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Detection of insecticides resistance status in *Culex quinquefasciatus* and *Aedes aegypti* to four major groups of insecticides

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Abstract. The resistance to various insecticides from 4 major groups (organochlorine, organophosphate, carbamate and pyretroid) was investigated in a field strain of *Culex quinquefasciatus* from Baan Suan community, Nonthaburi province, Thailand by using a standard World Health Organization susceptibility test. The Baan Suan strain was completely resistant to DDT and highly resistant to deltamethrin, permethrin, fenitrothion and propoxur but this strain was still found to be highly susceptible to malathion. This strain displayed high resistance to cypermethrin since the result revealed that the resistance ratio of the 50% lethal concentration value (RR_{50}) between the field and the laboratory strains (NIH strain) was 16. The study indicated that mosquitoes were resistant to almost all insecticide tested except malathion and this should be an alternative for *Cx. quinquefasciatus* control in this area. Moreover, *Aedes aegypti*, which is a main dengue vector in Baan Suan community was also tested with deltamethrin, permethrin and fenitrothion. The results showed that dengue mosquitoes are clearly resistant to permethrin and tolerant to deltamethrin, but was 100% susceptible to fenitrothion. The cause of insecticide resistance in *Cx. quinquefasciatus* may be due to the continuous use of insecticide for dengue vector control programme in Baan Suan community.

INTRODUCTION

Culex quinquefasciatus is a common nuisance mosquito that lives close to people of Baan Suan community, Nonthaburi, Thailand because a large number of *Cx. quinquefasciatus* larvae have been found in ditches around homes in this community. Although in Thailand, *Cx. quinquefasciatus* is not a vector of bancroftian filariasis it has been proved to be positive for *Wuchereria bancrofti* in the laboratory (Sucharit *et al.*, 1981). This may lead to future outbreak of filariasis due to immigrant workers coming in from filariasis endemic areas in Myanmar (Swaddiwudhipong *et al.*, 1996).

The species, *Aedes aegypti* is the important vector of dengue fever in Baan Suan community and some dengue cases were reported by The Health Care Center of Ta-sai District. The local authorities have routinely used permethrin and deltamethrin for control of dengue vector since 1998. Continuous use of insecticides for long period may cause the development of insecticide resistance in mosquito. In some areas, *Ae. aegypti* mosquitoes are resistant to permethrin and deltamethrin such as in Ratchaburi Province (Paeporn *et al.*, 2004), Nan and Chiang Mai Provinces (Somboon *et al.*, 2003). While insecticides are used for the control of *Ae. aegypti* it is possible for *Cx. quinquefasciatus* to become resistant. *Cx. quinquefasciatus* from Chiang Mai province, was also reported to be tolerant to permethrin, deltamethrin, malathion and fenitrothion and resistant to DDT and etofenprox (Somboon *et al.*, 2003). Also, cross-resistance between pyrethroid and non-pyrethroid compounds may occur in mosquito vectors since they share the same target-site mechanisms such as cross-resistance in *Cx. quinquefasciatus* was observed to pyrethroid (lambda-cyhalothin) and organophosphate (malathion) caused by overproduced esterase (Bisset *et al.*, 1997). Moreover, *Ae. aegypti* in northern Thailand was reported to have cross-resistance to deltamethrin, permethrin and etofenprox (Somboon *et al.*, 2003). Therefore, the investigation of insecticide resistance in mosquitoes should be monitored.

This study was conducted to detect the susceptibility status of *Ae. aegypti* and *Cx. quinquefasciatus* populations from Baan Suan community to different insecticides to plan an effective mosquito control programme.

MATERIALS AND METHODS

Mosquito Species

A laboratory strain of *Cx. quinquefasciatus* (NIH strain) from National Institute of Health, Ministry of Public Health, Nonthaburi, Thailand has been maintained in the insectary for 27 years. Field strain of *Cx. quinquefasciatus* and *Ae. aegypti* were collected from Baan Suan community, Nonthaburi province in November 2005 and December 2004, respectively. The collected larvae and pupae of both *Cx. quinquefasciatus* and *Ae. aegypti* strains were maintained under laboratory conditions. Three to five day old female mosquitoes were used for susceptibility tests.

Insecticides

Filter papers (Whatman No 1) were impregnated using technical grades of malathion (95% a.i.), propoxur (97% a.i.) and cypermethrin (93.04% a.i.) for preparing malathion 5%, propoxur 0.1% and cypermethrin 0.5% using WHO (1996) impregnation procedure. The insecticide was dissolved in mixture of acetone and silicone (Dow Corning 556). Two ml of insecticide solution was applied to the filter paper. The treated papers were air dried for 24 hours. The concentration of each insecticide was as recommended by WHO (1992).

The standard WHO diagnostic dosages, DDT 4%, fenitrohtion 1%, deltamethrin 0.05% and permethrin 0.75% treated papers were purchased from Vector Control Research Unit, University Sains, Malaysia.

Susceptibility test

Sugar fed female, aged 3-5 days old mosquitoes were exposed to DDT 4% treated paper for 4 hours and were exposed for 1 hour to diagnostic concentrations of fenitrothion 1%, malathion 5% for Culex mosquito and malathion 0.8% for Aedes mosquito, propoxur 0.1%, deltamethrin 0.05% and permethrin 0.75% impregnated papers, these concentrations were recommended by WHO (1992). For cypermethrin test, the mosquitoes were exposed to cypermethrin 0.5% at different periods from 1 hour to 3 hours to find the median lethal time 50 (LT_{50}) and the 95% of lethal time (LT_{95}) to this compound. After the exposure time, the tested mosquitoes were transferred to the holding tubes and cotton pad soaked in 10% sugar solution was provided. Four replicates were conducted for each insecticide. The mortality was recorded after 24 hours.

Analysis

Adult mortality data against cypermethrin were analyzed with the regression-probit in SPSS program for the lethal time (LT_{50} and LT_{95}) values for NIH and Baan Suan strains. The resistance ratio was defined as the ratio of LT of field strain to LT of NIH strain (Laboratory mosquito).

RESULTS

The results of the susceptibility test revealed that adults of *Cx. quinquefasciatus* from Baan Suan community were highly resistant to DDT, deltamethrin, fenitrothion and permethrin with the percentage mortality of 0%, 11.0%, 21.2% and 10.1%, respectively (Table 1). The Baan Suan strain was moderately resistant to propoxur with 66% mortality. A 100% mortality was obtained for malathion indicating that this strain was susceptible to malathion. The results of cypermethrin tests with Baan Suan strain compared with NIH strain is shown in Table 2. The results showed that Baan Suan strain induced a 16-fold resistance to cypermethrin in comparison to NIH strain.

Table 3 shows the mortality of *Ae. aegypti* from Baan Suan community against fenitrothion, malathion, deltamethrin and permethrin. *Ae. aegypti* adults tested were resistant to permethrin and tolerant to deltamethrin, but were highly susceptible to fenitrothion.

The comparison of mortalities between *Cx. quiquefasciatus* and *Ae. aegypti* is shown in Figure 1. *Cx. quiquefasciatus* mosquitoes displayed higher resistance to deltamethrin, permethrin and fenitrothion compared to *Ae. aegypti* mosquitoes. But both species were susceptible to malathion.

DISCUSSION

Culex quinquefasciatus, displayed resistance to permethrin and deltamethrin that have been used by the local authorities for dengue vector control

Table 1. Mortality of Baan Suan *Culex quinquefasciatus* strain at 24 hours after exposure to DDT, fenitrothion, malathion, propoxur, deltamethrin and permethrin

Insecticide	Dose (%)	Exposure period (hours)	No of tested	Mortality (%)	Control Mortality (%)
DDT	4	4	100	0	1
Fenitrothion	1	1	99	21.2	0
Malathion	5	1	99	100	1
Propoxur	0.1	1	100	66.0	1
Deltamethrin	0.05	1	100	11.0	0
Permethrin	0.75	1	99	10.1	0

Table 2. Resistance ratios observed at LT_{50} (RR_{50}) and LT_{95} (RR_{95}) of Baan Suan *Culex quinquefasciatus* strain to cypermethrin

Strain	LT_{50} (minute)	LT_{95} (minute)	RR_{50}	RR_{95}
NIH	7.52	33.80	1	1
Baan Suan	120.87	445.58	16.07	13.18

LT_{50} and LT_{95} values = Median lethal time in minute
RR_{50} and RR_{95} = $LT_{50(95)}$ of Baan Suan strain / $LT_{50(95)}$ of NIH strain

Table 3. Mortality of Baan Suan *Aedes aegypti* strain at 24 hours after exposure to fenitrothion, malathion, deltamethrin and permethrin

Insecticide	Dose (%)	Exposure period (hours)	No of tested	Mortality (%)	Control Mortality (%)
Fenitrothion	1	1	50	100	0
Malathion	0.8	1	50	100	4
Deltamethrin	0.05	1	50	82.7	0
Permethrin	0.75	1	50	34.8	0

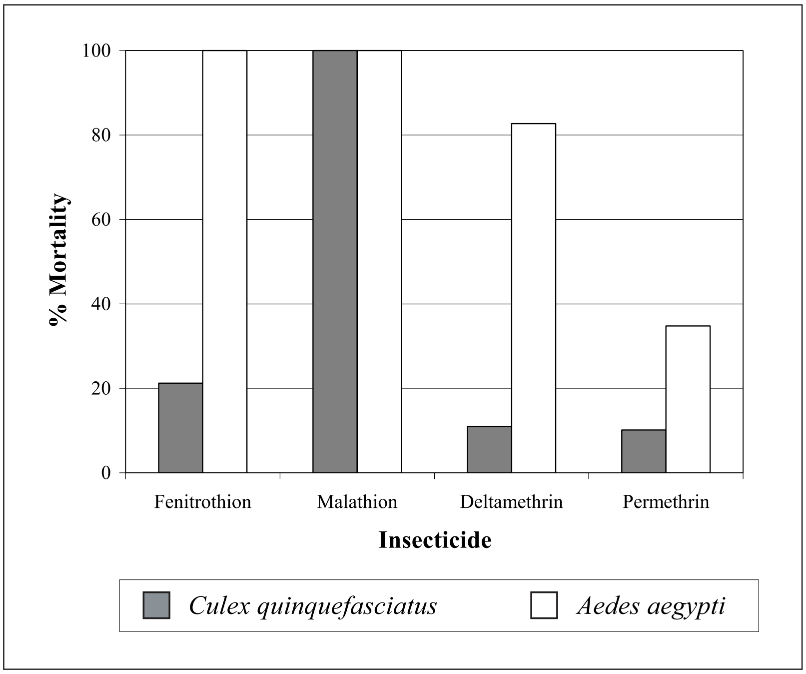

Figure 1. Comparison of mortality of *Culex quinquefasciatus* and *Aedes aegypti* strains after exposure fenitrothion, malathion, deltamethrin and permethrin.

program in Baan Suan community for a long term. This species was also resistant to DDT, fenitrothion, malathion, propoxur and cypermethrin and these compounds have never been applied in this community. This could be due to cross-resistance between insecticides. It is found that organophosphate and carbamate insecticides share the same target site is acetylcholinesterase in nerve synapses, and the target of organochlorines (DDT) and synthetic pyrethroids are the sodium channels of the nerve sheath (Brogdon & McAllister, 1998). Moreover, The breeding sites of *Cx. quinquefasciatus* may be contaminated (Canyon & Hii, 1999) with insecticides that have been used for *Ae. aegypti* control. Interestingly, *Cx. quinquefasciatus* species can develop resistance rapidly to insecticides than most other mosquitoes (Hamon & Mouchet, 1967). According to WHO (1996) *Cx. quinquefasciatus* mosquitoes have developed resistance to various insecticides in many countries. This species has been reported to be resistant to organophosphate, carbamate and synthetic pyrethroid compounds in America, Saudi Arabia and Northern Thailand (Georghiou *et al*., 1980; Amin & Peiris, 1990; Somboon *et al*., 2003).

The result of this study showed that both *Cx. quinquefasciatus* and *Ae. aegypti* are still susceptible to malathion. Thus this can be another choice for mosquito control in Baan Suan community. Moreover, bioinsecticide can also be used as an alternative in mosquito control program. *Bacillus thuringiensis* is highly active against *Ae. aegypti* larvae (Monnerat *et al.*, 2005) whereas *Bacillus sphaericus* is toxic to Culex larvae (Wirth *et al.*, 2000). These bacteria produce a toxic crystal protein that is effective for mosquito larvae (Aly *et al.*, 1987). However, integrated vector control strategy should be considered and resistance surveillance in mosquito need to be conducted regularly.

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