938</td>
<td>0.3082</td>
</tr>
</tbody>
</table>

LD₅₀ = 50% lethal concentration; 95% CI = 95% confidence interval; LD₉₀ = 99% lethal concentration.

**Table 3** Insecticide susceptibility level of four strains of *Aedes aegypti*.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Diagnostic Dose (%)</th>
<th>USDA</th>
<th>Kanchanaburi</th>
<th>Khon Kaen</th>
<th>Nong Khai</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>Dead (%)</td>
<td>No. tested</td>
<td>Dead (%)</td>
<td>No. tested</td>
</tr>
<tr>
<td>α-Cypermethrin</td>
<td>0.086</td>
<td>100 (100)</td>
<td>92 (97.83)</td>
<td>104 (98)</td>
<td>98 (97.96)</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.094</td>
<td>97 (100)</td>
<td>92 (89.39)</td>
<td>93 (9.68)</td>
<td>99 (14.14)</td>
</tr>
</tbody>
</table>
diseases such as malaria (Krogstad, 1996; WHO, 2002), dengue/dengue hemorrhagic fever (Gubler et al., 1995; Brogdon et al., 1998) and filariasis (Thompson et al., 1996) among others. One of the keys to preventing disease transmission is the reduction of human-vector contact through the use of insecticides (Roberts et al., 1997; Jirakanjanakit et al., 2007). However, the long-term use of insecticides may result in insecticide resistance in an insect population of, for example, mosquitoes, body lice, bedbugs, fleas, ticks, flies and cockroaches (WHO, 1992; Roberts et al., 1994; Thanispong et al., 2010). The information on insecticide resistance in Ae. aegypti, which is a vector of dengue/dengue haemorrhagic fever, is relatively scarce due to a shortage of comprehensive, sustainable and effective monitoring systems used within the framework of the national public health vector control program in Thailand. Aedes aegypti is one of the most efficient, well-adapted and widely distributed mosquito species found throughout the tropical and sub tropical zone and has proven extremely difficult to control. Among several available control techniques, chemical control remains the most effective proven method to reduce dengue transmission. Of the chemicals available to choose from, synthetic pyrethroids are the most common and extensively used in both the government and private sectors. Pyrethroids have the potential to be effective adulticides at present (Chareonviriyaphap et al., 1999; Kongmee et al., 2004, Jirakanjanakit et al., 2007; MOPH, 2010). In vector control programs, ultra-low volume (ULV) application of deltamethrin has been effective at interrupting dengue transmission when used soon after the first dengue case is reported. For household use, several synthetic pyrethroids are currently available for homeowners to control household arthropod pests. However, long-term, continuous and repetitive contact with insecticides has resulted in some degree of insecticide resistance in Ae. aegypti mosquito populations.

Although a number of studies on pyrethroid resistance in Ae. aegypti have subsequently been published, most have reported findings that follow the WHO standard criteria used for monitoring resistance in Anopheles spp. mosquitoes which may not be appropriate or reliable for all vector species. Only diagnostic dosages of lambda-cyhalothrin (0.03%) and permethrin (0.25%) have been reported for detecting the resistance status of Ae. aegypti populations (WHO, 2011). Diagnostic tests for insecticide resistance to the currently labeled chemicals used in vector control are of paramount importance in order to monitor and evaluate their effectiveness after long-term use in a vector control program. In recent years, diagnostic doses of two commonly used synthetic pyrethroids—permethrin (0.9%) and deltamethrin (0.06%)—were established for a susceptible reference strain of Ae. aegypti in Bora, French Polynesia (Jirakanjanakit et al., 2007). However, these two diagnostic doses were obtained from an analytical process of log-probit mortality relationships which gave a very high chi-square and an extremely low P-value (P < 0.005), indicating a poor goodness of fit (Jirakanjanakit et al., 2007). It is still believed that conclusive information on susceptibility baselines and diagnostic doses for various compounds used to control Ae. aegypti are lacking and that information on the susceptibility status of Ae. aegypti to pyrethroids may not be completely accurate. Furthermore, data on insecticide susceptibility levels are limited to only small focal areas of Thailand (Chareonviriyaphap et al., 1999; Prapanthadara et al., 2002; Ponlawat et al., 2005; Jirakanjanakit et al., 2007; Thanispong et al., 2008; Chuaycharoensuk et al., 2011). In addition, WHO (2005) recommended that baseline insecticide susceptibility levels should be determined on a reference strain prior to evaluating the susceptibility level in any field mosquito populations. A global dengue control program may not succeed without adequate information on insecticide susceptibility levels in Ae. aegypti populations.
The current study used three field-collected *Ae. aegypti* strains to determine the possibility of applying the established diagnostic doses for two synthetic pyrethroids. The results indicated that the physiological resistance in Thai *Ae. aegypti* populations varies depending upon the mosquito strain and the test chemical. A strain from Kanchanaburi province demonstrated similar mortality levels to the susceptible USDA reference strain with only low levels of resistance to α-cypermethrin and bifenthrin as reported by Thanispong *et al.* (2008) and Chuaycharoensuk *et al.* (2011). Furthermore, the current findings show similar results to previous reports on the resistance levels of various strains of *Ae. aegypti* to synthetic pyrethroids (Charoenviriyaphap *et al.*, 1999; Prapanthadara *et al.*, 2002; Ponlawat *et al.*, 2005; Jirakanjanakit *et al.*, 2007; Thanispong *et al.*, 2008; Chuaycharoensuk *et al.*, 2011).

Synthetic pyrethroids are currently the insecticides of choice for dengue control in Thailand. The monitoring of insecticide resistance should be increased in consistency, periodicity, and geographic coverage and should be an integral part of any vector/public health program. The investigation of cross resistance to the same, similar or related synthetic compounds should also be included in any insecticide evaluation program. Knowledge of vector/pest susceptibility to pesticides, changing trends of resistance and their operational implications are basic requirements to guide pesticide use in vector-borne disease and pest control programs.

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