AN AUTOMATED FIELD DEVICE FOR CONDUCTING BEHAVIORAL TESTS ON TWO MOSQUITO POPULATIONS, *Aedes aegypti* L. AND *Anopheles harrisoni* HARBACH AND MANGUIN (DIPTERA: CULICIDAE)

INTRODUCTION

Many areas of the world are at risk for a wide variety of arthropod-borne diseases with millions of people suffering each year (World Health Organization (WHO), 2007). A significant growth in human population, demographic movement from rural to more crowded urban areas and an increase in tourism-based facilities have been documented as major contributors to an increasing trend in disease transmission. As yet no effective multi-valent vaccine or anti parasitic drugs are commercially available for several major vector borne diseases. Prevention of these diseases remains almost entirely dependent on various methods of vector control. Control of the vector by chemicals remains the most effective means of reducing transmission potential and preventing mosquito bites (Reiter and Gubler, 1997; WHO, 1999).

Mosquito behavior is of epidemiological importance because by altering host seeking behavior one can inhibit a mosquito from preferentially feeding on a human, ingesting an infectious blood meal and transmitting a pathogen to a susceptible hosts (Elliott, 1972). The natural reaction for mosquitoes to avoid insecticide-treated surfaces is a general phenomenon, yet behavioral responses of adult insects exposed to insecticides remains poorly studied or understood. Behavioral avoidance is critical to our understanding of how various vector control methods function thereby allowing better decisions on pesticide selection and application (Muirhead-Thomson, 1960; Roberts *et al*., 2000).

A ‘free choice’ test system was designed that has enabled investigators to distinguish between two distinct types of behavioral responses in mosquitoes, contact

irritancy and noncontact repellency (Roberts *et al*., 1997). Modifications and improvements have been made to the original system to allow greater ease and accuracy in demonstrating the innate behavioral response of mosquitoes exposed to varying doses of residual insecticides (Chareonviriyaphap *et al*., 2002; Tanasinchayakul *et al*., 2006).

Relatively large amounts of data have been gathered on the impact of insecticides on *Anopheles* species responsible for malaria transmission, whereas fewer observations have been attempted to describe the function and response of chemicals on other mosquito genera. Relatively little attention has been paid to the response of *Ae. aegypti* to insecticides (Kennedy, 1947; Brown, 1964; Lal *et al*., 1965; Moore, 1977). Behavioral responses of Thai field and laboratory populations of *Ae. aegypti* to insecticides have recently been assessed under different nutritional and physiological conditions (Chareonviriyaphap *et al*., 2006). However, this study did not control for the confounding influence of age as a potential cause of variation in behavioral responses combined with other intrinsic physiological conditions (Hamon and Eyraud, 1961; Busvine, 1964; Kaschef, 1970). To measure the effects of insemination, gonotrophic status and blood feeding on female *Ae. aegypti* exposed to deltamethrin, I used same age specimens to compare behavioral patterns more accurately.

Chemicals protect humans from the bite of mosquitoes through three different actions: irritation after making contact, repelling prior to contact, or by killing the insects (toxicity) (Grieco *et al*., 2007). Most research has focused on the toxic function of chemicals whereas comparatively few have concentrated on non-toxic chemical characteristics. Non-toxic action can be categorized into two distinct mechanisms, contact irritancy and noncontact repellency. Irritant responses result from physical contact with chemical-treated surfaces, whereas repellency is an avoidance response devoid of making actual contact with the chemical (Chareonviriyaphap *et al*., 1997; Roberts *et al*., 1997). Much of the early research on behavioral responses was concentrated on the synthetic chemicals (Evans, 1993, Chareonviriyaphap *et al*., 2001; Kongmee *et al*., 2004; Pothikasikorn *et al*., 2005,

2007; Grieco *et al*., 2005, 2007). In Thailand, synthetic compounds, including organophosphates, carbamates, and pyrethroids have been used with varying degrees of success in national public health vector control programs (Reiter and Gubler, 1997). Since 1994, the Ministry of Public Health (MOPH) in Thailand has recommended the use of deltamethrin in public health to control malaria and dengue haemorhagic fever. Recent studies have reported the spread of deltamethrin resistance in several field *Culex quinquefastiatus* and *Ae. aegypti* populations from Thailand (Somboon *et al*., 2003; Jirakanjanakit *et al*., 2007; Sathantriphop *et al*., 2006). Alternative compounds or new methods of controlling mosquito vectors are needed. One source of alternatives lies in botanical compounds which are commonly used as "insect repellents". These compounds are effective, safe and increasingly available for domestic use against indoor and outdoor biting mosquitoes and arthropod pests.

One option for preventing the transmission of a vector-borne pathogen to a host is the use of insect repellents. N, N-diethyl-3-methylbenzamide (DEET), one of the most common insect repellents, is effective at protecting humans from mosquito bites (Qiu *et al*., 1998). Recently, several botanical extracts, such as eucalyptus (*Eucalyptus citriodora* Hook), citronella grass (*Cymbogon nardus* Rendle), thyme (*Thymus vulgaris* L.), clove (*Syzygium aromaticum* L.), and catnip (*Nepeta cataria* (L.)) were tested as alternative mosquito repellent (Barnard, 1999; Tawatsin *et al*., 2001; Zhu *et al*., 2006). Among these, the essential oil from catnip showed to be a safe and promising insect repellent. This oil contains two stereoisomer forms of nepetalactone (E,Z and Z,E isomer) which have been reported to function as insect repellents against 13 families of insects (Eisner, 1964). The E,Z-nepetalactone form showed to be a stronger repellent against German cockroaches than the Z,E-nepetalactone one (Peterson *et al*., 2002). Catnip oil was also reported to be a good repellent compound for the short term protection of house flies and American cockroaches (Schultz *et al*., 2004). Additionally, catnip oil was found to be a good spatial repellent compound in protecting humans from mosquito bites for at least six hours past treatment (Bernier *et al*., 2005; Zhu *et al*., 2006). However, no investigation has been performed to identify the two distinct categories of behavioral

responses, irritancy and repellency, to catnip oil by mosquitoes. I investigated the active properties of catnip oil using two species of mosquitoes, *Aedes aegypti*, a vector of dengue and *Anopheles harrisoni*, a vector of malaria in Thailand. Irritant and repellent responses were quantitatively assessed using an automated excito repellency (ER) test system (Tanasinchayakul *et al*., 2006).

OBJECTIVES

1. To identify behavioral responses in six-day-old cohorts of *Aedes aegypti* under different physiological states in a lab based behavioral assay.

2. To characterize the behavioral responses to catnip oil (*Nepeta cataria*) by two field-collected mosquito species using an automated excito-repellency test system.

LITERATURE REVIEW

1. Dengue and dengue situation

Dengue fever (DF) and dengue heamorrhagic fever (DHF) are the most important mosquito-borne viral diseases of public health in tropical and subtropical regions of the world (Gubler, 1997). Dengue fever and DHF are transmitted to humans by the bite of infective mosquitoes. *Aedes aegypti*, the primary vector of DF and DHF, typically resides very near or inside human dwellings and preferentially feeding on humans (Christophers, 1960; Polawat and Harrington, 2005). Billions of people are at risk of DF or DHF and millions of people are infected annually (Jacobs, 2000). Dengue is endemic to most tropical countries whereas DHF is endemic to over 100 countries. In tropical and subtropical regions, about 2.5 billion people (40% of the population in the world) are at risk. Annually, an estimated 50 million cases of DF and approximately 400,000 cases of DHF are report. Records of dengue cases in the South-East Asia region (SEAR) have been kept since 1985. The highest numbers of dengue cases in Thailand occurred between 1985 and 2003, while Indonesia reported its highest numbers of dengue cases between 2004 and 2006. One hundred percent of the dengue cases that occurred in 2006 were reported by ten countries in the SEAR (Figure 1) (WHO, 2006).

In Thailand, the first recorded outbreak of dengue occurred in 1958, with 2,158 cases and 300 deaths. The majority of DF and DHF cases occurred in children below the age of 14. Dengue reported in Thailand from 1985 to 2006 show high numbers of cases until 2003 (Figure 2). In 2007, weekly reports of DHF cases between January to December showed 35,950 cases and 28 deaths. The central region of Thailand reported the highest number of cases while the least number of cases were reported from the southern region (Figure 3). *Aedes aegypti* is the primary vector in the country. Currently, as no dengue vaccine is yet available for prevention and treatment of dengue infection, the control of the mosquito vector is an important method to prevent dengue virus transmission. Several methods are available for the

control of mosquitoes to include chemical control (larvicide and adulticide), biological control, source reduction or environmental management, etc. Chemical compounds in four classes of insecticide including organochlorines, organophosphates, carbamates and pyrethroids have been used to control both the adult and larval forms of mosquito vector (Chareonviyaphap *et al*., 1999). DDT has long been shown to induce strong behavioral avoidance responses in many species of mosquito (Kennedy, 1947; Roberts and Alecrim, 1991) and is still an excellent standard by which comparisons with other compounds can be made (Evans, 1993; Chareonviriyaphap *et al*., 2001). Deltamethrin has been effective and widely used for the control of household nuisance mosquitoes and disease vectors. Since 1994, the Ministry of Public Health (MOPH) in Thailand has recommended deltamethrin as the compound to be used in public health to control malaria and dengue haemorrhagic fever. Deltamethrin has been applied as a space spray to reduce dengue transmission in active areas (MOPH, 2006).

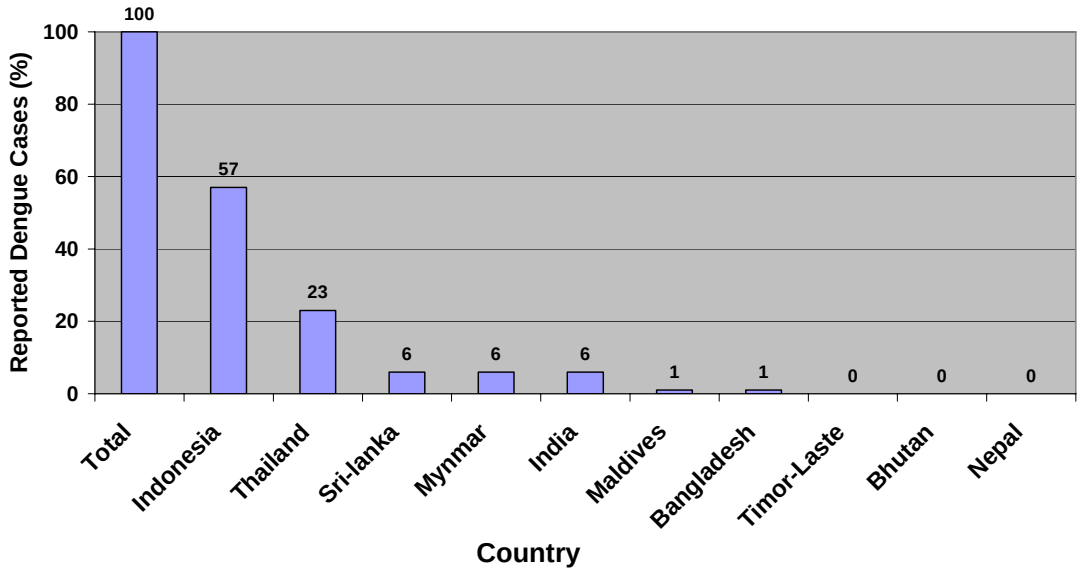


Figure 1 Percentage of dengue cases reported by ten countries in SEAR in 2006

Source: World Health Organization (2006)

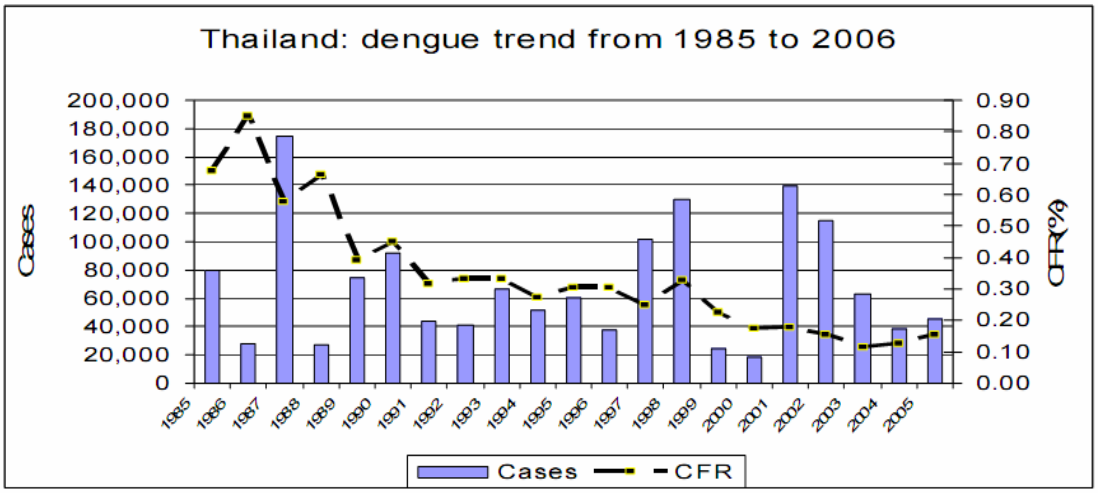


Figure 2 Dengue cases reported in Thailand from 1985 to 2006

Source: World Health Organization (2006)

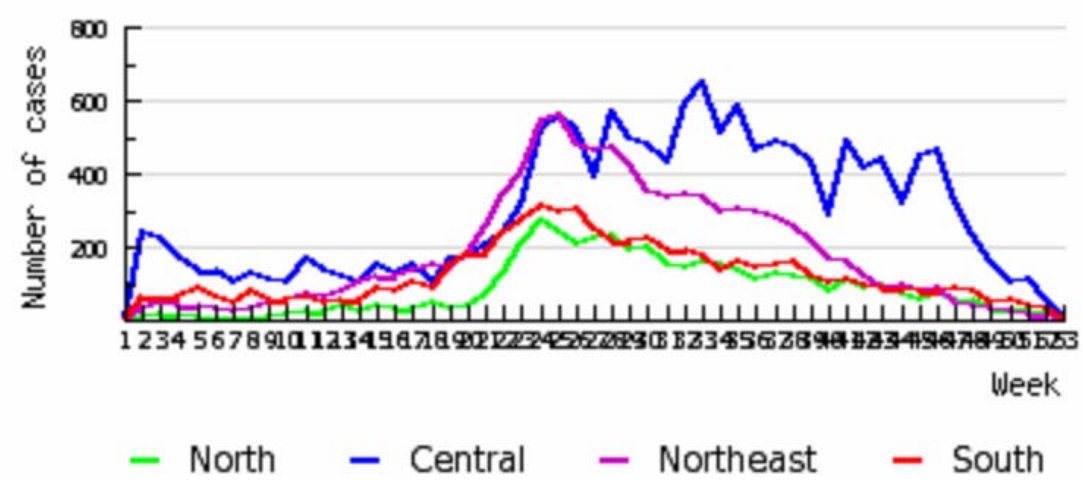


Figure 3 Number of DHF cases by region in Thailand

Source: Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health (2007)

2. Malaria situation and malaria vectors

2.1 Malaria situation

Malaria is still one of the most serious vector borne diseases found throughout the tropical and subtropical regions of the world with estimates of transmission occurring in over 107 countries (Roll back malaria, 2006). Currently, approximately 70% of malaria cases are found in African countries whereas 30% of cases are found in the Americas and Asia (WHO, 2006). Vector control programs, however, have been successful in reducing both malaria morbidity and mortality (MOPH, 2006). Malaria remains a disease of major importance in Thailand. The highest number of malaria cases occur in the rural communities in forested and hilly areas along the border of eastern Myanmar, western Cambodia and northern Marlaysia (Chareonviriyaphap *et al*, 2000) (Figure 4). Since 1999, reported cases of malaria have dropped from 131,055 to 44,555 in 2002, to 37,355 in 2003 and a further reduction to 26,690 in 2004. In 2005, the numbers of malaria cases in Thailand increased slightly to 29,782 with 71 deaths (WHO, 2007). A reduction in morbidity and mortality rates have also been observed (Figure 5). In 2007, weekly reported cases of malaria from January to December represented 29228 cases and 39 deaths. The southern region of Thailand showed the highest number of cases (Figure 6) (Bureau of Epidemiology, 2007). Furthermore, the Thai population at risk of malaria can be divided into two main groups, Thai and Non-Thai (refugee, migrant laborer and treatment seeking groups along the border) (Natsathapana, 2005).

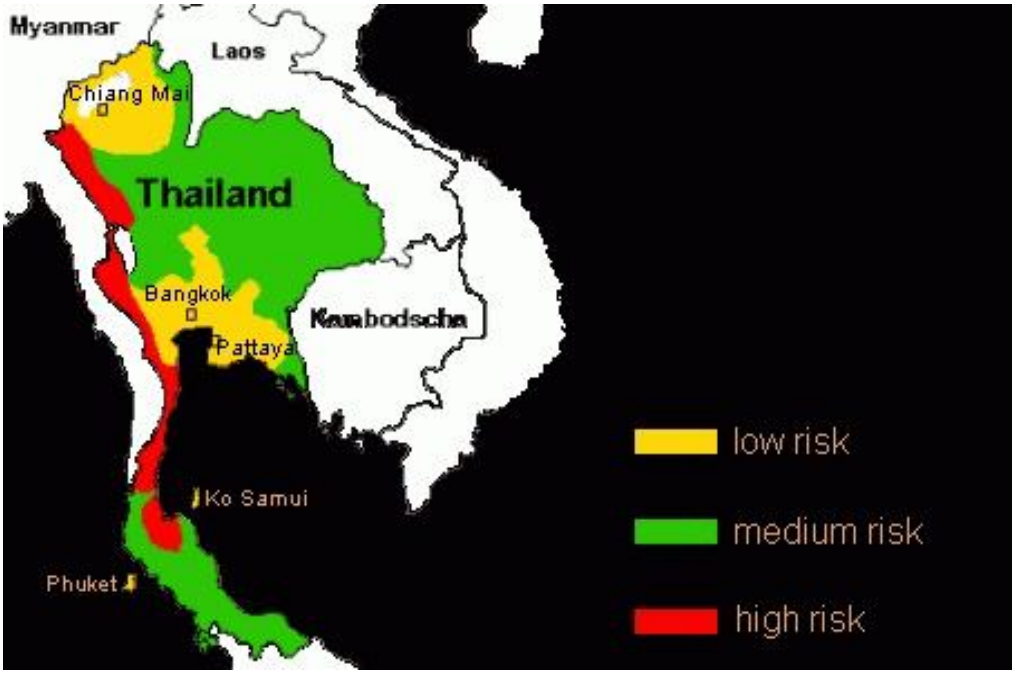


Figure 4 The ranges with risk of malaria in Thailand.

Source: Anonymous (2007)

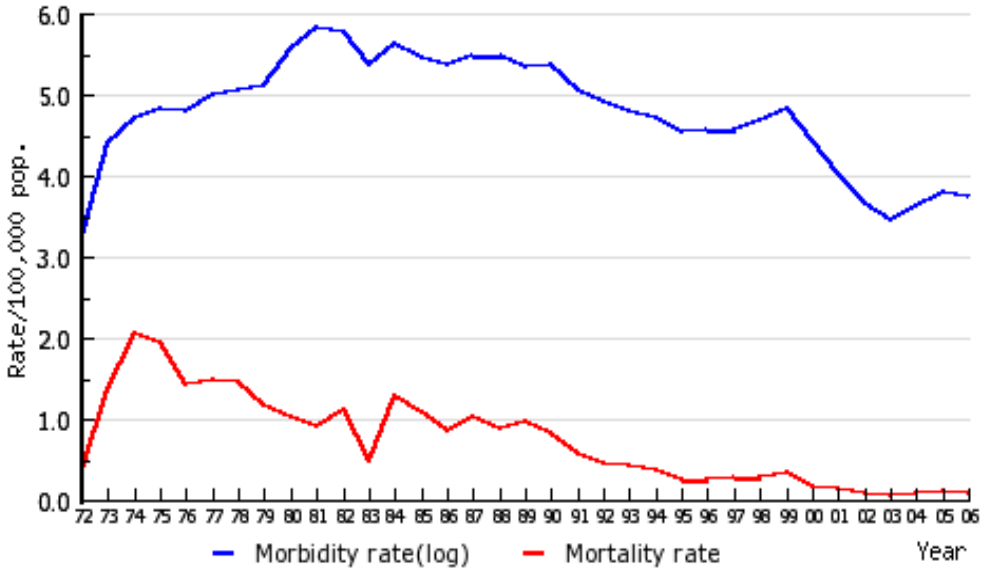


Figure 5 Morbidity and mortarlity rates (per 100,000 populations) of malaria cases

Source: Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health (2007)

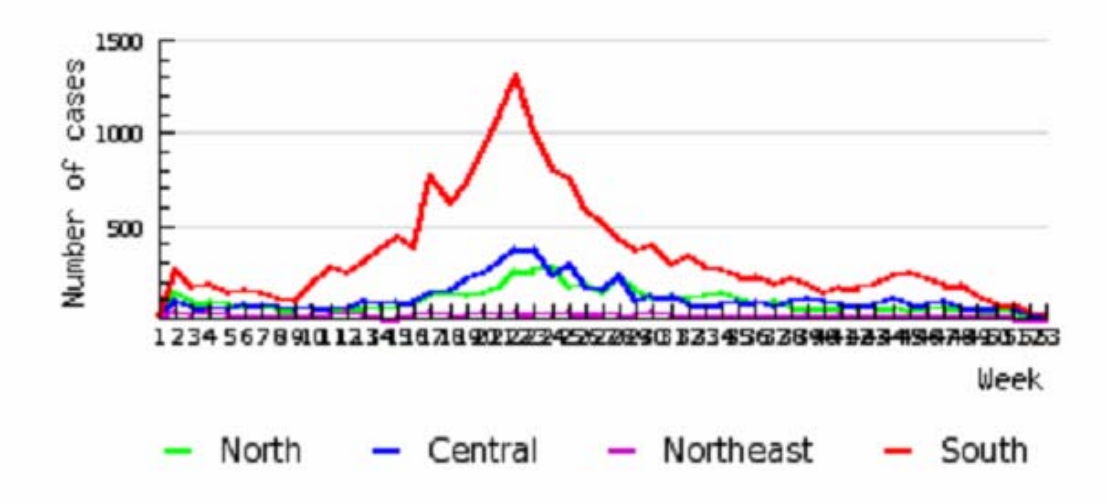


Figure 6 Number of malaria cases by region of Thailand

Source: Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health (2007)

2.2 Malaria vectors in Thailand

The malaria parasite is transmitted to humans by the bite of various infective species of *Anopheles* mosquitoes, which bite mainly between sunset and sunrise. Five species of *Anopheles* mosquitoes are considered to be the primary malaria vectors in Thailand and include *Anopheles dirus, Anopheles minimus, Anopheles baimaii, Anopheles pseudowillmori* and *Anopheles aconitus*. *Anopheles. dirus* and *An. minimus* represent a species complex which can not clearly be identified by morphology (Rattanarithikul and Panthusiri, 2006). These species present both endophagic and exophagic behavioral patterns and both play a role in malaria transmission (Chareonviriyaphap *et al*., 2000).

Anopheles minimus Theobald, an important vector of malaria, is found mainly in the forested hilly areas. The breeding sites of *An. minimus s.l.* are streams and canals with clear, shallow, slow running water in heavily shaded areas (Harrison, 1980). Members of the *An. minimus* complex consists of 3 sibling species, *An. minimus* s.s. (species A), *An. harrisoni* (*An. minimus* species C) and *An. minimus* species E. *Anopheles minimus s.s.* and *An. harrisoni* are found on the Southeast Asian mainland, while species E is found on the Ishigaki Island in the Ryukyu Archipelago of Japan (Somboon *et al*., 2001; Harbach, 2004). In Thailand, *An. minimus* is found predominantly throughout the country, whereas *An. harrisoni* seems to be localized along the northern and western Thai-Myanmar border, especially in Kanchanaburi, Tak and in the northern, Chiang-Mai Province in conjunction with *An. minimus* (Sucharit *et al*., 1988; Green et *al*., 1990; Kengluecha *et al*., 2005). In western Thailand, a remote sensing – based Geographic Information System (GIS) characterized breeding habitats of *An. minimus* and *An. harisoni*. *Anopheles minimus* was found in a wide variety of habitats ranging from densely canopied forest to open agricultural fields, while *An. harrisoni* showed a narrow habitat preference (Rongnoparut *et al*.,2005). Seasonal abundance and blood feeding activity of *An. minimus s.l.* reported peak population density during the wet season and a bimodal pattern of night time feeding: primary peak occurring after sunset (18.00-21.00 h), with a secondary peak occurring before sunrise (03.00-06.00 h). However, low levels

of blood feeding continued throughout the night (Chareonviriyaphap *et al*., 2003). *Anopheles minimus s.l.* exhibited exophagic, exophilic and zoophilic behaviors after DDT was introduced to control malaria transmission (Ismail *et al*., 1978; Ratanatham *et al*., 1988). Although *An.minimus* and *An. harrisoni* are found together in Thailand, differences in host feeding and seeking behaviors may influence differences in vectoral capacity between the two species of this taxon. In Vietnam, the behavior of *An. harrisoni* showed a greater tendancy towards exophagy and zoophily as compared to *An. minimus* (Van Bortel *et al.*, 2000). The outdoor feeding activity of *An. harrisoni* has also been observed in Thailand (Sungvornyothrin *et al*., 2006b).

Anopheles harrisoni Harbach and Manguin is the reclassifiedname for *An. minimus* C. Previously it was classified as a member of the *An. minimus* complex. *An. minimus* species C was determind to be a new species and differs from *An. minimus* by molecular markers, available information on bionomics and distribution (Harbach *et al*., 2007). Morphological characters can differentiate *An. harrisoni* from *An. minimus* by the presence or absence of a hemeral pale spot (HP) and presector pale spot (PSP) on the wings. *Anopheles minimus* maintains only the presector pale spot (PSP) on the wing costa, while *An. harrisoni* has both the presector pale spot (PSP) and hemeral pale spot (HP) on the wing costa (Harrison, 1980; Rattanarithikul *et al*., 2006; Sungvornyothin *et. al*., 2006a). Morphological identification alone, however, cannot clearly distinguish *An. harrisoni* from *An. minimus*. Molecular methods must be employed to confirm the identification of *An. harrisoni* (Sharpe *et al*., 1999; Van Bortel *et al*., 2000; Garros *et al*., 2004; Harbach *et al*., 2007).

2.3 Malaria vector control

Control of mosquito vectors is an important tool for reducing transmission of malaria. Drug distributions and personal protective measures are also available for reducing disease transmission (MOPH, 2006). In Thailand, DDT was introduced for indoor residual spraying (IRS) into the national malaria control program in 1949 (Prasittisuk, 1985; Chareonviriyaphap *et al*., 2000). DDT was the chemical of choice and was used extensively in malaria-endemic areas until 1995.

Since that time, however, DDT has been banned by the government as a result of political pressure due to the chemicals long residual life in the environmental. The use of DDT for malaria control was officially stopped in 2000 and was replaced by synthetic pyrethroids (Chareonviriyaphap *et al*., 2000; MOPH, 2006). Synthetic pyrethroids have been widely accepted for controlling disease vectors based on their low mammalian toxicity (Elliot, 1972). Deltamethrin and lambdacyhalothrin were the only two alternative synthetic pyrethroids available at the time for use in public health programs, as IRS compounds to combat malaria transmission in Thailand (Pothikasikorn *et al*., 2005; MOPH, 2006). Currently, there are various alternative methods available to control malaria vectors and include thermal fogging in outbreak areas, indoor residual spray and personal protective measures which include impregnated mosquito nets, mosquito repellents and screen barriers (MOPH, 2006).

3. Mosquito repellent compounds

Mosquitoes are known as more than nuisance insects. Some species are disease vectors that cause serious human diseases such as dengue, malaria, yellow fever and filariasis. Each year, millions of people worldwide suffer from mosquito-borne diseases (WHO, 2007). As of yet no effective commercially available vaccine or antiviral agents have been produced for prevention and treatment of several major vector borne diseases. Control of the mosquito vectors and protection from mosquito bites remains the most important methods for reducing disease transmission and preventing mosquito-borne diseases.

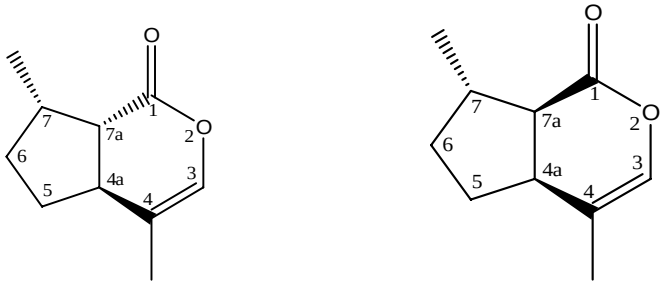
There are many compounds currently labeled as mosquito repellents such as plant oils, smoke, tars, chemicals, etc (Peterson and Coats, 2001). Furthermore, several compounds and natural products have been evaluated as topical insect repellents. In rural areas, smoke from burning waste plant materials and mosquito coils are still the most widely used to repel mosquitoes (Debboun *et al*., 2007). Currently, there are many types of mosquito repellent formulations on the market that contain synthetic compounds or botanicals. DEET (N, N- diethyl-m-toluamide) is still the most effective and commercially successful product on the market. DEET is a

common ingredient found in many mosquito repellent formulations (Peterson, 2000), however, adverse reactions to DEET vary from mild to severe after application on sensitive people (Qiu *et al*., 1998; Peterson, 2000). The search for alternatives to DEET have led to several studies on insect repellents to investigate the effectiveness of plant extracts. Some plant extracts have been reported as repellents against mosquitoes such as citronella grass (*Cymbogon nardus* Rendle), tumeric (*Curcuma longa* Linn.), hairy basil (*Ocimum americanum* L.) (Tawatsin *et al*, 2001), Thyme (*Thymus vulgaris* L.), and clove (*Syzygium aromaticum* L.) (Barnard, 1999). Recently, catnip oil has been tested as an alternative repellent by different methods.

4. Catnip oil

Catnip, *Nepeta cataria* L., is a native perennial herb of Europe and Asia in the family Labiatae (mint family) and was recently introduced to North America. It grows wild in most parts of the United States. Other names commonly used for catnip are catnep, catrup, catwort, cataria or catmint. Leaves and stems produce a mint odor when they are crushed or wilted (http:// www.oardc.ohio-state.edu). The leaves and flowers are often used in herbal tea for medical treatment such as fevers, colds, cramps and migraines (Simon *et al*., 1984). Catnip odor has long been recognized to have an intoxicant effect on most cats (Peterson and Coat, 2001). The active component of catnip oil is nepetalactone which has two forms: Z,E - nepetalactone and E,Z- nepetalactone (Figure 7). Nepetalactone in catnip oil has been documented as a repellent to 13 families of insects to include beetles, weevils, plant hoppers and spittlebugs (Eisner, 1964). In addition, catnip oil has shown to be repellent to cockroaches, house flies (*Musca domestica* L.) and mosquitoes (Peterson *et al*., 2002; Schultz et al., 2004). Peterson *et al*. (2002) also found that nepetalactone from catnip oil could repel German cockroaches, *Blattella germanica* L. and E,Z-nepetalactone showed greater activity than Z,E-nepetalactone on some receptor cites. Catnip oil was also found to be effective as a spatial repellent (Bernier *et al*., 2005) and feeding deterrent on *Ae. aegypti* (Chauhan *et al*., 2005). Nepetalactone in catnip oil was more effective than DEET with repellent activity ranging from the same to ten times greater than DEET (American Chemical Society (ASC), 2001). Additionally, catnip oil was

found to repel *Ae. albopictus* up to 6 hours post treatment (Zhu *et al*., 2006) while protection times, ranged from 0 min for *Ae. aegypti* up to 240 ± 60 min for *Cx. quinquefasciatus*. Other studies showed catnip oil also provided a long protection time to *Ae.vigilax, Cx. annulirostris* and *Cx. quinquefasciatus* in Australia (Webb and Russell, 2007).



E,Z-nepetalactone (~48%) Z,E-nepetalactone (~40%)

Figure 7 Major constituents of catnip oil (*Nepeta cataria*)

Source: Chauhan *et al*. (2005)

Figure 8 Catnip foliage (*Nepeta cataria*)

Source: Ombrello (2007)

MATERIALS AND METHODS

1. Physiological conditions of *Aedes aegypti* for pesticide avoidance assay

1.1 Mosquito population

A population of *Aedes aegypti* was established from immature stages collected from Pu Teuy Village, Sai Yok District, Kanchanaburi Province (14° 17’ N, 99° 17’ E), approximately 100 km northwest of Bangkok, between June- August 2006. Species identification and subsequent colonization was managed at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Thailand.

1.2 Mosquito rearing and conditioning

Mosquito colonization and rearing method followed those described by Kongmee et al. (2004) with only minor modifications to meet testing requirements. All life stages were maintained under controlled insectary conditions (25 ± 5° C and 80 ± 10% relative humidity) before, during and after testing in the insectary at the Department of Entomology, Kasetsart University, Bangkok, Thailand. Larval stages were reared in plastic trays and fed by fish food, under identical physical and nutritional conditions throughout the study period. Larvae and adults were reared under a 12:12 h light: dark photophase regime. Upon emergence, all adults were provided with 10% sucrose solution soaked on cotton pads and were held 12 h before testing. Detail conditioning requirements followed methods described by Chareonviriyaphap *et al*. (2006) with appropriate modifications described herein.

Six -day- old female *Ae. aegypti* representing four different physiological states were used for this study : parous, nulliparous, unmated and full bloodfed.

1. Parous females were allowed to feed on blood of guinea pigs on day 2

post- emergence after being held with males. Only fully bloodfed females were selected and segregated into containers for oviposition on day 4. Females on day 6 after oviposition were provided with water only up to time of testing.

2. Nulliparous (mated) mosquitoes were denied blood and held with males up to day 2 post-emergence. Females were provided with 10% sucrose solution soaked on cotton pads and only water 12 hours prior to testing. Spermatheca were dissected on a small sample to determine the proportion that had successfully mated.

3. Unmated (infertile) mosquitoes came from separating individual pupae into containers until emergence after which the females were selected and held together. A 10% sucrose solution was provided until 12 hours prior to testing when only water was provided.

4. Fully bloodfed (mated) mosquitoes were held with males and selected after feeding on guinea pigs three hours before testing. Dissection of spermatheca followed mating to confirm nulliparous status.

All four different conditions were deprived of sucrose solution and water 12 h before testing. All four conditions were kept separately before, during and after testing.

1.3 Insecticide impregnated papers.

1. Deltamethrin [(S)-alpha-cyan-3-phenoxybenzyl (1R, 3R)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropanecarboxylate] (85% purity). This chemical was supplied by BASF (pyrethroid, BASF Corporation, Ludwigshafen, Germany - CAS#67375-30-8Bayer)

2. DDT [1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane] (92.5 % purity). This chemical was purchased from Sigma-Aldrich Inc. (organochlorine, Sigma-Aldrich Inc., S. Louis, MO- CAS#59-29-3).

Based on current policy of the Thai national vector control program, a standard field dose of chemical of 0.02 g/m^2 was selected for testing deltamethrin and 2 g/m^2 for DDT. Treated papers, both standard size (12 x 15 cm) of filter-papers (Whatman® No. 1) for susceptibility tests and 15 x 17.5 cm dimensions for use in the Excito-repellency chambers, were prepared at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand, according to World Health Organization specification (WHO, 1996). Insecticide-impregnated papers were treated at the rate of 2.75 ml insecticide solution per 360 cm^2 (susceptibility tests and excito repellency were treated at the rate 2.0 ml. and 2.8 ml., respectively). Control papers were treated with acetone solvent (analytical grade) plus silicone oil (a non-volatile carrier) and at a rate of 0.66 ml/paper (WHO, 2006).

1.4 Insecticide susceptibility tests.

The susceptibility of each test population with deltamethrin (0.05%) and DDT (4%) was assessed by direct tarsi contact exposure to a single diagnostic dose on insecticide-treated test papers, following standard testing procedures for *Ae. aegypti* (WHO, 1998). For each test, five cylinders (two for controls and three for treatments) were used. Control cylinders contained filter paper impregnated with solvent only; whereas, treatments contained filter paper impregnated with the diagnostic concentration of insecticide in solvent. For each test population, 25 female mosquitoes were exposed for 1 h to deltamethrin or 30 min to DDT. Following test and control exposures, knockdown was recorded and all mosquitoes transferred to separate clean holding containers and provided 10% sucrose solution. Total knockdown and mortality was recorded after 24 h post-exposure. Each matched test-control series was repeated 3 times per test population and the mean adjusted susceptibility (% mortality) was derived per test population.

1.5 Excito-repellency tests.

In this study, I used the improved excito-repellency test chamber as described in a recent publication (Tanasinchayakul *et al*., 2006) but without the automated system for the counting of escaped mosquitoes. The main supporting structure is fabricated using stainless steel with each side wall measuring 23x23 cm^2. The chamber walls have an aluminum side tongue and groove configuration on joining ends that make it easier and faster to set up and disassemble for transportation and storage. The frame of the inner chamber is constructed of 22.5x19 cm stainless steal beams, which include metal holders for securing test papers on either of two sides for the dual purpose of providing either a contact or a noncontact exposure configuration. For noncontact tests, a thin sheet of fine mesh iron screening secured on the opposite side of the test paper allows for a 1.5 cm gap that prevents mosquito tarsal contact with the test paper. A Plexiglas™ panel at the rear of the chamber is equipped with a 11.5 cm diameter hole sealed with overlapping dental dam to prevent escape during handling. There is a forward exit portal (13.5x2cm) connected to a funnel projecting from the box (Figure 9).

Each test series consisted of 2 chemically treated test chambers and two paired control chambers fitted with appropriate papers. Female mosquitoes were held in 473 cm^3 (16 fl. oz.) capacity cups for approximately 8-10 h prior to testing and were provided with only water soaked cotton pads. For each test chamber, 15 mosquitoes were carefully introduced into each of 4 chambers using a mouth aspirator. Mosquitoes were allowed a 3 min adjustment period inside the test chamber before opening the escape funnel to begin the observation period. A receiving cage was connected to the exit portal for collecting exiting mosquitoes. Mosquitoes escaping were recorded at 1 min intervals for a period of 30 min. All tests were conducted between 0800-1600 hours and replicated 4 times per test population.

Immediately following the 30-min exposure, the number of dead or knockdown specimens remaining inside the chamber and those that had escaped into the receiving cage were recorded for each of the 4 chambers. Also, all live specimens that had escaped or remained inside the test chamber were tranferred to clean holding cups and provided a 10% sucrose solution. All test mosquitoes were maintained separately in lots for 24 h post-exposure evaluation followed by recording of mortality.

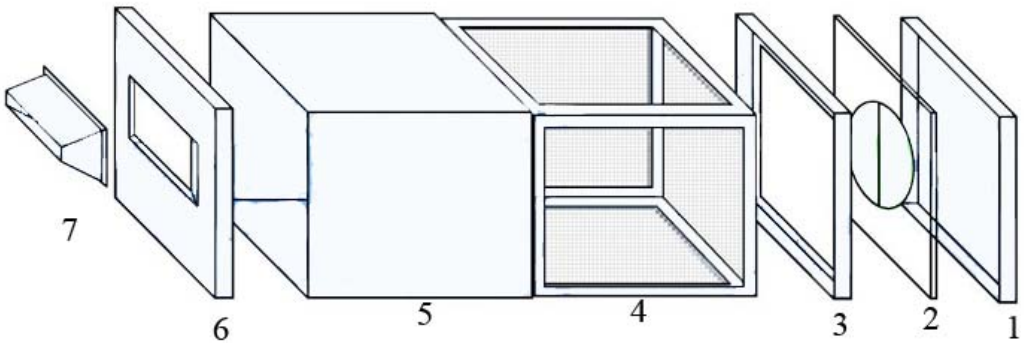


Figure 9 Illustration of the excito-repellency test chamber for study of insecticide avoidance behavior of mosquitoes.
1 = rear door cover, 2 = Plexiglas panel with rubber-sealed door,
3 =Plexiglas holding frame, 4 = screened inner chamber, 5 = outer chamber,
6 = front panel, 7 = exit portal.

1.6 Data analysis.

In contact susceptibility tests, control mortalities exceeding 5% were corrected and adjusted in order to determine baseline susceptibility in each test population (Abbott, 1925). For excito-repellency data, I used a life table survival analysis approach (Roberts et al. 1997) to estimate mosquito escape rates (number of mosquitoes staying in the chambers) and then compared differences in mosquito escape rates between test populations and insecticides. Survival analysis provides a robust statistical treatment of sequential excito-repellency data, and relative to other

quantitative methods describing behavioral avoidance, survival curves minimize loss of valuable information while estimating temporal mosquito escape probability (Roberts et al. 1997). The time in minutes for 25, 50 and 75% of the test population to escape was estimated with he life table method and these estimates were used as the "escape time" summary statistics (ET_{25}, ET_{50}, and ET_{75}).

A log-rank method is used to compare patterns of escape behavior. This test is designed to detect differences between survival curves that result when the death (or escape) rate in one group is consistently higher than the corresponding rate in the 2^{nd} group and the ratio of this rate is consistent over time. With excito-repellency data, the basic idea underlying the log-rank test involves examining escape observations by 1-min intervals. The log-rank method was proposed by Mentel and Haenzel (1959). The discriminating level for statistical significance was set at 0.05%.

2. Behavioral responses of two field-collected mosquito species to catnip oil (*Nepeta cataria*) using an automated excito-repellency test system.

2.1 Mosquito population

Populations of *Ae. aegypti* and *An. harrisoni* were used in this study. *Aedes aegypti* was established from immature stages whereas *An. harrisoni* were collected by cow bait from 1800-2400 hours between April-September 2006. For cow baited collections, one cow was kept in a net trap and mosquitoes were collected from inside the net for 15 min/hour. The captured mosquitoes were kept in mosquito cups and provided with 10% sugar solution. *Anopheles harrisoni* mosquitoes were identified using the morphological keys of Rattanarithikul *et al*., (2006) (Figure 10) in the following morning.

Anopheles minimus *Anopheles harrisoni*

Figure 10 Wing pattern between *Anopheles minimus* and *Anopheles harrisoni*

Source: Rattanarithikul *et al*. (2006)

2.2 Mosquito conditioning

Unfed three-five day- old female *Ae. aegypti* mosquitoes were used in this study. All female mosquitoes were deprived of sucrose solution and water 12 h before testing. With *Anopheles harrisoni*, only field collected mosquitoes were subjected to testing.

2.3 Insecticide impregnated papers

Different concentrations (1%, 2.5%, 5% and 10%) of essential oil from catnip were impregnated onto test papers measuring 12 by 15 cm for susceptibility tests and 15 by 17.5 cm for excitio-repellency test, following the standard WHO procedure (WHO 1998). Catnip oil was received from the Chemicals Affecting Insect Behavior Lab (CAIBL) at the United States Department of Agriculture, Beltsville, Maryland. Nepetalactones (E,Z ~ 48% and Z,E ~ 40% isomers) and β-caryophyllene (~9%) are the major constituents in catnip oil. *E,Z* and *Z,E* nepetalactone isomers were 99% pure chemically and 95-98% pure stereo-chemically according to capillary gas-liquid chromatography (Chauhan and Zhang 2004). The structures of nepetalactone isomers were confirmed by GC-mass spectroscopy (GC-MS) and nuclear magnetic resonance spectral analysis (Eisenbraun et al. 1980). Racemic nepetalactone was formulated by mixing 1:1 ratio of *E,Z* and *Z,E*-nepetalactones, and homogeneity was confirmed by GC.

2.4 Dose response assay

The standard WHO tarsal contact test (WHO, 1996) was used in this study. For each test, five cylinders (two for controls and three for treatments) were used. Control cylinders contained filter paper impregnated with solvent (acetone) whereas, treatments contained filter paper impregnated with the different concentrations of catnip oil in solvent. For each test population, 25 female mosquitoes were exposed for 1 h to catnip oil. Following test and control exposures, knockdown was recorded and all mosquitoes transferred to separate clean holding containers and provided with 10% sucrose solution. Total knockdown and mortality was recorded after 24 h post-exposure. Each matched test-control series was repeated 4 times per test population

2.5 Excito-repellency tests

In this study, I used an automated field excito-repellency test system as described in a recent publication (Tanasinchayakul *et al*., 2006). The main supporting structure was fabricated using stainless steel, each side wall measuring 23x23 cm^2. The chamber walls were constructed with an aluminum side tongue and groove configuration on adjoining ends which made the assay easier and faster to set up and disassemble for transportation and storage. The frame of the inner chamber was constructed of 22.5x19 cm stainless steal beams. The frame included metal holders for securing test papers on either of two sides for the dual purpose of providing a contact or a noncontact exposure configuration. For noncontact tests, a thin sheet of fine mesh iron screening secured on the opposite side of the test paper allows for a 1.5 cm gap that prevented mosquitoes from makings tarsal contact with the test paper. A Plexiglas^{TM} panel at the rear of the chamber was equipped with a 11.5 cm diameter hole sealed with overlapping dental dam to prevent escape during handling. Each assay chamber contained a forward exit portal (13.5x2cm) connected to a funnel projecting from the box (Figure 11).

The photoelectric sensor (FX-301, SUNX Limited, Aichi, Japan) detects and counts escaping mosquitoes (Figure 11, #2). The sensor has two operational

mode switches (#3), a jog switch, and a MODE key require for operating the system. To record data during testing, the DATA Logger CL 123 (#5) is connected to the photoelectric sensor and records values at three signal channels, one analog and two digital. The DATA Logger CL123 is a small, battery-operated device (#4) with software to record and transfer data in tabular and graphic form to the computer system (#6) (Tanasinchayakul *et al*., 2006).

Each test series consisted of two chemically-treated test chambers and two paired control chambers fitted with appropriate papers. Female mosquitoes were held in 473 cm^3 (16 fl. oz.) capacity cups for approximately 8-10 h prior to testing and were provided with only water soaked cotton pads. For each test chamber, 15 mosquitoes were carefully introduced into each of the four chambers using a mouth aspirator. Mosquitoes were allowed a 3 min adjustment period inside the test chamber prior to opening the escape funnel to begin counting. A receiving cage was connected to the exit portal for collecting exiting mosquitoes. Escaping mosquitoes were recorded at 1 min intervals for a period of 30 min. All tests were conducted between 0800-1600 hours and replicated 4 times per test population.

Immediately following the 30-min exposure, the number of dead or knockdown specimens remaining inside the chamber and those that had escaped into the receiving cage were recorded for each of the four chambers. Also, all live specimens that had escaped or remained inside the test chamber were transferred to clean holding cups and provided with a 10% sucrose solution. All test mosquitoes were maintained separately in lots for 24 h post-exposure at which time mortality was recorded.

2.6 Data analysis

In contact susceptibility tests, control mortalities exceeding 5% were corrected and adjusted for determining baseline susceptibility in each test population (Abbott, 1925). For excito-repellency data, a life table survival analysis approach was used to estimate mosquito escape rates and compared differences in mosquito escape

rates between test populations and insecticides (Roberts *et al*., 1997). Survival analysis provides a robust statistical treatment of sequential excito-repellency data, and relative to other quantitative methods describing behavioral avoidance, survival curves minimize loss of valuable information while estimating temporal mosquito escape probability (Roberts *et al*., 1997). The time in minutes for 25, 50 and 75% of the test population to escape was estimated using life table analysis and these estimates were used as the "escape time" summary statistics (ET_{25}, ET_{50}, and ET_{75}).

A log-rank method is used to compare patterns of escape behavior. This test is designed to detect differences between survival curves that result when the death (or escape) rate in one group is consistently higher than the corresponding rate in the 2^{nd} group and the ratio is consistent over time. With excito-repellency data, the basic idea underlying the log-rank test involves examining escape observations by 1-min intervals. The log-rank method was proposed by Mentel and Haenzel (1959). The discriminating level for statistical significance was set at 0.05%.

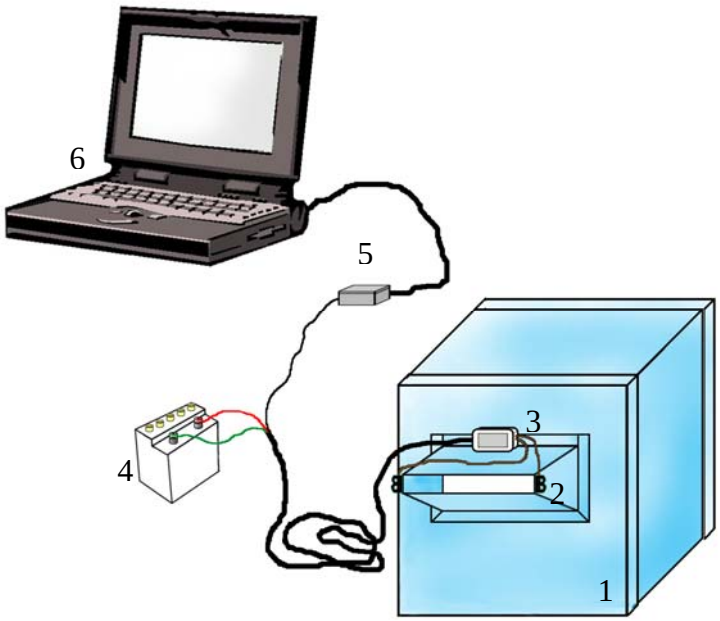


Figure 11 Illustration of an automated excito-repellency test system.1 = excito-repellency chamber, 2 = photoelectric sensor, 3 = operation mode, 4 = battery, 5 = DATA Logger CL 123, 6 = Computer system

RESULTS AND DISCUSSION

Results

Two experiments were performed in this study. The first experiment was to test the effect of physiological condition of a single age group *Aedes aegypti* population on insecticide avoidance behavior. Secondly, evaluate the biological effect of catnip oil (*Nepeta cataria*) on the behavioral response of two field-collected mosquito species characterized by using an automated excito-repellency test system.

1. Effect of physiological condition of a single age group of *Aedes aegypti* on insecticide avoidance behavior.

1.1 Insecticide susceptibility test

Six-day-old *Ae. aegypti* test specimens at different physiological states were exposed to a diagnostic dose of deltamethrin (0.05%) and DDT (4%) to access susceptibility level (Table 1). All four test populations were found completely (100% mortality) susceptible to deltamethrin whereas high levels of resistance to DDT were detected in all states (3-10% mortality). Between the physiological states, DDT produced the highest mortality (10%) in infertile, non-bloodfed mosquitoes and the lowest mortality (3%) in blood engorged females (Table 1).

1.2 Excito-repellency test on different physiological conditions of six-day old females

Total mortalities and percent escape responses were recorded for each physiologically different test population exposed to deltamethrin and DDT in contact and noncontact trials (Tables 2 and 3). Only slightly higher mortality was recorded for escape mosquitoes in contact trials exposed to deltamethrin (range 0-10%) as compared to DDT (0-8.8%) (Table 2). Similarly, nonescaped mosquitoes from

deltamethrin-treated contact chambers resulted in higher (range 8.3-30%) mortality rates as compared with DDT (range 0-4%), with one exception, a lower percent mortality in escaped and nonescaped mosquitoes (11.54% and 37.31%) was seen in noncontact trials when exposed to either compound (Table 3).

A dramatic escape response after contact either with deltamethrin or DDT was observed compared to both paired controls and noncontact trials, regardless of the physiological condition at the time of test ($P < 0.05$) (Table 2). Additionally, significant differences in the pattern of escape were found in all noncontact trials compared to paired controls ($P < 0.05$) (Table 3). In contact trials, unmated, non-bloodfed mosquitoes produced the greatest escape responses, 91.7% (DDT) and 78.9% (deltamethrin), followed by nulliparous mosquitoes, 74.6% (DDT) and 78% (deltamethrin) (Table 2). Similarly, in noncontact trials, higher numbers of unmated (56.9% DDT and 42.4% deltamethrin) and nulliparous mosquitoes (48.3% DDT, 46.5% deltamethrin) escaped from chambers treated with either deltamethrin or DDT compared to parous and blood engorged mosquitoes (Table 3). Bloodfed mosquitoes had the lowest rate of escape response from both contact and noncontact trials regardless of test insecticide.

Mean times in minutes for 25, 50 and 75% of the exposed test population to escape the treated chambers as designated by ET25, ET50 and ET75, respectively are provided in Table 4. In deltamethrin contact trials, both unmated and nulliparous females had near identical escape times with a maximum ET75 of 16 min. The number of parous and bloodfed specimens escaping were relatively low and ET50 and ET75 values could not be obtained for either test population exposed to deltamethrin. For DDT, ET values for unmated mosquitoes were similar to deltamethrin, whereas ET50 and ET75 values for nulliparous were approximately two times greater than those of unmated. Both parous and bloodfed produced far slower responses with only parous females managing 50% escape within 30 minutes.

Survival statistics show the rate of mosquito escape during a 30 min exposure (Figures 12-13). The patterns are indicative of escape probabilitiess

between the four test populations in contact and noncontact trials with deltamethrin (Figure 12), and contact and noncontact trials with DDT (Figure 13). No significant differences ($P < 0.05$) in escape patterns were seen between unmated and nulliparous females exposed to deltamethrin in contact and noncontact trials (Figure 12). However, escape responses were markedly different for unmated and nulliparous compared to parous and bloodfed mosquitoes ($P > 0.05$). Escape patterns were similar for parous and bloodfed test populations in deltamethrin contact trials. DDT contact produced a significant ($P < 0.05$) escape rate in unmated females compared to all others (Figure 13). In DDT noncontact trials, there was no significant difference in escape response between unmated, nulliparous and parous mosquitoes, while the bloodfed produced the slowest overall response ($P > 0.05$) (Figure 13). Pairwise comparisons of escape responses between test conditions found no significant difference in either irritability ($P = 0.148$-0.539) or repellency ($P = 0.108$-0.606) between the two chemicals.

Table 1 Percent mortality of unmated, nulliparous, parous, and bloodfed six day-old *Aedes aegypti* after contact with deltamethrin and DDT using standard WHO susceptibility test procedures.

Insecticides[1]	Conditions	Treatment		Control	
		No. tested	%Mortality ±SE	No. tested	%Mortality
Deltamethrin					
	Parous	75 [2]	100	75	0
	Nulliparous	75	100	75	0
	Unmated	75	100	75	0
	Bloodfed	75	100	75	0
DDT					
	Parous	75	6.7 ± 3.64	75	0
	Nulliparous	72	9.3 ± 3.17	75	0
	Unmated	75	10.0± 3.61	75	0
	Bloodfed	75	3.0± 1.58	75	0

[1] Diagnostic dosage DDT (4%) and deltamethrin (0.05%)

[2] Three replicates (25 mosquitoes/replicate)

Table 2 Percent escape and total mortality of pre-conditioned six-day-old *Aedes aegypti* after contact with deltamethrin and DDT in excito-repellency tests.

Insecticides[1]	Conditions	Treatment Chamber		Control Chamber		% Mortality			
						Treatment		Control	
		No. Tested	% Esc	No. Tested	% Esc	Esc[2]	Not[3] Esc	Esc	Not Esc
Deltamethrin									
	Parous	95	45.3	94	14.0	9.3	13.5	0	0
	Nulliparous	59	78.0	59	33.9	4.4	15.4	0	2.6
	Unmated	57	78.9	60	30.0	0	8.3	0	0
	Bloodfed	58	51.7	58	6.9	10.0	30.0	0	0
DDT									
	Parous	59	57.6	56	17.9	8.8	4.0	0	0
	Nulliparous	59	74.6	59	22.0	2.3	0	0	0
	Unmated	60	91.7	59	30.5	0	0	0	0
	Bloodfed	60	40.0	59	0	0	0	0	0

[1] DDT = 2 g/m^2, Deltamethrin = 0.02 g/m^2

[2] Esc = Escaped mosquitoes

[3] Not Esc = Not escaped mosquitoes

Table 3 Percent escape and total mortality of pre-conditioned six-day-old *Aedes aegypt* after noncontact with deltamethrin and DDT in excito-repellency tests.

Insecticides[1]	Conditions	Treatment Chamber		Control Chamber		% Mortality			
						Treatment		Control	
		No. Tested	% Esc	No. Tested	% Esc	Esc[2]	Not[3] Esc	Esc	Not Esc
Deltamethrin									
	Parous	93	28.0	93	10.7	11.5	37.3	0	0
	Nulliparous	58	46.5	57	21.1	0	0	0	0
	Unmated	59	42.4	58	24.1	0	0	0	0
	Bloodfed	59	5.1	58	3.4	0	0	0	0
DDT									
	Parous	57	42.1	54	7.4	8.3	0	0	0
	Nulliparous	58	48.3	58	13.8	0	0	0	0
	Unmated	58	56.9	58	24.1	0	4.0	0	0
	Bloodfed	59	13.6	60	0.0	0	0	0	0

[1] DDT = 2 g/m^2, Deltamethrin = 0.02 g/m^2

[2] Esc = Escaped mosquitoes

[3] Not Esc = Not escaped mosquitoes

Table 4 Escape time for 25%, 50% and 75% of six-day-old *Aedes aegypti* at different physiological conditions to escape insecticide-treated chambers.

Insecticides	Condition	Contact			Noncontact		
		ET25 [2]	ET50 [3]	ET75 [4]	ET25	ET50	ET75
Deltamethrin							
	Parous	5	-[1]	-	20	-	-
	Nulliparous	1	3	16	2	-	-
	Unmated	1	4	16	8	-	-
	Bloodfed	5	-	-	-	-	-
DDT							
	Parous	3	19	-	16	-	-
	Nulliparous	2	8	28	4	-	-
	Unmated	2	5	14	8	21	-
	Bloodfed	8	-	-	-	-	-

[1] Very few mosquitoes escaped from exposure chambers so that the ET values could not be estimated for a 30-min exposure period.

[2] ET 25 = Escape time = Time in minutes for 25% of female mosquitoes to escape from excito-repellency test chambers

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

[4] ET 75 = Escape time = Time in minutes for 75% of female mosquitoes to escape from excito-repellency test chambers

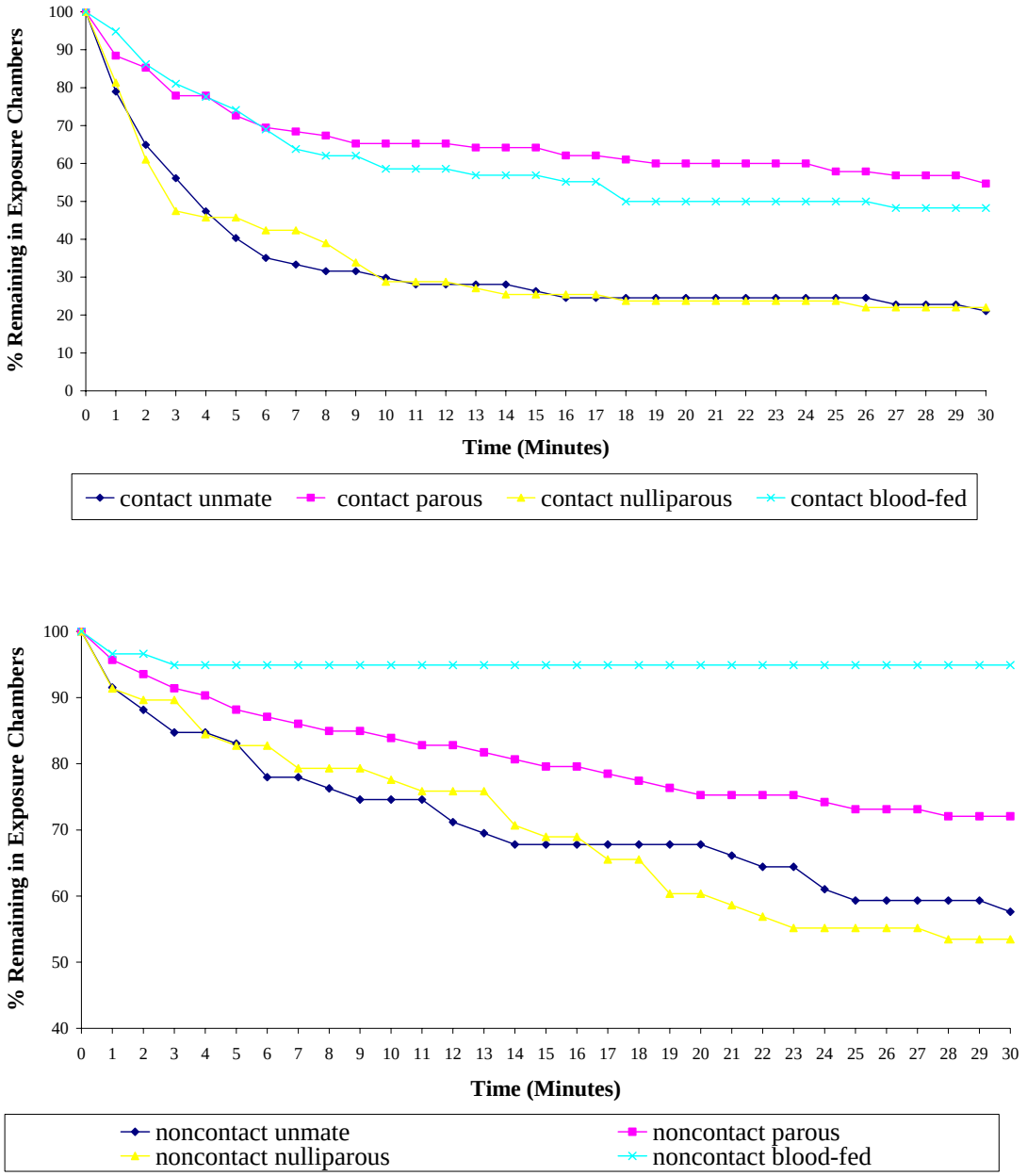


Figure 12 Escape patterns of four different physiological conditions of female *Aedes aegypti* in contact and noncontact trials with 0.02 g/m^2 deltamethrin

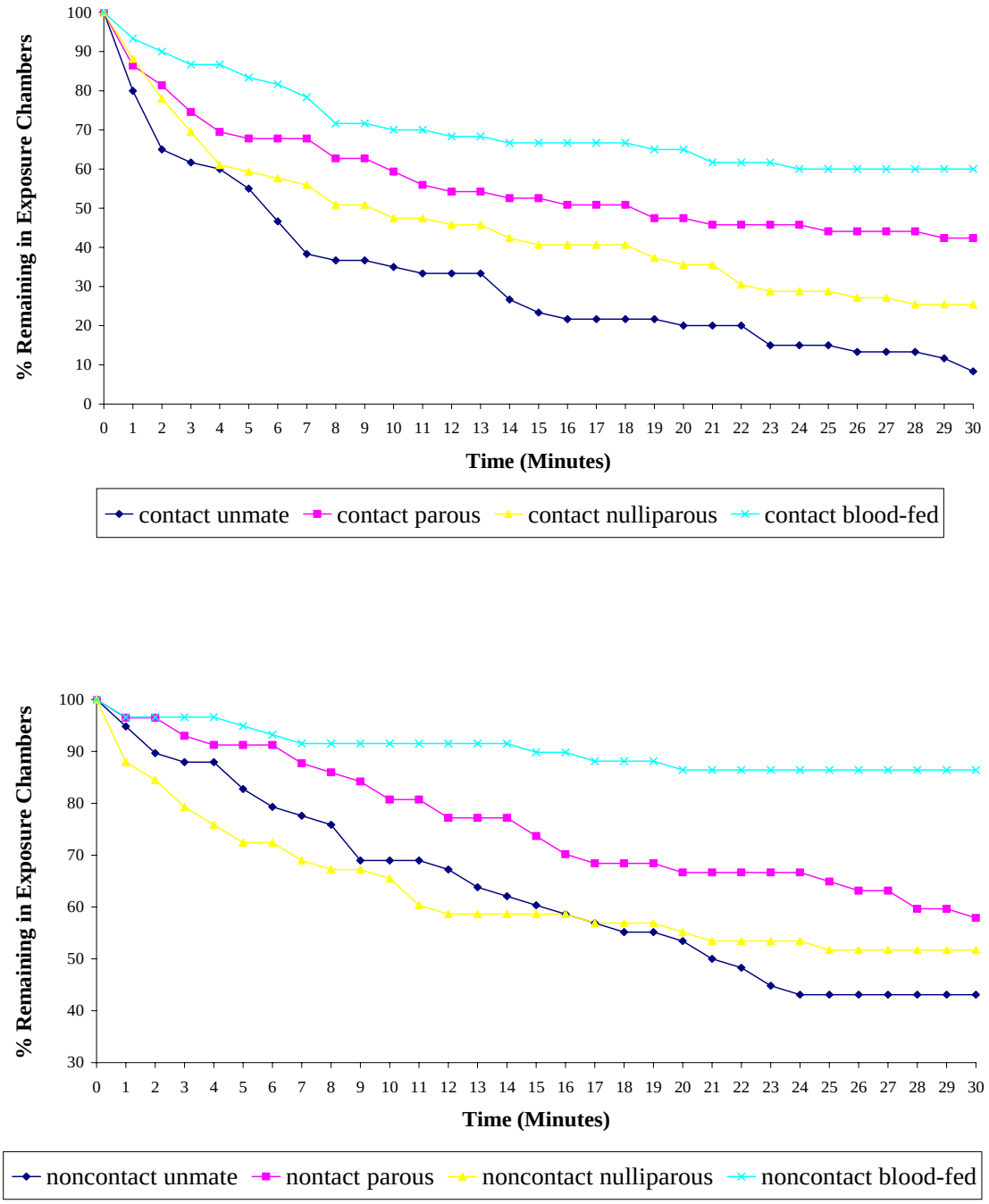


Figure 13 Escape patterns of four different physiological conditions of female *Aedes aegypti* in contact and noncontact trials with 2 g/m^2 DDT.

2. Behavioral responses of two field-collected mosquito species to catnip oil (*Nepeta cataria*) using an automated excito-repellency test system.

2.1 Dose response assay

Bioassays were conducted to obtain the dose response mortality on test populations of two mosquito species (*Ae. aegypti* and *An. harrisoni*), collected from Kanchaburi Province, western Thailand, using the WHO susceptible test for adult mosquitoes (WHO 1998). From preliminary screening, three appropriated concentrations of catnip oil (1%, 5% and 10% for *Ae. aegypti* and 1%, 2.5% and 5% for *An. harrisoni*) were selected for the bioassay and behavioral assay. Results revealed the low toxicity of catnip oil on two test populations (Table 5). Percent mortality of two test populations was comparatively low, regardless of test concentrations. Mortality varied between 0-3% for *Ae. aegypti* and 0-7% for *An. harrisoni* (Table 5). With *Ae. aegypti*, 94% percent knockdown at 1 hour was observed from 5% catnip oil and a 43% knockdown at 10% catnip oil whereas a 55% percent knockdown of *An. harrisoni* was observed from 5% catnip oil.

2.2 Excito-repellency test

Percent escape responses of the two test populations exposed to different concentrations of catnip oil were recorded in contact and noncontact trials (Tables 6 and 7). With *Ae. aegypti* in contact trials, the greatest escape responses were observed from 5% catnip oil (80%) whereas the lowest escape responses were observed from 1% catnip oil (35%). At the highest concentration (10%), a high percentage of knockdown specimens was observed from those that had escaped (21.21%) and those that remained in the test chamber (40%). In noncontact trials, the highest escape responses were observed from 10% catnip oil (53.57%) and the lowest was seen in 1% catnip oil (31.03%). Percent knock down was not as high as that observed from the contact trials. The highest knockdown rate was seen from those nonescaped specimens at 10% catnip oil (34.61%) whereas the percent of knockdown was comparatively low for those females that had escaped, ranging from 0-6.67%. With

An. harrisoni in contact trials, a marked escape response was observed at 2.5% catnip oil (71.19%), compared to 5% catnip (58.62%) and 1% catnip (16.95%). In noncontact trials, escape responses were comparatively high at 2.5% catnip oil (63.16%) and 5% catnip oil (67.87%) compared to 1% catnip oil (15%). In general, high percent knockdown was observed at the higher concentrations of catnip oil. Contact trials produced higher numbers of knock down specimens than those from noncontact trials. The greatest percent of knockdown was observed from females failing to escape at 5% catnip oil in contact trials (62.50%).

Twenty four hour mortalities of *Ae. aegypti* and *An. harrisoni* females after exposure in contact and noncontact trials with catnip oil are given in Tables 6 and 7. Lower mortality rates were recorded for *Ae. aegypti* as compared to *An. harrisoni* when tested against different concentrations of catnip oil. With *Ae. aegypti* in contact trials, percent mortalities of escape and nonescape females varied from 0-8%. No mortality was observed from non-contact trials for all test concentrations (Table 6). With *An. harrisoni* in contact trials, the percent mortality of nonescaping females was high (2.04-20.83%) compared to escaping females (9.52-14.70%). Similarly, high mortality rates were observed from noncontact trials in both escaping and nonescaping females, ranging from 2.78 to 10.53% for escaping and 1.96-16.67% for nonescaping females (Table 7).

Escape times (ET) from chambers treated with different concentrations of catnip oil, measured in 1-min intervals, are designated based on the percentage of the test population escaping, 25% (ET25), 50% (ET50) and 75% (ET75), the treated chamber within 30 min (Table 8). For 1% catnip oil, the *Ae. aegypti* test population had an ET25 value of 15 min in contact trials and of 18 min in noncontact trials whereas an ET25 value could not be calculated for *An. harrisoni* in both contact and noncontact trials due to the lack of mosquito movement. At 2.5% catnip oil, ET25 and ET50 for *An. harrisoni* values were 4 and 9 min, respectively, for contact trials and 3 and 11 min, respectively, for noncontact trials. At 5% catnip oil, the ET25 value was 2 min for *Ae. aegypti* and 4 min for *An. harrisoni* in contact trials whereas the ET25 values in noncontact trials were 8 and 6 min for *Ae. aegypti* and *An. harrisoni*,

respectively. The ET50 value was also low (4 min) for *Ae.aegypti* whereas it was comparatively high for *An. harrisoni* in contact (14 min) and noncontact trials (12 min) (Table 8). At 10% catnip oil, *Ae. aegypti* had a low ET25 values of 2 min in contact trials and 3 min in noncontact trials whereas ET 50 values of 16 and 20 min in contact and noncontact trials, respectively. ET75 values for both contact and noncontact trials at different concentrations of catnip oil could not be estimated because too few specimens departed the exposure chamber (Table 8).

Contact vs. noncontact escape responses of *Ae. aegypti* to 1%, 5% and 10% catnip oil were compared (Table 9). Escape probabilities in contact and noncontact trials were significantly higher than in controls for all cases *(P <0.05),* except for 1% catnip oil when the contact trials were not significantly different from the control. Significant differences in escape responses were observed in 5% catnip oil between contact and noncontact trials *(P <0.05).* Likewise, the contact vs. noncontact escape response of *An. harrisoni* to 1%, 2.5% and 5% catnip oil were compared. No significant differences in escape response were observed in all pairs when contact trials was compared to noncontact trial, regardless of test concentration *(P > 0.05).* Statistically significant differences in escape responses were observed at 2.5% and 5% catnip oil when control was compared to contact and noncontact trials.

Statistical comparisons between 2 doses of catnip oil (1%, 5% and 10% for *Ae. aegypti* and 1%, 2.5% and 5% for *An. harrisoni*) in contact and noncontact trials demonstrated that there were significant differences between all pairs ($P < 0.05$), except when catnip oil at 2.5% was compared to 5% in *An. harrisoni* test population ($P > 0.05$) (Table 10).

Figures 14 and 15 show the proportions of mosquitoes remaining in the exposure chambers at different test concentrations. These proportions are used to show patterns of escape rates. The patterns are used to compare escape probabilities between contact and noncontact trials for *Ae. aegypti* (Figure 13) and *An. harrisoni* (Figure 14). A higher escape response of *Ae. aegypti* was observed when exposed to 5% catnip oil in contact trials compared to non-contact trials. Significantly lower

escape responses were found at 1% and 10% catnip oil in both contact and non-contact trials when tested against *Ae. aegypti* (Figure 14). The patterns of escaped females of *An. harrisoni* were significantly greater at 2.5% and 5% catnip oil than at 1% catnip oil (Figure 15).

Table 5 Percent knockdown and mortality of *Aedes aegypti* and *Anopheles harrisoni* populations from Kanchanaburi expose to different doses of catnip oil using standard WHO susceptibility test procedures.

Mosquito	Dosage	Number Tested	%KD	% Mortality ± SE
Ae. aegypti				
	1%	100	0	0
	5%	100	4	0
	10%	100	43	3 ± 0.75
An. harrisoni				
	1%	100	0	0
	2.5%	100	3	3 ± 0.48
	5%	100	55	7 ± 0.63

Table 6 Escape response and percent mortality of female *Aedes aegypti* from Kanchanaburi after contact and non-contact with catnip oil in excito-repellency tests.

Conditions	Dosage	Treatment Chamber				Control Chamber		% Mortality			
				% KD				Treatment		Control	
		No. Tested	% Esc	Esc	Not Esc	No. Tested	% Esc	Esc[1]	Not[2] Esc	Esc	Not Esc
Contact											
	1%	60	35.00	0	0	56	21.43	0	0	0	0
	5%	55	80.00	6.81	18.18	58	13.79	2.27	0	0	0
	10%	58	56.90	21.21	40.00	58	18.97	3.03	8.00	0	0
Non-contact											
	1%	58	31.03	0	0	57	14.04	0	0	0	0
	5%	55	40.00	0	9.09	59	10.17	0	0	0	0
	10%	56	53.57	6.67	34.61	59	11.86	0	0	0	0

[1] Esc = Escaped mosquitoes

[2] Not Esc = Not escaped mosquitoes

Table 7 Escape response and percent mortality of female *Anopheles harrisoni* from Kanchanaburi after contact and non-contact with catnip oil in excito-repellency tests.

Condition	Dosage	Treatment Chamber				Control Chamber		% Mortality			
				% KD				Treatment		Control	
		No. Tested	% Esc	Esc	Not Esc	No. Tested	% Esc	Esc[1]	Not[2] Esc	Esc	Not Esc
Contact											
	1%	59	16.95	0	0	56	1.79	0	2.04	0	0
	2.5%	59	71.19	11.36	18.18	59	8.47	9.52	17.64	0	0
	5%	58	58.62	35.29	62.50	58	8.62	14.70	20.83	0	0
Non-contact											
	1%	60	15.00	0	0	55	1.82	0	1.96	0	0
	2.5%	57	63.16	0	9.09	58	10.34	2.78	14.04	0	0
	5%	56	67.86	5.26	38.89	54	5.56	10.53	16.67	0	0

[1] Esc = Escaped mosquitoes

[2] Not Esc = Not escaped mosquitoes

Table 8 Escape time (ET) in minutes for 25%, 50% and 75% of two species of field caught mosquito exposed to treated chambers with catnip oil

Mosquitoes	Dosage	Contact			Non-contact		
		ET 25	ET 50	ET 75	ET 25	ET 50	ET 75
Ae. aegypti							
	1%	15	-[1]	-	18	-	-
	5%	1	4	16	8	-	-
	10%	2	16	-	3	20	-
An. harrisoni							
	1%	-	-	-	-	-	-
	2.5%	4	9	-	3	11	-
	5%	4	14	-	6	12	-

[1] Very few mosquitoes escaped from exposure chambers so that the ET values could not be estimated for a 30-min exposure period.

Table 9 Comparison of the escape response between paired control and noncontact trials, control and contact trials, and paired contact and noncontact trials for two species of field caught mosquito with catnip oil in excito-repellency tests.

Mosquitoes	Dosage	Control[1] vs. Non-contact (*P*)	Control vs. Contact (*P*)	Contact[1] vs. Non-contact (*P*)
Ae. aegypti	1%	0.040*	0.131	0.558
	5%	0.000*	0.000*	0.000*
	10%	0.000*	0.000*	0.593
An. harrisoni	1%	0.169	1.000	0.998
	2.5%	0.000*	0.000*	0.335
	5%	0.000*	0.000*	0.568

[1] The * identifies results of log-rank tests with statistically significant (0.05 level of probability) diffences in escape response between paired trials.

Table 10 Comparison of escape response between doses for 2 species of field collected mosquito with catnip oil after contact and non-contact trials in excito repellency tests.

Mosquitoes	Dosage	Contact trial (*P*)	Non-contact trial (*P*)
Ae. aegypti			
	1% vs. 5%	0.000*	0.008*
	1% vs. 10%	0.012*	0.000*
	5% vs. 10%	0.030*	0.009*
An. harrisoni			
	1% vs. 2.5%	0.000*	0.000*
	1% vs. 5%	0.000*	0.000*
	2.5% vs. 5%	0.128	0.858

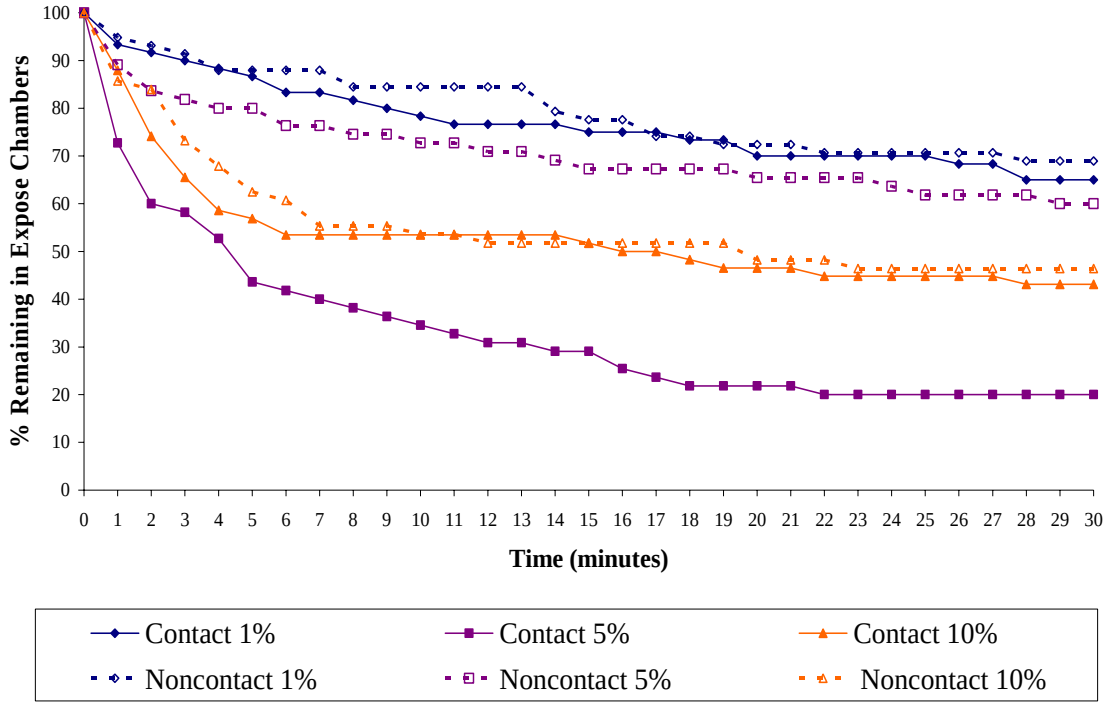


Figure 14 Comparison of escape patterns of female *Aedes aegypti* from Kanchanaburi in contact and non-contact trials exposed to different doses of catnip oil.

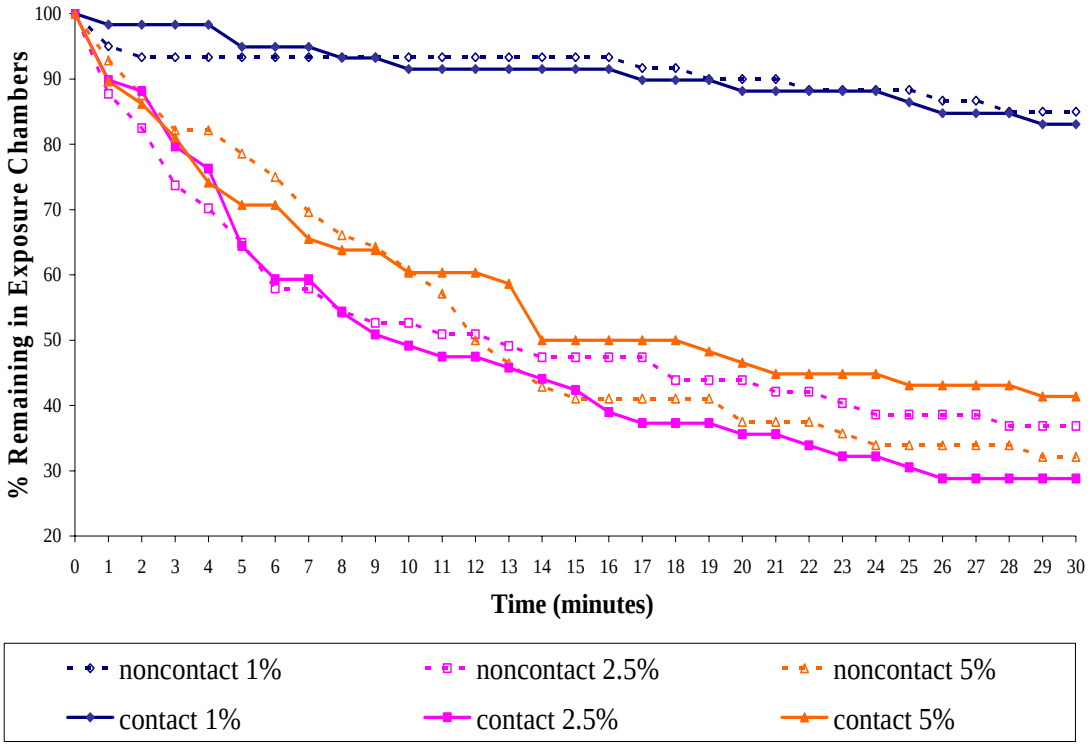


Figure 15 Comparison of escape patterns of female *Anopheles harrisoni* from Kanchanaburi in contact and non-contact trials exposed to different doses of catnip oil.

Discussion

1. Effects of physiological condition of a single age group of *Aedes aegypti* on insecticide avoidance behavior

The physiological status of mosquitoes is an important factor influencing escape movement from chemical-treated surfaces (Roberts *et al*., 1984; Sungvornyothin *et al*., 2001; Chareonviriyaphap *et al*., 2006). Intrinsic factors known to influence susceptibility and behavioral responses include carbohydrate (energy) reserves, age, blood feeding and gonotrophic condition of female mosquitoes (Hadaway and Barlow, 1956; Busvine, 1964; Xue and Barnard, 1999). In our study, age and sucrose availability were controlled and environmental factors such as temperature, humidity and light were maintained within a reasonably defined range so as not to cause disparate responses. In particular, age can influence both susceptibility (Raffaele *et al*., 1958; Lines and Nassor, 1991) and irritability to insecticides (Busvine, 1964). For *Ae. aegypti*, David and Bracey (1946) noted a decline in DDT tolerance with advancing age. Glutathione-*S*-transferase mediated resistance to DDT has also shown a marked decline in activity with increased age (Hazelton and Lang, 1983). Few investigations have been performed to investigate the effect of age on behavior, but generally, older mosquitoes have been found less irritable than younger ones, possibly related to lower or depleted energy reserves (Hamon and Eyraud, 1961; Kaschef, 1970).

The *Aedes aegypti* test colony was collected from an area with perennial malaria transmission (MOPH, 2006). For over 40 years DDT was commonly used as an indoor residual spray (IRS) to control anopheline vectors in the Pu Teuy area. DDT use for malaria control in Thailand ceased over a decade ago. Deltamethrin, on the other hand, is a much more recent introduction and the insecticide of choice for non-residual space spray during dengue outbreaks in Thailand (Kongmee *et al*., 2004). However, very little of this compound or other synthetic pyrethroid has been applied in Pu Teuy, due to an apparent absence of dengue transmission (MOPH, 2006).

Regardless of conditioning, all individuals were completely susceptible to deltamethrin, whereas the same test population was found highly resistant to DDT. Previous studies had also documented a high degree of resistance to DDT (> 90%) in this mosquito population despite the long interval of time since its last use (Chareonviriyaphap *et al*., 2006; Suwonkerd *et al*., 2006). As cross-resistance between the two chemical classes was not observed, these results indicate that the resistance is not based on the *kdr* (knockdown resistance) or *kdr*-like genetic mutation associated with the physical alteration of the target sodium channel protein. Although biochemical assays were not performed to determine the precise metabolic mechanism(s) responsible, it may involve elevated enzyme levels of glutathione *S*-transferases (GST) resulting in increased DDT-dehydrochlorinase activity, a common mechanism for conferring physiological resistance to organochlorine insecticides (Prapanthadana *et al*., 1995). Another possible detoxification mechanism present in parallel with GST, could involve oxidation of DDT by elevated cytochrome *P*- 450 dependent microsomal monooxygenase systems (Wilkinson, 1983). Susceptibility patterns varied depending upon physiological state. Although not particularly striking, higher mortality to DDT was seen in unmated and nulliparous test population compared to parous (previous bloodfed) and engorged test population. As blood can serve as an additional nutritional reserve and exogenous energy source of glycogen and fat, unmated and non-bloodfed mosquitoes may have had less vigor or tolerance to residues compared to parous/ bloodfed females (Clements, 1992).

Background physiological resistance has been associated with either increased or reduced irritability depending on the mosquito species, chemical concentration and test conditions (de Zulueta, 1959; Brown, 1958; Elliott, 1964; Gaaboud and Dawood, 1974). In many cases, *Ae. aegypti* has either been suspected or proven significantly less irritable in DDT-resistant strains (Hecht *et al*., 1960; Cullen and de Zulueta, 1962; Busvine, 1964; Brown, 1964). Although I did not compare our DDT-resistant strain with a susceptible population, DDT produced significant excito-repellency in the face of high levels of resistance. Moreover, the dramatic contrast in resistance profile between chemicals in this population had no significant effect on differing escape responses between exposure to deltamethrin and DDT.

DDT produced stronger repellent activity than deltamethrin, presumably because of inherently greater fumigant (vapor pressure) properties than the later. This is in agreement with previous findings on the repellency of DDT and pyrethroids (Roberts *el al*., 2000; Chareonviriyaphap *et al*., 2004, 2006). The lower escape responses of blood engorged mosquitoes are likely the result of the additional physical burden (weight) of the blood meal, resulting in a greater reluctance to take flight. Reduced irritability of mosquitoes soon after a blood meal is a common finding (Hecht *et al*., 1960; Busvine, 1964; Brown and Pal, 1971; Roberts *et al*., 1984; Sungvornyothin *et al*., 2001), although Brown (1964) reported very little difference in *Ae. aegypti* before and 1 h after blood feeding. Jones (1981), on the other hand, observed blood-engorged mosquitoes with greatly reduced flight activity and only became active again around the third day when fully gravid. Under similar test conditions, unfed mosquitoes (mated or unmated) often demonstrated stronger irritant/repellent behavior than bloodfed (Busvine, 1964). As in other studies, I have consistently seen decreased excito-repellency in recently bloodfed *Ae. aegypti* (Kongmee *et al*., 2004; Chareonviriyaphap *et al*., 2006).

The strong anthropophagic and endophilic behaviors of *Ae. aegypti* have presented an enormous challenge to vector control specialists to devise new or improved methods to more effectively reduce mosquito populations and disease transmission risk (WHO, 1999). Since the early 1990s, synthetic pyrethroids, including deltamethrin, have been in common use in Thailand as space sprays for controlling household nuisance and vector mosquitoes, including *Ae. aegypti* (Chareonviriyaphap *et al*., 1999). Pyrethroids have also been widely used in an attempt to interrupt virus transmission in communities reporting dengue cases. Outdoor/peridomestic space spraying alone has often failed to achieve any meaningful control of indoor adult *Ae. aegypti* populations (and virus transmission) because the chemical fails to reach the intended target resting sites inside homes (Reiter and Gubler, 1997; Mani *et al*., 2005). Moreover, without simultaneous attention to larval habitats and source reduction, adult populations often quickly rebound. However, when residual insecticides are applied indoors using conventional portable space spray devices (ultra-low-volume units, mist blowers and thermal foggers) more

effective and longer lasting control of adult *Aedes* has been achieved compared to more conventional outdoor application (Sulaiman *et al*., 1993; Lee *et al*., 1997; Perich *et al*., 2001). Pant *et al*. (1974) reported up to 7 months of effective control of indoor *Ae. aegypti* using fenitrothion applied by an aerosol mist blower.

Greater tendency for unengorged mosquitoes to escape a treated surface or area alters the normal behavioral patterns and reduces the opportunity for blood feeding and potential for cyclic transmission of virus. This greater hyper-activation response in non-bloodfed compared to the more subdued reaction of bloodfed mosquitoes has several implications for the epidemiological consequences of excito-repellency caused by insecticides for controling vector populations. Nevertheless, a residual chemical with sufficiently strong irritant and repellent properties applied to indoor surfaces has the potential to both decrease longevity of mosquitoes acquiring a sufficient toxic dose and in reducing vector-human contact by a behavioral avoidance mechanism. Even if the majority of indoor populations of *Ae. aegypti* rest on unsprayed surfaces, such as hanging clothing, repellency alone could be responsible for reducing transmission risk by disrupting the normal host resting and feeding patterns of a vector (Roberts *et al*., 2000). This is in line with arguments supporting the view that individuals sleeping in rooms with pyrethroid-treated bednets or curtains are afforded adequate protection because of significant ‘deterrency’ of vectors from entering the house (Miller *et al*., 1991). Deltamethrin, acting as a potent deterrent (repellent) that would also inhibit successful blood feeding, is deemed advantageous for enhancing personal protection. Furthermore, our findings with deltamethrin do not support the notion that deterrency is necessarily independent of excito-repellency as stimulated by the insecticide active ingredient (Lindsay *et al*.,1991). Although I would not disagree that certain commercial product formulations may contain ‘inert ingredients’ (e.g., aromatic hydrocarbon solvents) that influence behavior, but as our paired treatment – control assays clearly attest, deterrency is the result of hyper-activation by irritant and repellent properties caused by the parent chemical and not a function of the solvent or oil-based carrier.

Differences in the physiological condition of mosquitoes have considerable bearing on the results of behavioral avoidance assays. Even when tests are carried out on apparently homogenous material, there can be a number of other factors responsible for unexplained variations in response (Busvine, 1964). Because we carefully controlled for age in this study, extrapolation of laboratory findings to heterogenous field populations under less controlled conditions is cautioned. A better understanding of the behavioral avoidance that interfers with vector feeding must be considered when assessing the epidemiological effect of insecticides on disease transmission, given the view that the primary measure of success is the reduction of transmission (disease incidence), not simply the quantitative reduction of vector densities. Susceptibility tests alone should not be the sole criteria or evidence for making critical decisions on the continued usefulness of a chemical or its replacement (Davidson and Zahar, 1973). The continued refinement and use of excito-repellency assays offers a better means to objectively evaluate an integral component of the full attributes of an insecticide and its potential to suppress disease transmission.

2. Behavioral responses of two field-collected mosquito species to catnip oil (*Nepeta cataria*) using an automated excito-repellency test system.

Understanding the behavioral responses of mosquito vectors, especially avoidance behavior to test compounds, is of paramount importance to any mosquito control program. There have been numerous attempts to accurately measure the behavioral responses of mosquitoes to insecticides using several types of excito-repellency test system (Roberts *et al*., 1984; Chareonviriyaphap *et al*., 1997; Rutledge *et al*., 1999; Sungvornyothin *et al*., 2001). Due to the inherent complexities of accurately measuring excito-repellency in mosquitoes, no test method had been adequate and fully accepted. No test recommended by the WHO will discriminate between the two types of behavioral responses, contact irritancy and noncontact repellency (Roberts *et al*., 1984). However, an experimental test system described by Roberts *et al*. (1997) addresses a number of deficiencies attributed to behavioral test systems. This test system was first used to test the avoidance behavior of *An. abimanus* from Belize, Central America (Chareonviriyaphap *et al*., 1997). This

prototype test system has been modified further into the collapsible chamber designed for the greater ease of use (Chareonviriyaphap *et al*., 2002) and has proved valuable in the evaluation of behavioral responses in several laboratory and field populations of mosquitoes in Thailand and Indonesia (Chareonviriyaphap *et al*., 2004; Kongmee *et al*., 2004; Pothikasikorn *et al*., 2005, 2007; Muenworn *et al*., 2006; Chareonviriyaphap *et al*., 2006). However, this system was still cumbersome and required a minimum of two investigators to observe and record data during the 30-min testing period.

Recently, an assay for evaluating the three types of chemical actions, contact irritancy, spatial repellency and toxicity, in adult mosquitoes was developed (Grieco *et al*., 2007), but this system was not designed as a field-adaptable assay. To overcome these technical problems when conducting field studies, a more compatible design was developed and is referred to as an "automated, field-compatible device for testing excito-repellency behavior (Tanasinchayakul *et al*., 2006). This system consists of two major modifications from the previous model: a substantial reduction in the size of the test box and the use of an electronic sensor for automated counting of mosquitoes as they departed the test chamber through the opened gate into the external holding box. This device has been successfully used to measure the behavioral responses of *Ae. aegypti* from Bangkok, Thailand to deltamethrin (Tanasinchayakul *et al*., 2006). Moreover, an automated excito repellency test system provides the advantage it makes it easier for automatically counting escaping mosquitoes from the chamber and recording data by computer system. This system can eliminate error from confounding factors by human such as human odor, body heat, and carbon dioxide. An additional advantage is system requires only one investigator to observe and collect escaped mosquitoes from the receiving cage.

In this study, I observed the behavioral responses of two field collected mosquito species, *Ae. aegypti* and *An. harrisoni* collected from Kanchanaburi, western Thailand, to catnip oil, a promising plant derived compound from catnip (Peterson and Coat, 2001).

Chemicals protect human from the bite of mosquitoes in three different ways, irritate, repel or kill the mosquitoes (Grieco *et al*., 2007). In this study, *Ae. aegypti* demonstrated clear behavioral escape responses to catnip oil in both contact and noncontact trials compared to the control trials. Greater contact irritancy escape responses from 5% catnip oil were documented in *Ae. aegypti*, compared with 1% and 10% catnip oil. All tests showed mosquitoes successfully departed treated surfaces and chambers before receiving a lethal dose of test compound. Higher knockdown rates were observed at the higher doses, regardless of test condition, indicating a strong vapor from the test chemical affected the test specimens. However, a high percent of recovery (>92%) was observed, indicating no toxic action of catnip oil. Recently, there were several studies to examine the repellency effect of catnip oil in mosquito species and other insects (Bernier *et al*., 2005; Chauhan *et al*., 2005; Peterson and Coat, 2001; Schultz *et al*., 2004; Webb and Russell, 2007; Zhu *et al*., 2006). With *An. harrisoni*, strong contact irritancy and noncontact repellency were quite high, especially at 2.5% catnip. Knockdown rates were somewhat greater at the higher doses with greater percent mortality of both contact and noncontact mosquitoes, suggesting *An. harrisoni* were more sensitive to the toxic action of catnip oil.

The protection time of catnip oil has been reported elsewhere. Catnip oil was shown to be an effective repellent up to 6 hours against *Ae. albopictus* (Zhu *et al*., 2006). In Australia, catnip oil demonstrated mean protection times, ranging from 0 min for *Ae. aegypti* up to 240 ± 60 min for *Cx. quinquefasciatus* (Webb and Russell, 2007). In contrast, catnip oil showed a long protection time to *Ae.vigilax, Cx. annulirostris* and *Cx. quinquefasciatus* compared to other potential natural plant extracts (Webb and Russell, 2007). In this study, the protection time of catnip oil on mosquito populations was not evaluated. However, we found that catnip oil has strong irritant and repellent actions on mosquito test populations as indicated by the comparatively low escape time (ET).

In summary, several studies have investigated mosquitoes repellents derived from plant extracts (Tawatsin *et al*., 2001; Suwonkerd and Tantrarongroj, 1994), but

none have described the true behavioral responses of mosquitoes to various potential plants. With the existance of a field-automated excito-repellency test system, the true behavioral actions of catnip oil on two field collected mosquito species were identified and the resulting data will facilitate mosquito control personnel in deciding the proper use of test compounds to combat mosquito in the future.

CONCLUSION

1. Effects of physiological condition of a single age group of *Aedes aegypti* on insecticide avoidance behavior

Behavioral responses of six -day-old *Aedes aegypti* females in different physiological conditions when expose to deltamethrin and DDT were evaluated using a free-choice excito-repellency test system. This study showed high escape rates from contact and non-contact trials with deltamethrin in non-bloodfed groups (unmated and nulliparous) compared to bloodfed groups (parous and bloodfed). There were no significant differences in escape responses between unmated and nulliparous ($P>$ 0.05). High escape responses were observed in the unmated condition compared to other physiological conditions while moderate escape responses were shown in nulliparous and parous mosquitoes and the lowest response was observed in the full bloodfed condition. *Aedes aegypti* test populations were completely susceptible to deltamethrin but showing high resistance to DDT. Moreover, pre- and post-bloodfed conditions exhibited influence on the behavioral response of mosquitoes to insecticides.

2. Behavioral responses of two field-collected mosquito species to catnip oil (*Nepeta cataria*) using an automated excito-repellency test system.

The biological effect of catnip oil (*Nepeta cataria*) on the behavioral response of field collected *Aedes aegypti* and *Anopheles harrisoni* were characterized using an automated excito-repellency test system. *Aedes aegypti* showed a greater irritant behavior at 5% catnip oil (80%) than repellency. There were no significant difference between repellency and irritancy at 1 and 10% catnip oil. *Anopheles harrisoni* showed a high escape response at 2.5% catnip oil in contact trial (71.19%), while in noncontact trials showed a high escape response at 5% catnip oil (67.86%). No significant difference was observed between contact and non-contact trials at each concentration and similar responses were observed between concentrations.

LITERATURE CITED

Abbott, W.S. 1925**.** A method of computing the effectiveness of an insecticide. **J. Econ. Entomol.** 18: 265-267.

Anonymous. 2007. **Map of malaria risk in Thailand.** Available Source:http://www.break-fresh-ground.com, December 20, 2007.

American Chemical Society (ACS). 2001. **Catnip a mosquitoes repellent.** 222nd Annual meeting ACS, Chicago, IL, August 27, 2001.

Barnard, D. R. 1999. Repellency of essential oils to mosquitoes (Diptera: Culicidae). **J. Med. Entomol.** 36: 625-629.

Bernier, U. R., K. D. Furman, D. L. Kline, S. A. Allan and D. R. Barnard. 2005. Comparison of contact and spatial repellency of catnip oil and *N,N*-diethyl-3-methylbenzamide (deet) against mosquitoes. **J. Med. Entomol.** 42(3): 306-311.

Brown, A.W.A. 1958. Laboratory studies on the behaviouristic resistance of *Anopheles albimanus* in Panama. **Bull.Wld Hlth Org.** 19: 1053-1056.

_____. 1964. Experimental observation governing the choice of a test method for determining the DDT-irritability of adult mosquitos. **Bull.Wld Hlth Org.** 30: 97-111.

_____ and R. Pal. 1971. **Insecticide resistance in arthropods.** 2nd ed. Monograph Ser. 38. World Health Organization, Geneva. 1-49.

Bureau of Epidemiology. 2007a. **A morbidity and mortality rates of malaria and Dengue.** Avialable Source: http://epid.moph.go.th, December 17, 2007.

Bureau of Epidemiology. 2007b. **Numbers of Dengue and malaria cases in Thailand.** Avialable Source: http://epid.moph.go.th, December 17, 2007.

Busvine, J.R. 1964. The significance of DDT-irritability tests on mosquitos. **Bull. Wld. Hlth. Org.** 31: 645-656.

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

_____, _____, _____ and B. Aum-Aung. 2003. Seasonal abundance and blood feeding activity of *Anopheles minimus* Theobald (Diptera: Culicidae) in Thailand. **J. Med. Entomol.** 40: 876-881.

_____, _____, _____, M. Kongmee, S. Sathantriphop, V. Meunworn, W. Suwonkerd and P. Akratanakul. 2006. Influence of nutritional and physiological status on behavioral responses of *Aedes aegypti* (Diptera: Culicidae) to deltamethrin and cypermethrin. **J. Vector Ecol.** 31: 89-101.

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

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

_____, D.R. Roberts, R.G. Andre, H. Harlan, S. Manguin and M.J. Bangs. 1997. Pesticide avoidance behavior in *Anopheles albimanus* Wiedemann. **J. Am. Mosq. Control Assoc.** 13: 171-183.

Chareonviriyaphap, T., M. J. Bangs and S. Ratanatham. 2000. Status of malaria in Thailand. **Southeast Asian J. Trop. Med. Public Health**. 31(2): 225-237.

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

Chauhan, K. R. and A. Zhang. 2004. Methods of separating Z,E and E,Z nepetalactones from catnip oil. **U.S Patent application** # 11/007,078 .

_____, J. A. Klun, M. Debboun and M. Kramer. 2005. Feeding deterrent effects of catnip oil components compared with two synthetic amides against *Aedes aegypti*. **J. Med. Entomol.** 42(4): 643-646.

Christophers, R. 1960. ***Aedes aegypti* (L.), the yellow fever mosquito: its life history, bionomics and structure.** Cambridge University Press, London.

Clements, A.N. 1992. Adult energy metabolism, pp. 292-303. *In* **The biology of mosquitoes, vol. 1, Development, nutrition and reproduction.** Chapman & Hall, London.

Cullen, J.R. and J. de Zulueta. 1962. Observations on the irritability of adult mosquitos to DDT in Uganda. **Bull.Wld Hlth Org.** 27: 239-250.

David, W. A. L. and P. Bracey. 1946. Factors influencing the interaction of insecticidal mists on flying insects**. Bull. Entomol. Res.** 37: 177-190

Davidson, G.and A.R. Zahar. 1973. The practical implications of resistance of malaria vectors to insecticides. **Bull.Wld Hlth Org.** 49: 475-483.

Debboun, M., S.P. Frances and D. Strickman. 2007. **Insect repellents: principle, method, and uses.** CRC Press., Florida.

de Zulueta, J. 1959. Insecticide resistance in *Anopheles sacharovi*. **Bull.Wld Hlth Org.** 20: 797-822.

Eisenbraun, E.J., C.E. Browne, R.L. Irvin-Willis, D.J. McGurk, E.L. Eliel and D.L. Harris. 1980. Structure and stereochemistry of 4a*B*, 7α,7a*B*-Nepetalactone from *Nepeta mussini* and its relationship to the 4a*α*,7*α*,7a*α*- and 4a*α*,7*α*,7a*B*-Nepetalactones from *Nepeta cataria*. **J. Org. Chem.** 45: 3811-3814.

Eisner, T. 1964. Catnip: its reason. **Science**. 146: 1318

Elliott, R. 1964. Studies on the kinetic response of mosquitos to chemicals. **Bull.Wld Hlth Org.** 31: 657-667.

_____. 1972. The influence of vector behavior on malaria transmission. **Am. J. Trop. Med. Hyg.** 21: 755-763.

Evans, R.G. 1993. Laboratory evaluation of the irritancy of bendiocarb, lambdacyhalothrin, and DDT to *Anopheles gambiae*. **J. Am. Mosq. Control Assoc.** 9: 285-293.

Gaaboub, I.A. and M.R. Dawood. 1974. Irritability status of adults of *Culex pipiens* L. under selection pressure with lethal concentrations of DDT and malathion**. Z. Angew. Entomol.** 77 : 126-132.

Garros, C., L.L. Koekemoer, M. Coetzee, M. Coosemans and S. Manguin. 2004. A single multiplex assay to identify major malaria vectors within the African *Anopheles funestus* and the Oriental *An. minimus* groups. **Am. J. Trop. Med. Hyg.** 70: 583-590.

Green, C.A., R.F. Gass, E. Munstermann and V. Baimai. 1990. Population genetic evidence for two species in *Anopheles minimus* in Thailand. **Med. Vet. Entomol.** 4:25-34.

Grieco, J.P., N.L. Achee, M.R. Sardelis, K.R. Chauhan and D.R. Roberts. 2005. A novel high throughput screening system to evaluate the behavioral response of adult mosquitoes to chemicals. **J. Am. Mosq. Control Assoc.** 21: 404-411.

_____, _____, T. Chareonviriyaphap, W. Suwonkerd, K. Chauhan, M.R. Sardelis and D.R. Roberts. 2007. A new classification system for the actions of IRS chemical traditionally used for malaria control. **PLos one.** 2(8): e716. doi: 10.1371/journal. pone. 0000716.

Gubler, D.J. 1997**.** Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem, pp. 1-22. *In*: D.J. Gubler and G. Kuno [eds.], **Dengue and dengue hemorrhagic fever**. CABI, Wallingford, United Kingdom.

Hadaway, A.B. and F. Barlow. 1956. Effects of age, sex and feeding on the susceptibility of mosquitoes to insecticides. **Ann. Trop. Med. Parsitol.** 50: 438-443.

Hamon, J. and M. Eyraud. 1961. Etude des facteurs physiologiques conditionnant, chez les *Anopheles*, l'irritabilite au DDT. **Riv. Malariol.** 40: 219-242.

Harrison, B.A. 1980. Medical entomology studies XII. The Myzomyia Series of *Anopheles* (Cellia) in Thailand (Diptera: Culicidae), with emphasis on intra-interspecific vaiations. **Contrib. Am. Entomol. Inst. (Ann Arbor).** 17(4): 1-195.

Harbach R.E. 2004. The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationship. **Bull. Entomol. Res.** 94:537-553.

_____., C. Garros, N. D. Manh and S. Manguin. 2007. Formal taxonomy of species C of the *Anopheles minimus* sibling species complex (Diptera: Culicidae). **Zootaxa.** 1654: 41-54.

Hazelton, G.A. and C.A. Lang. 1983. Glutathione *S*-transferase activities in the yellow-fever mosquito (*Aedes aegypti*) during growth and aging. **Biochem. J.** 210: 281-287.

Hecht, O., O. Mancera and A. Barrera. 1960. Relation of DDT-irritation threshold to knockdown of three species of anopheline mosquitoes. **J. Econ. Entomol.** 53: 634-640.

Jacobs, M. 2000. Dengue: emergence as a global public health problem and prospects of control. **Trans. R. Soc. Trop. Med. Hyg.** 94: 7-8.

Jirakanjanakit, N., P. Rongnoparat, S. Saengtharatip, T. Chareonviriyaphap, S. Duchon, C. Bellec and S. Yokgan. 2007. Insecticide susceptible/resistance status in *Aedes* (Stegomyia) *aegypti* and *Aedes* (Stegomyia) *albopictus* (Diptera: Culicidae) in Thailand during 2003-2005. **J. Econ Entomol.** 100(2): 545-550.

Jones, M.D.R. 1981. The programming of circadian flight-activity in relation to mating and the gonotrophic cycle in the mosquito, *Aedes aegypti*. **Physiol. Entomol.** 6: 307-313.

Kaschef, A.H. 1970. Effects of temperature on the irritability caused by DDT and DDT-analogues in anopheline mosquitos. **Bull.Wld Hlth Org.** 42: 917-930.

Kengluecha, A., P. Rongnoparut, S. Boonsuepsakul, R. Sithiprasasna, P. Rodpadit and V. Baimai. 2005. Geographical distribution of *Anopheles minimus* A and C in western Thailand. **J. Vector Ecol.** 30:225-230.

Kennedy, J.S. 1947. The excitant and repellent effects on mosquitoes of sub-lethal contacts with DDT. **Bull. Entomol. Res.** 37: 593-607.

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

Lal, H., S. Ginocchio and E.J. Hawrylewicz. 1965. Effect of allethrin on feeding behavior of insects. **Proc. Soc. Exp. Biol. Med.** 120: 441-443.

Lee, H.L., M.S. Khadri and Y.F. Chiang. 1997. Preliminary field evaluation of the chemical adulticidal, larvicidal, and wall residual activity of ULV-applied bifenthrin against mosquitoes. **J. Vector Ecol.** 22: 146-149.

Lines, J.D. and N.S. Nassor. 1991. DDT resistance in *Anopheles gambiae* declines with mosquito age. **Med. Vet. Entomol.** 5: 261-265.

Lindsay, S.W., J.H. Adiamah, J.E. Miller and J.R.M. Armstrong. 1991. Pyrethroid-treated bednet effects on mosquitoes of the *Anopheles gambiae* complex in The Gambia. **Med. Vet. Entomol.** 5: 477-483.

Mani, T.R., N. Arunachalam, R. Rajendran, K. Satyanarayana and A.P. Dash. 2005. Efficacy of thermal fog application of deltacide, a synergized mixture of pyrethroids, against *Aedes aegypti*, the vector of dengue. **Trop. Med. Inter. Health.** 10: 1298-1304.

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

Miller, J. E., S.W. Lindsay and J.R.M. Armstrong. 1991. Experimental hut trails of bednets impregnated with synthetic pyrethroid and organophosphate insecticides for mosquito control in The Gambia. **Med. Vet. Entomol.** 5: 465-476.

Ministry of Public Health. 2006a. **Annual report on vector-borne disease in Thailand.** Department of Disease Control, Ministry of Public Health, Thailand.

_____. 2006b. **Malaria control programme in Thailand.** Available source: http//eng.moph.go.th/, December 17, 2007.

Moore, C.G. 1977. Insecticide avoidance by ovipositing *Aedes aegypti*. **Mosq. News**. 37: 291-293.

Muenworn, V., P. Akaratanakul, M.J. Bangs, A. Parbaripai and T. Chareonviriyaphap. 2006. Insecticide-induced behavioral responses in two populations of *Anopheles maculatus* and *Anopheles sawadwongporni*, malaria vectors in Thailand. **J. Am. Mosq. Control Assoc.** 22(4): 689-698.

Muirhead-Thomson, R.C. 1960. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. **Wld. Hlth Organ.** 22: 721-734.

Nutsathapana, S. 2005. **Malaria situation in Thailand.** Malaria cluster, Bureau of Vector-borne disease, Department of Disease Control, Ministry of Public Health, Thailand.

Ohio State University. 2007. **Catnip (*Nepeta cataria*).** Available Source: http:// www.oardc.ohio-state.edu. November 20, 2007.

Ombrello, T. 2007. **Catnip foliage (*Nepeta cataria* L.).** Available Source: http://faculty.ucc.edu/ biology -n mbrello/POW/ catnip. html. November 20, 2007.

Pant, C.P., H. L. Mathis, M. J. Nelson and B. Phanthumachinda. 1974. A large-scale field trial of ultra-low-volume fenitrothion applied by a portable mist blower for the control of *Aedes aegypti.* **Bull.Wld Hlth Org.** 51: 409-415.

Perich, M.J., C. Sherman, R. Burge, E. Gill, M. Quintana and R.W. Wirtz. 2001. Evaluation of the efficacy of lambda-cyhalothrin applied as ultra-low-volume and thermal fog for emergency control of *Aedes aegypti* in Honduras. **J. Am. Mosq. Control Assoc.** 17: 221-224.

Peterson, C.J. 2000. Mosquito repellents. **Winter** . 11-13.

_____ and J. Coats. 2001. Insect repellents- past, present and future. **Pestic. Outlook.** 12: 154-158.

_____, L.T. Nemetz, L.M. Jone and J.R. Coats. 2002. Behavioral activity of catnip (Lamiaceae) essential oil components to the German cockroach (Blattodea: Blattellidae. **J. Econ. Entomol.** 95(2):377-380.

Polawat, A. and L.C. Harrington. 2005. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. **J. Med. Entomol.** 42: 844-849.

Pothikasikorn, J., H. Overgard, C. Ketavan, S. Visetson, M. J. Bangs and T. Chareonviriyaphap. 2007. Behavioral responses of malaria vectors, *Anopheles minimus* complex, to three classes of agrochemical in Thailand. **J. Med. Entomol.** 44(6): 1032-1039.

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

Prasittisuk, C. 1985. Present status of malaria in Thailand. **Southeast Asain J. Trop. Med. Public Health.** 16: 141-145.

Qui, H., H. W. Jun and J. W. McCall. 1998. Phamacokinetics, formulation, and safty of insect repellent N,N-diethyl-3-methylbenzamide (deet): A review. **J. Mosq. Control Assoc.** 14: 12-27.

Raffaele, G., A. Coluzzi and J. de Zulueta. 1958. Observations on the effect of numbers and age on the susceptibility of mosquitoes to DDT. **Bull.Wld Hlth Org.** 18: 464-468.

Rattanarithikul, R., B.A. Harrison, R.E. Harbach, P. Panthusiri and R.E. Coleman. 2006. Illustrated keys to the mosquitoes of Thailand IV. Anopheles. **Southeast Asian J. Trop. Med. Public Health.** 37 (suppl. 7): 1-128.

Reiter, P. and D.J. Gubler. 1997. Surveillance and control of urban dengue vectors, pp. 425-462. *In* D.J. Gubler and G. Kuno (eds.], **Dengue and dengue hemorrhagic fever**. CABI, Wallington, United Kingdom.

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

_____ and W.D. Alecrim. 1991. Behavioral response of *Anopheles darlingi* to DDT sprayed house walls in Amazonia. **Pan Am. Health Organ. Bull.** 25: 210-217.

Roberts, D. R., W.D. Alecrim, A.M. Tavares and K.M. McNeil. 1984**.** Influence of physiological condition on the behavioral response of *Anopheles darlingi* to DDT**. Mosq News.** 44: 357-361.

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

Roll Back Malaria (RBM). 2006. **World Malaria Report.** Available source: http:// www. rollbackmalaria.org/ December 17, 2007.

Rongnoparut, P., D.M. Ugsang, V. Baimai, K. Honda and R. Sithiprasasna. 2005. Use of Remote Sensing-based Geographic Information System in the characterizing spatial patterns for *Anopheles minimus* A and C breeding habitats in Western Thailand. 2005. **Southeast Asian J Trop Med Pub Health.** 36:1145-1152.

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

Sathantriphop, S., C. Ketavan, A. Prabaripai, S. Visetson, M.J. Bangs, P. Akratanakul and T. Chareonviriyaphap. 2006. Susceptibility and avoidance behavior by *Culex quinquefasciatus* Say to three classes of residual insecticides. **J. Vector Ecol.** 31: 266-274.

Schultz, G., E. Simbro, J. Belden, J. Zhu and J. Coats. 2004. Catnip, *Nepeta cataria* (Lamiales: Lamiaceae) – A close look: seasonal occurrence of nepetalactone isomers and comparative repellency of the terpenoids to insects. **Environ Entomol.** 33: 1562-1569.

Sharpe, R.G., M.M. Hims, R.E. Harbach and R.K. Butlin. 1999. PCR-based methods for identification of species of the *Anopheles minimus* group: allele-specific amplification and single- strand conformation polymorphism. **Med. Vet. Entomol.** 13: 265-273.

Simon, J.E., A.F. Chadwick and J.E. Cracker. 1984. Herbs, an indexed bibliography, 1971-1980. The scientific literature on selected herbs and aromatic and medicinal plants of the Temperate zone. **Archon Books**, Hamden, CT.

Somboon, P., C. Walton, R.G. Sharpe, Y. Higa, N. Tuno, Y. Tsuda and M. Takagi. 2001. Evidence for a new sibling species of *Anopheles minimus* from the Ryukyu Achipelago, Japan. **J. Am. Mosq. Control Assoc.** 17(2): 98-113.

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

Sucharit, S. N. Komalamisra, S. Leemingsawat, C. Apiwathnasorn and S. Thongrungkiat. 1988. Population genetic studies on the *Anopheles minimus* complex in Thailand. **Southeast Asian J. Trop. Med. Public Health.** 19: 717-723.

Sulaiman, S., M.A. Karim, B. Omar, J. Jeffery and F. Mansor. 1993. The residual effects of the synthetic pyrethroids lambda-cyhalothrin and cyfluthrin against *Aedes aegypti* (L.) in wooden huts in Malaysia. **Mosq. Borne Dis. Bull.** 10: 128-131.

Sungvornyothin, S., C. Garros, T. Chareonviriyaphap and S. Manguin. 2006. How reliable is the humeral pale spot for identification of cryptic species of the Minimus complex ?. **J. Am. Mosq. Control Assoc.** 22(2): 185-191.

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

_____, V. Muenvorn, C.Garros, S. Manguin, A. Prabaripai, M.J. Bangs and T. Chareonviriyaphap. 2006b. Trophic behavior and biting activity of the two sibling species of the Anopheles minimus complex in western Thailand. **J. Vector Ecol.** 31(2): 252-261.

Suwonkerd, W. and K. Tantrarongroj. 1994. Efficacy of essential oil against mosquito biting. **Commun. Dis. J.** 20: 4-11.

_____, P. Mongkalangoon, A. Parbaripai, J. Grieco, N. Achee, D.R. Roberts and T. Chareonviriyaphap. 2006. The effect of host type on movement patterns of *Aedes aegypti* (Diptera: Culicidae) into and out of experimental huts in Thailand. **J. Vector Ecol.** 31: 311-318.

Tanasinchayakul, S., S. Polsomboon, A. Prabaripai and T. Chareonviriyaphap. 2006. An automated, field-compatible device for excito-repellency assays in mosquitoes. **J. Vector Ecol.** 31: 210-212.

Tawansin, A., S. D. Wratten, R. R. Scott, U. Thavara and Y.Techadamronsin. 2001. Repellency of volatile oils from plants against three mosquito vectors. **J Vector Ecol.** 26(1): 76-82.

Van Bortel, W., H.D. Trung, P. Roelants, R.E. Harbach, T. Backeljau and M. Coosemans. 2000. Molecular identification of *Anopheles minimus s.l.* beyond distinguishing the members of the species complex. **Insect Mol Biol.** 9: 335-340.

Webb, C.E. and R. C. Russell. 2007. Is the extract from plant catmint (*Nepeta cataria*) repellent to mosquitoes in Australia. **J. Am. Mosq. Control Assoc.** 23(3): 351-354.

Wilkinson, C.F. 1983. Role of mixed-function oxidases in insecticide resistance, pp. 175-205. *In* G.P. Georghiou and T. Saito [eds.], **Pest resistance to pesticides**. Plenum Press, New York.

World Health Organization. 1996. Operational manual on the application of insecticides for control of the mosquito vectors of malaria and other diseases. **WHO/CTD/VBC/96.1000. Wld. Hlth Organ.** Geneva, Switzerland. 1-198.

_____. 1998. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. **Report of WHO Informal Consultation, WHO/CDS/CPC/MAL/98.12. Wld. Hlth Organ.** Geneva, Switzerland. 1-43.

_____. 1999. Prevention and control of dengue and dengue haemorrhagic fever: comprehensive guidelines. **WHO Regional Publication, SEARO, No. 29.** New Delhi. 1-134.

_____. 2006. **Dengue/DHF.** Available Source: http://www.searo.who.int/ en/Section10/Section332_1104.htm, November 20, 2007.

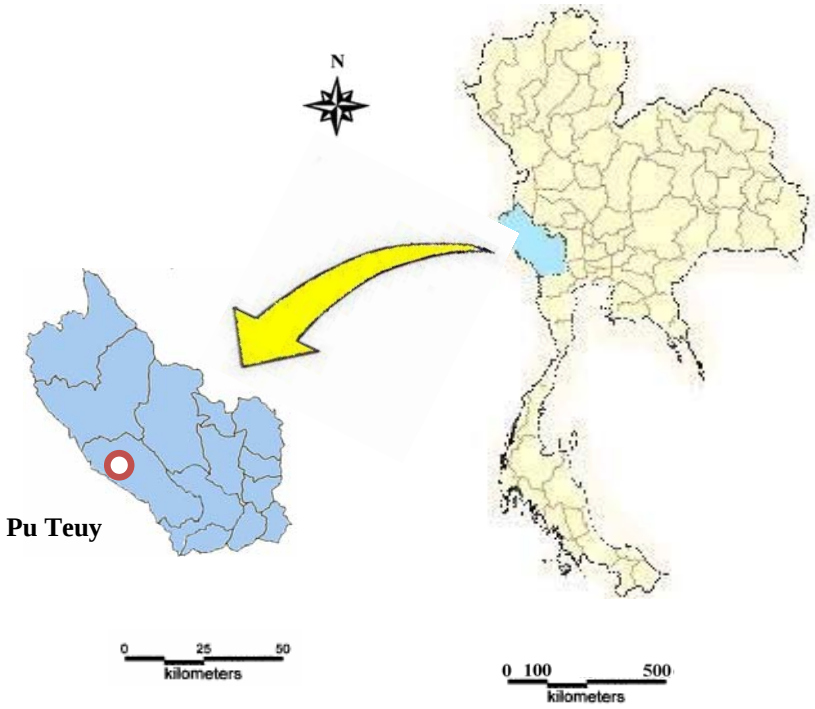
_____. 2007. **Health topic.** Available Source: http://www.who.int/topics/en/, November 20, 2007.

_____. 2007. **Malaria.** Available Source: http://www. searo.who.int/en/ Section10/Section21/Section1370.htm, November 20, 2007.

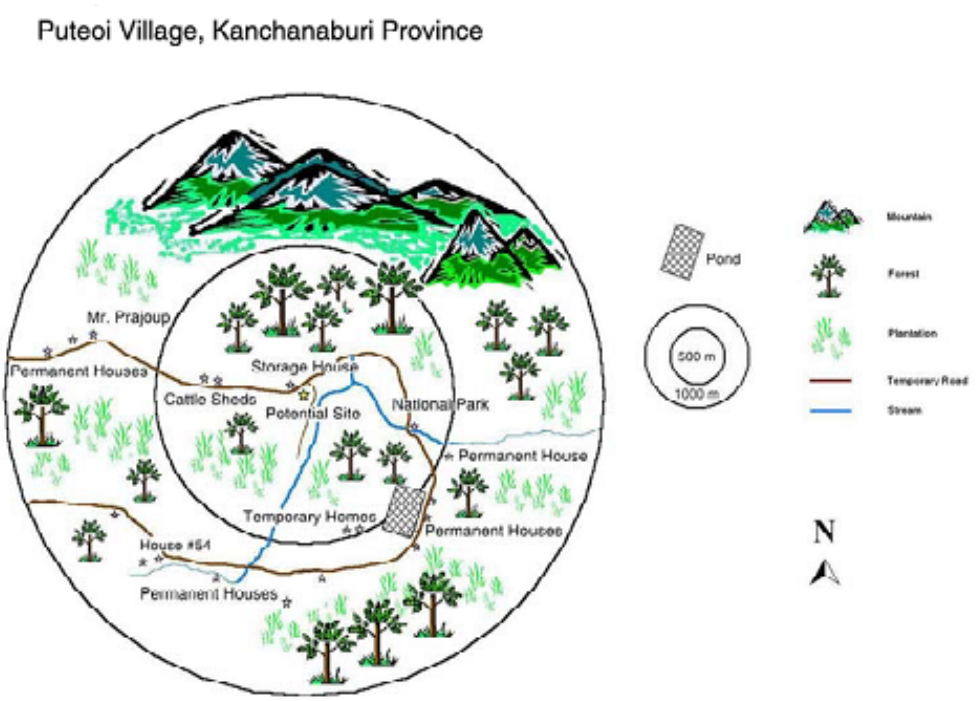
Xue, R.D. and D.R. Barnard. 1999. Effects of partial blood engorgement and pretest carbohydrate availability on the repellency of deet to *Aedes albopictus*. **J. Vector Ecol.** 24: 111-114.

Zhu, J., X. Zeng, Yanma, T. Liu. K. Qian, Y. Han, S. Xue, B. Tucker, G. Schultz, J. Coats, W. Rowley and A. Zhang. 2006. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. **J. Am. Mosq. Control Assoc.** 22(3): 515-522.

APPENDIX



Appendix Figure 1 Map of Kanchanaburi Province and study site



Appendix Figure 2 Map of Puteoi village

CURRICULUM VITAE

NAME : Miss Suppaluck Polsomboon

BIRTH DATE : December 4, 1984

BIRTH PLACE : Bangkok, Thailand

EDUCATION :

YEAR	INSTITUTE	DEGREE
2004	Kasetsart University	B.Sc. (First class Honor)

WORK PLACE : Student research assistance at the Department of Entomology

PUBLICATION :

Tanasinchayakul, S., S. Polsomboon, A. Prabaripai and T. Chareonviriyaphap. 2006. An automated, field-compatible device for excito-repellency assays in mosquitoes. **J. Vector Ecol.** 31: 210-212.

Polsomboon, S., P. Poolprasert, M.J. Bangs, W. Suwonkerd, J.P. Grieco, N.L. Achee, A. Prabaripai and T. Chareonviriyaphap. 2008. Effects of physiological conditioning on behavioral avoidance using a single age group of *Aedes aegypti* (L.) exposed to Deltamethrin and DDT. **J. Med. Ent.** 45(2): (in press).