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THESIS

EXPERIMENTAL HUTS STUDY TO EVALUATE THE BEHAVIORAL RESPONSES OF *AEDES AEGYPTI* TO SYNTHETIC PYRETHROIDS USED IN VECTOR CONTROL UNDER FIELD CONDITION

PITI MONGKALANGOON

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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The main objective of this study was to find the optimal dosage of deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin that would elicit repellency and irritability responses in *Aedes aegypti*. The F1-F3 generations of field mosquitoes collected from Pu Teuy Village, Sai-Yok District, Kanchanaburi Province, Thailand, were tested with four pyrethroids to determine the LC_{25}, LC_{50}, and LC_{99}. These concentrations were 0.010%, 0.020%, and 0.055%, respectively, for deltamethrin; 0.113%, 0.167%, and 0.353%, respectively, for cyphenothrin; 2.091%, 2.770%, and 5.114%, respectively, for d-tetramethrin; and 2.377%, 4.251%, and 10.715%, respectively, for tetramethrin. All dosages were tested in the excito-repellency system. Survival analysis was used to compare each chamber of the test. Cyphenothrin had a stronger repellent effect than the other pyrethroids, while the contact irritant effect was similar among compounds tested. The LC_{50} of each pyrethroid was found to be the optimal dose for repelling *Ae. aegypti*. There was no significant difference when compared with LC_{99} values for either noncontact or contact trials for each pyrethroid, $p>.05$, 0.077 and 0.624, respectively, for deltamehrin; 0.266 and 0.916, respectively, for cyphenothrin; 0.610 and 0.280, respectively, for d-tetramethrin; and 0.276 and 0.291, respectively, for tetramethrin. In the field test, we used two experimental huts, 4 x 5 x 2.5 m in width, length and height. The results indicated that the impact of insecticides on vector populations is much more complex than just toxicity. They can function as repellents (spatial repellency) and as irritants (contact irritancy). Even though, all four insecticides tested in the excito-repellency system demonstrated a contact irritancy effect, the experimental hut tests showed only two insecticides had this property, i.e. deltamethrin and cyphenothrin. We found that cyphenothrin had both a spatial repellent and contact irritant effect in the field tests while deltamethrin did not. D-tetrametrin and tetramethrin data from the field were not in agreement with the results from the excito-repellency system tests.

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Piti Mongkalangoon

March, 2011

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LIST OF ABBREVIATIONS

Ae.	=	Aedes
C1	=	Numbers of entrance mosquitoes in control hut, pre-treatments
C2	=	Numbers of entrance mosquitoes in control hut, post-treatments
CDC	=	Communicable Disease Control
Con	=	Control
Cyp	=	Cyphenothrin
Del	=	Deltamethrin
DEN	=	Dengue
D-tet	=	D-tetramethrin
ET	=	Escape time
hr	=	Hour
Hut 1	=	Treated hut
Hut 2	=	Control hut
LC	=	Lethal concentration
P	=	Probability value
T1	=	Numbers of entrance mosquitoes in treated hut, pre-treatments
T2	=	Numbers of entrance mosquitoes in treated hut, post-treatments
Tet	=	Tetramethrin
WHO	=	World Health Organization

EXPERIMENTAL HUTS STUDY TO EVALUATE THE BEHAVIORAL RESPONSES OF *AEDES AEGYPTI* TO SYNTHETIC PYRETHROIDS USED IN VECTOR CONTROL UNDER FIELD CONDITION

INTRODUCTION

Medically important insects can cause mortality and morbidity in human populations. Some of them can transmit pathogenic agents whereas the others may be considered as an irritance/nuisance. In the case of vector borne diseases, transmission is completely driven by a complex interaction between three different components; the parasitic agents, insect vectors and humans. Successful control of these diseases requires a complete understanding of the links between these components in addition to the environmental, agricultural and socio-economic factors associated with disease transmission. Any control effort must also rely on the participation of governmental and private sectors to provide sufficient manpower, financial support, and a long-term commitment to a well organized disease control program. These vector borne diseases include malaria, filariasis, and several arthropod borne viruses ie. dengue fever (DF), dengue haemorrhagic fever (DHF), and Japanese encephalitis.

People in many areas of the world are at risk from arthropod borne viruses, including DF and DHF. It is estimated that 50-100 million people are infected with dengue viruses worldwide (Gubler, 1997). In Southeast Asia, DHF cases have been increasing from an annual rate of <10,000 in the 1960s to >200,000 in the 1990s (Gibbons and Vaughn, 2002). Currently, the annual rate of DHF in Southeast Asia is estimated to be approximately 212,123 (World Health Organization (WHO), 2008). In Thailand, there were approximately 30,000-50,000 cases of dengue and dengue like illness during the last five years, but the trend of dengue cases appears to be increasing, with 56,651 cases being reported in 2009 (Communicable Disease Control (CDC), 2009). In 2010, however, a dengue outbreak occurred in Thailand and the number of cases reaches 114,100 by December 22, 2010.

Four serotypes of dengue viruses (DEN-1, 2, 3, and 4) are transmitted primarily by *Aedes aegypti* (L.), a notoriously efficient vector mosquito that often resides in and around human dwellings. This mosquito-borne disease causes tremendous morbidity and mortality each year in Thailand. Currently, there are no viable prevention and control methods or commercial available multi-valent dengue vaccine to limit the spread of this disease. At the moment, prevention of disease remains dependent on various methods of vector control (Roberts *et al*., 1997; Chareonviriyaphap *et al*., 2004; Grieco *et al*., 2007). Vector control has proven the most common and practical means of reducing virus transmission (Reiter and Gubler, 1997; WHO, 1999; Grieco *et al*., 2007). Unfortunately, *Ae. aegypti* has proven extremely difficult to control due to its close association with humans and is ability to exploit both the domestic and peridomestic environments. The available tools for vector control in Thailand include mechanical, chemical, and biological methods, as well as larval habitat elimination. These approaches can be more expensive (and less efficient), especially with some of the current chemical or biological products. Previously, studies on how chemicals function have focused primarily on toxicity. Little is known about several behavioral avoidance responses elicited by these chemicals: irritability and repellency (Chareonviriyaphap *et al*., 1997; Grieco *et al*., 2007). Irritability takes place when an insect is stimulated to move away from an insecticide after making direct physical contact with the chemical residue, whereas repellency occurs when the insect detects chemicals from a distance and diverts out of the treated area without making physical contact with the chemical (Roberts *et al*., 1997). In the last decade, these types of responses have been documented in both field and laboratory mosquito populations. The outcome of either form of behavioral avoidance can be quantified using a specially designed excito-repellency test system (Roberts *et al*., 1997; Chareonviriyaphap *et al*., 1997, 2004; Sungvornyothin *et al*., 2001; Tanasinchayakul *et al*., 2006).

Many chemical compounds, including synthetic pyrethroids have long been used in national vector control programs (Reiter and Gubler, 1997). In Thailand, deltamethrin is widely used as an indoor residual spray for controlling household nuisance mosquitoes and disease vectors, including *Ae. aegypti* (Chareonviriyaphap *et*

al., 1999; Somboon *et al*., 2003; Thanispong *et al*., 2008, 2010). Deltamethrin, applied as a space spray, has also been used in attempts to break disease transmission in dengue active areas (CDC, 2010.). The impact of pyrethroids on disease vectors requires continued investigation and serves as a stimulus for future studies on the mode of action and epidemiological significance of avoidance behavior (Chareonviriyaphap *et al*., 2001).

Recent studies have reported the spread of deltamethrin resistance in several field *Ae. aegypti* populations from Thailand (Jirakanjanakit *et al*., 2007; Thanisphong *et al*., 2008). The spread of resistance is raising awareness for the need of alternative insecticides or new methods of controlling mosquito vectors in Thailand. New, effective and safe synthetic pyrethroids, are readily available and are becoming more common for domestic protection against indoor biting mosquitoes and other arthropod pests. Therefore, it is important to investigate how these new synthetic pyrethroids might behaviorally impact *Ae. aegypti* populations before large scale use. Careful monitoring of the behavioral responses of *Ae. aegypti* to test compounds is extremely important and is facilitated by using an excito-repellency test system (Tanasinchayakul *et al*., 2006). Behaviroral responses of wild caught *Ae. aegypti* were compared using three different concentrations (LC_{25}, LC_{50} and LC_{99}) of four synthetic pyrethroids; deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin, and with or without physical contact with insecticides.

OBJECTIVES

1. To determine the optimum dose of four synthetic pyrethroids against *Ae. aegypti* using an excito repellency test,

2. To compare the behavioral responses of field collected *Ae. aegypti* to four synthetic pyrethroids, and

3. To characterize under field conditions that primary modes of action of four promising synthetic pyrethroids using an experimental hut study design.

LITERATURE REVIEW

1. Overview of dengue (DF) and dengue haemorrhagic fever (DHF)

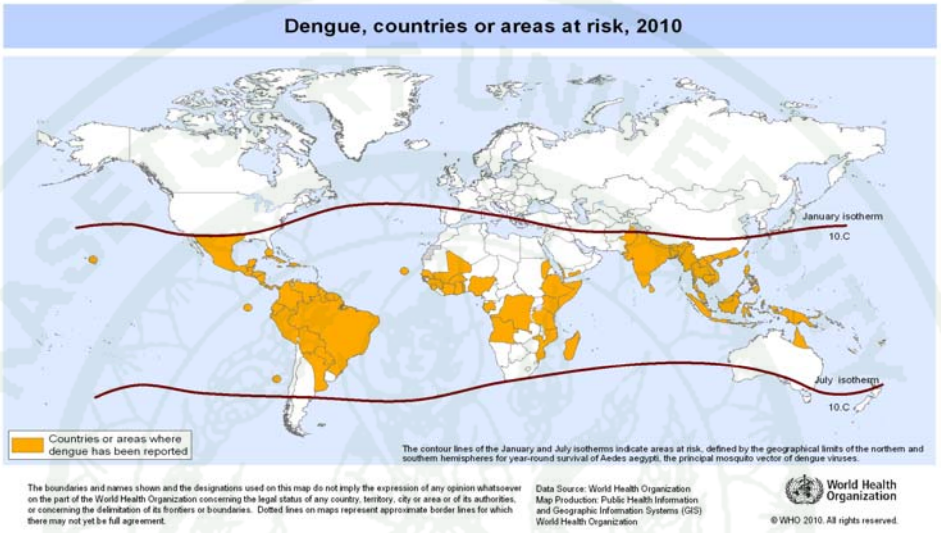


Figure 1 Zone of at risk areas for dengue transmission (WHO, 2010).

Source: World Health Organization (WHO), 2010. Map production: Public Health Information and Geographic Information System (GIS). World Health Organization.

Dengue has two forms, dengue fever (DF) and dengue haemorrhagic fever (DHF) (WHO, 2006a). DHF is known as the more severe form of DF. This form causes haemorrhaging, and in some cases may proceed to dengue shock syndrome which can result in death. Both DF and DHF are now considered as one of the most important viral diseases of Thailand as well as in many other countries of the world. Geographical distribution of DF and DHF covers almost all tropical and subtropical countries (WHO, 2010). This dengue virus belongs to the Flavivirus group in the family Flaviviridae. There are four dengue serotypes, DEN-1, DEN-2, DEN-3, and DEN-4, in which their antigens are commonly related (Nimmannitya, 1998).

It is estimated that at least 100 countries can be classified as DF/DHF endemic areas. At least 40% of the world population (approximately 2.5 billion people) live in areas at risk for dengue with most of these people living entirely in tropical and subtropical areas. Over 50 million infections with approximately 400,000 cases of DHF are reported annually in Asian countries (WHO, 2008)

DF/DHF is the most important emerging viral disease which affects nearly half of the world population. It is estimated that there are about 50 to 100 million cases of DF each year, and 500,000 cases of DHF, which require hospitalization. DF/DHF is found in tropical and sub-tropical region around the world, predominantly in urban and semi-urban areas. Even though dengue-like illnesses have been reported for many years, the etiology of the disease has only been known since 1944. The first dengue virus serotypes to be discovered were DEN-1 and DEN-2. They were isolated from India, New Guinea and Hawaii. In 1954 the first documented outbreak of DF occurred in Southeast Asia, in the country of Philippines. In 1956, a second outbreak occurred and once again it was in Philippines. However, the second outbreak was caused by DEN-3 and DEN-4 (Hammon *et. al*., 1960).

After that, Dengue outbreaks occurred in the other countries of Southeast Asia including Thailand. Thailand's first outbreak occurred in Bangkok-Thonburi in 1958. Both the Thailand and Philippines outbreaks resulted in the majority of patients being children under the age of ten years old. The primary symptoms characterized in this outbreak were the acute onset of high fever, petechial haemorrhage and shock symptoms. In fact, Thailand had been keeping clinical records at Siriraj Hospital since 1949. The outbreak in 1958 was the largest epidemic and focused both the public and the government's attention on the problem. After the first outbreak of DF in Southeast Asia, an epidemic occurred every year in Philippines, Thailand, Myanmar, Malaysia, Singapore, Indonesia and Viet Nam. Although the outbreaks were similar, they did exhibit changing patterns from year to year (WHO, 1993; Gubler, 1997).

The number of cases in Thailand demonstrated a yearly increase for 50 years. In 1960, there were 1,851 cases (morbidity 7 per 100,000 population), in 2001 there were 139,355 cases (morbidity 224 per 100,000 population), indicating that the number of cases in Thailand was increasing every year through five decades. The only exception occurred in 1987 when the highest number of cases was 174,285 (morbidity 325 per 100,000 population). In 2002, there were 114,833 cases (morbidity 185 per 100,000 population), even though it was less than 2001, the number of cases still remained high. These data suggest that the Dengue control program in Thailand is having little success in controlling the disease. It must be stated, however, that the trend in deaths from DHF has shown a continuous decrease from 10 per 100,000 population during the first outbreak in 1958 to 0.08 per 100,000 population in 2004 (Figure 2).

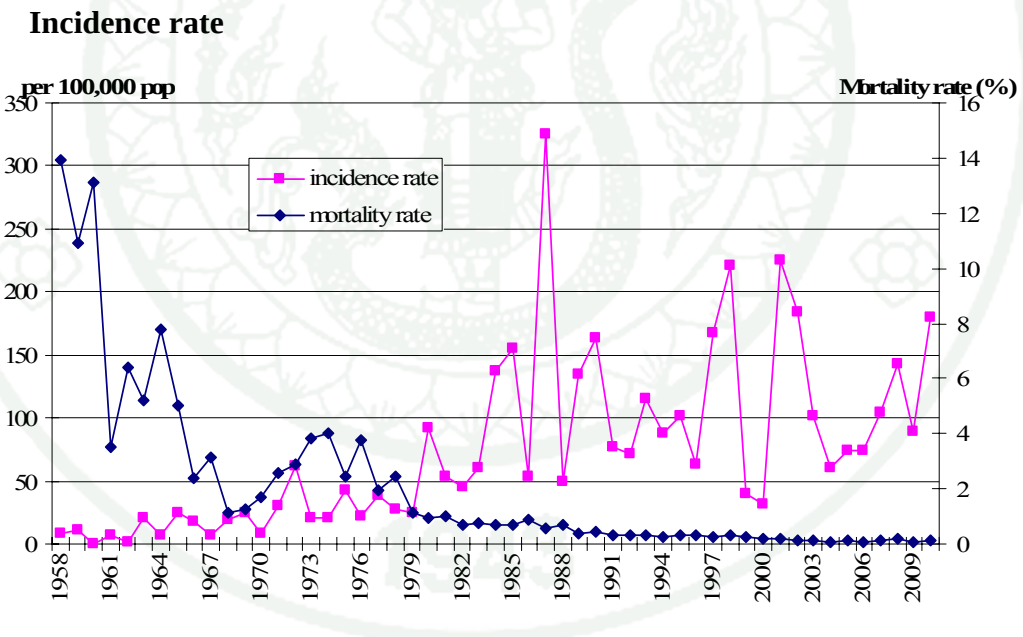


Figure 2 Incidence and mortality rate of DF and DHF, Thailand, 1958-2010.

Source: Bureau of Epidemiology, Department of Diseases Control, Ministry of Public Health.

During the last five years, the age group that had the highest morbidity occurred in the 15-24 years old followed by the 5-9 and 10-14 years old, respectively (Figure 3).

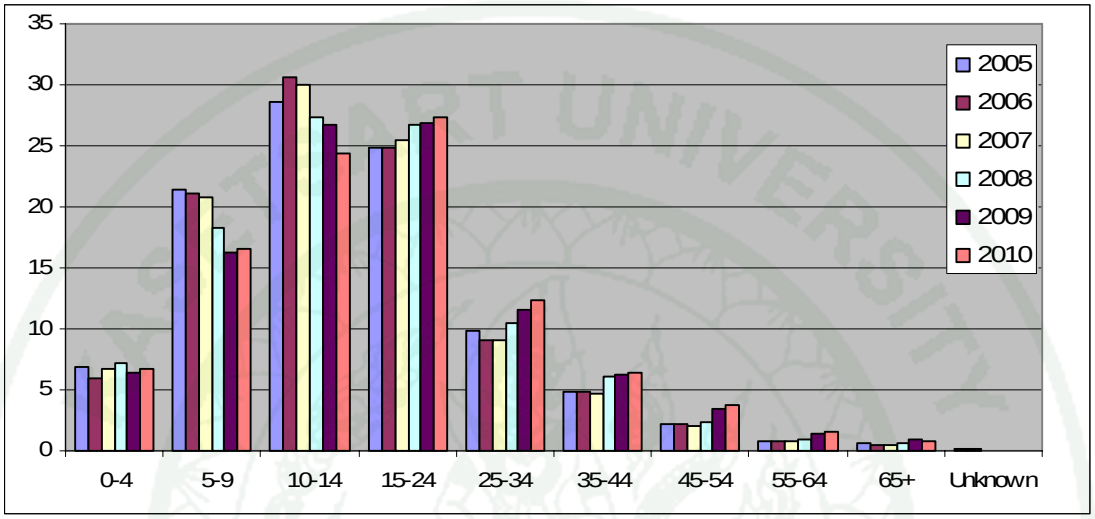


Figure 3 The morbidity rate of DF and DHF by age group, Thailand, 2005-2010.

Source: Bureau of Epidemiology, Department of Diseases Control, Ministry of Public Health

Even though the mortality rates have shown a steady decrease over the past few years (1958), the incidence rate has remained high every year. However, the number of dead cases remains high when compared with the past, particularly in the remote areas that lack health care centers or medical doctors. The cause of death from dengue is the result of the rapid loss of plasma during the condition known as Dengue Shock Syndrome (DSS). There is no drug therapy for dengue and the treatment focuses only on reducing the symptoms.

There are a number of reasons why dengue transmission present an interesting and challenging research question to include 1) Thailand is a dengue endemic area, 2) the climatic conditions and local environment support the vectors and increase survivorship long enough for the virus to be transmitted, 3) vector surveillance and

vector control are very difficult to implement and maintain, 4) the dengue vaccine is still many years away from development, and 5) dengue is unlike other communicable diseases that are susceptible to therapeutic drugs which can kill the pathogen. DF/DHF often affects children and frequently causes mortality in children when they present with severe symptoms. Their mortality rates are higher than other age groups. The important measurement for prevention and control is reducing of vector biting. Therefore, this study was interested in the examining of the following factors.

2. Biology of *Ae. aegypti*

2.1 Distribution and medical importance

All known vectors of the four dengue serotypes belong to the genus *Aedes*. *Ae. aegypti* is the most important in the group of dengue vectors. It is believed that species originates from somewhere in Africa. From Africa, *Ae. aegypti* spread to the western hemisphere in the seventeenth century and then to the Mediterranean basin in the eighteenth century followed by a movement to tropical Asia in the nineteenth century, and finally to the Pacific Islands in the late nineteenth and beginning of the twentieth centuries (Rodhain and Rosen, 1997). Its spread to other countries has been facilitated by human travel. It is widely distributed between isotherms 10°C January (Latitude 40°N) and 10°C July (Latitude 40°S), such as South East Asia, Western Pacific, Africa, Central and South America and some areas of Europe (Figure 4) (WHO, 1993). It is a vector of many diseases such as, dengue and dengue haemorrhagic fever, urban yellow fever in Africa, and Chikungunya in areas of Africa and Asia.

In the South-East Asia Region, *Ae. aegypti* is the principal epidemic vector of DF and DHF (Gould *et al*., 1968; Chan *et al.*, 1971; Rai, 1986), and *Aedes albopictus* has been recognized as a secondary vector. *Ae. aegypti* is common in most urban areas. Urbanization is the major factor in the spread of *Ae. aegypti*. In recent years there have been many cases of dengue transmitted by *Ae. aegypti* which

spreaded to rural areas of Thailand. This means that this mosquito has spread to all areas of Thailand.

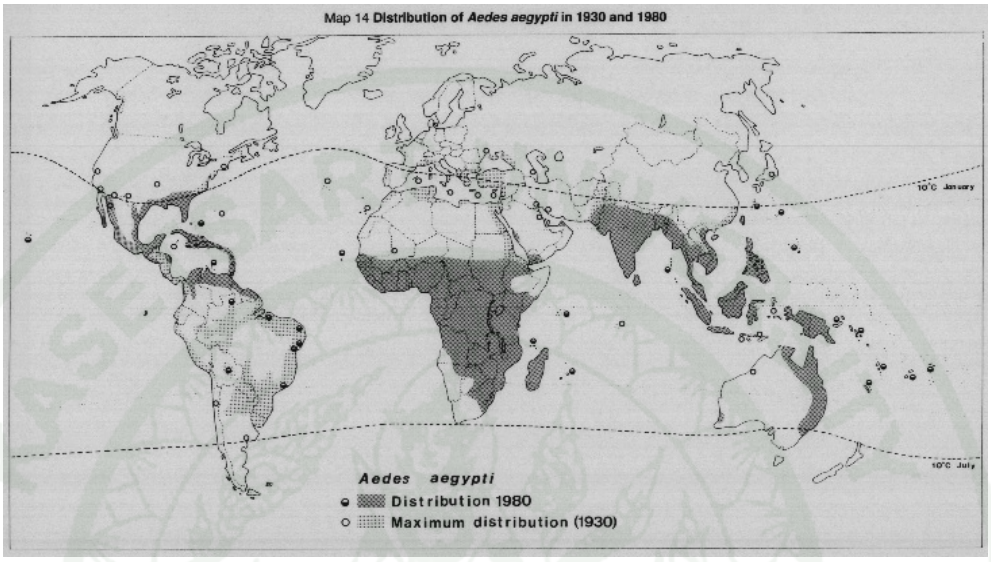


Figure 4 Distribution of *Ae. aegypti*.

Source: World Health Organization (WHO), 1989. Geographical distribution of arthropod-borne diseases and their principal vectors. WHO/VBC/89.967. Geneva: World Health Organization.

Thailand lies in a zone influenced by monsoons which determine the amount and distribution of precipitation over the entire country. The rains begin in May and continue through September each year. Because of a population explosion and rapid growth of cities, these heavy rains lead to poor sanitary conditions. This in turn has led to an increase of man-made containers for drink which enhances breeding sites for *Ae. aegypti.* Drinking water is very rare and necessary in the rural area. Thai people have an important habit of collecting rainwater in containers around the home to use for drink throughout the year. They prefer rainwater because of its light sweet taste and believe that it is clean. Some people in urban area also collect rainwater for drinking, although there is a good water supply system. These water storage containers promote *Ae. aegypti* breeding everywhere in the country. Furthermore,

temperatures in Thailand are conducive for mosquitoes to develop and be active. Temperatures are high throughout the year, with monthly averages ranging between 22°C and 30°C. Northern Thailand shows a different temperature pattern, with temperatures that fall below 22°C and may even reach 16°C in winter. Low temperatures establish the northern limit of *Ae. aegypti*. This mosquito will stop its development cycle when temperatures drop below 20°C (Aiken and Leigh, 1978). At these temperatures, it will survive but its population number will remain low.

2.2 Ecology and Bionomics

2.2.1 Egg and oviposition

Ae. aegypti likes to oviposit in water containers in and around houses. Gravid *Ae. aegypti* females prefer to lay egg on the inner wall of water containers near the water surface. The inner wall above the water line is usually damp surface. The eggs are approximately 1 mm long, and are elongated in shape. They are deposited singly. At first, the eggs are pale in color, then change to black color in a few minutes. However, if the wall of the container is not suitable for resting during egg laying, the mosquito will lay its eggs on the surface of the water. These eggs will hatch within three to five days. There will be no delay in hatching because the water will be absorbed into the eggs at all times. So the embryos inside the eggs will continue to develop until they are ready to hatch. However, if the eggs on the water surface are disturbed and sink below the water surface before hatching, all developing embryos will die. For those eggs that are deposited on the damp wall of the container, they will receive enough moisture from the wall to complete embryonic development. Embryonic development is usually completed in 48 hours after deposition under warm and humid conditions (WHO, 1999), however, most will require three days or more. After three days of developing, the eggs can survive for greater than one year. Eggs that have completed embryonic development will hatch within 5-30 minutes if they are flooded by water. Hatching usually occurs at warm temperatures after the eggs have been submerged into water. The number of hatching eggs corresponds to the percent of water which evaporates from the eggs (Christophers, 1960). If the eggs

continue to lose water until they adopt a flatten appearance greater than 50% of the fully inflated eggs, they will not hatch.

In the insectary, we use filter paper to collect *Aedes* eggs. Egg papers are left in the laying container with water at least three days after finding eggs. The newly laid eggs are fertilized outside the female (Clements, 1992). During the short period of ovulation, when each egg passes the spermathecal duct, one sperm from the spermathecae will penetrate into the micropyle of each egg. Therefore, cell differentiation occurs outside the female. This process also requires moisture for approximately two or three days but this depends on each species of mosquitoes. If there is a lack of water before completing development, the embryos inside will die. Egg papers which have cured for three days must also be dried in the air slowly. After that they are kept in a sealed container in a cool room with high humidity to protect the eggs from losing too much moisture.

2.2.2 Larvae stage and pupae stage

Larvae go through four stages. The duration of larval development depends on temperature, availability of food, and larval density. Under optimal conditions, the time from hatching to adult can be as short as seven days (WHO, 1999). At low temperatures, it may take several weeks for adults to emerge. However, Matini (1923) noted that mosquito larvae subjected to warm temperatures produced smaller-sized adults than those subjected to cooler temperatures. Larvae of *Ae. aegypti* grow equally well in both presence and absence of light (Jobling, 1937).

2.2.3 Adult stage

Males can be distinguished from females by their plumose antennae and the longer palpi, which in males are almost as long as the proboscis, whilst in female the palpi are very short and antennae are pilose. The weight of a female is greater than that of a male by nearly twice as much. Male mosquitoes usually emerge before the females by about one or two days. This ensures that the males avoid

mating with their sisters and allows for a proper genetic mixing within the population. After emergence, both males and females must rest on the walls of the larval container for a few hours to allow the exoskeleton and wings to harden. For the male mosquito, it is capable of mating within 24 hrs after emergence. Males are incapable of mating within the first 24 hrs due to the position of the genitalia which is twisted 180^{o} at the time of emergence. Therefore, the male has to wait until the genitalia rotate 180^{o} to be in their normal position.

Females cannot take a blood meal during the first 24 hrs of their adult life. Their mouthparts do not harden and are not sufficiently strong to penetrate the hosts' skin. After 24 hrs of emergence, both sexes can mate and the female can take a blood meal (WHO, 1993). More often, inseminated females feed on honeydew before they are in a proper physiologic state to feed on blood. They usually take a blood meal within 24-36 hours after mating. Seaton and Lumsden (1941) studied uninseminated *Ae. aegypti* females and found that a large proportion of mosquitoes fed in the three to four day old range. Furthermore, they also found that there was no difference in the feeding rate between virgin and fertilized females.

2.2.4 Feeding behavior

Dispersal of *Ae. aegypti* females is influenced by many factors including finding a blood meal, an oviposition site, a resting area, etc. The common flight range of a female is usually within 100 m from the site of emergence. However, Cumming (1931) and Shannon and Davis (1930) found that this mosquito could fly continually for 400-1000 m. Bonnet and Worcestor (1946) studied *Aedes albopictus* and found that wind had little effect on the dispersal of mosquitoes. The mosquitoes would fly into the wind when the velocity was low, otherwise they would cling to vegetation. Wolfinsohn and Galun (1953) released 28,000 gravid females of *Ae. aegypti* to determine their location of oviposition. They found eggs laid up to 2.5 km from the liberation point, and all places experienced wind. Even though females can fly very far, in nature they do not disperse farther than 100 m from their emergence location (Muir and Kay, 1998; Harrington *et al*., 2005).

Female mosquitoes use volatile chemicals of their hosts to locate where they are. Carbon dioxide, lactic acid, and octenol are the best known host attractants. Moreover, skin emanations are also important. The odors from living hosts are more attractive than the combination of these chemicals in warm, humid air stream alone. These odors commonly have a combined effective range of 7-30 m, but in some species, the range can be up to 60 m. Vision is also important for diurnal mosquitoes, such as *Ae. aegypti*, especially in an open environment. Dark, contrasting, and moving objects are particularly attractive. At the range of 1-2 m, heat and humidity surrounding the host will serve as an attractant to host seeking females. The organs which female mosquitoes use to detect these attractants are the sensilla on the antennae and palpi (Bowen, 1991). However, when females land on the host, the receptors on the proboscis, tarsi, and elsewhere on the legs are important. Probing by the mosquito is the final check by the mosquito to ensure that the host is suitable.

2.2.5 Mosquito chemoreceptor

When we try to explain the behaviors of an animal, we must know which factors the animal selects through its sense organs out of the complexity of the physical and biological environment. In addition, we have to know which information the sensory system selects and sends to the brain. The stimulus, which releases a specific behavioral response, is not limited to visual stimuli but also includes mechanical stimuli, heat/temperature stimuli, and chemical signals. Mechanoreceptors are used to detect pressure changes such as air movement (including sound waves) and touching by the insect. Thermoreceptors are used to detect heat (or changes in temperature). When present, these receptors will usually be found on the antennae, but can also occur on the legs (e.g. American cockroach) or mesothorax (Melanophila beetles). Chemoreceptors are used by insects and are involved in the detection of airborne or substrate chemicals (senses of taste and smell). Chemoreceptors are primarily located in the mouthparts, antennae, and legs. Moreover, they are found in the other parts, e.g. buccal cavity (in several insects), tarsi, and ovipositor. Antennae carry olfactory receptors, but can also contain contact

chemoreceptors. Gustatory receptors (feeding-stimulant receptor) and some olfactory organs are located on the mouthparts in many insects. In *Ae. aegypti* gustatory receptor cells are sensitive to salts, sugars and amino acids and are located on the proboscis and tarsi. We also know that *Ae. aegypti* is attracted by lactic acid, fatty acids, essential oils, pyruvic acid, butyric acid, and ammonia. Adenosine triphosphate (ATP) acts as a phagostimulant. All of these compounds affect olfactory cells in the antennae. Many blood-sucking insects are very sensitive to heat, and *Ae. aegypti* is not different. It shows maximal neuronal spike changes in the cold and hot receptors of its antennal sensilla when a temperature change of only 0.2°C is detected (Davis and Sokolove, 1975).

If various types of chemoreceptor cells are distributed on the antennal surface or over other body parts, we can imagine that any chemical stimulus will be detected by the insect and messages sent to the central nervous system (CNS). Chemically induced behavioral responses often can be inhibited or reinforced by certain compounds. By chemical stimuli we mean any compound that excites the receptor cell. Excitation comprises a sequence of changes in the receptor cell, which eventually lead to a change in the spontaneous rate of nerve impulses. In addition, if the compound decreases the impulse activity, it is called inhibition. An increased impulse rate is produced by a lowered membrane potential or a depolarization of the receptor cell, and a decreased impulse rate follows a hyperpolarization. Both depolarization and hyperpolarization can be achieved via a change in permeability of the receptor-cell membrane. When Na^+ in the axon is increased, it produces depolarization, whereas an increase of K^+ induces hyperpolarization (Katz, 1966). In several insects, olfactory cells can be depolarized or hyperpolarized by some compounds. In considering the mechanism of exitation in chemoreceptors, Kaissling (1971) stated that the outer dendrite of the cell has special molecular receptor (acceptor) units that bind to its membrane. These receptor molecules are thought to become activated and induce changes across the cell membrane if a stimulus molecule is bound to them in an appropriate manner. The surrounding receptor cite wall, which has to be penetrated by the stimulus molecule, is constructed by a special cell, i.e. the trichogen cell, and specific receptor cells inside the hair. The cuticle of insect is

extremely impermeable to water and water-soluble compounds, except, at the tip of taste-receptor bristles. Moreover, lipophilic odor molecules can penetrate the cuticle easily. Most cuticular pore channels are found in the wall of most olfactory hairs, which facilitate the penetration of the stimulus molecules to the receptor cell membrane.

2.2.6 Mosquito chemoreceptor response to repellent

When considering the behavioral aspects of repellency it is necessary to mention several things, such as the orientation mechanism involved in attraction, etc. Mosquitoes must follow the stimulating odor by the shortest upwind path between their starting point and the source of the attractant whether this is in a vertical or horizontal path. Dethier *et al*. (1960) and Kennedy (1977) defined the attractant as 'a chemical or mixture of chemicals which, acting in the vapor phase, cause an insect to behave in way which result in its moving toward the source of the material or toward a zone of preferred concentration'. Conversely, an insect repellent is a chemical or mixture of chemicals which, acting in the vapor phase, cause an insect to behave in way which result in its moving away from the source of the material. In addition, the repellent prevents an insect from reaching a target to which it would otherwise be attracted. Daykin *et al*. (1965) found *Ae. aegypti* was disrupted from its host finding by the presence of repellents. Wright (1968) showed that the presence of a piece of repellent-soaked paper on the floor of a cage reduced to zero the number of mosquitoes biting a human arm to which no repellent had been applied. He ascribed that the repellent prevented biting of mosquitoes that had been activated by carbon dioxide arising from the human arm. It seems that the effect of the repellent might be due to other types of disruption of the host-finding action.

There are two available methods in which repellents can be used. The first is applying the repellent to the potential target (i.e. topical repellent). Second, to apply in the area that contains potential hosts to permeate the area with repellent vapor (i.e. area repellent). However, the active zone of the plume of repellent corresponds more or less, depends on the relative emission rates and insect

sensitivity, with that of the host attractants. For the area repellent, the permeation would be effective if the repellent elicits negative anemotaxis or if it causes the insect to ignore host stimuli, and if a major degree of adaptation of insects to the repellent does not occur. However, we know very little about how repellents affect behavioral responses and how they prevent insects from finding their hosts. As the repellent molecules are volatile their residual life can be greatly reduced by temperature, humidity and wind. Many effective repellents have a high vapor pressure. At high mosquito densities, a heavy dose of a low vapor pressure repellent may be required, whereas repellents with high vapor pressures may offer protection at low concentration. Therefore, a repellent that has a lower evaporation rate and produces a low vapor pressure means that it will continue to repel for a longer time period (Spencer, 1974).

3. Vector control

The objectives of a vector control program are 1) to reduce vector density, 2) to reduce longevity, 3) to reduce man-vector contact, and 4) to reduce transmission rate. Because there is no vaccine or specific drug therapy for the treatment of dengue, control of the disease is dependent on the control of the vector. Many attempts at eradication have failed because of a number of reasons, such as inefficient and unsustainable vertical programs, ineffective outdoor space spraying, unaccepted larviciding by communities and insufficient educational messages presented to the public. Of particular concern is space spraying, which people rely heavily on, shows little success in controlling dengue. In order to maintain a successful dengue control program, it is essential to focus on larval habitat source reduction. However, the present aim of dengue vector control is to reduce population densities below a certain threshold level, rather than completely eliminate vector populations. There are many necessary and appropriate methods to achieve long-term, sustainable control of the vectors (WHO, 1999, 2006a).

4. Insecticides used for vector control

Chemicals protect humans from the bite of insects through three different actions; irritancy, repellency, and toxicity (Grieco *et al*., 2007). Recently, it has been reported that some synthetic chemicals, such as *N*, *N*-Diethyl-*meta*-toluamide (DEET), demonstrate the forth action by successfully masking the presence of a host through the inhibition of an odor activated receptor (Ditzen *et al*., 2008). Most studies have concentrated on the toxic actions of these chemicals whereas little focus has been placed on non-toxic properties of these compounds (Roberts *et al*., 1997, 2000; Chareonviriyaphap *et al*., 1997). Well documented behavioral responses of vectors to chemicals began with the use of DDT to control *Anopheles* mosquitoes in 1953 (Kennedy, 1947) and subsequently two different types of non-toxic actions have been recognized; contact irritancy and noncontact repellency (Davidson, 1953; Dethier *et al.*, 1960; Lockwood *et al*., 1984; Roberts and Andre, 1994; Roberts *et al*., 2000; Grieco *et al*., 2000, 2007; Achee *et al*., 2009).

In Thailand, many synthetic compounds have been extensively used for the control of medically important insects in both private businesses and in government programs. In the private sector, several forms of household products are commercially available (Thanispong *et al*., 2008; Jirakanjanakit *et al*., 2007). These products have been formulated in several forms i.e. aerosols, mosquito coils, mats and liquid all of which may contain one or more active insecticide ingredients. Over 80% of synthetic compounds used in homes belong to the pyrethroid group, and the less extent is organophosphates and carbamates. The most popular use of these synthetic pyrethroids is to control mosquitoes as compared to ants and cockroaches. Permethrin appears to be the major synthetic pyrethroid available in the commercial market, followed by cypermethrin. In the government sector, several important groups of synthetic compounds, including organophosphates, carbamates, pyrethroids and other so called biorational pesticides (biological agents and botanical repellents) were recommended for use in public health vector control programs (Reiter and Gubler, 1997; Chareonviriyaphap *et al*., 1999; Paeporn *et al*., 2004). For routine dengue control, temephos, an organophosphate larvicide, has been used in the control

of *Ae. aegypti* larvae in Thailand since 1950 (Chareonviriyaphap *et al*., 1999). Although still effective for *Aedes* larval control, a trend of temephos resistance in *Ae. aegypti* was recently observed in some localities of Thailand (Jirakanjanakit *et al*., 2007). In 1994, deltamethrin, a promising pyrethroid, was introduced to Thailand for controlling indoor biting mosquitoes, including *Ae. aegypti* (Chareonviriyaphap *et al*., 1999) and this compound remains the insecticide of choice to use during a dengue outbreak. The other organophosphates such as malathion, fenitrothion and pirimiphos-methyl were heavily used in the past prior to being replaced by the synthetic pyrethroids (Chareonviriyaphap *et al*., 1999). Deltamethrin and permethrin are currently the major compounds used in the malaria control program in Thailand (CDC, 2008). Deltamethrin has been used for indoor residual spaying and is applied once or twice a year, depending upon the endemic malaria zoning as determined by the Bureau of Vector Borne Disease. Permethrin has been used for impregnation of fabric materials such as clothes, blankets, and bed nets. These impregnated materials have been shipped, in place of spray teams, to malaria endemic areas, especially in the four southern provinces of Thailand in which the insurgency continues to intensify. As a consequence, an increase in malaria cases has been documented in these southern provinces since 2004.

Due to the financial crisis in 1996, Department of Disease Control reorganized the Vector Borne Disease Control Program structure by merging the malaria control unit with other vector borne disease control units. The new structure has been effective since October 1996. The policy for the restructuring has facilitated the utilization of human resources, budget and equipment for control of all vector-borne diseases, and minimized the relatively high costs associated with running a vector borne disease control program.

At the National level, the Bureau of Vector Borne Disease falls under the direction of Department of Disease Control in the Ministry of Public Health. At the country level the program comprises 12 regions and each is directed by a Medical Officer, Director of the Office of Vector-Borne Disease Control (VBDO) who is directly responsible to the Director-General, Department of Disease Control. Thirty-

nine Vector-Borne Disease Control Centers (VBDC) and 302 Vector-Borne Disease Control Units (VBDU) are set up at the provincial and district levels, respectively. In addition, there is a number of district and sub district municipalities that are controlled by Ministry of Interior.

The Ministry of Interior brought about a new policy for pest control activities, including planning, evaluation, budget allocation, and monitoring. Each municipality office maintains autonomy with regard to deciding how they will implement their vector control operations, including allocation of money for purchasing chemical insecticides to control disease vectors. As a result, each office can be directly approached by insecticide companies without any consultations from Center of Disease Control, Ministry of Public Health. These synthetic compounds include chemicals from a number of chemical classes with the majority being synthetic pyrethroids such as cypermethrin, alphacypermethrin, tetramethrin, resmethrin, and metofluthrin.

5. Types of Behavioral Responses to Insecticides

Two principal forms of responses to insecticides are recognized, physiological and behavioral. Physiological resistance is referred to the ability of mosquito to survive the effect of insecticides by various mechanisms such as detoxifying enzymes. Behavioral responses comprise two major types of responses, irritability and repellency (Roberts *et al.*, 1997; Chareonviriyaphap *et al*., 1997; Grieco *et al*., 2007). Irritability occurs when an insect is stimulated to move away from an insecticide after making direct physical contact with the chemical residue, whereas repellency occurs when the insect detects chemicals from a distance and diverts away from the treated area without making physical contact with the chemical (Roberts *et al*., 1997). Both types of behavioral responses of mosquitoes to insecticides are significant components of the insecticide-malaria control program. In the past, both types of behavioral responses were overlooked in the impact of the national control activity and the majority of the attention was placed on the toxic responses of insects to these chemicals. Currently, both types of behavioral responses can be experimentally

differentiated by using the excito-repellency (ER) test system (Chareonviriyaphap *et al.*, 1997, 2002) and a modular, high-throughput laboratory-based assay system (Grieco *et al*., 2007). The former is relatively convenient and can be used in both the field and laboratory whereas the latter is limited to laboratory use.

MATERIALS AND METHODS

Part 1 To determine the optimum dose of four synthetic pyrethroids against *Ae. aegypti* using an excito repellency test assay.

Mosquito population

Ae. aegypti larvae and pupae were collected from various containers located in and around human dwellings from Pu Teuy Village, Sai Yok District, Kanchanaburi Province (14^{O} 17' N, 99^{O} 11'E, 310 m above sea level), western Thailand in December 2008. The rural site is located in mountainous terrain generally surrounded by intact forest (Figure 5). There are approximately 150 houses with the population of 15,000 in the village. Agricultural practice is a major occupation. Mosquito larvae and pupae were brought back to the insectary at Kasetsart University, Bangkok, Thailand and reared to the adult stage. Adult mosquitoes were identified as *Ae. aegypti* and were introduced into the insectary for continued rearing. Test populations of F1-F3 *Ae. aegypti* mosquitoes were reared and used for testing.

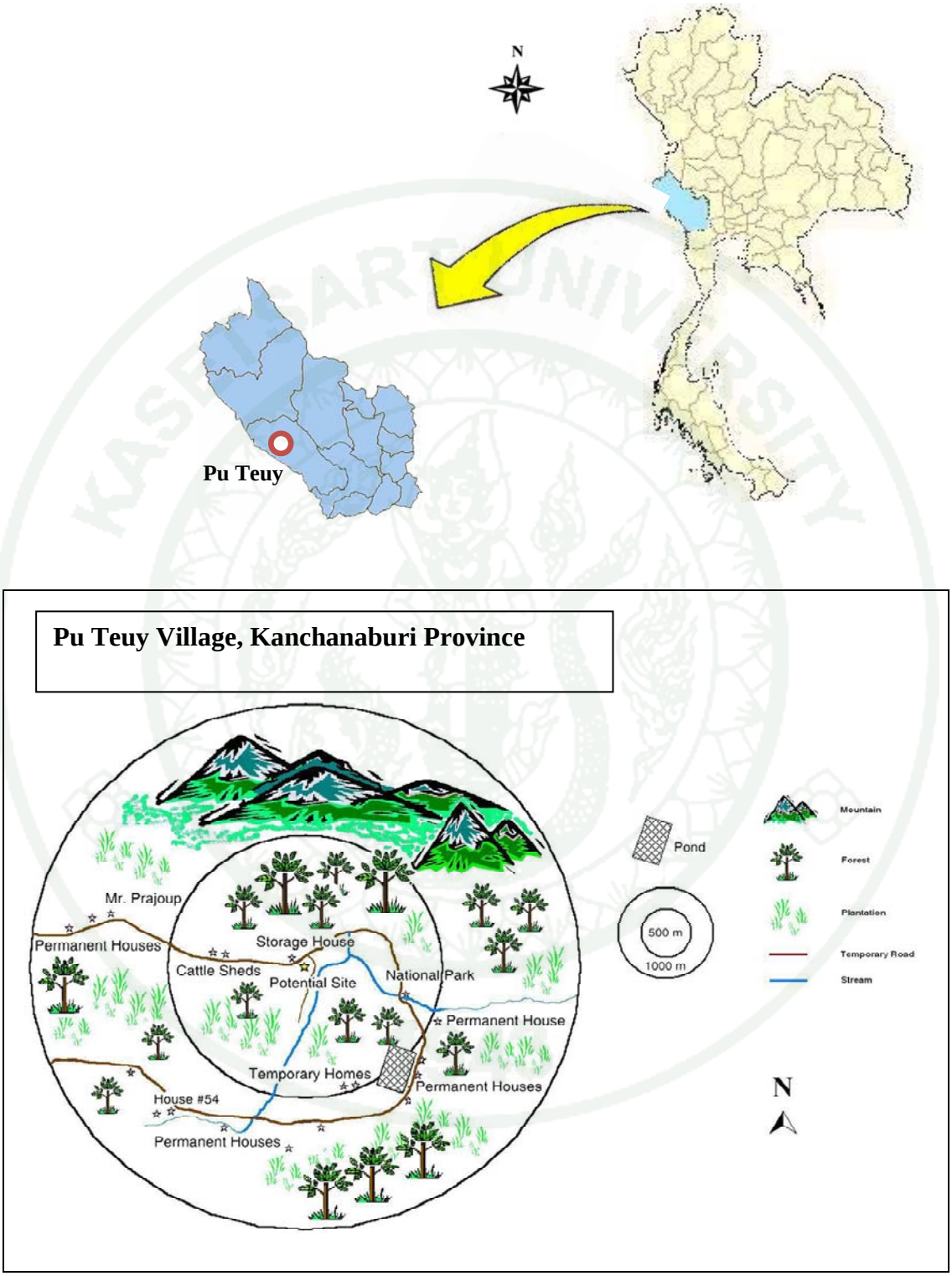


Figure 5 Map of Pu Teuy Village, Sai Yok District, Kanchanaburi Province, Western Thailand.

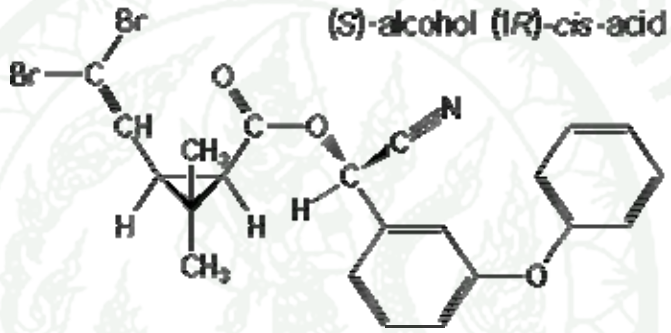
Mosquito rearing

All life stages of mosquitoes were maintained at 25±5°C and 80±10% relative humidity (RH) in the insectary at the Department of Entomology, Faculty of Agriculture, Kasetsart University. Larvae were reared in plastic boxes, 20 cm wide x 30 cm long x 8 cm high, which were covered by net to protect them from contamination with other mosquito species. They were fed on fish food until pupation. Pupae were collected daily and transferred to small bowls containing clean water by pipette. The bowls were placed in cages for adult emergence. Adults were provided with cotton pads soaked with 10% sugar solution, from the day of emergence and adults were maintained in a 12 x 12 x 12 in. screened cage. Female mosquitoes were permitted to feed on a guinea pig on the third day post emergence. Oviposition bowls, made by putting a piece of Whatman filter paper No.1 around the inside of the bowl were filled about half way with tap water and were placed in the cages with two day post-blood fed females. After one or two days, the gravid females would lay their eggs in the oviposition bowls. Subsequently, the egg papers were left for at least three days in this condition to allow the embryos to mature. Eggs were dried at room temperature for 1-2 days, before being immersed in water in individual hatching trays.

Insecticides

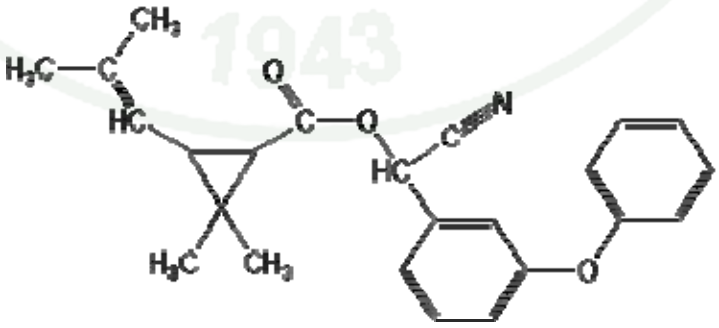
Four synthetic pyrethroid insecticides were used to determine dose response assays:

1. Deltamethrin [(S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] was received from BASF company (CAS# 52918-63-5),



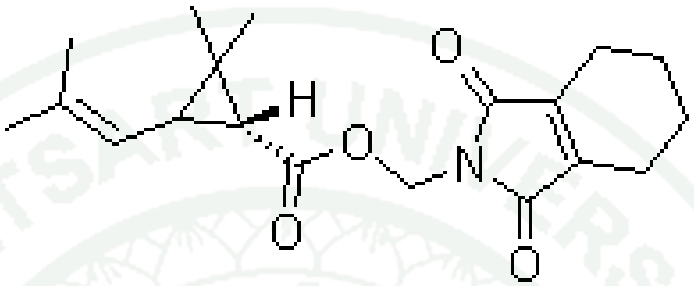
Deltamethrin

2. Cyphenothrin [(RS)-α-cyano-3-phenoxybenzyl (1R,3R;1R,3S)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane carboxylate] was received from TJC, Bangkok, Thailand (CAS#39515-40-7),



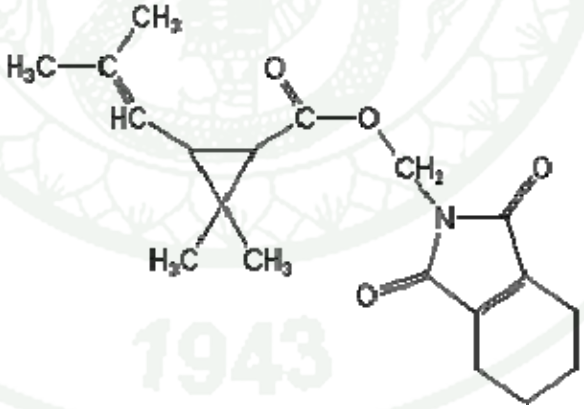
Cyphenothrin

3. D-Tetramethrin [(cyclohex-1-ene-1,2-dicarboximidomethyl (1R,3R; 1R,3S)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate] was received from TJC, Bangkok, Thailand (CAS#1166-46-7),



D-Tetramethrin

4. Tetramethrin [(cyclohex-1-ene-1,2-dicarboximidomethyl (1RS,3RS; 1RS,3SR)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate] was received from TJC, Bangkok, Thailand (CAS#7696-12-0).



Tetramethrin

Dose-response Assay

The biological assay was performed using Whatman (12 x15 cm) filter paper impregnated with 2 ml of technical grade insecticide suspended in acetone solution plus silicone oil according to the World Health Organization protocol and

specification (WHO, 1996, 2006b). Test papers (12 x 15 cm) were impregnated with deltamethrin at 0.005%, 0.0125%, 0.025%, 0.05%, and 0.1%, with cyphenothrin at 0.05%, 0.1%, 0.5%, and 1%, with d-tetramethrin at 0.25%, 0.5%, 1%, 2%, 4%, and 8%, and with tetramethrin at 0.8%, 1.6%, 2%, 4%, 8%, 16%, and 32%, respectively. All papers were treated at a rate of 2 ml of insecticide solution per 180 cm^2. The control cylinders contained paper impregnated with solvent and carrier. The treatment cylinders contained paper impregnated with insecticides plus solvent and carrier. Twenty-five non-blood-fed females were introduced into each cylinder and maintained for 1 hr in a normal vertical position. After 1 hr, mosquitoes were transferred to holding containers and provided with cotton pads soaked with 10% sucrose solution. Mortality was recorded at 24 hrs and each set was replicated 4 times.

Data analysis

Abbott's formula was used to correct for the observed mortality. The LC_{25}, LC_{50}, and LD_{99} values of all four synthetic pyrethroids were estimated from dosage-mortality regression using probit analysis from SPSS for windows version 13 (SPSS Inc., Chicago, Illinois.).

Part 2 To compare the behavioral responses of field collected *Ae. aegypti* to four synthetic pyrethroids.

Mosquito population

Test populations of F1-F3 *Ae. aegypti* mosquitoes originally collected from Pu Teuy Village, Sai Yok District, Kanchanaburi Province as described in Part 1 were used for excito-repellency testing.

Mosquito rearing

Mosquito larvae and pupae were raised to the adults under controlled laboratory conditions (25 ± 5 ^{o}C, 80 ± 10% RH, and 12:12 light: dark photoperiod). Female and male adults were identified as *Ae. aegypti* and were provided with cotton pads soaked with 10% sugar solution after the first day of emergence. The mated female mosquitoes were permitted to feed on blood from restrained live guinea pigs on day 4 post-emergence. Two days after blood-feeding, 10-cm diameter oviposition dishes containing moist filter paper were placed in the cages for egg deposition. Eggs were dried at room temperature for 1-2 days, before being immersed with water in individual hatching trays. At two days post-hatch, approximately 250 larvae were transferred to individual plastic rearing trays (20 cm wide x 30 cm long x 8 cm high) containing 1,500 ml of tap water and one teaspoon (~2.5 gm) of ground fish pellets. Pupae were transferred daily from larval trays to emergence cups and placed directly into 12 x 12 x 12 in. screened cages. Adults were provided cotton soaked with 10% sucrose solution from time of eclosion until 24 hrs prior to testing in the assays. Only the F1 to F3 generations were used in assay trials.

Insecticides

Four synthetic pyrethroid insecticides (as described in Part 1) were used in the excito repellency assays at LC_{25}, LC_{50}, and LC_{99}.

Insecticide impregnated paper

Test papers (15 x 17.5 cm) were chemically impregnated with the established LC_{25}, LC_{50}, and LC_{99} of deltamethrin, cyphenothrin, d-tetramethrin and tetramethrin. Papers were impregnated with deltamethrin at 0.010%, 0.020%, and 0.055%, with cyphenothrin at 0.113%, 0.167%, and 0.353%, with d-tetramethrin at 2.091%, 2.770%, and 5.114%, and with tetramethrin at 2.377%, 4.251%, and 10.715%, respectively. Treated papers were prepared at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand, according to WHO protocol and specifications (WHO 1996, 2006). All insecticide papers were treated at the rate of 2.9 ml of insecticide solution per 262.5 cm^2 surface area. Control papers were treated with acetone (solvent) plus silicone oil (carrier).

Behavioral tests

Tests were carried out by using the excito-repellency test box assay. The complete system consisted of four chambers which are made from stainless steel. Each chamber is comprised of 1) a rear door cover, 2) a Plexiglas panel with an 11.5 cm diameter hole, 3) a Plexiglas holding frame, 4) an inner screen chamber that measures 22.5 x 19 cm, 5) the outer chamber that measures 23 x 23 cm, 6) a front door with an exit portal slot, and 7) the exit portal. A receiving box was constructed from a paper carton (8 in wide x 8 in long x 8 in high) and was attached to the exit portal for collecting all escaping mosquitoes.

Each treatment was paired with a matched control in the following configurations: a control noncontact chamber with a noncontact chamber, and a control contact chamber with a contact chamber (Appendix Figure 1 – 2) (Chareonviriyaphap *et al*., 2002). Non-blood-fed, 3-5 day old females were used in the tests. Mosquitoes were deprived of a sugar meal for 24 hrs prior to testing, but were provided with water soaked cotton pads. All tests were performed between 0800 and 1630 hr. For each test, 15 female mosquitoes were introduced into each of four chambers via a hole in the Plexiglas panel, after which the rear of the outer chamber

was shut. The mosquitoes were allowed to acclimate to the inside of the chamber for 3 min (Chareonviriyaphap *et al*., 1997). Subsequently, each exit portal slot was opened to begin testing. The numbers of mosquitoes that escaped from the exposure chamber into the receiving cage were recorded at 1 min intervals for a period of 30 min (Chareonviriyaphap *et al*., 2002). All mosquitoes escaping during each 1 min interval were transferred to separate holding cups.

Ambient temperatures and relative humidity were recorded during the experiment. All tests were performed during the day time and each test series was replicated 4 times. After each test period, the numbers of dead or knockdown specimens were recorded separately from each exposure chamber. Live escaped specimens and those remaining inside the treatment and control chambers were collected and held separately in small holding containers topped with cotton soaked with 10% sugar solution until 24 hrs mortalities were recorded.

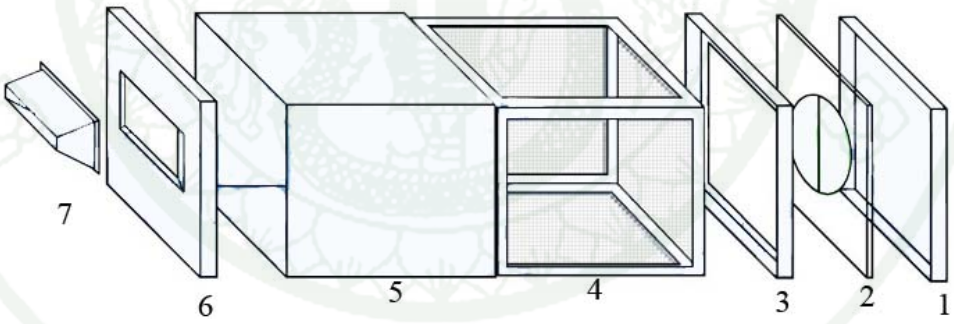


Figure 6 Illustration of the excito-repellency test chamber for study of insecticide avoidance behavior of mosquitoes.
1 = rear door cover, 2 = Plexiglas panel with rubber-sealed door,
3 =Plexiglas holding frame, 4 = screened inner chamber, 5 = outer chamber,
6 = front panel, 7 = exit portal.

Data analysis

The Kaplan-Meier survival analysis method was used to analyze and interpret the rates of escaping mosquitoes from each chamber of the excito-repellency system (Roberts *et al*., 1997; Chareonviriyaphap *et al*., 1997). Mosquitoes that escaped out of the test chamber were treated as "deaths", and those still remaining in the test chamber from one minute to the next were treated as "survivals". At the end of the test, the remaining mosquitoes in the exposure chambers were treated as "censored". Survival analysis was also used to estimate escape time (ET) when the percents of escaping mosquitoes reached 25%, 50% and 75 % (ET_{25}, ET_{50} and ET_{75}). The log-rank method was used to compare patterns of escape behavior within the test groups from the chambers and between different treatment groups (Mantel and Haenzel, 1959). Statistical significances for all tests were determined at $P < 0.05$. The SAS system for windows V. 6.12 analysis (SAS program package (SAS Release 6.12, SAS Institute, Cary, NC) was used in the analysis.

Part 3 To study the movement patterns of *Ae. aegypti* into and out of the experimental huts treated with synthetic pyrethroids as compared to a matched control.

Mosquito population

Test populations of *Ae. aegypti* mosquitoes originally collected from Pu Teuy Village, Sai Yok District, Kanchanaburi Province as described in Part 1 were used for the experimental hut study. Only the F1 to F3 generations were used in this study.

Mosquito rearing

A group of 300-400 larvae was collected from Pu Teuy Village, Sai Yok District, Kanchanaburi Province. Colonization of *Ae. aegypti* from field collected material followed established methods (Kongmee *et al*., 2004) with only minor modifications to meet testing requirements. All life stages were maintained under insectary conditions before, during, and following testing. Larval and adult insects were kept under a 12:12 light : dark photoperiod regime. Adults were provided cotton pads soaked with 10% sucrose solution from the first day of emergence. Adults were held in 12 x 12 x 12 in. screened cages. Depending on required experimental conditions, female mosquitoes were permitted to have a blood meal (live hamster) on the third or fourth day post-emergence. Two days after blood feeding, oviposition dishes were placed in the cage for gravid females to deposit eggs.

Insecticides

Based on the excito-repellency test assay in which there were no significant differences in response patterns between LC_{50} and LC_{99} levels for each test pyrethroid, synthetic pyrethroids at the LC_{50} level were selected for experimental hut studies: 0.020% deltamethrin, 0.167%cyphenothrin, 2.770% d-tetramethrin and 4.251% tetramethrin.

Insecticide-impregnated netting material

Polyester netting material was impregnated with 0.020% deltamethrin, 0.167% cyphenothrin, 2.770% d-tetramethrin, and 4.251% tetramethrin following the method of Thanispong *et al*. (2008). Netting was soaked with each test solution in individual metal pans topped with a smaller weighted pan, thus allowing complete and even absorption of the chemical solution. Control netting was prepared in the same manner but without insecticides.

Experimental Huts

The experimental huts used in this study were previously described (Chareonviriyaphap *et al*., 2005; Grieco *et al*., 2007). Two identical huts were constructed in an isolated area (Appendix Figure 8). Each hut, measuring 4 m wide x 5 m long x 3.5 m high, and each hut had three windows and one door. The dimensions of the windows and door were 1.125 x 1.175 m, and 0.8 x 2 m, respectively. Each portal was constructed in such a way as to allow them to be affixed with entrance and exit traps. Huts were built of similar material and in a similar fashion to the indigenous Thai homes. Huts were constructed from pieces of untreated plank wood of 1 m wide x 2.5 m long and pieces of zinc roofing of 0.75 m x 3 m in size. Hut frames used to support the walls were made from galvanized iron pipe measuring 1 m in width x 2.5 m in length and were custom – welded to accommodate each wall. The apex of the angled roof measured 3.5 m from leveled ground. The hut maintained three windows, one on each of three sides, and a northward – facing door which were all affixed with either entrance or exit traps (Appendix Figs 3 - 4).

The dimension of the window traps was 0.84 m long x 1.065 m wide x 1.065 m high. They were constructed using an iron frame. Louvers made of 3/8 in. non-treated plywood and fixed vertically at 60 degree angles were placed over the front opening of each of the three window traps, 1.065 x 1.065 m with a horizontal row of 10 cm wide slit. The louvers were placed in the open position producing a series of

horizontal, which mosquitoes could enter (Appendix Figure 4-5). A door trap, measuring 1.2 m long x 0.845 m wide x 2.10 m high, was fixed to the door opening. Twenty plywood louvers identical to those used in the window traps were installed over the front opening and were again fixed at 60 degree angles to the vertical (Appendix Figure 4). These were arranged to facilitate the movement of mosquitoes from the hut into the trap. Both trap types were covered by nylon insect netting (Appendix Figure 6). Cotton sleeve material was sewn over several holes in both types of trap to facilitate the removal of mosquitoes. Additional details pertaining to the experimental huts were given in Suwonkerd et al. (2006) and Grieco et al. (2007).

Mosquito marking and releasing technique

Only the F1-F3 adult generation was used in this study. Two groups of 3-5 day old, non-blood-fed *Ae. aegypti* females were marked with luminous marking powder (BioQuip Products, Rancho Dominquez, CA) following the method of Achee *et al*. (2005). Each group of 125 females consisted of 100 females that were used as experimental test populations and 25 females which were used as controls. Marked mosquitoes were sugar starved for 24 hrs, placed in a humidified chamber that consisted of an ice chest covered with water soaked towels, and provided with water soaked cotton pads until the time of release.

For the entrance experiment, 100 marked mosquitoes were released 10 m outside of each hut (Appendix Figure 7), and for the exit experiment, 100 marked mosquitoes were released inside of each hut. The released time was at 0500 hr, 1 hr before the start of the collection. The louvers of traps were turned to outside when tested the entrance experiment, and turned to inside of the hut when tested the exit experiment (Appendix 6). All traps were open the louvers at 0540 hr to let the test mosquitoes fly into the traps.

All experiments were replicated three times in both huts and in each month. Human hosts were placed under an untreated mosquito net to protect them from being bitten during the study. Two human hosts were used in each hut for entrance

experiment. Only one human host was used in each hut for exit experiment. Entrance and exit traps were sampled at 20 min intervals, from 0600 – 1800 hr. The collections were made by 2 collectors per hut. Mosquitoes collected from the traps were placed into plastic cups that were topped with mesh netting affixed with rubber bands. All cups were labeled with the location and time of collection. All mosquitoes from the traps were examined for fluorescent using stereomicroscope. The ambient temperature and relative humidity were observed by human hosts inside the hut every 20 min.

Data analysis

The Kaplan-Meier survival analysis method was used to analyze and interpret the rates of escaping mosquitoes from each hut of the exit experiments (Roberts *et al*., 1997; Chareonviriyaphap *et al*., 1997). Mosquitoes that escaped from the huts when testing for the contact irritant effect of insecticides (exit experiment) were treated as "deaths", and those still remaining in the huts after every twenty minute period were treated as "survivals". At the end of the test, the remaining mosquitoes in the huts were treated as "censored". Survival analysis was also used to estimate escape time (ET) when the percent of escaping mosquitoes reached 25, 50 and 75 % (ET_{25}, ET_{50} and ET_{75}). The log-rank method was used to compare patterns of escape behavior between the matched control and treatment experimental huts (Mantel and Haenzel, 1959).

For entrance experiment, The Kaplan-Meier survival analysis method was also used to analyze and interpret the rates of entrance mosquitoes into each pair of experimental huts, control and treatment. Mosquitoes that entered into the huts during testing with a spatial repellency insecticide (entrance experiment) were treated as "deaths", and those still remaining outside both huts during testing from one twenty minute sample period to the next were treated as "survivals". At the end of the test, all mosquitoes that did not fly into the control hut were treated as "censored of control". In the same way, all mosquitoes that remained outside of treated hut or flew into another hut were treated as "censored of treatment".

Statistical significances for all tests were determined at $P < 0.05$. The SAS system for windows V. 6.12 analysis (SAS program package (SAS Release 6.12, SAS Institute, Cary, NC) was used in the analysis.

RESULTS

Part 1 To determine the optimum dose of four synthetic pyrethroids against *Ae. aegypti* in the excito repellency test.

Data on dose-mortality relationships for all four synthetic pyrethroids, deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin against *Ae. aegypti* are given in Table 1. The LC_{25}, LC_{50}, and LC_{99} values of deltamethrin were lower when compared to those of the other three chemicals at the same levels. The slopes of the regression lines for test data from each chemical were computed. The highest slope was obtained from deltamethrin (24.587) and the lowest value was from tetramethrin (0.121) (Table 1). Results of the susceptibility tests established from the single diagnostic doses (LC_{99} x 2) of deltamethrin (2 x 0.055 = 0.111%), cyphenothrin (2 x 0.353 = 0.707%), d-tetramethrin (2 x 5.114 = 10.228%), and tetramethrin (2 x 10.715 = 21.430%) showed that *Ae. aegypti* test population was completely susceptible to all four insecticides (100% mortality).

Table 1 Toxicity data for four insecticides tested against adult *Ae. aegypti.*

Parameters	Insecticides			
	deltamethrin	cyphenothrin	d-tetramethrin	tetramethrin
Slope ± SE	24.587 ± 0.001	3.113 ± 0.002	0.190 ± 0.002	0.121 ± 0.001
LC_{25} (%)	0.010	0.113	2.091	2.377
LC_{50} (%)	0.020	0.167	2.770	4.251
LC_{99} (%)	0.055	0.353	5.114	10.715

Part 2 To compare the behavioral responses of field collected *Ae. aegypti* to four synthetic pyrethroids.

Mortalities of *Ae. aegypti* at three levels (LC_{25}, LC_{50}, and LC_{99}) of deltamethrin, cyphenothrin, d-tetramethrin and tetramethrin were tested in contact and noncontact exposure chambers (Tables 2-5). In general, higher mortalities were observed in contact trials than in noncontact trials and in controls. Within treatment trials, higher mortalities were observed in mosquitoes that did not escape as compared with those that were able to escape. A high level of mortality can be seen in mosquitoes that did not escape from the LC_{99} compared with the LC_{25} and LC_{50} values. Comparatively, a low percent mortality was observed from escaping females in contact trials, ranging from 0 to 7.69% for deltamethrin, from 2-8% for cyphenothrin, from 2.13-2.27 % for d-tetramethrin and 0% for tetramethrin. A high mortality from nonescaping females from the treated chambers with cyphenothrin at LC_{99} (60%) and d-tetramethrin at LC_{99} (31.25%) were also recorded. Mortality levels in noncontact insecticide trials were low, ranging from 0-4.76% for escaped specimens and 0-7.69% for nonescaped specimens.

The percent of escaping females in response to the four test chemicals were separated based on contact irritancy and noncontact repellency to provide the information in Tables 2-5. In general, significantly greater escape responses were found in contact trials compared with noncontact trials ($P<0.05$). In the deltamethrin contact trials, higher escape responses were seen at LC_{50} (88.33%) and LC_{99} (86.67%) compared to LC_{25} (63.33%). Cyphenothrin produced a stronger escape response (80.36-83.33%) compared to d-tetramethrin (73.33%-79.66%) and tetramethrin (60.00-72.41%), regardless of test concentrations (Tables 2-5). Escape responses for noncontact trials were low, however, in all cases there was a significant difference as compared to the matched control ($P <0.05$).

Time in minutes for *Ae. aegypti* females to escape from the treated chamber with deltamethrin, cyphenothrin, d-tetramethrin and tetramethrin at three different doses are listed in Table 6. The escape patterns from chambers treated with chemicals

were defined as times for 25% (ET_{25}), 50% (ET_{50}), and 75% (ET_{75}) of a test population to depart the treated chambers (Chareonviriyaphap *et al*. 1997). In contact trials, the ET_{25} value for all four chemicals was 1 min, except for deltamethrin at LC_{25} (4 min). ET_{50} values for three concentrations of cyphenothrin and d-tetramethrin were recorded at < 4 min. In general, ET_{99} value for d-tetramethrin was somewhat high compared with deltamethrin and cyphenothrin. Due to insufficient numbers of escaping mosquitoes after 30 min, the ET_{99} value for tetramethrin could not be calculated. In noncontact trials, ET_{25} values for all four chemicals were comparatively high (>18 min). The ET_{50} and ET_{99} values for all chemicals in noncontact trials could not be estimated due to low escape numbers (Table 6).

Comparison between contact vs. noncontact and control vs. contact responses demonstrated significant differences in escape response of *Ae. aegypti* across all doses for all four insecticides (P <0.01). Significant differences in escape patterns were not observed between paired noncontact vs. control trials, except in the case of LC_{50} and LC_{99} values for cyphenothrin (P <0.05) (Table 7). Multiple comparisons among three different doses for all four chemicals in contact, noncontact, and control trials were evaluated. The pattern of escape response was analyzed with the log-rank method, and statistical significance was established at the 0.05 level of probability. No significant differences in escape patterns between any two doses from contact trials, and noncontact trials were found, except in two comparisons of deltamethrin in contact trials (LC_{25} vs. LC_{50} and LC_{25} vs. LC_{99}) (Table 9) and in one comparison of cyphenothrin in noncontact trials (LC_{25} vs. LC_{99}) (Table 8).

Figure 7-10 demonstrated the proportions of mosquitoes remaining in the insecticide treated chambers under different test conditions. These proportions are used to demonstrate patterns of escape rate. These patterns are indicative of escape probabilities of *Ae. aegypti* females between contact and noncontact trials with three doses of deltamethrin (Figure 7), cyphenothrin (Figure 8), d-tetramethrin (Figure 9) and tetramethrin (Figure 10). Significant differences in escape patterns were seen when contact trials were compared to noncontact trials for all tests (P <0.01) (Table 7).

Table 2 Escape rate and mortality of *Ae. aegypti* in response to three lethal concentrations of deltamethrin, LC_{25}, LC_{50}, and LC_{99}.

Test condition	LC	Treatment		Control		%Mortality in 24hrs			
						Treatment		Control	
		No. tested	% Escaped	No. tested	% Escaped	Escape	Not escape	Escape	Not escape
Contact	25	60	63.33 (38)	60	30.00 (18)	0	0	0	0
	50	60	88.33 (53)	60	16.67 (10)	1.89 (1)	28.5 (2)	0	0
	99	60	86.67 (52)	60	21.67 (13)	7.69 (4)	12.5 (1)	0	0
Non contact	25	60	25.00 (15)	59	16.95 (10)	0	0	0	0
	50	60	13.33 (8)	60	6.67 (4)	0	0	0	0
	99	60	26.67 (16)	59	22.03 (13)	0	2.27 (1)	0	0

Table 3 Escape rate and mortality of *Ae. aegypti* in response to three lethal concentrations of cyphenothrin, LC_{25}, LC_{50}, and LC_{99}.

Test condition	LC	Treatment		Control		%Mortality in 24hrs			
						Treatment		Control	
		No. tested	% Escaped	No. tested	% Escaped	Escape	Not escape	Escape	Not escape
Contact	25	60	83.33 (50)	60	20.00 (12)	2.00 (1)	0	0	0
	50	56	80.36 (45)	60	16.67 (10)	2.22 (1)	0	0	0
	99	60	83.33 (50)	60	28.33 (17)	8.00 (4)	60.00 (6)	0	0
Non contact	25	60	18.33 (11)	60	15.00 (9)	0	2.04 (1)	0	0
	50	60	23.33 (14)	59	8.47 (5)	0	0	0	0
	99	60	35 (21)	60	16.67 (10)	4.76 (1)	7.69 (3)	0	0

Table 4 Escape rate and mortality of *Ae. aegypti* in response to three lethal concentrations of d-tetramethrin, LC_{25}, LC_{50}, and LC_{99}.

Test condition	LC	Treatment		Control		%Mortality in 24hrs			
						Treatment		Control	
		No. tested	% Escaped	No. tested	% Escaped	Escape	Not escape	Escape	Not escape
Contact	25	59	76.27 (45)	60	26.67 (16)	2.22(1)	21.43 (3)	0	2.27 (1)
	50	59	79.66 (47)	60	26.67 (16)	2.13(1)	0	(1)	0
	99	60	73.33 (44)	59	25.42 (15)	2.27(1)	31.25 (5)	0	0
Non contact	25	60	23.33 (14)	59	15.25 (9)	0	2.17 (1)	0	0
	50	59	23.73 (14)	58	15.52 (9)	0	2.22 (1)	0	2.04 (1)
	99	59	20.34 (12)	60	13.33 (8)	0	4.26 (2)	0	0

Table 5 Escape rate and mortality of *Ae. aegypti* females in response to three lethal concentrations of tetramethrin, LC_{25}, LC_{50}, and LC_{99}.

Test condition	LC	Treatment		Control		%Mortality in 24hrs			
						Treatment		Control	
		No. tested	% Escaped	No. tested	% Escaped	Escape	Not escape	Escape	Not escape
Contact	25	60	60.00 (36)	60	28.33 (17)	0	4.17 (1)	0	0
	50	59	61.02 (36)	60	20.00 (12)	0	8.70 (2)	0	0
	99	58	72.41 (42)	60	23.33 (14)	0	6.25 (1)	0	0
Non contact	25	60	28.33 (17)	60	18.33 (11)	0	0	0	0
	50	59	20.34 (12)	60	15.00 (9)	0	0	0	0
	99	60	30.00 (18)	60	18.33 (11)	0	2.38 (1)	0	0

Table 6 Time in minutes for 25% (ET_{25}), 50% (ET_{50}), and 75% (ET_{75}) of *Ae. aegypti* females to escape from noncontact and contact chambers treated with three lethal concentrations (LC_{25}, LC_{50}, and LC_{99}) of deltamethrin, cyphenothrin, d-tetramrthrin, and tetramethrin.

Chemical	LC	Noncontact (min.)			Contact (min.)		
		ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}
Del	25	29	-	-	4	9	-
Del	50	-	-	-	1	3	8
Del	99	23	-	-	1	2	7
Cyp	25	-	-	-	1	1	10
Cyp	50	-	-	-	1	1	11
Cyp	99	23	-	-	1	1	8
D-tet	25	-	-	-	1	2	26
D-tet	50	-	-	-	1	1	12
D-tet	99	-	-	-	1	2	-
Tet	25	18	-	-	1	10	-
Tet	50	-	-	-	1	5	-
Tet	99	26	-	-	1	4	-

Table 7 Log-rank comparisons of escape responses between noncontact vs. control, contact vs. control and contact vs. noncontact trails of each lethal concentration, LC_{25}, LC_{50}, and LC_{99} of deltamethrin, cyphenothrin, d-tetramrthrin, and tetramethrin.

Chemical	LC	Noncontact vs. Control (*P*-value)	Contact vs. Control (*P*-value)	Contact vs. Noncontact (*P*-value)
Del	25	0.2971	0.0002*	0.0001*
Del	50	0.2123	0.0001*	0.0001*
Del	99	0.5698	0.0001*	0.0001*
Cyp	25	0.6313	0.0001*	0.0001*
Cyp	50	0.0223*	0.0001*	0.0001*
Cyp	99	0.0309*	0.0001*	0.0001*
D-tet	25	0.2633	0.0001*	0.0001*
D-tet	50	0.2387	0.0001*	0.0001*
D-tet	99	0.268	0.0001*	0.0001*
Tet	25	0.2223	0.0002*	0.0001*
Tet	50	0.4185	0.0001*	0.0001*
Tet	99	0.1508	0.0001*	0.0001*

* identifies result of log-rank tests with statistically significant differences ($P<0.05$).

Table 8 Log-rank comparisons of escape responses from noncontact chamber between LC_{25}, LC_{50}, and LC_{99} of each insecticide, deltamethrin, cyphenothrin, d-tetramrthrin, and tetramethrin.

Chemical	LC_{25} vs. LC_{50} (*P*-value)	LC_{25} vs. LC_{99} (*P*-value)	LC_{50} vs. LC_{99} (*P*-value)
Del	0.1053	0.8821	0.0766
Cyp	0.3845	0.0364*	0.2662
D-tet	0.8720	0.7088	0.6104
Tet	0.3328	0.9016	0.2763

* identifies result of log-rank tests with statistically significant differences ($P<0.05$).

Table 9 Log-rank comparisons of escape responses from contact chamber between LC_{25}, LC_{50}, and LC_{99} of each insecticide, deltamethrin, cyphenothrin, d-tetramrthrin, and tetramethrin.

Chemical	LC_{25} vs. LC_{50} (*P*-value)	LC_{25} vs. LC_{99} (*P*-value)	LC_{50} vs. LC_{99} (*P*-value)
Del	0.0001*	0.0001*	0.6239
Cyp	0.7213	0.7307	0.9160
D-tet	0.3271	0.8653	0.2801
Tet	0.7208	0.1308	0.2905

* identifies result of log-rank tests with statistically significant differences ($P<0.05$).

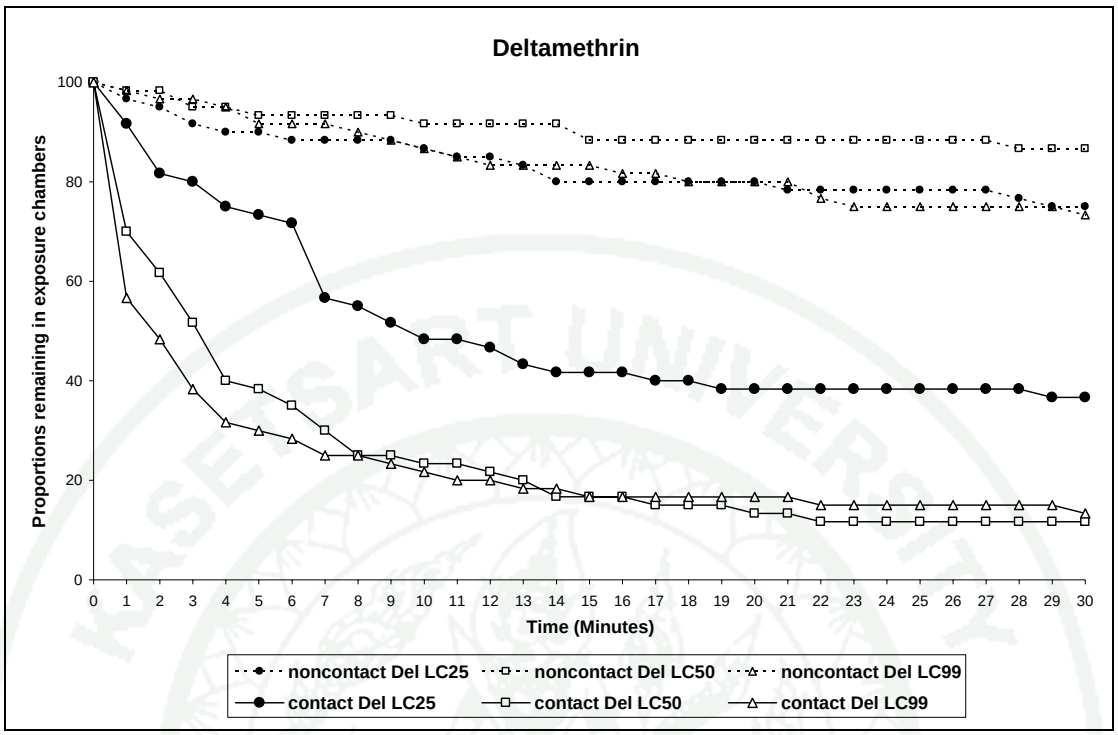


Figure 7 Escape rate of *Ae. aegypti* exposed to deltamethrin.

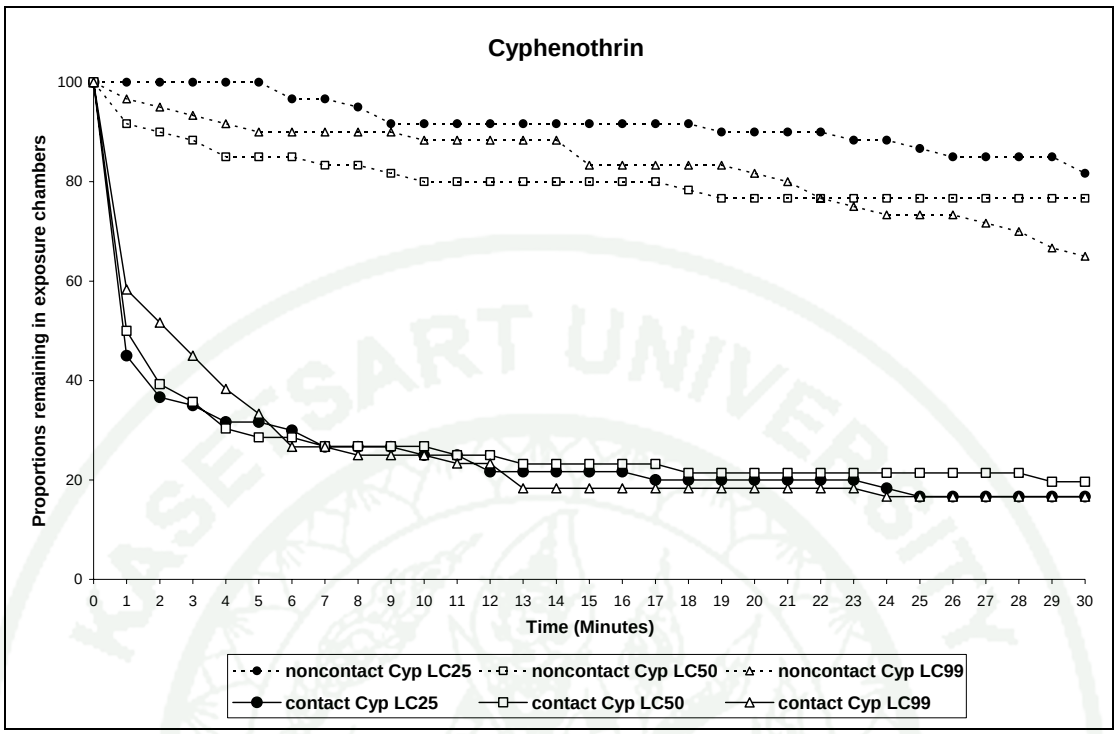


Figure 8 Escape rate of *Ae. aegypti* exposed to cyphenothrin.

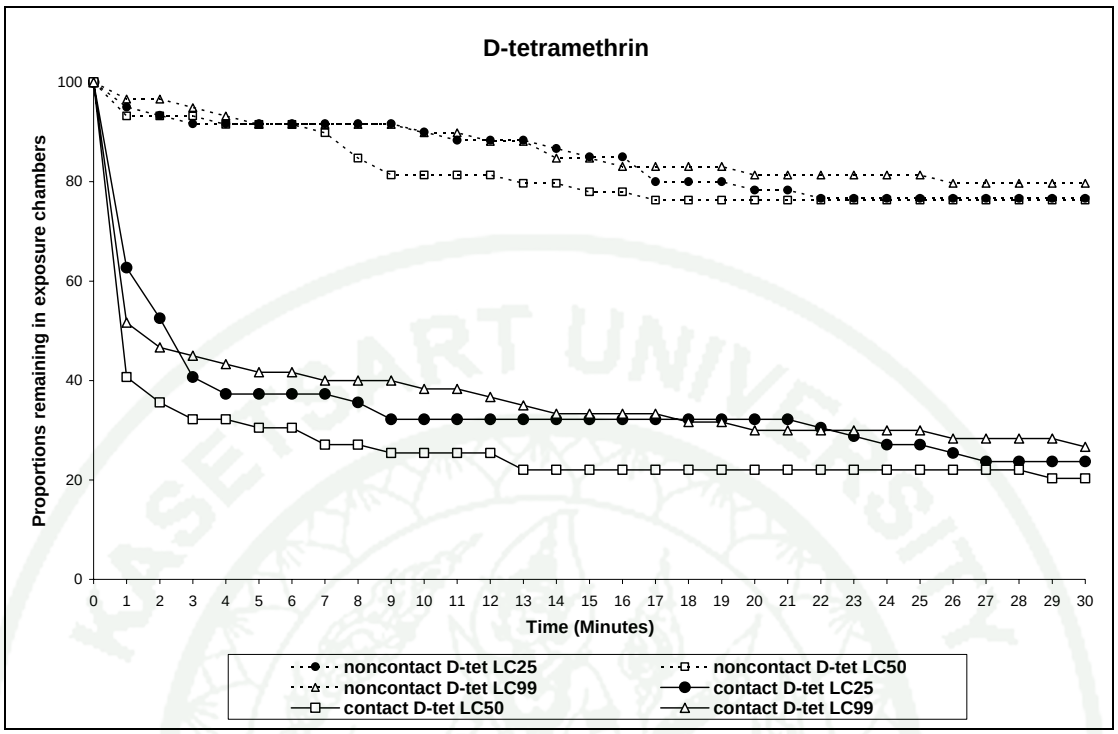


Figure 9 Escape rate of *Ae. aegypti* exposed to d-tetramethrin**.**

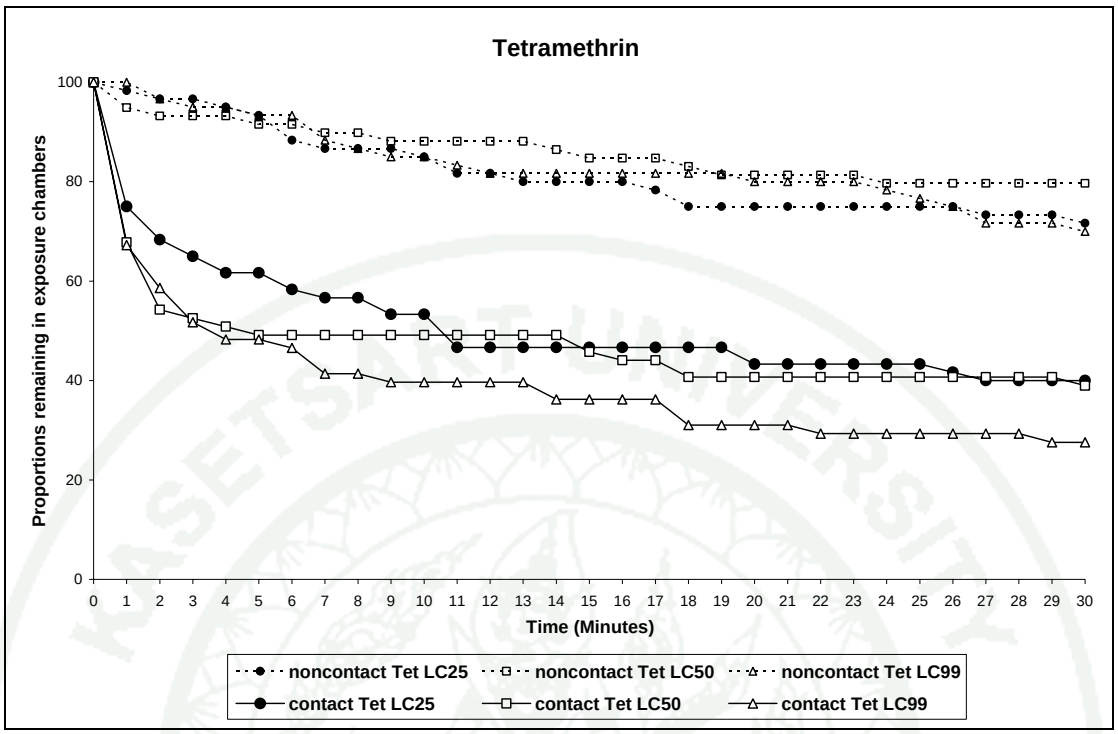


Figure 10 Escape rate of *Ae. aegypti* exposed to tetramethrin.

Part 3 To characterize the function of four synthetic pyrethroids by using the experimental hut study.

The LC_{50} for all four pyrethroids were tested in the field trials at the rate of one insecticide per month. The first test was deltamethrin followed by cyphenothrin, d-tetramethrin, and tetramethrin, respectively. Each insecticide was tested for six days. On the first three days of each trial the chemicals were tested in an entrance experiment, and the remaining three days were tested in the exit experiment.

Entrance experiments

We found that the two groups of one hundred marked mosquitoes that we released each day, there was indiscriminant movement to both the control and treated huts. However, the data from the two colors in each day that were released were used for statistical calculation. The difference in the percent of mosquitoes entering the two huts during entrance experiments revealed that cyphenothrin had the greatest difference, with the number of mosquitoes entering the control hut was higher than in the treated hut 16.28 % (Table 17). The percent differences with the other insecticides were as follows: 2.76 % in deltamethrin, and 7.18 % in tetramethrin. For d-tetramethrin, the entrance pattern was different from the other chemicals, with the number entering the control hut being lower than that recorded from the treated hut 6.16 %. However, the results of entrance experiments showed that there were no significant differences between the control hut and treated hut for three of the insecticides, with the only exception being with cyphenothrin at the LC_{50} (P = 0.0073) (Table 13).

When we divided the twelve hour collection into six equal periods, we found that the peaks of entrance behavior occurred during 0800 – 1000 hr, in both control and treated huts during all trials, except in the tetramethrin trial, where entrance behavior occurred in the early morning during 0600 – 0800 hr. Despite this difference, all trials resulted in only one peak that occurred in the morning with no additional afternoon peak.

Exit experiments

One hundred unfed mosquitoes were released at 0500 hr. At 0540 hr, all exit traps were opened to let the mosquitoes fly out via the window or door traps. Both huts maintained a human-bait who stayed under an untreated bed net in order to establish a host presence in the huts. Those mosquitoes affected by the chemical would fly out of the hut because of the irritant effect of the insecticide impregnated net on the wall. Moreover, the effect of temperature and humidity inside the hut also caused the mosquitoes to fly out resulting in movement out of the control hut, as well. The difference between the two huts could be quantified as the intensity of the exiting response or a shift in time for the mosquitoes to exit the treated hut earlier than they would under control conditions.

The results showed the different patterns of escape times of mosquitoes between the control hut and treated hut. Each insecticide elicited a different exit pattern. Deltamethrin and cyphenothrin both showed significant differences between the control and treated hut, $P = 0.0257$ and 0.0101, respectively (Table 13). The mosquitoes in the treated huts would fly out before the mosquitoes in the control huts. Because of this we would know by the percent numbers of escaping mosquitoes in the treated huts were higher than in the control huts since 0800 hr (Table 18). The ET_{25} and ET_{50} in Table 14 also supported these results.

As in the entrance experiment, the d-tetramethrin trial did not show a significant difference in the escape pattern between control and treated huts, $P = 0.1418$ (Table 13). However, the escape time in the control hut was quicker than the treated hut (Table 14). The results from the tetramethrin trials showed a significant difference between control and treated huts, $P = 0.0001$ (Table 13). However, the pattern of movement was different from the other insecticides. The pattern of escape from the control hut showed a higher percent and faster escape response as compared to the treated hut (Table 14 and 15).

Table 10 Escape rate and mortality of *Ae. aegypti* in response to deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin, LC_{50}, in the field condition of avoidance response experiment by using experimental huts.

Chemical	LC	Treated hut		Control hut		%Mortality in 24hrs			
						Treated hut		Control hut	
		No. tested	% Escaped	No. tested	% Escaped	Escape	Not escape	Escape	Not escape
Del	50	241	73.03 (176)	261	63.22 (165)	5.11 (9)	20.00 (13)	0	0
Cyp	50	252	66.67 (168)	243	55.14 (134)	2.98 (5)	14.44 (13)	0	0
D-tet	50	239	64.44 (154)	274	70.07 (192)	7.10 (11)	8.33 (7)	1.04 (2)	0
Tet	50	248	57.26 (142)	269	75.46 (203)	4.23 (6)	1.89 (2)	0.5 (1)	0

Table 11 Mortality rate of knock down mosquitoes on floor during the tests of exit experiments.

Chemical	LC	Treated hut		Control hut		%Mortality of knock down in 24hrs	
						Treated hut	Control hut
		No. tested	% Knock down	No. tested	% Knock down		
Del	50	241	0.05 (11)	261	0 (0)	90.90 (10:11)	0 (0)
Cyp	50	252	0.02 (6)	243	0 (0)	100 (6:6)	0 (0)
D-tet	50	239	0.02 (4)	274	0 (0)	50 (2:4)	0 (0)
Tet	50	248	0 (0)	269	0 (0)	0 (0)	0 (0)

Table 12 Entrance and mortality rate of *Ae. aegypti* in response to deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin, LC_{50}, in the field condition of host seeking experiment by using experimental huts.

Chemical	LC	Treated hut		Control hut		%Mortality in 24hrs	
		No. tested	% Entrance	No. tested	% Entrance	Treated hut	Control hut
Del	50	600	8.83 (53)	600	9.33 (56)	0	0
Cyp	50	600	15 (90)	600	20.83 (125)	0	0
D-tet	50	600	11.50 (69)	600	10.17 (61)	0	0
Tet	50	600	25.83 (155)	600	29.83 (179)	0	0

Table 13 Log-rank comparisons of escape responses between control hut and treated hut of exit experiment, and entrance experiment, which treated hut was treated with the lethal concentration 50, (LC_{50}) of deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin.

Chemical	LC	Exit experiment (*P*-value)	Entrance experiment (*P*-value)
Del	50	0.0257*	0.7732
Cyp	50	0.0101*	0.0073*
D-tet	50	0.1418	0.4342
Tet	50	0.0001*	0.1138

* identifies result of log-rank tests with statistically significant differences ($P<0.05$).

Table 14 Time in hours for 25% (ET_{25}), 50% (ET_{50}), and 75% (ET_{75}) of *Ae. aegypti* females to escape from control hut and treated hut, which each treated hut was treated with lethal concentration 50, (LC_{50}) of deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin.

Chemical	LC	Treated hut (min.)			Control hut (min.)		
		ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}
Del	50	11.30	13.50	NA	11.50	14.50	NA
Cyp	50	12.10	16.10	NV	14.10	17.10	NA
D-tet	50	14.10	16.30	NA	12.50	16.10	NA
Tet	50	11.10	16.20	NA	9.30	12.00	17.50

Table 15 Number of *Ae. aegypti* which collected from entrance traps by collections conducted for 3 days in untreated huts (pre-treatments).

Experiment	Hut	Number of *Ae. aegypti*							
		0600-0800	0800-1000	1000-1200	1200-1400	1400-1600	1600-1800	Total	Ratio
Entrance	Hut 1	0	46	15	4	0	2	67	0.91
	Hut 2	0	48	20	4	1	1	74	1.00

(Hut 1 was used as treated hut (T1), and hut 2 as control hut (C1).)

Table 16 Total numbers of *Ae. Aegypti* which collected from the entrance traps during four time periods during 0600 to 1800 (data from three days) in entrance experiments, between treated hut and their matched control for three days per an insecticide.

Experiment	Hut	Number of entrance *Ae. aegypti* into traps							
		0600-0800	0800-1000	1000-1200	1200-1400	1400-1600	1600-1800	Total	% Reduction
Entrance	Del	6	19	18	4	5	1	53	-4.531
	Con	1	28	10	5	6	6	56	
	Cyp	6	56	19	2	5	2	90	20.478
	Con	14	68	25	3	7	8	125	
	D-tet	8	25	25	5	2	4	69	-24.933
	Con	1	26	18	5	6	5	61	
	Tet	86	42	13	7	5	2	155	4.361
	Con	108	46	14	7	4	0	179	

% reduction = (100 – (((C1 x T2) / (T1 x C2)) x 100))

- C1 and T1 were the number of entrance mosquitoes in control hut and treated hut, respectively, when we did the pre-treatments.
- C2 and T2 were the number of entrance mosquitoes in control hut and treated hut, respectively, when we did the post-treatments for each insecticide.

Table 17 Percent ratios of *Ae. aegypti* which entered the entrance traps during 6 periods of 12 hrs collection (data from three days) compared between hut treated with deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin and the pair control.

Experiment	Hut	Percents of entrance *Ae. Aegypti* in each periods						Total
		0600-0800	0800-1000	1000-1200	1200-1400	1400-1600	1600-1800	
Entrance	Del	5.50	17.43	16.51	3.67	4.58	0.91	48.62
	Con	0.91	25.68	9.17	4.58	5.50	5.50	51.38
	Cyp	2.79	26.04	8.83	0.93	2.32	0.93	41.86
	Con	6.51	31.62	11.62	1.39	3.25	3.72	58.14
	D-tet	6.15	19.23	19.23	3.84	1.53	3.07	53.08
	Con	0.76	20.00	13.84	3.84	4.61	3.84	46.92
	Tet	25.74	12.57	3.89	2.09	1.49	0.59	46.41
	Con	32.33	13.77	4.19	2.09	1.19	0	53.59

Table 18 Percent of *Ae. aegypti* recaptured in exit traps in huts treated with deltamethrin, cyphenothrin, d-tetramethrin, and tetramethrin as compared to a matched control during 6 periods of 12 h collection (data from three days).

Experiment	Hut	Percents of entrance *Ae. Aegypti* in each periods						Total
		0600-0800	0800-1000	1000-1200	1200-1400	1400-1600	1600-1800	
Exit	Del	2.49	7.88	19.91	20.74	15.35	6.63	73.03
	Con	1.91	8.42	14.94	18.77	14.55	4.59	63.21
	Cyp	0.78	3.10	14.34	18.21	10.85	17.82	65.12
	Con	1.23	2.88	8.23	11.52	16.04	15.22	55.14
	D-tet	4.18	5.44	6.27	8.36	19.66	20.92	64.85
	Con	2.55	5.10	10.58	11.67	16.42	23.72	70.07
	Tet	2.42	10.48	20.16	6.85	8.46	8.87	57.26
	Con	7.43	25.65	17.10	7.06	7.80	10.40	75.46

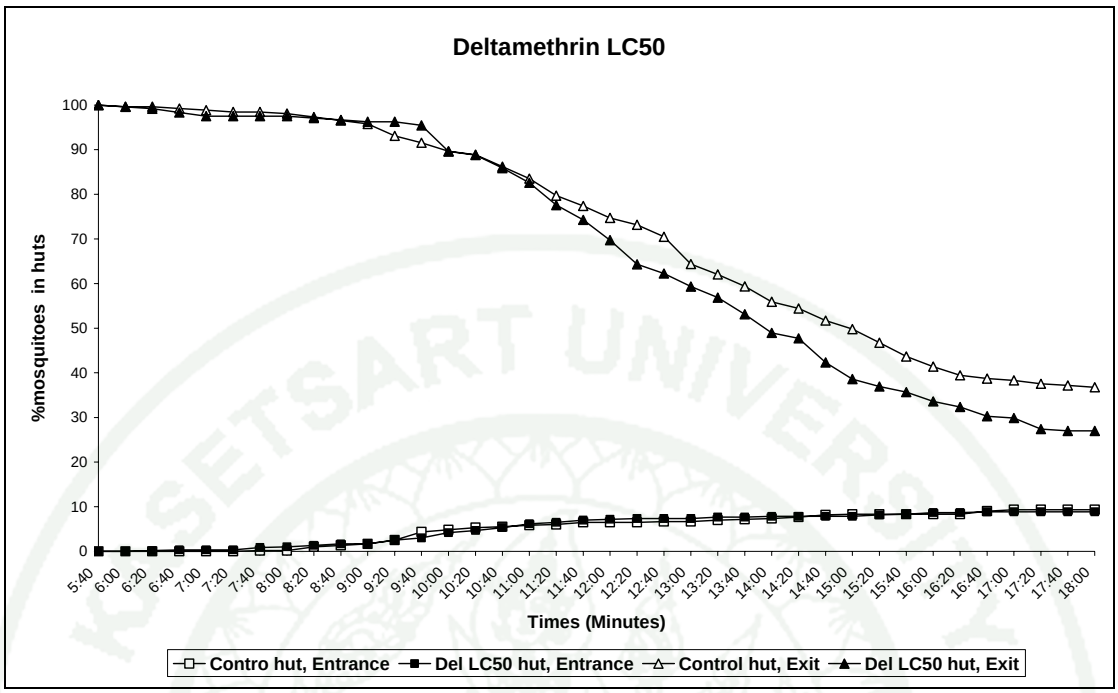


Figure 11 Escape rate and entrance rate of *Ae. aegypti* in exit experiment and entrance experiment exposed to a pair of control and treated huts (treated hut was treated with deltamethrin LC_{50}).

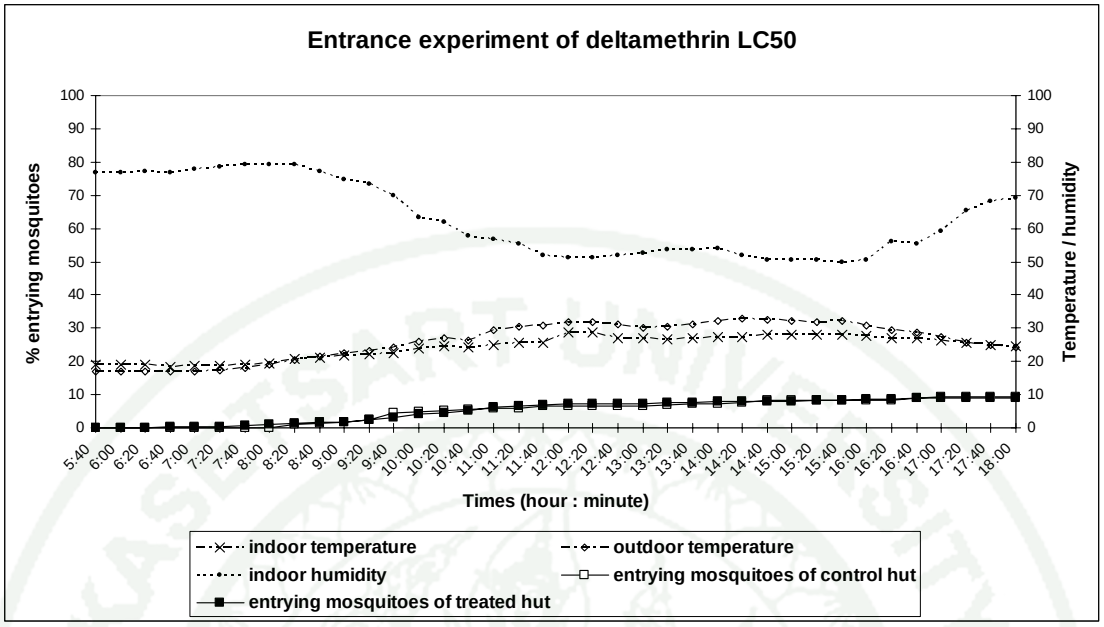


Figure 12 Entrance rate of *Ae. aegypti* between control and deltamethrin LC_{50} - treated hut in entrance experiment, and environmental data during exposure time.

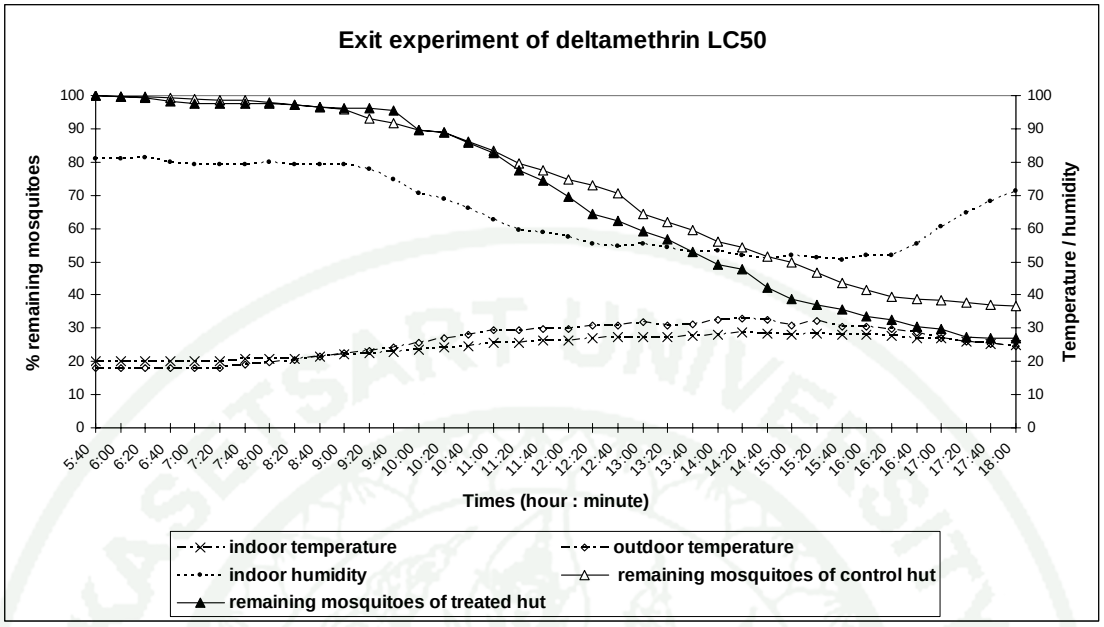


Figure 13 Escape rate of *Ae. aegypti* between control and deltamethrin LC_{50} - treated hut in exit experiment, and environmental data during exposure time.

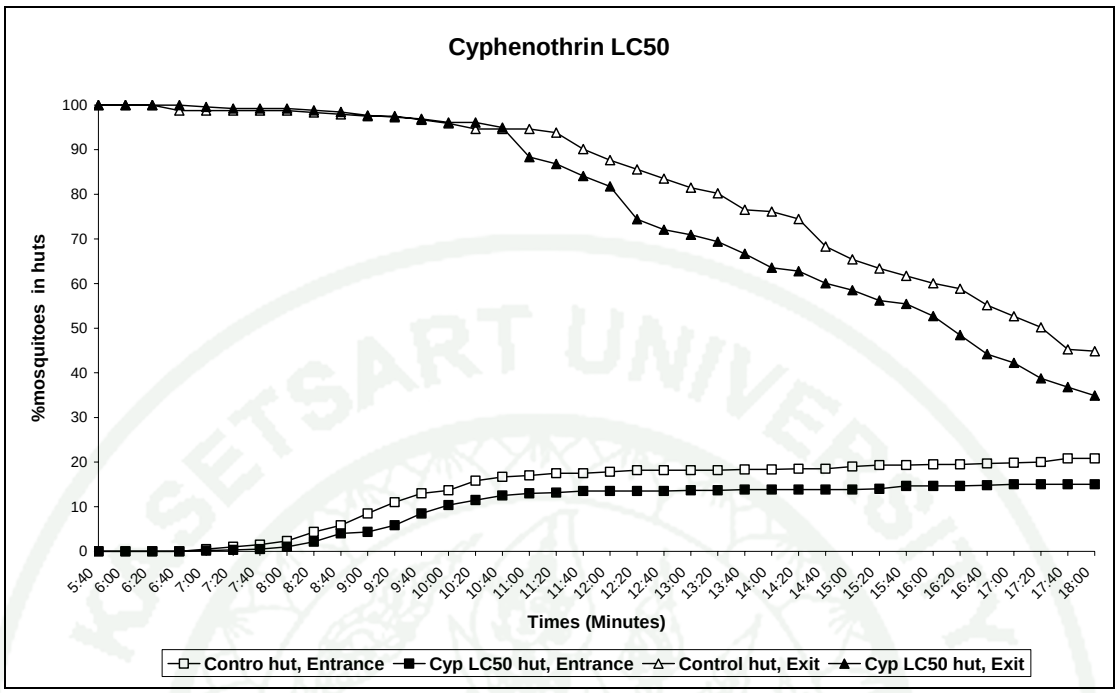


Figure 14 Escape rate and entrance rate of *Ae. aegypti* in exit experiment and entrance experiment exposed to a pair of control and treated huts (treated hut was treated with cyphenothrin LC_{50}).

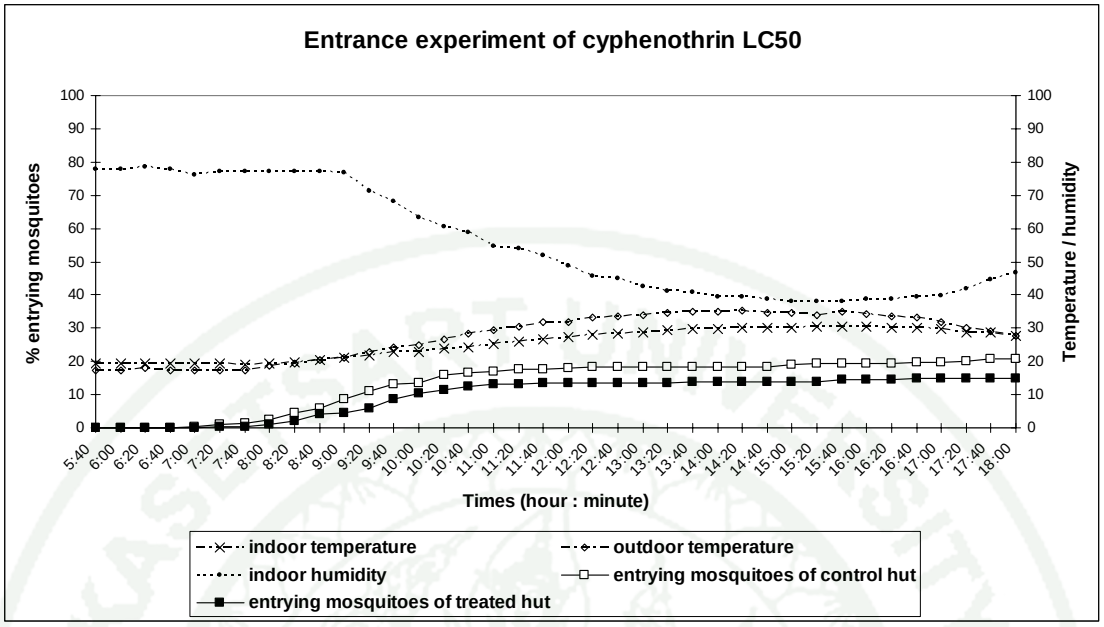


Figure 15 Entrance rate of *Ae. aegypti* between control and cyphenothrin LC_{50} - treated hut in entrance experiment, and environmental data during exposure time.

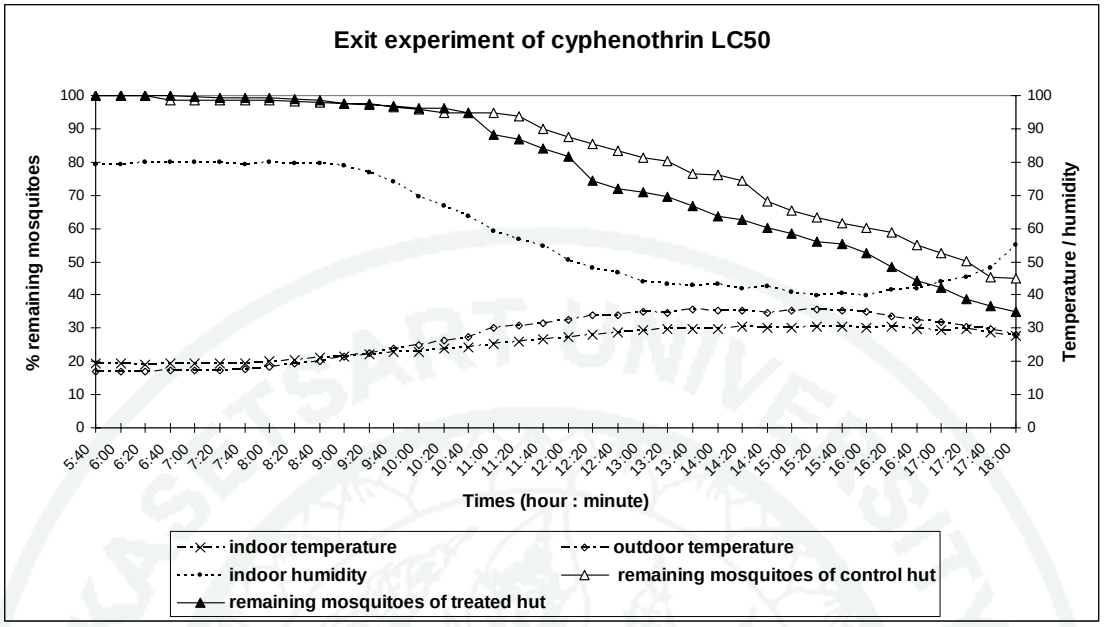


Figure 16 Escape rate of *Ae. aegypti* between control and cyphenothrin LC_{50} - treated hut in exit experiment, and environmental data during exposure time.

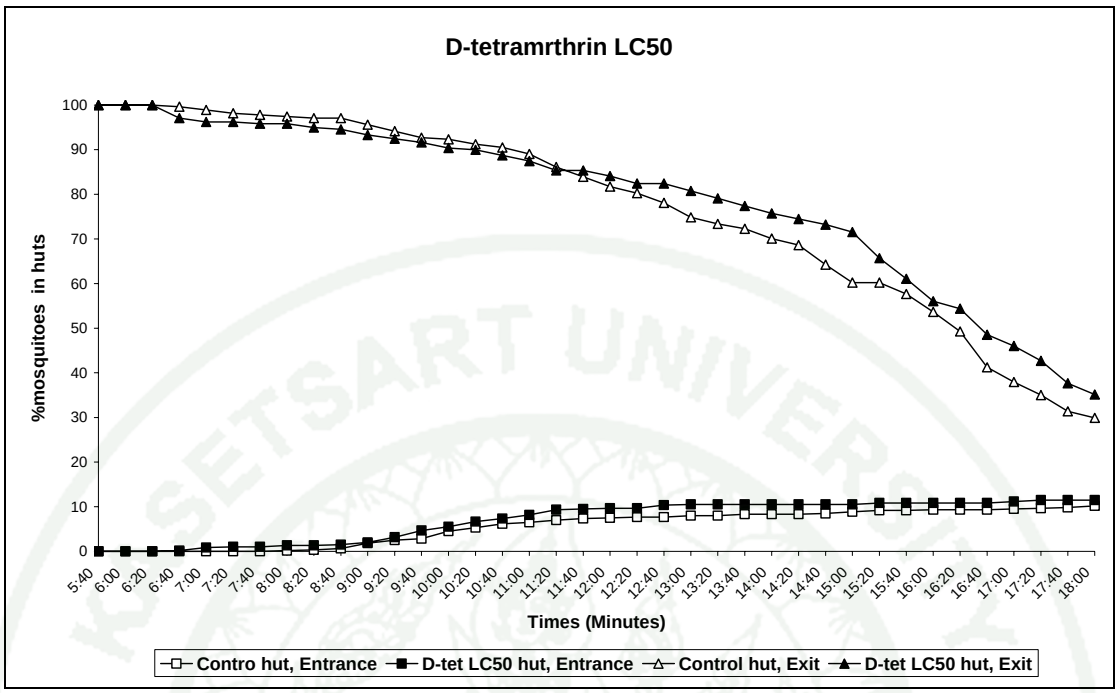


Figure 17 Escape rate and entrance rate of *Ae. aegypti* in exit experiment and entrance experiment exposed to a pair of control and treated huts (treated hut was treated with d-tetramethrin LC_{50}).

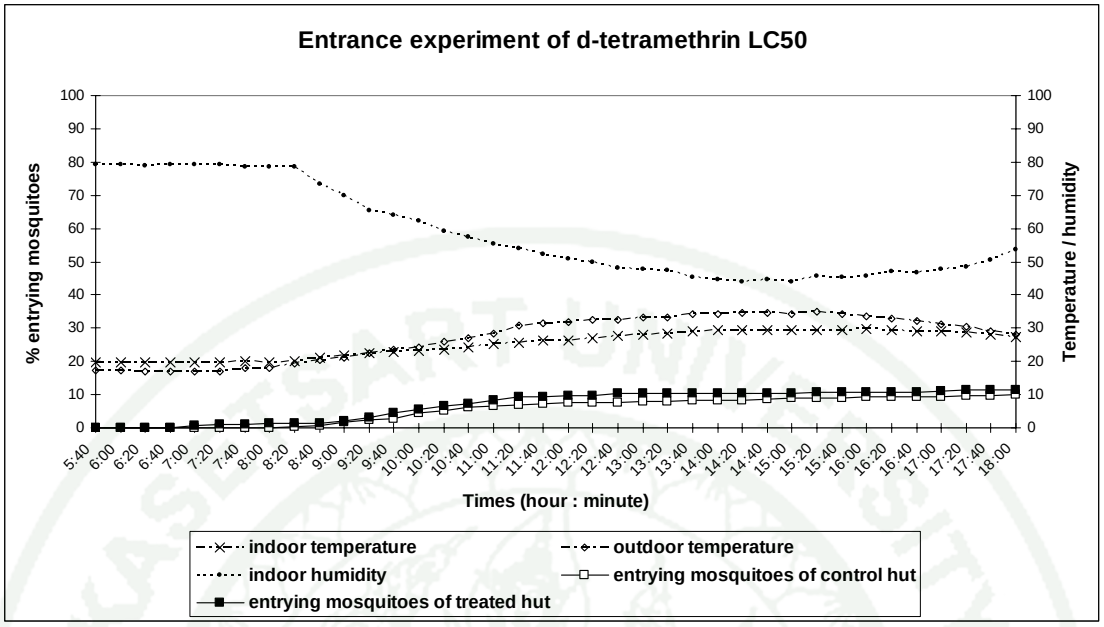


Figure 18 Entrance rate of *Ae. aegypti* between control and d-tetramethrin LC_{50} - treated hut in entrance experiment, and environmental data during exposure time.

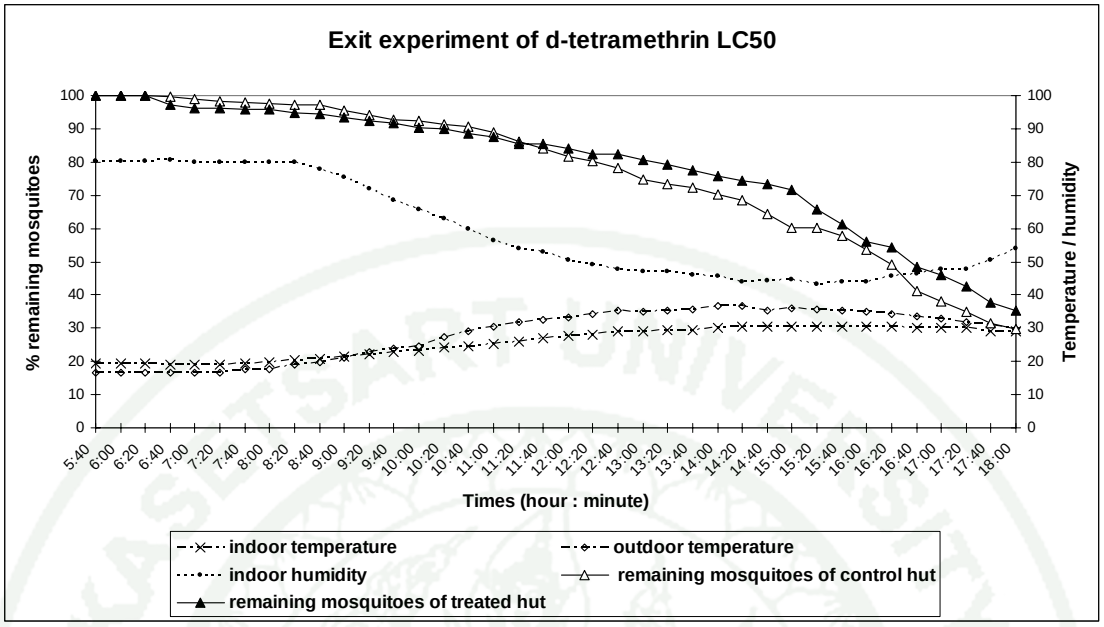


Figure 19 Escape rate of *Ae. aegypti* between control and d-tetramethrin LC_{50} - treated hut in exit experiment, and environmental data during exposure time.

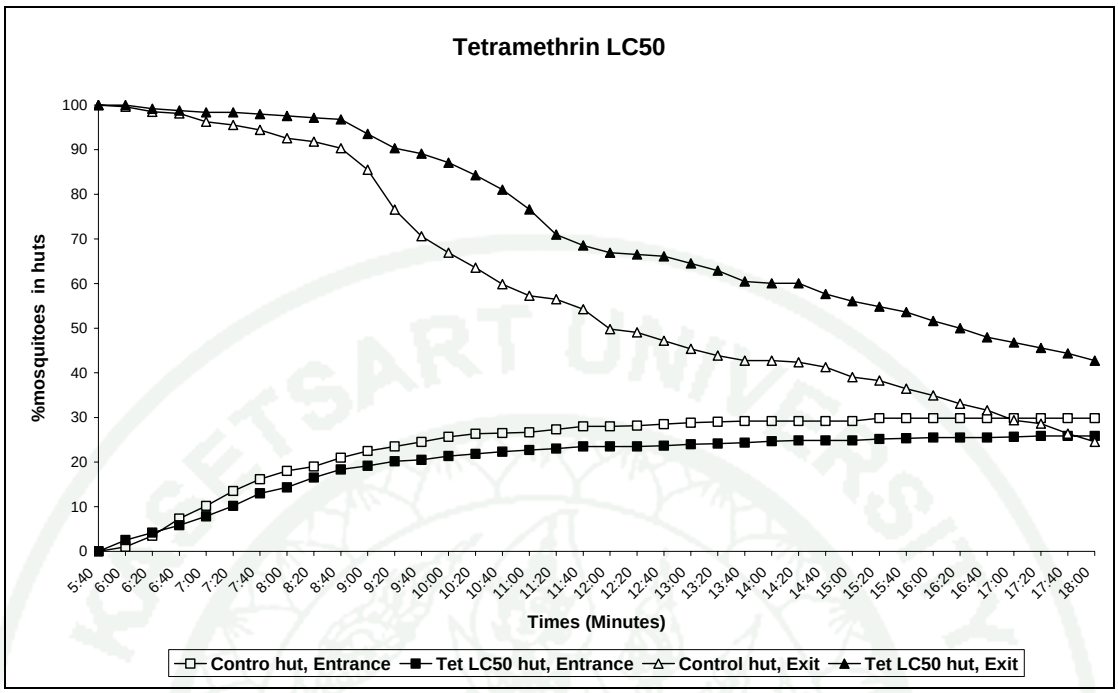


Figure 20 Escape rate and entrance rate of *Ae. aegypti* in exit experiment and entrance experiment exposed to a pair of control and treated huts (treated hut was treated with tetramethrin LC_{50}).

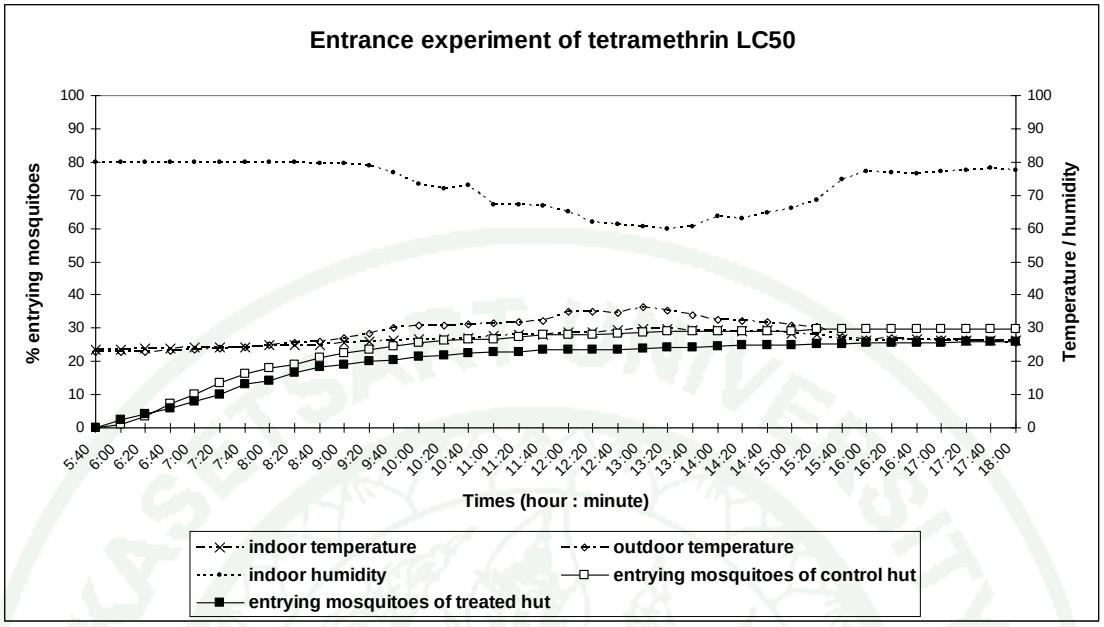


Figure 21 Entrance rate of *Ae. aegypti* between control and tetramethrin LC_{50} - treated hut in entrance experiment, and environmental data during exposure time.

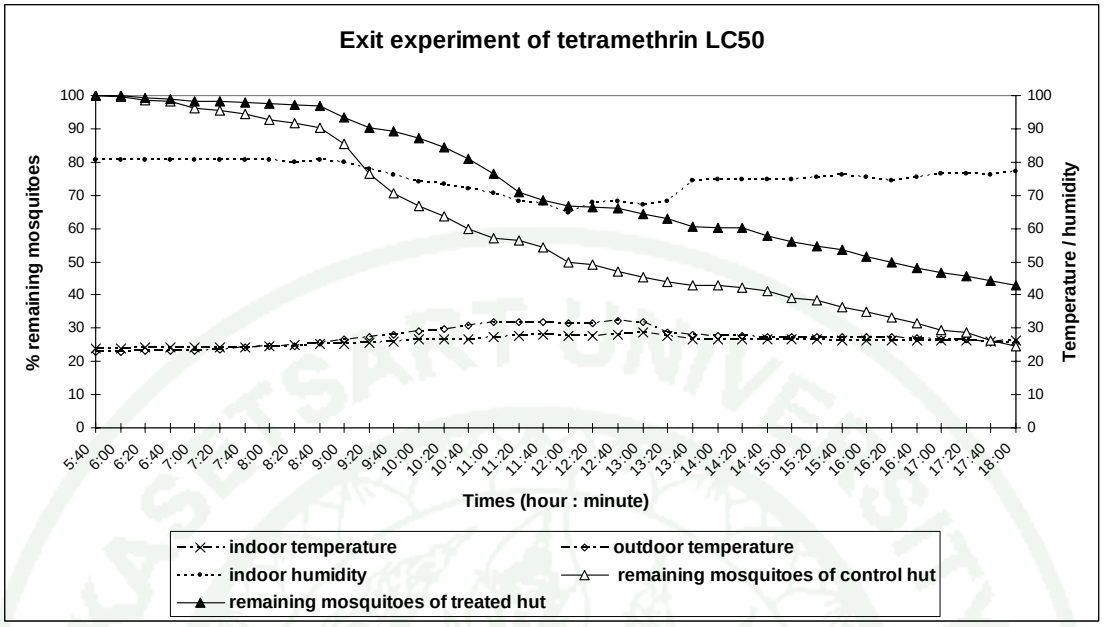


Figure 22 Escape rate of *Ae. aegypti* between control and tetramethrin LC_{50} - treated hut in exit experiment, and environmental data during exposure time.

DISCUSSION

Part 1 To determine the optimum dose of four synthetic pyrethroids against *Ae. aegypti* in the excito repellency test.

From table 1, deltamethrin elicited the highest toxic response in the mosquitoes when compared with the other three insecticides. This is based on the slope of the regression lines for the test data. High toxicity means that only a small amount of insecticide will result in high mosquito mortality. Conversely, if we have to add too much insecticide for killing the same number of mosquitoes, it means, that insecticide has very low toxicity. The higher slope occurs because the LC_{25} , LC_{50} , and LC_{99} are very close together on the X axis, so it will raise the slope up. Moreover, if each concentration of LC_{25}, LC_{50}, and LC_{99} are far out, that insecticide will pull the slope down too. The ranges between each lethal concentration; LC_{25}, LC_{50}, and LC_{99} of each insecticide are different quantity of active ingredient. D-tetramethrin and tetramethrin seem to be the lowest toxicity, because their slopes are very small, 0.190, and 0.121, respectively. So, these two insecticides must be used in high concentrations to prepare the impregnated papers for any tests.

This part was established to find the lethal concentrations of each insecticide, then used LC_{25}, LC_{50}, and LC_{99} to test behavioral responses of *Ae. aegypti* later in part2.

Part 2 To compare the behavioral responses of field collected *Ae. aegypti* to four synthetic pyrethroids.

Behavioral responses to chemicals by mosquitoes have long been recognized (Roberts and Andre, 1994). In the past, responses to chemicals by mosquitoes were often ignored when selecting compounds for vector control programs. Most works focused on the toxic action (killing) of test chemicals on insect populations. Relatively little has been done on the behavioral responses of mosquitoes to

chemicals. We believe that at least two different categories of behavioral responses in mosquito vectors exist; contact irritancy and noncontact spatial repellency (Rawlings and Davidson, 1982; Roberts and Andre, 1994; Chareonviriyaphap *et al.*, 1997). Irritability occurs when an insect is stimulated to move away from an insecticide treated surface after making direct physical contact with the insecticide residue. In contrast, spatial repellency takes place when the insect detects and avoids a treated surface without making physical contact (Roberts and Andre, 1994; Chareonviriyaphap *et al*., 1997).

Both types of behavioral responses can be experimentally differentiated by using the excito-repellency (ER) test system (Chareonviriyaphap *et al*., 1997, 2002). In 2000, Roberts *et al*. (2000) proposed a mathematical model for better understanding the repellent, irritant and toxic functions of insecticides to control vector-borne disease. Recently, a modular, high-throughput laboratory-based assay system for rapid detecting the three actions, irritancy, repellency and toxicity, of insecticides was developed (Grieco *et al*., 2007). Since the development of the two systems for evaluating the behavioral responses in mosquitoes and with the development of a quantified mathematical framework, reports on behavioral responses by mosquitoes to public health insecticides have been progressively increasing (Chareonviriyaphap *et al.*, 2001, 2004; Grieco *et al*., 2007; Polsomboon *et al*., 2008). Most of these studies have focused on the behavioral response of *Anopheles* species to insecticides whereas comparatively little has been published on the avoidance behavior of *Ae. aegypti* exposed to test chemicals (Kennedy, 1947; Kongmee *et al*., 2004; Grieco *et al*., 2007; Poolsomboon *et al*., 2008).

In the past, we observed the clear insecticide responses by several *Anopheles* mosquitoes (Chareonviriyaphap *et al*., 1997, 2001, 2004; Sungvornyothin *et al*., 2001), *Aedes* mosquitoes (Kongmee *et al*., 2004; Grieco *et al*., 2007), and *Culex* mosquitoes (Sathantriphop *et al*., 2006). In this study, two different types of behavioral responses are examined in *Ae. aegypti* to four synthetic pyrethroids, deltamethrin, cyphenothrin, d-tetramethrin and tetramethrin. We found that contact irritancy was the primary behavioral response elicited by these compounds. However,

we found that there was a low but statistically significant noncontact spatial repellent escape response in the pairs of noncontact vs. control trials of LC_{50} and LC_{99} values for cyphenothrin. Significant behavioral avoidance responses were observed in all contact trials when compared with their paired controls, regardless of insecticide or concentration used. Greater escape responses after physical contact were observed from deltamethrin at LC_{50} and LC_{99} compared with the other three chemicals. In general, no significant differences in escape responses to the three doses of four different chemicals in *Ae. aegypti* females were found, suggesting that using insecticides at sublethal doses may be appropriate in controlling disease vectors. Applying the minimal dose to elicit a behavioral response can also help to prevent or delay insecticide resistance in insect populations (Grieco *et al*., 2007).

Deltamethrin is currently one of the most commonly used insecticides for public health and has been the mainstay for emergency control of *Ae. aegypti* adults in Thailand since 1994 (Chareonviriyaphap *et al*., 1999; Kongmee *et al*., 2004). In addition to deltamethrin, several other synthetic pyrethroids, *i.e.* cyphenothrin and tetramethrin, are commonly used by home owners to control household mosquitoes and other arthropod pests (Sathantripop *et al*., 2006). The continued demand for synthetic pyrethroids serves as the stimulus for further studies to evaluate the avoidance behavior of pyrethroids to *Ae. aegypti* mosquitoes. In addition, little is known about the role of the irritant and repellent actions of pyrethroids on *Ae. aegypti* and how they function to break disease transmission (Kongmee *et al*., 2004).

We now have a laboratory based system to evaluate these two behavioral actions, irritancy and repellency (Chareonviriyaphap *et al*., 2002; Grieco *et al*., 2005). A mathematical framework for understanding the true function of chemicals in controlling disease transmission has also been developed (Roberts *et al*., 2000). Grieco *et al*. (2007) have demonstrated the three actions of chemicals using experimental hut studies. However, this model must be evaluated using different mosquito species from endemic areas as well as other chemicals in order to gain a better understanding of how to improve our vector control strategies. In the future, a

greater focus should be placed on the role of excito-repellency in the control of dengue.

Part 3 To characterize the function of four promising synthetic pyrethroids by using experimental huts.

Experimental huts are commonly used to evaluate a variety of characteristics of insecticides, such as the efficiency of residual spraying on house walls and the evaluation of insecticide-impregnated nets on mosquito behavior (WHO, 2000). In addition, experimental huts equipped with entrance and exit traps can be used to evaluate other mosquito behaviors. Most experimental huts must be constructed to mimic typical housing structures in the area of study. The specific aim of this study was to identify modified behaviors of *Ae. aegypti* in response to different kinds of pyrethroids and to evaluate their efficacy in reducing risks of mosquito entering a house (noncontact repellency). Moreover, the optimum dosage (minimum) of each insecticide can help delay mosquito resistance. In this study, two experimental huts were constructed to house insecticide-impregnated nettings (Chareonviriyaphap *et al.*, 2005).

Each mosquito species characteristically bites at a certain time of day (Clements, 1999). The time of biting is decided by the physiological rhythm of the species and by reactions to daily cycles of light, temperature, and humidity. Moreover, it is well known that different species of mosquitoes show different characteristic host preferences (Clements, 1999). *Ae. aegypti* is a day time biting mosquito which prefers to feed on human inside dwelling. The most important factor from human skin that attracts mosquitoes to come is human odor (Khan and Maibach, 1966). Gillies and Wilkies (1969, 1970, 1972) found that the human odors were effective at attracting mosquitoes from a greater distance than CO_2. Moreover, mosquitoes will fly upwind toward the hosts even against a strong wind whenever they are searching for their preferred hosts (Rudolfs, 1922; Happold, 1965; Hocking, 1971).

In this study we demonstrated that the impact of insecticides on vector populations is much more complex than just toxicity. Grieco *et al*. (2007) indicated that insecticides can function as repellents (spatial repellency) and as irritants (contact irritancy). In this study, the optimum dosage of each pyrethroid was used in order to observe these two properties. Our previous results (from part 2) indicated that all three lethal concentrations at LC_{25}, LC_{50}, and LC_{99} of the four pyrethroids did not have a spatial repellent effect, except for cyphenothrin at the LC_{50}, and LC_{99}. However, we found that the LC_{50} dosage of all four pyrethroids elicited contact irritancy resulting in the LC_{50} being selected for use in the experimental hut studies.

Entrance experiment

Even though, we marked two groups of mosquitoes with two different colors we found that the two groups of mosquitoes would mix and travel to both the treatment and control huts in equal numbers. Therefore, when conducting survival analysis the two release populations were summed for a total release of 200 mosquitoes. All mosquitoes that did not enter the control hut, including those that entered a hut not where they were released, would be called “the censored of control hut”, conversely, all mosquitoes that did not enter the treated hut were called “the censored of treated hut”. The factors that led to the mosquitoes entering one hut or the other were 1) a host presence without chemical in hut (control hut), and 2) a host presence with insecticide in hut (treated hut). In the control hut, there were only the host attractants such as, carbon dioxide, fatty acids, lactic acid in human sweat, and human odor etc. that were released into the air, while the treated hut contained a mixture of host attractants and volatilized insecticide. Several statistical methods were used in addition to survival analysis to analyze the experimental hut data because it was important to not only evaluate the number of mosquitoes entering each hut but also the time of entering. Even though the total number of mosquitoes entering each hut might be equal at the end of the day, the time of entering might be different demonstrating an impact by the chemical.

All of the tests showed only one peak of host seeking which occurred in the morning. This was interpreted as the mosquitoes being interrupted by the strong sunlight. The majority of the trials were conducted during the winter, except for with tetramethrin which were conducted during the summer. During the winter the morning temperature was cool with temperatures ranging between 17.00°C-17.33°C at 0600 hr and 18.00°C-19.00°C at 0800 hr (Appendix Table 1-3). Therefore, the mosquitoes that were released outdoors at 0540 hrs remained in a resting state and were not active. So, the numbers of entering mosquitoes from 0600-0800 hr were very few. The temperature increased continuously from 18.00°C-19.00°C after 0800 hr to be 24.33°C-26.00°C at 1000 hr. Moreover, during this period, there was a light mist covering most of the study area until the ground was exposed to the sunlight. We noticed that the entering mosquitoes were very active when the sunlight was not strong. If the sunlight was intensely strong and hot, it would decrease the activity of the mosquitoes and they would avoid flying and rest in the shades of plants found around the huts. The resting mosquitoes would then enter the huts when the sun was less intense until 1800 hr. Moreover, we found that humidity and temperature maintained an inverse relationship, i.e. in the morning humidity would be high and gradually decrease throughout the day, until about 1600-1700 hr at which point the humidity would raise up again (Appendix Table 1-3).

The results of d-tetramethrin and tetramethrin in the experimental huts were different from the results from the excito-repellency test system. This may be due to inherent differences between these two methods. When we tested with the excito-repellency system, we did not use any baits inside the contact chambers for both the control and treatment. Moreover, the sizes of the two test platforms were different resulting in greater volumes of air in the experimental huts that may have contributed to differences in results.

In addition, of all entering mosquitoes into the traps from all tests showed a 0% mortality rate after 24 hrs. This means that all test mosquitoes remained healthy outside the huts, and were not affected by the vapor phase of the insecticides.

After reviewing all the data from the entrance experiments, we summarize that *Ae. aegypti* will emigrate from other places outside of the huts only once a day, i.e. in the morning. In fact, if there are dengue cases in the community, it is probable that the infectious vectors are flying from the patient's house to the other houses in the early morning hours. The outbreak of dengue usually occurs by this way. If the report of cases is delayed, the spraymen will come to control the vectors late too. So, insecticides which have a repellent effect may help to protect the people from these infectious vectors, and thereby slow transmission and reduce the number of cases. In our study, the time when mosquitoes are most active is the time when people are working, which may help to reduce the risk of man vector contact. Only people who stay in the houses at that time will be at risk for dengue infection. In the morning the people who remain in the houses should stay under mosquito nets or use window screens.

Exit experiment

Everyone who studies mosquito behavior in the field must face the same issue of the loss of test subjects during a test. Loss of mosquitoes can result from mistakes in counts before release; escape to the outside of the hut when hosts switch positions; loss due to knockdown that were not observed before being walked on or carried off by other insects. Only mosquitoes that could be accounted for at the end of the study were considered in the analysis of the results.

In general, the excito-repellency system did not result in significantly different findings in escape responses between the three doses of these four chemicals in *Ae. aegypti* females. So, this suggests that using insecticides at sublethal doses may be appropriate in controlling disease vectors. Applying the minimal dose to elicit a behavioral response can also help to prevent or delay resistance buildup in insect populations (Grieco *et al*., 2007). In the experimental huts, we found that these sublethal doses (LC_{50}) might not be enough to establish an effective vapor phase to elicit a behavior response in mosquitoes. Deltamethrin and cyphenothrin at sublethal doses seemed to repel a proportion of the mosquitoes but this proportion was small

compared with the matched controls. This may be the result of the much larger volume of air present in the experimental hut as compared to the excito-repellency chamber while the vaporizing rate of each insecticide remained constant. The result would be less volatilized insecticide in the hut as compared to the excito-repellency chamber. Despite this fact, the results show that both deltamethrin and cyphenothrin maintained the same trends as seen in the excito-repellency test system, however deltamethrin did show a minor repellent effect. For the optimum dose we selected the LC_{50}, which was calculated based on their killing action against mosquitoes. Deltamethrin had the highest toxicity, resulting in the smallest amount of active ingredient to achieve the LC_{50}. The level of repellency for an insecticide, however, may depend on its evaporation rate. Some insecticides have a low vapor pressure and may require a higher dose to elicit a repellent response. Conversely, if the insecticide has high vapor pressures, they might repel mosquitoes at a much lower dose.

As mentioned above, these four insecticides all had the effect of contact irritancy, so if the mosquitoes did not contact the impregnated nets, they would not fly out of the huts. Because there was a host in the huts, some mosquitoes tried to bite the host and did not rest on the impregnated nets or avoid the huts. However, most of the mosquitoes would escape from the huts in the afternoon, especially 1200-1400 hr. The humidity and temperature would track together as observed in the entrance experiment, i.e. in the morning humidity would be high and after that reduce thoroughly day, until about 1600-1700 hr the humidity would raise up again (Appendix Table 4-6). Finally, very few mosquitoes would remain in the huts in the evening. It was suggested that the morning was the most dangerous time for humans to get dengue infected. Therefore, insecticides should be used which have a repellent effect to chase the indoor mosquitoes out.

For d-tetramethrin and tetramethrin the results showed that both insecticides had no significant irritant response when compared with their match control hut. The reason for this may once again be the result of the larger surface in the experimental hut. It was noticed, however, that with these two chemicals the mosquitoes appeared drunk after exposure in the huts. This could be explained as a side effect of the

mosquitoes making contact with the toxic compounds and altering the health status of the mosquitoes. This resulted in the mosquitoes becoming weak and being unable to fly out of the huts. Because d-tetramethrin and tetramethrin are both members of a noncyano-containing pyrethroid group, the irritancy effect may be lower than for deltamethrin and cyphenothrin, which are members of a cyano-containing pyrethroid group. The cyano-containing pyrethroids will contain an α-cyano-3-phenoxybenzyl alcohol, which increases insecticidal activity about 10 folds from the noncyano-containing pyrethroids. At sublethal concentrations the chemicals used made the mosquitoes that contacted the impregnated net appear drunk.

In addition, the mortality rates for mosquitoes collected from the traps were different for the exit collections as compared to the entrance experiments, i.e. deltamethrin and cyphenothrin killed the escaping mosquitoes at 20% and 14.44%, respectively. Of the mosquitoes that were knockdown in the huts, 90.90-100% mortality rates were recorded after 24 hrs. These data demonstrate the high toxicity as well as the high level of irritancy elicited by these two insecticides. Both d-tetramethrin and tetramethrin showed lower toxicity. These knockdown and mortality results from the exit experiments indicate that the mosquitoes occasionally contact the insecticide residues on the walls and will occasionally rest long enough to pick up a lethal dose of chemical.

The optimal situation would be to find insecticides that have both a killing effect and can also chase mosquitoes out of the hut. The insecticides that we select should have at least, a contact irritant or repellent effect. Both deltamethrin and cyphenothrin are interesting insecticides that require more evaluation. However, they should be used at higher concentrations than the $LC_{50,}$ because in a real house, this concentration is not enough. Closer consideration should be paid to the ratio of vapor pressure of the intended chemical to the air volume of the intended treated space. For d-tetramethrin and tetramethrin, a larger amount of active ingredient was required to achieve the LC_{50} due to their lower toxicity. No further evaluation of these chemicals is warranted given the field results and the high cost of these two chemicals.

Malaria control programs in Thailand use deltamethrin as the frontline insecticide for indoor residual spraying (IRS). The used target dose is 20 mg a.i. / m^2. In this experiment we used deltamethrin at a dosage of only 7.5867 mg a.i. / m^2. Therefore, the houses sprayed with deltamethrin for malaria control should also provide a level of protection from *Ae. aegypti*.

CONCLUSION

1. To determine the optimum dose of synthetic pyrethroids against *Ae. aegypti* in excito repellency test.

Deltamethrin had a higher toxicity than the other three insecticides based on the slope of the regression line (24.587). The remaining chemicals in order of toxicity from highest toxicity to lowest were cyphenothrin (3.113), d-tetramethrin (0.190), and tetramethrin (0.121), respectively. Results of susceptibility tests of deltamethrin found that its LC_{25}, LC_{50}, and LC_{99} were 0.010%, 0.020%, and 0.055%, respectively. Cyphenothrin were 0.113%, 0.167%, and 0.353%, respectively. D-tetramethrin were 2.091%, 2.770%, and 5.114%, respectively. Tetramethrin were 2.377%, 4.251%, and 10.715%, respectively. All values were used in Part 2 of this study.

2. To compare the behavioral responses of field collected *Ae. aegypti* to four synthetic pyrethroids.

All four pyrethroids clearly showed contact irritancy in the excito-repellency assay tests. It was also found that cyphenothrin had a stronger repellent effect than the other pyrethroids, while the contact irritant effect was similar among all compounds tested. Mortality rates of *Ae. aegypti* at all three levels (LC_{25}, LC_{50}, and LC_{99}) of each insecticide were observed in both contact and noncontact exposure chambers. In general, higher mortalities were observed in contact trials than in noncontact trials and in controls. Within treatment trials, higher mortalities were observed from nonescaped mosquitoes compared with those that were able to escape. A level of high mortality can be seen in nonescaped mosquitoes from the LC_{99} compared with the LC_{25} and LC_{50} values. Comparatively, low percent mortality was observed from escaping females in contact trials, ranging from 0 to 7.69% for deltamethrin, from 2-8% for cyphenothrin, from 2.13-2.27 % for d-tetramethrin and 0 % for tetramethrin. A high mortality of nonescaped females from the treated chambers with cyphenothrin at LC_{99} (60%) and d-tetramethrin at LC_{99} (31.25%) was

recorded. Mortalities in noncontact insecticide trials were low, ranging from 0-4.76% for escaped specimens and 0-7.69% for nonescaped specimens.

Significantly greater escape responses were found in contact trials compared with noncontact trials ($P<0.05$). In the deltamethrin contact trials, higher escape responses were seen at LC_{50} (88.33%) and LC_{99} (86.67%) compared to LC_{25} (63.33%). Cyphenothrin produced a stronger escape response (80.36-83.33%) compared to d-tetramethrin (73.33%-79.66%) and tetramethrin (60.00-72.41%), regardless of test concentrations (Tables 2-5). Escape responses for insecticide noncontact trials were low, but significantly different from the controls in all cases ($P<0.05$).

The escape patterns from chambers treated with chemicals were defined as times for 25% (ET_{25}), 50% (ET_{50}), and 75% (ET_{75}) (Chareonviriyaphap et al. 1997). In contact trials, the ET_{25} value for all four chemicals was 1 min, except for deltamethrin at LC_{25} (4 min). ET_{50} values for three concentrations of cyphenothrin and d-tetramethrin were recorded at < 4 min. In general, ET_{99} value for d-tetramethrin was somewhat high compared with deltamethrin and cyphenothrin. Due to insufficient numbers of escaping mosquitoes after 30 min, the ET_{99} value for tetramethrin could not be calculated. In noncontact trials, ET_{25} values for all four chemicals were comparatively high (>18 min). The ET_{50} and ET_{99} values for all chemicals in noncontact trials could not be estimated due to low escape numbers.

Comparison between contact vs. noncontact and control vs. contact responses demonstrated significant differences in the escape response of *Ae. aegypti* across all doses for all four insecticides ($P<0.01$). Significant differences in escape patterns were not observed between paired noncontact vs. control trials, except the pairs of LC_{50} and LC_{99} values for cyphenothrin ($P<0.05$). Multiple comparisons among three different doses for all four chemicals in contact, noncontact, and control trials were evaluated. The pattern of escape response was analyzed with the log-rank method, and statistical significance was established at the 0.05 level of probability. No significant differences in escape patterns between any two doses from contact and

noncontact trials were found, except in two comparisons of deltamethrin in contact trials (LC_{25} vs. LC_{50} and LC_{25} vs. LC_{99}) and in one comparison of cyphenothrin in noncontact trials (LC_{25} vs. LC_{99}).

However, the LC_{50} of each pyrethroid was found to be the optimal dose for repelling *Ae. aegypti*. There was no significant difference in LC_{99} values for either noncontact or contact trials for each pyrethroid.

3. To characterize the function of promising synthetic pyrethroids by using the experimental hut study.

The results from part2 and part3 make us confident that some insecticides have behavior modifying actions against mosquitoes that go far beyond their killing effect. Even though, all four insecticides tested in the excito-repellency system demonstrated a contact irritancy effect, the experimental hut tests showed only two insecticides with this property, i.e. deltamethrin and cyphenothrin. In general, the results from the experimental huts and the excito-repellency system showed the same trend. The results from the entrance experiments matched the noncontact experiments conducted in the excito-repellency test system. We found that cyphenothrin still had the spatial repellency effect in the field tests. Moreover, we found that deltamethrin also had a repellency effect, but it was less than cyphenothrin. This may have been the result of other factors in the field such as the wind, humidity, temperature, etc. that facilitated the chemical in the vapor phase to spread outside the treated hut. Other possible reasons could be that there was a greater quantity of deltamethrin in the treated hut, the hut is much larger than the laboratory assay and has three windows and a door, and the test period is much longer in the field studies which might have impact on the repellency effect. The results of deltamethrin conformed with the results obtained by Grieco *et al*. (2007).

D-tetrametrin and tetramethrin were not comfromed with the results of excito-repellency system tests, it was probably in the excito-repellency system with the limit

of area, so the test mosquitoes felt to be detained and forced to contact with the chamber wall. Moreover, their irritancy effects might be very low.

However, the adult dengue vectors are controlled through the use of space spraying which has no residual effect. If we would like to use this method, we would be better to use techniques that employ insecticide treated materials, such as impregnated curtain, etc.

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APPENDIX

Appendix Table 1 Outdoor temperature for entrance experiments

Times	Del LC_{50}	Cyp LC_{50}	D-tet LC_{50}	Tet LC_{50}
5:40	17	17.33333	17.33333	22.66667
6:00	17	17.33333	17.33333	22.66667
6:20	17	18	17	23
6:40	17	17.33333	17	23.33333
7:00	17	17.33333	17	23.66667
7:20	17.33333	17.33333	17	24
7:40	18	17.33333	18	24.33333
8:00	19	18.66667	18	25
8:20	20.33333	19.33333	19.33333	25.66667
8:40	21.33333	20.33333	20.33333	26
9:00	22.33333	21	21	27
9:20	23.33333	23	22.33333	28.33333
9:40	24.33333	24.33333	23.66667	30
10:00	26	25	24.33333	30.66667
10:20	27	26.66667	26	30.66667
10:40	26.33333	28.33333	27	31
11:00	29.33333	29.33333	28.33333	31.33333
11:20	30.33333	30.33333	30.66667	31.66667
11:40	30.66667	32	31.33333	32.33333
12:00	31.66667	32	31.66667	35
12:20	32	33.33333	32.66667	35
12:40	31	33.66667	32.66667	34.66667
13:00	30	34	33.33333	36.33333
13:20	30.33333	34.66667	33.33333	35.33333
13:40	31	35	34.33333	34
14:00	32.33333	35	34.33333	32.66667
14:20	33	35.33333	34.66667	32.33333
14:40	32.66667	34.66667	34.66667	31.66667
15:00	32.33333	34.66667	34.33333	30.66667
15:20	32	34	35	30.33333
15:40	32.33333	35	34.33333	27.33333
16:00	30.66667	34.33333	33.66667	26.66667
16:20	29.33333	33.66667	33	27
16:40	28.66667	33.33333	32.33333	26.66667
17:00	27.33333	31.66667	31	26.66667
17:20	25.66667	30	30.33333	26.66667
17:40	25	29	29	26
18:00	24.33333	27.66667	28	25.66667

Appendix Table 2 Indoor temperature for entrance experiments

Times	Del LC_{50}	Cyp LC_{50}	D-tet LC_{50}	Tet LC_{50}
5:40	19	19.33333	19.66667	23.66667
6:00	19	19.33333	19.66667	23.66667
6:20	19	19.33333	19.66667	24
6:40	18.33333	19.33333	19.66667	24
7:00	18.66667	19.33333	19.66667	24.33333
7:20	18.66667	19.33333	19.66667	24.33333
7:40	19	19	20	24.33333
8:00	19.33333	19.33333	19.66667	25
8:20	20.66667	19.66667	20	25
8:40	21	20.33333	21	25
9:00	21.66667	21	21.66667	25.66667
9:20	22	21.66667	22.33333	26
9:40	22.33333	22.66667	22.66667	26.33333
10:00	24	23	23.33333	26.66667
10:20	24.66667	24	23.66667	26.66667
10:40	24.33333	24.33333	24.33333	27
11:00	25	25.33333	25.33333	27.66667
11:20	25.66667	26	25.66667	28
11:40	25.66667	26.66667	26.33333	28
12:00	28.66667	27.33333	26.33333	28.66667
12:20	28.66667	28	27	28.66667
12:40	27	28.33333	27.66667	29.33333
13:00	27	28.66667	28	29.66667
13:20	26.66667	29.33333	28.33333	29.66667
13:40	27	29.66667	29	29.33333
14:00	27.33333	29.66667	29.33333	29.33333
14:20	27.33333	30	29.33333	29
14:40	28	30	29.33333	29.33333
15:00	28	30	29.33333	28.33333
15:20	28	30.33333	29.33333	28
15:40	28	30.33333	29.33333	26.66667
16:00	27.66667	30.33333	29.66667	26.33333
16:20	27	30	29.33333	26.33333
16:40	27	30	29	26.66667
17:00	26.33333	29.66667	29	26.33333
17:20	25.66667	28.66667	28.66667	26.33333
17:40	25	28.66667	28	26.33333
18:00	24.66667	27.66667	27.33333	26.33333

Appendix Table 3 Indoor humidity for entrance experiments

Times	Del LC_{50}	Cyp LC_{50}	D-tet LC_{50}	Tet LC_{50}
5:40	76.66667	78	79.33333	80
6:00	76.66667	78	79.33333	80
6:20	77.33333	78.66667	79	80
6:40	76.66667	78	79.33333	80
7:00	78	76	79.33333	80
7:20	78.66667	77	79.33333	80
7:40	79.33333	77.33333	78.66667	80
8:00	79.33333	77.33333	78.66667	80
8:20	79.33333	77.33333	78.66667	80
8:40	77.33333	77.33333	73.33333	79.66667
9:00	74.66667	76.66667	70	79.66667
9:20	73.33333	71.33333	65.33333	79
9:40	70	68	64	76.66667
10:00	63.33333	63.33333	62.33333	73.33333
10:20	62	60.66667	59.33333	72
10:40	57.66667	58.66667	57.33333	73
11:00	56.66667	54.66667	55.33333	67
11:20	55.33333	54	54	67
11:40	52	52	52.33333	66.66667
12:00	51.33333	48.66667	51	65
12:20	51.33333	45.66667	50	62
12:40	52	45	48	61.33333
13:00	52.66667	42.66667	47.66667	60.66667
13:20	53.66667	41.33333	47.33333	60
13:40	53.66667	40.66667	45.33333	60.66667
14:00	54	39.33333	44.66667	63.66667
14:20	52	39.33333	44	63
14:40	50.66667	38.66667	44.66667	64.66667
15:00	50.66667	38	44	66
15:20	50.66667	38	45.66667	68.66667
15:40	50	38	45.33333	74.66667
16:00	50.66667	38.66667	45.66667	77.33333
16:20	56	38.66667	47	76.66667
16:40	55.33333	39.33333	46.66667	76.33333
17:00	59.33333	39.66667	47.66667	77
17:20	65.33333	42	48.33333	77.66667
17:40	68	44.66667	50.66667	78.33333
18:00	69.33333	46.66667	53.66667	77.66667

Appendix Table 4 Outdoor Temperature for exit experiments

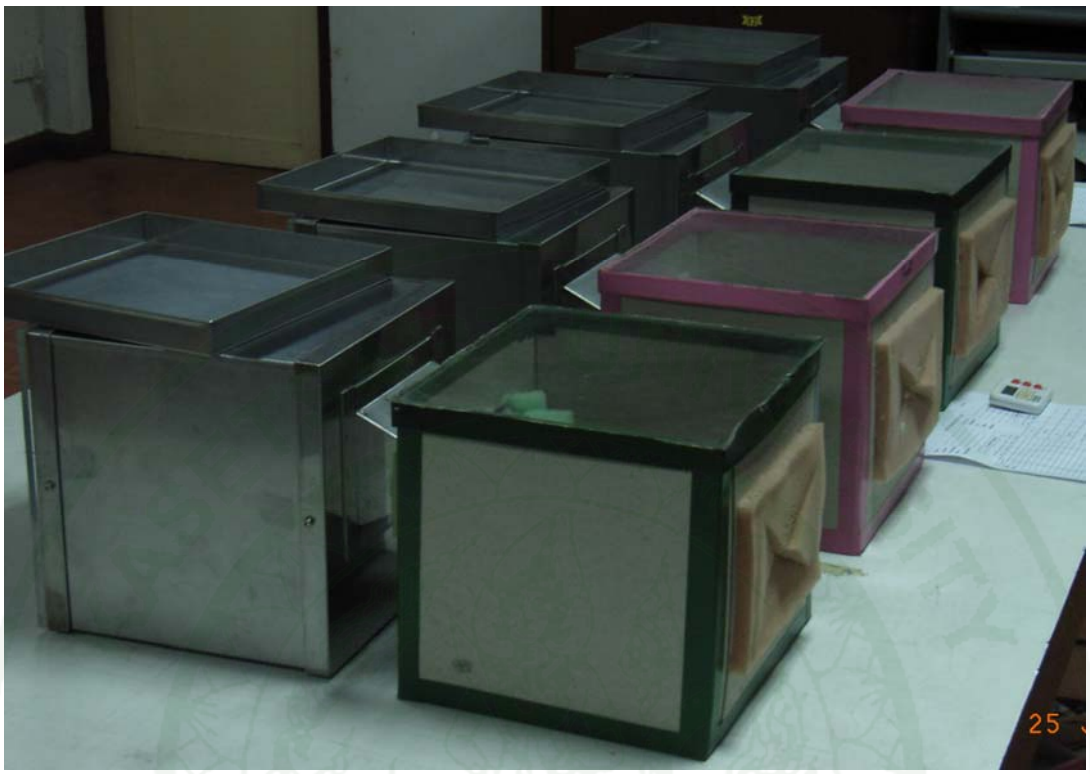
Times	**Del LC_{50}**	**Cyp LC_{50}**	**D-tet LC_{50}**	**Tet LC_{50}**
5:40	18	17	16.66667	23
6:00	18	17	16.66667	23
6:20	18	17	16.66667	23.33333
6:40	18	17.33333	16.66667	23.33333
7:00	18	17.33333	16.66667	23.33333
7:20	18	17.33333	16.66667	23.66667
7:40	19	17.66667	17.66667	24.33333
8:00	19.66667	18.33333	17.66667	24.66667
8:20	20.33333	19.33333	19	24.66667
8:40	21.33333	20	19.66667	25.66667
9:00	22.33333	21.33333	21	26.66667
9:20	23.33333	22.33333	22.66667	27.33333
9:40	24.33333	24	24	28
10:00	25.66667	25	24.66667	29
10:20	27	26.33333	27.33333	29.66667
10:40	28	27.33333	29	30.66667
11:00	29.33333	30	30.33333	31.66667
11:20	29.33333	30.66667	31.66667	31.66667
11:40	29.66667	31.33333	32.66667	31.66667
12:00	29.66667	32.66667	33.33333	31.33333
12:20	30.66667	34	34.33333	31.33333
12:40	30.66667	34	35.33333	32.33333
13:00	32	35	35	32
13:20	30.66667	34.66667	35.33333	28.66667
13:40	31	35.66667	35.66667	28
14:00	32.66667	35.33333	36.66667	27.66667
14:20	33	35.33333	36.66667	27.66667
14:40	32.66667	34.66667	35.33333	27.33333
15:00	30.66667	35.33333	36	27.33333
15:20	32.33333	35.66667	35.66667	27.33333
15:40	30.33333	35.33333	35.33333	27.33333
16:00	30.33333	35	35	27.33333
16:20	29.66667	33.66667	34.33333	27.33333
16:40	28.66667	32.66667	33.66667	27
17:00	27	31.66667	33	26.66667
17:20	26	30.33333	32	26.66667
17:40	25.33333	29.66667	31	25.66667
18:00	24.66667	28	29.66667	25.66667

Appendix Table 5 Indoor Temperature for exit experiments

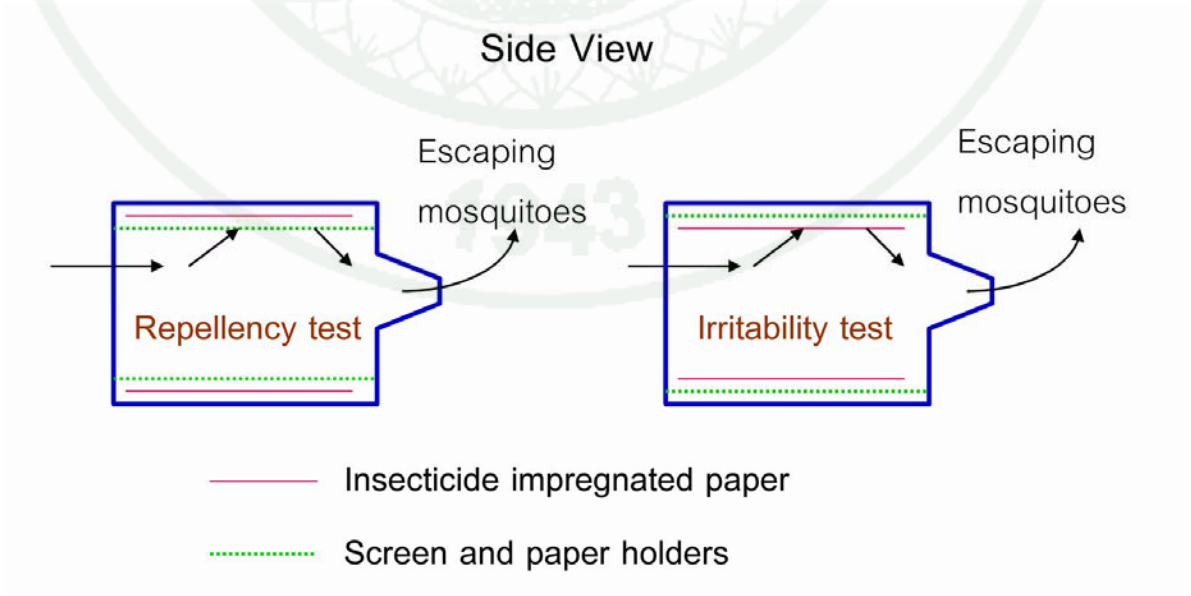
Times	Del LC_{50}	Cyp LC_{50}	D-tet LC_{50}	Tet LC_{50}
5:40	20	19.33333	19.33333	24
6:00	20	19.33333	19.33333	24
6:20	20	19	19.33333	24.33333
6:40	20	19.33333	19	24.33333
7:00	20	19.33333	19	24.33333
7:20	20	19.33333	19	24.33333
7:40	20.66667	19.33333	19.33333	24.33333
8:00	20.66667	20	19.66667	24.66667
8:20	20.66667	20.33333	20.33333	25
8:40	21.33333	21	20.66667	25.33333
9:00	22	21.33333	21.33333	25.33333
9:20	22.33333	22	22	25.66667
9:40	23	22.66667	22.66667	26
10:00	23.66667	23	23.33333	26.66667
10:20	24.33333	24	24.33333	26.66667
10:40	24.66667	24.33333	24.66667	26.66667
11:00	25.66667	25.33333	25.33333	27.33333
11:20	25.66667	26	26	27.66667
11:40	26.33333	26.66667	27	28
12:00	26.33333	27.33333	27.66667	27.66667
12:20	27	28	28	27.66667
12:40	27.33333	28.66667	29	28
13:00	27.33333	29.33333	29	28.66667
13:20	27.33333	29.66667	29.33333	27.66667
13:40	27.66667	29.66667	29.33333	26.66667
14:00	28	29.66667	30	26.66667
14:20	28.66667	30.33333	30.33333	26.66667
14:40	28.33333	30	30.33333	26.66667
15:00	28	30	30.33333	26.66667
15:20	28.33333	30.33333	30.33333	26.66667
15:40	28	30.33333	30.33333	26.33333
16:00	28	30	30.33333	26.33333
16:20	27.66667	30.33333	30.33333	26.33333
16:40	27	29.66667	30	26.33333
17:00	27	29.33333	30	26.33333
17:20	26	29.66667	30	26.33333
17:40	25.33333	28.66667	29	26
18:00	25	27.66667	29	26.33333

Appendix Table 6 Indoor Humidity for exit experiments

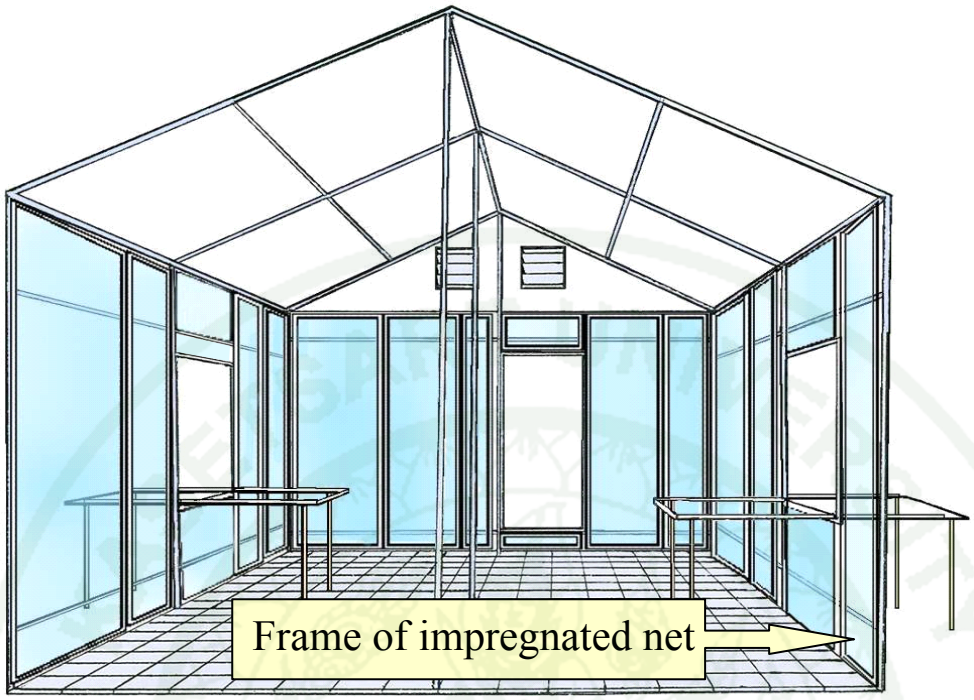
Times	Del LC_{50}	Cyp LC_{50}	D-tet LC_{50}	Tet LC_{50}
5:40	81	79.33333	80.33333	80.66667
6:00	81	79.33333	80.33333	80.66667
6:20	81.33333	80	80.33333	80.66667
6:40	80	80	80.66667	80.66667
7:00	79.33333	80	80	80.66667
7:20	79.33333	80	80	80.66667
7:40	79.33333	79.33333	80	80.66667
8:00	80	80	80	80.66667
8:20	79.33333	79.66667	80	80
8:40	79.33333	79.66667	78	80.66667
9:00	79.33333	79	75.33333	80
9:20	78	76.66667	72	78
9:40	74.66667	74	68.66667	76
10:00	70.66667	69.66667	65.66667	74
10:20	69	66.66667	63	73.33333
10:40	66	63.66667	60	72
11:00	62.66667	59.33333	56.33333	70.66667
11:20	59.66667	56.66667	54	68
11:40	58.66667	54.66667	53	67.33333
12:00	57.33333	50.66667	50.66667	64.66667
12:20	55.33333	48	49	67.66667
12:40	54.66667	46.66667	47.66667	68
13:00	55.33333	44	47	67
13:20	54.33333	43.33333	47	68.33333
13:40	52.66667	43	46	74.33333
14:00	53.33333	43.33333	45.66667	74.66667
14:20	52	42	44	74.66667
14:40	51	42.66667	44.33333	74.66667
15:00	52	40.66667	44.66667	74.66667
15:20	51.33333	39.66667	43.33333	75.33333
15:40	50.66667	40.33333	44	76
16:00	52	39.66667	44	75.33333
16:20	52	41.66667	45.66667	74.33333
16:40	55.33333	42	46.33333	75.33333
17:00	60.66667	44	47.66667	76.33333
17:20	64.66667	45.33333	47.66667	76.33333
17:40	68	48	50.66667	76
18:00	71.33333	55	54	77



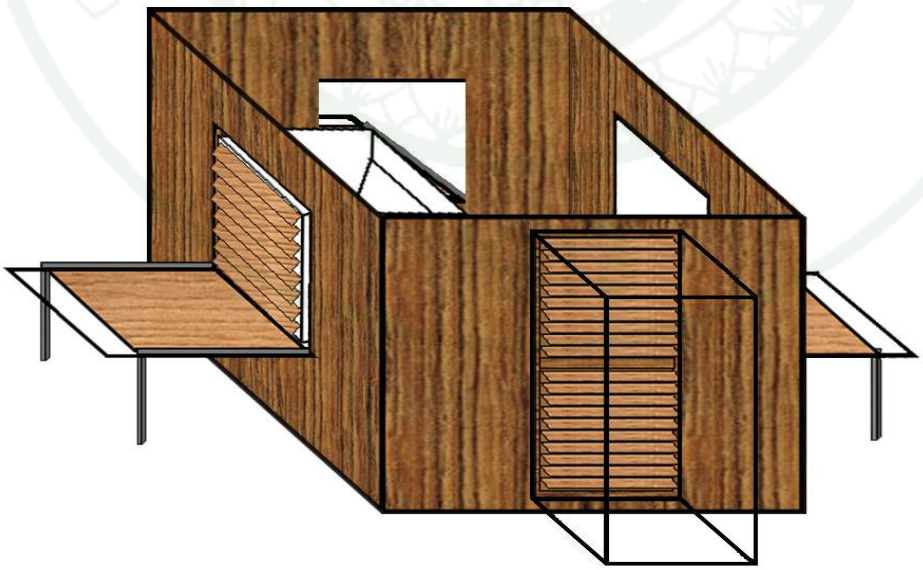
Appendix Figure 1 The picture shows four chambers in a set of each replication of excito-repellency assay. The escaping mosquitoes will fly into the carton, then be collected each minute of thirty minutes.



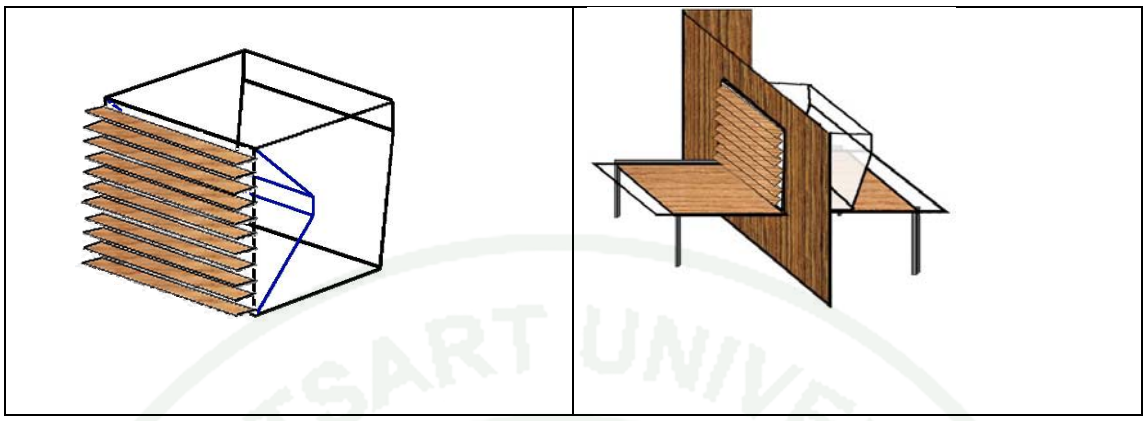
Appendix Figure 2 The left picture is non-contact repellency chamber, and the right picture is contact irritancy chamber.



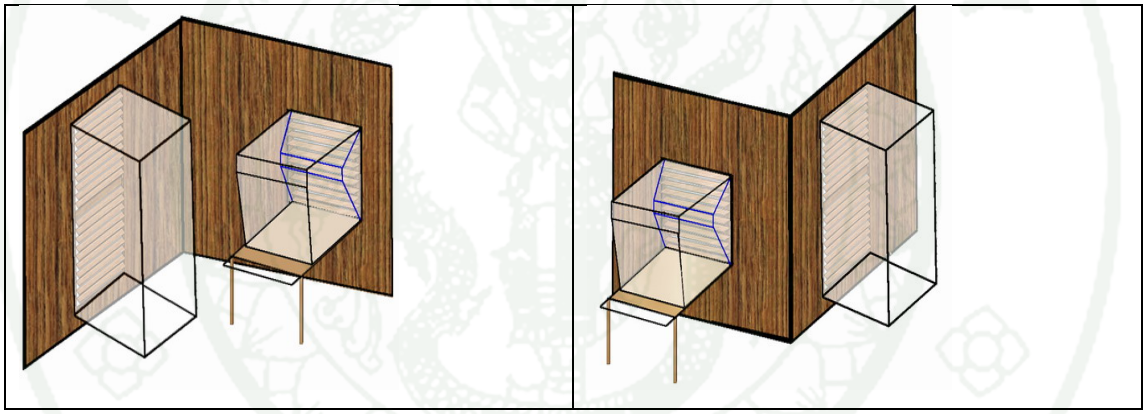
Appendix Figure 3 The picture shows impregnated nets which hang with their frames against inside wall of each experimental hut, except the windows, the roof, and the door.



Appendix Figure 4 The picture shows three windows and a door of each hut, which each of them is closed with a mosquito trap (louver trap).



Appendix Figure 5 The picture of removable window trap, it can be reversed by changing the cage into or out of the hut.



Appendix Figure 6 The left picture shows the method to prepare window traps and door trap for entrance experiment, which the part of the mosquito cage of the traps will be put inside of the hut, and will be put outside the hut in exit experiment (right picture).

Appendix Figure 7 The picture shows the releasing points of mosquitoes, which are 10 m far from each hut in entrance experiment.

Appendix Figure 8 The pictures show the real huts which their doors turn to the west. The farer one is the treated hut, behind them is the forest while in front of them is the grassland.

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