THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Entomology)

DEGREE

Entomology	Entomology
FIELD	**DEPARTMENT**

TITLE: Irritancy and Repellency by *Culex quinquefasciatus* Say (Diptera: Culicidae) to Three Different Classes of Compounds

NAME: Ms. Sunaiyana Sathantriphop

THIS THESIS HAS BEEN ACCEPTED BY

C. Ketavan **THESIS ADVISOR**

(Associate Professor Chitapa Ketavan, Docteur d'Universite (Hons))

T. Chareonviriyaphap **COMMITTEE MEMBER**

(Associate Professor Theeraphap Chareonviriyaphap, Ph.D.)

S. Visetson **COMMITTEE MEMBER**

(Associate Professor Suraphon Visetson, Ph.D.)

Surachate Jamornmarn **DEPARTMENT HEAD**

(Mr. Surachate Jamornmarn, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON 9th March 2006

Vinai Artkongharn **DEAN**

(Associate Professor Vinai Artkongharn, M.A.)

THESIS

IRRITANCY AND REPELLENCY BY *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE) TO THREE DIFFERENT CLASSES OF COMPOUNDS

SUNAIYANA SATHANTRIPHOP

A Thesis Submitted in Partial Fulfilment of

the Requirements for the Degree of

Master of Science (Entomology)

Graduate School, Kasetsart University

2006

ISBN 974-9844-78-5

Sunaiyana Sathantriphop 2006: Irritancy and Repellency by *Culex quinquefasciatus* Say (Diptera: Culicidae) to Three Different Classes of Compounds. Master of Science (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Associate Professor Chitapa Ketavan, Docteur d'Universite (Hons) 45 pages.
ISBN 974-9844-78-5

Insecticide susceptibility and behavioral responses of three *Culex quinquefasciatus* strains, two field strains from Nonthaburi and Tak Provinces, and a laboratory strain from the National Institute of Health (NIH), Nonthaburi, Thailand by deltamethrin, propoxur and fenitrothion were characterized. All strains were resistant to deltamethrin and tolerant to propoxur, but were completely susceptible to fenitrothion. Great escape responses were observed from the NIH strain under direct contact with deltamethrin and fenitrothion whereas those with weak responses were observed in the two field strains. Propoxur demonstrated the least irritancy effect against all three strains of *Cx. quinquefasciatus*. Repellent responses were observed when all three strains were tested against deltamethrin. The irritant and repellent responses exhibited by *Cx. quinquefasciatus* to these insecticides in the current study contribute significant information to disease control programs in Thailand.

In addition, the irritancy tests of deltamethrin, propoxur and fenitrothion by *Cx. quinquefasciatus* were also investigated using irritancy test system at the Laboratoire de Lutte Contre les Insects Nuisibles (LIN), Montpellier, France. Deltamethrin demonstrated the highest irritancy effect against both susceptible strain from LIN (S-Lab strain) and Nonthaburi strain. This result revealed that the irritant property of deltamethrin could cause irritation of *Cx. quinquefasciatus* by contact with this insecticide.

Sunaiyana Sathantriphop   C. Ketavan   2 Mar 2006

Student's signature   Thesis Advisor's signature

ACKNOWLEDGMENTS

I would like to express my most gratitude to my advisor, Assoc. Prof. Dr. Chitapa Ketavan for her kindness, invaluable advice and giving me the chance to be trained the useful techniques on mosquito testing at Laboratoire de Lutte Contre les Insects Nuisibles (LIN), France.

I would like to express sincere gratitude to my co-advisor, Assoc. Prof. Dr. Theeraphap Chareonviriyaphap for his valuable guidance, patiently correcting the manuscript and for his permission to use the facilities, supporting materials and equipment and constructive criticisms throughout the thesis. I also wish to extend my grateful appreciation to Assoc. Prof. Dr. Suraphon Visetson, my co-advisor for his kindness, valuable guidance and great encouragement.

I am deeply grateful to the Thailand Research Fund Organization for providing the excito-repellency test chambers and thank to Bayer and Ladda companies for supporting technical insecticides. Also, sincere thanks go to Assoc. Prof. Atchariya Prabaripai for her precious help in the statistical analysis of the data.

I would like to thanks to Mr. Kasin Suphapathom, Dr. Pungasem Paeporn and Mr. Thumrong Phonchevin for their kindness, support and helpful guidance.

I wish to extend my appreciation to Ms. Jinrapa Phothikasikorn and Mr. Suprada Sukonthabhirom na Pattalung for their generous advices and understanding. Also thanks for the friendly help of Mr. Rosdee Waekachi. Special thanks go to Ms. Monthathip Kongmee for her friendship and helping about computer to the eventual improvement of my thesis.

Finally, I would like to express my heartfelt gratitude to my lovely family for their love and understanding. They're always helping and cheering me to complete this thesis.

Sunaiyana Sathantriphop

March 2006

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Cx	=	*Culex*
W	=	*Wuchereria*
B	=	*Brugia*
F_1	=	Filial 1
F_2	=	Filial 2
AchE	=	Acetylcholinesterase
°C	=	Degree (s) Celsius
cm	=	Centimeter
cm^3	=	Cubic Centimeter
m^2	=	Square Meter
hr	=	Hour
g	=	Gram
mg	=	Milligram
ml	=	Milliliter
%	=	Percent
P	=	Probability Value
Fig	=	Figure
WHO	=	World Health Organization
MOPH	=	Ministry of Public Health
NIH	=	National Institute of Health

IRRITANCY AND REPELLENCY BY *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE) TO THREE DIFFERENT CLASSES OF COMPOUNDS

INTRODUCTION

Lymphatic filariasis is known as elephantiasis and considered as one of important public health disease problems in Southeast Asia (Harinasuta, 1984). There are at least 5 endemic countries in Southeast Asia that are suffering with this disease, namely Malaysia, the Philippines, Indonesia, Myanmar and Thailand. In Thailand, there are two major filarial nematode parasites that cause lymphatic filariasis, *Wuchereria bancrofti* and *Brugia malayi*. Malayan filariasis is restricted to the area of the South whereas Bancroftian is endemic to the West along the Thai-Myanmar border. *Culex quinquefasciatus* has been incriminated as one of the potential filarial vectors (Swaddiwudhipong *et al*., 1996; Jitpakdi *et al*., 1998).

Several environmental factors including temperature, humidity as well as rainfall have favored the increase of disease vectors. There is a number of potential vectors that can experimentally transmit the disease. Under the field condition, *Cx. quinquefasciatus* has been proved to transmit bancroftian filariasis in Thailand (Triteeraprapab *et al*., 2000). To control the vector, several groups of synthetic compounds, including organophosphates, carbamates and synthetic pyrethroids, have been used (Chareonviriyaphap *et al*., 1999; Somboon *et al*., 2003). Among these, deltamethrin (synthetic pyrethroids), propoxur (carbamate) and fenitrothion (organophosphate) represent the commonly used compounds for controlling *Cx. quinquefasciatus* mosquito (Ministry of Public Health (MOPH), 1989). These insecticides are also commercially available to the general public for private use in homes for the protection against indoor biting mosquitoes. These circumstances increase the probability of insecticide resistance and resistance in *Cx. quinquefasciatus* in Thailand has been documented with several major groups of compounds, including deltamethrin, propoxur and fenitrothion (MOPH, 2004; Somboon *et al*., 2003).

Although several reports of resistance have been published (Curtis and Pasteur, 1981; Roberts and Andre, 1994; Chandre *et al*., 1998; Nazni *et al*., 1998; Chareonviriyaphap *et al.*, 1999), the impact of these compounds on *Cx. quinquefasciatus*, in terms of behavioral responses, remain unclear. Field and laboratory studies often fail to differentiate between the two forms of repellency (Roberts *et al.,* 1997). Generally, behavioral responses, known as insecticide avoidance (Potikasikorn *et al*., 2005), can be separated into two important and distinct categories: contact irritancy and non-contact repellency. Irritancy results from physical contact with chemical-treated surfaces whereas repellency is a response to avoid making physical contact with insecticides. There are some difficulties to overcome in quantifying these two forms of behavioral responses, including an appropriate test system.

Excito-repellency test designed to study the irritant (contact) effect of insecticides on mosquitoes was first used in 1962 (Rachou *et al*., 1963). Subsequently, investigations have been conducted on numerous malaria vectors using modified versions of the initial WHO excito-repellency (E-R) test boxes (Bondareva *et al*., 1986; Pell *et al*., 1989; Quinones and Suare, 1989; Ree and Loong, 1989). The complexities of excito-repellency testing, including analysis and interpretation of results, have resulted in no test system being completely accepted (Chareonviriyaphap *et al.*, 1997). However, a useful test chamber that can separate these two forms of behavioral responses was developed (Roberts *et al.*, 1994) and has been successfully used to study the responses of *Anopheles albimanus* to insecticides by Chareonviriyaphap *et al.* (1997). Subsequently, a more field-friendly test system has been developed to facilitate transportation and set-up (Chareonviriyaphap *et al*., 2002). This test system provides information on both contact irritability and non-contact repellency behavior.

Although behavioral responses have preciously been recorded with various mosquito species using the excito-repellency test box (Chareonviriyaphap *et al*., 1997, 2001, 2004; Sungvornyothin *et al*., 2001; Kongmee *et al*., 2004; Potikasikorn *et al*., 2005), no studies have been performed to compare the behavioral responses among *Cx. quinquefasciatus* strains. Described herein are observations using the excito-repellency test system to quantitatively measure behavioral responses between field and National Institute of Health (NIH) strains of *Cx.*

quinquefasciatus exposed to recommended field concentrations of deltamethrin, propoxur and fenitrothion. Understanding the behavioral responses of *Cx. quinquefasciatus*, a night biting mosquito, to insecticides will provide vital information for the vector control officers to implement the effective management interventions.

The objective of this study is:

To characterize behavioral responses of *Culex quinquefasciatus* to three commonly test compounds, deltamethrin, propoxur and fenitrothion.

LITERATURE REVIEWS

Culex quinquefasciatus is called the house haunting mosquito because of its close relationship with man's environment. This is one of the most widely distributed species in the world (Chow *et al*., 1957; Nazni *et al*., 1998). It is the predominant vector of filariasis caused by *Wuchereria bancrofti* in African and Southeast Asia Regions (WHO, 1992; Singh, 1967).

Biology and behaviour of *Culex quinquefasciatus*

Culex quinquefasciatus is a medium size mosquito, comprising four polymorphic stages in its life. The first three are aquatic. An adult female lays as many as 150-200 eggs in clusters called "rafts", which float on the water surface until they hatch in one to two days. The female lays eggs in standing and polluted still water such as sewage, street drainage, septic tanks, and cesspools (Singh, 1967).

The eggs hatch into larvae which feed on small organic and microorganisms in the water. *Culex* larvae hang on the water surface by tip of their siphon. Larval development requires four to six days. At the end of the larval stage, the mosquito molts and becomes the pupa. The pupa is active whenever it is disturbed. This takes about two-three days during which time feeding does not occur. When the transformation is completed, the new adult splits the pupal skin and emerges at the surface. Under optimum conditions, it takes about one week to complete an aquatic stage. However, mosquito development cycle is dependent on the day length period, temperature and nutrient in which they mature (Jocelyn *et al.*, 1992).

Culex quinquefasciatus may survive for two or three weeks in the summer, but the females may live for several months under cooler conditions. In the temperate areas, this species usually survives the winter by hibernating females in protected natural or artificial shelters such as cellars, outbuildings, wood piles, culverts etc (source: http://www.fresnomosquito.org).

Culex quinquefasciatus is mainly indoor resters (Raghavan, 1961). They rest on clothing and furnishings in houses as well as walls. Over 50% of *Cx. quinquefasciatus* resting indoor during daytime were collected from behind and underneath furniture, bed, and closet (Chow and Thevasagavan, 1957). *Culex quinquefasciatus* is nocturnal in its habits, being most active towards dusk and during the first part of the night (Indian Council of Medical Research, 1959). Females feed primitively on human blood and also on a variety of other mammals and birds, depending on the availability of hosts. Males feed on nectar and plant juices. Females may also feed on plant juices for energy reserve, but definitively require blood to develop their eggs (Clements, 1992).

Culex quinquefasciatus **as a tendentious vector in Thailand**

Culex quinquefasciatus is a urban mosquito which is extremely abundant and widespread in tropical countries, including those in Southeast Asia. *Culex quinquefasciatus*, the main vector of *Wuchereria bancrafti*, found mostly in Myanmar and Indonesia (Harinasuta, 1984). Although *Aedes niveus* group are the major vectors for *W. bancrofti* in Thailand (Swaddiwudhipong *et al*., 1996), Thai *Cx. quinquefasciatus* has been shown to be positive for *W. bancrofti* (Sucharit *et al.*, 1981; Potikasikorn *et al*., 2005). This might be possible transmission of Bancroftian filariasis related to the influx of illegal immigrant workers in many urban and periurban areas of Thailand (Swaddiwudhipong *et al*., 1996). Moreover, large cities have rapid extension of urbanization and industrialization that may cause the increase of the vector breeding sites and potential risk of transmission of the disease.

Lymphatic Filariasis

Lymphatic filariasis (elephantiasis) caused by nematodes is localized in the lymphatic system of the infected person. Adult worms may block lymphatic vessels and prevent the circulation of the lymph fluid. This may lead to swellings of the genitalia, legs, and arms. Although lymphatic filariasis is not a lethal disease, it appears a major cause of health suffering.

There are currently over 600 million people living in endemic areas of Lymphatic filariasis. In the Southeast Asia regions, there are over 70 million who have various forms of the disease. In all countries of this region, both urban and rural areas are affected by Brugian filariasis which is predominantly a rural disease (WHO, 1998a). Furthermore, *Cx. quinquefasciatus* is not only an urban species but also becoming widespread rural areas in East Africa (de Meillon, 1977). All three parasites that cause Lymphatic filariasis (*Wucheraria bancrofti*, *Brugia malayi* and *Brugia timori*) are presented in the Southeast Asia regions. *Wuchereria bancrofti* is predominantly transmitted by *Cx. quinquefasciatus* and subperiodic forms of the parasite are transmitted by *Aedes niveus*. Nocturnal period *B. malayi* is transmitted by Anopheles and Mansonia mosquitoes in this region while nocturnal subperiodic Brugia has Mansonia mosquito as the vector (WHO, 1998a).

Classification of tested insecticides

Organophosphate insecticides (OP)

Organophosphate compounds are the most widely used for agricultural purposes but, due to vector resistance to organochlorine groups, they are now commonly used in public health practices. These compounds are much less persistent in the environment, but are toxic to non-target creatures such as birds, aquatic organisms and some beneficial insects (bees). Most OP insecticides are esters or amides of organically bound phosphoric or pyrophosphoric acid (Eto, 1974). Fenthion, temephos, chloropyrifos, fenitrothion, and malathion are the most important compounds of phosphorothionates. Malathion, organophosphate, was widely used in malaria control program. Fenitrothion is another organophosphate compound of longer residual effect as compared to malathion. It was used in residual spraying for mosquito control (WHO, 1982).

Carbamate insecticides (C)

Carbamate is one of the most common group of insecticides that is used in agriculture, as insecticides, fungicides, herbicides, nematocides, or sprout inhibitors. In addition, they are used as biocides for industrial or other applications, and as household products. The carbamate ester derivatives, used as insecticides (and nematocides), are generally stable with a low vapour pressure and low water solubility. The carbamate herbicides and sprout inhibitors are aromatic and/or aliphatic moieties whereas carbamate fungicides contain a benzimidazole group. A potential use of the carbamate esters is in public health vector control. These chemicals are part of the large groups of synthetic pesticides that have been developed, and used on a large scale in the last 40 years. Example of this group is propoxur which was used in malaria control program, particularly in areas where resistance to OPs.

Synthetic pyrethroid insecticides (PY)

Synthetic pyrethroids are an important class of insecticides that have been proved to be effective in controlling arthropods of medical and veterinary importance (Zerba, 1988). The pyrethroids derived from a group insecticidal esters, of which both the alcohol and carboxylic acid moieties may have isomeric forms so that each pyrethroids may have several isomers. These insecticides show remarkably high toxicity and rapid action against a wide range of insects, but relatively low mammalian toxicity (Elliot, 1989). Pyrethroids are highly selective insect toxicity compared with mammal toxicity. They also have low volatility and low polarity, properties that restrict their movement in the air and soil from the site of application. The pyrethroids have been commonly used for mosquito control programmes such as permethrin, cypermethrin, deltamethrin and lambdacyhalothrin.

The mode of action of insecticides

Most insecticides in a current use disturb and interfere the nervous system of insects, either the passage of nerve impulse or the blockage of nerve transmitter.

Mode of action of organophosphate insecticides on insects

Organophosphate insecticides inhibit esterases. The result of this inhibition is the accumulation of acetylcholine in the synapses so that nerve function is impaired. This leads ultimately to the death of the insect. Enzyme kinetics analysis has identified insensitive forms of acetylcholinesterase in insecticide resistant strains (Hemingway *et al*., 1986).

Mode of action of carbamate insecticides on insects

Carbamate insecticides are nervous system poison. They bind to active site of the enzyme acetylcholinesterase (AchE), which is necessary for normal function of nerve impulses to other nerves and muscles. This causes an accumulation of acetylcholine, an excitatory neurotransmitter, at nerve muscle sites resulting in poisoning symtoms. Unlike the organophosphates, carbamtes do not bind permanently to the enzyme and can be "dislodged" from the active site, so that poison effects reversible, upon administration of the antidote atropine.

Mode of action of pyrethroid insecticides on insects

These compounds act on the nervous system by modifying the gating kinetics of voltage sensitive sodium (Na^+) channels (Bloomquist, 1994). Arthropod resistance to pyrethroids is characterized by a marked reduction in the intrinsic sensitivity of the insect nervous system to these compounds.

Insecticide resistance in *Culex quinquefasciatus*

Culex quinquefasciatus has developed resistances to many types of organochlorines, organophosphates, carbamates and pyrethroids which found in many countries (WHO, 1992b and WHO, 1996). In tropical areas, the first clear evidence of the development of DDT-resistance in the field was obtained in 1952 from a village in Delhi State where houses were treated with DDT for the proceeding 6 years (Brown and Pal, 1971). The development of resistance to DDT in nature and laboratory, as reported by several authors (Brown, 1958; Suzuki and Mizutani, 1962), led to the use of alternative chemicals such as dieldrin, organophosphates and carbamates. The use of organophosphate larvicides in urban regions in many parts of Southeast Asia, has resulted in multiple resistances to a range of organophosphate insecticides such as malathion, diazinon and fenthion (WHO, 1976).

High levels of resistance to organophosphate insecticides have been reported in California (EL-Khatib and Georghiou, 1985), Cuba (Bisset *et al.*, 1997), Colombia (Bisset *et al.*, 1998) and several other areas of the World. Resistance potential of *Cx. quinquefasciatus* toward carbamate (propoxur) and pyrethroids (permethrin) was reported (Amin and Peiris, 1990; Chandre *et al*., 1998).

Insecticide resistance in mosquito involves the avoidance behavior when exposed to insecticide. Individual mosquitoes that are less susceptible to a specific insecticide have a better chance to survive exposure and reproduce. Normally, the most common types of insecticide resistance are grouped within four categories (Miller, 1988), 1. behavioral resistance, results from behavior changes which mosquito avoid to contact with the insecticide deposit 2. penetration resistance, is another mechanism of resistance, where the composition of the insect's exoskeleton becomes modified in a ways that inhibit insecticide penetration 3. site-insensitivity, the chemical site of action for the insecticide becomes modified to have reduced sensitivity to the active form of the insecticide and 4. metabolic resistance, occurs when detoxification enzyme are used to break down the insecticide so it is no longer toxic to mosquitoes.

Control of *Culex quinquefasciatus*

Four classes of insecticides, ie. organochlorines, organophosphates, carbamates and pyrethroids have been used for control both adult and larval *Cx. quinquefasciatus*. Unfortunately, some of them have already been found inactive against *Cx. quinquefasciatus* due to rapid resistance in the population. However, It was found that deltamethrin could be an effective control agent if used in a proper way. In 1986, Thomas and Pillai suggested that deltamethrin could be used as a very effective mosquito control agent because it was found to be irritating to mosquitoes, including *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Materials

Experiment in Thailand

1. Mosquitoes

All mosquito strains were maintained in the insectary at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand in the course of research.

1.1 The NIH strain was originally collected as larvae and pupae from Pom Prab Satru Phai, Bangkok, Thailand in 1978 and has been maintained in a colony at the National Institute of Health (NIH), Ministry of Public Health, Nonthaburi for at least 27 years.

1.2 The Nonthaburi strain was collected as larvae and pupae from Sai Thong Community, Tambol Tasai, Maung District, Nonthaburi Province (13º 50’ 30” N, 100º 29’ 45” E) in December 2004.

1.3 Tak strain was collected as larvae and pupae in an urban area of Mae Sot District, Tak Province (16º 42’ 30” N, 97º 34’ 30” E) in November 2004. The collection site is near to the Thai-Myanmar border where a number of filariasis has been found.

Females of the F_1 and F_3 generations were used for all tests. Mosquitoes were maintained at 25 $\pm$ 5 ºC and 80 $\pm$ 10 % relative humidity before using in susceptibility tests and excito-repellency studies.

2. Insecticides

Three insecticides, deltamethrin, propoxur and fenitrothion, were subjected to susceptibility and behavioral tests.

2.1 Deltamethrin (88% active ingredient) : (S)-alpha-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylate-technical grade was received from Bayer Thailand in January 2004.

2.2 Propoxur (97% active ingredient) : 2-isopropoxy-phenyl-N-methycarbamate-technical grade was obtained from Bayer Thailand in January 2004.

2.3 Fenitrothion (95% active ingredient) : O,O-dimethyl-O-4-nitro-m-tolyl phosphorothioate-technical grade was supplied by Ladda company, Thailand in July 2004.

3. Excito-repellency test chamber

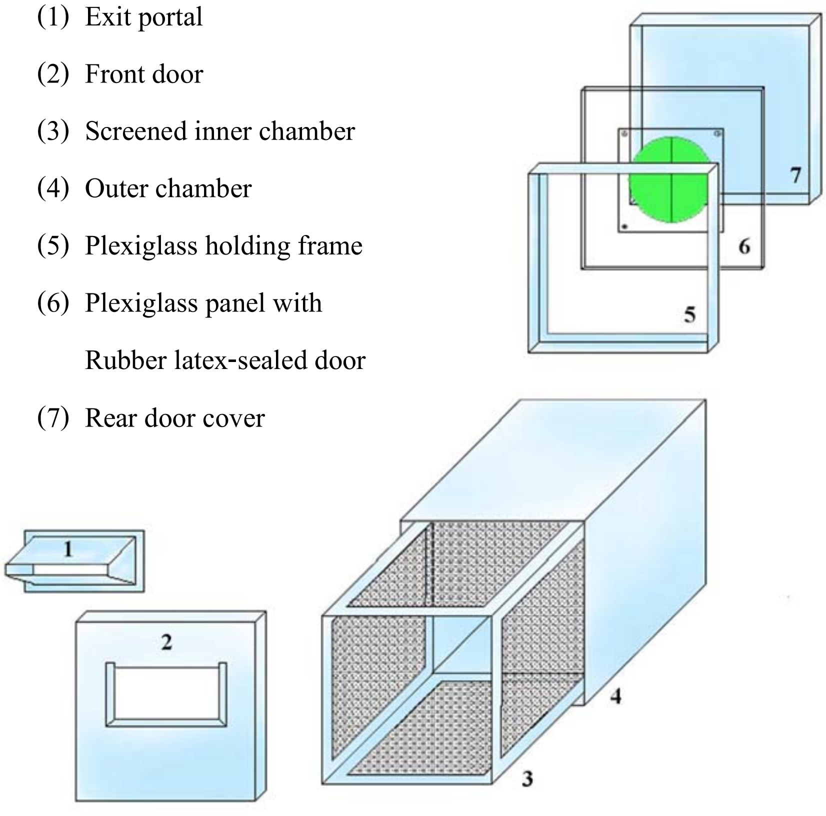


Figure 1 Excito-repellency test chamber

Methods

1. Rearing of mosquitoes

The egg rafts were placed in plastic tray (24×32×6 cm^3), containing 1,500 ml of chlorine-free water. Larvae were hatched within 48 hours after immersion. Approximately 150 larvae were placed in each rearing tray and about 0.05 g of powdered cat biscuits and yeast in the ratio of 3:2 were put every morning as larval food. The scum was removed daily from the surface of the water with a strip of filter paper before feeding the larvae. The water of rearing tray was changed every 2 or 3 days. The duration of larvae development lasted about 7-8 days. The pupae were separated from the larvae daily with a pipette and put in a small plastic cup containing water and placed in the adult cage (30×30×30 cm^3) for emergence.

The adults emerged in two days after pupation and were fed with 10% sugar solution soaked in cotton wool. Three to five days old females were used for the tests. The other adult female mosquitoes were fasted for 24 hours before providing a blood source (the quinea-pig was restricted in a small cage and was put into mosquito cage). The egg-collecting cup with chlorine-free water was placed in the cage on the second day after blood feeding to collect the egg rafts.

2. Paper impregnated for susceptibility test

Impregnated papers were prepared according to protocol of The Laboratoire de Lutte Contre les Insects Nuisibles (LIN), Montpellier, France. The technical insecticide was dissolved in a mixture of silicone (Dow Corning 556) and acetone in the ratio of 0.34:1.66 and diluted to give a concentration as remcommened by the World Health Organization (WHO, 1992b); 0.1% propoxur and 1% fenitrothion. For deltamethrin, 0.05% was selected as an appropriate concentration for the susceptibility test as very few mosquitoes were killed by 0.025% deltamethrin as WHO (1992b) recommendation. Whatman No.1 (12×15 cm^2) was then impregnated with 2 ml of the insecticide solution per 180 cm^2. The papers were air dried for 24 hours.

3. Insecticide susceptibility test

The test was followed the standard WHO tarsal contact test (WHO, 1996). Group of 20 to 25 female mosquitoes per exposure tube was exposed to the insecticide impregnated papers for 1 hour to deltamethrin (0.05%), and for 2 hours to propoxur (0.1%) and fenitrothion (1%). After the exposure period, the mosquitoes were transferred into the holding tube and cotton pad soaked in 10% sugar solution was given as food source. If control mortality was between 5 and 20% the percentage mortality should be corrected by Abbott's formula. When mortality in the controls was exceed 20%, the tests was discarded.

$$\text{Abbott's formula} = \frac{\%\text{ Test mortality} - \%\text{ Control mortality}}{100 - \%\text{ Control mortality}} \times 100$$

Based on WHO standard criteria on discriminating dosages (WHO, 1998b), the susceptibility level to insecticide can be classified as follows:

99-100% mortality	=	susceptible
80-98% mortality	=	verification required
$<$ 80% mortality	=	resistant individuals present

4. Paper impregnated for behavioral test

Insecticide solution was impregnated on filter paper (Whatman No.1) at operational field concentration for deltamethrin 0.02 g/m^2 (WHO, 1992a). Prior to selecting concentrations of propoxur and fenitrothion, preliminary screening was performed with 10 times higher concentrations of propoxur (2 g/m^2) and fenitrothion (2 g/m^2) following the WHO protocol (WHO, 1992a) in order to find the appropriate concentration that gave the proper number of mosquitoes to insecticide response study. Thus, the concentrations of 0.2 g/m^2 propoxur and 0.2 g/m^2 fenitrothion were selected for behavioral tests.

5. Behavioral test

Tests were designed to compare the behavioral responses among three strains of *Cx. quinquefasciatus*, NIH, Nonthaburi, and Tak strains, in contact versus non-contact exposures to three different groups of compounds using excito-repellency chambers. Test system was previously described by Chareonviriyaphap *et al*., (2002). The chamber (size 40.5 cm × 32.5 cm × 32.5 cm) is made up of several components numbered from 1 to 7; (1) an exit portal, (2) a front door, (3) a screened inner chamber, (4) an outer chamber, (5) a plexiglass holding frame, (6) a plexiglass panel with rubber latex-sealed door, and (7) a rear door cover as shown in Figure 1. It is assembled by initially attaching the exit portal (1) to the front door (2). The screened inner chamber (3) is slided into the outer chamber (4) before connecting them to component (1) and (2) by the back side of the front door (2). Plexiglass holding frame (5), plexiglass panel with rubber latex-sealed door (6), and rear door cover (7) are respectively put in place to complete the unit. To gather the escaped mosquitoes from each test chamber, a receiving box is used by connecting it to the exit portal (1). The complete test system consisted of 2 treated test chambers and 2 paired control chambers. Prior to the exposure, mosquitoes were deprived from all nutrition supply for at least 24 hours. Twenty-five mosquitoes were carefully transferred into each of the 4 test chambers by using a mouth aspirator. Mosquitoes were allowed a 3-min resting period to permit adjustment to the chamber conditions. Observations were taken at 1 min intervals for 30 mins. After each test was completed, the number of dead or knockdown specimens was recorded separately for each exposure chamber, paired control chamber and external holding cage which was receiving box connected to the exit portal for collecting escaped mosquitoes. Escaped specimens and those remaining inside the chambers, for both controls and treatments, were held separately in small holding containers with cotton pads soaked in 10% sugar solution as food. Mortality count were made at the end of the 24 hours observation period. Each chemical was replicated 4 times.

6. Data analysis

A Kaplan-Meier survival analysis method was used to analyze and interpret the behavioral response data (Roberts *et al*., 1997). Survival analysis was used to estimate the probability of escape time (ET) and compare differences in behavioral responses among the mosquito strains and insecticides (Kleinbaum, 1995). In analysies, mosquitoes that escaped were treated as "deaths" and those remaining in the test chamber considered as "survivals" (Chareonviriyaphap *et al*., 1997). The time in minutes for 30, 50 and 70% of the test population to leave the test chamber, ET_{30}, ET_{50} and ET_{70} respectively, were estimated from data collected at 1-min intervals. Differences in escape responses among strains by type of trial (contact vs. non-contact) were determined using the log-rank method with statistical significance observed of the 0.05 level of probability (Mantel and Haenzel, 1959).

Experiment in France

Irritancy test training at LIN

<u>Materials</u>

1. Mosquitoes

Culex quinquefasciatus mosquitoes were maintained in the insectary at Laboratoire de Lutte Contre les Insects Nuisibles (LIN), Montpellier, France during the study period.

S-Lab strain: The laboratory strain of *Cx. quinquefasciatus*, originating from the University of California, have been colonized for 10 years in the insectary at LIN and served as a reference colony.

Nonthaburi strain: The field strain was collected from Sai Thong community, Tombol Tasai, Maung District, Nonthaburi Province on January 2004. Details on this strain was previously described.

2. Impregnated papers

Papers impregnated with deltamethrin, propoxur and fenitrothion at dosage which gave 100% mortality to susceptible strains were prepared, including 0.2% deltamethrin, 0.2% propoxur and 1% fenitrothion.

3. Equipment for irritancy test

Figure 2 Equipment for irritancy test

Methods

1. Irritancy test

World Health Organization standard plastic cone was used along with insecticide-impregnated paper as the test system. Individual 3 to 5 days old non-blood fed female mosquito was gently introduced into the cone. After an adaptation time of exactly 60 seconds, the time elapsed between the first landing and the following take-off of the mosquito was recorded as the "time to first take-off" (FT). The observation did not continue for the very few mosquitoes that did not take-off at least once after 256 sec. For each test, 50 mosquitoes were used. A simple program using the internal clock of a laptop computer was developed to run the test and to analyze the results by grouping mosquitoes by classes of first take-off time.

2. Data analysis

The time to first take-off regression lines from the results were constructed by probit analysis program. The regression line was fitted by eye and the FT_{50} and FT_{95} were read from the graph.

RESULTS

Results from susceptibility tests on *Culex quinquefasciatus* strains against deltamethrin, propoxur and fenitrothion are given in Table 1. All strains were susceptible to fenitrothion and propoxur with slightly tolerence to propoxur in Nonthaburi strain. In contrast, high resistance to deltamethrin was observed in Nonthaburi strain whereas moderate resistance was observed in NIH and Tak strains.

Escape responses of *Cx. quinquefasciatus* to deltamethrin, propoxur and fenitrothion were investigated on both contact and non-contact chamber configurations (Tables 2 and 3). The *Cx. quinquefasciatus* escape responses varied between strains and insecticides. In contact trials, NIH strain demonstrated dramatic escape responses to both deltamethrin (75%) and fenitrothion (47%) whereas showed low behavioral contact responses to propoxur (14%). Nonthaburi strain showed similar degree of contact responses to all three compounds, ranging from 23 to 30%. Tak strain demonstrated a higher contact escape response to deltamethrin (44%) than in response to that of propoxur (14%) and fenitrothion (16%) (Table 2). In non-contact trials, obvious escape responses, especially with NIH (23%) and Nonthaburi (18%) strains, were observed when tested against fenitrothion (Table 3). Overall, the number of mosquitoes escaping from the control test chambers in both contact and non-contact trials was significantly lower than from treated chambers.

Mortalities of escaped and non-escaped females after a 24-hour holding period are presented in Tables 2 and 3. In contact trials with propoxur, precent mortality of non-escaped NIH mosquitoes (69.8%) were higher compared to non-escaped Nonthaburi (10.4%) and Tak (36.1%) mosquitoes. With deltamethrin and fenitrothion, percent mortality of non-escaped females from the three strains were 4.3% or less with the highest mortality occurring in the NIH strain. Surprisingly, 35.7% of escpaed NIH mosquitoes from the test chamber treated with propoxur were obtained (Table 2). Generally, percent mortality of escaped and non-escaped mosquitoes in control chambers from three strains were low (0-3.2%). In non-contact trial, the highest mortality was obtained from Tak (55.8%) and NIH (28.1%) mosquitoes in chambers treated with propoxur (Table 3).

Time to escape of test mosquitoes from treated chambers was observed (Table 4). The time required for 30, 50 and 70% of mosquitoes to escape the treated chamber was defined as ET_{30}, ET_{50} and ET_{70}, respectively. Because of the low number of escapes in propoxur assays, the ET_{30}, ET_{50} and ET_{70} values could not be calculated. However, the ET_{30}, ET_{50} and ET_{70} values for deltamethrin were 2, 5 and 18, respectively for the NIH strain. The value for fenitrothion was low (5 min) in NIH strain whereas was rather high in Nonthaburi strain (26 min). All other deltamethrin and fenitrothion time patterns could not be calculated.

Multiple comparisons of escape responses among three strains in contact, non-contact and control trials also were performed (Tables 5 and 6). In contact trial, significant differences were found in all cases, except for those paired strains tested against propoxur (Table 5). For non-contact trials, differences in escape responses were seen between strains exposed to propoxur. With fenitrothion, Tak strain showed significantly low escape response compared to both NIH and Nonthaburi strains (Table 2). When the escape response in control chambers were compared to treated chambers, significant differences were observed for all contact trials, except for the Tak strain exposed to propoxur (Table 6). Significant differences between control and treatment chambers in non-contact trial were also found in the Tak strain exposed to deltamethrin and propoxur as well as the NIH and Nonthaburi strains exposed to fenitrothion (Table 6). As expected, there are quick responses in contact trial for all assays except there from propoxur treated against NIH and Tak strains which were showed similar numbers of escape (Table 2).

The percent of mosquitoes remaining in the exposure chambers under contact and non-contact conditions was calculated to determine the escape probabilities for each chemical (Figs 3 and 4). The greatest escape responses of NIH strain were observed when exposed to deltamethrin and fenitrothion in contact trials and were significantly different from control escape rates. With propoxur, escape responses for all three compounds were rather low compared to deltamethrin and fenitrothion (Fig 3). In non-contact vs. control, delayed escape responses were observed in both Tak and NIH strains tested against propoxur (Fig 4). Fenitrothion showed the fastest escape responses for both Nonthaburi and NIH strains.

<u>Table 1</u> Percent mortality of *Culex quinquefasciatus* strains from NIH, Nonthaburi and Tak exposed to deltamethrin (0.05%), propoxur (0.1%) and fenitrothion (1.0%)

Strain[1]	Deltamethrin		Propoxur		Fenitrothion	
	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality
NIH	100	66	100	97	100	100
Nonthaburi	100	12	100	77	100	98
Tak	75	54.7	75	93.3	75	100

[1] NIH = Mosquito strain from National Institute of Health

Table 2 Escape response and percent mortality of female *Culex quinquefasciatus* from NIH, Nonthaburi and Tak exposed to 0.02 g/m^2 deltamethrin, 0.2 g/m^2 propoxur and 0.2 g/m^2 fenitrothion in contact excito-repellency test chambers.

Insecticide[1]	Strain[2]	Treatment Chamber		Control Chamber		% Mortality			
						Treatment		Control	
		Tested	% Esc	Tested	% Esc	Esc[3]	Not[4] Esc	Esc	Not Esc
Del									
	NIH	93	75	100	14	0	4.3	0	1.2
	Non	100	27	99	6	7.4	1.4	0	0
	Tak	99	44	98	7	0	0	0	0
Pro									
	NIH	100	14	99	0	35.7	69.8	0	0
	Non	100	23	100	4	4.3	10.4	0	0
	Tak	69	14	100	7	7.7	36.1	0	2.2
Fen									
	NIH	99	47	99	13	0	1.9	0	0
	Non	100	30	100	12	0	0	0	0
	Tak	100	16	100	5	0	0	0	0

[1] Del = 0.02 g/m^2 Deltamethrin, Pro = 0.2 g/m^2 Propoxur, Fen = 0.2 g/m^2 Fenitrothion

[2] NIH = Mosquito strain from National Institute of Health

Non = Mosquito strain from Nonthaburi province

[3] Esc = Escaped mosquitoes

[4] Not Esc = Not Escaped mosquitoes

Table 3 Escape response and percent mortality of female *Culex quinquefasciatus* from NIH, Nonthaburi and Tak exposed to 0.02 g/m^2 deltamethrin, 0.2 g/m^2 propoxur and 0.2 g/m^2 fenitrothion in non-contact excito-repellency test chambers.

Insecticide[1]	Strain[2]	Treatment Chamber		Control Chamber		% Mortality			
						Treatment		Control	
		Tested	% Esc	Tested	% Esc	Esc[3]	Not[4] Esc	Esc	Not Esc
Del									
	NIH	98	5	99	3	0	0	0	0
	Non	99	5	100	8	0	2.1	0	0
	Tak	100	5	99	0	0	0	0	0
Pro									
	NIH	98	9	99	0	0	28.1	0	2
	Non	100	1	100	1	0	7.1	0	0
	Tak	96	20	98	4	5.3	55.8	0	3.2
Fen									
	NIH	99	23	93	4	0	0	0	2.2
	Non	100	18	99	3	5.6	0	0	0
	Tak	100	7	99	4	0	0	0	0

[1] Del = 0.02 g/m^2 Deltamethrin, Pro = 0.2 g/m^2 Propoxur, Fen = 0.2 g/m^2 Fenitrothion

[2] NIH = Mosquito strain from National Institute of Health

Non = Mosquito strain from Nonthaburi province

[3] Esc = Escaped mosquitoes

[4] Not Esc = Not Escaped mosquitoes

Table 4 Time in minutes for 30% (ET_{30}), 50% (ET_{50}) and 70% (ET_{70}) of *Culex quinquefasciatus* from NIH, Nonthaburi and Tak escape from test chambers treated with 0.02 g/m^2 deltamethrin, 0.2 g/m^2 propoxur and 0.2 g/m^2 fenitrothion within 30 minutes of contact trials.

Strain[1]	Insecticide	ET_{30}[2]	ET_{50}[3]	ET_{70}[4]
NIH				
	Deltamethrin	2	5	18
	Propoxur	-	-	-
	Fenitrothion	5	-	-
Nonthaburi				
	Deltamethrin	-	-	-
	Propoxur	-	-	-
	Fenitrothion	26	-	-
Tak				
	Deltamethrin	11	-	-
	Propoxur	-	-	-
	Fenitrothion	-	-	-

[1] NIH = Mosquito strain from National Institute of Health

[2] ET_{30} = Escape time = Time in minutes for 30% of female mosquitoes to escape from excito-repellency test chambers

[3] ET_{50} = Escape time = Time in minutes for 50% of female mosquitoes to escape from excito-repellency test chambers

[4] ET_{70} = Escape time = Time in minutes for 70% of female mosquitoes to escape from excito-repellency test chambers

(-) Indicates insufficient number escaped from exposure chambers to estimate ET_{30}, ET_{50} and ET_{70} during the 30 minutes exposure period

Table 5 Comparison of escape patterns among three test strains of *Culex quinquefasciatus* from NIH, Nonthaburi and Tak in contact and non-contact trials with 0.02 g/m^2 deltamethrin, 0.2 g/m^2 propoxur and 0.2 g/m^2 fenitrothion.

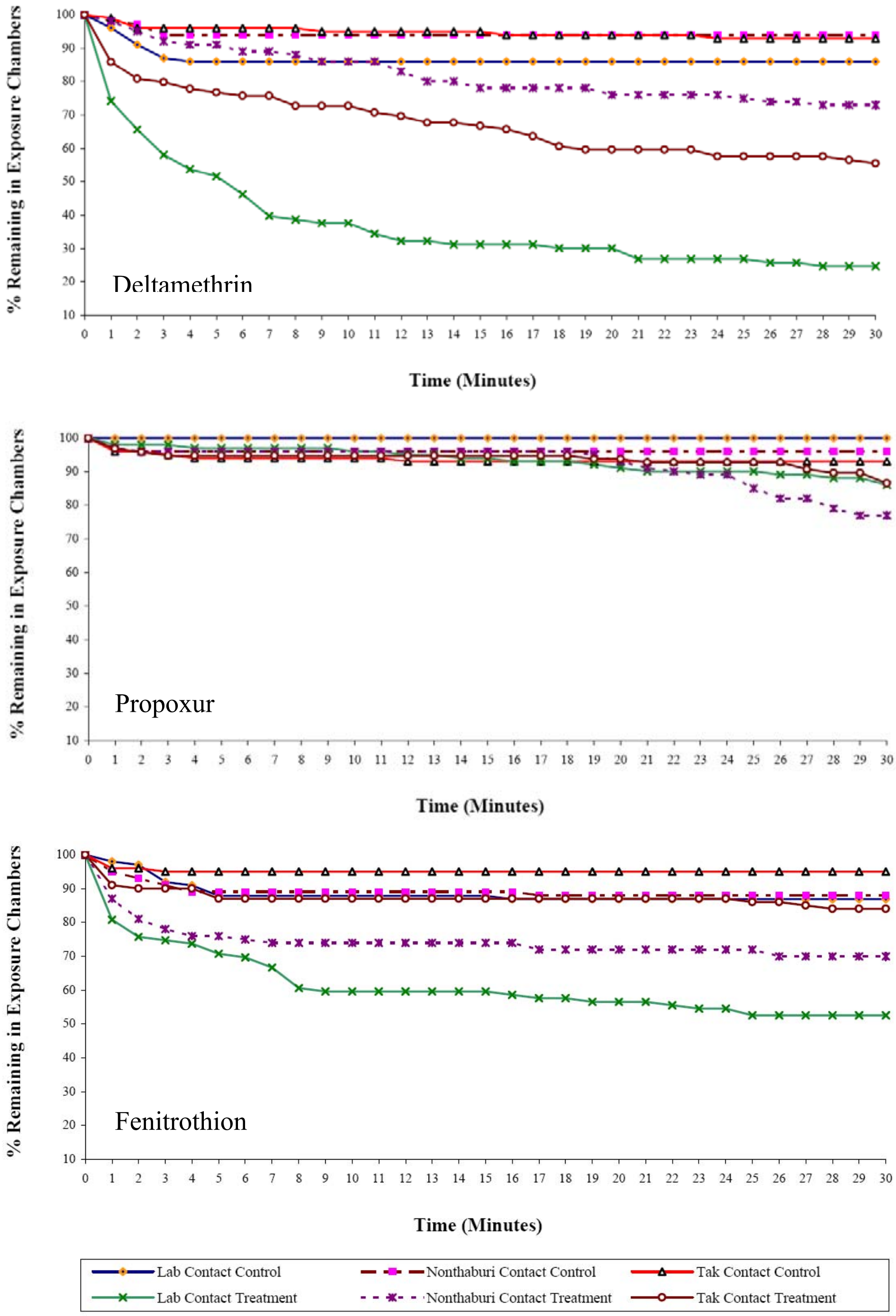
Insecticide	Test strain[1]	Contact trial (P)	Non-contact trial (P)
Deltamethrin			
	NIH vs. Nonthaburi	$<$ 0.0001	0.9869
	NIH vs. Tak	$<$ 0.0001	0.9530
	Nonthaburi vs. Tak	0.0069	0.9662
Propoxur			
	NIH vs. Nonthaburi	0.1190	0.0084
	NIH vs. Tak	0.9111	0.0346
	Nonthaburi vs. Tak	0.0882	$<$ 0.0001
Fenitrothion			
	NIH vs. Nonthaburi	0.0168	0.3987
	NIH vs. Tak	$<$ 0.0001	0.0014
	Nonthaburi vs. Tak	0.0185	0.0188

[1] NIH = Mosquito strain from National Institute of Health

Table 6 Within-strain comparison of escape response between paired control and contact trials, contact and non-contact trials, and paired control and non-contact trials for 3 test strains of *Culex quinquefasciatus* from NIH, Nonthaburi and Tak in contact and non-contact trials with 0.02 g/m^2 deltamethrin, 0.2 g/m^2 propoxur and 0.2 g/m^2 fenitrothion.

Insecticide	Test strain[1]	Control vs. Contact (*P*)	Control vs. Non-contact (*P*)	Contact vs. Non-contact (*P*)
Deltamethrin				
	NIH	< 0.0001	0.4651	< 0.0001
	Nonthaburi	0.0001	0.4136	< 0.0001
	Tak	< 0.0001	0.0246	< 0.0001
Propoxur				
	NIH	0.0001	0.0777	0.2621
	Nonthaburi	0.0001	0.9972	< 0.0001
	Tak	0.1457	0.0010	0.2677
Fenitrothion				
	NIH	< 0.0001	0.0002	0.0002
	Nonthaburi	0.0018	0.0006	0.0438
	Tak	0.0120	0.3629	0.0454

[1] NIH = Mosquito strain from National Institute of Health



<u>Figure 3</u> Escape patterns of unfed female *Culex quinquefasciatus* from NIH, Nonthaburi and Tak strains in contact trials with 0.02 g/m^2 deltamethrin, 0.2 g/m^2 propoxur and 0.2 g/m^2 fenitrothion

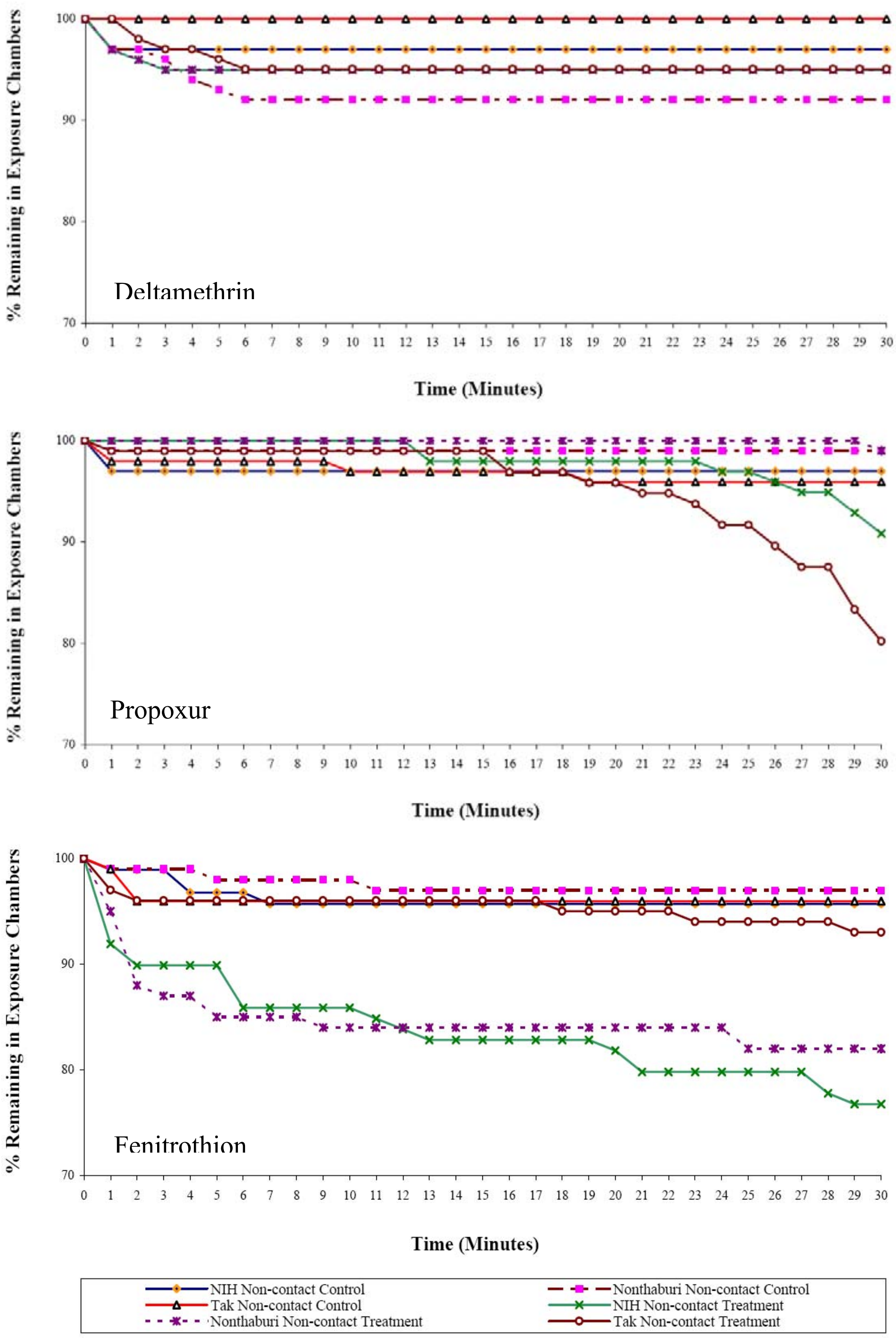


Figure 4 Escape patterns of unfed female *Culex quinquefasciatus* from NIH, Nonthaburi and Tak strains in non-contact trials with 0.02 g/m^2 deltamethrin, 0.2 g/m^2 propoxur and 0.2 g/m^2 fenitrothion

<u>Result of irritancy test</u>

Deltamethrin displayed the greatest irritant response to both S-Lab strain and Nonthaburi strain as indicated by the low FT_{50} and FT_{95} values (Table 7, Figs 4 and 5). With all insecticides, S-Lab strain took longer time than Nonthaburi strain to obtained 50% of mosquitoes took-off from the surface of impregnated papers.

<u>Table 7</u> Time to first take-off of 50% (FT_{50}) and time to first take-off of 95% (FT_{95}) of *Culex quinquefasciatus* from LIN and Nonthaburi exposed to 0.2% deltamethrin, 0.2% propoxur and 1% fenitrothion

Insecticide	Time to first take-off of *Culex quinquefasciatus* (second)			
	S-Lab strain		Nonthaburi strain	
	FT_{50}	FT_{95}	FT_{50}	FT_{95}
Deltamethirn	21	180	13	80
Propoxur	150	4,000	70	380
Fenitrothion	200	10,000	70	1,900

FT_{50} and FT_{95} = Time to first take-off of 50% and 95% of female *Cx. quinquefasciatus,* respectively

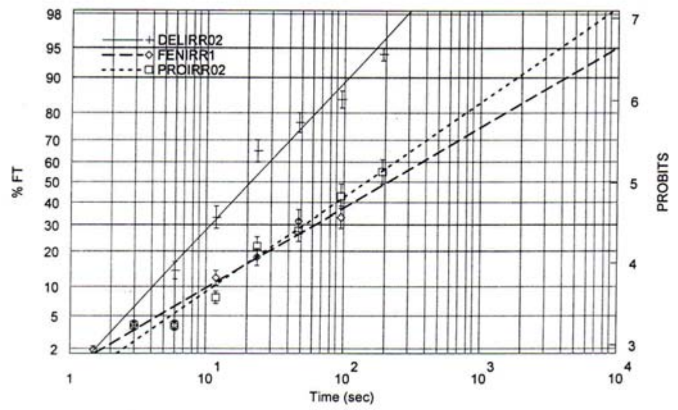


Figure 5 Time to first take-off regression line for S-Lab strain of *Culex quinquefasciatus* exposed to 0.2% deltamethrin, 0.2% propoxur and 1% fenitrothion

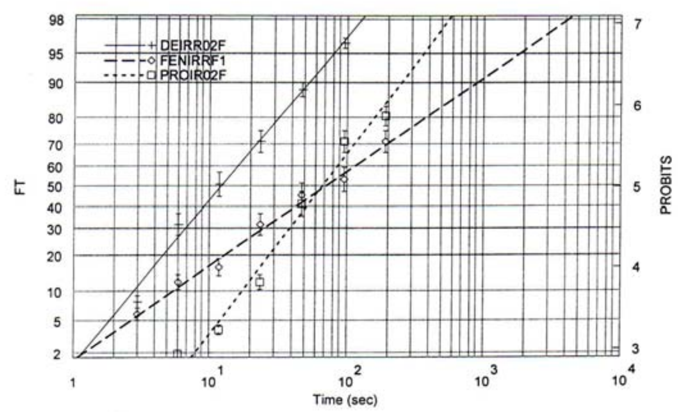


Figure 6 Time to first take-off regression line for Nonthaburi strain of *Culex quinquefasciatus* exposed to 0.2% deltamethrin, 0.2% propoxur and 1% fenitrothion

DISCUSSION

This is the first report to distinguish two forms of behavioral responses of *Culex quinquefasciatus*, a nuisance night biting mosquitoes to field dose concentrations of deltamethrin (0.02 g/m^2), propoxur (0.2 g/m^2) and fenitrothion (0.2 g/m^2). These behavioral responses include both contact irritability and non-contact repellency (spatial repellency) (Davidson 1953, Rawlings and Davidson 1982, Roberts *et al*., 2000). Irritability takes place when insects actually make physical contact with an insecticide prior to eliciting a stimulus-mediated response whereas repellency is referred to as a stimulus functioning from a far distance from the insecticide-treated surface that prevent insects from entering a treated area (Roberts *et al*., 1997). Although several studies have been conducted to examine the behavioral responses of mosquito species to various insecticides, none has been performed to describe to contact irritability and spatial repellency responses in *Cx. quinquefasciatus* (Ree and Loong 1989; Chareonviriyaphap *et al.,* 1997; Roberts *et al*., 1997; Rutledge *et al*., 1999; Kongmee *et al*., 2004 and Potikasikorn *et al*., 2005). One of the complexities in understanding these two types of behavioral responses in mosquitoes is the availability of an adequate test system. Roberts *et al*. (1997) provided a test system that can be used to distinguish between contact and non-contact responses. In this study, the mode of actions of the three most widely used compounds against *Cx. quinquefasciatus* in the Thailand vector control program were investigated.

Culex quinquefasciatus females from three different strains demonstrated dramatic escape responses from exposure chambers that permitted direct contact with deltamethrin and fenitrothion treated surfaces, regardless of background insecticide susceptibility status. Most specimens, except for those within the chamber treated with propoxur, departed the treated chambers without receiving a lethal dose of test compounds, indicating rapid behavioral avoidance. Previous studies examining several *Anopheles* species from both field and laboratory populations have also shown dramatic contact irritancy responses to deltamethrin compared. Our study observed the greatest contact escape responses to deltamethrin and fenitrothion were observed in specimens from the National Institute of Health (NIH) colony that has been maintained for at least 27 years. A similar phenomenon of rapid escape responses to test compounds was also observed in earlier studies with a Bora Bora strain of *Aedes aegypti* that was maintained as a colony for at least 25 years (Kongmee *et al*., 2004). However, several previous works have documented no behavioral responses to test compounds in other long-standing colonized populations of *Anopheles albimanus* Wiedemann from El Salvador (Chareonviriyaphap *et al*.,1997) and *Anopheles dirus* Peyton and Harrison from Thailand (Chareonviriyaphap *et al*., 2004). A same occurrence was also seen in a colony of *An. albimanus* from Panama (Brown, 1958). The reason for the rapid escape responses to test compounds in specimens from the *Cx. quinquefasciatus* colony maintained at the NIH, in the present study is unclear but most likely reflects differences in genetic backgrounds.

Delayed escape responses were observed in NIH and Tak strains when exposed to propoxur although the escape pattern was significantly different from the control. A small group of female mosquitoes (Nonthaburi strain) began to leave the propoxur treated chamber after a long exposure (23 min) to this compound. Overall, percent mortality were highest in contact trials. The large numbers of escaped and non-escaped specimens killed in contact trials with propoxur indicates the toxic function of this chemical. The reason for the high percent mortality in *Cx. quinquefasciatus* females in non-contact trials with propoxur that may have resulted from released volatiles in association with a rise in as reported by US Environmental Protection Agency (1988). Miller and Shafik (1974) reported the volatilization process of propoxur was based on the humidity condition as well and documented a higher concentration of propoxur during conditions of high relative humidity. More importantly, previous studies have confirmed similar levels of toxicity of propoxur in natural populations of several mosquito species (WHO, 1972).

Chemical control using various groups of insecticides, including organophosphates, carbamates, and synthetic pyrethroids have long been used in public health vector control programs (Roberts and Andre, 1994; Reiter and Gubler, 1997). For years, propoxur and fenitrothion have been used in Thailand to control mosquitoes that enter houses such as *Aedes spp*. and *Culex* species. In 1994, deltamethrin was first used in Thailand for controlling malaria as well as household nuisance mosquitoes (Chareonviriyaphap *et al.*, 1999). Subsequently, this compound has been accepted as the chemical of choice for mosquito control during epidemics. Additionally, these compounds have been made readily available in the public sector for use in homes. The wide use of chemicals such as deltamethrin, propoxur and fenitrothion make it necessary to evaluate residual activity. Such information will guide field operational techniques for adult mosquito control.

Discussion of irritancy test

Susceptible-Lab strain (S-Lab strain) from LIN showed slower responses to all three compounds as compared to those from Nonthaburi strain, regardless susceptibility status. S-Lab strain was found high resistance to deltamethrin and propoxur as observed from WHO susceptible test. Thus S-Lab strain was tested with 8 and 2 times diagnostic dosage of deltamethrin and propoxur, respectively for receiving 100% mortality. The reason for this are unclear. But it could be originated from the contamination of this strain during maintain in the insectary. Another reason it may have lost some ability to respond normally to insecticides because mosquitoes have been reared in the laboratory for many years (Chareonviriyaphap *et al*., 2004).

Even though, the result of irritancy test and behavioral test are similar in distinct irritancy effect of deltamethrin. The irritancy test and behavioral test with contact trial could not be compared because the tests were operated in different conditions and test methods.

CONCLUSION

Irritable and repellent functions of deltamethrin, propoxur and fenitrothion were investigated on three strains of female *Culex quinquefasciatus* from National Institute of Health (NIH), Nonthaburi and Tak provinces using excito-repellency test chambers.

The study showed 0.02 g/m^2 deltamethrin to be irritating than 0.2 g/m^2 propoxur and 0.2 g/m^2 fenitrothion observed from percent escaped of NIH, Nonthaburi and Tak strains. Whereas, among them, propoxur showed toxic effect with high mortality on the three test strains. These data were probably correlative with their insecticide susceptibility status. But irritant and repellent effects were not showed clearly in three strains of mosquitoes with fenitrothion.

Therefore, behavioral test with 0.2 g/m^2 fenitrothion should be confirmed for obtain the perfect data and during the test all conditions have to be controlled carefully. For further study on behavioral response in adults of *Cx. quinquefasciatus* should be done with other insecticides such as permethrin, cypermethrin, malathion and so on for being useful data in *Culex* control.

LITERATURE CITED

Amin, A.M. and H.T. Peris. 1990. Detection and selection of organophosphate and carbamate resistance in *Culex quinquefasciatus* from Saudi Arabia. **Med. Vet. Entonol.** 4(3): 269.

Bisset, J., M.M. Rodriguez, A. Soca, N. Pasteur and M. Raymond. 1997. Cross resistance to pyretroid and organophosphorus insecticides in the Southern House Mosquito (Diptera: Culicidae) from Cuba. **J. Med. Entomol.** 34: 244.

_______, _______, C. Diaz and A. Soca. 1998. Resistance in a strain of *Culex quinquefasciatus* coming from Medellin, Colombia. **Rev. Cubana. Med. Trop.** 50(2): 133-137.

Bloomquist, J. R. 1994. Cyclodiene resistance at the GABA receptor-chloride channel complex confers broad cross-resistance to convulsants and experimental phenyl pyrazole insecticides. **Arch Biochem Physiol**. 26(1): 69-79.

Bondareva, N.I., M.M. Artem'ev. and G.V. Gracheva. 1986. Susceptibility and irritability caused by insecticides to malaria mosquitoes in UUSR. Part 1. *Anopheles pulcherrimus*. **Med Parazitol Parazit Bolezni.** 6: 52-55.

Brown, A.W.A. 1958. Laboratory studies on the behavioristic resistance of *Anopheles albimanus* in Panama. **Bull. Wld. Hlth. Org.** 19:1053-1061.

_______ and R. Pal. 1971. Insecticide resistance in arthropods. 2ed. Monograph Ser. 38. **Wld. Hlth. Org.** pp. 491.

Chandre, F., F. Darriet, M. Darder, A. Cuany, J.M.C. Doannio, N. Pasteur and P. Guillet. 1998. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. **Med. and Vet. Entomol.** 12: 359-366.

Chareonviriyaphap, T., A. Prabaripai, and M. J. Bangs. 2004. Excito-repellency of deltamethrin on the malaria vectors, *Anopheles minimus*, *Anopheles dirus*, *Anopheles swadiwongporni*, and *Anopheles maculates*, in Thailand. **J. Am. Mosq. Control Assoc.** 20: 45-54.

_______, _______, and S. Sungvornyothin. 2002. An improved excito-repellency for mosquito behavioral test. **J. Vector Ecol.** 27: 250-252.

_______, B. Aum-Aung, and S. Ratanatham. 1999. Current insecticide resistance patterns in mosquito vectors in Thailand. **Southeast Asian J. Trop. Med. Public Health** 30: 184-194.

_______, D.R. Robberts, R.G. Andre, H.H. Harlan, S. Manguin, and M.J. Bangs. 1997. Pesticide avoidance behavior in *Anopheles albimanus*, a malaria vector in the Americas. **J. Am. Mosq. Control Assoc.** 13: 171-183.

_______, S. Sungvornyathin, S. Ratanatham, and A. Prabaripai. 2001. Pesticide-induced behavioral responses of *Anopheles minimus*, a malaria vector in Thailand. **J. Am. Mosq. Control Assoc.** 17: 13-22.

Chow, C.Y. and E.S.Thevasagayam. 1957. Bionomics and control of *Culex pipiens fatigans* Wied. in Ceylon. **Bull. Wld. Hlth. Org.** 16(3): 609-632.

Clements, A.N. 1992. **The biology of mosquitoes**. 1st ed. Chapman&Hall, London.

Curtis, C.F. and N. Pasteur. 1981. Organophosphate resistance in vector populations of the complex of *Culex pipiens* L.)Diptera: Culicidae). **Bull. Ent. Res.** 71: 153-161.

Davidson, G. 1953. Experiments on the effect of residual insecticides in houses against *Anopheles gambiae* and *Anopheles funestus*. **Bull. Entomol. Res.** 44: 231-255.

De Meillon, B. 1977. **The changing pattern in transmission of Bancroftian filariasis.** 500-501.

El-Khatib, Z.I. and G.P. Georghiou. 1985. Geographic variation of resistance to organophosphates, propoxur and DDT in the southern house mosquito, *Culex quinquefasciatus*, in California. **J. Am. Mosq. Control Assoc.** 1(3): 279-283.

Elliot, M. 1989. The pyrethroids: Early discovery, recent advantages and the future. **Pestic. Sci.** 27: 337-351.

Eto, M. 1974. **Organophosphorus pesticides:** organic and biological chemistry, p.387. CRC Press, Cleveland. Ohio. USA.

Fresno Mosquito and Vector Control District. n.d. Mosquito information: Southern house mosquito (*Culex quinquefasciatus*). Available Source: http://www.fresnomosquito.org, October 14, 2005.

Harinasuta, C. 1984. Mosquito-Borne diseases in Southeast Asia. **Mosquito-Borne Dis. Bull.** 1(1): 1-11.

Hemingway, J., Smith. C., Jayawardena, K. G. I. & Jerath, P. R. 1986. Field and laboratory detection of the altered acetylcholinesterase resistance genes which confer organophosphate and carbamate resistance in mosquitoes (Diptera: Culicidae). **Bull. Entomol. Res.** 76: 559-565.

Indian Council of Medical Research. 1959. Report on behaviour of vector species enquiry under the Director, Malaria Institute of India. In: **Technical Report of the Scientific Advisory Board for 1959 (in press).**

Jitpakdi, A., W. Choochote, P. Panart, B. Tookyang, K. Panart, and S. Prajakwong. 1998. Possible transmission of two types of *Wuchereria bancrofti* in Muang district, Chiang Mai, Northern Thailand. **Southeast Asian J. Trop. Med. Public Health** 29: 141-143.

Jocelyn, G.M., J.D. Chaney, and M.S. Mulla. 1992. Identification of oviposition attractants for *Culex quinquefasciatus* from fermented bermuda grass infusions. **J. Am. Mosq. Control Assoc.** 8:11-17.

Kleinbaum, D. G. 1995. **Survival analysis.** Springer, New York.

Kongmee, M., A. Prabaripai, P. Akratanakul, M. J. Bangs, and T. Chareonviriyaphap. 2004. Behavioral responses of *Aedes aegypti* (Diptera: Culicidae) Exposed to deltamethrin and possible implications for disease control. **J. Med. Entomol.** 41: 1055-1063.

Mantel, N., and W. Haenzel. 1959. Statistic aspects of the analysis of data from retrospective studies of disease. **J. Natl. Cancer Inst.** 22: 719-748.

Millar, J.G., J. D. Chaney and M. S. Mulla. 1992. Identification of oviposition attractants for Culex quinquefasciatus from fermented Bermuda grass infusions. **J. Am. Mosq. Control Assoc.** 8(1): 11-17.

Miller, C. W., and T. M. Shafik. 1974. Concentrations of propoxur in air following repeated indoor applications. **Bull. WHO.** 51: 41-44.

Miller, T.A. 1988. Mechanism of resistance to pyrethroids insecticides. **Parasitology Today**. S8-S12.

Ministry of Public Health. 1989. **Vector-borne Disease Annual Report.** CDC, Ministry of Public Health, Nonthaburi, Thailand.

Ministry of Public Health. 2004. **Vector-borne Disease Annual Report.** CDC, Ministry of Public Health, Nonthaburi, Thailand.

Nazni W.A., H.L. Lee and Sa diyah I. 1998. Rate of resistance development in wild *Culex quinquefasciatus* (Say) selected by malathion and permethrin. **Southeast Asian J. Trop. Med. Public Health** 29 (4):849-855.

Pell, J.K., M.A. Spinney and K.J. Ward. 1989. Observations on the behavior of adult *Anopheles gambiae* encountering residual deposits of lambda-cyhalothrin compared with the other major classes, p.18. **Fourth Annual Conference of the Society for Vector Ecology, European Region.**

Potikasikorn, J., T. Chareonviriyaphap, M, J. Bangs, and A. Prabaripai. 2005. Behavioral responses to DDT and Pyrethroids between *Anopheles minimus* species A and C, malaria vectors in Thailand. **Am. J. Trop. Med. Hyg.** 73(2): 343-349.

Quinones, M.L. and M.F. Suare. 1989. Irritability to DDT of natural populations of the primary malaria vectors in Colombia. **J. Am. Mosq. Assoc.** 5: 56-59.

Rachou, R.G., M. Moura Lima, J.P. Duret and J.R. Kerr. 1963. Experiments with the excito-repellency test box-model OPS. **Proceedings of the 50th Annual Meeting of the American Mosquito Control Association.** 442-447.

Raghavan, N.G.S. 1961. The vectors of human infections by *Wuchereria* species in endemic areas and their biology. **Bull. Org. mond. Sante.** 24: 177-195.

Rawlings, P., and G. Davidson. 1982. The dispersal and survival of *Anopheles culicifacies* Giles (Diptera: Culicidae) in a Sri Lanka village under malathion spraying. **Bull. Entomol. Res.** 72: 139-144.

Ree, H.I. and K.P. Loong. 1989. Irritability of *Anopheles farauti*, *Anopheles maculatus*, and *Culex quinquefasciatus* to permethrin. **Jpn. J. Sanit. Zool.** 40: 47-51.

Reiter, P., and D. J. Gubler. 1997. Surveillance and control of urban dengue vectors, pp. 425-462. In D. J. Gubler and G. Kuno, eds. **Dengue haemorrhagic fever.** New York: CAB International.

Roberts, D.R., T. Chareonviriyaphap, H.H. Harlan and P. Hshieh. 1997. Methods of testing and analyzing excito-repellency responses of malaria vectors to insecticides. **J. Am. Mosq. Control Assoc.** 13:13-17.

_______, and R. G. Andre. 1994. Insecticide resistance issues in vector-borne disease control. **Am. J. Trop. Med. Hyg.** 50: 21-34.

_______, T. Chareonviriyapphap, H. H. Harlan, and P. Hshieh. 1997. Methods for testing and analyzing excito-repellency responses of malaria vectors to insecticides. **J. Am. Mosq. Control Assoc.** 13: 13-17.

_______, W. D. Alecrim, P. Hshieh, J. Grieco, M. J. Bangs, R. G. Andre, and T. Chareonviriyaphap. 2000. A Probability model of vector behavior: effects of DDT repellency irritability, and toxicity in malaria control. **J. Vector Ecol.** 25: 48-61.

Rutledge, L. C., N. M. Echana, and R. K. Gupta. 1999. Responses of male and female mosquitoes to repellents in the World Health Organization insecticide irritability test system. **J. Am. Mosq. Control Assoc.** 15: 60-64.

Singh, D. 1967. The Culex pipiens fatigans problem in South-East Asia. **Bull. Wld. Hlth. Org.** 37: 239-243.

Somboon, P., L. Prapanthadara, and W. Suwanakerd. 2003. Insecticide susceptibility tests of *Anopheles minimus*, *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* in northern Thailand. **Southeast Asian J. Trop. Med. Public Health** 34: 87-93.

Sungvornyothin, S., T. Chareonviriyaphap, A. Prabaripai, V. Thirakhupt, S. Ratanatham, and M. J. Bangs. 2001. Effects of nutritional and physiological status on behavioral avoidance of *Anopheles minimus* (Diptera: Culicidae) to DDT, deltamethrin and lambdacyhalothrin. **J. Vector Ecol.** 26: 202-215.

Sucharit, S., C. Harinasuta, K. Surathin, T. Deesin, S. Vutikes, and Y. Rongsriyam. 1981. Some aspects on biting cycles of *Culex quinquefasciatus* in Bangkok. **Southeast Asian J. Trop. Med. Public Health** 12(1): 74-78.

Suzuki, T. and D. Mizutani. 1962. Studies on insecticide resistance on mosquitoes of Japan. **Jap. J. Exp. Med.** 32: 297.

Swaddiwudhipong, W., Y. Tatip, M. Meethong, P. Preecha and T. Kobasa. 1996. Potential transmission of Bancroftian filariasis in urban Thailand. **Southeast Asian J. Trop. Med. Public Health.** 27(4): 847-849.

Thomas A., and Pillai M.K.K. 1986. Toxicity and irritability of K-othrine against four species of mosquitoes. **Entomon.** 11(1): 29-33.

Triteeraprapab, S., K. Kanjanopas, S. Suwannadabba, S. Sangprakarn, Y. Poovopawan, and A. L. Scott. 2000. Transmission of the nocturnal periodic strain of *Wuchereria bancrofti* by *Culex quinquefasciatus* : establishing the potential for urban filariasis in Thailand. **Epidemiol. Infect.** 125: 207-212.

US Environmental Protection Agency. 1988. **Pesticide fact sheet: Metiram.** Office of pesticides and toxic substances, US EPA, Washington, DC.

WHO. 1982. Manual on environmental management for mosquito control with special emphasis on malaria vectors. **WHO offset publication No 66.** Geneva. Switzerland.

_______. 1998a. Control of Lymphatic filariasis in the South-East Asia. **Report of an intercountry workshop.** New Delhi. India.

_______. 1998b. Insecticide resistance in mosquito vectors of disease. **Report of a regional working group meeting Salatiga (Indonesia).** New Delhi. India.

_______. 1996. Report of the WHO informal consultation on the evaluation and testing of insecticides. Document **CTD/WHOPES/IC/96.1.** Geneva. Switzerland.

_______. 1992. Report of the WHO informal consultation in the evaluation and testing of insecticides. Document **CTD/WHOPES/IC/96.1.** Geneva. Switzerland.

_______. 1972. Residual toxicity of propoxur and fenitrothion to adult mosquitos in animal shelters in Korea. Document **WHO/VBC/72.397.** Seoul, Korea.

_______. 1976. Resistance of vectors and reservoirs of disease to pesticides. **WHO Tech. Rep. Ser.** 585.

_______. 1992. Vector resistance to pesticides. **Fifteenth report of the WHO expert committee on vector biology and control.** Geneva. Switzerland.

Zerba, E. 1988. Insecticidal activity of pyrethroids on insects of medical importance. **Parasitology Today.** 4: 53-57.

APPENDIX

<u>Appendix Table 1</u> Collection sites of *Culex quinquefasciatus* for this study

Test populations	Collection site	Latitude / Longitude
Nonthaburi	Sai Thong Community, Maung District, Nonthaburi	13º 50’ 30” N, 100º 29’ 45” E
Tak	Mae Sot District, Tak	16º 42’ 30” N, 97º 34’ 30” E