THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Entomology)

DEGREE

Entomology	Entomology
FIELD	**DEPARTMENT**

TITLE: Behavioral Responses of *Aedes aegypti* to Insecticides Using Two Assays Systems and The Influence of Insecticide Resistance Mechanisms

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THESIS

BEHAVIORAL RESPONSES OF *AEDES AEGYPTI* TO INSECTICIDES USING TWO ASSAYS SYSTEMS AND THE INFLUENCE OF INSECTICIDE RESISTANCE MECHANISMS

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Entomology)
Graduate School, Kasetsart University
2008

Kanutcharee Thanispong 2008: Behavioral Responses of *Aedes aegypti* to Insecticides Using Two Assays Systems and The Influence of Insecticide Resistance Mechanisms. Doctor of Philosophy (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Professor Theeraphap Chareonviriyaphap, Ph.D. 139 pages.

This study was designed to quantify irritancy (contact) and repellency (non-contact) responses of six field strains of female *Aedes aegypti* adults to alphacypermethrin, deltamethrin, permethrin and DDT and to describe strain-specific insecticide susceptibility status and resistance mechanisms. Field strains were collected from various geographical regions in Thailand; Chiang Mai (north), Kanchanaburi (west), Khonkaen (northeast), Nonthaburi (central), Songkhla and Satun (south). Susceptibility bioassays were performed to evaluate the degree of background insecticide resistance in all 6 strains of *Ae. aegypti*. All strains were found highly resistant to DDT and permethrin, with one exception (Chiang Mai susceptible to permethrin). In contrast, the majority of test strains were found susceptible to deltamethrin, alphacypermethrin and malathion, exceptions being Nonthaburi strain showing incipient (low) resistance to alphacypermethrin and Khonkaen strain showing marked resistance to malathion. One mechanism of insecticide resistance, metabolic detoxification, was investigated. The findings found that monooxygenase activity was elevated in two permethrin- resistant *Ae. aegypti* strains and one susceptible strain. Elevated esterase activity in the Khonkaen strain appears to be associated with malathion resistance.

In addition, all strains exhibited strong contact irritancy responses when exposed to synthetic pyrethroids but significantly weaker irritant responses when exposed to DDT. The degree of non-contact repellency varied, depending upon the *Ae. aegypti* strain and assay type. Pronounced repellency to DDT was found in the three *Ae. aegypti* strains from Chiang Mai, Kanchanaburi and Khonkaen when evaluation was performed using an excito-repellency test system. In contrast, five strains of *Ae. aegypti* showed strong repellency response when a high throughput screening system was used. Although differences in response outcomes were seen depending on assay type, both test systems remain appropriate for evaluating the behavioral responses of *Ae. aegypti* to residual insecticides used in vector control. Differences in repellency responses among the *Ae. aegypti* strains between the two test systems are discussed. We conclude that irritant/repellent responses of *Ae. aegypti* females to insecticides are important components of vector control. Results from this study indicate physiological resistance and behavioral responses may not be associated.

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Student's signature Thesis Advisor's signature

ACKNOWLEDGMENTS

I would like to express my sincere gratitude and deep appreciation to my thesis advisor, Prof. Dr. Theeraphap Chareonviriyaphap for his helpful guidance, suggestion, invaluable comment, patiently correcting the manuscript, great encouragement and supporting materials and equipments throughout the study. I would sincerely like to thank Assistant Dr. Nicole Achee of the Uniformed Services University of Health Science, Bethesda, MD, USA, for her valuable suggestion and English usage. I also wish to extend my grateful appreciation to Assoc. Prof. Dr. Chitapa Ketavan, for her kindness and giving me the chance to travel to LIN, Montpellier, France for entomological training. Sincere thanks go to Assoc. Prof. Monchan Maketon for her critical comment on this thesis improvement. Special thanks are also due to Dr. Vincent Corbel and Mrs. Brengues Cecile for their kindness and technical support during my insecticide resistance mechanisms study at LIN, Montpellier, France.

I would like to express my appreciation to the Thailand Research Fund Organization, Kasetsart University Research and Development Institute, Thailand, Thai-French Cooperation Program for Higher Education and Research and the National Institute of Health, Bethesda, Maryland, United State of America for their financial support.

Finally, I would like to thank Dr. Wannapa Suwankerd of Office of Disease Prevention and Control, 10 Chiang Mai for her kindness in providing *Aedes aegypti* eggs. Thanks also go to Mr.Virat Wanghirunrat and Mr. Siangdhamvimol Rojruthai of Office of Disease Prevention and Control at 12 Songkhla and 1 Nonthaburi, respectively, for helping me in collecting to mosquito larvae.

Kanutcharee Thanispong

October 2008

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LIST OF ABBREVIATIONS

Ae. aegypti	=	*Aedes aegypti*
^{o}C	=	degree Celsius
cm	=	centimeter
cm^2	=	square centimeter
cm^3	=	cubic centimeter
e.g.	=	example gratia
h	=	hour
i.e.	=	id est
kg	=	kilogram
mg	=	milligram
min	=	minute
ml	=	milliliter
mosq	=	mosquito
m^2	=	square meter
nm	=	nanomole
RH	=	Relative Humidity
sec	=	second
µl	=	microliter

BEHAVIORAL RESPONSES OF *AEDES AEGYPTI* TO INSECTICIDES USING TWO ASSAYS SYSTEMS AND THE INFLUENCE OF INSECTICIDE RESISTANCE MECHANISMS

INTRODUCTION

Dengue is one of the most significant infectious diseases that has a major public health impact in many tropical and subtropical countries (Gubler, 1997; Guzman and Kouri, 2002). The disease is transmitted by *Aedes aegypti,* a notoriously efficient vector that invariably resides in close association with humans (WHO, 1999). Typically, *Ae aegypti* breeds in household man-made water-storage containers and preferentially feeds indoors, especially in the morning hours and in the late afternoon (Christophers, 1960; Gubler, 1997). *Aedes aegypti* prefers to rest indoors in secured and undisturbed places of the closet or dark corner of the house (Reiter *et al*., 1995; Scott *et al*., 2000a). Generally, *Ae. aegypti* has a flight range of less than 400 m, although recent studies indicated that *Ae. aegypti* might disperse over much longer distances in search of oviposition sites (Reiter *et al*., 1995; Scott *et al*., 1993, 2000b; Harington *et al*., 2005). Despite research progress, a completely effective and commercially available dengue vaccine is not yet available. For this reason, the prevention and control of this disease is currently dependent on vector surveillance and vector control methods. Most vector surveillance strategies rely solely or only on indicators that have been designed to detect the presence or absence of mosquito larvae or pupae. Elimination of *Ae. aegypti* through source reductions has been proposed but this approach is somewhat expensive, needs full community participation and is invariably unsuccessful (Kongmee *et al*., 2004). Furthermore, ultra-low-volume (ULV) and thermal fogging applications of synthetic pyrethroids are commonly used, especially during the peak period of adult populations. Additionally, many synthetic pyrethroids are commonly used by home owners to control household mosquitoes. Chemical application could be an important cause of insecticide resistance in the house-haunting mosquito like *Ae. aegypti*.

Mosquito behavior in response to insecticide exposure is a critical component in the epidemiology of vector- borne disease transmission. The use of chemical barriers have historically been used to exploit these behavioral responses for the purpose of inhibiting mosquitoes from preferentially feeding on humans, ingesting infectious blood meals, or transmitting pathogens to susceptible hosts (Elliott, 1972). The natural reaction of mosquitoes to avoid insecticide-treated surfaces is a general phenomenon, yet behavioral responses of adult mosquitoes exposed to insecticides remains unclear and poorly studied. This is true despite the fact that quantifying behavioral responses to insecticides other than toxicity is an important aspect in understanding how various vector control chemicals function. Large amounts of data have been gathered on the impact of test compounds on *Anopheles* species responsible for malaria transmission, whereas fewer attempts have been made to describe the function and response of chemicals on other mosquito species (Kennedy, 1947; Brown, 1964; Lal *et a*l., 1965; Moore, 1977). This knowledge will allow better decision-making on pesticide selection and application (Muirhead-Thomson, 1960; Roberts *et al*., 2000a; Grieco *et al*., 2007).

Synthetic compounds, including organophosphates, carbamates, and pyrethroids have been used with varying degrees of success in national public health vector control programs (Reiter and Gubler, 1997). Since 1994, the Ministry of Public Health [MOPH] (1990) in Thailand has recommended the use of deltamethrin as the compound to be used for emergency vector control during dengue outbreaks and, to this day remains the only compound used in public health control programs for dengue control (Chareonviriyaphap *et al*., 1999). Recent work has reported the spread of deltamethrin resistance in several field populations of *Ae. aegypti* from Thailand (Jirakanjanakit *et al*., 2007). The spread of resistance is raising awareness of the need for alternative insecticides or new methods of controlling mosquito vectors. Alphacypermethrin and permethrin, an effective and safe synthetic pyrethroid, are currently being used in homes for the protection against indoor biting mosquitoes and other arthropod pests. Therefore, it is of importance to quantify the chemical actions of alphacypermethrin and permethrin against various *Ae. aegypti* populations from

Thailand prior to its large scale use in public health programs. This will generate the production of innovative control methodologies.

Excito-repellency test chamber system and a high throughput screening system are practical experiment tools that available to investigate behavioral responses of mosquitoes to chemical compounds. The excito-repellency test system is a tool that can be used to evaluate contact irritancy and non-contact repellency behavioral responses of mosquitoes (Chareonviriyaphap *et al*., 1997; Roberts *et al*., 1997b). In addition, the high throughput screening system (HTSS) is a tool which compact in size and require a minute quality of chemical compounds, and allows to observe the three types of behavioral responses include contact irritancy, spatial repellency and toxicity responses. The HTSS provide consistent, quantifiable measures of behavioral responses with a relatively low number of replications (Grieco *et al*., 2005). Since the introduction of the assay, modifications and improvements have been made to these systems to allow greater ease and accuracy in evaluating the innate behavioral response of mosquitoes exposed to varying doses of residual insecticides (Chareonviriyaphap *et al*., 2002; Grieco *et al*, 2005, 2007; Tanasinchayakul *et al*., 2006). Both test systems were used to evaluate the behavioral responses of six field strains of *Ae. aegypti* to alphacypermethrin, deltamethrin and permethrin. In this study, DDT is a repellency excellent standard for using the chemicals comparison purposes. In addition, insecticide susceptibility status and the influence of insecticide resistance mechanisms in the six *Ae. aegypti* strains were determined. Evidence for the insecticide resistance status and behavioral response of *Ae. aegypti*, as a vector of dengue, to insecticide have been the importance information to chooses the appropriate methods in the dengue vector control program in Thailand.

OBJECTIVES

The objectives of this study were

1. to determine the insecticide susceptibility status and resistance mechanisms in the *Aedes aegypti* strains and

2. to identify the two types of behavioral responses of *Aedes aegypti* strains to various test compounds.

LITERATURE REVIEW

Thailand is located in the heart of Southeast Asia, with an area of 514,000 km^2. The geographic location lies between 5°37′ to 20 °27′ N and longitudes 97° 22′ to 105 ° 37′ E. Thailand shares international borders with Myanmar to the west and north, Laos to the northeast, Cambodia to the west, and Malaysia to the south. Thailand has a tropical monsoon climate with high relative humidity (average 73 – 80 %) and temperature (average 27 ° C). Three seasons, rain (May to October), dry (November to February), and hot (March to April) are recognized. Temperatures may exceed 38 ° C in the summer. Rainfall varies but is generally heaviest in the southeast with 4,000 mm annual (Thai Meteorological Department [TMD], 2008).

1. Dengue situation in Thailand

Dengue is a serious public health problem throughout the tropics and subtropics (Gubler, 1997; Guzman and Kouri, 2002). There are four antigenically distinct types of dengue viruses (dengue 1, 2, 3 and 4) which are transmitted by mosquitoes (Gubler, 1997; Guzman and Kouri, 2002). Infection in man varies considerably in severity ranging from asymptomatic to shock and death, depending on host immunological responses from prior exposure to dengue viruses. The severe forms are known as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Guzman and Kouri, 2002; Deen *et al*., 2006). Dengue was first recognized as an arthropod-borne virus early in 1900 (Gubler and Clark, 1995; Gubler, 1997). Several early epidemics of dengue in Asia, the Americas and Europe are thought to have lead to associated sever haemorrhagic manifestation, but the relationship between DHF and dengue was not identified until 1956, after an epidemic in Manila, the Philippines (Hammon *et al*., 1960; Ehernkranz *et al*., 1971; Gubler, 1997; Pinheiro and Corber, 1997). Since then, DSS has spread to many areas of the Southeast Asian countries, the Pacific and the Americas (Gubler, 1997; Pinheiro and Corber, 1997; Guzman and Kouri, 2003; Mairuhu *et al.*, 2004).

In Thailand, dengue fever and DHF are major health problems. The first case of DHF in Thailand was recognized in 1958 in Bangkok (Jatanasea, 1966). Since then, the disease has distributed throughout the country. Although dengue was originally thought of as a rural disease and continues to rise (MOPH, 2003). Risk seems to be higher in urban and suburban areas. In Thailand, there were 65,581 reported cases and 95 deaths in 2007 (MOPH, 2007b), although the case-fatality rate is generally reducing annually. Generally, the number of dengue cases starts to increase in April, at the end of the dry season. The highest case numbers occur in June and August, during the rainy season, with a significant decline occurring in September (MOPH, 2007b; WHO, 2008).

The distribution of the dengue is not only considerable by vectors and viruses, but included human as well. Harrington *et al*. (2005) reported that the human-movement factor is more important in spreading of dengue than the flight range of *Ae. aegypti*. Except that factor, daily, seasonal, and variable in temperature, atmospheric moisture and rainfall, environmental factor, all factors were influenced the dengue system in a variety of ways (Kuno, 1997; WHO, 2008). It was supported by the study of Thongrungkiat *et al*. (2003), that the environmental conditions of each season in Thailand might be a temporal change of mosquitoes competence.

2. Dengue vector

Aedes aegypti is the vector of the virus that causes dengue, DHF and DSS in most parts of the world (Gubler, 1997, 1998). Important epidemics have occurred in areas where *Ae. aegypti* presents. In other areas of the Western Pacific and Southeast Asia, *Aedes albopictus* has also played a secondary role in transmitting dengue viruses during outbreaks (Gubler, 1998; Guzman and Kouri, 2002; Effler *et al*., 2005). Therefore, the recent finding of *Ae. albopictus* in the United States and Brazil, and the possibility of its infestation in other countries in South and Central America, may have a great impact in an epidemiological importance (Hornby *et al*., 1995; Knudsen, 1996; Moore, 1999).

Aedes aegypti is strongly anthropophilic and recognized as the most efficient vector due to its close association with humans. In most areas of Southeast Asia countries *Ae. aegypti* breeds almost entirely in and outside human habitation in freshwater. The majority of *Ae. aegypti* breeding places is primarily in man-made containers, i.e., earthenware jars, concrete cisterns, ant traps containers, and other items that collect rainwater nearby houses (Swaddiwudhipong *et al*., 1992; Chansaeng *et al*., 1993; Kittayapong and Strickman, 1993; Chareonviriyaphap *et al*., 2003a). *Aedes aegypti* prefers to rest inside houses, typically in sheltered places such as dark corners, undersides of furniture, hanging objects such as clothes and curtains, and on dark walls (Reiter, 1991; Reiter *et al*., 1995; WHO, 1999; Scott *et al*., 2000a). Although patterns of biting may vary by season and location (Lumsden, 1957; Sheppard *et al.,* 1969; Nelson *et al.,* 1978), feeding patterns have been associated with multiple bloodmeal during gonotrophic cycle (Scott *et al*., 1993, 2000b) and the preference to feed on humans (Edman *et al*., 1992).

Aedes aegypti deposits their eggs on clean moist surfaces (Riter and Gubler, 1997) and eggs hatch when flooded (Riter and Gubler, 1997). Their eggs appear viable for many months after they are dried. Embryonic development is usually completed in 48 hours in a warm and humid environment (Klowden, 2002). Under optimal conditions, the time taken from hatching to adult emergence can be as short as seven days (Clements, 2000). The adults of *Ae. aegypti* do not fly far, dispersing probably no more than 100 meters beyond the emergence location (Reiter *et al*., 1995; Muire and Kay, 1998; Scott *et al*., 2000a; Honorio *et al*., 2003; Harrington *et al.,* 2005; WHO, 2008). *Aedes aegypti* population dynamics vary in geographical areas, depending upon the altitude, seasonal, temperature, humidity and rainfall. Kalra *et al*. (1997) found that altitude is a limiting factor of the distribution of *Ae. aegypti* in India. In countries of Southeast Asia, the attitude of 1000 to 1500 meters appears to be limited factor for *Ae. aegypti* distribution (WHO, 1999). Scott *et al*. (2000a), found that the high temperature has an influence on female adult abundance.

Aedes albopictus, the other major vector of dengue disease, breeds in tree holes, bamboo stumps, and other natural containers and in many of the same man-

made habitats where *Ae. aegypti* breeds (Christophers, 1960; Hawley, 1988; Ratlanarithikul and Panthusiri, 1994; O′ Meara *et al*., 1995). Several strains of *Ae. albopictus* in the United States and Brazil (Hobbs *et a*l., 1991; Tabachnick, 1991; Hornby *et al*., 1995; Barks *et al*., 2004), which were probably introduced through importation of tires from Asia (Hawley *et al*., 1987), seem equally capable of maintaining themselves in natural habitats some distance away from human dwellings and in man-made habitats (Hawley, 1988; O′ Meara *et al*., 1995). Introduction of this species has complicated control efforts, which has been directed against *Ae. aegypti*. The threat of northern areas of the United States is increased by the fact that the introduced strain of *Ae. albopictus* is able to survive freezing temperature (Hanson *et al*., 1993; Hanson and Graig, 1994; Moore, 1999).

3. Test compounds

Test compounds can be classified into three main groups by their chemical nature (Handa, 2000). These are inorganic, organic, and botanical compounds (Handa, 2000). Of three, organic compound is somewhat important and include organophosphates, carbamates and pyrethroids synthetic chemicals. These compounds are available for use in dengue vector control program in Thailand (WHO, 2006a; MOPH, 2007a). Presently, pyrethroid insecticides (include alphacypermethrin, deltamethrin and permethrin) are insecticides of choice for dengue vector control program in Thailand (MOPH, 2007a).

3.1 Organochlorine (Chlorinated hydrocarbons: OC)

Organochlorine insecticides were the first modern synthetic insecticide group (Mellanby, 1992). This group contains the cyclodienes (i.e., aldrin), the substituted ethanes (e.g., DDT) and the complex mixture of chlorinated terpinoids collectively referred to as “toxaphene”. The only chemical feature in common is the presence of chlorine substituents on an organic parent compound. Chemical within the organochlorine groups are environmentally persistence (Hodgson *et al.,* 1998).

- DDT

DDT [1,1,1-trichloro-2,2-bis(4-chloropheney) ethane] was developed as the first modern synthetic insecticides in the 1940's. It was initially used with great effect to combat malaria, typhus, and other insect-borne human diseases among both military and civilian populations. DDT was also used for insect control in crop and livestock production, institutions, homes, and gardens (Mellanby, 1992; Agency for Toxic Substances and Diseases Registry [ATSDR], 2002) (Figure 1). DDT is prepared by the condensation of chloral and chlorobenzene in the presence of excess concess concentrated sulphuric acid (Handa, 2000).

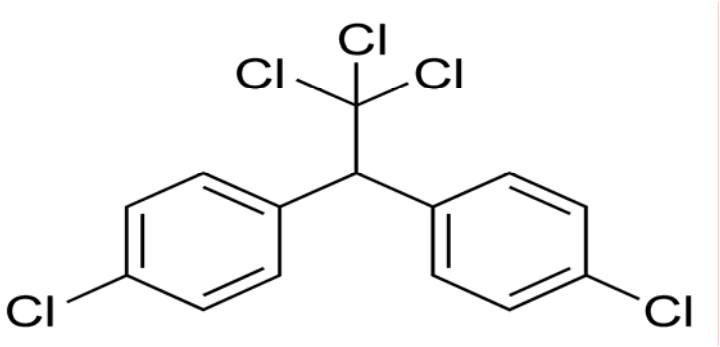

Figure 1 DDT chemical structure

Source: David (2008).

The mode of action of DDT is a neuron toxicant. The acute toxicity of DDT is attributed to a direct action on nerve axon membranes, increasing excitability and resulting in multiple impulses, tremors and tetanus. More specifically, DDT increases sodium conductance across nerve cell membranes, probably by a direct interaction with the sodium channel protein (Corbett *et al*., 1984; Mellanby, 1992; Hodgson *et al.,* 1998).

DDT is a highly hydrophobic, white amorphous powder, crystalline solid with a weak, chemical odor and moderate stability to sunlight (Wasserman *et al*, 1982). It is nearly insoluble in water but has a good solubility in most organic

solvents, fats, and oils. The relative molecular mass of DDT is 354.51(Wasserman *et al*., 1982). DDT is classified as moderately hazardous by WHO (2005b), with rat LD_{50} of 113 mg/kg. It has a broad spectrum of activity and low acute mammalian toxicity, but is persistent in the environment (Augustijn-Beckers *et al*., 1994; ATSDR, 2002).

3.2 Organophosphate (OPs)

Organophosphate insecticides are the second class of synthetic insecticides. They were developed in 1941 during research on nerve gases in Germany (Chamber, 1992). Generally, organophosphate insecticides are highly toxic to mammals as well as target insects, however, they rapidly hydrolyze. Most organophosphate insecticides are esters or amids of organically bound phosphoric or pyrophosphoric acid (Chamber, 1992). These compounds can be divided into five classes according to their phosphorous moiety (Eto, 1974). Among the five classes, two contain important insecticides which have been widely used in mosquito control programs. These are phosphorothionates and phosphorothiolothionate esters.

- Malathion

Malathion [S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate] was one of the earliest organophosphate insecticides developed (Figure 2). It is a non-systemic, wide-spectrum organophosphate insecticide, used in agriculture to control a wide range of sucking and chewing insect pests in a variety of field crops and is also used for insect control on livestock, in stables and on stored products (Handa, 2000; Environmental Protection Agency [EPA], 2008). It is widely used in public health, including the control of dengue and other vector-borne diseases (WHO, 2003a). Malathion is a neurotoxicant, after activation to its oxygen analog, malaoxon, by cytochrome P450, by virtue of malaxon to act as an acetylcholinesterase (AChE) inhibitor. The inhibition blocks the hydrolysis of the AChE substrate acetylcholine (ACh), a neurotransmitter. Their action results in excess stimulation at

the neuromuscular junction by an accumulation of ACh, although all synapses using ACh are affected and nerve function is impaired (EPA, 2008).

Malthion is a yellow to brown liquid with a strong smell. It is slightly soluble in water, soluble in alcohols and aromatic solvents, and of limited solubility in petroleum oils (ATSDR, 2007). It is generally stable to photolysis (EPA, 2008). In the environment, microbes and water often degrade malathion into compounds of lower toxicity, however, malathion may be converted into more toxic substrate under some conditions (Brown *et al*, 1993; EPA, 2008). The relative molecular mass of malathion is 303.36 (ATSDR, 2007). Malathion is classified as slightly hazardous by WHO (2005b).

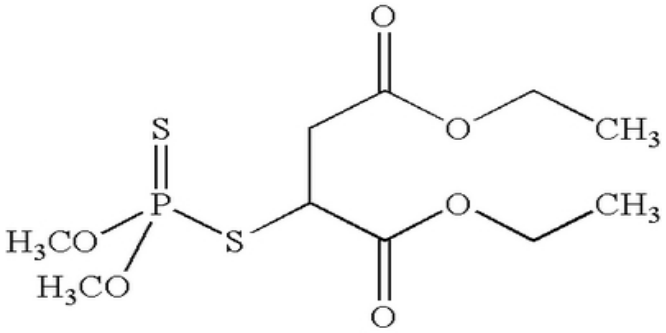

Figure 2 Malathion chemical structure.

Source: Anonymous (2008).

3.3 Carbonates

Carbamate insecticides [*N*-methyl or *N*, *N*-dimethyl] are derivations of esters of carbamic acids. They exert their insecticidal activity, as well as their toxicity to other animals, by virtue of their ability to act as potent cholinesterase inhibitors. They are biodegradable, moderately volatile (vapor pressure between 10^{-4} and 10^{-6} mmHg), moderately soluble in water, and susceptible to hydrolysis (Handa, 2000). Some of the more common carbamtes include propoxur, bediocarb, carbaryl,

methonyl, aldicarrd, and thiocarb. These insecticides exhibit toxicities that range from class I to III (Hodgson *et al*., 1998).

3.4 Synthetic pyrethroids (PY)

Synthetic pyrethroid insecticides are a relatively new class of vector control compounds. They were developed from the basic structure of the pyrethrins, insecticides of botanical origin (Davies, 1985). The active principles of these are esters of chrysanthemumic acid (R^1=CH_3) or pyrethric acid (R^1=$COOCH_3$) combined with the cyclopentenolone alcohols pyrethone, cinerolone and jasmolone. Structural modifications to one or other of these moieties have produced a diversity of pyrethroid compounds (Soderlund *et al*., 2002). Pyrethroids have been historically divided into two types, according to their chemical structure: type I pyrethroids, which do not contain an alpha-cyano group in their molecule, and type II pyrethroids, which do contain an alpha-cyano group (Tordoir *et al.,* 1994). The mode of action of pyrethroids is similar to that of DDT (Henk *et al.,* 1982). Pyrethroids are a neuron toxic insecticide interacting with sodium channels. Opening and closing of the sodium channel is slowed, resulting in increased sodium permeability and depolarization, causing rapid paralysis or knockdown and death at a later stage in a variety of insects (Soderlund and Bloomquist, 1989; Mueller-Beilschmidt, 1990; Vijverberg and Van den Bercken, 1990; Pollack *et al*., 1999; Reigart and Roberts, 1999). At present, the class of pyrethroids includes 42 active ingredients, differing in chemical structure or in relative stereoisomer composition (ATSDR, 2003; WHO, 2005a). This class includes compounds such as alphacypermethrin, deltamethrin and permethrin (WHO, 2005a).

- Alphacypermethrin

Alphacypermethrin [(*S*)-α-cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate and (*R*)-α-cyano-3-phenoxybenzyl-(1*S*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate)] consist of 2 cis-isomers from the 8 isomers present in cypermethrin

Alphacypermethrin is a type II synthetic pyrethroid and contains an alpha-cyano group (Figure 3). Alphacypermethrin is a non-systemic, broad spectrum compound. It is used in to control a wide range of agriculture pests, public health pest control, and also used in animal health as an ectoparasiticide (Department of Health and Ageing, 2007). The mode of action of alphacypermethrin is by preventing transmission of nerve impulses, by blocking the passage of sodium ions through channels in nerve membranes, thus preventing signals passing down axons. Typically this intoxication results in a rapid knockdown activity and mortality (WHO, 2007b).

Alphacypermethrin is white to pale yellow powder with a weak aromatic odor. The relative molecular mass is 413.6. It is very stable in neutral and acid media but hydrolyzed in strongly alkaline media. Thermally stable up to 220^{o}C, it is stable to air and light(International Program on Chemical Safety [IPCS], 2004). The WHO hazard classified of alphacypermethrin is moderately hazardous, class II (WHO, 2005b).

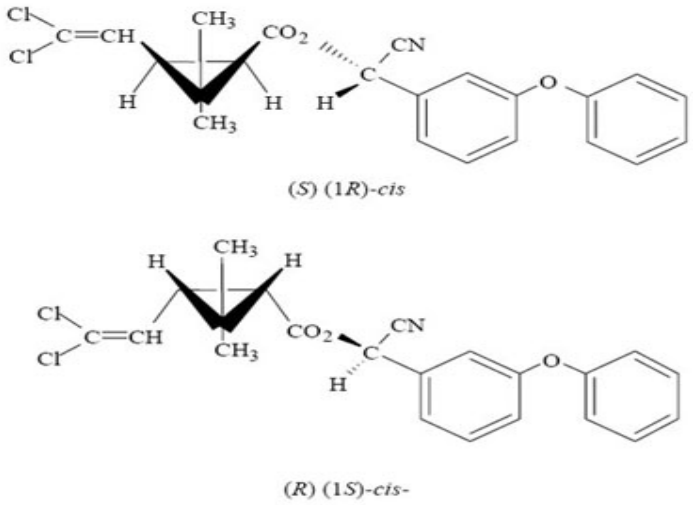

Figure 3 Alphacypermethrin chemical structure.

Source: Department of Health and Ageing (2007).

- Deltamethrin

Deltamethrin [(S)-α-cyyano-3-phenoxybenzyl(1R,3R)-3(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate], is a single stereoisomer pyrethroid (Hodgson *et al*., 1998) (Figure 4). It contains an alpha-cyano group, designating in as a type II pyrethroid. Deltamethrin is effective against a wide range of insect and widely used in the control of agriculture pests, for public health and insect pests of livestock (Hodgson *et al.,* 1998). It acts as a neurotoxicant modifying sodium channels in the nerve membrane there by increasing sodium permeability leading to depolarization and nerve blocked (Hang and Hoffiman, 1990; Eriksson and Fredriksson, 1991). There is evidence that deltamethrin also acts as the gamma-aminobutyric acid (GABA) receptor/ ionophore complex (Ray and Forshaw, 2000).

Deltamethrin is a colorless to white crystalline powder. It is a lipophilic compound of high molecular weigh, 505.24, and consequent low volatility. The WHO hazard classification of deltamethrin is moderately hazardous, class II (WHO, 2005b; IPCS, 2001). Deltamethrin is a non-systemicchemical with contact and stomach action

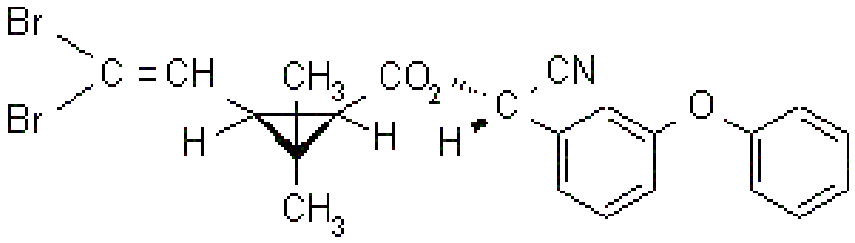

Figure 4 Deltamethrin chemical structure.

Source: Anonymous (2008).

- Permethrin

Permethrin [3-phenoxybenzyl-3-(2,2-dichlorovineyl)-2,2-dimethyl-cyclopropanecarboxylate] is a type I synthetic pyrethroid in that it is without an alpha-cyano groups. It has four stereoisomers (two enantiomeric pairs), molecules made up of the same atoms with different three-dimensional structures (Cox, 1998) (Figure 5). 1R, cis-permethrin is the most insecticidally active isomer (WHO, 2005a). Permethrin is a broad spectrum insecticide, used in agriculture, public health and by homeowners for control of insect pests. It is extremely toxic to fish, and also highly toxic to cats. Permethrin acts predominantly on the central nervous system, and kills insects by strongly exciting their nervous systems. It affects the neuron membrane by prolonging sodium channel activation, causing continuous blocks the movement of sodium ions from outside to inside of the nerve cell (Vijverberg and Bercken, 1990; IPCS, 2000).

Permethrin is a lipophilic substance lacking fumigant action. The pure isomers are colorless crystals at ambient temperatures changing to a clear, pale yellow. The molecular mass is 391.30. Permethrin is a moderately to practically non-toxic pesticide I EPA toxicity class II or III, depending on the formulation (EPA, 2000). It is very effective as a direct contact poison or as a residual substance (IPCS, 2000).

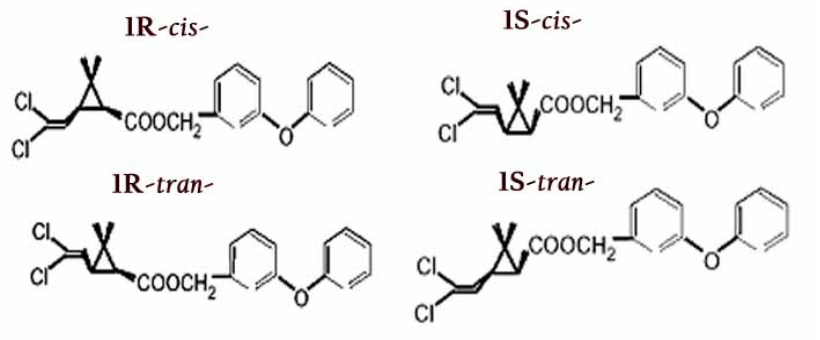

Figure 5 Permethrin chemical structure.

Source: Cox (1998).

4. Dengue vector control

Dengue viruses are transmitted to humans through the bites of infective female *Aedes* mosquitoes. Primary and secondary vectors include: *Ae. aegypti* and *Ae. albopictus*, respectively. After virus incubation for 8 to 10 days, an infected mosquito is capable, during probing and blood feeding, of transmitting the virus for the rest of its life (WHO, 1999, 2008). Despite developmental progress, an effective vaccine is not available to prevent dengue viruses. As such, present dengue control methods focus on reducing human-vector contact (Chareonviriyaphap *et al*., 1997; Roberts *et al*., 1997a; Somboon *et al.,* 2003; Jirakanjanakit *et al.*, 2007). This includes measures to reduce *Ae. aegypti* breeding sites and adult biting in and outside households. The decentralized dengue control program in Thailand is based on the National Dengue Prevention and Control Plan developed from the Dengue Haemorrhagic Fever Epidemic Control Scheme of the National Public Health Development Plan 6(1987-1991). The control program emphasizes community participation in larval control through health education in conjunction with space spraying with pyrethroid insecticides to control adults (Phuanukoonnon *et al.,* 2005). However, the activities are under the government responsibility, especially insecticide space spraying for reducing mosquito abundance during disease outbreaks (MOPH, 2007a).

To date, biological, physiological and chemical control have coexist methods in the country, the primary method which used upon the situations of disease in each area (WHO, 1999; MOPH, 2007a). Although, the chemical control is a principal methods that used in dengue vector control program in Thailand. Several synthetic insecticides, including organochlorines, organophosphates, carbamates and synthetic pyrethroids, have been used in dengue control programs in Thailand (Christophers, 1960; Ziam and Guillet, 2002). The organochlorine, DDT was widely used to control *Ae. aegypti* after the first dengue epidemic in 1958, with a successful (Jatanasen, 1966; Ponlawat *et al*., 2005). In 1970, the program switch to using organophosphate insecticides, fenitrothion and malathion, followed by the synthetic pyrethroids in the late 1980's (Paeporn *et al*., 2005). However, DDT was used during this time for emergency control malaria but removed from use in the public health control program

in Thailand since 2003 (MOPH, 2003; Paeporn *et al*., 2005). Since 1992, synthetic pyrethroids deltamethrin, cypermethrin and permethrin have been the primary insecticides used in control of disease vectors, in particular during endemic seasons (MOPH, 2003, 2007a). Currently, deltamethrin is one of the most commonly used insecticides in public health programs and has been the mainstay ULV and thermal fogging applications for the emergency control of *Ae. aegypti* adults in Thailand since 1994 (Chareonviriyaphap *et al.,* 1999; MOPH, 2003; Kongmee *et al*., 2004). Permethrin, another synthetic pyrethroid insecticide has been used in many areas of Thailand for routine space spraying during dengue outbreak (Paeporn *et al*., 2005). Temephos an organophosphate is commonly used in water containers for the control of *Ae. aegypti* larvae. Additionally, ultra-low-volume (ULV) applications of fenitrothion and malathion are also used during the peak period of adult *Aedes* populations, especially during the rainy season (Paeporn *et al.,* 1996, 2005; Chareonviriyaphap *et al.,* 1999; MOPH, 2003).

A part from government activities, preventing mosquito contact through the use of insecticides by homeowners is common in urban households. Presently, household insecticide products are a popular mode of personal protection against mosquitoes in several areas in Thailand. There are several formulations of household products available for homeowners use including aerosols, mosquito coils, vaporizing mats, liquid vaporizing and bats (Paeporn *et al.,* 2004). Synthetic pyrethroids such a permethrin, d-tetramethrin, d-allethrin, s-bioallethrin, cypermethrin and deltamethrin are commonly used in these household insecticide products, (WHO, 2006a).

5. Behavioral responses of mosquitoes to insecticides

There are two principal types of responses of mosquitoes to insecticides, one is physiological and the other is behavioral (avoidance). Physiological and behavioral responses are represents an interrelated spectrum of biological responses. For example, a physiological response such as altered nerve sensitivity is the sum total of a series of biochemical events potentially involving changes in nerve structure. Likewise, a behavioral response is the sum total of the series of physiological and

biological changes (Sparks *et al*., 1989). Both physiological and behavioral effects of insecticides are important in vector-borne disease transmission because they interrupt human-vector contact. The use of chemical barriers have historically been used to exploit these behavioral responses for the purpose of inhibiting mosquitoes from preferentially feeding on humans, ingesting infectious blood meals, or transmitting pathogens to susceptible hosts (Elliott, 1972). There are two different types of behavioral avoidance responses by mosquitoes that are generally recognized: irritancy and repellency (Davidson, 1953; Roberts and Andre, 1994; Roberts *et al*., 1997b; Rutledge *et al*., 1999). Contact irritancy occurs when insects actually make physical contact with chemical residues on a treated surface before eliciting a stimulus-mediated response, whereas repellency is elicited by stimulus acting from a distance, without the mosquito making physical contact to an insecticide-treated surface. Both behavioral responses result in the insect moving away from treated surfaces (i.e. escape or deterred entry) or otherwise disrupting normal patterns of behavior (Mattingly, 1962; Georghiou, 1972; Lockwood *et al*., 1984; Roberts *et al*., 1997b, 2000b; Chareonviriyaphap *et al*., 1997, 2004; Potikasikorn *et al*., 2005). Another definition for repellency is associated with the area from which the biting insect is repelled within a set distance of the source of repellent molecules; spatial repellency (Nolen *et al*., 2002; Kline *et al*., 2003). Spatial repellents have been defined as inhibiting compounds, dispensed into the atmosphere of a three dimensional space which inhibits the ability of mosquitoes to locate and track a target such as human or livestock (Nolen *et al*., 2002).

The natural reaction of mosquitoes to avoid insecticide-treated surfaces is a general phenomenon (Spark *et al*., 1989; Klowden, 1996), however, understanding the behavioral responses of vectors to treated surface is important to the success of vector control programs. This is because innovative control methodologies will rely on understanding how various vector control chemicals function outside of toxicant effects. There have been numerous attempts to accurately measure the behavioral responses of mosquitoes to insecticides, through both laboratory and field experimentation. Chareonviriyaphap *et al*. (2001) studied behavioral responses of *Anopheles minimus* to DDT 2g/m^2, deltamethrin 0.0625 g/m^2 and lambdacyhalothrin

0.0369 g/m^2 and found that *An. minimus* rapidly escaped from direct contact with DDT, deltamethrin and lambdacyhalothrin. Among these 3 compounds, lambdacyhalothrin exhibited the strongest irritant effect on female mosquitoes. The effect of these insecticides, however, depends on the physiological condition at test populations (Sungvornyothin *et al.,* 2001). Likewise, in studied with *Ae. aegypti*, the degree of response to deltamethrin (0.02 g/m^2) and DDT (2 g/m^2) varied according to physiological conditioning (Polsomboon *et al*., 2008). In 2004, Kongmee *et al.* tested *Ae. aegypti* to 0.02 g/m^2 of deltamethrin, contact irritancy response is a major behavioral of *Ae. aegypti*. Additionally, contact irritancy and non-contact repellency responses in *Anopheles sawawongporni* was observed in DDT treated, whereas only contact irritancy response found in permethrin treated (Muenworn *et al.,* 2006).

Large amounts of data have been gathered on behavioral responses of *Anopheles* and *Aedes* species to vector control compounds by using varying assays (Kennedy 1947; Brown, 1964; Lal *et al.,* 1965; Moore, 1977; Kongmee *et al*., 2004; Muenworn *et al.,* 2006; Polsomboon *et al.,* 2008). This includes a developed by Chareonviriyaphap *et al*. (1997), an excito-repellency test system and a high throughput screening system (Grieco *et al*., 2005, 2007).

6. Resistance of mosquitoes to insecticides

The development of insecticide resistance by arthropod vectors is a primary concern for the management of human disease control. A few published papers on insecticide resistance in *Ae. aegypti* population have been reported in Thailand (Somboon *et al*., 2003; Jirakanjanakit *et al*., 2007; Ponlawat *et al*., 2005; Sathatriphop *et al*., 2006) however, these studies failed to investigate the susceptibility status of varying geographic strain on a large scale. A clear understanding of the dynamics between insecticides used and the susceptibility level of the mosquito population is required for the development of successful vector control activities. A better understanding of the insecticide susceptibility level will throughout *Ae. aegypti* strains will allow for greater efficiency in program design for targeting mosquito vectors in specific geographic areas. Development of insecticide resistance in mosquito vectors

is common (Hemingway and Ranson, 2000). It is a complex and dynamic process and depends upon many factors (Insecticide Resistance Action Committee [IRAC], 2008). Resistance is defined as the developed ability in a strain of insects to tolerate doses of toxic chemicals which would prove lethal to the majority of individuals in a normal population of the same species (WHO, 1957). Insecticide resistance can be classified into three modes; biochemical, physiological and behavioral. Although a convenient way of viewing resistance, in reality these three modes represent an interrelated spectrum of biological responses (Georghiou, 1986).

Resistant populations of *Ae. aegypti* have been detected in several areas in Thailand. The bulk of the data available are from WHO susceptibility assay using insecticide impregnated paper. Neely (1964) reported *Ae. aegypti* in Bangkok and Nakhon Ratchasrima resistant to DDT since the early 1960's. Currently, DDT resistance is reported throughout the northern (Somboon *et al.,* 2003), and many regions of Thailand have reported resistance of *Ae. aegypti* to temephos, malathion fenitrothion, permethrin and deltamethrin (Chareonviriyaphap *et al*., 1999; Somboon *et al*., 2003; Ponlawat *et al.*, 2005; Sathantriphop *et al*., 2006; Jirakanjanakit *et al.,* 2007). The resistant phenotype, an insect that survives a dose of insecticide that would normally have killed, it is relatively easy to monitor with direct insecticide bioassays. However, in many cases the actual biochemical or molecular mechanisms responsible for the resistant phenotype are still unknown.

There are two main mechanisms of insecticide resistance in mosquitoes: metabolic and target-site resistance. Metabolic resistance is the most common resistance mechanism that occurs in insects. This mechanism is based on detoxification enzymes which degrade the active ingredient of the insecticide. There are three categories of enzymes that are primarily implicated in effecting increased levels of insecticide degradation: esterases, cytochrome P450 monooxygenases and glutathione-S-transferases, which catalys a wide range of detoxification reactions (IRAC, 2008). The second most common resistance mechanism encountered in insects is target-site resistance. Insecticides generally act at a specific site within the insect, typically within the nervous system. The site of action can be modified in

resistant insects such that the insecticide no longer binds effectively at that site, or reduces the binding of the insecticide (Hemingway *et al.,* 2004; IRAC, 2008). There are three target-site mechanisms that have been described: knockdown resistance (*kdr*), a mutation in the voltage-gated sodium channel (pyrethroid and DDT resistance), modified acetylchlolinesterase, (organophosphate and carbamate resistance) and modified GABA- gated chlorine channel, (chlorinated hydrocarbons resistance other than DDT resistance) (Nauen, 2007).

Resistance mechanisms in *Ae. aegypti* have been extensively studied in Thailand. The study of Pethuan *et al*. (2007) found mixed-function oxidases (MFO) was responsible for pyrethroid resistance in Chonburi, Nakhon Sawan, Nakhon Ratchasrima and Chantaburi Provinces, in the contrast, non-specific esterase was responsible for fenitrothion resistance in Nakhon Sawan Province. In addition, *Ae. aegypti* from Bangkok and Pathum Thani the central Thailand showed elevation of MFO enzyme activity, leading to resistance to deltamethrin and cross-resistance to DDT (Yaicharoen *et al*., 2005). While both of the non-specific esterases, alpha and beta esterase, and insensitive acetylchlolinesterase (AChE) are documented to play role in fenitrothion (organophosphate) resistance in *Ae. aegypti* population in Nakhon Ratchasrima (Pethuan *et al*., 2007). Previous studies reported DDT resistance in *Ae. aegypti* populations form the Chiang Mai was due to increased DDTase activity and cytochrome P450 (Prapanthadara *et al*., 1995). Other studies have reported that various *Ae. aegypti* populations in Thailand have developed resistance to pyrethroids, such a Brenges *et al*. (2003), reported in the low level resistance to permethrin of *Ae. aegypti* from Mae Kud and Mae Kasa districts, Chiang Mai Province that found reducing monooxgenase level compare to the standard strain.

MATERIALS AND METHODS

1. Mosquito collection sites

Aedes aegypti larvae and pupae were collected from the natural breeding habitats (containers) located in and outside the houses in six different provinces of Thailand: Chiang Mai, Kanchanaburi, Khonkaen, Nonthaburi, Songkhla and Satun (Figure 6). The mosquito collection was done twice in each site. All larvae and pupae brought back to the insectary at the Kasetsart University. GPS coordinates and brief descriptions of locations are provided herein (Table 1).

1.1 Chaing Mai strain. This strain was collected from Ban Pang Mai Deang Village in Mae Teang District, Chiang Mai Province, northern Thailand (elevation 600 m). Ban Pang Mai Deang Village is located in a mountainous area, surrounded by dry forest and cultivated vegetable fields. A stream runs through the village during the dry season, increasing dramatically in water volume during the wet season.

1.2 Kanchanaburi strain. This strain was collected from the Pu Teuy Village in Sai Yok district, Kanchanaburi Province, western Thailand (elevation 292 m). The village consists of mixed residential, fruit orchards, vegetable plantation and/or forest. A small stream runs through the village throughout the year.

1.3 Khonkaen strain. This strain was collected from Ban Non Ton Village in Muang district, Khonkaen Province, northeastern Thailand (elevation 48 m). The village is located in a semi-urban area, piped water supply. However, residents practical storing water in various type of containers in and outside of houses.

1.4 Nonthaburi strain. This strain was collected from Tar Sai sub district of Muang district, Nonthaburi Province, central Thailand (elevation 1 m). This collection site is classified as an urban area, belong to the Irrigation Department. There is a piped water supply available in the community, though, there are many

water-storage still in use in and outside houses, some of which are permanently opened.

1.5 Songkhla strain. This strain was collected from Bon Wua Village in Bor Yang subdistrict of Muang district, Songkhla Province, southern Thailand (elevation 7m). The collection site is a community of fishermen residential. This location site is classified as an urban area. It is a slum community. The availability of a piped water supply is limited. Several water-storage containers are within the surrounding area, in particular in the front of the houses. Almost of the water-storage container are reused plastic boxes of gasoline. The containers are used for storage of rain water and are often left open.

1.6 Satun strain. This strain was collected from Pimarn subdistrict of Muang district, Satun Province, southern Thailand (elevation 8m). The location site is an urban area within a mixed use of residential and fruit orchards. Water-storage containers exist some of the around the houses.

1.7 USDA strain. This strain was received an egg from, Gainesville, Florida, USA. This strain as referred to the USDA strain (United State Department of Agriculture). This is a Laboratory colony was served as reference strain in the susceptibility test.

1.8 Bora Bora strain. This strain was originated in French Polynesia Island. This strain was maintained at the Laboratoire de Lutte contre les Insectes Nuisibles (LIN), Institut de Resecherche pour le Developpment (IRD), Montpeelier, France. This is a Laboratory colony was served as reference strain in the resistance mechanisms assay.

2. Mosquito rearing

Mosquito larvae and pupae that were collected form each of the six sites (Figure 7 A-C), were reared to the adult stage in the insectary at the Department of

Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. The insectary was maintained at 25 ± 5 ^{o}C and 80 ± 10% relative humidity with a photoperiod of 12:12 light : dark (L:D). Adults were identified as *Aedes aegypti* (Figure 7D) and provided with cotton pads soaked with 10% sucrose solution from the day of emergence and were maintained in separate 30 cm x 30 cm x 30 cm screen cages. These used for colony purposes. The female mosquitoes were permitted to feed on guinea-pig blood on the fourth day after emergence. Two days after blood-feeding, oviposition dishes (10-cm diameter) containing moist filter paper were placed in the cages with the gravid females for egg deposition. Eggs in oviposition dishes were dried at room temperature for 24 – 48 h. Egg papers were then floated on the water of individually labeled hatching trays. The first and second day after hatching approximately 250 larvae of each strain were transferred to individual plastic trays (20 cm x 30 cm x 5 cm) containing 1,500 ml of tap water and 5 – 6 of fish granules (0.5 cm diameter). Pupae were transferred daily from larval trays to emergence cups using a hand-pipet and placed directly into separate screened 30 cm^3 cages. These were used for insecticide susceptibility and behavioral assays. Adult were provided a 10% sugar meal until 24 h prior to use in assays. Only F1 and F2 generations of each test strains were used in assay trials.

Table 1 Village Names and GPS coordinates of field sites where *Aedes aegypti* larvae and pupae collections were made in 2006–2007.

Province	Village	District	GPS coordinates
Chiang Mai	Pang Mai Deang	Mae Teang	19 ^{o}14′ N 98 ^{o} 82′ E
Kanchanaburi	Pu Teuy	Sai Yok	14^{o} 20′ N 98 ^{o} 59′ E
Khonkaen	Non Ton	Muang	16^{o} 25’ N 102 ^{o} 50′ E
Nonthaburi	Tha Sai	Muang	13^{o} 53′ N 100 ^{o} 29′ E
Songkhla	Bon Wua	Muang	7^{o} 11′ N 100 ^{o} 35′ E
Satun	Pi-marn	Muang	6^{o} 37′ N 100 ^{o} 03′ E

Figure 6 Map of the six localities of *Aedes aegypti* mosquitoes collection sites in Thailand.

Figure 7 *Aedes aegypti* mosquito collection procedure; (A) mosquito larvae and pupae were removed from natural breeding sites, (B) *Aedes* species removed by hand-pipet, (C) transferred to Kasetsart University insectary (D) reared to adult. Emerged adults were identified (D) and place into 30 cm^3 screened-cages.

3. Insecticide test compounds

Three synthetic pyrethroids: alphacypermethrin, deltamethrin and permethrin, one organochlorine: DDT, and one organophosphate: malathion, were used in this study.

3.1 Alphacypermethrin [(a race-mate comprising (S) alpha-cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2 dichlorovinyl) 2, 2-dimethyl cyclopropane carboxylate), purity 95%] was provide from BASF. The Chemical Company. USA.

3.2 Deltamethrin [(cyano-(3-phenoxyphenyl)-methyl] 3-(2, 2-dibromoethenyl)-2, 2-dimethyl-cyclopropane-1-carboxylate), purity 99%] was purchased from BASF, USA.

3.3 Permethrin [(3 phenoxybenzyl (1 RS, 3 RS, 1RS, 3 SR)-3-(2, 2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate), purity 92%] was provided by Ladda Company, Thailand.

3.4 DDT [(1,1 Bis(4-chlorophenyl)-2,2,2-trichloroethane), purity 98%], was purchase from a Sigma-Aldrich, CAS 50-29-3, Product #: 386340.

3.5 Malathion [(diethyl (dimethoxy thiophosphorylthio) succinate), purity 95%] was provided by Ladda Company, Thailand.

Part 1. Susceptibility test of female *Aedes aegypti* strains to insecticide compounds

1. Insecticide impregnated papers

Individual test paper (Whatman 12 x 15 cm, filter paper) was prepared with technical grade active ingredient samples following World Health Organization (WHO) protocol (WHO, 2006b). Individual papers were treated with 2-ml of diagnostic doses for each chemical as suggested by WHO for *Ae. aegypti* (Figure 8A). This includes 0.05 % for alphacypermethrin (WHO, 1998a), 0.05 % for deltamethrin,

0.25% for permethrin, 4.0 % for DDT and 0.8 % for malathion (WHO, 1998b). Other pieces of filter-paper were impregnated with 2- ml of acetone mixed with carrier (silicon oil) to serve as untreated control. The papers were left to air dry for 24 h, and then inserted into corresponding treatment or control WHO standard filter-paper test tubes.

2. World Health Organization filter-paper test

Aedes aegypti of USDA susceptible strain and each field strain were exposed to a single 'diagnostic' dosage on insecticide-treated test papers as recommended by WHO following standard testing procedures and exposure times (WHO, 1998b, 2006b) (Figure 8). Batches of 25 starved 3- 4 day-old female mosquitoes were introduced into the holding tube and maintained for 1 h. Subsequently, test and control mosquitoes were transferred into the exposure tube lined treated papers. The exposure tubes were placed vertically for 30 min for DDT and 60 min for alphacypermethrin, deltamethrin, permethrin and malathion assay (Figure 8B). The number of knocked down mosquitoes at the bottom of the tubes (except the malathion assay) was recorded every 5 min after the start of insecticide exposure until the end exposure time. After exposure, test and control were carefully transferred to separate clean holding tube and provided cotton pads socked with 10% of sucrose solution (Figure 8C, D). Morality was recorded after 24 h post exposure. Each chemical was replicated four times per mosquito strain. A simultaneous control was conducted for each test tube.

3. Data analysis

Interpretation of results of the susceptibility test were determined according to WHO criteria; a strain was considered susceptible if 24 h mortality rates were 98 – 100%, resistant if 24 h mortality was less than 80%, and possibility of resistance if mortality was 80 – 97% (WHO, 1998b). If mortality exceeded 20% in the control strain, the replicate was repeated. If control mortality was 5 – 20% Abbott's formula was used to correct for mortality in the treatment strains (Abbot,

1925). The time after which 50% and 95% of each test strain was knockdown (KT_{50} and $KT_{95,}$ respectively) and 95% confidence time was calculated using log probit analysis (SAS, 2002). Analysis of variance (ANOVA) was used to compare 24 h mortality rates and 1 h knockdown effect among test strains (SAS, 2002).

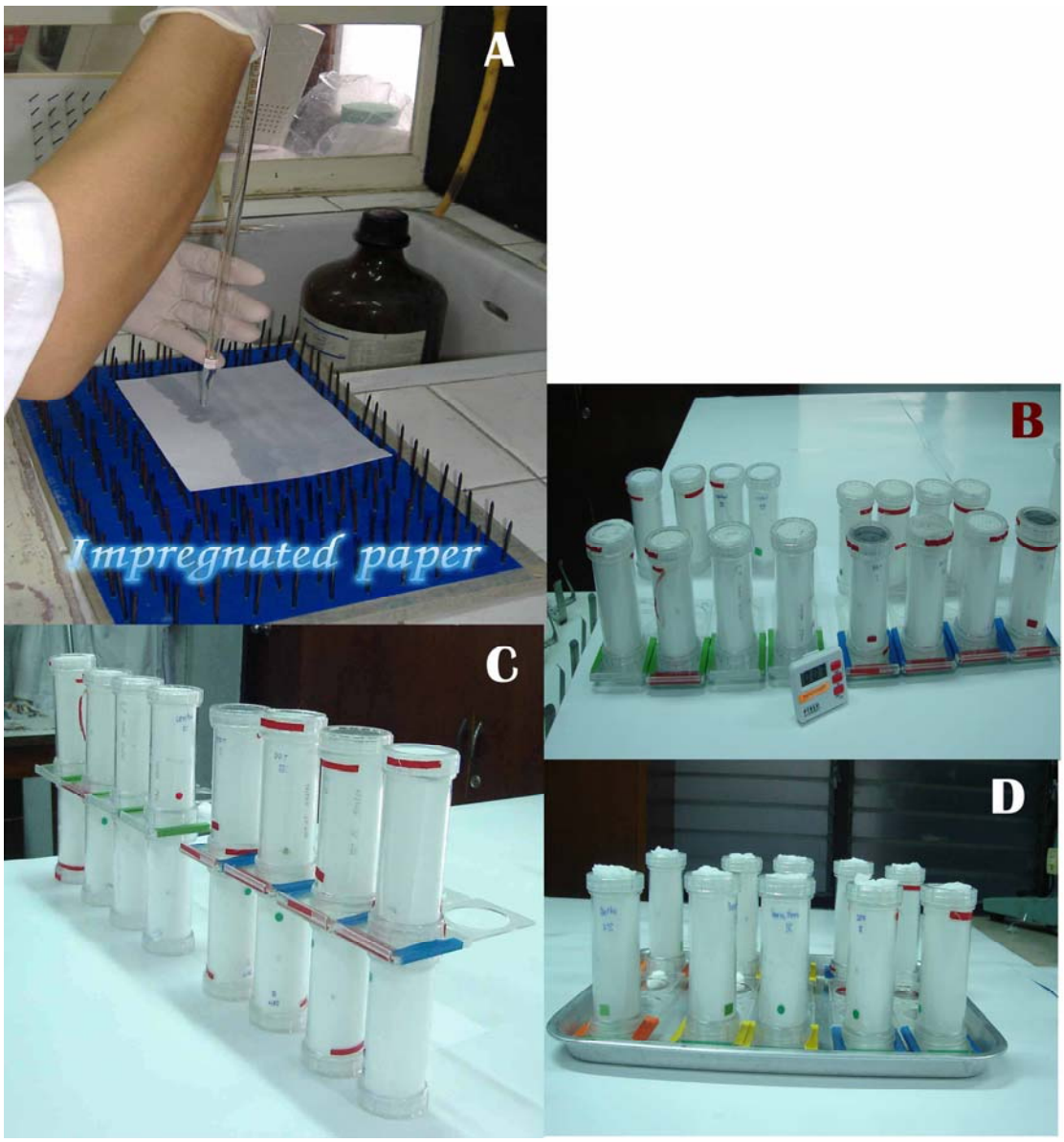

Figure 8 World Health Organization susceptibility assay using insecticide impregnated paper technique. (A) treatment of papers, (B) susceptibility test with 1h exposure to treated papers, (C) mosquitoes transferred into holding tubes, (D) sugar pads are placed on top of tubes and 24 h post-exposure mortality observed (WHO, 1998b, 2006b).

Part 2. Insecticide resistance mechanisms of *Aedes aegypti* strains

1. Biochemical assay preparation

Forty mosquitoes were used from Bora Bora the susceptible strain and six field strains. New emerged mosquitoes each strain were kept at -80 ^{o}C prior to subjection to biochemical analysis. Biochemical assays was used to detect monooxygenase and non-specific esterase involved in insecticide resistance as described by Hemingway *et al.* (1998) with slight modification.

2. Mosquito homogenates

One day before testing, mosquito homogenizing were prepared and kept at -40^{o}C. Individual mosquito each strain was homogenized in 200 µl of distilled water on ice (Figure 9) and spin at 14,000 rpm for 2 min. Then, 20 µl in duplicate was passed to microplate for monooxygenase assay. Another 4 plates were prepared with 10 µl in duplicates for proteins and general esterase assay (Figure 10A).

3. Protein assay

The total protein content of individual *Ae. aegypti* mosquitoes were determined using a BioRad protein assay system (Hercules, California). Microplate of the mosquito homogenate was transferred for assay. A volume of 290 µl of Coomassie Plus Protein Assay Reagent (CPPAR) in distilled water (dH_2O) at a ratio of 1:1 (15 ml CPPAR plus with 15 ml dH_2O) was then added to each well of a microplate. The microplate was incubated at 25 ^{o}C for 5 min and then read at 590 nm end point. The estimated protein in 10 µl of each mosquito was automatically made by Biolynx software.

4. Monooxygenase assay

The assay for monooxygenase activity was performed according to Hemingway *et al.* (1998) with slight modification. The microplate was placed on ice, 80 µl of a 0.0625 M Potassium Phosphate ($KHPO_4$) was added with buffer at pH 7.2 in each well. A volume of 0.01 g of 3, 3, 5', 5'-Tetramethly Benzidine (TMBZ) in 5 ml of methanol was prepared and a 0.25 M Sodium Acetone ($NaCzH_3Oz$). Buffer (pH 5.0) was added. Then, a 200 µl volume of this TMBZ solution was added into each well followed by 25 µl of 3% hydrogen peroxide. The microplate was then covered incubated for 30 min at room temperature. Monooxygenase levels determined using density values recorded at 630 nm wave lengths. Enzyme levels were determined from cytochrome c standard curve by using Biolynx software.

5. Non-specific esterase assay

The reaction was undertaken in Phosphate saline Buffer (PBS) (pH 6.5) containing 90 µl of 1% Triton following the method of Hemingway *et al*. (1998). A volume of 500 µl of 0.3M alpha-naphthyl acetate (or beta-naphthyl acetate) in 2.5 ml 1% triton PBS (pH 6.5) in 7ml distilled water was prepared. A 100 µl volume of this solution was added into each well. The microplate was incubated for 30 min at 25 ^{o}C. After 30 min the reaction was stopped by adding 100 µl of Fast Garnett solution (0.008 g of fast Garnett salt (PGBC) in 10 ml distilled water) (Figure 10B). The microplate was read immediately after 10 min at 550 nm wavelength (Figure 10C). Absorbance values converted to nm naphthol produced/min/mg protein by using naphthol standard curves and automatically made by using Biolnx software.

6. Data Analysis

Least Significant Differences (LSD) was used to compare the protein content and enzyme expression levels of the susceptible strain to each field strain each. All levels of statistical significance was determined at $P<0.05$ (SAS, 2002)

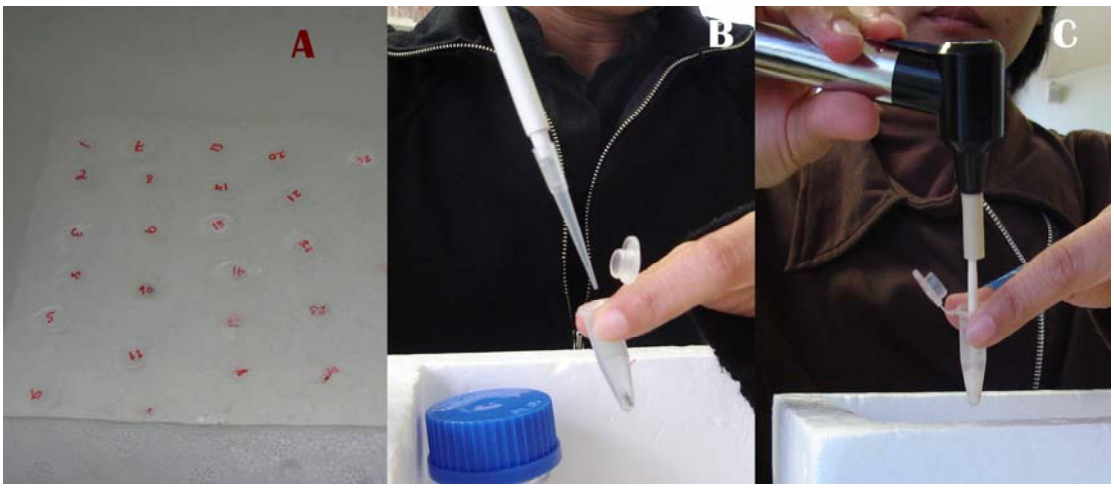

Figure 9 Mosquito homogenization preparation, (A) mosquito micro tube were place on ice, (B) distilled water was put into the micro tube, (C) mosquito was homogenized individually.

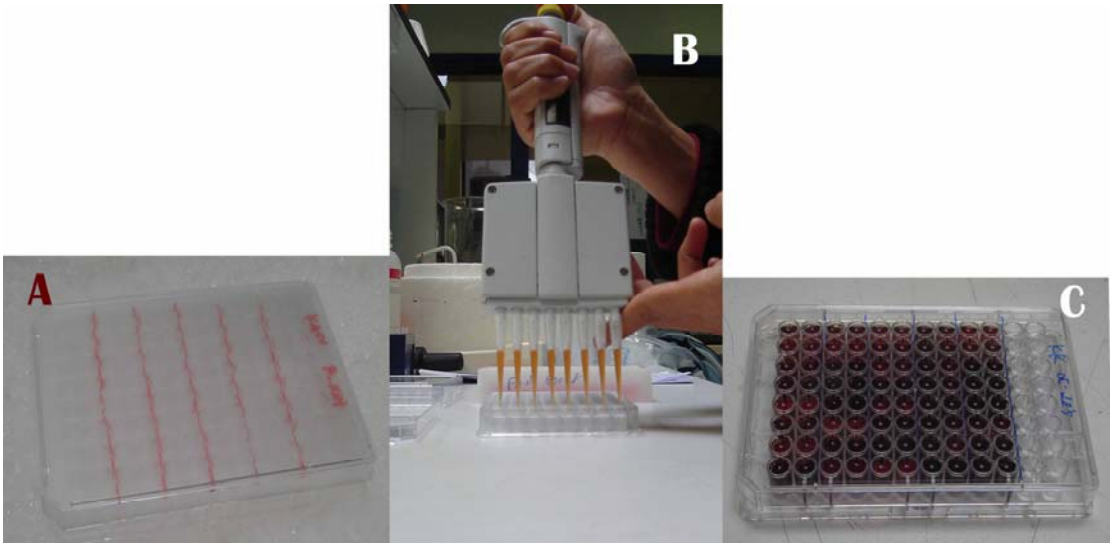

Figure 10 Biochemical assay, (A) microtiter plate preparation, (B) assay specific solutions and homogenate was placed into wells of the microtiter plate, (C) reaction product used to measure enzyme levels.

Part 3. Contact irritancy and non-contact repellency behavioral responses of female *Aedes aegypti* strains to insecticide compounds using an excito-repellency system

1. Insecticide-impregnated nets

The field application rate of alphacypermethrin, deltamethrin, permethrin and DDT were used in this investigation. Nettings were impregnated with alphacypermethrin at 6.0 nm/cm^2, deltamethrin at 4.9 nm/cm^2, permethrin at 127.8 nm/cm^2 and DDT at 564.2 nm/cm^2 using acetone diluents. Each treatment net (252 cm^2) was soaked with treatment solutions (1.0 ml) within individual glass Petri dishes (9 cm diameter) (Figure 11 A, B) then smaller glass Petri dish was placed over through the dry period (Figure 11C, D). Additional nets were treated with acetone to serve as untreated controls. All nets were allowed to air-dry for at least 20 min before use in an assay (Figure 11E).

2. Mosquitoes preparation

Testing females were sorted into groups of 15's for contact and non-contact assay. Mosquitoes were aspirated form cages and sorted into groups of 15's within individual plastic cups accordingly. The plastic cups were then placed into individual trays labeled by strain, age and the assay. On the day of assay testing, the plastic cups were transferred from the insectary to the testing.

3. Excito-repellency test

An excito-repellency test system was used to evaluate the behavioral responses of *Ae. aegypti* to field application rates of alphacypermethrin, deltamethrin, permethrin and DDT. The chamber comprises, an exit slot (1), a front door (2), an outer chamber (3), a screened inner chamber (4), a Plexiglass holding frame (5), an inner Plexiglass panel with a rubber latex-sealed door (6) and a rear door cover (7) (Figure 12). The modified test chamber remains similar to the previous version

Chareonviriyaphap *et al*. (2002) with the only modification being a reduction in size (Tanasinchayakul *et al*., 2006) thereby reducing the amount of chemical required.

To assemble chamber, the 4 side walls of the outer chamber are put together by connecting the aluminum tongue and groove elements. A screened inner chamber is prepared in the same manner. A Plexiglass holding frame and panel are attached to one end and an exit portal slot is attached to the opposite end of the box. A cover can be placed the Plexiglass holding frame if desired.

Each test series consisted of 2 insecticide test chambers and 2 paired control boxes (Figure 13). Fifteen 4 – 5 day-old of starved mosquitoes were introduced into each test chamber, after which the outer rear door were closed and secured. A receiving cage (20 cm × 27 cm × 24 cm paper box) was connected to the exit portal for collecting escaping mosquitoes (Figure 13). Mosquitoes were allowed a 3 min resting period to permit adjustment to test chamber conditions, after which the escape funnel was opened to begin the observation period. Mosquitoes escaping from the chamber into the receiving cage were recorded at 1 min intervals for a period of 30 min. The tests were performed between 0800 to1600. All trials were replicated 4 times for each particular test combination. After the 30 min observation time, the number of dead mosquitoes, the number remaining inside the chamber and those that escaped to the receiving cage was recorded for each treatment and control chamber. Additionally, all mosquitoes alive that escaped and remained inside the chamber, for both control and treatment chambers were maintained in individual holding plastic cups and provided 10% sugar solution. Plastic cups were maintained in the insectary at 27^{o}C and 80% RH for 24 h to monitor mortality rates.

4. Data analysis

A Kaplan-Meier survival analysis method was used to analyze and interpret the behavioral response data (Kleinbaum, 1995; Roberts *et al*., 1997b). Survival analysis was used to estimate the probability of escape time (ET) and compare differences in mosquito response among the test strains and four insecticides

(Kleinbaum, 1995). For analysis, mosquitoes that escaped were treated as death and those remaining in the exposure chambers were considered survivors (Chareonviriyaphap *et al*., 1997). The $ET_{25,}$ ET_{50} and ET_{70}, time in minutes for 25%, 50% and 70% of the mosquitoes to escape, respectively, were estimated from data collected at one minute intervals. Comparison escape response patterns between different treatments were determined using the log-rank method (Mantel and Haenzel, 1959). Statistical significance for all tests was set at $P<0.05$ (SAS, 2002).

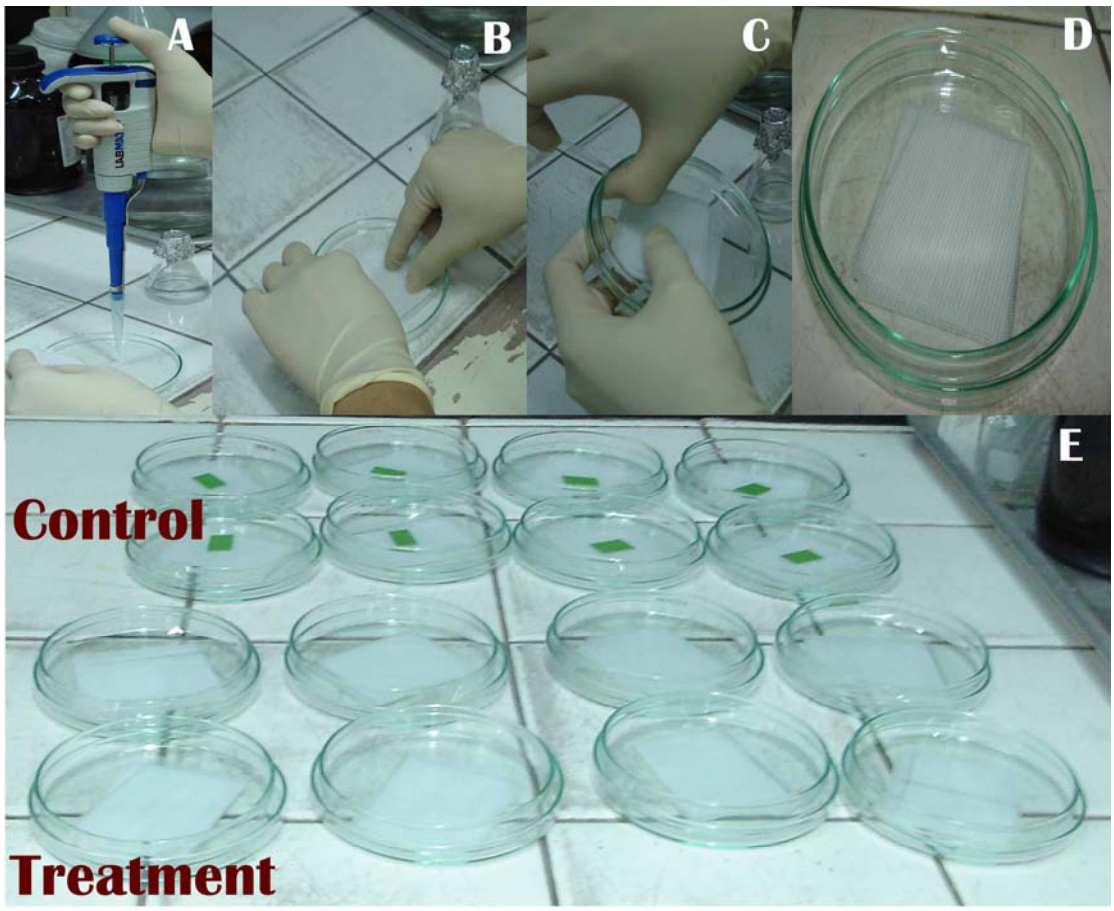

Figure 11 Insecticide impregnated nets used in the excito-repellency assay, (A) chemical solution was put into Petri dish, (B) impregnation net was done within the Petri dish, (C) a smaller of Petri dish was place over the impregnated net, (D) the impregnated net was prepared individually, (E) nets remained in solution for 20 min.

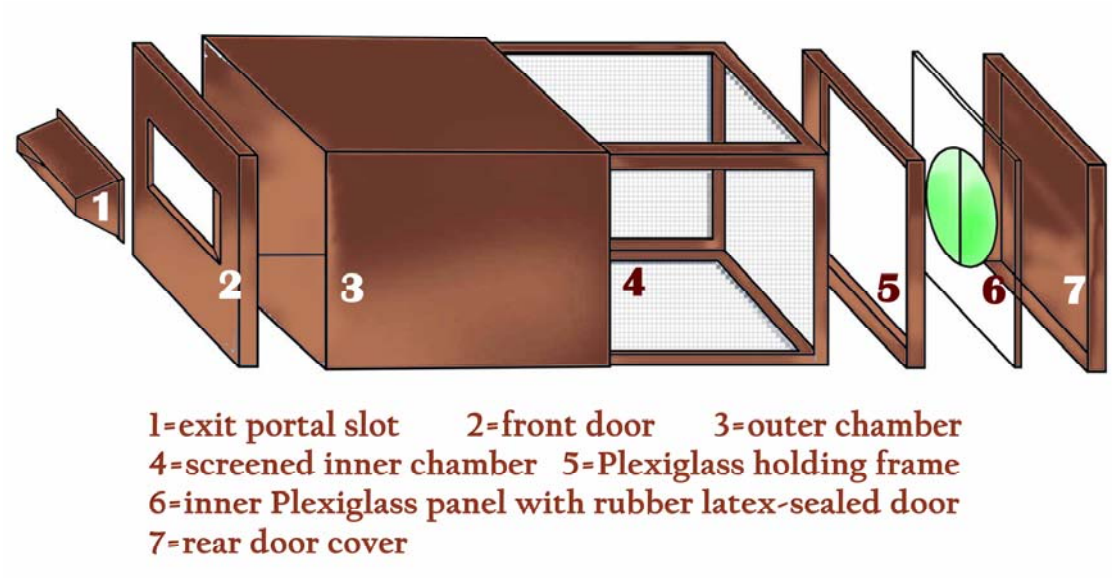

Figure 12 Schematic drawing of the excito-repellency test chamber.

Figure 13 Excito-repellency assay showing one test series that consisted of (A) contact, (B) noncontact trial.

Part 4. Behavioral responses of female *Aedes aegypti* strains to insecticide compounds using a High Throughput Screening System assay (HTSS)

1. Insecticide-treated netting strips

Nylon-organdy netting was cut into strips (11 cm x 25 cm) and treated either with chemical (treatment) or solvent only (acetone). Three concentrations of alphacypermethrin, deltamethrin, permethrin and DDT were used in this investigation based on field application rates. Netting strips were treated with alphacypermethrin at 0.06, 0.6 and 6.0 nm/cm^2, deltamethrin at 0.049, 0.49 and 4.9 nm/cm^2, permethrin at 1.27, 12.7 and 127.8 nm/cm^2 and DDT at 5.6, 56.4 and 564.2 nm/cm^2. All netting strips were treated using a micropipette at the rate of 1.5 ml of solution per 275 cm^2 and allowed to air dry for at least 15 minutes prior to use in the assay (Figure 14). Once dry nets were placed inside corresponding treatment and control cylinders, and remained there during the entire test day. New treatment and control nets were prepared at the beginning of each test day.

2. Mosquitoes preparation

Testing females were sorted into groups of 10's for Contact Irritancy Assay (CIA) and 20's for Spatial Repellency Assay (SRA) and Toxicity Assay (TOX). Mosquitoes were aspirated form cages and sorted into groups of 10's or 20's within individual plastic cups accordingly. The plastic cups were then placed into individual trays labeled by strain, age and the number of 10 or 20 grouping. On the day of assay testing, the plastic cups were transferred from the insectary to the testing and specimens aspirated from each plastic cup into individual miniaturized "holding tubes" (i.e., modified disposable 25 ml pipets) with a cork placed on the open end. Holding tubes were then placed into corresponding organizing trays to separate control and treatment CIA, SRA and TOX.

3. High throughput screening system assay

The HTSS has a modular design that allows for the examination of three behavioral responses, contact irritancy and spatial repellency, as well as toxicity using the same component (Grieco *et al*., 2005, 2007). The modular configuration will vary depending on the type of assay to be performed. The order of behavioral assays performed were the contact irritancy assay, spatial repellency assay and the toxicity assay (Figure 15). All trials were conducted under controlled laboratory conditions of temperature 25 ± 2 ^{o} C and relative humidity 70 – 80 %.

Figure 14 Insecticide impregnation of netting strips for the high-throughput screening system assay. (A) chemical application of 1.5 ml solution using a micropipette, (B) netting strips are allow to dry for 15 min before use in assay.

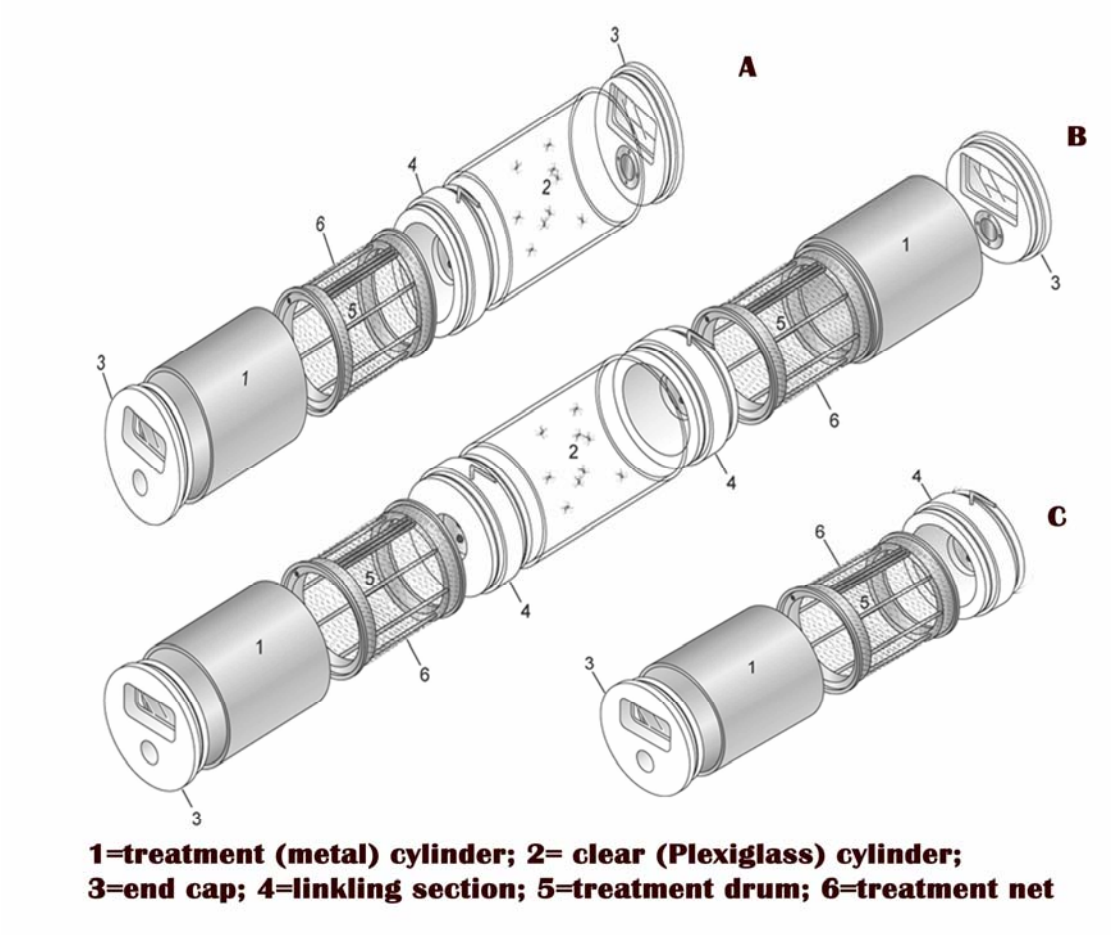

Figure 15 Schematic drawing of the high throughput screening system showing (A) the contact irritancy assay, (B) spatial repellency assay, (C) toxicity assay (Grieco *et al*., 2005, 2007).

3.1 Contact Irritancy Assay (CIA)

The components for the CIA consist of clear cylinders and metal chambers (control and treatment). A clear cylinder and a metal chamber are connected using a linking section which is a funnel cap. The narrow end of the funnel pointed towards the clear cylinder. The linking section's butterfly valve is initially turned to the closed position. An end cap is placed on the open end of all clear cylinders and opaque felt cloth pieces are wrapped around the clear cylinder and placed over the viewing port of the end caps to prevent eliciting any type of phototactic pressure on the mosquitoes in the chamber. An inner cylinder, with treatment net (control and chemical) affixed to it, is inserted into corresponding metal chambers and an end-cap installed. The viewing port of this end-cap is also covered with opaque felt cloth. The entire assembly fits into a holding cradle. Ten mosquitoes are introduced into each metal chamber using a mechanical aspirator and air compressor (Figure 16A) and, after a 30 sec rest period, the butterfly valve is placed into the open position. After 10 min the valve is closed, and the number of mosquitoes exiting into the clear cylinders (i.e. number escaping) is recorded, as well as the number of knock down in both metal and clear cylinders. For all trials, a second assay was simultaneously run within an acetone-treated net to serve as a control. The ratio of treatment to control assays was 1:2 (Figure 16B). To prepare for the next replicate, the mosquitoes were released from the assay system into individual control and treatment screened cages (Figure 16C). Six replicates were performed for each chemical treatment concentration and mosquito strain.

3.2 Spatial Repellency Assay (SRA)

The components the SRA consist of one clear cylinder and two metal chambers. Each metal chamber contains either test-insecticide-treated netting or netting treated with solvent similar to that described for CIA. The center clear cylinder is attached to each of the metal chambers using a linking section (i.e., funnel cap) with the narrow end of the funnel cap oriented toward the inside of each metal chamber. The entire assembly is placed in a holding cradle during the assay. Opaque

felt cloth is covers the viewing ports in the end caps but the clear chamber is left uncovered to initiate the light activated movement in test mosquitoes (Figure 17A). The butterfly valves of each funnel cap are set to the closed position, then twenty mosquitoes are introduced into the clear (central) cylinder using a mechanical aspirator and an air compressor. After the mosquitoes are introduced, a 30 sec "setting down" or "acclimation" period follows then both butterfly valves are simultaneously opened. After 10 min, the valves are simultaneously closed and the number of mosquitoes in each of the metal chambers is counted. In addition, the number of mosquitoes that are knocked down in each metal chamber and clear cylinder is recorded. The mosquitoes are then released using an air compressor (Figure 17B). Between replicates, the assembly is partially disassembled (the clear cylinder detached from the treated and control metal chambers) and the end cap section removed from the control metal chamber to allow ventilation of potential chemical saturation (Figure 17C). Nine replicates were performed for each chemical treatment concentration and mosquito strain.

3.3 Toxicity Assay (TOX)

The assembly configuration for this assay consists of individual metal chambers only (control and treatment) fitted with an end cap and funnel section. The test unit sits in a holding cradle (Figure 18A). Each metal chamber holds a treated netting strip (insecticide or control) similar to that described for the CIA and SRA. Twenty mosquitoes are introduced into each metal chamber using a mechanical aspirator and an air compressor. After a 1 h exposure period, the number of knocked down mosquitoes is first recorded in each metal chamber and then all specimens (knocked down and those still mobile) are transferred to individual control and treatment holding cups (Figure 18B). Holding cups are then placed into individual trays which are properly labeled by strain, dates, insecticide of use and dosage. The holding cups are then transferred to an insectary maintained at 25^{o}C and 80% RH where a cotton pad soaked with 10% sugar solution is placed on top of each holding cup (Figure 18C). Mortality is recorded after 24 h.

4. Data Analysis

Data analysis for CIA, SRA and TOX assays followed that described by Grieco *et al*. (2005, 2007). CIA data was analyzed using the Wilcoxon two-sample test (PROC NPARI WAY, SAS, 2002) to examine the differences between the number escaping from treated and control chambers. A spatial activity index (SAI), based upon the oviposition activity index of Kramer and Mulla (1979), was used to evaluate the responses of the female mosquitoes in the SRA. The calculation of SAI for each experimental replication as SAI= $(N_c - N_t)/(N_c + N_t)$, in which N_c is the number of females in the control chamber of the spatial repellency assay device and N_t is the number of females in the treated chamber of the spatial repellency assay device. The SAI is a measure of the proportion of females in the control chamber over the treated chamber after correcting for the proportion of females in the control chamber. The SAI varies from -1 to 1, with 0 indicating no response. Spatial repellency assay data was analyzed by a non parametric signed-rank test (Proc UNIVARIATE, SAS, 2002) to determine if the mean SAI for each treatment was significantly different from zero. For the toxicity data, percent knockdown and mortality values were corrected using Abbott's formula (Abbott, 1925) and transformed to arcsine square root for analysis of variance (ANOVA). For each insecticide mortality at each treatment concentration was compared and separated using Tukey's honestly significant difference (HSD) test at P=0.05 (SAS, 2002).

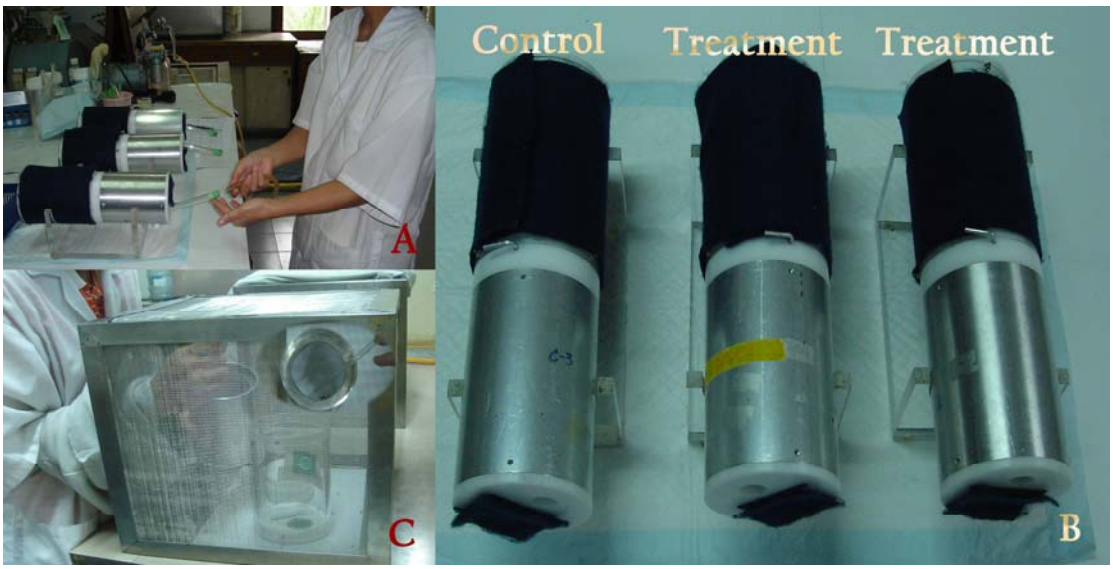

Figure 16 Contact irritancy assay procedure (A) introducing mosquitoes into test chamber, (B) a complete trails with one control and two treatment chambers, (C) releasing mosquitoes from test chambers.

Figure 17 Spatial repellency assay procedures: test preformed (A) a metal chamber containing treated netting is attached to clear cylinder that is attached to a metal chamber housing a solvent-treated netting strip, (B) mosquitoes aspirated from clear chamber at end of 10 min assay, (C) the control chamber and clear cylinder are allowed to ventilate for 3 min between each replicate.

Figure 18 Toxicity assay procedures: (A) test preformed tested, (B) mosquitoes released into screened cages, (C) all mosquitoes housing into metal chambers were transferred to individual control and treatment cups and held with sugar ad libitum for 24 h to monitor mortality rates.

RESULTS AND DISCUSSION

Results

Four different experiments were performed in this study. The first experiment evaluated the susceptibility status of seven *Ae. aegypti* strains, six field and one laboratory strain (USDA). The second experiment characterized resistance mechanisms of these six *Ae. aegypti* field strains compared to a susceptible strain (Bora). The third experiment involved quantifying the contact irritancy and non-contact repellency behavioral of each test strain responses using the excito-repellency test chamber. The last experiment evaluated similar behavioral responses of the same *Ae. aegypti* strains using a high-throughput screening system laboratory assay.

Part 1. Susceptibility test of female *Aedes aegypti* strains to insecticide compounds

Results of susceptibility tests with the single diagnostic concentration of alphacypermethrin (0.05%), deltamethrin (0.05%), permethrin (0.25%), DDT (4%) and malathion (0.8%) for different *Ae. aegypti* strains are given in Tables 2 - 5.

Of the three synthetic pyrethroids, alphacypermethrin and deltamethrin, produced consistent high levels of knock down effect after 1 h exposure (range from 89.9 – 100 %) (Table 2). However, after a 1 h exposure to permethrin, knock down was lower ranging from 0.33% to 61.2 % knockdown although significantly different compared to the standard USDA strain (Table 2). After 30 min exposure to DDT, there was no the knockdown mosquito effect within most of the test strains (Table 3). The Chiang Mai strain showed 21.2% of female knock down but this was significantly different than the reference USDA strain which had 96.1% knock down. For malathion, the knock down effect after 1 h exposure was varied upon the test strains (range from 23.0 – 96.0%) (Table 3).

The ability of mosquitoes to survive the diagnostic concentration after 24 h is indicative of resistance in the strain as defined by percent mortality in the test strain.

Based on WHO recommendations (WHO, 1998b), the results of susceptibility tests can be interpreted into three categories: 1). Mosquitoes are susceptible to insecticides if the percent mortality ranges from 98 – 100 %, 2) a possibility of incipient insecticide resistance if mortality varies from 80 – 97 %, and 3) insecticide resistant if percent mortality is <80% (WHO, 1998b). In the present study, no mortality was recorded in the untreated control over a 24 h holding period for all paired tests. Upon exposure to alphacypermethrin, only the Nonthaburi (85.7% mortality) and Songkhla (91.0% mortality) strain indicated tolerance/resistance. Conversely, all remaining test strains were susceptible to deltamethrin (98.0 – 100 % mortality) (Table 2). Following exposure to permethrin, various levels of physiological resistance was indicated (5.00 – 72.6% mortality) (Table 2). The Chiang Mai (98% mortality) and USDA standard susceptible test strain (100% mortality) showed complete susceptibility to permethrin.

Aedes aegypti from different localities showed strongly physiological resistance to DDT, except the standard susceptible USDA strain (100% mortality) (Table 3). Test strains demonstrated various levels of tolerance/resistance to malathion. Resistance status to malathion was seen in the Khonkaen strain (77.7% mortality) whereas the Chiang Mai, Nonthaburi, Songkhla and Satun were all susceptible to malathion (98.0 – 100 % mortality). The USDA reference strain was found to be susceptible to malathion (100% mortality) (Table 3). The possibility of incipient malathion resistance was observed in the Kanchanaburi strain (97.0% mortality) (Table 3).

The respective KT_{50} and KT_{95} observed for the seven strains are presented in Table 4. The mosquitoes knock down was s substantial in both the KT_{50} and KT_{95} in all strains following exposure to the pyrethroid test compounds. Overall mosquitoes from Nonthaburi strain showed high KT_{50} and KT_{95} following exposure to the pyrethroid insecticides, the KT_{50} of 0.05% alphacypermethrin, 0.05% deltamethrin and 0.25% permethrin, were 36.6, 20.8 and >60 min, respectively (Table 4). On the other hand, there were no mosquitoes knocked down at 50 and 95 min when exposure to DDT, except the Chiang Mai strain KT_{50} was 39.5 min (Table 5).

Table 2 Percent 1 h knockdown and 24 h mortality rates of seven *Aedes aegypti* [1] strains after exposure to diagnostic concentrations of alphacypermethrin, deltamethrin and permethrin in four replications.

Chemical (diagnostic concentration)	Strain	No. of (mosq.)	% Knockdown[2] (mean ± SE)	% Mortality[2] (mean ± SE)
alphacypermethrin	Chiang Mai	(75)	100 a	100 a
(0.05 %)	Kanchanaburi	(100)	97.0 ± 1.00 ab	98.0 ± 1.16 a
	Khonkaen	(100)	100 a	100 a
	Nonthaburi	(99)	89.9 ± 3.44 c	85.7 ± 4.05 b
	Songkhla	(100)	97.0 ± 3.83 ab	91.0 ± 1.91 b
	Satun	(97)	93.8 ± 2.06 bc	98.0 ± 2.17 a
	USDA [3]	(104)	100 a	100 a
deltamethrin	Chiang Mai	(99)	100 a	100 a
(0.05 %)	Kanchanaburi	(98)	100 a	100 a
	Khonkaen	(98)	100 a	100 a
	Nonthaburi	(100)	100 a	100 a
	Songkhla	(98)	99.0 ± 1.00 ab	98.0 ± 1.54 b
	Satun	(97)	97.9 ± 1.20 b	100 a
	USDA	(100)	100 a	100 a
permethrin	Chiang Mai	(100)	61.2 ± 6.08 b	98.0 ± 1.15 a
(0.25 %)	Kanchanaburi	(100)	6.50 ± 0.70 ef	9.00 ± 3.42 d
	Khonkaen	(97)	14.5 ± 1.44 de	38.3 ± 9.66 c
	Nonthaburi	(100)	0.33 ± 0.16 f	5.00 ± 1.91 d
	Songkhla	(99)	27.5 ± 2.97 c	72.6 ± 3.20 b
	Satun	(96)	17.2 ± 1.68 d	65.4 ± 6.46 b
	USDA	(100)	82.5 ± 4.84 a	100 a

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

[2] means with same letters are not significantly different at the 0.05 level using Duncan

[3] USDA Beltsville, Florida USA strain

Table 3 Percent 1 h knockdown and 24 h mortality rates of seven *Aedes aegypti* [1] strains after exposure to diagnostic concentrations of DDT and malathion in four replications.

Chemical (diagnostic concentration)	Strain	No. of (mosq.)	% Knockdown[2] (mean ± SE)	% Mortality[2] (mean ± SE)
DDT	Chiang Mai	(99)	21.2 ± 3.40 b	37.2 ± 5.54 b
(4 %)	Kanchanaburi	(100)	0 b	2.00 ± 2.00 c
	Khonkaen	(100)	0 b	3.00 ± 1.00 c
	Nonthaburi	(100)	0 b	0 c
	Songkhla	(100)	0 b	0 c
	Satun	(100)	0 b	0 c
	USDA [3]	(101)	96.1 ± 2.71 a	100 a
malathion	Chiang Mai	(100)	23.0 ± 5.30	98.0 ± 1.60 a
(0.8 %)	Kanchanaburi	(100)	69.0 ± 4.43	97.0 ± 1.91 a
	Khonkaen	(99)	42.3 ± 2.30	77.7 ± 2.54 b
	Nonthaburi	(100)	34.0 ± 5.03	100 a
	Songkhla	(100)	52.0 ± 11.4	99.0 ± 1.00 a
	Satun	(99)	65.0 ± 2.36	100 a
	USDA	(100)	96.0 ± 1.63	100 a

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

[2] means with same letters are not significantly different at the 0.05 level using Duncan

[3] USDA Beltsville, Florida USA strain

Table 4 Knockdown times[1] 50(KT_{50}) and 95(KT_{95}) in minutes of seven *Aedes aegypti*[2] strains to alphacypermethrin, deltamethrin and permethrin in four replications.

Chemical	Strain	KT_{50} (95% CL)	KT_{95} (95% CL)
alpha-	Chiang Mai	11.9 (11.1-12.7)	21.2 (19.5-23.5)
cypermethrin	Kanchanaburi	27.5 (26.5-28.4)	45.6 (43.4-48.4)
(0.05%)	Khonkaen	15.3 (14.5-16.2)	29.3 (27.4-31.7)
	Nonthaburi	36.6 (35.3-37.8)	>1 h
	Songkhla	18.7 (17.6-19.8)	43.0 (40.0-46.7)
	Satun	19.0 (17.6-20.3)	46.5 (42.6-51-7)
	USDA [3]	4.75 (4.09-5.30)	10.7 (9.51-12.7)
deltamethrin	Chiang Mai	9.81 (9.17-10.4)	17.6 (16.2-19.5)
(0.05%)	Kanchanaburi	15.5 (13.0-17.8)	25.7 (21.8-34.7)
	Khonkaen	13.9 (13.1-14.7)	26.9 (25.0-29.3)
	Nonthaburi	20.8 (19.9-21.7)	36.8 (34.7-39.4)
	Songkhla	16.8 (15.8-17.8)	38.1 (35.4-41.5)
	Satun	6.98 (5.82-8.08)	26.7 (23.4-31.3)
	USDA	8.49 (5.43-11.0)	14.8 (11.3-30.5)
permethrin	Chiang Mai	24.8 (23.9-25.7)	39.2 (37.4-41.5)
(0.25%)	Kanchanaburi	>1 h	>1 h
	Khonkaen	>1 h	>1 h
	Nonthaburi	>1 h	>1 h
	Songkhla	53.1	>1 h
	Satun	>1 h	>1 h
	USDA	12.4 (11.8-13.1)	19.3 (18.1-21.1)

[1] The time in minute at which 50% and 95% of total mosquitoes were knocked down by strains and chemicals.

[2] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

[3] USDA Beltsville, Florida USA strain

Table 5 Knockdown times[1] 50(KT_{50}) and 95(KT_{95}) in minutes of seven *Aedes aegypti*[2] strains to DDT in four replications.

Chemical	Strain	KT_{50} (95% CL)	KT_{95} (95% CL)
DDT	Chiang Mai	39.5 (34.6-53.7)	>1 h
(4%)	Kanchanaburi	-	-
	Khonkaen	-	-
	Nonthaburi	-	-
	Songkhla	-	-
	Satun	-	-
	USDA[3]	20.9	33.9

[1] The time in minute at which 50% and 95% of total mosquitoes were knocked down by strains and chemicals.

[2] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

[3] USDA Beltsville, Florida USA strain

(-) No knock down effect observed after 30 minutes exposure

Part 2. Insecticide resistance mechanisms of *Aedes aegypti* strains

Seven strains of *Ae. aegypti* mosquitoes were measured independently for susceptibility to alphacypermethrin, deltamethrin, permethrin and DDT using the WHO filter paper (Table 6 – 7). The result first showed a strong resistance to permethrin was found in Kanchanaburi (91.0% survival rate) and Nonthaburi (95.0% survival rate). Survival rate of permethrin was 61.7%, 27.4%, and 34.6% in Khonkaen, Songkhla, and Satun strains, respectively (Table 6). Strong resistance to DDT (>97.0% survival rate) was detected in all strains of *Ae. aegypti*, except those from the Chiang Mai (62.8% survival rate) and the reference standard strain (Bora Bora) (Table 7). For malathion, resistance was found in Khonkaen strain (22.3% survival rate) (Table 7).

A one way analysis of variance (ANOVA) was then perform to assess the enzymatic activity (oxidase, esterases) of each field strain of *Ae. Aegypti* comparatively to the susceptible reference Bora Bora strain (Table 5). Approximately 30 specimens per strain were used to assess the activities of monooxygenase and non-specific esterases according to the procedures of Hemingway *et al*. (1998). No significant differences in the total protein content were reported between the six mosquito field strains (Table 8). Significant increase in monooxygenase activity was found in Chiang Mai, Songkhla and Satun compared to the susceptible Bora Bora strain ($P<0.05$). No significant increase in monooxygenase activity was however observed in the strains of Kanchanaburi, Khonkean and Nonthaburi ($P>0.05$).

Alpha and beta-esterase activities differed among the field strains of *Ae. agypti*. Elevated α-esterase activity was shown in Khonkaen and Satun compared to the reference susceptible strain ($P<0.05$). There were no significant differences in α-esterase activities between Chiang Mai, Kanchanaburi, Nonthaburi compared to the Bora Bora ($P>0.05$) (Table 8). Higher activity of β-esterase activity was found in Khonkaen strain compared to the reference susceptible strain ($P<0.05$). However, there was no significant differences in β-esterase activity between the Bora Bora and the other four field strains, i.e., Chiang Mai, Kanchanaburi, Nonthaburi, and Satun

(P>0.05). Both α and β-esterase activities were significantly lower in the Songkhla strain compared to the susceptible Bora Bora strain (P<0.05) (Table 8).

Table 6 Percent 24 h mortality and survival rates of seven *Aedes aegypti*[1] strains after exposure to diagnostic concentrations of alphacypermethrin, deltamethrin and permethrin.

Chemical	Strain	No. of (mosq.)	% Mortality [3] (mean ± SE)	% Survival
alphacypermethrin	Chiang Mai	(75)	100 a	0
(0.05 %)	Kanchanaburi	(100)	98.0 ± 1.16 a	2.00
	Khonkaen	(100)	100 a	0
	Nonthaburi	(99)	85.7 ± 4.05 b	14.3
	Songkhla	(100)	91.0 ± 1.91 b	9.00
	Satun	(97)	98.0 ± 2.17 a	2.00
	Bora Bora [2]	(100)	100 a	0
deltamethrin	Chiang Mai	(99)	100 a	0
(0.05 %)	Kanchanaburi	(98)	100 a	0
	Khonkaen	(98)	100 a	0
	Nonthaburi	(100)	100 a	0
	Songkhla	(98)	98.0 ± 1.54 b	2.00
	Satun	(97)	100 a	0
	Bora Bora	(100)	100	0
permethrin	Chiang Mai	(100)	98.0 ± 1.15 a	2.00
(0.25 %)	Kanchanaburi	(100)	9.00 ± 3.42 d	91.0
	Khonkaen	(97)	38.3 ± 9.66 c	61.7
	Nonthaburi	(100)	5.00 ± 1.91 d	95.0
	Songkhla	(99)	72.6 ± 3.20 b	27.4
	Satun	(96)	65.4 ± 6.46 b	34.6
	Bora Bora	(100)	100 a	0

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

[2] Bora Bora; French Polynesia strain, the reference susceptible strain

[3] means with same letters are not significantly different at the 0.05 level using Duncan

Table 7 Percent 24 h mortality and survival rates of seven *Aedes aegypti*[1] strains after exposure to diagnostic concentrations of DDT and malathion.

Chemical	Strain	No. of (mosq.)	% Mortality[3] (mean ± SE)	% Survival
DDT	Chiang Mai	(99)	37.2 ± 5.54 b	62.8
(4 %)	Kanchanaburi	(100)	2.00 ± 2.00 c	98.0
	Khonkaen	(100)	3.00 ± 1.00 c	97.0
	Nonthaburi	(100)	0 c	100
	Songkhla	(100)	0 c	100
	Satun	(100)	0 c	100
	Bora Bora[2]	(100)	100 a	0
malathion	Chiang Mai	(100)	98.0 ± 1.60 a	2.00
(0. 8 %)	Kanchanaburi	(100)	97.0 ± 1.91 a	3.00
	Khonkaen	(99)	77.7 ± 2.54 b	22.3
	Nonthaburi	(100)	100 a	0
	Songkhla	(100)	99.0 ± 1.00 a	1.00
	Satun	(99)	100 a	0
	Bora Bora	(100)	100 a	0

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

[2] Bora Bora; French Polynesia strain, the reference susceptible strain

[3] means with same letters are not significantly different at the 0.05 level using Duncan

Table 8 Mean values and standard deviation of levels of monooxygenase, alpha and beta esterases in *Aedes aegypti* [1] field strains compared with a susceptible strain.

Strain	Total protein Mean ± SD mg protein/ml per mosquito(n)	Monooxygenase Mean ± SD nmole product/min/mg protein(n)	α Esterase Mean ± SD nmole α naphthol/min/mg protein(n)	β Esterase mean ± SD nmole β naphthol/min/mg protein(n)
Chiang Mai	0.0038 ± 0.0012(39)	0.0765 ± 0.0213(39) *	0.0845 ± 0.0276(39)	0.0836 ± 0.0189(39)
Kanchanaburi	0.0058 ± 0.0004(40)	0.0603 ± 0.0040(40)	0.1040 ± 0.0109(40)	0.0805 ± 0.0055(40)
Khonkaen	0.0061 ± 0.0006(40)	0.0568 ± 0.0053(40)	0.2892 ± 0.1173(40) *	0.2171 ± 0.0994(40) *
Nonthaburi	0.0060 ± 0.0004(40)	0.0484 ± 0.0028(40)	0.1058 ± 0.0108(40)	0.0733 ± 0.0045(40)
Songkhla	0.0044 ± 0.0006(39)	0.1241 ± 0.0351(39) *	0.0561 ± 0.0146(39) *	0.0282 ± 0.0070(39) *
Satun	0.0054 ± 0.0007(40)	0.0701 ± 0.0061(40) *	0.1126 ± 0.0143(40) *	0.0860 ± 0.0066(40)
Bora Bora [2]	0.0067 ± 0.0003(40)	0.0538 ± 0.0034(40)	0.0895 ± 0.0098(40)	0.0752 ± 0.0066(40)

[1] F1 – F2 female, 1 day-old fed

[2] Bora Bora; French Polynesia strain, the reference susceptible strain

* Significantly different from the Bora Bora strain at 95% confidence interval

Part 3. Contact irritancy and non-contact repellency behavioral responses of *Aedes aegypti* strains to insecticide compounds using an excito-repellency system

Results of excito-repellency test chamber trials of six *Ae. aegypti* field strains exposed to field operation rate of alphacypermethrin (6.0 nm/cm^2), deltamethrin (4.9 nm/cm^2), permethrin (127.8 nm/cm^2) and DDT (564.2 nm/cm^2) in contact (Table 9 – 11) and non-contact (Table 12 – 14) assays indicate varying levels of behavioral responses between strains.

Overall, the number of mosquitoes escaping from control chambers in both contact and noncontact trials were lower than treated chamber for all six strains evaluated. The *Ae. aegypti* escape responses varied significantly, depending on test strains, insecticides and test assays. In contact trials, all test strains demonstrated dramatic escape responses, ranging from 52.4 – 80.0%, 43.1 – 81.4% and 52.5 – 85.0% which exposure to alphacypermethrin, deltamethrin and permethrin, respectively (Table 9 – 11). All test strains demonstrated weaker contact responses to DDT than the three synthetic pyrethroids test compounds, (18.3 – 65.0% escaped during a 30 min exposure). In non-contact trials, escape responses of all test strains were lower than in contact trials (Table 12 – 14), however, significant escape over matched controls was indicated in the Chiang Mai (all chemicals), Kanchanaburi (deltamethrin and DDT) and Khonhaen (DDT) strains (Table 12 and 13).

Mortality rates of *Ae. aegypti* strains exposed to alphacypermethrin, deltamethrin, permethrin and DDT in both contact (Table 9 – 11) and non-contact (Table 12 – 14) behavioral assay indicated higher mortality rates in contact trials than in non-contact trials as expected. In addition, higher mortality was observed in treatment chambers upon exposure to alphacypermethrin, deltamethrin and permethrin than following exposure to DDT. Overall, mortality rates of test strains exposed to alphacypermethrin, deltamethrin, permethrin and DDT in contact trials were generally low. The one exception was the Chiang Mai strain that showed 25.0%, 39.3%, 50.0% and 4.76% mortality remaining mosquitoes which exposure to alphacypermethrin, deltamethrin, permethrin and DDT, respectively (Table 9). In

non-contact trials, no mortality was observed in either the escaped or remaining mosquito strains, except those the remaining mosquitoes of Nonthaburi strain were observed 2.27% mortality in the alphacypermethrin treated chamber (Table 12 – 14).

The time recorded in minutes for female mosquitoes to escape from the treated chamber within a 30 min sampling period was calculated for alphacypermethrin, deltamethrin, permethrin and DDT during contact trials (Table 15). Escape time was defined as the time for 25% (ET_{25}), 50% (ET_{50}) and 75% (ET_{75}) of an individual test strain to escape from the test chamber containing insecticide-treated (or solvent only) nets (Chareonviriyaphap *et al.,* 2001). In contact trials, the ET_{25} and ET_{50} values of alphacypermethrin observed in the Chiang Mai and Kanchanaburi *Ae. aegypti* strains ranged from 4-7 min and 9-22 min, respectively, with the ET_{75} observed for Chiang Mai strain at 22 min. The ET_{25} responses in contact trials using deltamethrin, ranged from 1 – 7 min and the ET_{50} values ranged from 3 – 29 min (Table 15). The Khonkaen strain showed the quickest response ET_{75} with 19 min. Result with deltamethrin indicate an overall quicker time of response compared to alphacypermethrin. The ET_{25} and ET_{50} values of permethrin observed in all test strains ranged from 1 – 3 min and 2 – 26 min, respectively. Khonkaen and Songkhla strains were observed the ET_{75} values 17 min and 18 min, respectively (Table 15). Additionally, the ET_{25} value for DDT was observed in almost all strains, between 3-16 min, whereas the ET_{50} was observed only in Chiang Mai strain (11 min) (Table 15). The ET_{75} values for all test strains cloud be not calculated for DDT in the 30 min sampling period (Table 15). In non-contact trials the time for escape was much longer than contact trials. ET_{50} and ET_{75} were >30 min for all strains and chemicals. Interestingly, the ET_{25} value observed in the Chiang Mai strain when using DDT (11 min) was the only calculation that could be conducted for within the ET_{25} 30 min observation period for DDT. In addition, the ET_{25}, ET_{50} and ET_{75} for all pyrethroid test compounds and test strains were >30 min.

Multiple comparisons among six test strains were performed by test compounds and assay type (contact and noncontact) (Tables 16 – 17). Comparison of the patterns of escape responses were examined with the log-rank method, and

significance was determined at the 0.05 level of probability. Escape probabilities in treatment chambers in contact trials was significantly different from the control for all strains and all insecticides ($P<0.05$), except those from Khonkaen against to DDT (Table 17). Additionally, significant differences were indicated in test strains when contact trials were compared with non-contact trials ($P<0.05$), except escape responses from DDT treated chamber of Khonkaen and Nonthaburi ($P>0.05$). Conversely, a high number of significant differences in escape patterns in non-contact trials were found in the Chiang Mai, Kanchanaburi and Khonkaen strains against DDT ($P<0.05$) (Table 17). For the Chiang Mai strain, escape probabilities in treatment chambers in non-contact trials was significantly different from the control for all test compounds ($P<0.05$) (Table 16 – 17).

The proportions of mosquitoes remaining in the test chambers during a 30-min exposure period for alphacypermethrin, deltamethrin, permethrin and DDT in contact and non-contact trials are show in Figure 19 – 22. These proportions are used to show patterns in escape rates. The patterns are indicative of escape probabilities in contact treatment and non-contact treatment with the six strains of *Ae. aegypti*. The pattern of escape from contact chambers for all insecticide compounds was similarly (Figures 19A, 20A, 21A and 22A). A significantly weaker escape pattern from both contact and non-contact chambers treated with alphacypermethrin was found in the Kanchanaburi strain compared with all other strains ($P<0.05$) (Figure 19A). In the contrast, quickly escape response in non-contact trials in the Kanchanaburi strain was observed in deltamethrin (Figure 20B). Overall, no significant difference in escape patterns were seen in non-contact trials using permethrin ($P>0.05$) (Figure 21B). While, escape patterns in both contact and non-contact trials were significantly faster in the Chiang Mai strains compared with all other strains when against to DDT ($P<0.05$) (Figure 22A and B).

Table 9 Percent escaping and 24 h mortality rates of *Aedes aegypti*[1] from Chiang Mai and Kanchanaburi strains after contact with alphacypermethrin, deltamethrin, permethrin and DDT in an excito-repellency test chamber.

Strain	Chemical	Dose	No. replicates	%Escaped	% Mortality	
		(nm/cm^2)	(mosq.)	(No. of Escaped)	Escaped	Remain
Chiang Mai	α-cypermethrin	6.0	4 (60)	80.0 (48) *	0	25.0
	control		4 (58)	3.46 (2)	0	0
	deltamethrin	4.9	4 (58)	43.1 (25) *	12.0	39.3
	control		4 (60)	1.67 (1)	0	0
	permethrin	127.8	4 (59)	52.5 (31) *	9.67	50.0
	control		4 (60)	10.0 (6)	0	0
	DDT	564.2	4 (60)	65.0 (39) *	0	4.76
	control		4 (60)	10.0 (6)	0	0
Kanchanaburi	α-cypermethrin	6.0	4 (61)	52.5 (32) *	0	10.3
	control		4 (58)	5.17 (3)	0	0
	deltamethrin	4.9	4 (58)	62.1 (36) *	1.82	5.00
	control		4 (60)	8.33 (5)	0	0
	permethrin	127.8	4 (60)	61.6 (37) *	0	4.35
	control		4 (60)	6.67 (4)	0	0
	DDT	564.2	4 (60)	43.3 (26) *	0	0
	control		4 (58)	8.62 (5)	0	0

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

* Log rank tests with statistically significant differences ($P < 0.05$) in escape patterns

Table 10 Percent escaping and 24 h mortality rats of *Aedes aegypti*[1] from Khonkaen and Nonthaburi strains after contact with alphacypermethrin, deltamethrin, permethrin and DDT in an excito-repellency test chamber.

Strain	Chemical	Dose	No. replicates	%Escaped	% Mortality	
		(nm/cm^2)	(mosq.)	(No. of Escaped)	Escaped	Remain
Khonkaen	α-cypermethrin	6.0	4 (58)	72.4 (42) *	2.37	6.25
	control		4 (60)	20.0 (12)	0	0
	deltamethrin	4.9	4 (59)	81.4 (48) *	0	0
	control		4 (60)	20.0 (12)	0	0
	permethrin	127.8	4 (60)	85.0 (31) *	0	0
	control		4 (59)	10.2 (6)	0	0
	DDT	564.2	4 (60)	26.6 (16)	0	0
	control		4 (57)	15.7 (9)	0	0
Nonthaburi	α-cypermethrin	6.0	4 (60)	61.6 (37) *	0	4.35
	control		4 (60)	20.0 (12)	0	0
	deltamethrin	4.9	4 (57)	49.1 (29) *	0	3.57
	control		4 (59)	3.45 (2)	0	0
	permethrin	127.8	4 (58)	56.9 (33) *	0	0
	control		4 (60)	8.33 (5)	0	0
	DDT	564.2	4 (60)	18.3 (11) *	0	0
	control		4 (60)	1.67 (1)	0	0

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

* Log rank tests with statistically significant differences ($P < 0.05$) in escape patterns

Table 11 Percent escaping and 24 h mortality rate of *Aedes aegypti*[1] from Songkhla and Satun strains after contact with alphacypermethrin, deltamethrin, permethrin and DDT in an excito-repellency test chamber.

Strain	Chemical	Dose	No. replicates	%Escaped	% Mortality	
		(nm/cm^2)	(mosq.)	(No. of Escaped)	Escaped	Remain
Songkhla	α-cypermethrin	6.0	4 (60)	66.6 (40) *	0	0
	control		4 (60)	13.3 (8)	0	0
	deltamethrin	4.9	4 (59)	67.8 (40) *	0	0
	control		4 (60)	8.33 (5)	0	0
	permethrin	127.8	4 (61)	83.6 (51) *	0	0
	control		4 (60)	11.6 (7)	0	0
	DDT	564.2	4 (60)	30.0 (18) *	0	0
	control		4 (60)	11.6 (7)	0	0
Satun	α-cypermethrin	6.0	4 (61)	63.9 (39) *	0	0
	control		4 (60)	16.6 (10)	0	0
	deltamethrin	4.9	4 (60)	50.0 (30) *	0	0
	control		4 (60)	11.6 (7)	0	0
	permethrin	127.8	4 (61)	59.0 (36) *	0	0
	control		4 (60)	1.67 (1)	0	0
	DDT	564.2	4 (60)	46.6 (28) *	0	0
	control		4 (60)	10.0 (6)	0	0

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

***** Log rank tests with statistically significant differences ($P < 0.05$) in escape patterns

Table 12 Percent escaping and 24 h mortality rates of *Aedes aegypti*[1] from Chiang Mai and Kanchanaburi strains after non-contact with alphacypermethrin, deltamethrin, permethrin and DDT in an excito-repellency test chamber.

Strain	Chemical	Dose (nm/cm^2)	No. replicates (mosq.)	%Escaped (No. of Escaped)	% Mortality	
					Escaped	Remain
Chaing Mai	α-cypermethrin	6.0	4 (60)	36.6 (22) *	0	0
	control		4 (58)	3.45 (2)	0	0
	deltamethrin	4.9	4 (60)	13.3 (8) *	0	0
	control		4 (60)	3.33 (2)	0	0
	permethrin	127.8	4 (59)	16.9 (10) *	0	0
	control		4 (59)	3.39 (2)	0	0
	DDT	564.2	4 (60)	33.3 (20) *	0	0
	control		4 (60)	3.00 (5)	0	0
Kanchanaburi	α-cypermethrin	6.0	4 (59)	3.39 (2)	0	0
	control		4 (59)	3.39 (2)	0	0
	deltamethrin	4.9	4 (56)	35.7 (20) *	0	0
	control		4 (58)	3.45 (2)	0	0
	permethrin	127.8	4 (59)	11.8 (7)	0	0
	control		4 (60)	5.00 (3)	0	0
	DDT	564.2	4 (60)	21.3 (13) *	0	0
	control		4 (58)	3.39 (2)	0	0

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

* Log rank tests with statistically significant differences ($P < 0.05$) in escape patterns

Table 13 Percent escaping and 24 h mortality rates of *Aedes aegypti*[1] from Khonkaen and Nonthaburi strains after non-contact with alphacypermethrin, deltamethrin, permethrin and DDT in an excito-repellency test chamber.

Strain	Chemical	Dose (nm/cm^2)	No. replicates (mosq.)	%Escaped (No. of Escaped)	% Mortality	
					Escaped	Remain
Khonkaen	α-cypermethrin	6.0	4 (60)	20.0 (12)	0	0
	control		4 (58)	18.6 (11)	0	0
	deltamethrin	4.9	4 (60)	21.6 (13)	0	0
	control		4 (60)	20.0 (12)	0	0
	permethrin	127.8	4 (61)	13.1 (8)	0	0
	control		4 (60)	8.33 (5)	0	0
	DDT	564.2	4 (60)	13.3 (8) *	0	0
	control		4 (60)	3.33 (2)	0	0
Nonthaburi	α-cypermethrin	6.0	4 (58)	24.1 (14)	0	2.27
	control		4 (59)	18.6 (11)	0	0
	deltamethrin	4.9	4 (58)	5.17 (3)	0	0
	control		4 (60)	0	0	0
	permethrin	127.8	4 (61)	4.92 (4)	0	0
	control		4 (60)	3.33 (2)	0	0
	DDT	564.2	4 (60)	8.33 (5)	0	0
	control		4 (60)	5.00 (3)	0	0

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24h pre-test

* Log rank tests with statistically significant differences ($P < 0.05$) in escape patterns

Table 14 Percent escaping and 24 h mortality rates of *Aedes aegypti*[1] from Songkhla and Satun strains after non-contact with alphacypermethrin, deltamethrin, permethrin and DDT in an excito-repellency test chamber.

Strain	Chemical	Dose (nm/cm^2)	No. replicates (mosq.)	%Escaped (No. of Escaped)	% Mortality	
					Escaped	Remain
Songkhla	α-cypermethrin	6.0	4 (60)	21.6 (13)	0	0
	control		4 (60)	11.6 (7)	0	0
	deltamethrin	4.9	4 (58)	12.1 (7)	0	0
	control		4 (60)	10.0 (6)	0	0
	permethrin	127.8	4 (59)	13.6 (8)	0	0
	control		4 (60)	15.0 (9)	0	0
	DDT	564.2	4 (60)	8.33 (5)	0	0
	control		4 (60)	5.00 (3)	0	0
Satun	α-cypermethrin	6.0	4 (59)	13.1 (8)	0	0
	control		4 (61)	10.1 (6)	0	0
	deltamethrin	4.9	4 (60)	6.67 (4)	0	0
	control		4 (60)	8.33 (5)	0	0
	permethrin	127.8	4 (61)	8.20 (5)	0	0
	control		4 (60)	13.3 (8)	0	0
	DDT	564.2	4 (62)	10.0 (6)	0	0
	control		4 (60)	3.23 (2)	0	0

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

* Log rank tests with statistically significant differences ($P < 0.05$) in escape patterns

Table 15 Time in minutes for 25%(ET_{25}), 50%(ET_{50}) and 75%(ET_{75}) of six *Aedes aegypti*[1] strains to escape from exposure chambers treated with chemical compounds during a 30 minutes observation period in contact trails.

Strain	Alphacypermethrin (6.0 nm/cm^2)			Deltamethrin (4.9 nm/cm^2)			Permethrin (127.8 nm/cm^2)			DDT (564.2 nm/cm^2)		
	ET_{25}[2]	ET_{50}[2]	ET_{75}[2]	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}
Chiang Mai	4	9	22	6	-	-	3	26	-	3	11	-
Kanchanaburi	7	22	-	3	11	-	1	15	-	5	-	-
Khonkaen	-	-	-	1	3	19	1	2	17	3	-	-
Nonthaburi	-	-	-	7	29	-	3	15	-	-	-	-
Songkhla	-	-	-	1	6	-	1	3	18	16	-	-
Satun	-	-	-	2	14	-	1	8	-	6	-	-

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

[2] ET_{25}, ET_{50} and ET_{75} ; Escape Time, Time in minutes for 25%, 50% and 75% of mosquito each strain to escape from excito-repellency test chambers.

(-) Indicates insufficient number escaped from exposure chambers to estimate ET_{25}, ET_{50} and ET_{75} during 30 min exposure period.

Table 16 Comparison of escape responses between contact vs. control, contact vs. non-contact and non-contact vs. control trials for six field strains of *Aedes aegypti* [1] against to alphacypermethrin and deltamethrin.

Chemical	Strain	Contact vs. control	Contact vs. non-contact	Non-contact vs. control
alphacypermethrin (6.0 nm/cm^2)	Chiang Mai	<0.0001*	<0.0001*	<0.0001*
	Kanchanaburi	<0.0001*	<0.0001*	0.9999
	Khonkaen	<0.0001*	<0.0001*	0.8721
	Nonthaburi	<0.0001*	<0.0001*	0.4664
	Songkhla	<0.0001*	<0.0001*	0.1385
	Satun	<0.0001*	<0.0001*	0.5910
deltamethrin (4.9 nm/cm^2)	Chiang Mai	<0.0001*	0.0002*	0.0466 *
	Kanchanaburi	<0.0001*	0.0029*	<0.0001*
	Khonkaen	<0.0001*	<0.0001*	0.8310
	Nonthaburi	<0.0001*	<0.0001*	0.0755
	Songkhla	<0.0001*	<0.0001*	0.7175
	Satun	<0.0001*	<0.0001*	0.7234

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

The* identifies results of log-rank tests with statistically significant ($P < 0.05$) differences in escape patterns.

Table 17 Comparison of escape responses between contact vs. control, contact vs. non-contact and non-contact vs. control trials for six field strains of *Aedes aegypti* [1] against to permethrin and DDT.

Chemical	Strain	Contact vs. control	Contact vs. non-contact	Non-contact vs. control
permethrin	Chiang Mai	<0.0001*	<0.0001*	0.0174*
(127.8 nm/cm^2)	Kanchanaburi	<0.0001*	<0.0001*	0.1750
	Khonkaen	<0.0001*	<0.0001*	0.4094
	Nonthaburi	<0.0001*	<0.0001*	0.6303
	Songkhla	<0.0001*	<0.0001*	0.8703
	Satun	<0.0001*	<0.0001*	0.3815
DDT	Chiang Mai	<0.0001*	<0.0001*	<0.0001*
(564.2 nm/cm^2)	Kanchanaburi	<0.0001*	0.0040*	0.0030*
	Khonkaen	0.1569	0.0679	0.0467*
	Nonthaburi	0.0026*	0.1020	0.4805
	Songkhla	0.0112*	0.0028*	0.4434
	Satun	<0.0001*	<0.0001*	0.1303

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

The* identifies results of log-rank tests with statistically significant ($P < 0.05$) differences in escape patterns.

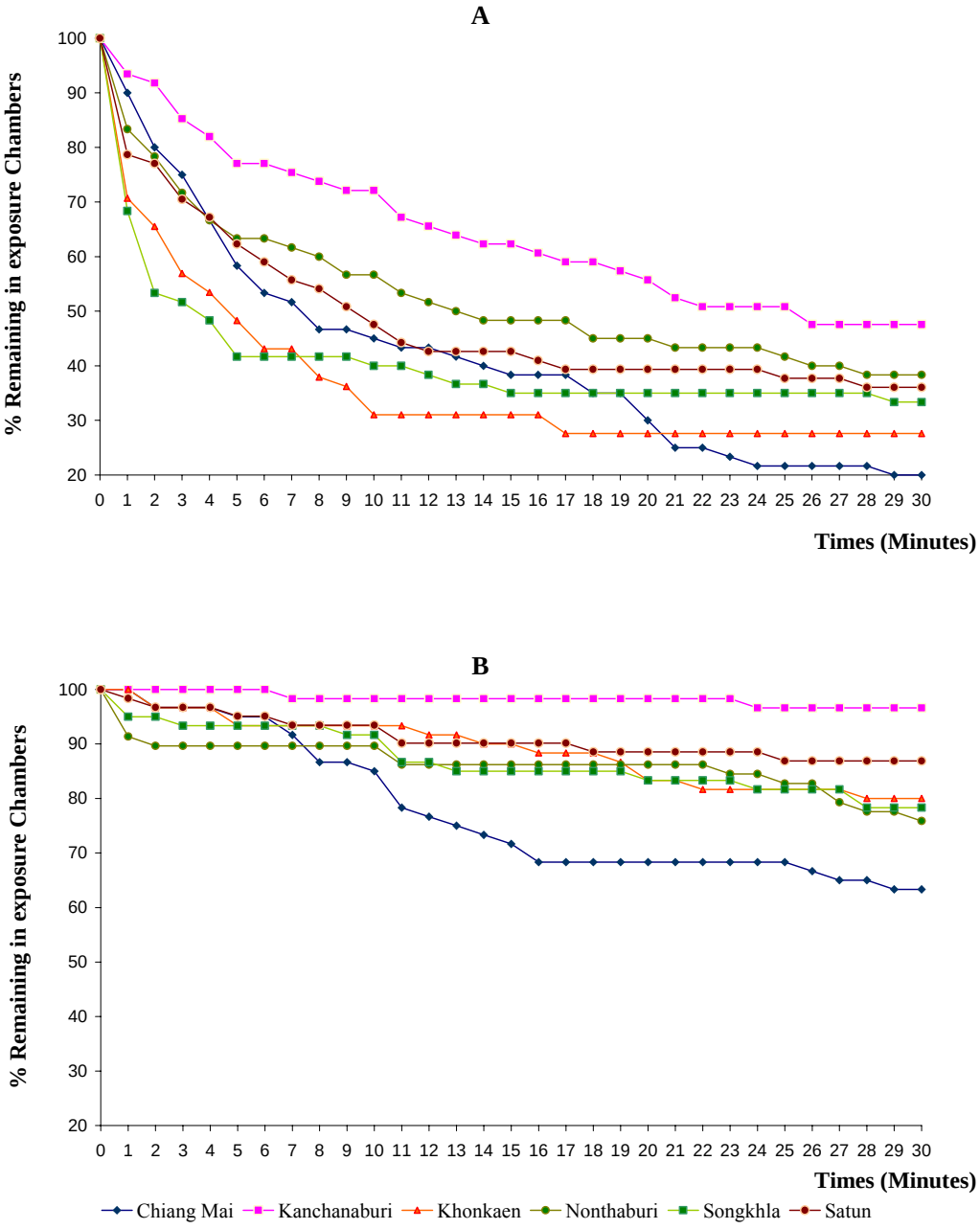

Figure 19 Escape patterns of six field *Aedes aegypti* strains (female, 4-5 day-old) in (A) contact and (B) non-contact assays using an excito-repellency test system against alphacypermethrin at 6.0 nm/cm^2.

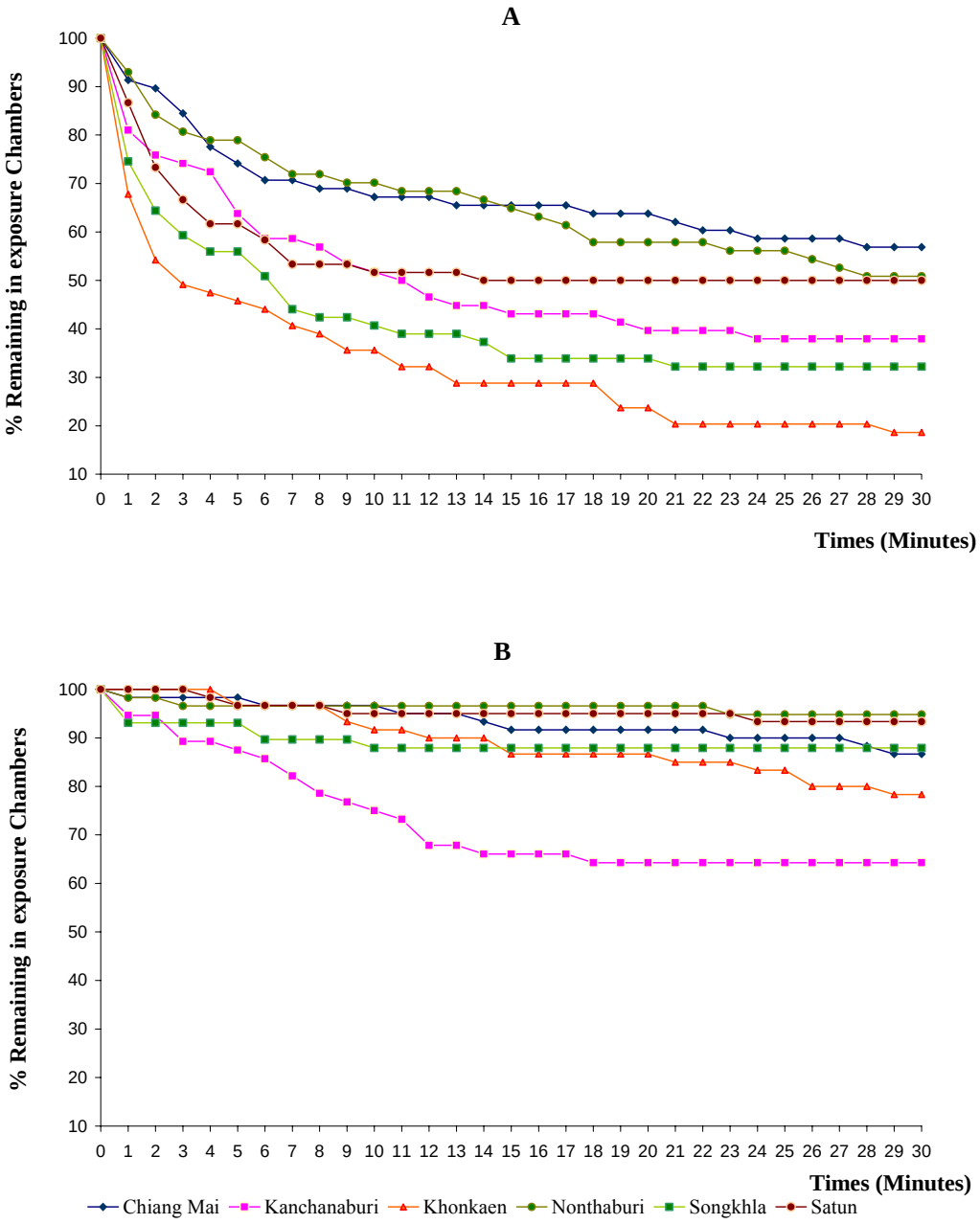

Figure 20 Escape patterns of six field *Aedes aegypti* strains (female, 4-5 day-old) in (A) contact and (B) non-contact assays using an excito-repellency test system against deltamethrin at 4.9 nm/cm^2.

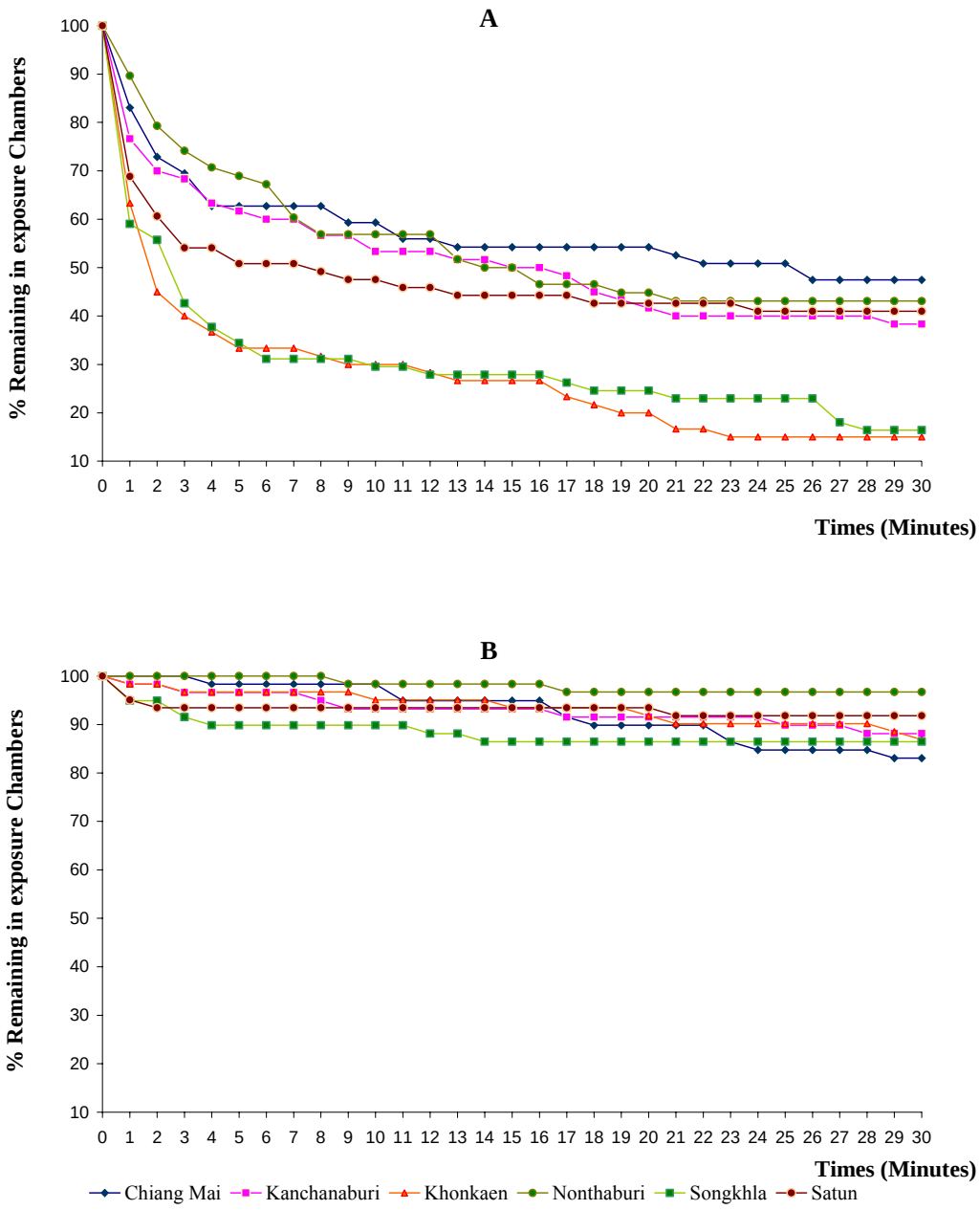

Figure 21 Escape patterns of six field *Aedes aegypti* strains (female, 4-5 day-old) in (A) contact and (B) non-contact assays using an excito-repellency test system against permethrin at 127.8 nm/cm^2.

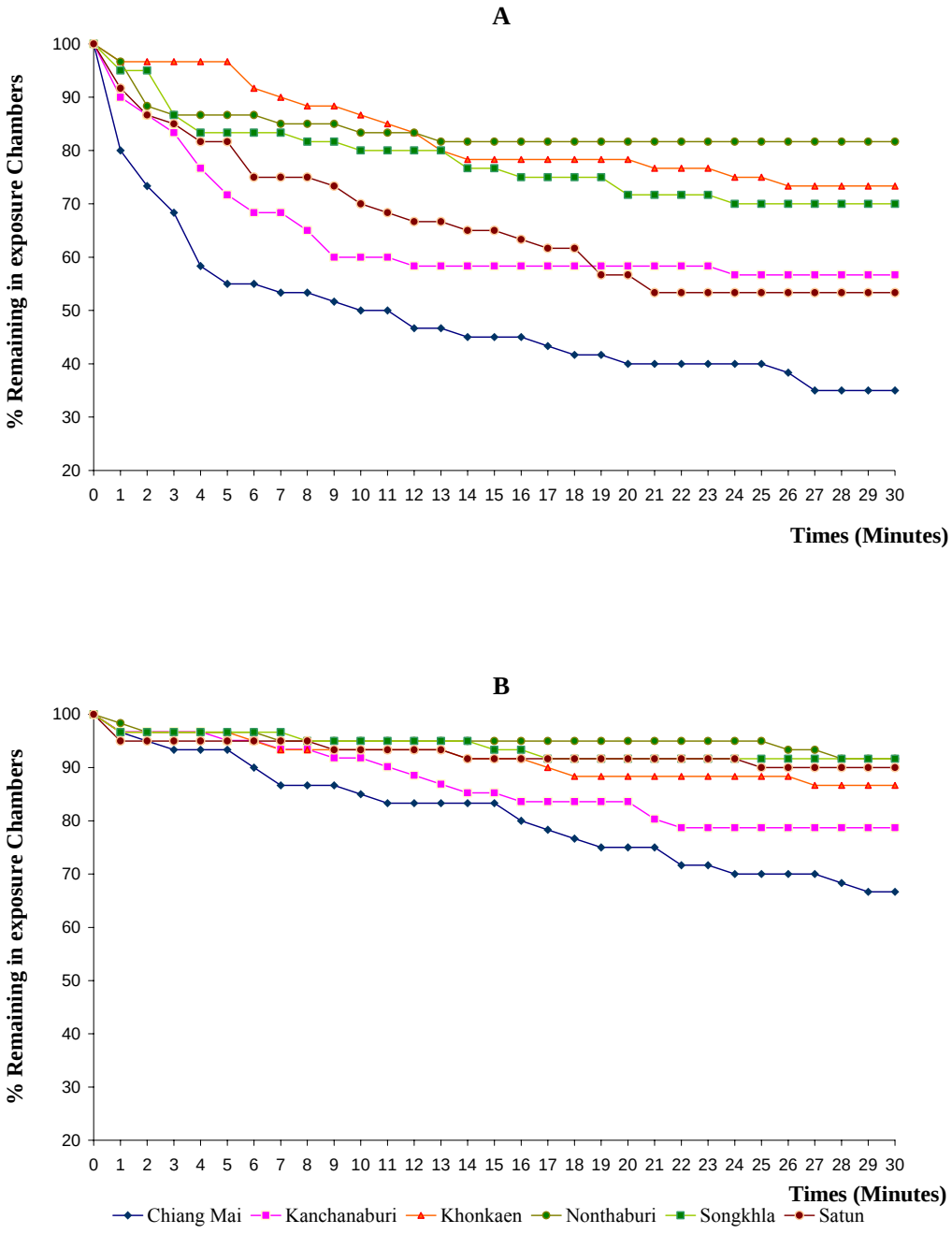

Figure 22 Escape patterns of six field *Aedes aegypti* strains (female, 4-5 day-old) in (A) contact and (B) non-contact assays using an excito-repellency test system against DDT at 564.2 nm/cm^2.

Part 4. Behavioral responses of female *Aedes aegypti* strains to insecticide compounds using a High Throughput Screening System assay

1. Contact irritancy assay

The escape response of *Ae. aegypti* in the contact irritancy assay are presented in Table 18 – 21 and Figure 23. In general, the percent escaping in all strains in increased with increasing concentration of alphacypermethrin, deltamethrin and permethrin treatment ($P<0.05$), although significant escape response was indicated at the all concentrations. Similar trends in escape responses were indicated against DDT (Table 21). However, significant responses in all strains were seen at field application rates while only the Chiang Mai and Songkhla had significant responses at the lowest dose. Mean number of escaping *Ae. aegypti* from the Chiang Mai, Kanchanaburi, Khonkaen, Songkhla and Satun strains against alphacypermethrin ranged from 2.00 to 8.67 (Table 18), against deltamethrin the rang was 4.50 to 7.67 (Table 19), and against permethrin from 2.50 to 7.67 (Table 20). DDT trials had escape from treated chambers ranging from, 1.17 to 4.50 (Table 21). Highest escape responses of *Ae. aegypti* from all test strains were observed in the chambers treated with alphacypermethrin deltamethrin and permethrin compared to response against DDT treated chambers (Table 18 – 21). In general, the highest percent escape was observed in the *Ae. aegypti* strain from Chiang Mai when exposed to alphacypermethrin, permethrin DDT at all doses (Figure 23). For DDT, all test strains showed lower irritant responses compared with the other test insecticides (Figure 23). Most importantly, significant contact irritant responses were observed in strains that were indicated as resistant to alphacypermethrin and permethrin (see Part 1).

Table 18 Escape responses of six *Aedes aegypti*[1] strains in the contact irritancy assay against three doses of alphacypermethrin.

Chemical	Dose (nm/cm^2)	Strain[2]	No. of trials (mosq.)	Number escaping (mean ± SE)		% $Escaping_c$[3] (mean ± SE)	P[4]
				Treated	Control		
alphacypermethrin							
	0.06	CM	6(60)	7.67 ± 0.71	1.00	74.1 ± 7.94	0.0022
		KAN	12(121)	4.80 ± 0.36	0.16 ±0.11	49.1 ± 5.26	<0.0001
		KK	6(60)	6.00 ± 0.68	0.67 ± 0.21	58.8 ± 6.52	0.0022
		NT	6(60)	4.33 ± 0.21	0	43.3 ± 2.10	0.0022
		SK	6(60)	8.33 ± 0.21	1.33 ± 0.21	80.5 ± 2.68	0.0022
		ST	6(60)	2.00 ± 0.73	0.33 ± 0.21	16.1 ± 9.39	0.0801
	0.6	CM	6(59)	8.50 ± 0.56	1.33 ± 0.21	98.2 ± 1.85	0.0022
		KAN	12(120)	5.00 ± 0.83	0.66 ± 0.22	49.1 ± 8.31	<0.0001
		KK	6(59)	5.50 ± 0.76	0	56.6 ± 6.67	0.0022
		NT	6(60)	4.67 ± 0.76	0	46.6 ± 7.06	0.0022
		SK	6(59)	5.33 ± 1.02	0.67 ± 0.21	67.5 ± 8.54	0.0022
		ST	6(60)	2.17 ± 0.79	0	28.3 ± 9.46	0.0152
	6.0	CM	6(60)	8.67 ± 0.49	0.33 ± 0.21	96.4 ± 2.23	0.0022
		KAN	6(59)	6.00 ± 0.73	0	79.0 ± 5.78	0.0022
		KK	6(60)	5.67 ± 0.67	0	80.0 ± 5.16	0.0022
		NT	6(60)	5.67 ± 0.67	0.33 ± 0.21	62.4 ± 3.77	0.0022
		SK	6(59)	6.33 ± 1.02	0	93.3 ± 4.94	0.0022
		ST	6(59)	6.50 ± 0.56	0.33 ± 0.21	78.8 ± 6.31	0.0022

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] CM, Chiang Mai strain; KAN, Kanchanaburi strain; KK, Khonkaen strain; NT, Nonthaburi strain; SK, Songkhla strain; ST, Satun strain.

[3] For each trail, percent escaping after correction using Abbott's formula.

[4] *P*-values are from Wilcoxon 2-sample test for difference between the number escaping in a chemical treated assembly and in an acetone-treated (control) assembly.

Table 19 Escape responses of six *Aedes aegypti*[1] strains in the contact irritancy assay against three doses of deltamethrin.

Chemical	Dose (nm/cm^2)	Strain [2]	No. of trials (mosq.)	Number escaping (mean ± SE)		% Escaping[3] (mean ± SE)	*P*[4]
				Treated	Control		
deltamethrin							
	0.049	CM	6(60)	5.50 ± 1.12	1.00 ± 0.37	58.5 ± 10.7	0.0022
		KAN	6(59)	4.50 ± 0.90	0	46.6 ± 9.88	0.0022
		KK	6(60)	5.02 ± 0.77	0.67 ± 0.21	46.8 ± 7.54	0.0022
		NT	6(60)	6.00 ± 0.37	0.33 ± 0.21	58.7 ± 3.44	0.0022
		SK	6(60)	4.50 ± 0.85	0.33 ± 0.21	42.7 ± 9.60	0.0065
		ST	6(60)	5.66 ± 0.84	0.33 ± 0.21	57.0 ± 9.82	0.0022
	0.49	CM	6(60)	7.00 ± 0.45	0.33 ± 0.21	79.2 ± 3.89	0.0022
		KAN	6(60)	6.00 ± 0.58	0	66.6 ± 4.22	0.0022
		KK	6(60)	6.00 ± 0.93	0.33 ± 0.21	67.4 ± 6.91	0.0022
		NT	6(59)	5.33 ± 0.42	0.33 ± 0.21	64.7 ± 6.91	0.0022
		SK	6(60)	6.67 ± 0.80	0	78.3 ± 7.03	0.0022
		ST	6(60)	6.50 ± 0.43	1.33 ± 0.42	66.6 ± 4.36	0.0022
	4.9	CM	6(59)	7.35 ± 0.76	0.33 ± 0.21	84.8 ± 6.88	0.0022
		KAN	6(60)	6.50 ± 0.81	0	78.3 ± 6.54	0.0022
		KK	6(59)	6.00 ± 0.93	0.33 ± 0.21	86.3 ± 7.37	0.0022
		NT	6(60)	7.67 ± 0.61	0.33 ± 0.21	95.0 ± 3.64	0.0022
		SK	6(60)	7.50 ± 0.62	0	86.3 ± 4.35	0.0022
		ST	6(58)	6.83 ± 0.79	0.67 ± 0.42	91.2 ± 3.15	0.0022

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] CM, Chiang Mai strain; KAN, Kanchanaburi strain; KK, Khonkaen strain; NT, Nonthaburi strain; SK, Songkhla strain; ST, Satun strain.

[3] For each trail, percent escaping after correction using Abbott's formula.

[4] *P*-values are from Wilcoxon 2-sample test for difference between the number escaping in a chemical treated assembly and in an acetone-treated (control) assembly.

Table 20 Escape responses of six *Aedes aegypti*[1] strains in the contact irritancy assay against three doses of permethrin.

Chemical	Dose (nm/cm^2)	Strain[2]	No. of trials (mosq.)	Number escaping (mean ± SE)		% Escaping[3] (mean ± SE)	P[4]
				Treated	Control		
permethrin							
	1.27	CM	6(59)	6.83 ± 0.95	0	76.6 ± 8.03	0.0022
		KAN	6(60)	3.17 ± 0.60	0	36.6 ± 9.94	0.0022
		KK	6(60)	3.00 ± 0.73	0.33 ± 0.21	27.2 ± 8.09	0.0022
		NT	6(59)	2.50 ± 0.81	0.67 ± 0.21	20.0 ± 7.80	0.0022
		SK	6(60)	3.83 ± 0.60	0.67 ± 0.21	37.5 ± 4.79	0.0022
		ST	6(60)	4.17 ± 0.83	1.33 ± 0.42	33.7 ± 7.09	0.0238
	12.7	CM	6(59)	6.33 ± 0.67	0.67 ± 0.42	82.5 ± 6.29	0.0022
		KAN	6(59)	5.33 ± 0.88	0.67 ± 0.21	66.2 ± 1.00	0.0022
		KK	6(60)	3.83 ± 1.01	0	38.3 ± 10.4	0.0065
		NT	6(60)	4.33 ± 0.67	0.33 ± 0.21	44.6 ± 7.38	0.0541
		SK	6(59)	5.00 ± 0.58	0	75.0 ± 3.42	0.0022
		ST	6(60)	6.33 ± 0.33	0.67 ± 0.21	62.0 ± 5.17	0.0022
	127.8	CM	6(60)	4.67 ± 1.05	0.33 ± 0.21	100	0.0022
		KAN	6(60)	4.33 ± 0.56	0	66.6 ± 5.58	0.0022
		KK	6(60)	5.83 ± 0.48	0.33 ± 0.21	84.6 ± 3.31	0.0022
		NT	6(60)	7.34 ± 0.33	0	83.3 ± 3.33	0.0022
		SK	6(60)	4.67 ± 0.47	0	93.3 ± 2.10	0.0022
		ST	6(61)	7.67 ± 0.42	0.33 ± 0.21	84.2 ± 4.48	0.0022

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] CM, Chiang Mai strain; KAN, Kanchanaburi strain; KK, Khonkaen strain; NT, Nonthaburi strain; SK, Songkhla strain; ST, Satun strain.

[3] For each trail, percent escaping after correction using Abbott's formula.

[4] *P*-values are from Wilcoxon 2-sample test for difference between the number escaping in a chemical treated assembly and in an acetone-treated (control) assembly.

Table 21 Escape responses of six *Aedes aegypti*[1] strains in the contact irritancy assay against three doses of DDT.

Chemical	Dose (nm/cm^2)	Strain[2]	No. of trials (mosq.)	Number escaping (mean ± SE)		% Escaping[3] (mean ± SE)	P[4]
				Treated	Control		
DDT							
	5.6	CM	6(60)	4.50 ± 0.72	1.00 ± 0.36	38.6 ± 7.99	0.0108
		KAN	6(60)	1.67 ± 0.61	1.00 ± 0.37	7.92 ± 4.10	0.5368
		KK	6(60)	1.55 ± 0.34	1.00 ± 0.37	5.37 ± 2.41	0.4697
		NT	6(60)	1.33 ± 0.61	0.67 ± 0.42	7.50 ± 4.03	0.4697
		SK	6(60)	2.83 ± 0.70	0.33 ± 0.21	25.0 ± 8.94	0.0216
		ST	6(60)	1.83 ± 0.60	0.33 ± 0.21	15.7 ± 5.32	0.0801
	56.4	CM	6(60)	4.33 ± 0.42	1.00 ± 0.36	38.0 ± 7.00	0.0022
		KAN	6(60)	1.67 ± 0.61	0.33 ± 0.21	11.6 ± 6.54	0.0455
		KK	6(60)	1.34 ± 0.21	0.33 ± 0.21	8.70 ± 3.12	0.0216
		NT	6(60)	1.17 ± 0.40	0	11.6 ± 4.01	0.0606
		SK	6(60)	3.00 ± 1.00	0.33 ± 0.21	29.4 ± 9.56	0.0130
		ST	6(59)	1.33 ± 0.33	0.67 ± 0.21	8.52 ± 5.58	0.2381
	564.2	CM	6(59)	3.33 ± 0.33	0.67 ± 0.42	31.2 ± 5.84	0.0065
		KAN	6(60)	2.00 ± 0.44	0	20.0 ± 4.42	0.0022
		KK	6(60)	2.17 ± 0.48	0.67 ± 0.21	19.8 ± 3.50	0.0108
		NT	6(60)	1.17 ± 0.31	0	11.6 ± 3.07	0.0152
		SK	6(60)	2.00 ± 0.26	0	18.5 ± 5.37	0.0022
		ST	6(60)	2.17 ± 0.60	0	20.5 ± 7.88	0.0152

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] CM, Chiang Mai strain; KAN, Kanchanaburi strain; KK, Khonkaen strain; NT, Nonthaburi strain; SK, Songkhla strain; ST, Satun strain.

[3] For each trail, percent escaping after correction using Abbott's formula.

[4] *P*-values are from Wilcoxon 2-sample test for difference between the number escaping in a chemical treated assembly and in an acetone-treated (control) assembly.

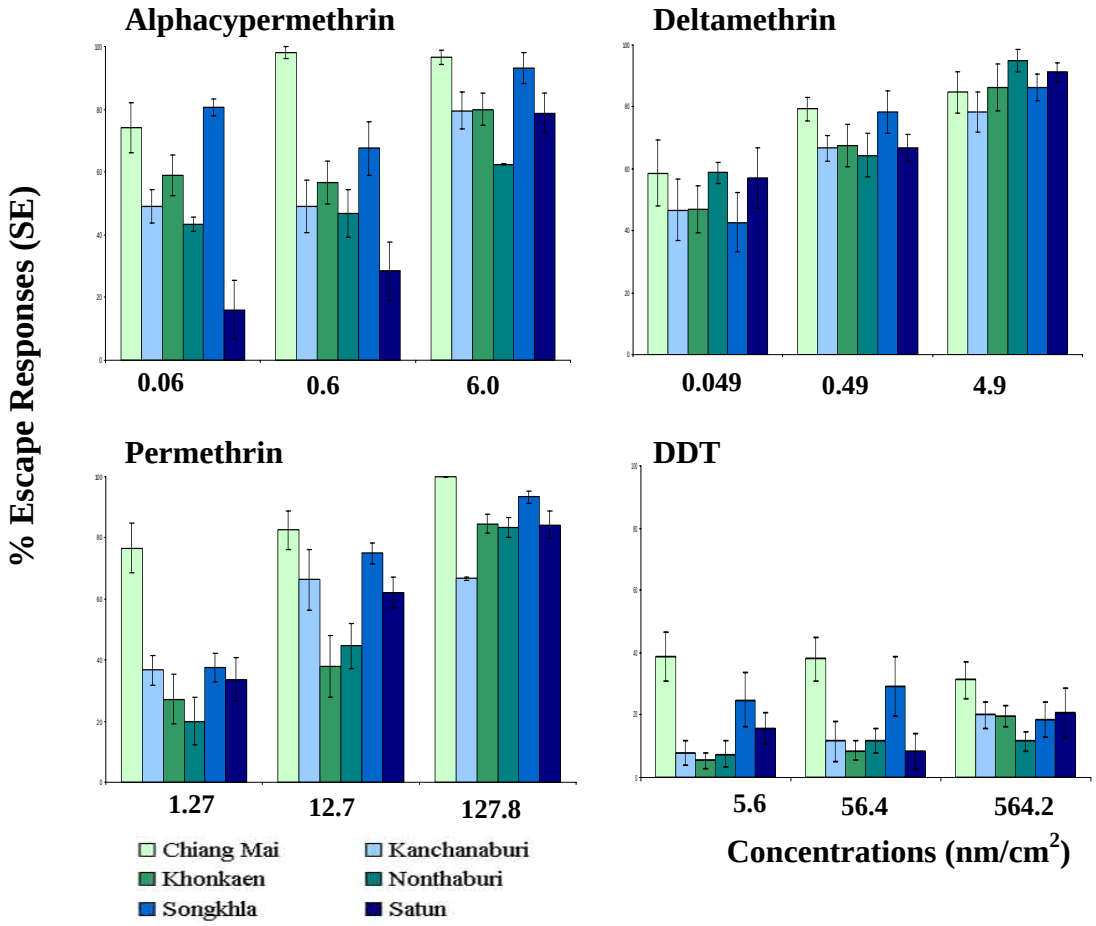

Figure 23 Escape responses of six *Ae aegypti* field strains (female, 4-6 day-old) in the contact irritancy assay to varying concentrations to alphacypermethrin, deltamethrin, permethrin and DDT.

2. Spatial repellency assay

Repellent responses of *Ae. aegypti* in the spatial repellency assay are presented in Tables 22 – 25 and Figure 24. The mean percent responding of *Ae. aegypti* test strains fluctuated among treatment concentrations and test compounds (Table 22 – 25). No statistically significant spatial repellent response was observed in any of the test strains for any treatment concentration of alphacypermethrin ($P>0.05$) (Table 22). In contrast, significant spatial repellent responses were observed at 0.49 and 4.9 nm/cm^2 against deltamethrin in the Satun strain ($P<0.05$) (Table 23). Permethrin elicited significant responses at 1.27 nm/cm^2 in the Songkhla strain, and at 12.7 nm/cm^2 in the Khonkaen strain and at 127.8 nm/cm^2 in both the Nonthaburi and Songkhla strains, respectively ($P<0.05$) (Table 24). In addition, permethrin was the only test compound to elicit a significant spatial repellent responses at the lowest test concentration for each chemical. On the other hand, a significant spatial repellent response was documented at the 564.2 nm/cm^2 treatment concentration of DDT in most of the test strains (Kanchanaburi, Khonkaen, Songkhla and Satun) (Table 25). It is important to note that although the Chiang Mai strain did not show significant at the 564.2 nm/cm^2 dose of DDT, the percent responding was still high with a positive SAI value. At the treatment concentration of 56.4 nm/cm^2 only the Chiang Mai strain showed significant spatial repellent response ($P<0.05$).

Table 22 Responses of six *Aedes aegypti*[1] strains in the spatial repellency assay against three doses of alphacypermethrin.

Chemical	Dose (nm/cm^2)	Strain [2]	No. of trials (mosq.)	Mean percent responding (SE)	Mean SAI[3] (SE)	SR[4]	*P>S*
alphacypermethrin							
-	0.06	CM	9(180)	17.7 (3.64)	-0.29 (0.20)	-8.50	0.2500
		KAN	9(181)	26.1 (3.57)	-0.18 (0.12)	-10.0	0.1875
		KK	9(176)	32.1 (3.94)	-0.06 (0.20)	-1.50	0.8672
		NT	9(181)	18.8 (4.71)	-0.25 (0.23)	-6.50	0.3438
		SK	9(182)	19.7 (2.12)	-0.38 (0.15)	-11.5	0.0625
		ST	10(198)	29.8 (3.38)	0.30 (0.19)	12.5	0.1719
	0.6	CM	9(181)	14.3 (3.68)	0.12 (0.22)	2.50	0.6250
		KAN	9(179)	23.9 (3.98)	0.10 (0.21)	2.00	0.8125
		KK	9(180)	18.8 (2.98)	0.23 (0.14)	9.00	0.1719
		NT	9(179)	26.8 (3.75)	0.17 (0.14)	6.00	0.3750
		SK	9(180)	23.2 (3.76)	0.16 (0.27)	4.50	0.6328
		ST	9(180)	17.2 (4.87)	0.24 (0.24)	6.50	0.4063
	6.0	CM	9(180)	18.9 (2.20)	0.17 (0.15)	5.00	0.4063
		KAN	9(180)	24.5 (4.62)	-0.19 (0.18)	-7.50	0.2344
		KK	9(180)	27.2 (4.72)	-0.02 (0.25)	-1.00	0.9531
		NT	9(179)	13.4 (3.04)	-0.19 (0.15)	-3.50	0.4375
		SK	9(181)	31.1 (5.29)	-0.28 (0.13)	-10.5	0.0938
		ST	9(179)	22.0 (3.64)	-0.02 (0.16)	-0.50	1.0000

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] CM, Chiang Mai strain; KAN, Kanchanaburi strain; KK, Khonkaen strain; NT, Nonthaburi strain; SK, Songkhla strain; ST, Satun strain.

[3] SAI, spatial activity index. see text for detail.

[4] SR, signed-rank statistic derived through PROC UNIVERIATE (SAS Institute 2002)

Table 23 Responses of six *Aedes aegypti*[1] strains in the spatial repellency assay against three doses of deltamethrin.

Chemical	Dose (nm/cm^2)	Strain[2]	No. of trials (mosq.)	Mean percent responding (SE)	Mean SAI[3] (SE)	SR [4]	*P>S*
deltamethrin							
	0.049	CM	9(183)	23.5 (3.45)	-0.07 (0.21)	-1.50	0.8672
		KAN	9(179)	23.5 (5.30)	0.14 (0.19)	4.00	0.6172
		KK	9(178)	32.4 (3.13)	0.13 (0.16)	3.50	0.6250
		NT	9(178)	24.7 (1.64)	0.05 (0.16)	0	1.0000
		SK	9(181)	23.8 (3.31)	0.11 (0.13)	6.00	0.3750
		ST	9(177)	23.9 (4.45)	0.33 (0.21)	8.00	0.2031
	0.49	CM	9(181)	22.6 (3.84)	-0.09 (0.16)	-3.00	0.6719
		KAN	9(179)	14.1 (3.91)	0.13 (0.27)	3.00	0.7656
		KK	9(179)	30.0 (3.43)	0.05 (0.15)	1.50	0.8438
		NT	9(179)	21.8 (3.29)	0.20 (0.13)	5.00	0.2500
		SK	9(179)	26.9 (4.92)	0.11 (0.22)	4.50	0.5625
		ST	9(177)	25.9 (4.80)	0.53 (0.12)	18.0	0.0078
	4.9	CM	9(180)	31.6 (2.64)	-0.22 (0.14)	-9.00	0.2500
		KAN	9(180)	26.1 (4.84)	0.11 (0.12)	3.50	0.5000
		KK	9(179)	23.0 (2.57)	0.30 (0.22)	8.50	0.3359
		NT	9(179)	26.7 (4.62)	0.03 (0.14)	4.50	0.4375
		SK	9(184)	25.7 (3.47)	0.35 (0.21)	12.5	0.1523
		ST	9(177)	28.6 (4.54)	0.53 (0.12)	18.0	0.0078

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] CM, Chiang Mai strain; KAN, Kanchanaburi strain; KK, Khonkaen strain; NT, Nonthaburi strain; SK, Songkhla strain; ST, Satun strain.

[3] SAI, spatial activity index. see text for detail.

[4] SR, signed-rank statistic derived through PROC UNIVERIATE (SAS Institute 2002)

Table 24 Responses of six *Aedes aegypti*[1] strains in the spatial repellency assay against three doses of permethrin.

Chemical	Dose (nm/cm^2)	Strain[2]	No. of trials (mosq.)	Mean percent responding (SE)	Mean SAI[3] (SE)	SR[4]	*P>S*
permethrin							
	1.27	CM	9(179)	30.6 (4.17)	0.06 (0.13)	2.50	0.7185
		KAN	9(179)	11.7 (2.37)	0.06 (0.29)	0.50	1.0000
		KK	9(181)	27.1 (4.20)	0.07 (0.17)	1.50	0.8750
		NT	9(178)	23.2 (4.42)	0.11 (0.16)	3.50	0.6719
		SK	9(179)	34.5 (5.83)	0.34 (0.06)	22.5	0.0039
		ST	9(177)	19.8 (3.26)	0.10 (0.23)	3.50	0.7227
	12.7	CM	9(181)	31.1 (4.43)	0.15 (0.14)	7.00	0.3672
		KAN	9(178)	20.8 (4.20)	0.14 (0.16)	4.00	0.5000
		KK	9(179)	25.1 (3.71)	0.38 (0.07)	18.0	0.0078
		NT	9(180)	22.2 (4.34)	0.30 (0.20)	9.00	0.1563
		SK	9(180)	27.9 (3.33)	0.38 (0.17)	14.0	0.0547
		ST	9(177)	21.8 (4.68)	0.21 (0.20)	5.50	0.3125
	127.8	CM	9(179)	29.1 (2.74)	0.35 (0.12)	12.0	0.1016
		KAN	9(178)	18.4 (4.86)	0.19 (0.21)	4.50	0.4375
		KK	9(180)	22.2 (3.70)	0.27 (0.21)	8.00	0.2187
		NT	9(179)	20.1 (4.62)	0.37 (0.12)	10.5	0.0313
		SK	9(180)	22.2 (4.70)	0.77 (0.08)	22.5	0.0039
		ST	9(179)	22.9 (2.30)	0.28 (0.19)	11.0	0.2266

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] CM, Chiang Mai strain; KAN, Kanchanaburi strain; KK, Khonkaen strain; NT, Nonthaburi strain; SK, Songkhla strain; ST, Satun strain.

[3] SAI, spatial activity index. see text for detail.

[4] SR, signed-rank statistic derived through PROC UNIVERIATE (SAS Institute 2002)

Table 25 Responses of six *Aedes aegypti*[1] strains in the spatial repellency assay against three doses of DDT.

Chemical	Dose (nm/cm^2)	Strain[2]	No.of trials (mosq.)	Mean percent responding (SE)	Mean SAI[3] (SE)	SR[4]	*P>S*
DDT							
	5.6	CM	9(180)	13.3 (3.91)	0.05 (0.19)	1.00	0.8750
		KAN	9(180)	19.4 (3.04)	0.09 (0.22)	2.50	0.7969
		KK	9(176)	32.2 (3.72)	0.05 (0.09)	1.50	0.8125
		NT	9(180)	19.1 (5.85)	0.61 (0.17)	3.00	0.5938
		SK	9(179)	34.1 (4.53)	0.04 (0.11)	1.00	0.9453
		ST	9(180)	26.6 (4.58)	-0.50 (0.14)	0	1.0000
	56.4	CM	9(179)	17.9 (3.28)	0.69 (0.16)	14.0	0.0156
		KAN	9(180)	23.8 (2.95)	0.22 (0.22)	6.50	0.4297
		KK	9(179)	32.4 (4.48)	0.30 (0.13)	11.0	0.0781
		NT	9(180)	16.6 (3.91)	0.16 (0.18)	3.50	0.3750
		SK	9(178)	33.7 (4.08)	0.17 (0.14)	7.00	0.3828
		ST	10(198)	22.2 (3.43)	0.04 (0.21)	1.00	0.9063
	564.2	CM	9(180)	27.2 (4.65)	0.43 (0.18)	15.5	0.0703
		KAN	9(180)	28.5 (4.06)	0.25 (0.09)	12.0	0.0469
		KK	9(179)	36.4 (4.81)	0.29 (0.09)	10.5	0.0313
		NT	9(179)	13.9 (3.31)	0.34 (0.22)	8.50	0.1719
		SK	9(178)	31.6 (5.35)	0.38 (0.14)	13.0	0.0313
		ST	9(178)	30.2 (5.31)	0.34 (0.12)	16.0	0.0234

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] CM, Chiang Mai strain; KAN, Kanchanaburi strain; KK, Khonkaen strain; NT, Nonthaburi strain; SK, Songkhla strain; ST, Satun strain.

[3] SAI, spatial activity index. see text for detail.

[4] SR, signed-rank statistic derived through PROC UNIVERIATE (SAS Institute 2002)

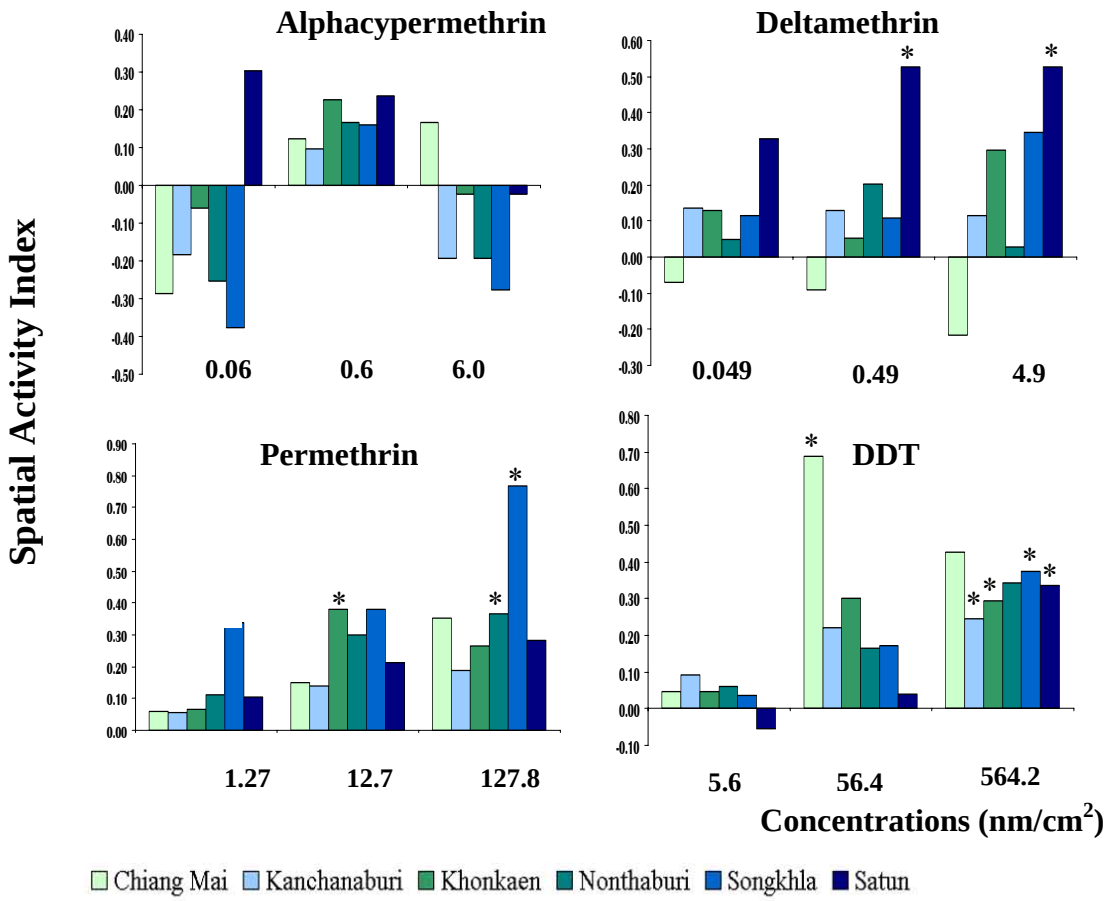

Figure 24 Spatial Repellent responses of six *Ae. aegypti* field strains (female, 4-6 day-old) to varying concentrations of alphacypermethrin, deltamethrin, permethrin and DDT.

* Statistic significant (P<0.05) differences in percent responding between control and treatment each test strain and compound.

3. Toxicity assay

Percent 1 h knockdown and 24 h mortality of six *Ae. aegypti* test strains against varying concentrations of alphacypermethrin, deltamethrin, permethrin and DDT are presented in Table 26 – 29 and Figure 25. The three synthetic pyrethroids evaluated were highly toxic to most strains at all doses while DDT showed comparatively lower toxic action to all test strains (Figure 25). In general, the 24h mortality rate of *Ae. aegypti* strains to alphacypermethrin, deltamethrin, permethrin and DDT, indicated an increase in mortality with increasing concentration (Table 26 – 29). Percent 24 h mortality for all test strains against the two highest concentrations of alphacypermethrin, deltamethrin and permethrin showed higher than 50%, the lowest mortality (52.6% mortality) was recorded at 0.6 nm/cm^2 of alphacypermethrin for Khonkaen strain (Table 26 – 29). However, 52.6% mortality of *Ae. aegypti* against to synthetic pyrethroids higher than the mortality (42.1% mortality) was recorded at the highest concentration of DDT. No statistically significant differences were indicated in mortality among the three treatment concentrations of DDT within most test strains (P>0.05), except the Khonkaen and Satun strains (P <0.05) (Table 31 – 32).

Among the synthetic pyrethroids tested the lowest concentration of deltamethrin showed higher rates of knockdown (59.6 – 92.4% range) when compared to alphacypermethrin (34.3 – 86.0% range) and permethrin (5.53 – 98.2% range) at the same dose (Table 26 – 28). Although, low knockdown occurred at the two lowest concentrations of permethrin, but high mortalities were recorded (Table 28). The highest concentrations of alphacypermethrin, deltamethrin and permethrin resulted in nearly 100% knockdown, ranging from 91.6 – 100%, 94.3 – 100% and 96.8 – 100%, respectively. Overall, the lowest and highest rates of knockdown at all treatment concentrations of alphacypermethrin, deltamethrin and permethrin were observed in the Satun and Chiang Mai strains, respectively, except at 6.0 nm/cm^2 of alphacypermethrin where the highest knockdown was observed in Kanchanaburi strain (Table 26 – 28). DDT gave consistent modest levels of knockdown at all treatment concentrations for all test strains (Table 29).

Table 26 Knockdown (KD) at 1 h and mortality (MORT) at 24 h of six *Aedes aegypti*[1] strains in the toxicity assay against varying doses of alphacypermethrin.

Chemical	Dose (nm/cm^2)	Strain	No. of trials (mosq.)	1 h KD (mean % ± SE)	24 h MORT (mean % ± SE)
alpha-cypermethrin	0.06	Chiang Mai	6(117)	86.0 ± 4.08	50.9 ± 9.92
		Kanchanaburi	6(110)	58.3 ± 8.86	17.1 ± 1.66
		Khonkaen	6(120)	60.0 ± 9.35	22.4 ± 6.15
		Nonthaburi	6(119)	39.6 ± 9.97	9.17 ± 5.07
		Songkhla	6(118)	58.9 ± 10.1	49.6 ± 10.5
		Satun	6(118)	34.3 ± 11.2	22.0 ± 7.00
alpha-cypermethrin	0. 6	Chiang Mai	6(120)	99.1 ± 0.83	100
		Kanchanaburi	6(119)	91.6 ± 2.11	80.0 ±5.63
		Khonkaen	6(119)	96.1 ± 2.81	52.6 ± 6.26
		Nonthaburi	6(120)	93.4 ± 1.55	65.2 ± 3.20
		Songkhla	6(118)	93.2 ± 2.47	63.5 ± 6.49
		Satun	6(128)	83.3 ± 4.37	61.9 ± 3.65
alpha-cypermethrin	6.0	Chiang Mai	6(120)	99.1 ± 0.83	100
		Kanchanaburi	6(120)	100	100
		Khonkaen	6(120)	99.1 ± 0.83	90.8 ± 3.00
		Nonthaburi	6(120)	91.6 ± 2.47	85.8 ± 3.75
		Songkhla	6(120)	96.6 ± 2.11	90.0 ± 4.47
		Satun	6(121)	93.5 ± 1.96	98.4 ± 0.98

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

Table 27 Knockdown (KD) at 1 h and mortality (MORT) at 24 h of six *Aedes aegypti*[1] strains in the toxicity assay against varying doses of deltamethrin.

Chemical	Dose (nm/cm^2)	Strain	No. of trials (mosq.)	1 h KD (mean % ± SE)	24 h MORT (mean % ± SE)
Deltamethrin	0.049	Chiang Mai	6(118)	92.4 ± 1.75	92.3 ± 1.76
		Kanchanaburi	6(119)	69.9 ± 7.37	62.2 ± 4.53
		Khonkaen	6(119)	66.2 ± 7.94	23.4 ± 3.96
		Nonthaburi	6(120)	60.0 ± 8.27	31.6 ± 7.15
		Songkhla	6(120)	76.6 ± 6.02	47.4 ± 2.88
		Satun	6(118)	59.6 ± 7.51	28.1 ± 6.06
Deltamethrin	0.49	Chiang Mai	6(120)	100	98.3 ± 1.67
		Kanchanaburi	6(119)	94.3 ± 2.07	77.7 ± 4.30
		Khonkaen	6(119)	89.7 ± 4.88	88.2 ± 4.58
		Nonthaburi	6(120)	94.1 ± 2.00	90.9 ± 3.00
		Songkhla	6(127)	80.4 ± 7.39	77.0 ± 5.75
		Satun	6(129)	80.8 ± 3.39	84.1 ± 2.08
Deltamethrin	4.9	Chiang Mai	6(120)	100	100
		Kanchanaburi	6(119)	99.1 ± 0.83	100
		Khonkaen	6(119)	96.6 ± 2.11	99.1 ± 0.83
		Nonthaburi	6(120)	97.5 ± 1.70	100
		Songkhla	6(119)	99.1 ± 0.83	100
		Satun	6(121)	94.3 ± 1.95	99.1 ± 0.83

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

Table 28 Knockdown (KD) at 1 h and mortality (MORT) at 24 h of six *Aedes aegypti*[1] strains in the toxicity assay against varying doses of permethrin.

Chemical	Dose (nm/cm^2)	Strain	No. of trials (mosq.)	1 h KD (mean % ± SE)	24 h MORT (mean % ± SE)
Permethrin	1.27	Chiang Mai	6(118)	98.2 ± 1.75	96.5 ± 2.16
		Kanchanaburi	69120)	52.5 ± 8.82	47.5 ± 7.27
		Khonkaen	6(117)	82.6 ± 6.65	49.3 ± 3.42
		Nonthaburi	6(120)	33.3 ± 4.77	17.5 ± 4.61
		Songkhla	6(119)	78.4 ± 2.00	54.9 ± 4.32
		Satun	6(121)	5.53 ± 2.54	7.04 ± 3.46
Permethrin	12.7	Chiang Mai	6(119)	100	100
		Kanchanaburi	6(119)	99.1 ± 0.83	100
		Khonkaen	6(119)	98.2 ± 6.65	98.3 ± 1.05
		Nonthaburi	6(118)	98.4 ± 1.00	98.4 ± 1.51
		Songkhla	6(119)	100	98.4 ± 1.51
		Satun	6(125)	54.2 ± 6.78	43.9 ± 4.76
Permethrin	127.8	Chiang Mai	6(119)	100	100
		Kanchanaburi	6(120)	100	100
		Khonkaen	6(119)	100	100
		Nonthaburi	6(119)	100	99.1 ± 0.83
		Songkhla	6(120)	99.1 ± 0.83	100
		Satun	6(119)	96.8 ± 3.44	96.7 ± 2.07

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

Table 29 Knockdown (KD) at 1 h and mortality (MORT) at 24 h of six *Aedes aegypti*[1] strains in the toxicity assay against varying doses of DDT**.**

Chemical	Dose (nm/cm^2)	Strain	No. of trials (mosq.)	1 h KD (mean % ± SE)	24 h MORT (mean % ± SE)
DDT	5.6	Chiang Mai	6(120)	45.9 ± 5.62	36.2 ± 5.75
		Kanchanaburi	6(120)	8.18 ± 2.09	3.26 ± 1.66
		Khonkaen	6(123)	3.25 ± 1.65	1.02 ± 1.02
		Nonthaburi	6(123)	0	0
		Songkhla	6(120)	44.1 ± 3.51	10.8 ± 4.73
		Satun	6(120)	11.6 ± 4.01	5.02 ± 1.30
DDT	56.4	Chiang Mai	6(119)	48.9 ± 14.0	33.4 ± 13.6
		Kanchanaburi	6(120)	5.00 ± 2.24	4.17 ± 2.01
		Khonkaen	6(119)	14.2 ± 5.67	10.0 ± 2.23
		Nonthaburi	6(122)	0	0.83 ± 0.83
		Songkhla	6(118)	10.0 ± 5.61	12.5 ± 5.71
		Satun	6(120)	4.17 ± 1.54	7.50 ± 2.81
DDT	564.2	Chiang Mai	6(117)	41.6 ± 4.16	38.9 ± 7.48
		Kanchanaburi	6(120)	8.33 ± 3.33	10.0 ± 2.58
		Khonkaen	6(121)	15.5 ± 6.28	13.1 ± 3.90
		Nonthaburi	6(120)	0	0.83 ± 0.83
		Songkhla	6(120)	2.50 ± 1.70	12.5 ± 1.12
		Satun	6(119)	32.9 ± 4.95	42.1 ± 6.72

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

Table 30 Percent 24 h mortality (MORT) of six *Aedes aegypti* [1] from Chiang Mai and Kanchanaburi strains in the toxicity assay against varying doses of alphacypermethrin, deltamethrin, permethrin and DDT.

Strain	Chemical	Dose (nm/cm^2)	No. of trials (mosq.)	24 h MORT[2] (mean % ± SE)
Chiang Mai	alpha-	0.06	6(117)	50.9 ± 9.92 b
	cypermethrin	0.6	6(120)	100 a
		6	6(120)	100 a
	deltamethrin	0.049	6(118)	92.3 ± 1.76 b
		0.49	6(120)	98.3 ± 1.67 a
		4.9	6(120)	100 a
	permethrin	1.27	6(118)	96.5 ± 2.16 a
		12.7	6(119)	100 a
		127.8	6(119)	100 a
	DDT	5.6	6(120)	36.2 ± 5.75 a
		56	6(119)	33.4 ± 13.56 a
		564.2	6(117)	38.9 ± 7.48 a
Kanchanaburi	alpha-	0.06	6(110)	17.1 ± 4.15 c
	cypermethrin	0.6	6(119)	80.0 ± 5.63 b
		6	6(120)	100 a
	deltamethrin	0.049	6(119)	62.2 ± 4.53 c
		0.49	6(119)	77.7 ± 4.30 b
		4.9	6(119)	100 a
	permethrin	1.27	6(120)	47.5 ± 7.27 b
		12.7	6(119)	100 a
		127.8	6(120)	100 a
	DDT	5.6	6(120)	3.26 ± 1.66 a
		56	6(120)	4.17 ± 2.01a
		564.2	6(120)	10.0 ± 2.58 a

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] means with same letters within each strain and chemicals treatment are not significantly different at the 0.05 level using Duncan

Table 31 Percent 24 h mortality (MORT) of six *Aedes aegypti* [1] from Khonkaen and Nonthaburi strains in the toxicity assay against varying doses of alphacypermethrin, deltamethrin, permethrin and DDT.

Strain	Chemical	Dose (nm/cm^2)	No. of trials (mosq.)	24 h MORT[2] (mean % ± SE)
Khonkaen	alpha-	0.06	6(120)	22.4 ± 6.15 c
	cypermethrin	0.6	6(119)	52.6 ± 6.26 b
		6	6(120)	90.8 ± 3.00 a
	deltamethrin	0.049	6(119)	23.4 ± 3.96 c
		0.49	6(119)	88.2 ± 4.58 b
		4.9	6(119)	99.1 ± 0.83 a
	permethrin	1.27	6(117)	49.3 ± 3.42 b
		12.7	6(119)	98.3 ± 1.05 a
		127.8	6(119)	100 a
	DDT	5.6	6(123)	1.02 ± 1.02 b
		56	6(119)	10.0 ± 2.23 a
		564.2	6(121)	13.1 ± 3.90 a
Nonthaburi	alpha-	0.06	6(119)	9.10 ± 5.07 c
	cypermethrin	0.6	6(120)	65.2 ± 3.20 b
		6	6(120)	85.8 ± 3.75 a
	deltamethrin	0.049	6(120)	31.6 ± 7.15 c
		0.49	6(120)	90.9 ± 3.00 b
		4.9	6(120)	100 a
	permethrin	1.27	6(120)	17.5 ± 4.61 b
		12.7	6(118)	98.4 ± 1.51a
		127.8	6(119)	99.1 ± 0.83 a
	DDT	5.6	6(123)	0 a
		56	6(122)	0.83 ± 0.83 a
		564.2	6(120)	0.83 ± 0.83 a

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] means with same letters within each strain and chemicals treatment are not significantly different at the 0.05 level using Duncan

Table 32 Percent 24 h mortality (MORT) of six *Aedes aegypti* [1] from Songkhla and Satun strains in the toxicity assay against varying doses of alphacypermethrin, deltamethrin, permethrin and DDT.

Strain	Chemical	Dose (nm/cm^2)	No. of trials (mosq.)	24 h MORT[2] (mean % ± SE)
Songkhla	alpha-	0.06	6(118)	49.6 ± 10.48 b
	cypermethrin	0.6	6(118)	63.5 ± 6.49 b
		6	6(120)	90.0 ± 4.47 a
	deltamethrin	0.049	6(120)	47.4 ± 2.88 c
		0.49	6(127)	77.0 ± 5.75 b
		4.9	6(119)	100 a
	permethrin	1.27	6(119)	54.9 ± 4.32 b
		12.7	6(119)	98.4 ± 1.51 a
		127.8	6(120)	100 a
	DDT	5.6	6(120)	10.8 ± 4.73 a
		56	6(118)	12.5 ± 5.71 a
		564.2	6(120)	12.5 ± 1.12 a
Satun	alpha-	0.06	6(118)	22.0 ± 7.00 c
	cypermethrin	0.6	6(128)	61.9 ± 3.65 b
		6	6(121)	98.4 ± 0.98 a
	deltamethrin	0.049	6(118)	28.1 ± 6.06 c
		0.49	6(129)	84.1 ± 2.08 b
		4.9	6(121)	99.1 ± 0.83 a
	permethrin	1.27	6(121)	7.04 ± 3.46 c
		12.7	6(125)	43.9 ± 4.76 b
		127.8	6(119)	96.7 ± 2.07 a
	DDT	5.6	6(120)	5.02 ± 1.30 b
		56	6(120)	7.50 ± 2.81 b
		564.2	6(119)	42.1 ± 6.72 a

[1] F1 – F2 female, 4-6 day-old unfed, sugar starved 24 h pre-test

[2] means with same letters within each strain and chemicals treatment are not significantly different at the 0.05 level using Duncan

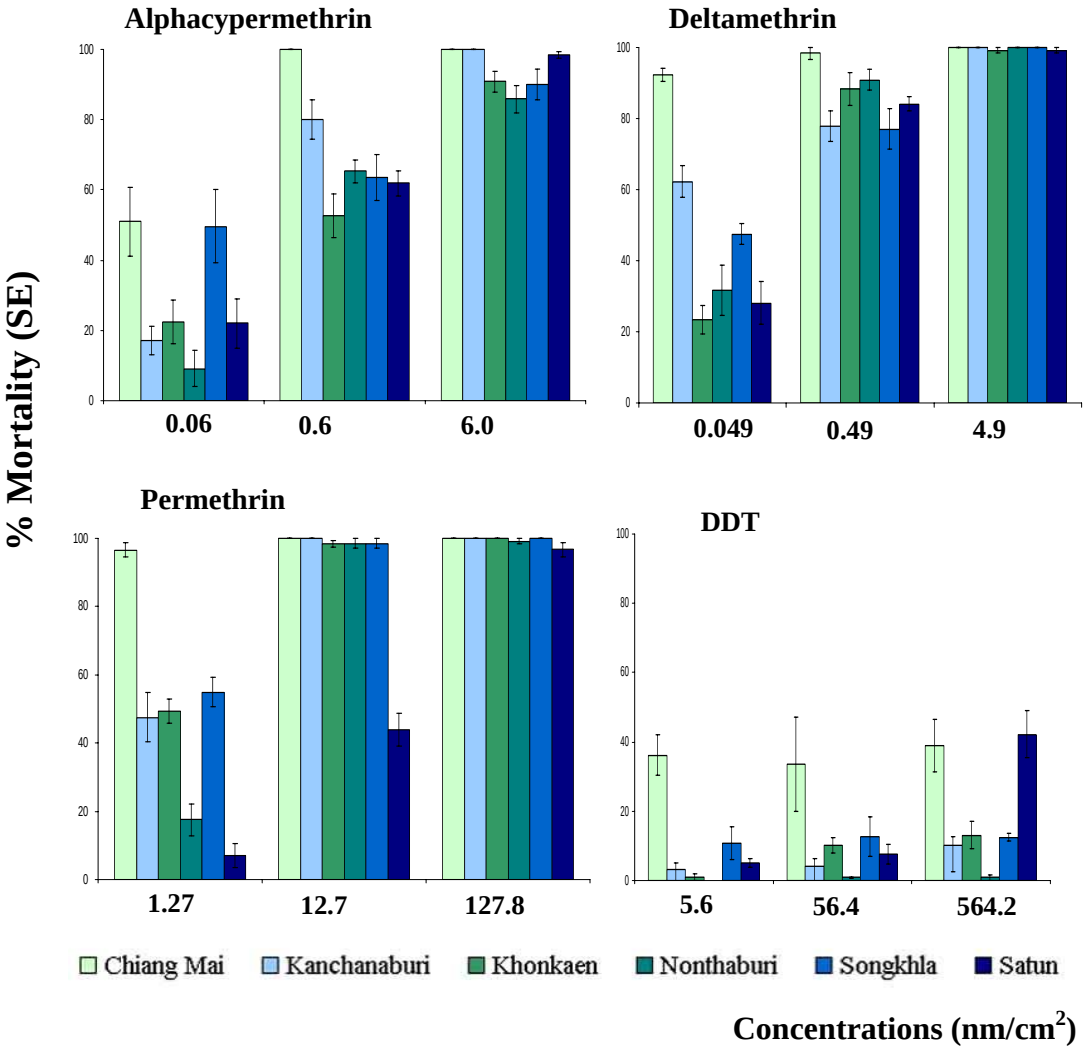

Figure 25 Twenty four hours mortality rates of six *Ae. aegypti* field strains (female, 4-6 day-old) in the toxicity assay to varying concentrations of alphacypermethrin, deltamethrin, permethrin and DDT.

Discussion

Part 1. Susceptibility test of female *Aedes aegypti* strains to insecticide compounds

Arthropod-borne diseases are an ever-increasing cause of death and suffering worldwide (WHO, 2007a). Thailand is endemic for several vector-borne diseases, including malaria, dengue fever (DF) and dengue haemorrhagic fever (DHF), Japanese encephalitis and lymphatic Filariasis (MOPH, 1990, 2003). Significant growth in the human population combined with demographic movement to urban residential areas and increased tourism-based facilities have led to tremendous deforestation, irrigation and urbanization all of which effect disease transmission dynamics. Changes in the surrounding environment due to global warming have also favored conditions for increasing mosquito populations that vector diseases (Chareonviriyaphap *et al*., 1999; Sathatriphop *et al*., 2006). Despite research progress, a completely effective vaccine against dengue and malaria is not yet available. The prevention of these diseases has relied mainly on the reduction of human-vector contact using chemical compounds.

The insecticide susceptibility level of mosquitoes is considered one of the major factors influencing the success of vector control. For years, chemical companies have been developing synthetic chemicals, especially synthetic pyrethroids. These synthetic pyrethroids have demonstrated great promise for mosquito vector control because of their low toxicity to humans and great potency at low doses, quickly immobilizing and killing insects (Prasittisuk, 1994; Grieco *et al*., 2007). However, overtime, resistance to these synthetic compounds has been recorded in several species of arthropods, including *Ae. aegypti* populations in Thailand (Chareonviriyaphap *et al*., 1999; Somboon *et al.,* 2003; Paeporn *et al*., 2005; Jirakanjanakit *et al*., 2007). In this study, it was clearly seen that the majority of the six most field-collected *Ae. aegypti* strains demonstrated comparatively high levels of resistance to permethrin. These finding are similar to these reported by Jirakanchanakit *et al*. (2007) and Ponlawat *et al*. (2005) that several strains of *Ae. aegypti* across Thailand were resistant to permethrin. The reason for this may be

related to household products used for pest control. Permethrin is a common compound that is regularly used in Thai households for pest control (Paeporn *et al.,* 2004; Ponlawat *et al*., 2005) and impregnated bed nets for mosquito control (Chareonviriyaphap *et al*., 1999). In contrast, most *Ae. aegypti* strains have been found to be susceptible to deltamethrin, suggesting that this compound is still effective in control programs during dengue outbreaks.

Surprisingly, incipient resistance to alphacypermethrin was also detected in *Ae. aegypti* from Nonthaburi and Songkhla, where deltamethrin remains the mainstay of the dengue vector control program. Alphacypermethrin incipient resistance may have been arisen from previous synthetic pyrethroid, such as permethrin impregnated bed net, indoor residue deltamethrin house spraying in malaria control program usage in the area. Cross-resistance as a consequence of unintentional or extensive use of the same or related groups of compounds in mosquito populations has been reported elsewhere (Chareonviriyaphap *et al*., 1999, 2003b; Kongmee *et al*., 2004).

A high level of physiological resistance to DDT in *Ae. aegypti* could be related to the previous use of DDT in agriculture and public health (Chareonviriyaphap *et al*., 1999). Although DDT has been completely stopped for public health use since 2000 in Thailand, frequent indoor residual spraying for over 40 years may have resulted in the development of resistant genes which contribute to physiological resistance in a certain mosquito population. Resistance to malathion in *Ae. aegypti* strain from Khonkaen could also be related to the tremendous use of this compound in public health and Thai households for domestic pest control in the form of aerosols; however, several *Ae. aegypti* strains are still susceptible to malathion, suggesting that this compound may still be effective in controlling *Ae. aegypti* in Thailand.

Insecticide resistance should be monitored and evaluated on a routine basis and over a wide geographical range to include as many known vector species as possible. This monitoring should be part of an insecticide evaluation program aimed at the success of disease control activity. Early detection of operationally unacceptable levels of resistance can prompt public health authorities to take

appropriate steps to counter the potential reduced control efforts. In addition, control programs should remain aware of cross-resistance to the same or related synthetic compounds against mosquito populations and agricultural pests.

Part 2. Insecticide resistance mechanisms of *Aedes aegypti* strains

Vector control in Thailand has relied mainly on the reduction of human-vector contact by cleaning of water containers that serve as mosquito breeding site and by using chemical compounds. Several insecticides have been used in dengue control program in Thailand. DDT was first used for dengue control as an indoor residual spraying in Bangkok metropolitan area during 1960's (Jatanasen, 1966). The following 40 years of intensive use of DDT to control mosquitoes has led to the extensive selection of DDT resistance in *Ae. aegypti* throughout Thailand (Chareonviriyaphap *et al*., 1999; Yaicharoen *et al*., 2005, Jirakanchanakit *et. al*., 2007). DDT was completely withdrawn for public health use in 2000 with the replacement of organophosphates and synthetic pyrethroids (Chareonviriyaphap *et al.,* 1999). Several synthetic pyrethroids are available in the market for controlling household nuisance and vector mosquitoes, i.e., *Ae. aegypti* (Kongmee *et al*., 2004). These household products (aerosols, mosquito coils, mats, and liquid forms) containing various synthetic pyrethroids such as permethrin, deltamethrin, bifenthrin, *d*-tetramethrin, esbiothrin and allethrin have been widely used in most Thai homes (Paeporn, 1996; Jirakanjanakit *et al*., 2007; Thanispong *et al*., 2008). Heavy use of these synthetic pyrethroids has resulted in the development of insecticide resistance in field mosquito populations (Chareonviriyaphap *et al.,* 1999).

Mosquito populations may survive the toxic effect of insecticides by different physiological mechanisms, including target site modifications, especially the kdr (knockdown resistance) mutation (Brengues *et al*., 2003; Saveedra-rodriguez *et al*., 2007) and metabolic detoxification, i.e., higher activity of enzyme involved in the detoxification of insecticides (monooxygenases, esterases, Glutathione-S-transferase (GSTs). These enzymes showed to be involved in pyrethroid resistance in several mosquito species (Brogdon and McAllister 1998; Vulule *et al*., 1999). As the whole,

quantitative increases in these enzymes, due to gene amplification and/or over-expression of target genes, can result in the high levels of insecticide resistance (Mouches *et al*., 1990; Hemingway and Ranson, 2004; Strode *et al*., 2007).

Our results showed that monooxygenase activity was higher in almost permethrin resistant *Ae. aegypti* field strains compared to the susceptible Bora Bora strain, except one Chiang Mai strain which susceptible to permethrin. Monooxygenases showed to be associated with pyrethroid resistance in several mosquito species (Ocampo *et al*., 2000; Hemingway and Ranson, 2000; Brooke *et al*., 2001; Chareonviriyaphap *et al.,* 2003b). In our study, there was a 2.2-fold increase in monooxygenase activity in Songkhla strain compared to the Bora Bora strain which may partly explain the high level of permethrin resistance in this strain (27.4% resistance to permethrin). Significant increases in monooxygenase activity were also detected in two strains of Chaing Mai (2% resistance to permethrin) and Satun (34.6% resistance to permethrin) compared to the susceptible Bora Bora strain. Although, monooygenase was present in Chiang Mai strain, it did not reveal a clear pattern in relation to permethrin resistance. However, this case has been reported (Casimiro *et al*., 2006), the *Anopheles funestus* from Mozal location, in Mozambiqe, had elevated P450 estimates but fully pyrethroid susceptible by bioassay. Increasing enzyme activity significant in the Chiang Mai strain compared to the susceptible strain may due to many factors be involves in the mechanism. Several studies have revealed the capacity of insect detoxification enzymes to be induced by xenobiotics and the relationship between elevated detoxifying enzyme levels and tolerance to chemical insecticides (Suwanchaichinda and Brattsten, 2001, 2002; Hemingway *et al*., 2004; Enayati *et al*., 2005: Feyreisen, 2005: Boyer *et a*l., 2006; Poupadin *et al*., 2008). And the other factor may due to the epoxidation reaction in the mosquito. The epoxidation consist of adding an oxygen atom between C atoms in an unsaturated system. Cytochrome P450 is involved in epoxidation reactions. The reaction has the effect of increasing the toxicities of permethrin compound and higher monooxygenase level in this strain. Thus, it is possible that monooxygenase activity in Chiang Mai strain may cause by xenobiotics induction or the epoxidation reaction in mosquito physiological.

Less attention has paid to the effect of exposure to the other xenobiotics on the mosquito in Thailand.

Resistance to pyrethroids is not developing because of a single mechanism. Non-specific esterases have been reported to be involved in pyrethroid metabolism in insect, including mosquitoes (Brogdon and Borber, 1990; Mourya *et al*., 1993; Vulule et al., 1999), and could play a role in the metabolism of permethrin in *Ae. aegypti*. Even in some case, there is evidence that the significantly elevated level of α-esterase in the population of *Ae. aegypti* from Baja California, Mexico that play a role in the detoxification of permethrin (Flores *et al*., 2005). In our study, over-production of α and β-esterase was also observed in some strains of *Ae. Aegypti* (Khonkaen and Satun). Because these enzymes were shown to be responsible for high level of resistance to organophosphate and carbamate in mosquiotes (Oppenoorth, 1985; Beach *et al*., 1989), their implications in insecticide resistance in *Ae. Aegypti* strains from Thailand need to be further investigated.

Although all strains of *Ae. aegypti* demonstrated a strong resistance to DDT, level of resistance to permethrin varied according to the strain considered. In addition, physiological factors may vary among the strains and this may contribute to differences in insecticide resistance. Based on our findings, elevated monooxygenase activity is probably the main metabolic factor responsible for permethrin resistance. The use of synergists (PBO, DEF) to confirm/infirm the role of these detoxification enzymes in insecticide resistance is urgently needed.

Part 3. Contact irritancy and non-contact repellency behavioral responses of female *Aedes aegypti* strains to insecticide compounds using an excito-repellency system

There are three main chemical actions used to protect people from adult vectors of arthropod-borne diseases. These include contact irritancy, non-contact repellency and toxicity (Roberts *et al.,* 2000a; Grieco *et al*., 2007). Collectively, most research has focused on toxicity of chemicals whereas comparatively little research

has been performed to evaluate the irritant/repellent action of these chemicals on disease vectors. The lack of an appropriate test system in the study of mosquito behavioral responses other than toxicity has been one shortcoming to this information gap.

In 1997 a behavioral test system was developed to distinguish two types of behavioral responses, contact irritancy and non-contact repellency (Roberts *et al*., 1997b; Chareonviriyaphap *et al*., 1997). Recently, a more field-friendly version of the test system was developed (Chareonviriyaphap *et al*., 2002; Tanasinchayakul *et al*., 2006). Using this modified system, behavioral responses of several major mosquito vectors to various test compounds have been quantitatively investigated (Chareonviriyaphap *et al*., 2002, 2004; Kongmee *et al*., 2004; Muenworn *et al*., 2006; Sathantriphop *et al*., 2006; Polsomboon *et al*., 2008). A detailed database has been accumulated for the synthetic pyrethroids, the most successful modern chemicals, including deltamethrin, permethrin and lambda-cyhalothrin. In this study, we investigated the contact irritancy and non-contact repellency actions of alphacypermethrin, deltamethrin, permethrin and DDT, commonly used insecticides for vector-borne diseases.

Using the excito-repellency test chamber, six strains of *Ae. aegypti* showed varying degrees of mortality and escape responses from both contact irritancy and non-contact repellency tests. Results indicate a strong contact irritant effect of alphacypermethrin, deltamethrin and permethrin in all six test strains. In addition, most mosquitoes escaped from the treatment chamber before receiving a lethal dose of insecticide. Three strains from Chiang Mai, Kanchanaburi and Khonkaen showed a significant non-contact repellent response to DDT. A few of the strains showed a significant non-contact repellent response to the pyrethroids, however this response was weaker than contact irritancy. Combined these results indicate contact irritancy as a primary function of alphacypermethrin, deltamethrin and permethrin.

The strong non-contact repellency response to alphacypermethrin was observed in mosquitoes collected from the rural area of Chiang Mai province (Chiang

Mai strain). The non-contact repellency response to this insecticide by the Chiang Mai strain is unclear. The Chiang Mai strain was susceptible to three pyrethroids while the other strains show levels of resistance to alphacypermethrin. The unexpectedly strong escape response of this strain may be related to natural variation in behavioral responses or its specific insecticide susceptibility status. Another strong non-contact repellency response was observed in the Kanchanaburi strain, also collected from a rural area. As this strain was found to be susceptible to deltamethrin, the strong non-contact repellency response observed to deltamethrin may also be due to innate behavioral response variation or specific insecticide susceptibility status.

Exposure to field application rate of DDT elicited both contact irritancy and non-contact repellency in Chiang Mai, Kanchanaburi and Khonkaen test strains. However, the contact irritant action of DDT was weaker than alphacypermethrin, deltamethrin and permethrin. The greatest escape response in both contact irritancy and non-contact repellency trials was elicited from the Chiang Mai strain, however, contact irritancy responses were also observed in the Kanchanaburi and Satun strains. The Khonkaen, Nonthaburi and Songkhla *Ae. aegypti* strains showed an attenuated behavioral response to DDT in both contact irritancy and non-contact repellency trials. This variation in behavior may be due to strain-specific levels of DDT resistant characteristic of the populations. The Chiang Mai strain exhibited lower resistance to DDT than other strains (i.e., higher 24 h mortality rates), and was the only strain observed to have mortality after chemical exposure in the contact trials. However, the Kanchanaburi and Satun strains were also found to have a high level of DDT resistance but showed strong contact irritancy response against DDT. Therefore, data indicate that resistance mechanisms may not be directly associated with the contact irritancy behavioral responses in the mosquitoes. It is unclear if the attenuation of the behavioral response to DDT in the Khonkaen, Nonthaburi, and Songkhla strains may be influenced by the physiological resistance status. Studies have documented a link between partial resistance to deltamethrin and *Ae. aegypti* Cepu strain from Indonesia subsequently a weak behavioral response (Kongmee *et al*., 2004). However, it is important to note that the escape response patterns for the DDT resistant strain was still significantly greater than in control test chambers for both trial types indicating

that insecticide resistance and behavior may have separate modes of action. Other studies have shown similar results (Grieco *et al.,* 2007). This area of research requires further extensive evaluation.

An understanding of the contact irritancy and non-contact repellency actions of chemicals that are used to interfere with vector feeding behavioral pattern is a necessity when assessing the true effect of these compounds on disease control. Quantification of a chemical's primary action will help drive the optimization of currently available public health tools and innovative control methodologies. More research is needed to verify the behavioral responses of insecticides by many known dengue vector populations from different geographical areas (Chareonviriyaphap *et al*., 1997; Bortel *et al*., 2004; Potikasikorn *et al*., 2005). The knowledge will allow better decision-making on pesticide selection and application (Muirhead-Thomson 1960; Roberts *et al.,* 2000a, b; Grieco *et al*., 2007).

Part 4. Behavioral responses of *Aedes aegypti* strains to insecticide compounds using a High Throughput Screening System assay

There is no effective vaccine for dengue and dengue hemorrhagic fevers. The only way to prevent the disease is to prevent contact between human hosts and the *Ae. aegypti* vectors. The current strategy for dengue vector control is emphasizing the use of insecticide compounds. Such chemical control is focused on the toxicity of insecticides to the vector. Other chemical actions, besides toxicity are not reported, these include contact irritancy and spatial repellency. Characterizing chemical actions is required to provide baseline information for choice of what insecticide to use in control programs and methodologies.

A High Throughput Screening System (HTSS) was developed in 2004 for use in screening chemical properties, and provide quantifiable measures of behavioral responses using a relatively low number of replicates. It is compact in size and quickly transitions between replicates and behavioral test type (Grieco *et al*., 2005). Previous studies using the HTSS have reported in *Ae. aegypti* from Thailand (Grieco

et al., 2005, 2007). These studies demonstrated that the impact of insecticides on mosquito behavior is much more complex than just toxicity.

Six *Ae. aegypti* field strains exposed to alphacypermethrin, deltamethrin, permethrin and DDT using the HTSS in the current study showed varying behavioral responses, depending on type of exposure (CIA, SRA, TOX), insecticides and treatment concentrations. In general, all *Ae. aegypti* strains showed significant contact irritancy responses, and no spatial repellency activity, to alphacypermethrin, deltamethrin and permethrin at all test doses. Although DDT also elicited contact irritant effects, significant spatial responses were observed only at the highest test concentration (i.e., field application rate). The pyrethroid compounds elicited higher toxic actions to all *Ae. aegypti* strains with higher mortality rates associated with increasing concentration. These results were similar to those of Grieco *et al*. (2007), in that contact irritancy is the primary action of the pyrethroids, and spatial repellency is the primary action of DDT.

Both behavioral responses, contact irritancy and spatial repellency, preclude toxicity since the mosquito may move away from the chemical before acquiring a lethal dose. This is especially true for spatial repellency as the response is elicited prior to direct tarsal contact with the treated surface. A spatial repellent compound will prevent house-entry, thereby reducing human-vector contact and prevent (or reduce the probability) of developing resistance to the chemical. The beneficial effect of contact irritancy will depend on the amount of time the vector spends indoors of a treated structure and therefore has higher potential for human biting.

Most importantly, results show *Ae. aegypti* strains either resistant to alphacypermethrin or permethrin elicited significant contact irritant responses at the lowest test concentration. Thus lower doses than field application rates may be operational important to reduce disease. Likewise, DDT elicited significant spatial repellent responses in resistant *Ae. aegypti* strains. These results indicate chemical resistance mechanisms in mosquitoes may utilize different modes of actions than those involved with behavioral responses to chemicals.

CONCLUSION

Aedes aegypti mosquitoes from different geographical areas in Thailand were found resistant to pyrethroids as detected by standard contact susceptibility assays. In contrast, the level of detoxification enzyme activity was elevated in both susceptible strains and those that showed the lowest level of resistance. It appears that increasing detoxification activity in resistant strains, although significant compared to the susceptible strain, may not be the only relevant mechanism contributing to insecticide resistance due to 1) there might be more than one mechanism involve in resistance, such as knockdown (*kdr* gene) resistance, or 2) the increase of enzyme activity may be related to resistance to other chemicals in the environment, or 3) the epoxidation reaction within the mosquito's body has the effect of increasing the toxicities of the parent compounds. Therefore, in terms of interpretation of physiological responses, these findings may only provide a rough idea of the mechanisms involved.

The higher level of DDT and pyrethroid resistance in *Ae. aegypti* mosquitoes in many areas may not have the assumed negative effect on dengue vector control programs in Thailand. Those areas that found permethrin or alphacypermethrin resistance, both compounds have remained effective in residual spraying or impregnated materials to reduce the number of mosquito-human contacts. Permethrin and alphacypermethrin provide sufficiently strong contact irritant action on *Ae. aegypti* at lower dosage. In this situation holds true, only a decrease in chemical dosage would be required with no change in application method or target site. Additionally, it might well delay cross resistance in the mosquito population. For DDT, used at the field operational rate as a residual spray against indoor resting anopheline mosquitoes, might also prove particularly effective in areas of high risk as this compound appears very effective in repelling mosquitoes away from the target zone.

Two behavioral avoidance test systems, the standard excito-repellency system and a high throughput screening system (HTSS), have been shown to be useful for screening the behavioral responses to insecticidal compounds in other mosquito

species. The excito-repellency system has proven convenient for investigations in field and laboratory, whereas the HTSS is especially useful with experiments under laboratory conditions and when the materials are not subjected to high temperatures ($> 26^{o}C$). The HTSS can work well with a number of different chemicals to observe the three primary actions of mosquito adulticides, namely contact irritancy, spatial repellency action and toxicity. Both systems are practical investigative tools for accurately measuring the behavioral responses of adult mosquitoes to chemical compounds.

Early detection of insecticide resistance in the target mosquito population is an important aspect of any control program and should be routinely monitored and evaluated. A better understanding of behavioral responses of mosquitoes to various insecticide compounds and their patterns and mechanisms of resistance should allow for greater efficiency and cost-effectiveness in program design and adjustment of strategies for targeting appropriate vectors. Careful targeting of control interventions will allow for more effective adult vector control and will minimize the amount of insecticides used to control dengue transmission in Thailand.

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APPENDIX

Appendix Table 1 Percent escaping and 24 h mortality rates of *Aedes aegypti* [1]strains after contact and non-contact with 6.0 nm/cm^2 of alphacypermethrin in an excito-repellency test chambers.

Strain	Contact Control			Contact Treatment			Non-contact Control			Non-contact Treatment		
	% Escaping	% Mortality[2]		% Escaping	% Mortality		% Escaping	% Mortality		% Escaping	% Mortality	
		ES	Non-ES		ES	Non-ES		ES	Non-ES		ES	Non-ES
Chiang Mai	3.46	0	0	80.0	0	25.0	3.45	0	0	36.6	0	0
Kanchanaburi	5.17	0	0	52.5	0	10.3	3.39	0	0	3.39	0	0
Khonkaen	20.0	0	0	72.4	2.37	6.25	18.6	0	0	20.0	0	0
Nonthaburi	20.0	0	0	61.6	0	4.35	18.6	0	0	24.1	0	2.27
Songkhla	13.3	0	0	66.6	0	0	11.6	0	0	21.6	0	0
Satun	16.6	0	0	63.9	0	0	10.1	0	0	13.1	0	0

[1] F1-F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test.

[2] ES ; Escaping mosquitoes, Non-ES; Remaining mosquitoes each test chamber

Appendix Table 2 Percent escaping and 24 h mortality rates of *Aedes aegypti* [1]strains after contact and non-contact with 4.9 nm/cm^2 of deltametnrın in an excito-repellency test chambers.

Strain	Contact Control			Contact Treatment			Non-contact Control			Non-contact Treatment		
	% Escaping	% Mortality[2]		% Escaping	% Mortality		% Escaping	% Mortality		% Escaping	% Mortality	
		ES	Non-ES		ES	Non-ES		ES	Non-ES		ES	Non-ES
Chiang Mai	1.67	0	0	43.1	12.0	39.3	3.33	0	0	13.3	0	0
Kanchanaburi	8.33	0	0	62.1	1.82	5.00	3.45	0	0	35.7	0	0
Khonkaen	20.0	0	0	81.4	0	0	20.0	0	0	21.0	0	0
Nonthaburi	3.45	0	0	49.1	0	3.57	0	0	0	5.17	0	0
Songkhla	8.33	0	0	67.8	0	0	10.0	0	0	12.1	0	0
Satun	11.6	0	0	50.0	0	0	8.33	0	0	6.67	0	0

[1] F1-F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test.

[2] ES ; Escaping mosquitoes, Non-ES; Remaining mosquitoes each test chamber

Appendix Table 3 Percent escaping and 24 h mortality rates of *Aedes aegypti* [1]strains after contact and non-contact with 127.6 nm/cm^2 of permethrin in an excito-repellency test chambers.

Strain	Contact Control			Contact Treatment			Non-contact Control			Non-contact Treatment		
	% Escaping	% Mortality[2]		% Escaping	% Mortality		% Escaping	% Mortality		% Escaping	% Mortality	
		ES	Non-ES		ES	Non-ES		ES	Non-ES		ES	Non-ES
Chiang Mai	10.0	0	0	52.5	9.67	50.0	3.39	0	0	16.9	0	0
Kanchanaburi	6.67	0	0	61.6	0	4.35	5.00	0	0	11.8	0	0
Khonkaen	10.2	0	0	85.0	0	0	8.33	0	0	13.1	0	0
Nonthaburi	8.33	0	0	56.9	0	0	3.33	0	0	4.92	0	0
Songkhla	11.6	0	0	83.6	0	0	15.0	0	0	13.6	0	0
Satun	1.67	0	0	59.0	0	0	13.3	0	0	8.20	0	0

[1] F1-F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test.

[2] ES ; Escaping mosquitoes, Non-ES; Remaining mosquitoes each test chamber

Appendix Table 4 Percent escaping and 24 h mortality rates of *Aedes aegypti* [1]strains after contact and non-contact with 564.2 nm/cm^2 of DDT ın an excito-repellency test chambers.

Strain	Contact Control			Contact Treatment			Non-contact Control			Non-contact Treatment		
	% Escaping	% Mortality[2]		%Escaping	% Mortality		%Escaping	% Mortality		% Escaping	% Mortality	
		ES	Non-ES		ES	Non-ES		ES	Non-ES		ES	Non-ES
Chiang Mai	10.0	0	0	65.0	0	4.76	3.00	0	0	33.3	0	0
Kanchanaburi	8.62	0	0	43.3	0	0	3.39	0	0	21.3	0	0
Khonkaen	15.7	0	0	26.6	0	0	3.33	0	0	13.3	0	0
Nonthaburi	1.67	0	0	18.3	0	0	5.00	0	0	8.33	0	0
Songkhla	11.6	0	0	30.0	0	0	5.00	0	0	8.33	0	0
Satun	10.0	0	0	46.6	0	0	3.23	0	0	10.0	0	0

[1] F1-F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test.

[2] ES ; Escaping mosquitoes, Non-ES; Remaining mosquitoes each test chamber

Appendix Table 5 Comparison of escape responses between test strains of *Aedes aegypti* [1] after contact and non-contact with 6.0 nm/cm^2 of alphacypermethrin in an excito-repellency test chambers.

Strain	Contact trials	Non-contact trials
Chiang Mai vs. Kanchanaburi	0.0009*	<0.0001*
Chiang Mai vs. Khonkaen	0.7835	0.0375*
Chiang Mai vs. Nonthaburi	0.0761	0.1438
Chiang Mai vs. Songkhla	0.8223	0.0740
Chiang Mai vs. Satun	0.1891	0.0035*
Kanchanaburi vs. Khonkaen	0.0015*	0.0049*
Kanchanaburi vs. Nonthaburi	0.1740	0.0011*
Kanchanaburi vs. Songkhla	0.0116*	0.0027*
Kanchanaburi vs. Satun	0.0724	0.0533
Khonkaen vs. Nonthaburi	0.0799	0.5843
Khonkaen vs. Songkhla	0.7488	0.7951
Khonkaen vs. Satun	0.1722	0.3389
Nonthaburi vs. Songkhla	0.2304	0.7589
Nonthaburi vs. Satun	0.6798	0.1308
Songkhla vs. Satun	0.3843	0.2285

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

The* identifies results of log-rank tests with statistically significant ($P < 0.05$) differences in escape patterns.

Appendix Table 6 Comparison of escape responses between test strain of *Aedes aegypti* [1] after contact and non-contact with 4.9 nm/cm^2 of deltamethrin in an excito-repellency test chambers.

Strain	Contact trials	Non-contact trials
Chiang Mai vs. Kanchanaburi	0.0359*	0.0030*
Chiang Mai vs. Khonkaen	<0.0001*	0.2315
Chiang Mai vs. Nonthaburi	0.5951	0.1305
Chiang Mai vs. Songkhla	0.0026*	0.9014
Chiang Mai vs. Satun	0.2708	0.2376
Kanchanaburi vs. Khonkaen	0.0142*	0.0519
Kanchanaburi vs. Nonthaburi	0.0891	<0.0001*
Kanchanaburi vs. Songkhla	0.3413	0.0045*
Kanchanaburi vs. Satun	0.3639	0.0001*
Khonkaen vs. Nonthaburi	<0.0001*	0.0107*
Khonkaen vs. Songkhla	0.1484	0.2145
Khonkaen vs. Satun	0.0008*	0.0219*
Nonthaburi vs. Songkhla	0.0090*	0.1853
Nonthaburi vs. Satun	0.5489	0.7472
Songkhla vs. Satun	0.0682	0.3034

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

The* identifies results of log-rank tests with statistically significant ($P < 0.05$) differences in escape patterns.

Appendix Table 7 Comparison of escape responses between test strains of *Aedes aegypti* [1] after contact and non-contact with 127.8 nm/cm^2 of permethrin in an excito-repellency test chambers.

Strain	Contact trials	Non-contact trials
Chiang Mai vs. Kanchanaburi	0.3447	0.4652
Chiang Mai vs. Khonkaen	<0.0001*	0.5742
Chiang Mai vs. Nonthaburi	0.7740	0.0424*
Chiang Mai vs. Songkhla	0.0002*	0.7129
Chiang Mai vs. Satun	0.3091	0.1739
Kanchanaburi vs. Khonkaen	0.0051*	0.8584
Kanchanaburi vs. Nonthaburi	0.5575	0.1817
Kanchanaburi vs. Songkhla	0.0033*	0.7405
Kanchanaburi vs. Satun	0.9122	0.5316
Khonkaen vs. Nonthaburi	0.0001*	0.1291
Khonkaen vs. Songkhla	0.7185	0.8800
Khonkaen vs. Satun	0.0044*	0.4069
Nonthaburi vs. Songkhla	0.0003*	0.1047
Nonthaburi vs. Satun	0.4270	0.4764
Songkhla vs. Satun	0.0089*	0.3548

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

The* identifies results of log-rank tests with statistically significant ($P < 0.05$) differences in escape patterns.

Appendix Table 8 Comparison of escape responses between test strains of *Aedes aegypti* [1] after contact and non-contact with 564.2 nm/cm^2 of DDT in an excito-repellency test chambers.

Strain	Contact trials	Non-contact trials
Chiang Mai vs. Kanchanaburi	0.0192 *	0.2534
Chiang Mai vs. Khonkaen	<0.0001*	0.0112*
Chiang Mai vs. Nonthaburi	<0.0001*	0.0008*
Chiang Mai vs. Songkhla	<0.0001*	0.0010*
Chiang Mai vs. Satun	0.0219*	0.0025*
Kanchanaburi vs. Khonkaen	0.0237*	0.2513
Kanchanaburi vs. Nonthaburi	0.0040*	0.0457*
Kanchanaburi vs. Songkhla	0.1023	0.0490*
Kanchanaburi vs. Satun	0.9499	0.0945
Khonkaen vs. Nonthaburi	0.3572	0.3764
Khonkaen vs. Songkhla	0.6170	0.3879
Khonkaen vs. Satun	0.0165*	0.5836
Nonthaburi vs. Songkhla	0.1808	0.9910
Nonthaburi vs. Satun	0.0018*	0.7395
Songkhla vs. Satun	0.0655	0.7510

[1] F1 – F2 female, 4-5 day-old unfed, sugar starved 24 h pre-test

The* identifies results of log-rank tests with statistically significant ($P < 0.05$) differences in escape patterns.

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