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THESIS

EVALUATION ON PLANT-BASED INSECT REPELLENTS AGAINST MOSQUITO VECTORS IN THAILAND

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Sunaiyana Sathantriphop 2015: Evaluation on Plant-Based Insect Repellents Against Mosquito Vectors in Thailand. Doctor of Philosophy (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Professor Theeraphap Chareonviriyaphap, Ph.D. 120 pages.

Escape response and toxicity studies of essential oils obtained from four plant species, citronella (*Cymbopogon nardus*), hairy basil (*Ocimum americanum*), catnip (*Nepeta cataria*) and vetiver (*Vetiveria zizanoides*) were characterized on two different mosquito species using two assay systems, high throughput screening system (HITSS) and excito-repellency (ER) test system. The HITSS was used to determine the different concentrations of plant essential oils compared with synthetic repellents (DEET and picaridin) to *Aedes aegypti* and *Anopheles minimus*. Results indicated that the two mosquito species exhibited significantly different escape responses between contact treatment and control across all concentrations of test compounds, except the lowest concentration of picaridin against *Ae. aegypti*. Spatial repellent responses were found in both mosquito species when exposed to all compounds but percent escape responses depend on compounds and their concentrations. The study showed that the higher concentrations of test compounds had greater toxicity effects to mosquitoes but toxic effects at all test concentrations of vetiver were not found on *Ae. aegypti* and picaridin on *Ae. aegypti* and *An. minimus*. In contrast excito-repellency test system (contact irritant and non-contact repellent) was performed on the four essential oils compared with two standard repellents (DEET and picaridin) and two synthetic pyrethroids (deltamethrin and permethrin) against *Ae. aegypti*, *An. minimus*, *Aedes albopictus* and *Culex quinquefasciatus*. Results revealed that *Cx. quinquefasciatus* and *An. minimus* exhibited much stronger behavioral responses to all test compounds compared to *Ae. aegypti* and *Ae. albopictus*. Synthetic pyrethroids displayed stronger contact irritant response than non-contact repellents across all four mosquito species. Picaridin had the least effect on all test mosquito species.

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Sunaiyana Sathantriphop

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LIST OF ABBREVIATIONS

Ae	=	*Aedes*
An	=	*Anopheles*
Cx	=	*Culex*
°C	=	Degree (s) Celsius
Cm	=	Centimeter
cm^2	=	Square Centimeter
m^2	=	Square Meter
mg/m^2	=	milligram per square meter
min	=	Minute
hr	=	Hour
RH	=	Relative Humidity
L:D	=	Light:Dark
µl	=	Microliter
ml	=	Milliliter
P	=	Probability Value
SE	=	Standard Error
ET	=	Escape Time
KD	=	Knock Down
USDA	=	United States Department of Agriculture
WHO	=	World Health Organization
MOPH	=	Ministry of Public Health
NIH	=	National Institute of Health

EVALUATION ON PLANT-BASED INSECT REPELLENTS AGAINST MOSQUITO VECTORS IN THAILAND

INTRODUCTION

The serious illnesses from dengue fever and malaria carried by mosquitoes are common inThailand. Every year, over 40,000 cases of dengue are reported in Thailand. In 2013, there were 136,058 cases of dengue fever (DF)/haemorrhagic dengue fever (DHF) as reported by Department of Disease Control, Ministry of Public Health (MOPH), Thailand. Recently, the number of dengue cases tend to increase when the weather is warmer due to the El Nino phenomenon (Hales *et al*., 2002). It was reported that mosquitoes breed faster in warmer climates and the rate of dengue virus infection is probably higher (Currie, 2001). Dengue is usually transmitted to humans by the *Aedes aegypti*, a day biting mosquito. This species is one of the most common mosquitoes found in Thailand and it prefers to feed on human hosts (Harrington, *et al.*, 2001). It has a biting activity cycle with 2 peaks, in the early morning and in the early evening (Thanispong *et al*., 2008). For its closely related species like *Aedes albopictus*, this species can transmit the dengue virus and plays a significant role in chikungunya transmission (Ponlawat *et al*., 2005; Thavara *et al.*, 2009). *Aedes albopictus* is usually found in the rubber and palm oil plantations (Ponlawat and Harrington, 2005). While *Ae. aegypti* prefers to breed in water-filled receptacles and lives in habitats close to humans (Jayasekera *et al*., 1983). *Aedes albopictus* is somewhat less discriminating in its species choice for biting and feeding. Consequently, *Ae. albopictus* has fewer opportunities to be infected with dengue and to transmit the virus to humans. Thus, *Ae. aegypti* species is regarded as the most important vector of dengue and is extensively spread throughout the country.

Like dengue, malaria remains one of the most serious public health problem. This disease can be transmitted from one person to another by various Anopheles vector species, depending on the area and the climatic environment (Chareonviriyaphap *et al*., 2004; Jaichapor *et al*., 2005). There are four different types

of human malaria parasite: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae*. Currently, the fifth human malaria parasite called *Plasmodium knowlesi* was discovered the cause of malaria in humans which was previously known to cause malaria only in macaques. The first case of knowlesi malaria in Thailand was identified in 2000 in which the patient was infected with *P. knowlesi* while stayed in a forest in Prachuap Khiri Khan Province in southern Thailand (Jongwutiwes *et al*., 2004). However, only two human malaria parasite species are common in Thailand, *P. falciparum* and *P. vivax*. These two parasites have been found along the north-western border of Thailand (Thai-Myanmar and Thai-Cambodia borders) while *P. vivax* is presented on Thai-Malaysia border (Mungthin *et al.*, 2014). Commonly, malaria occurs in rural forested areas that border Malaysia, Cambodia, and Myanmar (Chareonviriyaphap *et al*., 2000). For the year 2013, there were 15,138 Thai malaria cases and 12,007 foreigner cases living in Thailand (Bureau of Vector Borne Diseases, Department of Disease Control, Ministry of Public Health, 2013).

There are various methods to reduce mosquito populations such as chemical control (insecticide), biological control and mechanical control. The chemical control is considered as the most effective way in reducing or eliminating mosquito populations. Insecticide spraying to kill adult mosquitoes and larvae has been used in Thailand for many years. However, malaria and dengue outbreaks still recur every year. In the present, Anopheles and Aedes mosquitoes have developed resistance to all major groups of insecticides (organophosphates, carbamates and synthetic pyrethroids) that used for dengue and malaria vector control *(*Chareonviriyaphap *et al*., 1999; Paeporn *et al*., 2006*;* Jirakanjanakit *et al*., 2007*).* Using repellents as personal protection against mosquitoes is the alternative way to combat the diseases and these repellents have been occurred for decades. DEET (N, N-diethyl-3-methyl benzamide) as an example, was developed by the U.S. army in 1946 and has been available in the market since 1957. Now, DEET is still considered to be the most effective and common ingredient in many insect repellent products but may cause skin irritation in some people. Picaridin, 2-(2-hydroxyethyl)-1-piperidine carboxylic acid 1-methylpropylester, also known as KBR3023 has been developed as an

alternative to DEET by Bayer AG in 1980. This ingredient was as effective as DEET to repel mosquitoes (Yap *et al.*, 1998) but it is safer and does not dissolve plastics like DEET does (Nentwig *et al.*, 2002). From previous study, Barnard *et al.* (2002) reported the repellent effect of four synthetic repellents, including DEET, picaridin, PMD and IR3535. Furthermore, Frances *et al.* (2004) revealed that picaridin had similar repellent action to DEET against *Anopheles* spp.

Plant-derived essential oils are considered to be non-toxic alternative insect repellents. They are safe and have no long-term or serious side effects when used properly; many of them have properties of mosquito repellents. Citronella is the most commonly ingredient used in plant-based mosquito repellents. Phasomkusolsil and Soonwera (2010) reported that citronella had repellent activity against *An. minimus*, *Cx. quinquefasciatus* and *Ae. aegypti* when applied on human skin. Moreover, Bernier *et al.* (2005) revealed that catnip was promising spatial repellent against *Ae. aegypti*, *An. albimanus* and *An. quadrimaculatus*. Various plants have already been proved to be effective against many mosquitoes (Tawatsin *et al*., 2001; Zhu *et al.*, 2006; Phasomkusolsil and Soonwera, 2010). Therefore, essential oils obtained from plants can potentially play an important role in the fight against mosquito bites.

The purpose of this study was to determine behavioral responses of mosquitoes to different four plant species under laboratory condition. Citronella (*Cymbopogon nardus* (L.) Rendle), hairy basil (*Ocimum americanum* L.), catnip (*Nepeta cataria* L.) and vetiver oil (*Vetiveria zizamoides* (L.) Nash) were selected for the test. These plants have been reported as effective repellents against different species of mosquitoes such as *Ae. aegypti*, *Ae. vigilax*, *Culex annulirostris*, *Cx. quinquefasciatus*, *Anopheles dirus* and *An. minimus* (Tawatsin *et al*., 2001; Coats *et al*., 2003; Webb and Russell, 2007; Nuchuchua *et al.*, 2009; Phasokumsolsil and Soonwera, 2010). These plants contain active ingredients such as citronellal, citronellol, geraniol, limonene, 3-carene, caryophyllene, vetiveric acid, E,Z-nepetalactone, Z,E-nepetalactone and β-caryophellene that can repel mosquitoes. However, most studies on these plants have been conducted to determine their

repellent efficacy by direct application on human skin. While there have been only few studies to investigate a specific mechanism of plant essential oils that act as repellents or irritants against mosquitoes (Polsomboon *et al*., 2008; Suwansirisilp *et al*., 2012; Boonyuan *et al.*, 2014). Therefore, a clear understanding of mechanism of action of the plant essential oils with the use of two behavioral test equipments, namely the excito-repellency test system and the high throughput screening system.

OBJECTIVES

The objectives of the study were

1. To investigate the three effective properties of the spatial repellency, contact irritancy, and toxicity of plant essential oils and synthetic repellents against *Aedes aegypti* and *Anopheles minimus* using the high throughput screening system (HITSS).

2. To identify the two types of behavioral responses (contact irritant and non-contact repellent) of mosquitoes to plant essential oils, synthetic repellents and systhetic pyrethroids using the excito-repellency (ER) test system.

LITERATURE REVIEW

1. Mosquito

Mosquitoes belong to the order Diptera and family Culicidae. Mosquitoes differ from other flies in that mosquito wings contain scales. Males differ from females by having feathery antennae or plumose with receptors to help males locate females but mouthparts are not suitable for piercing skin. Female mosquitoes have short and a few small hair antennae which are called pilose. Normally, both male and female mosquitoes feed on nectar, honeydew and fruit juices to get sugar as their main source of energy supply. Only females require the protein in blood in order to develop their eggs. During blood feeding, mosquitoes inject saliva and anticoagulants into the blood steam which can spread disease-causing viruses or other parasites to humans. Therefore, female mosquitoes are the important vectors of many diseases such as dengue fever, yellow fever, chikungunya, malaria and Japanese encephalitis (JE), depending on the species of mosquito.

The mosquito life cycle is called holometabolous, consisting of egg, larva, pupa, and adult. Each stage is easily recognized by its special appearance. The developmental period of the life cycle depends on temperature, humidity, food and species. Larvae and pupae are aquatic stages, but the adults are active flying insects. Normally, adult female mosquito can live longer than a month, while male mosquito lives an average of one week (Centers for Disease Control and Prevention [CDC], 2012).

2. The medically important mosquitoes inThailand

There are over 4,000 different species of mosquitoes throughout the world. A total of 436 mosquito species was reported in Thailand (Rattanarithikul *et al.*, 2005). At least four major mosquito-borne diseases have been found in Thailand, including malaria, dengue, Japanese encephalitis (JE), and lymphatic filariasis (Rattanarithikul and Panthusiri, 1994). These diseases remain serious public health problems which

are related to four genera of mosquito (Aedes, Culex, Anopheles and Mansonia). The genus Aedes, usually *Ae. aegypti* is the main vector of dengue, but sometimes associated with *Ae. albopictus*. *Culex tritaeniorhynchus* species of the genus Culex is the major vector for JE transmission in Thailand. Malaria is transmitted by different Anopheles species (Tananchai *et al*., 2012; Chareonviriyaphap *et al*., 2013). Lymphatic filariasis (LF) caused by filarial nematode parasites in Thailand, mainly *Brugia malayi* and *Wuchereria bancrofti.* Brugia malayi is found in the south, especially Narathiwat province (Triteeraprapab *et al*., 2001). *Mansonia Indiana, M. uniformis*, *M. bonneae* and *M. annulata* are the main vectors for *B. malayi* (Harinasuta *et al*., 1970). While *W. bancrofti* is on the southwest to northwest Thai-Myanmar border, which *Ae. niveus* is the main vector (Zielke *et al*., 1993).

Most importantly, dengue and malaria are the serious mosquito-borne diseases in the country with the reporting number of cases and deaths have been increased annually, especially dengue fever. Currently, there are no effective vaccines to prevent dengue and malaria. In contrast, JE vaccine is available that can help to prevent infection by the JE virus. Vaccines are the main reason for low JE disease rates. Also, there are only a few LF cases reported in the country for example in 2009, only 158 LF cases were reported by Department of Disease Control, Ministry of Public Health, Thailand. Thus JE and LF are not serious problems in Thailand because these do not cause epidemics to spread across a large area.

3. Dengue

Dengue, known as a break bone fever, is considered by World Health Organization (WHO) to be one of the most common mosquito-borne viral diseases. The disease is a major public health problem worldwide. It is transmitted to humans by the bite of the infected Aedes mosquitoes. Dengue fever is caused by one of four virus serotypes: DEN-1, DEN-2, DEN-3 and DEN-4 which belong to the genus Flavivirus, family Flaviviridae (Tuiskunen *et al*., 2011). Dengue infection was divided into dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Mild dengue fever causes high fever, rash, and muscle and joint

pain. These symptoms can appear up to seven days after being bitten by an infected Aedes mosquito, and usually disappear after a week. A severe form of dengue fever, also called dengue hemorrhagic fever, can cause severe bleeding and this symptom can progress to the form of dengue shock syndrome cause shock and death due to severe drop in blood pressure (Singhi *et al.*, 2007).

3.1 Dengue in Thailand

Today, dengue becomes the global disease and has spread rapidly over the past few decades. As many as 50-100 million people are infected yearly during an outbreak of dengue fever estimated by World Health Organization (WHO, 2014). Dengue haemorrhagic fever (DHF) was first reported in the Philippines in 1953 (Kalayanarooj, 2011) and continues to spread the disease throughout Southeast Asia. The first outbreak of DHF was occurred in Bangkok, Thailand in 1958 and later spreading the disease to other cities (Hammon *et al*., 1960).The incidence rate in 1958 was 10.6 cases/100,000 populations with the death rate of 10.9 per 100,000 population. From the first year of the initial outbreak, dengue had continued until 1987 with the largest dengue outbreak at 174,285 confirmed cases (325/100,000 population) and 1,007 deaths (Thammapalo *et al.*, 2005). Recurring dengue outbreaks were occurred in 1997 with an incidence rate of 167 per 100,000 and cases remained high with 129,954 reported in 1998 (211 per 100,000). After the outbreak in 1998, the trends in morbidity and mortality have increased every two or three years. In 2013, over 150,000 cases and 133 deaths were report and the latest data by the Department of Disease Control recorded the number of dengue cases from January to May 2014 at over 8,000 cases.

Department of Disease Control (2013) reported that 43 blood samples of dengue patients from 11 provinces, contained three viruses - DEN-1 (37.21%), DEN-2 (34.88%) and DEN-3 (27.91%) serotypes. In Bangkok, the data from 1973-1999 was found DEN-3 was the most frequent serotype in dengue fever and DEN-2 in secondary and the same in dengue hemorrhagic fever (Nisalak *et. al*., 2003).

Vaughn *et al.* (2000) reported that 165 blood samples taken from dengue patients from two hospitals in Thailand contained 46 cases of DEN-1, 47 cases of DEN-2, 47 cases of DEN-3 and 25 cases of DEN-4 (Buathong *et al.*, 2006). During 1996-2005, most dengue cases were in children under the age of 15 years and most affected group of population from dengue was young students. In addition, the data from the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand showed that trend of dengue cases among adults aged over 15 years had been increasing from 9.2% in 1996 to 47.7% in 2005. Dengue epidemic in Thailand usually peaks in the rainy season (May-October) (Chareonsook *et al*., 1999).

3.2 Vaccination

Up to date there is no dengue vaccine or any specific treatment for dengue and patients. Recently it was announced that the first successful live attenuated tetravalent dengue vaccine has been developed by the collaboration of Mahidol University, Chiang Mai University and National Science and Technology Development Agency (NSTDA). This dengue vaccine candidate has been designed to produce a protective effect against all four types of dengue virus. The vaccines have begun testing in humans for safety and effectiveness. The vaccines are expected to become available in the near future (Thisyakorn and Thisyakorn, 2014).

3.3 Dengue vectors

Aedes aegypti is considered as the main vector of dengue virus and prefers to feed on a human host (anthropophilic). This mosquito is active in the early morning and in the early evening. Each year, thousands of Thai people are affected by dengue fever (DF) and dengue hemorrhagic fever (DHF). In addition, *Ae. albopictus* or an Asian tiger mosquito, can transmit the dengue disease but it plays more prominent role of chikungunya (CHIKV) (Thavara *et al.* 2009). *Aedes albopictus* is usually found in natural containers and mainly in rubber and palm oil plantations (Ponlawat and Harrington, 2005) whereas *Ae. aegypti* prefers to breed in artificial and natural

containers in and around house area. As a result, *Ae. albopictus* has fewer opportunities to be infected with dengue and to transmit the virus to humans. For the prevention and control program of DF/DHF in Thailand, the local health authorities have been putting an emphasis on reduction of the Aedes population based on breeding site reduction, space spray and community participation.

3.4 Insecticides for dengue vector control

Several different methods such as chemical, biological and physical techniques have been used for dengue vector control. However, the number of dengue cases are still high with 30,000 cases each year. Insecticides are one of the most common methods in the control of vector-borne diseases, including dengue fever. The first important synthetic insecticide was organochlorine, dichlorodiphenyl trichloroethane (DDT) which was discovered by a Swiss chemist, Paul Muller (Metcalf, 1973). In Thailand, DDT was first applied in 1950 as an insecticide in agriculture and public health (Kumblad *et al.*, 2001). In 1983, Thailand stopped using DDT because its impact on fish and bird and it was also broken down very slowly in the environment. Therefore, other insecticide groups have been replaced DDT and other organochlorines for malaria and dengue control such as organophosphates, carbamates and pyrethroids. Today pyrethroids are the most widely used insecticides in mosquito control program because these insecticides are safe to mammals and low environmental impact. In dengue control, temephos (organophosphate) is mainly used to control *Ae. aegypti* larvae. In addition, *Bacillus thuringiensis* israelensis (Bti) has been used as a biocontrol agent to control *Ae. aegypti* larvae.

3.5 Insecticide resistance in dengue vectors

In Thailand, *Ae. aegypti* has developed resistance to several groups of insecticides, including organochlorine (DDT), organophosphate (fenitrothion, malathion, and temephos or Abate®), carbamate (propoxur) and pyrethroid insecticides (deltamethrin, permethrin, lambdacyhalothrin and etofenprox) (Chareonviriyaphap *et al.*, 1999*;* Somboon *et al.*, 2003; Paeporn *et al.*, 2006;

Thanispong *et al.*, 2008*).* Currently, pyrethroid insecticides (deltamethrin, permethrin and cypermethrin) are the most used insecticides as households, thermal fog and ultra-low volume (ULV) against adult dengue mosquitoes but they are increasingly becoming less effective due to resistance development in vector populations (Chareonviriyaphap *et al.*, 2000; Jirakanjanakit *et al.*, 2007). Resistance of *Ae. aegypti* to pyrethroids has been reported across different parts of Thailand. Paeporn *et al.* (2006), Thanispong *et al.* (2008) and Somboon *et al.*, (2003) revealed that *Ae. aegypti* was resistance to deltamethrin, permethrin and alphacypermethrin in the central area of Thailand (Bangkok, Nonthaburi and Ratchaburi provinces), and some areas of northern Thailand (Nan and Chiang Mai provinces). Paeporn *et al.* (2005) found that *Ae. aegypti* populations from Ranong, Phang Nga, Phuket and Krabi provinces were highly resistant to deltamethrin and permethrin. Furthermore, there was a report of resistance in *Ae. albopictus* larvae to permethrin and temephos (Ponlawat *et al.*, 2005).

4. Malaria

4.1 Malaria in Thailand

Malaria is one of major public health problems worldwide including Thailand. Malaria is caused by the protozoan parasite Plasmodium. The parasite can be transmitted to humans through the bites of infected Anopheles mosquitoes. Human malaria is caused by four different human malaria parasite species of *Plasmodium falciparum*, *P.vivax*, *P.malariae*, and *P. ovale* (Sabbatani *et al*., 2010). In addition, *P. knowlesi* is confirmed to be the fifth species causing malaria in humans (Jongwutiwes *et al.*, 2004). *Plasmodium vivax* and *P. falciparum* are the most common malaria parasites in Thailand while the other three parasites cause fewer cases (Chaijaroenkul *et al.*, 2000). From a report of the Bureau of Vector Borne Diseases, Department of Disease Control, Thailand 15,138 Thai cases and 12,007 nonthai cases were documented.

4.2 Malaria vector control

There are nine incriminated malaria vectors in Thailand – *An. dirus, An. baimaii, An. minimus, An. pseudowillmori, An. maculatus, An. aconitus, An. sawadwongporni, An. epiroticus* and *An. campestris* (Cheong *et al*., 1968; Baimai *et al*., 1988; Green *et al*., 1991; Coleman *et al*., 2002; Rattanarithikul *et al*., 2006; Chareonviriyaphap *et al*., 2013; Ritthison *et al*., 2014). Among these Anopheline mosquitoes, *An. dirus* and *An. minimus* are the most abundant species in the forested and hilly areas along the borders of Thai-Myanmar, Thai-Cambodia and Thai-Malaysia borders (Chareonviriyaphap *et al.*, 2000; Muhamad *et al.*, 2013; Suwonkerd *et al.*, 2013). These species are mainly exophagic (outdoor biting) (Tainchum *et al*., 2014). They feed preferentially and frequently on human blood. Both species are nocturnal (active at night). The vectorial capacity of *An. dirus* was higher than *An. minimus* (Gingrich *et al*., 1990).

4.3 Insecticide resistance in malaria vectors

In Thailand, insecticides have been used for indoor residual spray (IRS) combined with long-lasting insecticidal nets (LLINs) to control malaria. Four groups of insecticide organochlorines, organophosphates, carbamates, and pyrethroids are recommended by WHO. DDT and several other organochlorine insecticides had been widely used in the past but they were banned in many countries because of their long persistence in the environment (Tsuda, 2012). However, DDT is still being used in malaria control programs in some developing countries in Asia and the South Pacific (Kannan *et al*., 1997). DDT has been banned in Thailand since 1994 (Kumblad *et al.*, 2001). Later, insecticides in organophosphates, carbamates, and pyrethroids have been replaced, respectively. In recent years, pyrethroid group is the only one currently used for IRS and LLINs in Thailand due to its rapid biodegradation and low mammalian toxicity (Khan, 1983).

Unfortunately, insecticide resistances among Anopheles mosquitoes were detected in several countries. Nwane *et al.* (2013) reported that DDT and pyrethroid

resistance in *Anopheles gambiae* in Cameroon, Central Africa. Pyrethroid resistance in Anopheles mosquitoes was also obtained by Kawada *et al.* (2011). These reports also indicated the cross-resistance between DDT and pyrethroid by reducing the sensitivity of the mosquito nervous system. In addition, other *Anopheles* species were resistant to several organophosphate and carbamate insecticides (Hemingway and Davidson, 1983; Aïzoun *et al*., 2013*).* Therefore, insecticide resistance in malaria vectors is the main obstacles against effective malaria control program. In Thailand, DDT resistance was first reported in *An. minimus* and *An. annularis* from the northern part of the country (Prapanthadara *et al*., 2000). The other three insecticide groups have not been documented in Thailand (Somboon *et al*., 2003; Overgaard *et al*., 2005; Chareonviriyaphap *et al*., 2013).

4.4 Antimalarial drug resistance

Plasmodium falciparum and *P. vivax* are common malaria parasite in Thailand. *Plasmodium falciparum* parasite has been found resistant to many antimalarial drugs such as chloroquine, mefloquine and quinine special on the Thailand-Cambodia border (Young *et al*., 1963). The antimalarial drug resistance has become a major problem in malaria control programme. Artemisinin-based combination therapies (ACTs) are the most effective drugs for treatment of *P. falciparum* recommended by World Health Organization (WHO) as the first-line treatment for *P. falciparum* malaria (Wongsrichanalai and Meshnick, 2008). *Plasmodium vivax* is mostly found at the Thailand-Myanmar border where Fansidar (pyrimethamine and sulfadoxine), primaquine, and artesunate have been used for treating *P. vivax* malaria. Wilairatana *et al.* (1999) revealed that the primaquine is effective in the treatment of *P. vivax* malaria.

5. Personal protection by mosquito repellents

Mosquito repellents are substance applied on to skin or clothes to repel mosquitoes and make humans unattractive to a mosquito so that mosquitoes will avoid areas of the body or clothes that have been treated with the repellents. Mosquito

repellents help prevent and control the outbreak of insect-borne diseases including dengue fever and malaria.

6. Mosquitoes - host attractants

The principal attractant is carbon dioxide (CO_2) which has been found in the human respiration. Mosquitoes detect smell with the olfactory receptor on their antennae and their sense of smell is extremely sensitive to carbon dioxide levels in the air. Mosquitoes have chemoreceptor neurons that are sensitive to carbon dioxide, lactic acid and human body temperature (Bernier, 2006). Mosquitoes will fly upwind when they detect carbon dioxide that released by mosquito hosts. As they get closer, the odors of other compounds, especially lactic acid emitted from the skin surface, allow them to find and identify a host. Also, their vision enables them to see the host and thermosensors located on their antennae and mouthparts enable them to find warm spots on human's body where blood capillary is closest to the skin surface. They pierce the blood vessel and inject their saliva that contains an anticoagulant, a compound that inhibits blood clotting. They suck the blood from the host and feed until their abdomens are entirely full. The female will find a protected area for laying eggs.

7. Chemoreceptors in mosquitoes

Chemoreceptors are sensory receptors that respond to chemical substances in food, host, air, and liquid. Chemoreceptors are divided into two main types - olfactory receptors and gustatory receptors.

7.1 Olfactory receptors (distant chemoreceptors)

Olfactory receptors (ORs) or smell receptors which belong to the olfactory sensory neurons (OSNs). They are mainly located on the antennae. Mosquitoes use these receptors to detect host odor, carbon dioxide, nectar as well as volatile chemicals at a distance (Peterson and Brown, 1952).

7.2 Gustatory receptors (contact chemoreceptors)

Gustatory receptors (GRs) or taste receptors are expressed in olfactory receptor neurons (ORNs) that are located on the internal mouthparts of the proboscis and on tarsal segments of legs. Mosquitoes use these receptors to taste chemicals and make important choices about foods, mates, and egg deposition sites. Sanford *et al.* (2013) revealed that *Ae. aegypti* responded to DEET and other insect repellents using gustatory receptor neurons that characterized for contact chemoreceptive sensilla on the labella.

8. Mode of action of repellent compounds

Most repellent substances function by interfering with the mosquito's ability to detect attractant chemicals that humans produce or they may prevent biting mosquitoes from landing. Bohbot *et al*. (2011) reported that these chemical receptors are activated by lactic acid, which naturally evaporates from the skin of humans. The mosquitoes have the innate ability to follow the lactic acid emissions to their source. When a repellent such as DEET is applied directly to the skin and it evaporates into the air. The repellent inhibits the binding of the lactic acid to the mosquito's chemical receptors (Dickens and Bohbot, 2013). This can protect human from the mosquito bites since the active ingredient must evaporate from the treated area to work. In general, mosquito repellents can be broadly divided into two types which are actions to repel insects by acting on the olfactory and gustatory senses. For the olfactory or vapor repellents, mosquitoes can detect vapor or spatial repellent such as DEET through the sense of smell, also called olfactory (smell) receptor while gustatory or irritant contact, mosquitoes must touch the treated surface before being repelled such as indalone (Garson *et al*., 1968; Kain *et al.*, 2013).

9. Mosquito repellents

There are two main types of mosquito repellents.

9.1 Synthetic repellents (chemical compounds)

DEET has been used for more than 50 years by worldwide people to repel mosquitoes, ticks, fleas, biting flies and chiggers (Fradin and Day, 2002). DEET has been proven effective in repelling a wide variety of mosquitoes such as Anopheles, Aedes and Culex mosquitoes (Frances *et al.*, 2009; Stanczyk *et al.*, 2010; Kramer *et al*., 2010). Although, DEET is very effective as an insect repellent, some people have irritation or an allergic reaction to DEET (Katz *et al*., 2008) especially small children. Picaridin (1-piperidinecarboxylic acid, 2-(2-hydroxy)ethyl)-,1-methylpropylester) is also known as icaridin (sold under the trade name Saltidin and formerly known as Bayrepel or KBR3023). It has been used worldwide since 1998. It is recommended by the World Health Organization as protection against malaria carrying mosquitoes (Tracy *et al*., 2008). Picaridin is an effective alternative to DEET that provides long lasting protection. It also has less smell, less greasy, and less toxic than DEET. It forms a barrier on the skin, blocking the mosquito's ability to locate host as DEET. DEET and picaridin are approved to use by the United States Environmental Protection Agency (EPA). However, insect repellents containing DEET or picaridin should not be used on children under 3 years (Iannelli, 2014). Synthetic permethrin is contact insecticide and repellent that belongs to the synthetic pyrethroid. This group is recommended for use only on clothing, bed nets, shoes, and tent, but should not be used on the skin (Fradin, 1998). The active ingredient permethrin not only repel insects but it can kill ticks, mosquitoes, chiggers and mites. This compound can cause eye and skin irritation, and tend to affect the immune system that results from exposure to the chemical (Alonso, 1991).

9.2 Natural repellents (plant-derived compounds)

Natural mosquito repellents are one of the best alternatives to synthetic repellents. They are eco-friendly, biodegradable and free from chemicals that can be harmful to the health (Fallatah *et al.*, 2010; Sutthanont *et al.*, 2010). There are thousands of plants which have been tested for their efficacy to repel mosquitoes with the most available ones such as citronella, lemon eucalyptus, eucalyptus, cinnamon, basil and geranium etc. (Barnard, 1999; Tawatsin *et al.*, 2001; Trongtokit *et al.*, 2005). IR3535 (ethyl butylacetyl aminopropionate) is another natural insect repellent and is classified in the same group as a biopesticide. This compound has been used in repellents in Europe and Asia. IR3535 formulas provided a good protection against mosquitoes and blacklegged ticks (Carroll, 2008). The three insect repellents (citronella, lemon eucalyptus/PMD and IR3535) are approved by the EPA for direct application to human skin.

10. Essential oil/volatile oil

Essential oil or volatile oil are organic compounds extracted from specific parts of plants such as roots, stems, leaves, seeds and flowers. These oils have individual chemical components and particular scent or fragrance. The factors affecting oil yields and essential oil quality are season collection, plant part (flower, seed, peels, leaves, stems, grasses, roots, bark or wood), plant age and storage (Singh and Sharma, 1976; Mejdoub and Katsiotis, 1998; Sefidkon *et al.*, 2009). They are easily evaporated or volatilized at or above room temperature compared with synthetic repellents. Therefore, essential oils should be developed and formulated with appropriate substances that can increase protection time from mosquitoes bite and improve mosquito repellent efficacy.

11. Chemical composition of plant essential oils

Each essential oil has different chemical components and odor. Most oils contain many components such as alcohols, aldehydes, ethers, esters, hydrocarbons

(terpenes, sesquiterpenes, etc.), ketones, lactones, phenols, and phenol ethers (Inouye *et al.*, 2006; Nerio *et al.*, 2010). Mosquito-repelling abilities of plants are due partly to their essential oil constituents. Each plant has different proportions of constituents that make its distinct and characteristic odor. For example, the main chemical compositions of eucalyptus oil are 1,8-cineole, citronellal, α-pinene, p-cymene, limonene, and α-terpineol (Eunae and Park, 2012). Terpines, alcohols and aldehydes arethe major components in citronella oil and the chemical compositions of geraniol Coeur are geraniol, nerol and citronellol (Novak and Gerberg, 2005).

The chemical compositions of essential oils were analyzed by gas chromatography coupled with mass spectrometry (GC/MS). Nuchuchua *et al.* (2009) reported that citronella essential oil (*Cymbopogon nardus*) contains limonene and citronellal as major components whereas 3-carene and caryophylleneare the main component in the oil of hairy basil (*Ocimum americanum*). A main constituent of vetiver oil (*Vetiveria zizanoides*) is vetiveric acid. The catnip consists of three main components, namely E, Z-nepetalactone, Z,E-nepetalactone and β-caryophellene (Chauhan and Zhang, 2004; Polsomboon *et al.*, 2008).

12. Chemical composition and mosquito repellent activities of test essential oils

Insect repellent properties of several essential oils are mainly composed of monoterpenes, sesquiterpenes, and oxygenated terpenes. Monoterpenes such as α-pinene, limonene, terpinolene, citronellol, citronellal, camphor and thymol are common constituents and found in a large number of essential oils. The major sesquiterpenes is β-caryophyllene that is widely distributed in essential oils of various plants. Trongtokit *et al.* (2005) and Jaenson *et al.* (2006) reported that β-caryophyllene has a strong repellent against *Ae. aegypti*. The oxygenated compounds such as phenylethylalcohol, β-citrunellol, citronamyl alcohol, geraniol, and *α*-pinene were isolated from essential oil of carnation flowers and showed strong repellent activities against ticks and *Ae. aegypti* mosquito (Tunon *et al*., 2006).

Moreover, Odalo *et al.* (2005) found that phytol (a linear diterpene alcohol) had high repellent activity against *An. gambiae*.

The four plant essential oils that were selected for the current study, including citronella, hairy basil, vetiver and catnip oils.

Citronella oil extracted from *Cymbopogon nardus* (L.) Rendle, belongs to the family Graminae (Poaceae). This essential oil is extracted from the grass through steam distillation method. Oil of citronella has been used as a flavoring for foods and beverages in very low concentration. Furthermore, this oil is a common ingredient in plant-based mosquito repellents (Hermes and James, 1961) and has been registered with the U.S. Environmental Protection Agency (EPA). Shasany (2000) discovered that mosquito repellent property of citronella oil was due to the presence of four main components, citronelal, eugenol, geraniol and limonene. Trongtokit *et al*. (2005) studied the repellent efficiency of 38 essential oils including citronella and found that citronella was effective against three species of mosquitoes. Moreover, Olivo *et al*. (2008) proved that citronella oil was effective to control the cattle ticks, the most important active principles being citronelal and geraniol.

Hairy basil oil (*Ocimum americanum* L.) belongs to the family Lamiaceae. This essential oil is extracted by steam distillation from leaves. Fresh or dried hairy basil is commonly used in food and traditional Thai medicine. Furthermore, essential oil of hairy basil was found to repel mosquitoes and to protect stored rice from damage by maize weevil (Tawatsin *et al*., 2001; Kerdchoechuen *et al*., 2010). Hairy basil oil was also reported to repel *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* under cage conditions up to 8 hours (Tawatsin *et al*., 2001). The oil from *Ocimum americanum* that was used in this study was found to contain 3-carene and caryophyllene as major components that were related to mosquito repellent property (Nuchuchua *et al.* 2009).

Catnip oil was extracted from *Nepeta cataria* L. that is an important medicinal plant belonging to the mint family, Lamiaceae. Fresh or dried leaves or young shoots

are sometimes used for flavoring sauces, soups, and cooked foods (Adiguzel *et al*., 2009). The repellent property of catnip oil to mosquitoes and other pest insects was reported by Peterson *et al*. (2002) and Schultz *et al.* (2006). Catnip contains two major components, E,Z- and Z,E-nepetalactone. These components showed highly effective repellent against german cockroach and house fly. Bernier *et al.* (2005) and Zhu *et al*. (2006) revealed that catnip was good spatial repellency against mosquitoes. In addition, catnip oil containing nepetalactons had antibacterial activity (Adiguzel *et al*., 2009).

Vetiver oil extracted from *Vetiveria zizanoides* (L.) Nash, belongs to the family Poaceae. The plant is used in traditional medicine and as botanical pesticide. The vetiver oil is obtained by steam distillation of roots. Vetiver contains small amounts of carboxylic acids. Vetiveric acid was found in vetiver oil as the main component (Nuchuchua *et al.*, 2009; Wellspring, 2010). The vetiver oil was reported to possess insect repellent properties against weevils and mosquitoes (Jain *et al.*, 1982). Additionally, vetiver oil was used as a larvicide against *Cx. quinquefasciatus* (Murty and Jamil, 1987), and as an insecticide to kill red flour beetles (Sujatha, 2010). This oil and its constituents were shown to repel and kill termites (Zhu *et al*., 2001).

13. Possible factors influrencing the behavioral responses of mosquitoes

13.1 Natural food source

In general, male and female mosquitoes feed on nectar flower and plant juices for energy reserve (Gillett *et al*., 1962). Sugar feedings go on throughout the adult stages of the mosquitoes (Yuval, 1992). Foster and Hancock (1994) found that floral odours play an important role in the attraction of mosquitoes to flowers. Sandholm and Price (1962) discovered that mosquitoes were attracted to light-colored flowers with unique odor. This odor appears to be primarily responsible for long distance attraction and visual cues play a role in short distance (Healy and Jepson, 1988). Bernays and Chapman (1994) found that insects use multiple sensory cues in flowering plant host selection, including visual, olfactory, gustatory, and tactile

stimulias well as humidity and light intensity. These cues stimulate receptors of insects, generating sensory input and finally behavioral responses. However, there are many plants that can be used to deter or repel insects because they have high vapour toxicity to mosquitoes. These plants do not serve as nectar sources for mosquitoes. For example, catnip flower is a good nectar source for honeybees (Pan and Herbert, 1996) but it demonstrates a good mosquito repellent property (Zhu *et al*., 2009). For basil plants (e.g., holy basil, sweet basil, hairy basil), their flowers attract pollinators (bees, butterflies, etc.), but repel flies and mosquitoes due to their strong smells.

13.2 Chemical resistance in mosquitoes

Mosquitoes have adapted to chemicals/insecticides by becoming physiological or behavioral resistances to them. Physiological resistance is referred to three factors, reduced penetration or absorption, enhanced detoxification, and target site insensitivity (Ames, 2011) (Appendix Figure 1). Behavioral resistance can be divided into stimulus dependent and stimulus independent (Georghiou, 1972; Chareonviriyaphap *et al*., 2013). Stimulus-dependent response (required sensory stimulation) sometimes known as behavioral avoidance includes both irritability, an insect is stimulated to leave the immediate toxic environment on contact with a treated surface, and repellency, an insect is stimulated to leave the immediate toxic environment before contact with a treated surface. Stimulus independent response (does not require sensory stimulation) refers to behavioral patterns that prevent exposure to the toxic area such as exophily (outdoor resting) or zoophily (feeding on animal blood) (Liu *et al*., 2006; Chareonviriyaphap *et al*., 2013).

13.3 Odorant binding proteins (OBPs) in mosquitoes

Mosquitoes use sense of smell for sugar feeding and oviposition as well as responding to human odors to find a blood meal (Foster and Hancock, 1994; Dekker *et al*., 2001). Volatile odorants are detected and discriminated by olfactory receptor neurons (ORNs) presented on sensory hairs, sensilla, which are found on the antennae and maxillary palps of mosquitoes. According to the model of olfaction,

odorants enter the sensillar lymph from the air through cuticular pores and are captured by odorant binding proteins (OBPs) that transport them through the sensillar lymph to odorant receptors (ORs) expressed in the cell membranes of olfactory neurons (Appendix Figure 2). After stimulation with cognate ligands, ORs transduce the odor signals to downstream effector molecules (Hallem *et al*., 2006). The OBPs and pheromone binding proteins (PBPs) are the first proteins to interact with odor and help determine odor responses (Pelosi *et al*., 2006). Moreover, the specificity of odor recognition is contributed by OBPs and ORs. Thus, OBPs are potentially key components of receptor cell specificity as defined by levels of sensitivity to specific odorants (Vogt *et al*., 1991; Biessmann *et al*., 2010).

13.4 Physiological status

The response of insects to pheromones and other semiochemicals may be affected by many factors, including the physiological status (e.g., mating and nutritional status) and behavioral changes in insects (Bauerfeind and Fischer, 2005; Wertheim *et al*., 2005; Weeks *et al*., 2013). Insects obtain energy and nutrients from food, so diet can be considered the main factor that potentially affects all of the insect life stages (Taylor *et al*., 2005). Singh and Sisodia (2012) reported larval nutrition affects development and life history traits as well as responses to environmental stress in adult. In addition, the nutritional status and physiological condition of mosquitoes as a result of blood feeding can significant influence avoidance/escape response test results (Sungvornyothin *et al.*, 2001). Polsomboon *et al*. (2008) found that the escape rates of *Ae. aegypti* to deltamethrin and DDT varied according to the physiological conditionings.

13.5 Laboratory colony and field population

Some mosquito species (e.g., *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*) are easily reared in laboratory conditions and have been maintained under the artificial conditions for several years. This can lead to modifications in the sensory array to allow adaption to the sensorial requirements of

different habitats (Catalá, 1997). Sathantriphop *et al.* (2014) showed that DEET and citronella produced the strongest behavioral avoidance response in the field population of *Ae. aegypti* compared to the laboratory populations tested. This was in agreement with Chareonviriyaphap *et al.* (1997) who showed that a long-established, insecticide-susceptible laboratory colony of *Anopheles albimanus* demonstrated virtually no avoidance behavior to pyrethroids compared to significant escape responses seen in natural populations. Similar findings were reported by Kongmee *et al.* (2004) that showed a long-established laboratory population of *Ae. aegypti* had much weaker responses to direct, sublethal contact with synthetic pyrethroids compared to recent wild-caught populations. Clark *et al.* (2011) found *Ae. aegypti* showed a lower response to host odors after four to six generations of colonization compared to wild-type, field populations. Additionally, Stanczyk *et al.* (2010) proposed that long colonized populations appear to lose a degree of sensitivity in olfactory sensory (receptor) neurones on the insect's antennal flagella that diminish the sensillum functions to more effectively detect volatile chemicals over time. Therefore, results from experimentation using laboratory-reared mosquitoes should be viewed with a degree of caution as it may be likely the responses do not reflect the responses of field mosquitoes.

14. Escape responses of *Aedes aegypti* and *Anopheles minimus* evaluated by using the high-throughput screening system and the excito-repellency test system

14.1 *Aedes aegypti*

14.1.1 Response to insecticides

Mosquitoes have the capacity to develop behavioral, physiological, and biochemical resistance mechanisms in response to chemical. Avoidance behavior or behavioral resistance to insecticides is one interesting mechanisms of mosquitoes for study. Several papers described escape responses of various mosquito species when exposed to different insecticides and natural compounds by using high throughput screening system (HITSS) and excito-

repellency (ER) test system (Polsomboon *et al*., 2008; Thanispong *et al*., 2010; Boonyuan *et al*., 2014). The HITSS was developed by Grieco *et al.* (2005) which is used to determine the three actions of contact irritant, spatial repellent, and toxic actions of compounds. Grieco *et al.* (2005) used the HITSS to evaluate contact irritancy and spatial repellency activities of *Ae. aegypti* to DEET, Bayrepel and SS220 and found that DEET displayed low contact irritant response and showed no spatial repellent activity at any of the doses tested. SS220 was more toxic than DEET. In addition, Grieco *et al.* (2007) demonstrated that DDT was repellent, alphacypermethrin was irritant, and dieldrin was toxic to *Ae. aegypti*. Many other insecticides were tested by Kongmee *et al.* (2010) and Thanispong *et al.* (2010) and discovered that pyrethroids were significant irritant whereas DDT was repellent against *Ae. aegpyti*. Achee *et al.* (2009) used the HITSS to evaluate contact irritant, spatial repellent, and toxic actions of many different types of insecticides against a field strain of *Ae. aegypti* collected in Kanchanaburi province, Thailand. The result of Achee *et al.* showed that pyrethroids and DDT had spatial repellent and contact irritant actions against *Ae. aegypti*.

The excito-repellency test boxes were early described by Rachou *et al.*, 1963; Roberts *et al.*, 1984, 1997; Chareonviriyaphap *et al.*, 1997. These boxes have been applied for insecticide avoidance behavior study of female mosquitoes in the forms of contact irritant and non-contact repellent by Chareonviriyaphap *et al*., 1997. Later, the test boxes were modified by Chareonviriyaphap *et al.* (2002a) to improve the efficacy of the excito-repellency test boxes and reduce the size to easily use in the field. There are several examples of behavioral studies of field and laboratory populations of different types of mosquitoes to insecticides by using the excito-repellency test system. Chareonviriyaphap *et al.* (2001) tested avoidance response of *An. minimus* to DDT and pyrethroids. The study by Thanispong *et al.* (2009) found that *Ae. aegypti* from Kanchanaburi and Chiangmai provinces exhibited good contact irritant to alphacypermethrin but Kanchanaburi strain had less response to DDT compared with Chiang Mai and laboratory strains. Kongmee *et al.* (2004) reported that nine field populations of *Ae. aegypti* showed moderate to high response to physical contact with deltamethrin. Another study by Potikasikorn *et al.* (2005) in

which they studied on escape response between *An. minimus* species A and C to DDT, deltamethrin, and lambdacyhalothrin and found both species displayed high escape rates in contact chambers. In addition, behavioral avoidance to insecticides was tested on *Cx. quinquefasciatus* (Sathantriphop *et al*., 2006).

14.1.2 Response to plant essential oils

There are a few studies which evaluate the contact irritant and non-contact repellent actions of essential oil derived from plants against mosquitoes. Polsomboon *et al.* (2008) studied the efficacy of catnip oil (*Nepeta cataria* L.) on the behavioral response of field strain *Ae. aegypti* and *An. harrisoni* using an improved excito-repellency test system with automated device for the counting of escaping mosquitoes as described by Tanasinchayakul *et al.*, 2006. The result of Polsomboon *et al.* (2008) found that *An. harrisoni* showed higher escape response to catnip in both contact and non-contact chambers than *Ae. aegypti* at the same concentration of catnip. Noosidum *et al.* (2008) also using these chambers with three native plants of Thailand, *Melaleuca leucadendron, Litsea cubeba,* and *Litsea salicifolia*. Result shows that these plants displayed significant irritant and repellent actions against field strain of *Ae. aegypti*. Other two different studies (Suwansirisilp *et al.*, 2012; Boonyuan *et al.*, 2014) evaluated several Thai plants such as hairy basil, ginger, lemongrass, citronella, plai, orange peel, cinnamon and clove for their irritant and repellent effects on *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. Suwansirisilp *et al.* (2012) found that orange oil had poor response to both *Ae. aegypti* and *Cx. quinquefasciatus*. In contrast, *Cx. quinquefasciatus* exhibited more actively escape response to tested plants than *Ae. aegypti*. Boonyuan *et al.* (2014) discovered that ginger, lemongrass and citronella had effective repellent and irritant actions against *Ae. aegypti* at 5% from three test concentrations (2.5%, 5% and 10%).

14.2 *Anopheles minimus*

14.2.1 Response to insecticides

There are few studies using excito-repellency test chambers to assess the behavioral response of *An. minimus* to insecticides. Chareonviriyaphap *et al.* (2002b) found that *An. minimus* and *An. dirus* had good contact response to DDT and deltamethrin. Potikasikorn *et al.* (2005) revealed that *An. minimus* species A exhibited strong contact irritancy and noncontact repellency to DDT, deltamethrin and lamba-cyhalothrin whereas *An. minimus* species C played a minor role in noncontact repellency to those insecticides. In 2011, Tisgratog *et al.* reported that two field strains of *An. minimus* displayed faster escape response to 5% DEET than the operational field dose of bifenthrin whereas *An. harrisoni* (former *An. minimus* species C) showed a contrast-response. Malaithong *et al.* (2011) also tested behavioral responses of *An. minimus* and *An. harrisoni* to alphacypermethrin and found that both Anopheles species had great responses to contact chambers. Other papers published for assessing the escape response of Anopheles mosquitoes by using the HITSS. Only one publication reported escape response of Anopheles mosquitoes (Dusfour *et al*., 2009). The study by Dusfour *et al.*was found that *An. albimanus* was significant irritant response to alphacypermethrin, deltamethrin, permethrin, DDT, and propoxur.

14.2.2 Response to plant essential oils

Deletre *et al.*(2013) reported the use of the HITSS (Grieco *et al*., 2005) on contact irritant and spatial repellent behavioral responses of *An. gambiae* to twenty plant extracts in laboratory testing conditions. The data revealed that most plants had irritant and repellent effects on female *An. gambiae*. While the use of an automated excito-repellency test system to evaluate the escape response of *An. harrisoni* to plant essential oil (catnip) was only reported by Polsomboon *et al.* (2008). The study demonstrated that *An. harrisoni* showed a high escape response to 2.5% catnip oil from the contact chamber while 5% catnip oil provided a high escape response in the noncontact treated chamber.

MATERIALS AND METHODS

Part 1 Behavioral responses of *Aedes aegypti* and *Anopheles minimus* to plant essential oils and synthetic repellents using high throughput screening system

1.1 Mosquito populations

Two laboratory colonies of *Ae. aegypti* and *An. minimus* were used in the study. The colony of *Ae. aegypti* is a long-standing (~70 years) laboratory colony originating from the USDA (U.S. Department of Agriculture), Gainesville, FL, USA. *Anopheles minimus* colony has been maintained in the Department of Entomology, Kasetsart University for more than 15 years without exposure to any insecticides. The colony originated from the Malaria Division, Department of Communicable Disease Control (CDC), Ministry of Public Health, Nonthaburi, Thailand, in 1998. Both mosquito species were reared in the insectary of entomological laboratory at Kasetsart University, Bangkok, Thailand under rearing conditions of 25 + 5 °C and 80 + 10% RH with a photoperiod of LD 12:12 h (Figures 1A and 1B). *Aedes aegypti* larvae were fed with fish food pellets and *An. minimus* larvae were fed finely ground TetraMin® fish food flakes. Adult mosquitoes were kept in clean screen cages and 10% sugar solution was provided. Three to five day-old female mosquitoes were used in the susceptibility and behavioral tests. For behavioral tests, mosquitoes were provided no access to sugar solution for approximately 24 hours before conducting the assays.

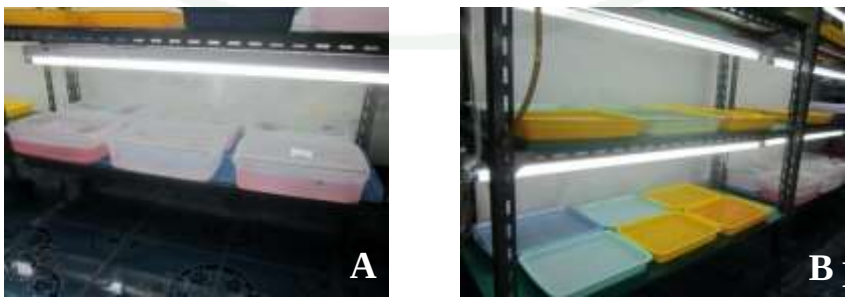


Figure 1 Mosquito larvae culture, (A) *Aedes aegypti*, (B) *Anopheles minimus*

1.2 Plant essential oils

Citronella oil, *Cymbopogon nardus* (Lot No: NO5410008-1/1910), hairy basil oil**,** *Ocimum americanum* (Lot No: 5308093/0408), catnip oil, *Nepeta cataria* (Lot No: 01022011) and vetiver, *Vetiveria zizanoides* (Lot No: 5506713/2706) were selected for the tests. Citronella, hairy basil and vetiver oils were supplied in 100% purity from Thai-China Flavours and Fragrances Industry (TCFF) Co. Ltd., Thailand. Catnip oil was received from the Chemicals Affecting Insect Behavior Lab, United States Department of Agriculture, Beltsville, MD.

1.3 Synthetic repellents

DEET (N, N-diethyl-3-methylbenzamide) and picaridin (2-(2-hydrozyethyl-1-piperidincarboxylic acid 1-methylpropyl ester) were used as standard repellents for comparison purposes. DEET (97% purity, Lot No: 0326/2009)) was obtained from the USDA, Beltsville, MD, USA. Picaridin (98.4% purity, (Lot No: CHCAEN0020) was obtained from Bayer Thai Company Limited.

1.4 Screening dosage using World Health Organization (WHO) susceptibility assay

The screening method was adapted from the standard WHO adult bioassay (WHO, 1998). Filter paper (Whatman No.1) sized 12 × 15 cm^2 was impregnated with series of essential oil solution and left the treated papers air dry for an hour (Figure 2A). Twenty-five female mosquitoes, aged 3-5 days were introduced into holding tube and then transferred into the exposure tube with treated filter paper (Figure 2B). After an hour in the exposure tube, mosquitoes were returned into the holding tube and provided a cotton pad soaked in 10% sugar solution (Figure 2C). Number of knockdown after 1-hour exposure and mortality at 24 hours in all four replicates were recorded.

The assay was conducted using the WHO susceptibility test (WHO 1998) and four concentrations (1, 3, 5, and 10%) were used. Twenty-five female mosquitoes, aged 3-5 days were introduced into holding tubes then transferred into the exposure tube with a treated filter paper. After 1 hour in the exposure tube, mosquitoes were returned to the holding tube and provided a 10% sugar solution. Knockdown after 1-hour exposure and mortality at 24 hours was recorded.

Concentrations were selected for HITSS tests based on knockdown response data which produced the high knockdown rates at the lowest concentration. At 3% concentration of most test compounds provided high knockdown rates of *Ae. aegypti* and *An. minimus* after one hour exposure, thus we selected test concentration ranges for the HITSS tests that were less than or equal to 3% for each test compound (0.5, 1, 2 and 3%).

Figure 2 WHO susceptibility assay, (A) paper impregnation, (B) one-hour exposure to the treated filter paper, (C) mortality recorded after 24 hours

1.5 A novel high-throughput screening system (HITSS)

HITSS device (Grieco *et al*., 2005) consists of three parts. The first part is spatial repellency assay, second part is contact irritancy assay and another part is toxicity assay (Figure 3).

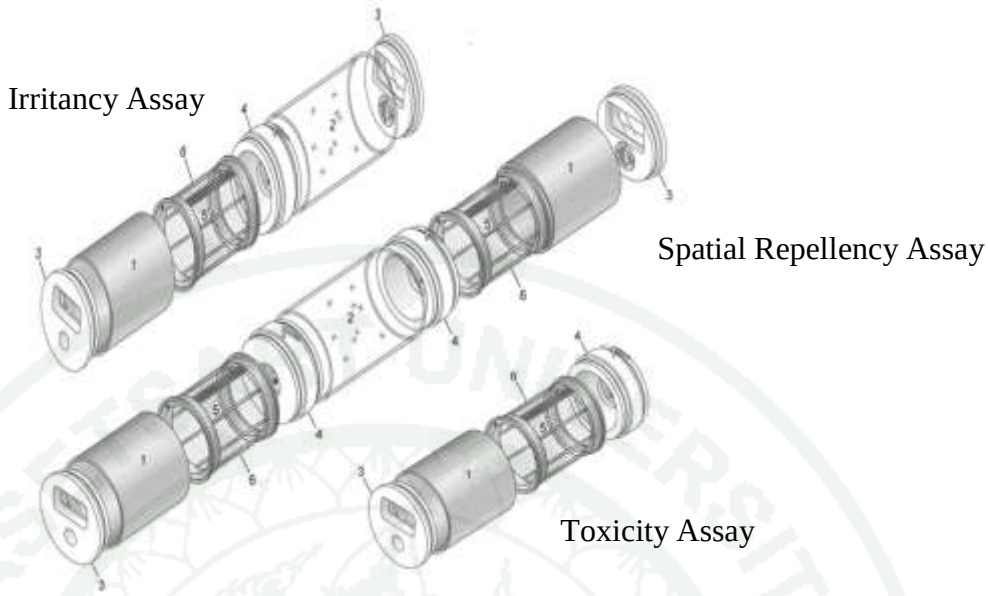


Figure 3 The high-throughput screening system (HITSS) showing the configuration for contact irritancy assay on the top, spatial repellency assay in the middle and toxicity assay on the bottom. The metal treatment cylinder can be used alone to evaluate chemical toxicity. Major components include: 1) treatment chamber 2) clear chamber 3) end cap 4) linking sections 5) inner metal spool and 6) treatment net (Thainispong *et al*., 2010)

1.6 Net impregnation

Essential oils and repellents were tested at concentration in the ranges 0.5-3.0% active ingredient in absolute ethanol. Nylon netting was cut into 11 × 25 cm^2 strips. Volume of 1.5 ml test solution was treated on a net strip by using a 1000 µl micropipette with a tip (Figure 4A). The treated nets were dried in air for 15 minutes (Figures 4B) before attaching it into the metal test cylinder (Figures 4C). Control nets were treated with ethanol only.

Figure 4 Net impregnation, (A) Using a micropipette evenly spread solution on the net, (B) The treated net allowed to air dry for 15 min, (C) After 15 min, a treated net was rolled inside the treatment drum

1.7 HITSS test assays

The HITSS was used to determine three actions of test compounds, contact irritant, spatial repellent and toxicity. The protocol and system was developed and optimized by Grieco *et al.* (2005, 2007).

1.7.1 Contact irritancy assay (CIA)

This assay consisted of two opposite placed cylinders- a clear cylinder and a metal cylinder with both ends capped. Both cylinders were connected to each other by a butterfly valve on linking section (Figure 5). Ten female mosquitoes were released into the metal cylinder which having the treated net (Figure 6A). The mosquitoes were allowed to rest for 30 second then a butterfly valve was turned in the open position for 10 minute (Figure 6B). The valve was turned back to the closed position. The number of mosquitoes escaping into the clear cylinder and those remaining in the metal cylinder as well as knocked down mosquitoes from each side was recorded. All tested mosquitoes were removed from the clear and metal cylinders (Figure 6C). Six replicates were conducted for treatments and controls.

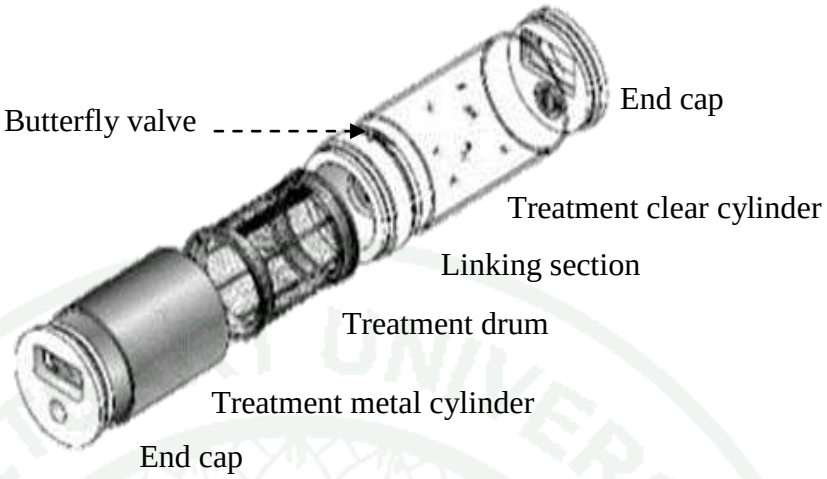


Figure 5 Test chamber for contact irritancy assay

Figure 6 Contact irritancy assay, (A) Transferring ten female mosquitoes into each test chamber, (B) Mosquitoes remained in the test chambers for 10 min, (C) After 10 min, data was recorded and mosquitoes were removed from the test chambers

1.7.2 Spatial repellency assay (SRA)

The SRA consisted of one clear and two metal cylinders. The each side of clear cylinder was connected to metal test chamber (control and treatment) (Figure 7). Twenty female mosquitoes were introduced into the clear cylinder (Figure 8A) and allowed to rest for 30 second then the butterfly valves on linking sections were open for 10 minute (Figure 8B). After the valves being close, number of mosquitoes in each cylinder was recorded including knocked down specimens.

All tested mosquitoes were removed from the clear and metal cylinders (Figure 8C). Nine replicates of each treatment and control were required to complete this assay.

A spatial activity index (SAI), based upon the oviposition activity index as described by Kramer and Mulla (1979), was applied to evaluate the escape response of mosquitoes in the spatial repellency assay.

$$SAI = \frac{(N_c - N_t)}{(N_c + N_t)}$$

N_c = the number of mosquitoes in the control cylinder

N_t = the number of mosquitoes in the treatment cylinder.

The SAI value ranges from -1 to 1, with zero represented no response. The SAI value of -1 is referred to that a higher number mosquitoes appeared in the treatment cylinder indicating an attractant response. SAI value of 1 is referred to that a higher number of mosquitoes remained in the control cylinder indicating a repellent response.

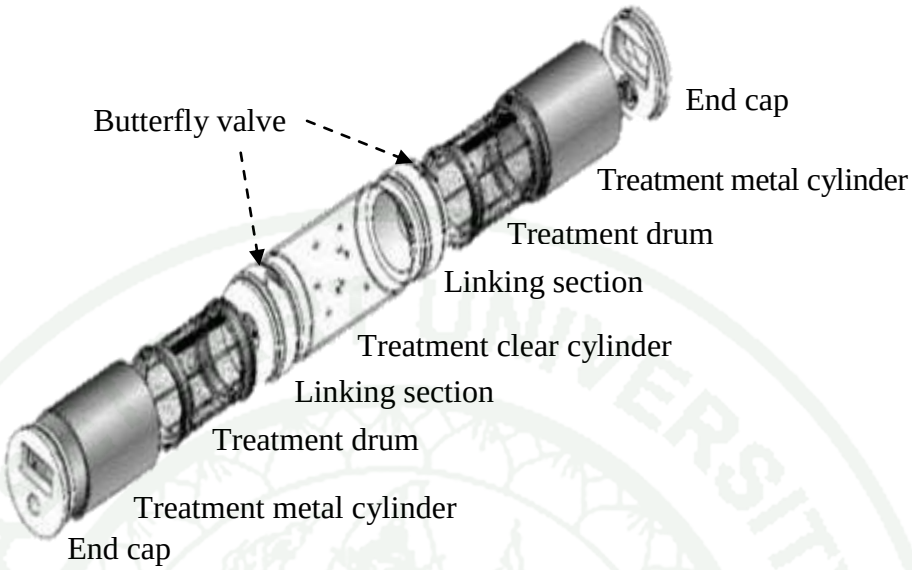


Figure 7 Test chamber for spatial repellency assay

Figure 8 Spatial repellency assay, (A) Transferring twenty female mosquitoes into the center clear chamber, (B) Mosquitoes remained in the test chambers for 10 min, (C) After 10 min, data was recorded and mosquitoes were removed from the test chambers

1.7.3 Toxicity assay (TOX)

The TOX assay was only used metal cylinder with end cap and butterfly valve for each side of cylinder (Figure 9). The treated net was rolled inside the cylinder. Twenty female mosquitoes were transferred into the test cylinder for

control and treatment for an hour (Figures 10A and 10B). After the exposure period, knocked down mosquitoes were noted and all mosquitoes were moved to clean plastic cups with a 10% sugar solution (Figure 10C). Mortality was monitored and recorded after the 24 hour holding period. Six replicates were required for treatment and control in this assay.

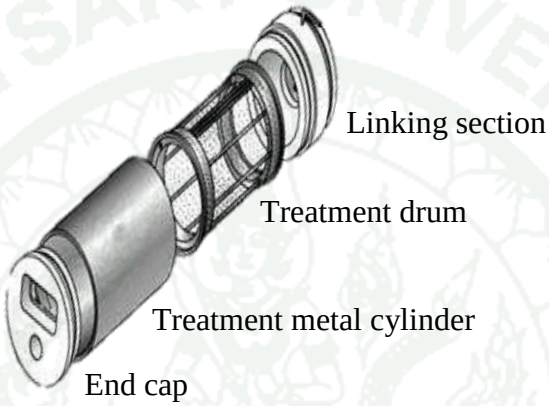


Figure 9 Test chamber for toxicity assay

Figure 10 Toxicity assay, (A) Transferring twenty female mosquitoes into each test chamber, (B) Mosquitoes remained in the test chambers for an hour, (C) Moving mosquitoes to clean plastic cups separately and recording mortality after 24 hour

1.8 Statistical analysis

Contact irritancy data were analyzed using the two-sample Wilcoxon test to evaluate the different number of escape mosquitoes between treated and control cylinders. Spatial repellency data were analyzed by the nonparametric Wilcoxon signed ranks test to calculate the SAI for each test treatment. Toxicity data, percentage of knockdown and mortality at 24 hour was corrected using Abbott's formula and transformed to arcsine square root for analysis of variance (ANOVA). The knock down and mortality of treatment at each concentration was compared and separated using Tukey's honestly significant difference test at P=0.05 (SAS Institute, 1999).

Part 2 Behavioral responses of *Aedes aegypti, Aedes albopictus, Anopheles minimus,* and *Culex quinquefasciatus* to plant essential oils, synthetic repellents and pyrethroid insecticides using excito repellency test system

2.1 Test mosquitoes

Laboratory test populations of *Ae. aegypti*, *Ae. albopictus*, *An. minimus* and *Cx. quinquefasciatus* were used in this study. The *Ae. aegypti*, insecticide susceptible test population (U.S. Department of Agriculture laboratory [USDA]) was obtained as eggs from the Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. The USDA colony of *Ae. aegypti* has been reared in the laboratory for many years. *Aedes albopictus* has been maintained in the entomological laboratory at Kasetsart University for 6 years. A colony of *Cx. quinquefasciatus* were obtained from the National Institute of Health (NIH), Ministry of Public Health, Nonthaburi, Thailand where the colony has been established since 1978. The *An. minimus* colony was originated from the Malaria Division, Department of Communicable Disease Control (CDC), Ministry of Public Health, Nonthaburi, Thailand in 1998. The colony has been maintained in the Department of Entomology, Kasetsart University for more than 15 years.

All four species of mosquitoes were reared in the insectary of the Department of Entomology, Faculty of Agriculture, Kasetsart University. All larvae and adults were held under laboratory conditions of 25 ± 5 °C and 80 ± 10% RH with a photoperiod of LD 12:12 h. Larvae were fed with fish food pellets whereas *An. minimus* larvae were fed finely ground fish food flakes (TetraMin®). Adult mosquitoes were reared in a screened cage and provided 10% sugar solution as food. Female mosquitoes aged 3-5 days old were starved for 24 h before testing.

2.2 Plant essential oils

Four plant essential oils were used in behavioral response study - citronella (*Cymbopogon nardus*), hairy basil (*Ocimum americanum*), catnip (*Nepeta cataria*), and vetiver (*Vetiveria zizanoides*). Two essential oils were provided by Thai-China Flavours and Fragrances Industry Co., Ltd. and catnip oil (*Nepeta cataria*) was received from the Chemicals Affecting Insect Behavior Lab, United States Department of Agriculture, Beltsville, MD.

2.3 Synthetic repellents

DEET and picaridin were selected as representative synthetic repellents. DEET was obtained from the USDA, Beltsville, MD (Lot No: 0326/2009). Picaridin was obtained from Bayer Thai Co., Ltd. (Lot No: CHCAEN0020).

2.4 Pyrethroid insecticides

Deltamethrin ([Cyano-[3-(phenoxy)phenyl]methyl] 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate) (98% purity) was obtained from BASF (Lot No: HDDLTK034). Permethrin (3-phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) (97.6% purity) was provided by Sherwood Chemicals Public Co., Ltd. (Lot No: ABJFPAP118).

2.5 Paper impregnation

Deltamethrin and permethrin were dissolved in a mixture of acetone and silicone (Dow Corning 556) to obtain doses of 25 mg/m^2 and 500 mg/m^2, the highest doses of insecticidal treated nets (ITNs), respectively (WHO 2006). DEET, picaridin and four essential oils were diluted in absolute ethanol to a concentration of 2.5% active ingredient, based on optimal concentrations identified from previous mosquito behavioral studies (Polsomboon *et al.*, 2008; Suwansirisilp *et al.*, 2012).

A 2.8 ml of the solution was dropped with a micropipette on Whatman No.1 filter paper, 14.7 × 17.5 cm^2. Impregnated papers were allowed to air dry one hour before testing (Licciardi *et al.*, 2006). Control papers were treated with only ethanol or solvent mixture for insecticide tests.

2.6 Excito repellency test system

The excito-repellency test assay used in this study has already been well described previously (Mongkalangoon *et al.*, 2009; Suwansirisilp *et al.*, 2012) as shown in Figure 11. Briefly, a set of four test chambers was used to evaluate both non-contact repellent and contact irritant behaviors (Figure 12). Each test chamber was connected with a receiving box for collecting mosquitoes exiting from the test chamber. A matched control was performed for each chemical treatment evaluation. Fifteen unfed female mosquitoes of 3-5 days old were released into each of four test chambers and mosquitoes were allowed to adjust themselves to environmental conditions inside the test chamber for 3 minutes before the exit door open. The number of escaping mosquitoes were recorded every minute for 30 minutes during exposure to test repellent compounds. At the end of the exposure period, escaped and non-escaped mosquitoes were transferred to individual containers and provided 10% sugar solution. Knockdown was observed after 30 minutes and mortality after 24 hours in both treatments and controls for both escaped and non-escaped cohorts. Each repellent compound was tested in four replicates between 0800 am and 1600 pm.

2.7 Statistical analysis

Kaplan-Meier survival analysis was used to evaluate escaping mosquitoes from each test chamber of the excito-repellency test system (Roberts *et al.*,1997). The time in minutes for 25% (ET_{25}), 50% (ET_{50}) and 75% (ET_{75}) of assay populations to escape was calculated for each product assessed and the log-rank test used to compare the escape responses of test populations. Observed percentage escape was corrected with Abbott's formula (Finney, 1971).

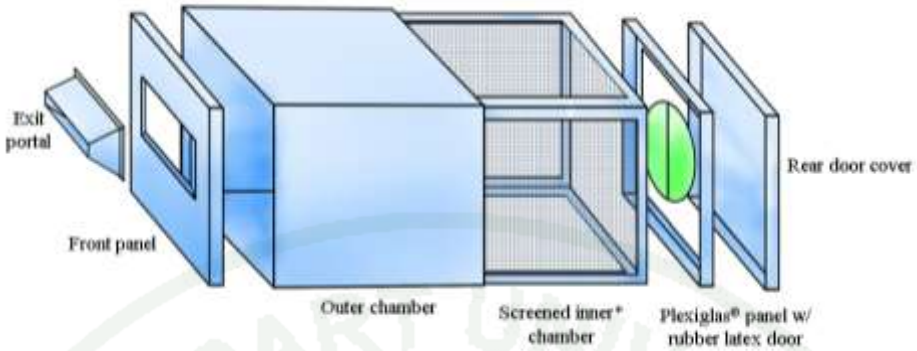


Figure 11 Blow-up diagram of the excito-repellency test chamber showing key components in apparatus design, including the screened inner chamber to measure spatial repellency

* Screened inner chamber: our treated papers were placed inside of the screened surfaces (top, bottom, 2 sides) of the inner chamber for contact trials, wherein mosquitoes were able to make direct contact with the trated surface of the papers and alternatively, papers were placed on the outside of the screens for non-contact trials, wherein mosquitoes were denied direct access to make physical contact the treated surfaces.

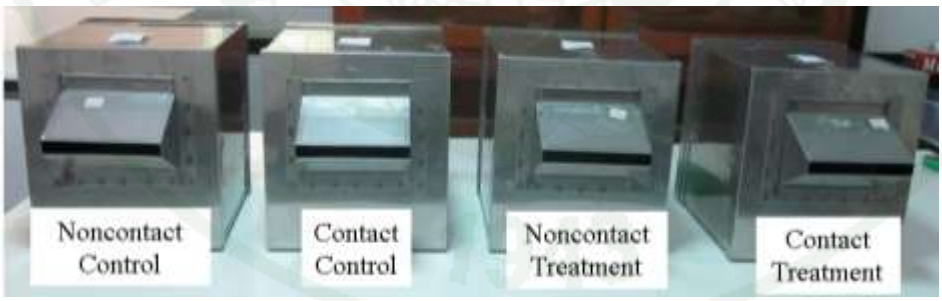


Figure 12 Excito-repellency test system showing the four chambers aligned in sequence for each trial run, one pair as contact and matched control, the other set as non-contact and control chamber.

RESULTS

Part 1 Behavioral responses of *Aedes aegypti* and *Anopheles minimus* to essential oils and synthetic repellents using the high throughput screening system

1.1 Knockdown and mortality responses test

Aedes aegypti showed high knockdown and mortality rates of 3 to 10% concentrations when exposed to DEET (80-92%) and hairy basil (100%), while 3% citronella and catnip gave high knockdown (100%) with medium mortality for citronella (48.6%) and low mortality for catnip (2.6%), respectively (Table 1). Knockdown and mortality were not observed with picaridin and very few mosquitoes were knocked down and no mortality occurred when exposed to 3% vetiver. At 3-10% DEET and hairy basil, *An. minimus* demonstrated 100% mortality. Citronella and catnip at 5-10% produced 100% knockdown and mortality. All concentrations of vetiver gave between 87-100% knockdown rates and 91-100% mortality rates with 3-10% concentrations. Picaridin tested on *An. minimus* and *Ae. aegypti* showed 0% knockdown and mortality (Figures 13 and 14).

Table 1 Percent knockdown and mortality of *Aedes aegypti* and *Anopheles minimus* exposed to DEET, picaridin, citronella, hairy basil, catnip, and vetiver (25 female mosquitoes per test)

Mosquito	Compound	Percent knockdown in 1 hour at indicated concentration (%)				Percent mortality at 24 hour at indicated concentration (%)			
		1	3	5	10	1	3	5	10
Ae. aegypti	DEET	0	92.0	100	100	0	80.0	100	100
	Picaridin	0	0	0	0	0	0	0	0
	Citronella	0	100	100	100	0	48.6	100	100
	Hairy basil	21.3	100	100	100	6.7	100	100	100
	Catnip	6.7	100	100	100	0	2.6	100	100
	Vetiver	0	2.0	9.0	22.2	0	0	2.0	2.0
An. minimus	DEET	0	100	100	100	0	100	100	100
	Picaridin	0	0	0	0	0	0	0	0
	Citronella	0	100	100	100	3.1	57.3	99.0	100
	Hairy basil	100	100	100	100	95.9	100	100	100
	Catnip	68.8	100	100	100	1.3	8.0	100	100
	Vetiver	86.5	95.7	100	100	50.0	91.4	98.6	100

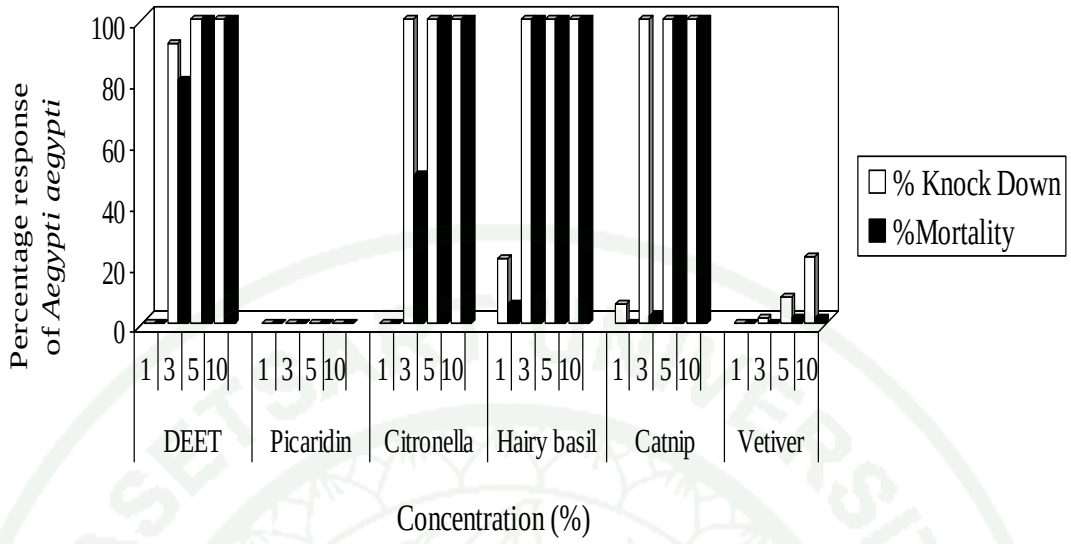


Figure 13 Percent knockdown and mortality of *Aedes aegypti* exposed to DEET, picaridin, citronella, hairy basil, catnip and vetiver

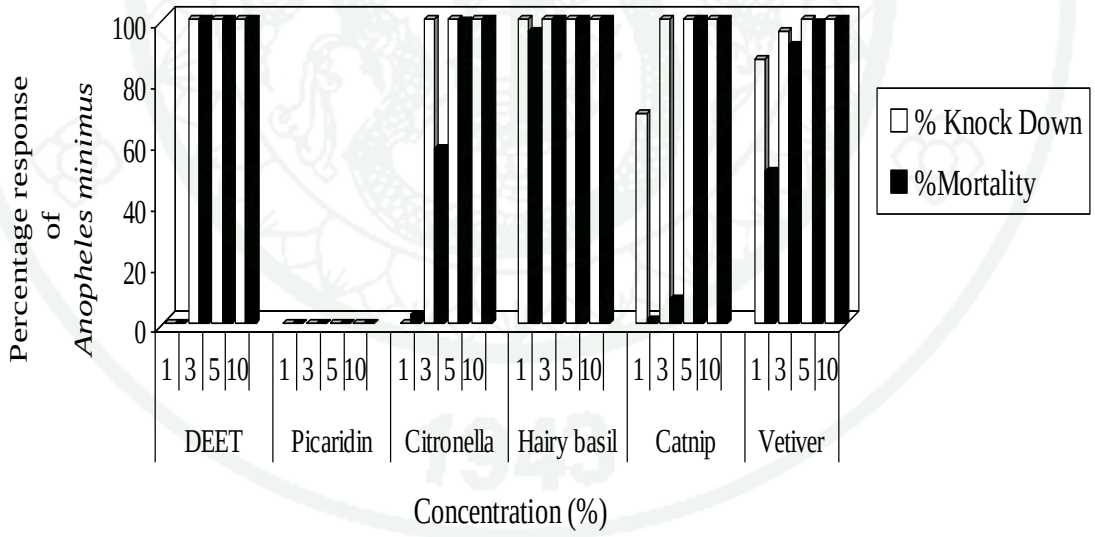


Figure 14 Percent knockdown and mortality of *Anopheles minimus* exposed to DEET, picaridin, citronella, hairy basil, catnip and vetiver

1.2 Contact irritancy assay

Escape response of *Ae. aegypti* and *An. minimus* varies dramatically depending on the compound tested, concentration (0.5-3%), and mosquito species (Tables 2 and 3). Most *Ae. aegypti* data indicated significant differences between the treatment and control escape responses, but no significant difference was observed at 0.5% picaridin ($P > 0.05$). Statistical significance ($P < 0.05$) between all pairs of treatment and control groups was seen in all *An. minimus* (Figure 15).

Table 2 Escape response of *Aedes aegypti* in the contact irritancy assay to citronella, hairy basil, catnip, vetiver, DEET and picaridin

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Number escaping (mean ± SE)		Percent escaping (mean ± SE)	Mean %KD in the treated metal chamber	*P*-value
				Treated	Control			
Citronella	0.5	6	60	7.2 ± 0.8	0.3 ± 0.2	70.0 ± 6.1	0	0.0022
	1	6	60	6.5 ± 0.6	0.3 ± 0.2	63.3 ± 6.6	0	0.0022
	2	6	60	3.5 ± 0.4	0.3 ± 0.2	54.8 ± 4.9	21.7 ± 11.7	0.0022
	3	6	60	2.3 ± 0.3	0.7 ± 0.2	71.7 ± 4.2	50.0 ± 15.5	0.0022
Hairy basil	0.5	6	60	8.0 ± 0.4	0	80.0 ± 3.7	0	0.0022
	1	6	56	5.8 ± 0.9	0.7 ± 0.2	62.8 ± 7.0	0	0.0022
	2	6	60	3.8 ± 0.7	0.3 ± 0.2	69.4 ± 8.0	31.7 ± 21.4	0.0022
	3	6	60	3.5 ± 0.6	0	65.0 ± 5.0	30.0 ± 17.9	0.0022
Catnip	0.5	6	60	7.3 ± 0.9	0	73.3 ± 8.8	0	0.0022
	1	6	60	8.3 ± 0.4	0	83.3 ± 4.2	0	0.0022
	2	6	59	0.8 ± 0.4	0	100 ± 2.6	90 ± 8.9	0.0022
	3	6	60	0.3 ± 0.2	0.3 ± 0.2	93.0 ± 4.5	90 ± 8.9	0.0022

Table 2 (Continued)

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Number escaping (mean ± SE)		Percent escaping (mean ± SE)	Mean %KD in the treated chamber	*P*-value
				Treated	Control			
Vetiver	0.5	6	60	6.5 ± 0.2	0.3 ± 0.2	63.7 ± 2.5	0	0.0022
	1	6	60	8.2 ± 0.5	0	81.7 ± 4.8	0	0.0022
	2	6	60	7.3 ± 0.3	0.3 ± 0.2	72.4 ± 3.4	0	0.0022
	3	6	60	7.5 ± 0.6	0	75.0 ± 6.2	0	0.0022
DEET	0.5	6	60	4.3 ± 0.8	0.3 ± 0.2	56.9 ± 5.4	15 ± 10.5	0.0022
	1	6	56	6.3 ± 0.8	0	78.3 ± 7.0	15 ± 10.5	0.0022
	2	6	60	4.3 ± 0.4	0.3 ± 0.2	61.9 ± 2.7	20 ± 11.0	0.0022
	3	6	60	4.0 ± 0.9	0	65.0 ± 6.7	25 ± 13.8	0.0022
Picaridin	0.5	6	60	1.0 ± 0.4	0	10.0 ± 4.5	0	0.0606
	1	6	60	3.6 ± 0.6	0.7 ± 0.2	31.7 ± 7.4	0	0.0108
	2	6	59	3.8 ± 0.8	0.7 ± 0.4	33.3 ± 8.6	0	0.0130
	3	6	60	2.2 ± 0.5	0.3 ± 0.2	18.9 ± 5.1	0	0.0130

P-value were from Wilcoxon two-sample test for difference between the number escaping in a treatment and a control chambers

Table 3 Escape response of *Anopheles minimus* in the contact irritancy assay to citronella, hairy basil, catnip, vetiver, DEET and picaridin

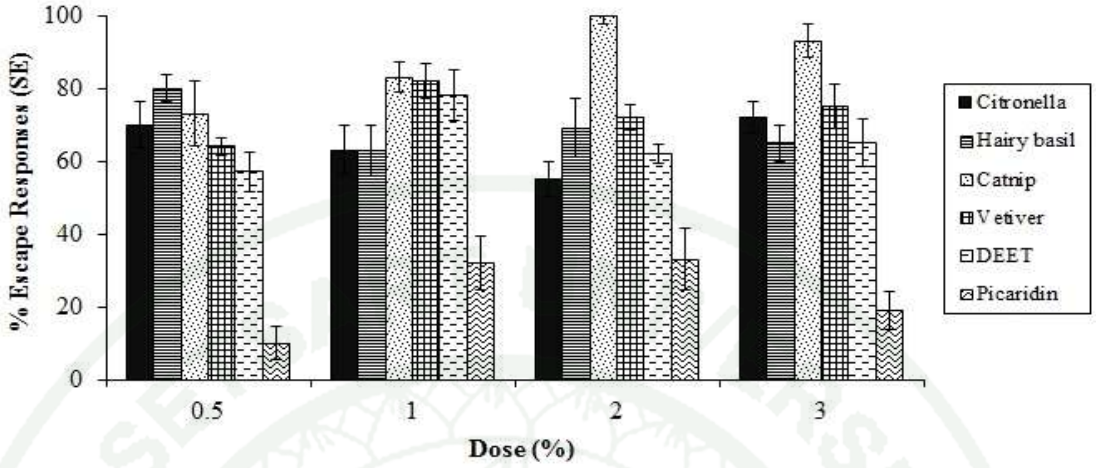
Compound	Dose (%)	No. of replicates	No. of mosquitoes	Number escaping (mean ± SE)		Percent escaping (mean ± SE)	Mean %KD in the treated metal chamber	*P*-value
				Treated	Control			
Citronella	0.5	6	60	3.5 ± 0.6	0	40.0 ± 4.5	6.7 ± 10.3	0.0022
	1	6	60	4.7 ± 0.5	0.7 ± 0.2	77.0 ± 4.8	31.7 ± 19.4	0.0022
	2	6	60	1.8 ± 0.4	0.3 ± 0.2	86.1 ± 2.3	68.3 ± 11.7	0.0022
	3	6	59	1.2 ± 0.3	0	96.7 ± 3.3	84.8 ± 5.3	0.0022
Hairy basil	0.5	6	60	8.5 ± 0.7	0.3 ± 0.2	84.8 ± 6.7	0	0.0022
	1	6	56	3.3 ± 0.7	0	88.3 ± 3.1	55.0 ± 18.7	0.0022
	2	6	60	2.5 ± 0.7	0.7 ± 0.2	100 ± 3.8	75.0 ± 16.4	0.0022
	3	6	60	2.2 ± 0.5	0.5 ± 0.2	94.4 ± 5.0	73.3 ± 13.7	0.0022
Catnip	0.5	6	60	5.7 ± 0.2	0	83.3 ± 9.2	26.7 ± 20.7	0.0022
	1	6	60	5.7 ± 0.7	0.7 ± 0.2	100 ± 0	43.3 ± 16.3	0.0022
	2	6	60	3.0 ± 0.3	0.7 ± 0.4	100 ± 0	70.0 ± 6.3	0.0022
	3	6	60	3.0 ± 0.7	0.7 ± 0.4	100 ± 0	70.0 ± 16.7	0.0022

Table 3 (Continued)

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Number escaping (mean ± SE)		Percent escaping (mean ± SE)	Mean %KD in the treated chamber	*P*-value
				Treated	Control			
Vetiver	0.5	6	60	6.8 ± 0.4	0.7 ± 0.2	66.3 ± 3.7	0	0.0022
	1	6	60	7.3 ± 0.5	0.7 ± 0.4	71.3 ± 5.1	0	0.0022
	2	6	60	5.7 ± 0.6	0.3 ± 0.2	58.2 ± 6.1	3.3 ± 5.2	0.0022
	3	6	60	5.3 ± 0.7	0	61.7 ± 6.0	8.3 ± 4.1	0.0022
DEET	0.5	6	60	6.0 ± 0.6	0.3 ± 0.2	78.9 ± 7.7	20.0 ± 17.9	0.0022
	1	6	60	4.8 ± 0.5	0.3 ± 0.2	84.3 ± 5.9	36.7 ± 5.2	0.0022
	2	6	60	4.2 ± 0.5	0	81.7 ± 7.5	40.0 ± 17.9	0.0022
	3	6	60	3.8 ± 0.8	0.7 ± 0.2	79.1 ± 5.7	41.7 ± 14.7	0.0022
Picaridin	0.5	6	60	6.0 ± 0.7	0.7 ± 0.4	55.0 ± 9.8	0	0.0022
	1	6	60	5.5 ± 0.9	0.3 ± 0.2	53.5 ± 8.7	0	0.0022
	2	6	60	3.3 ± 0.5	0.3 ± 0.2	30.9 ± 5.1	0	0.0065
	3	6	60	1.8 ± 0.3	0	18.3 ± 3.1	0	0.0022

P-value were from Wilcoxon two-sample test for difference between the number escaping in a treatment and a control chambers

Aedes aegypti



Anopheles minimus

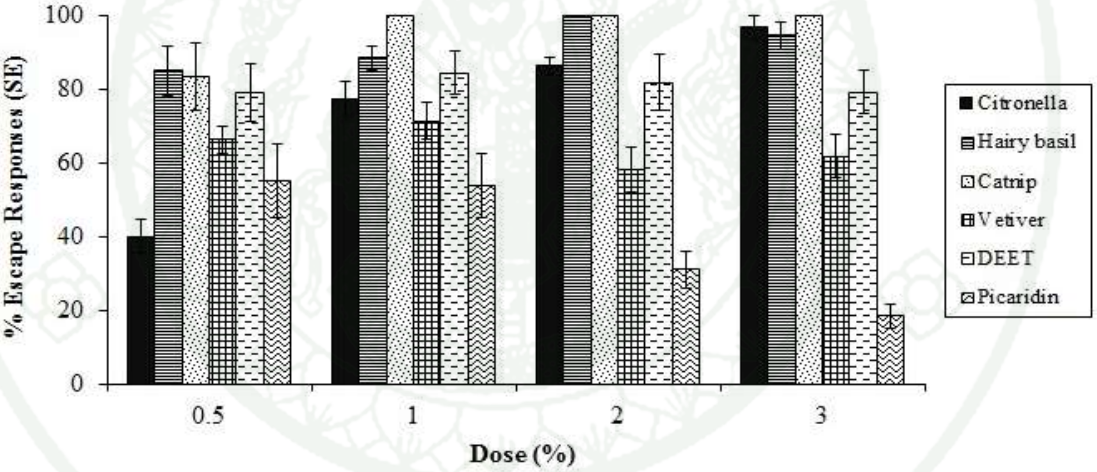


Figure 15 Contact irritant responses (CIA) with S.E. of *Aedes aegypti* and *Anopheles minimus* laboratory colonies exposed to four concentrations of citronella, hairybasil, catnip, vetiver and DEET and picaridin

1.3 Spatial repellency assay

Significant differences ($P< 0.05$) in escape responses of *Ae. aegypti* were found between all pairs of treatment and controls for catnip, vetiver, and DEET (Table 4). Citronella and hairy basil showed significant differences at 2 and 3% concentrations. No significant difference in spatial repellent response was observed at 0.5 and 1% citronella and hairy basil or with 0.5% picaridin. DEET exhibited SAI values between 0.97-1 for all concentrations whereas 0.5% picaridin had SAI negative value of -0.33 (Figure 16).

Anopheles minimus results indicated no statistical significance ($P> 0.05$) in 2 and 3% catnip, which also was found in picaridin 0.5% (Table 5).
All concentrations of DEET had SAI values of 1 while picaridin had values of SAI less than 1. Citronella at 1-3% indicated SAI of 1 and hairy basil had SAI values between 0.96-1. Catnip showed SAI closed to 1 at 0.5% while 3% catnip found a negative SAI value (Figure 16).

Table 4 Escape response of *Aedes aegypti* in the spatial repellency to citronella, hairy basil, catnip, vetiver, DEET and picaridin

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Mean percent responding (SE)	Mean SAI[1](SE)	SR[2]	*P* > *S*
Citronella	0.5	9	179	14.6 (2.73)	0.31 (0.28)	7.0	0.3359
	1	9	180	25.0 (1.67)	0.26 (0.18)	11.0	0.2383
	2	9	180	27.2 (5.66)	0.84 (0.08)	22.5	0.0039
	3	9	180	29.4 (4.12)	1.00 (0)	22.5	0.0039
Hairy basil	0.5	9	180	26.7 (5.59)	0.05 (0.23)	0.5	0.9766
	1	9	179	20.8 (4.45)	0.24 (0.24)	5.5	0.4375
	2	9	180	30.0 (3.61)	0.60 (0.23)	12.5	0.0781
	3	9	180	31.7 (5.77)	0.95 (0.03)	22.5	0.0039
Catnip	0.5	9	179	21.8 (3.03)	0.46 (0.16)	15.5	0.0313
	1	9	180	41.7 (4.86)	0.64 (0.15)	20.5	0.0117
	2	9	180	75.6 (5.30)	0.99 (0.01)	22.5	0.0039
	3	9	180	65.0 (5.59)	0.94 (0.05)	22.5	0.0039

Table 4 (Continued)

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Mean percent responding (SE)	Mean SAI[1] (SE)	SR[2]	*P* > *S*
Vetiver	0.5	9	179	28.9 (3.89)	0.56 (0.13)	21.5	0.0078
	1	9	180	29.1 (3.36)	0.66 (0.07)	22.5	0.0039
	2	9	180	33.9 (5.26)	0.58 (0.10)	18.0	0.0078
	3	9	180	33.7 (3.81)	0.74 (0.09)	22.5	0.0039
DEET	0.5	9	180	37.8 (7.37)	1.00 (0)	22.5	0.0039
	1	9	179	32.4 (5.05)	1.00 (0)	22.5	0.0039
	2	9	180	28.9 (4.0)	0.97 (0.03)	22.5	0.0039
	3	9	180	28.3 (2.89)	1.00 (0)	22.5	0.0039
Picaridin	0.5	9	179	13.9 (2.61)	- 0.33 (0.24)	- 9.0	0.2578
	1	9	180	28.1 (3.34)	0.36 (0.12)	16.0	0.0313
	2	9	180	32.8 (3.64)	0.35 (0.1)	18.0	0.0078
	3	9	180	27.2 (5.08)	0.32 (0.16)	13.0	0.0781

[1] SAI = spatial activity index [2] SR = signed-rank statistic derived through PROC UNIVARIATE

Table 5 Escape response of *Anophelesminimus* in the spatial repellency to citronella, hairy basil, catnip, vetiver, DEET and picaridin

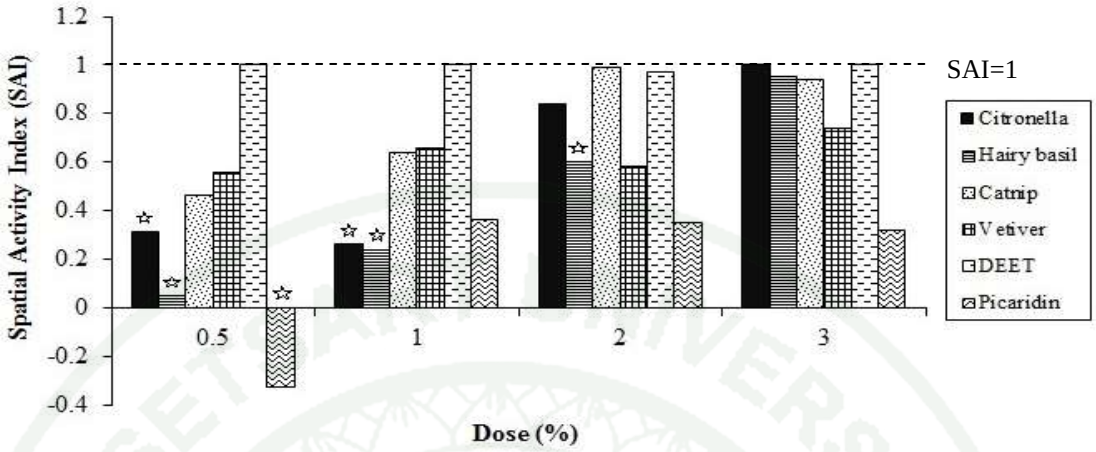
Compound	Dose (%)	No. of replicates	No. of mosquitoes	Mean percent responding (SE)	Mean SAI[1] (SE)	SR[2]	*P* > *S*
Citronella	0.5	9	180	10.0 (2.50)	0.89 (0.11)	18.0	0.0078
	1	9	180	42.8 (4.80)	1.00 (0)	22.5	0.0039
	2	9	180	53.3 (1.67)	1.00 (0)	22.5	0.0039
	3	9	180	60.0 (2.36)	1.00 (0)	22.5	0.0039
Hairy basil	0.5	9	180	51.1 (3.71)	0.86 (0.07)	22.5	0.0039
	1	9	180	66.1 (3.71)	0.97 (0.02)	22.5	0.0039
	2	9	180	71.7 (4.25)	0.96 (0.03)	22.5	0.0039
	3	9	180	84.4 (5.56)	1.00 (0)	22.5	0.0039
Catnip	0.5	9	180	73.3 (4.64)	0.96 (0.02)	22.5	0.0039
	1	9	181	55.8 (3.95)	0.45 (0.07)	22.5	0.0039
	2	9	180	62.2 (4.94)	0.26 (0.14)	12.5	0.1641
	3	9	180	68.3 (3.63)	- 0.02 (0.09)	- 2.0	0.7656

Table 5 (Continued)

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Mean percent responding (SE)	Mean SAI[1](SE)	SR[2]	*P* > *S*
Vetiver	0.5	9	180	27.2 (3.55)	0.91 (0.06)	22.5	0.0039
	1	9	180	62.8 (3.02)	0.96 (0.04)	22.5	0.0039
	2	9	180	42.2 (5.34)	0.79 (0.09)	22.5	0.0039
	3	9	180	48.3 (3.22)	0.54 (0.14)	20.5	0.0117
DEET	0.5	9	180	40.6 (6.15)	1.00 (0)	22.5	0.0039
	1	9	180	53.9 (7.67)	1.00 (0)	22.5	0.0039
	2	9	180	63.3 (5.34)	1.00 (0)	22.5	0.0039
	3	9	180	70.6 (5.10)	1.00 (0)	22.5	0.0039
Picaridin	0.5	9	180	29.4 (4.37)	0.38 (0.22)	11.5	0.1250
	1	9	180	38.9 (6.71)	0.53 (0.21)	17.0	0.0430
	2	9	180	45.6 (5.03)	0.76 (0.10)	22.5	0.0039
	3	9	180	46.7 (3.44)	0.88 (0.05)	22.5	0.0039

[1] SAI = spatial activity index [2] SR = signed-rank statistic derived through PROC UNIVARIATE

Aedes aegypti



Anopheles minimus

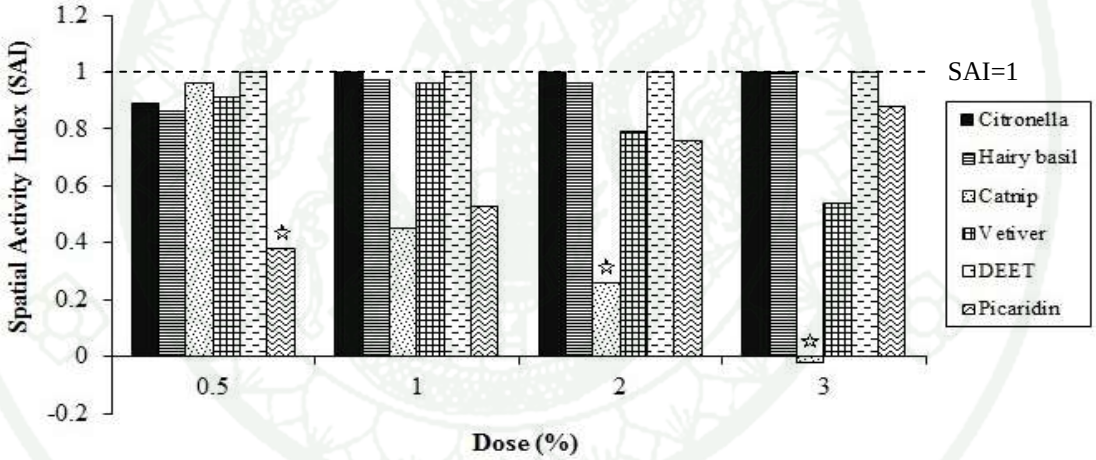


Figure 16 Spatial repellent responses (SRA) of *Aedes aegypti* and *Anopheles minimus* laboratory colonies exposed to four concentrations of citronella, hairy basil, catnip, vetiver, DEET and picaridin

☆ Denoted not statistically significant ($P > 0.05$) repellent response compared between treatment and control

1.4 Toxicity assay

In general, knockdown and mortality rates for both mosquito species increased with higher compound concentrations. In Figure 17, citronella, hairy basil, and catnip showed high mortality rates. DEET exhibited the highest knockdown and mortality rates at all concentrations for both mosquito species. Vetiver showed toxicity in

An. minimus with 59.1-93.7% mortality but was non-toxic and no mortality with *Ae. aegypti* (Table 6). For *Ae.aegypti*, 1-3% citronella and catnip showed 98.3-100% knockdown and mortality. Hairy basil at 2-3% gave high knockdown (98-100%) and mortality rate (99-100%) in *Ae. aegypti*. The results of *An. minimus* indicated that at 1-3% of citronella, hairy basil, and catnip displayed between 99.1-100% knockdown and 82.9-100% mortality (Table 7). No toxicity, knockdown, or mortality was detected with picaridin in both mosquito species.

Table 6 Knockdown and mortality response of *Aedes aegypti* in the toxicity assay to citronella, hairy basil, catnip, vetiver, DEET and picaridin

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Mean percent 1 h knockdown (mean ± SE)	Mean percent 24 h Mortality (mean ± SE)
Citronella	0.5	6	117	12.5 ± 6.7 B	1.7 ± 1.7 C
	1	6	120	98.3 ± 1.7 A	20.8 ± 9.7 B
	2	6	119	100 ± 0 A	98.3 ± 1.1 A
	3	6	120	100 ± 0 A	100 ± 0 A
Hairy basil	0.5	6	121	1.7 ± 1.1 C	2.5 ± 2.5 B
	1	6	121	8.3 ± 3.8 B	4.2 ± 3.3 B
	2	6	118	98.3 ± 1.1 A	99.2 ± 0.8 A
	3	6	120	100 ± 0 A	100 ± 0 A
Catnip	0.5	6	117	0.8 ± 0.8 B	0 D
	1	6	117	100 ± 1.4 A	9.3 ± 3.6 C
	2	6	119	100 ± 0 A	75.7 ± 3.9 B
	3	6	120	100 ± 0 A	87.5 ± 5.3 A

Table 6 (Continued)

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Mean percent 1 h knockdown (mean ± SE)		Mean percent 24 h Mortality (mean ± SE)	
Vetiver	0.5	6	115	4.6 ± 2.2	A	0	A
	1	6	116	4.5 ± 1.8	A	0	A
	2	6	120	5.8 ± 2.0	A	0	A
	3	6	119	10.2 ± 4.8	A	0	A
DEET	0.5	6	118	100 ± 0	A	96.7 ± 2.8	B
	1	6	119	100 ± 0	A	100 ± 0	A
	2	6	118	100 ± 0	A	100 ± 0	A
	3	6	119	100 ± 0	A	100 ± 0	A
Picaridin	0.5	6	119	0	A	0	A
	1	6	118	0	A	0	A
	2	6	119	0	A	0	A
	3	6	120	0	A	0	A

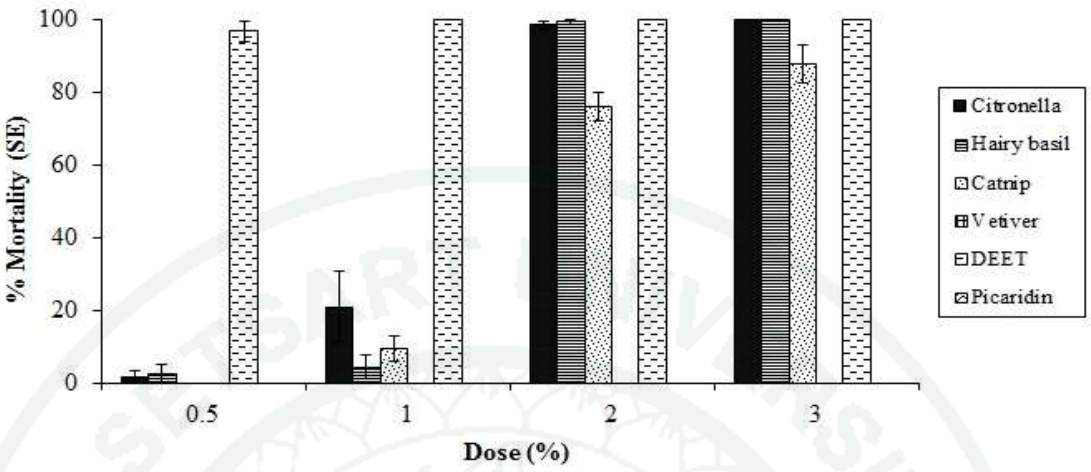
Table 7 Knockdown and mortality response of *Anopheles minimus* in the toxicity assay to citronella, hairy basil, catnip, vetiver, DEET and picaridin

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Mean percent 1 h knockdown (mean ± SE)		Mean percent 24 h Mortality (mean ± SE)	
Citronella	0.5	6	117	67.9 ± 9.0	B	31.8 ± 6.5	C
	1	6	116	100 ± 0	A	90.5 ± 4.9	B
	2	6	120	100 ± 0	A	100 ± 0	A
	3	6	116	100 ± 0	A	100 ± 0	A
Hairy basil	0.5	6	118	73.0 ± 8.9	B	14.3 ± 8.8	B
	1	6	116	99.1 ± 0.9	A	82.9 ± 10.8	A
	2	6	119	100 ± 0	A	100 ± 0	A
	3	6	120	100 ± 0	A	100 ± 0	A
Catnip	0.5	6	114	87.0 ± 7.0	B	5.7 ± 2.6	C
	1	6	118	100 ± 0	A	88.1 ± 3.7	B
	2	6	117	100 ± 0	A	96.5 ± 2.2	A
	3	6	119	100 ± 0	A	100 ± 0	A

Table 7 (Continued)

Compound	Dose (%)	No. of replicates	No. of mosquitoes	Mean percent 1 h knockdown (mean ± SE)		Mean percent 24 h Mortality (mean ± SE)	
Vetiver	0.5	6	114	92.9 ± 2.5	B	59.1 ± 9.0	B
	1	6	119	94.4 ± 1.5	B	91.1 ± 4.7	A
	2	6	114	100 ± 0	A	89.7 ± 2.1	A
	3	6	113	99.2 ± 0.8	A	93.7 ± 2.6	A
DEET	0.5	6	114	100 ± 0	A	98.9 ± 1.1	A
	1	6	115	100 ± 0	A	97.3 ± 1.9	A
	2	6	120	100 ± 0	A	100 ± 0	A
	3	6	119	100 ± 0	A	100 ± 0	A
Picaridin	0.5	6	119	0	A	0	A
	1	6	119	0	A	0	A
	2	6	117	0	A	0	A
	3	6	117	0	A	0	A

Aedes aegypti



Anopheles minimus

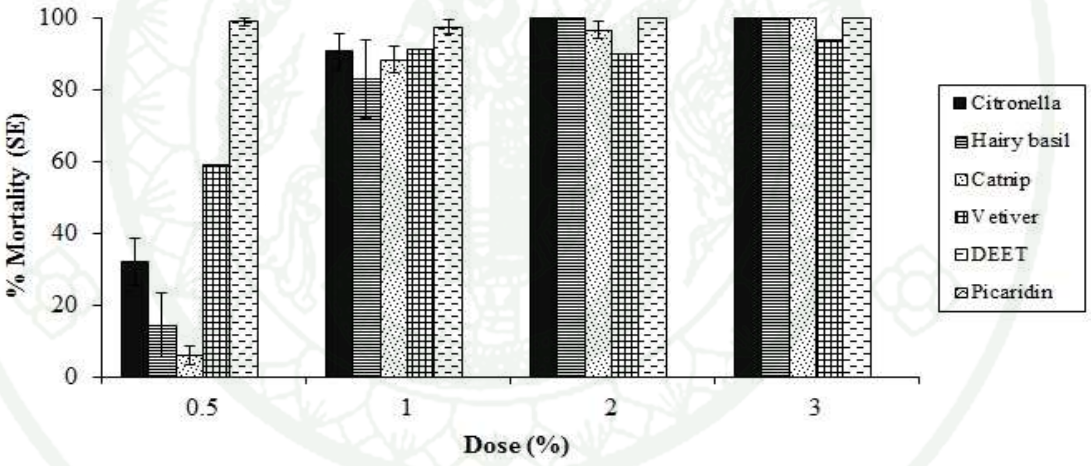


Figure 17 Percentage of 24-h mortality (TOX) with S.E. (Standard error) of *Aedes aegypti* and *Anopheles minimus* laboratory strains exposed to four concentrations of citronella, hairybasil, catnip, vetiver, DEET and picaridin

Part 2 Behavioral responses of *Aedes aegypti, Aedes albopictus, Anopheles minimus* and *Culex quinquefasciatus* to plant essential oils, synthetic repellents and pyrethroid insecticides using excito repellency test system

2.1 Escape response rates of mosquitoes

Results in contact trials indicate that responses of *Ae. albopictus* to the synthetic pyrethroids (deltamethrin and permethrin) were significantly greater than DEET, picaridin or essential oils ($P<0.05$). For *Ae. aegypti*, deltamethrin showed greater escape responses than others, except hairy basil ($P=0.0892$). Permethrin showed no significant difference in repelling effect as compared to all essential oils, $P>0.05$ (Tables 8 and 9). All compounds were found to elicit escape responses in both *Cx. quinquefasciatus* (69.2% to 96.6%) and *An. minimus* (65.4% to 97.8%), except picaridin in that comparatively low escape responses were observed (3.4% and 12.2% respectively). Moreover, two test populations, *Cx. quinquefasciatus* and *An. minimus* showed high escape responses to three essential oils, citronella, hairy basil and catnip in contact and non-contact trials (72.4% to 94.4%). Vetiver displayed extremely high escape responses from both species in the contact chambers (>95%).

2.2 Mortality rates of mosquitoes

Overall, mortality rates were low, except when *An. minimus* was exposed to catnip as 76.7% and 89.9% mortality was reported in contact and non-contact, respectively (Tables 10, 11).

2.3 Escape time

Escape time in minutes (ET) 25%, 50% and 75% during the 30-min exposure assay is shown in Tables 12 and 13. *Aedes aegypti* showed the greatest escape response to permethrin with an ET_{25} value of 1 min and an ET_{50} value of 4 mins in contact trial. No response was found in the non-contact trials. *Aedes*

albopictus had low ET_{25} values in both contact and non-contact trials (≤1 min) against deltamethrin, DEET, catnip and vetiver. For ET_{50}, this species displayed the value of <1 min only in contact trials for deltamethrin and permethrin. ET_{75} values of 2 mins and 4 mins appeared for deltamethrin and permethrin in contact treatment chambers, respectively. *Culex quinquefasciatus* displayed ET_{25} values of <1 min in contact trials against all four essential oils tested and ≤1 min in non-contact trials for citronella, hairy basil and catnip. *Anopheles minimus* showed fast escape responses of ≤1 min at ET_{25} to DEET, hairy basil and catnip. For *An. minimus,* catnip was found to have an ET_{25}, ET_{50} and ET_{75} for both contact and non-contact trials ≤4 mins. *Anopheles minimus* had a delayed escape response to permethrin, DEET, and citronella with ET_{50} values of 6, 6 and 7 mins, respectively.

2.4 Escape patterns

The escape patterns during 30-min exposures for the four mosquito species in response to four essential oils or two repellents or two pyrethroid insecticides in contact and non-contact trials are presented in Figures 18 and 19.

A comparison of escape responses among the mosquito test populations between contact and non-contact trials is shown in Table14. All species exhibited significant differences in response patterns between trials for deltamethrin and permethrin (P<0.0001). *Aedes aegypti* showed slightly different in patterns of escape among DEET (P=0.0460), hairy basil (P=0.0166) and catnip (P=0.0201), but there were no significant differences for picaridin, citronella and vetiver (P>0.05).
A significant difference was found between DEET and vetiver (P<0.0001) trials using *Cx. quinquefasciatus*. Escape responses were also significantly against citronella and vetiver trials using *An. minimus.*

The log-rank test for comparisons between two species of test mosquitoes in either contact or non-contact trials were conducted. The study found that there were very significant differences between *Ae. albopictus* and other three species in both test

conditions of deltamethrin (P<0.0001). The significant differences in escape responses between two species were most found in citronella, hairy basil, catnip and vetiver (P<0.05). While synthetic pyrethroids and repellents indicated some significant differences between species (Table 15).

Statistical tests for comparisons between pyrethroids and repellents/essential oils against four mosquito species are given in Table 15. *Anopheles minimus* and *Ae. albopictus* showed most comparison data significant differences (P<0.05) in contact trials. There were very significant differences between deltamethrin and other compounds except when paired with hairy basil in contact trials but no statistically significant differences between permethrin and all four essential oils in the same trials for *Ae. aegypti*.

Table 8 Escape response rates of four mosquito species exposed to deltamethrin (0.025 g/m^2), permethrin (0.5 g/m^2), DEET, picaridin, citronella, hairy basil, catnip and vetiver each at 2.5% (2.72 g/m^2) in contact trial

Compound[1]	*Ae. aegypti*		*Ae. albopictus*		*An. minimus*		*Cx. quinquefasciatus*	
	No. tested	% Esp	No. tested	% Esp	No. tested	% Esp	No. tested	% Esp
Deltamethrin-C	58	8.6	60	10.0	58	10.0	61	3.3
Deltamethrin	58	67.9	60	94.4	59	65.4	60	72.4
Permethrin-C	57	7.0	60	10.0	58	10.0	59	3.4
Permethrin	54	72.2	60	85.2	58	97.8	59	85.9
DEET-C	60	6.7	60	6.7	60	6.7	60	3.3
DEET	60	21.4	59	47.3	61	80.7	57	69.2
Picaridin-C	60	5.0	60	5.0	61	5.0	59	3.4
Picaridin	59	3.7	59	28.6	59	12.2	60	3.4
Citronella-C	60	0	60	6.7	59	6.7	60	5.0
Citronella	60	37.9	59	5.6	57	86.3	55	80.8
Hairy basil-C	60	6.7	60	6.7	59	6.7	59	5.1
Hairy basil	59	47.3	60	3.5	58	90.8	59	89.3
Catnip-C	59	5.1	60	10.0	59	10.0	59	1.7
Catnip	58	32.8	60	61.1	60	87.9	58	80.7
Vetiver-C	60	1.7	60	6.7	58	6.7	59	1.7
Vetiver	59	37.8	60	42.9	59	96.5	60	96.6

[1] C = control test without insecticide/repellent/essential oil

All treatment escapes were adjusted for control escapes by Abbott's formula (Finney, 1964)

Table 9 Escape response rates of four mosquito species exposed to deltamethrin (0.025 g/m^2), permethrin (0.5 g/m^2), DEET, picaridin, citronella, hairy basil, catnip and vetiver each at 2.5% (2.72 g/m^2) in non-contact trial

Compound[1]	*Ae. aegypti*		*Ae. albopictus*		*An. minimus*		*Cx. quinquefasciatus*	
	No. tested	% Esp	No. tested	% Esp	No. tested	% Esp	No. tested	% Esp
Deltamethrin-C	57	3.5	60	3.3	61	3.3	57	0
Deltamethrin	56	3.7	60	62.3	58	16.2	59	0
Permethrin-C	56	8.9	60	15.3	59	15.3	59	1.7
Permethrin	56	0	60	40.8	60	50.8	60	0
DEET-C	60	5.0	59	10.3	58	10.3	60	3.3
DEET	60	8.7	60	57.9	60	77.7	59	21.1
Picaridin-C	60	6.7	60	10.0	60	10.0	59	1.7
Picaridin	60	0	60	14.8	59	2.1	57	1.8
Citronella-C	59	3.4	60	6.6	61	6.6	60	3.3
Citronella	58	30.4	59	14.6	60	73.2	59	85.9
Hairy basil-C	60	1.7	60	11.7	60	11.7	60	1.7
Hairy basil	57	30.4	59	11.2	57	78.1	59	89.6
Catnip-C	60	1.7	60	8.3	60	8.3	61	0
Catnip	60	18.6	60	60.0	59	94.4	58	72.4
Vetiver-C	60	0	60	3.4	59	3.4	57	0
Vetiver	60	31.7	60	63.7	61	66.0	58	24.1

[1] C = control test without insecticide/repellent/essential oil

All treatment escapes were adjusted for control escapes by Abbott's formula (Finney, 1964)

Table 10 The 24 hour mortality rates of four mosquito species after exposure to deltamethrin (0.025 g/m^2), permethrin (0.5 g/m^2), DEET, picaridin, citronella, hairy basil, catnip and vetiver each at 2.5% (2.72 g/m^2) in contact trial

Compound[1]	*Ae. aegypti*		*Ae. albopictus*		*An. minimus*		*Cx. quinquefasciatus*	
	Es[2]	NEs[3]	Es	NEs	Es	NEs	Es	NEs
Deltamethrin-C	0	0	0	0	0	0	0	0
Deltamethrin	46.6	27.6	13.3	5.0	15.3	10.2	0	0
Permethrin-C	0	0	0	0	0	0	0	0
Permethrin	24.1	24.1	33.3	10.0	6.9	0	0	0
DEET-C	0	0	0	0	0	0	0	0
DEET	0	0	0	1.7	0	0	0	0
Picaridin-C	0	0	0	1.7	0	0	0	0
Picaridin	0	0	0	0	0	1.7	0	0
Citronella-C	0	0	0	0	0	0	0	0
Citronella	0	0	0	0	0	0	0	0
Hairy basil-C	0	1.7	0	0	0	0	0	0
Hairy basil	0	0	0	1.7	0	3.4	0	0
Catnip-C	0	0	0	0	3.4	0	0	0
Catnip	0	0	0	1.7	76.7	0	0	5.2
Vetiver-C	0	6.7	0	1.7	0	0	0	0
Vetiver	0	42.9	0	1.7	0	0	0	0

[1] C = control test without insecticide/repellent/essential oil

[2] Es = escaped mosquitoes, tested mosquitoes were found in the receiving box

[3] NEs =not escaped mosquitoes, tested mosquitoes remained inside the test chamber

Table 11 The 24 hour mortality rates of four mosquito species after exposure to deltamethrin (0.025 g/m^2), permethrin (0.5 g/m^2), DEET, picaridin, citronella, hairy basil, catnip and vetiver each at 2.5% (2.72 g/m^2) in non-contact trial

Compound[1]	*Ae. aegypti*		*Ae. albopictus*		*An. minimus*		*Cx. quinquefasciatus*	
	Es[2]	NEs[3]	Es	NEs	Es	NEs	Es	NEs
Deltamethrin-C	3.5	0	0	0	0	0	0	0
Deltamethrin	0	12.5	0	0	3.4	3.4	0	0
Permethrin-C	0	1.8	0	0	0	1.7	0	0
Permethrin	0	3.6	0	0	1.7	3.3	0	0
DEET-C	0	0	0	0	0	0	0	0
DEET	0	0	0	1.7	0	0	0	0
Picaridin-C	0	0	0	0	0	0	0	0
Picaridin	0	0	0	0	1.7	0	0	0
Citronella-C	0	0	0	0	0	0	0	0
Citronella	0	0	0	1.7	0	0	0	1.7
Hairy basil-C	0	1.7	0	1.7	0	0	0	0
Hairy basil	1.8	1.8	0	5.1	1.8	3.5	0	0
Catnip-C	0	0	1.7	0	5.0	0	0	0
Catnip	0	0	0	0	89.8	0	0	0
Vetiver-C	0	0	1.7	1.7	0	0	0	0
Vetiver	0	0	3.3	0	3.3	4.9	0	0

[1] C = control test without insecticide/repellent/essential oil

[2] Es = escaped mosquitoes, tested mosquitoes were found in the receiving box

[3] NEs =not escaped mosquitoes, tested mosquitoes remained inside the test chamber

Table 12 Time in minutes for 25% (ET_{25}), 50% (ET_{50}) and 75% (ET_{75}) of four mosquitoes test populations to escape from test chambers treated with deltamethrin, permethrin, DEET, picaridin, citronella, hairy basil, catnip and vetiver in contact trial

Compound	*Ae. aegypti*			*Ae. albopictus*			*An. minimus*			*Cx. quinquefasciatus*		
	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}
Deltamethrin	2	8	-	<1	<1	2	7	18	-	4	9	-
Permethrin	1	4	-	<1	<1	4	2	6	12	2	7	14
DEET	17	-	-	<1	28	-	1	6	21	2	12	-
Picaridin	-	-	-	10	-	-	-	-	-	-	-	-
Citronella	15	-	-	-	-	-	5	7	22	<1	4	19
Hairy basil	1	29	-	-	-	-	<1	2	4	<1	3	8
Catnip	21	-	-	<1	15	-	<1	2	4	<1	3	18
Vetiver	6	-	-	<1	-	-	3	5	11	<1	2	5

Table 13 Time in minutes for 25% (ET_{25}), 50% (ET_{50}) and 75% (ET_{75}) of four mosquitoes test populations to escape from test chambers treated with deltamethrin, permethrin, DEET, picaridin, citronella, hairy basil, catnip and vetiver in non-contact trial

Compound	*Ae. aegypti*			*Ae. albopictus*			*An. minimuus*			*Cx. quinquefasciatus*		
	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}
Deltamethrin	-	-	-	<1	24	-	-	-	-	-	-	-
Permethrin	-	-	-	5	-	-	10	22	-	-	-	-
DEET	-	-	-	<1	6	-	5	16	27	-	-	-
Picaridin	-	-	-	-	-	-	-	-	-	-	-	-
Citronella	26	-	-	-	-	-	8	10	30	<1	6	22
Hairy basil	15	-	-	-	-	-	<1	1	16	<1	2	6
Catnip	-	-	-	1	4	-	<1	2	4	1	7	-
Vetiver	9	-	-	<1	2	-	3	19	-	-	-	-

Table 14 Comparison of escape responses between paired contact and non-contact trials for 4 mosquito species exposed to deltamethrin, permethrin, DEET, picaridin, citronella, hairy basil, catnip and vetiver using the log rank test

Compound	*Ae. aegypti*	*Ae. albopictus*	*An. minimus*	*Cx. quinquefasciatus*
Deltamethrin	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
Permethrin	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
DEET	0.0460*	0.2170	0.0563	< 0.0001*
Picaridin	0.7091	0.3112	0.0845	0.4367
Citronella	0.4212	0.2039	0.0379*	0.9690
Hairy basil	0.0166*	0.1776	0.1963	0.4648
Catnip	0.0201*	0.4484	0.7397	0.1342
Vetiver	0.3783	0.0382*	< 0.0001*	< 0.0001*

* indicates significant differences (P<0.05) between contact and non-contact trials

Table 15 Comparison of escape responses between two species from the four species of mosquito exposed to deltamethrin, permethrin, DEET, picaridin, citronella, hairy basil, catnip and vetiver using the log rank test

Compound	Mosquito species	Contact	Non-contact
Deltamethrin	*Ae. aegypti* & *Ae. albopictus*	< 0.0001*	< 0.0001*
	Ae. aegypti & *Cx. quinquefasciatus*	0.7049	0.0374*
	Ae. aegypti & *An. minimus*	0.1060	0.0739
	Ae. albopictus & *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *An. minimus*	< 0.0001*	< 0.0001*
	Cx. quinquefasciatus & *An. minimus*	0.1469	0.0005*
Permethrin	*Ae. aegypti* & *Ae. albopictus*	< 0.0001*	< 0.0001*
	Ae. aegypti & *Cx. quinquefasciatus*	< 0.0001*	0.0824
	Ae. aegypti & *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *Cx. quinquefasciatus*	0.0656	< 0.0001*
	Ae. albopictus & *An. minimus*	0.5848	0.5167
	Cx. quinquefasciatus & *An. minimus*	0.1179	< 0.0001*

Table 15 (Continued)

Compound	Mosquito species	Contact	Non-contact
DEET	*Ae. aegypti* & *Ae. albopictus*	0.0068*	< 0.0001*
	Ae. aegypti & *Cx. quinquefasciatus*	< 0.0001*	0.1055
	Ae. aegypti & *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *Cx. quinquefasciatus*	0.0496	< 0.0001*
	Ae. albopictus & *An. minimus*	0.0482	0.5171
	Cx. quinquefasciatus & *An. minimus*	0.7556	< 0.0001*
Picaridin	*Ae. aegypti* & *Ae. albopictus*	0.0012*	0.0092*
	Ae. aegypti & *Cx. quinquefasciatus*	0.7233	0.4496
	Ae. aegypti & *An. minimus*	0.0237*	0.3217
	Ae. albopictus & *Cx. quinquefasciatus*	0.0004*	0.0018*
	Ae. albopictus & *An. minimus*	0.2554	0.0821
	Cx. quinquefasciatus & *An. minimus*	0.0106*	0.0990

Table 15 (Continued)

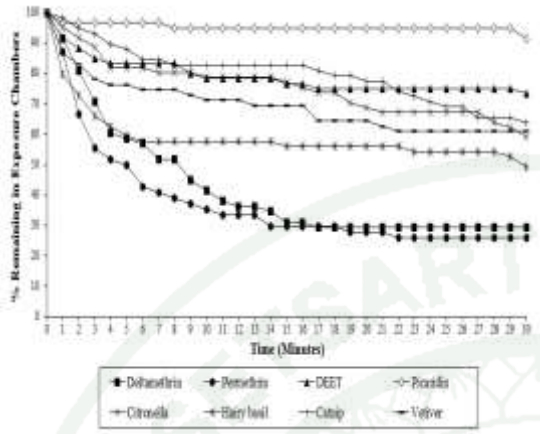
Compound	Mosquito species	Contact	Non-contact
Citronella	*Ae. aegypti* & *Ae. albopictus*	0.0003*	0.0910
	Ae. aegypti & *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. aegypti & *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *An. minimus*	< 0.0001*	< 0.0001*
	Cx. quinquefasciatus & *An. minimus*	0.2641	0.0091*
Hairy basil	*Ae. aegypti* & *Ae. albopictus*	< 0.0001*	0.1502
	Ae. aegypti & *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. aegypti & *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *An. minimus*	< 0.0001*	< 0.0001*
	Cx. quinquefasciatus & *An. minimus*	0.0344*	0.0440*

Table 15 (Continued)

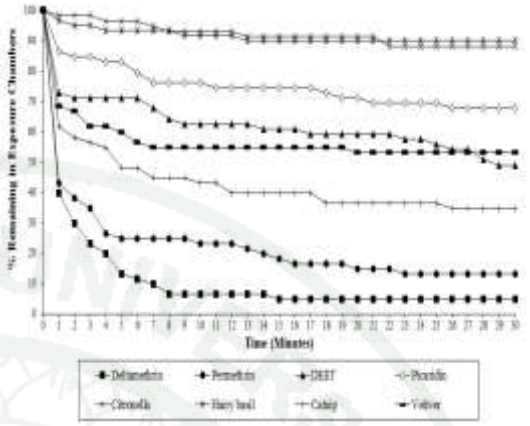
Compound	Mosquito species	Contact	Non-contact
Catnip	*Ae. aegypti* & *Ae. albopictus*	0.0001*	< 0.0001*
	Ae. aegypti & *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. aegypti & *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *Cx. quinquefasciatus*	0.0830	0.1852
	Ae. albopictus & *An. minimus*	0.0035*	< 0.0001*
	Cx. quinquefasciatus & *An. minimus*	0.2038	< 0.0001*
Vetiver	*Ae. aegypti* & *Ae. albopictus*	0.2709	< 0.0001*
	Ae. aegypti & *Cx. quinquefasciatus*	< 0.0001*	0.2246
	Ae. aegypti & *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. albopictus & *An. minimus*	< 0.0001*	0.3252
	Cx. quinquefasciatus & *An. minimus*	0.3733	< 0.0001*

* indicates significant differences (P<0.05) between two species

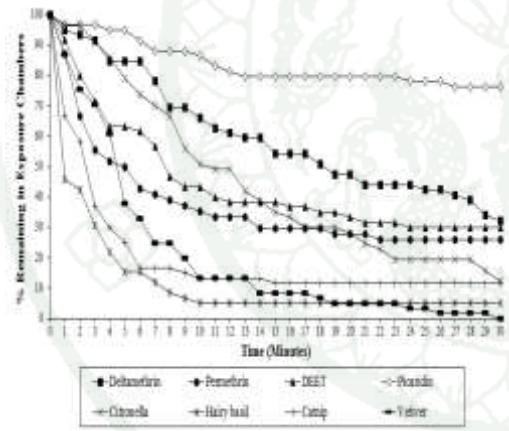
Aedes aegypti



Aedes albopictus



Anopheles minimus



Culex quinquefasciatus

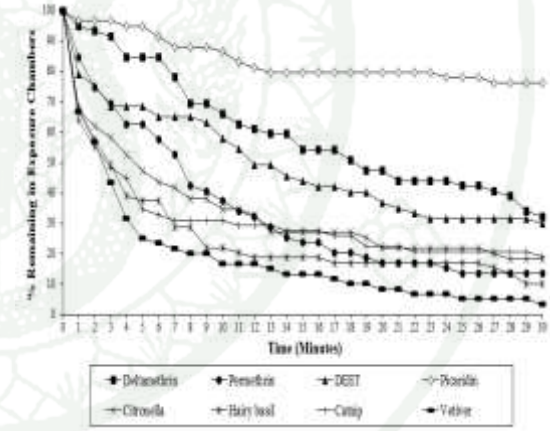
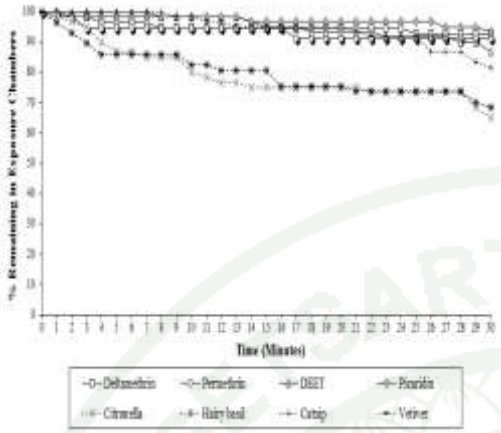
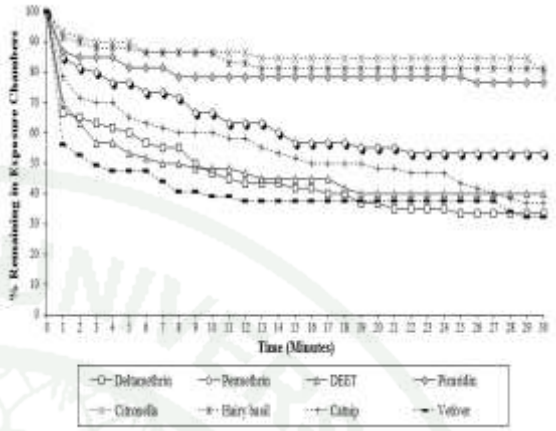


Figure 18 Kaplan-Meier survival curves indicating the percent of four mosquito species remaining inside a treated chamber during 30-minute exposure to eight test compounds in contact behavioral assays

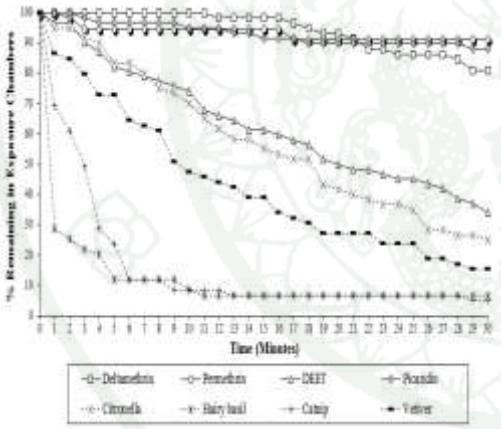
Aedes aegypti



Aedes albopictus



Anopheles minimus



Culex quinquefasciatus

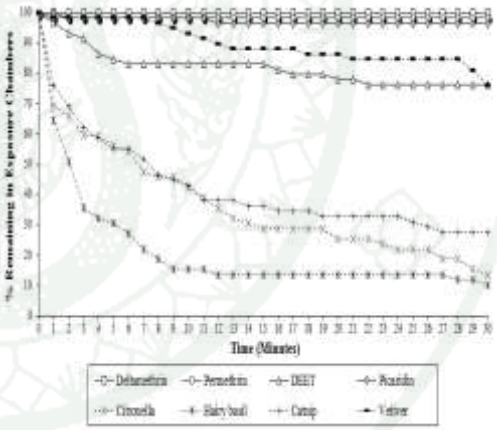


Figure 19 Kaplan-Meier survival curves indicating the percent of four mosquito Species remaining inside a treated chamber during 30-minute exposure to eight test compounds in non-contact behavioral assays

DISCUSSION

Part 1 Behavioral responses of *Aedes aegypti* and *Anopheles minimus* to plant essential oils and synthetic repellents using high throughput screening system

Mosquito-borne diseases, especially dengue and malaria, are serious worldwide, especially in tropical and sub-tropical areas. Many control programs have been conducted to reduce transmission for decades, but they are unsuccessful. The dengue and malaria vaccines are not approved for safe and effective use (Wan *et al.*, 2013) and malaria parasites have become resistant to antimalaria drug (Chareonviriyaphap *et al.*, 2000).

Synthetic pyrethroids have been heavily applied in mosquito control programs but there are increasing reports of pyrethroid resistance in *Ae. aegypti* (Paeporn *et al*., 2006; Thanispong *et al*., 2008; Chareonviriyaphap *et al*., 2013). *Anopheles minimus* in Thailand is still widely susceptible to pyrethroids (Wangroongsarb *et al*., 2007). With high cases of dengue fever and malaria have been reported annually thus individual personal protection method has become an important tool against mosquitobites.There are two types of mosquito repellent products available in markets, synthetic and natural. Plant-based repellents are sometimes used as natural alternatives to synthetic repellents. There are many of plant-based oils that contain the mosquito repellent properties and some of them are registered as mosquito repellents, such as citronella, lemon eucalyptus, geranium, neem, and clove (Barnard, 1999; Novakand *et al*., 2005; Paluch *et al*., 2010).

Most previous studies on the repellent properties of plants against mosquitoes have used a cage or hand in cage method, which tests against mosquito bites (Barnard *et al.*, 1999; Misni *et al*., 2008; Phasomkusolsil *et al*., 2010). An excito-repellency test

system can investigate mosquito escape response in the form of non-contact repellent and contact irritant actions to plant essential oils. With this assay, Polsomboon *et al*. (2008) discovered catnip with strong contact irritant and non-contact repellent actions against *Ae. aegypti* and *An. harrisoni* at different concentrations. Suwansirisilp *et al.* (2012) found *Cx. quinquefasciatus* displayed stronger escape responses to citronella and clove than *Ae. aegypti*. In addition, high-throughput screening system (HITSS) was used to characterize the spatial repellent, contact irritant, and toxicant chemical actions against mosquitoes. Achee *et al*. (2009) used HITSS to evaluate insecticides and concluded that DDT had all three actions of spatial repellency, contact irritancy, and toxicity. Alphacypermethrin, cypermethrin, permethrin, and deltamethrin generated greater contact irritantion than a repellent effect on *Ae. aegypti*. Similarly, an escape response test using HITSS by Thanispong *et al.* (2010) demonstrated that six *Ae. aegypti* field strains exhibited significant contact irritant responses to alphacypermethrin, deltamethrin and permethrin and found good repellency responses to DDT.

This study is one of few studies to identify the three major actions of plants by using HITSS. Understanding the type of repelling action of plants against mosquitoes is important to selecting appropriate essential oils for mosquito control. This study found that catnip showed high irritant contact to *Ae. aegypti* and *An. minimus*. Hairy basil was a better contact irritant to *An. minimus* than *Ae. aegypti*. Citronella and DEET were moderate irritants to *Ae. aegypti*. Picaridin displayed the least irritancy to *Ae. aegypti* and *An. minimus*. For spatial repellency, DEET exhibited the highest SAI in both mosquito species. Citronella and hairy basil at 1-3% concentrations gave a similar effect as DEET when tested against *An. minimus* with SAI values close to 1. Only the maximum concentration of hairy basil showed good repellency to *Ae. aegypti*. Deletre *et al.* (2013) tested the spatial repellency action of 20 plant extracts against *An. gambiae* using HITSS and found that citronella was one of twelve plants that had a repellent effect. The result showed that catnip had the highest repellency against *Ae. aegypti* at 2-3% concentrations but the greatest repellency to

An. minimus was found at 0.5 % concentration. Bernier *et al.* (2005) used an attraction and attraction inhibitor bioassay and found that catnip is demonstrated an effective spatial repellent. Picaridin showed the weakest repellency effect to both mosquitoes in this study with low SAI values and 0.5% picaridin was potentially and attractant to *Ae. aegypti* because the SAI value was less than zero. Additional results for toxicity assays found DEET was the most toxic to *Ae. aegypti* and *An. minimus.* Other studies have shown that DEET has insecticidal properties (Licciardi *et al.*, 2006). At high concentrations (2-3%) citronella and hairy basil were toxic to both test species. There were no toxic effects for picaridin against either mosquitoes. Vetiver showed no toxic effect on *Ae. aegypti*. Licciardi *et al.* (2006) found that picaridin did not have insecticidal activity. Phasomkusolsil and Soonwera (2011a) demonstrated using WHO test kit (WHO 1998), that lemongrass and citronella had lethal effects against *Ae. aegypti*, *Cx. quinquefasciatus,* and *An. dirus*. Deletre *et al*. (2013) found that cinnamon, citronella, savory, and thyme exhibited a toxic effect on *An. gambiae*.

DEET was highly effective in spatial repellency, irritancy and toxicity on *Ae. aegypti* and *An. minimus* in the current study. The highest percent active ingredient tested with citronella, hairy basil, and catnip displayed effective achievement of all three tested reactions as well. These plants properties can be developed as potential mosquito repellents and insecticides. However, plant essential oils are short-lived in their effectiveness since they can rapid evaporate compared with DEET. A previous study by Yang and Ma (2005) revealed that adding 5% vanillin gave longer the protection time against *Ae. albopictus*. Kongkaew *et al*. (2011) reported that citronella with 5% vanillin added, was effective for up to six hours. Recently, using nanotechnology in plant-based repellents can improve the capacity of mosquito repellancy and extend protection time (Nuchuchua *et al*., 2009).

The study provides contributory data on the mosquito-repellent property of plants against *Ae. aegypti* and *An. minimus*. This will be useful with the screening of plants for mosquito repellents. The data that obtained from these laboratory tests

should be confirmed in the field study to further support the effectiveness of the tested compounds because human and environmental factors could impact repellent efficacy. Further studies using human volunteers could be performed to obtain a complete data set.

Part 2 Behavioral responses of *Aedes aegypti, Aedes albopictus, Anopheles minimus,* and *Culex quinquefasciatus* to plant essential oils, synthetic repellents and pyrethroid insecticides using excito repellency test system

Insecticides commonly used to control the vectors of diseases, particulary. synthetic pyrethroids. This group included deltamethrin and permethrin. Recently, several papers have reported pyrethroid resistance in *Ae. aegypti*, *Ae.albopictus* and *An. minimus*, and in the nuisance-biting *Cx. quinquefasciatus* in Thailand (Ponlawat *et al.*, 2005; Paeporn *et al.*, 2006; Jirakanjanakit *et al.*, 2007; Chareonviriyaphap *et al.*, 2013). Thus, topical repellents could be used in combination with traditional indoor residual spraying (IRS) and bed net strategies for preventing mosquito bites. Nowadays, plant-based mosquito repellents are more popular to use for self-protection because of their safety to humans and natural scents. Many plants have been studied for their efficacy in repelling mosquitoes. Phasomkusolsil *et al.* (2010) reported that citronella essential oil (*Cymbopogon nardus*), phlai (*Zingiber cassumunar*) - a famous Thai herbal medicine belonging to the same family as ginger and sweet basil (*Ocimum basilicum*) provided protection against *Cx. quinquefasciatus*, *An. minimus* and *Ae. aegypti* when applied to the skin. Misni *et al.* (2008) also found that there was no significant difference in efficacy between *Piper aduncum* essential oil (Malaysian plant) and DEET when tested against *Ae. aegypti*. Also, Tawatsin *et al.* (2001) reported that turmeric, citronella grass and hairy basil combined with 5% vanillin provided improved the protection time against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus.* Such studies are encouraging that a plethora of natural repellent products are still to be discovered as effective tools for personal protection.

The current study measured irritant contact and repellent non-contact characteristics of eight compounds; citronella, hairy basil, catnip and vetiver (plant essential oils), DEET and picaridin (synthetic repellents) and two synthetic

pyrethroids (deltamethrin and permethrin) using the excito-repellency test system. The results showed that both pyrethroid insecticides were effective in eliciting an irritant rather than a repellent response against test vector populations, similar to previous findings reported by Chareonviriyaphap *et al.* (2004), Kongmee *et al.* (2004), and Dusfour *et al.* (2009). In these papers found that pyrethroids had an irritant effect when mosquitoes came into contact with the treated surface. Additionally, the results indicated that pyrethroids, particularly deltamethrin induced a knockdown effect and mortality against *Ae. aegypti* and *Ae. albopictus* in contact trials. In general, synthetic pyrethroids have a knock down and killing effect through direct contact (Elliott *et al.*, 1965).

Most essential oils were effective against 4 mosquito vector species, especially *Cx. quinquefasciatus* and *An. minimus* but some of them were much weaker *t*han DEET and/or deltamethrin and permethrin when tested against *Ae. aegypti* and *Ae. albopictus*. Previous study using the same excito-repellency test system found that *Cx. quinquefasciatus* had a strong behavioral escape response to clove, citronella and cinnamon whereas *Ae. aegypti* was less responsive and some were knocked down with clove (Suwansirisilp *et al*., 2012). Phasomkusolsil *et al.* (2010) revealed that *An. minimus* and *Cx. quinquefasciatus* were more sensitive to several different oils than *Ae. aegypti*. While Polsomboon *et al.* (2008) reported that *Ae. aegypti* showed increased escape rates in the contact chamber with 5% catnip but *An. harrisoni* had greater irritancy escape response to 2.5% catnip. Thus, the irritant/repellent efficacy of essential oils depends upon active ingredient, concentration, and mosquito species tested. Moreover, some plants have a toxic property to mosquitoes. Deletre *et al.* (2013) reported that 1% of cinnamon, citronella and thyme essential oils exhibited a toxic effect to *An. gambiae* and Phasomkusolsil and Soonwera (2011a) found that 10% lemongrass gave 100% mortality of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. dirus*. In this study, hairy basil and citronella achieved over 50% knockdown of *Ae. albopictus*. Similarly, Boonyuan *et al.* (2014) using the excito-repellency test system demonstrated that 10% hairy basil

gave almost 90% mortality of *Ae. aegypti* for contact assay and 69% mortality for non-contact assay while 10% citronella showed very high knockdown in *Ae. aegypti*. Therefore, hairy basil and citronella are potential candidates for the development as natural insecticides.

Behavioral responses against 2.5% DEET in the present study indicate that this compound elicited both a moderate repellent action against *Ae. albopictus* and *An. minimus* and an irritant effect against *Ae. albopictus*, *Cx. quinquefasciatus* and *An. minimus*. Tisgratog *et al.* (2011) found that DEET at 5% was greater escape response to *An. minimus* than bifenthrin at 25 mg/m^2.

Overall, this study showed the synthetic pyrethroids are the most effective against all four mosquito species tested. In addition, each mosquito species responded in different escape patterns to the various test compounds. Understanding how alternative natural products function to prevent mosquito human contact is vital in optimizing tools for personal protection. Insecticide resistance phenotypes in many important vector species will continue to pressure the scientific community to develop new and improved vector control strategies. Combination approaches are a good way to ensure the beneficial qualities of the various tools implemented (Gratz, 1993; Yap *et al.*, 1994; Lee *et al.*, 2010). Essential oils are one such area for exploration. As a result of the current study, citronella, hairy basil, catnip and vetiver essential oils could be served as potential mosquito repellent products against *Cx. quinquefasciatus* and *An. minimus*. Further testing with the higher concentrations of all essential oils should be performed to find an effective repellent dose against *Ae. aegypti*. According to Phasomkusolsil and Soonwera (2011b), increased concentration of essential oil can increase the repellent activity.

However, development of a natural repellent formulation with long lasting protection is necessary to be comparable to the effectiveness of the standard topical

repellent DEET. The irritant and/or repellent effects of test compounds described here must be validated with tests on field mosquitoes for confirmation.

CONCLUSION

Understanding of mechanistic actions of four plant essential oils, citronella oil, hairy basil oil, catnip oil and vetiver oil against mosquitoes is comepletely needed. The two test systems, high throughput screening (HITSS) and excito-repellency (ER) test systems are the appropriate designed for further study of behavioral response patterns in mosquitoes to volatile compounds. The HITSS was applied to evaluate through three behavioral modes of action (contact irritant, spatial repellent and toxic) of four plant essential oils on *Ae. aegypti* and *An. minimus*. While the excito-repellency test system was used in the testing of contact irritant action and non-contact repellent action of the plant essential oils against four mosquito species, *Ae. aegypti*, *Ae. albopictus*, *An. minimus* and *Cx. quinquefasciatus*. As a result, the behavioral responses of mosquitoes to four plant essential oils indicated that some plant essential oils had spatial repellent, contact irritant and/or toxic properties but some of these plant compounds performed better repellent than irritant to test mosquitoes (Appendix Tables 1 and 2). The study also found that each test mosquito species responded differently in escape patterns to the various test compounds. Therefore, the plant efficacy against mosquitoes depends on the type, chemical composition and concentration of the essential oils as well as the species of mosquito used in the test.

The recent study also found that the high throughput screening system and excito-repellency test system are useful tools to perform screening of plants for repellent activity. The high throughput screening system is suitable for working in the laboratory where temperature and humidity operating can be controlled because the materials are affected by high temperature (above 26 °C). This system is required a small amount of test compound and each assay of the HITSS is attained within a short time. In contrast, excito-repellency test system can be applied in both the laboratory and field experiments due to the whole tool set can be carried in convenient bags and

materials are not affected by surrounding temperature. These two systems are effective tools to help evaluate the potential of plant essential oils or extracts as natural mosquito repellents. This will lead to developed formulations of plant based repellents with long-lasting mosquito protection.

The study provides supportive data on the mosquito-repellent property of plants against *Ae. aegypti*, *Ae. albopictus*, *An. minimus* and *Cx. quinquefasciatus*. This will be useful with the screening of plants for mosquito repellents. The data that obtained from these laboratory tests should be confirmed in the field study to further support the effectiveness of the tested compounds because human and environmental factors could impact repellent efficacy.

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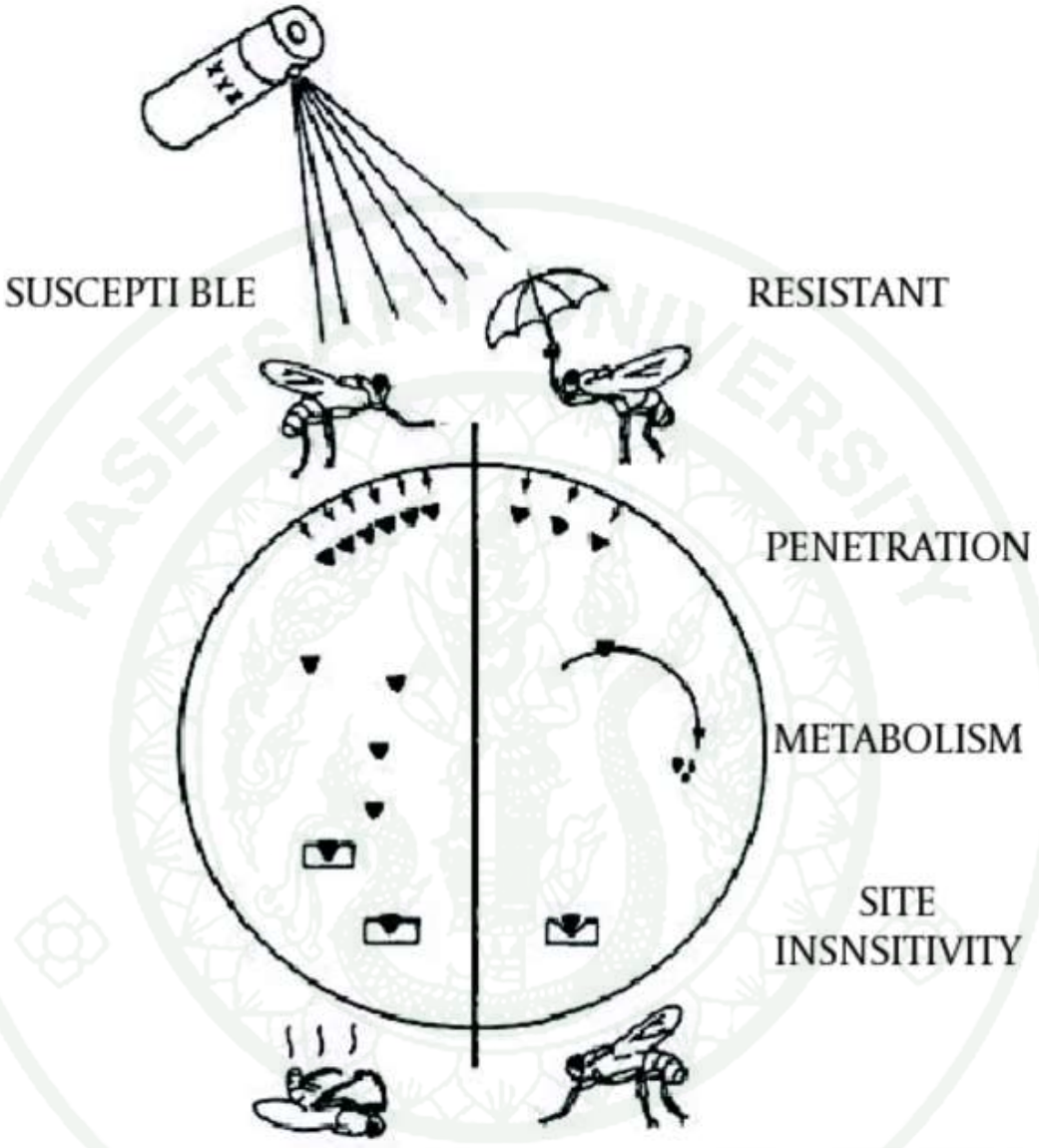
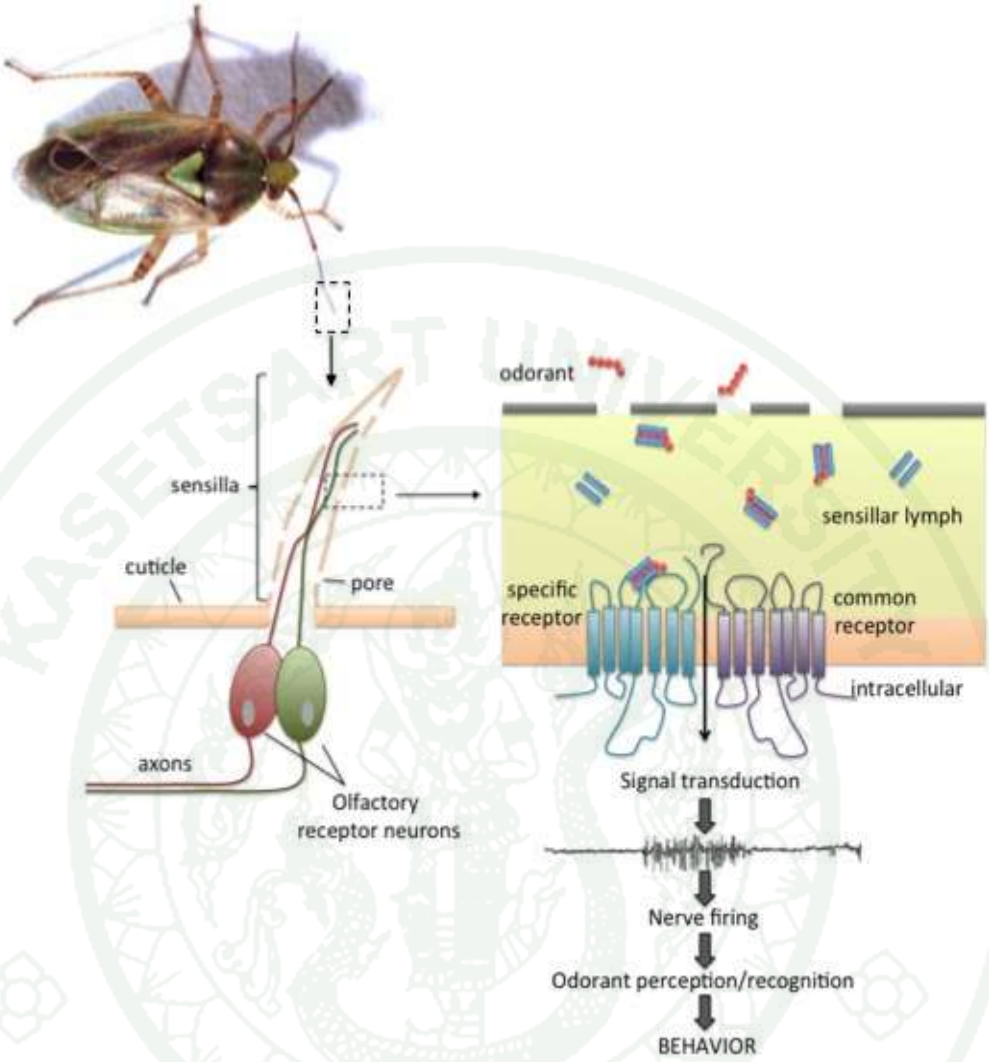
APPENDIX



Appendix Figure 1 Physiological resistance mechanisms in susceptible and resistant insects

Source: Georghiou,1986



Appendix Figure 2 Schematic of insect olfaction

Source: USDA,2012

Appendix Table 1 Essential oils as repellency for *Aedes aegypti*, *Aedes albopictus*, *Anopheles minimus* and *Culex quinquefasciatus*

Mosquito	Essential oil	Repellency to mosquitoes		
		Strong (>70%)	Moderate (50-70%)	Weak (<50%)
Ae. aegypti	Citronella			×
	Hairy basil			×
	Catnip			×
	Vetiver			×
Ae. albopictus	Citronella			×
	Hairy basil			×
	Catnip		×	
	Vetiver		×	
An. minimus	Citronella	×		
	Hairy basil	×		
	Catnip	×		
	Vetiver		×	
Cx. quinquefasciatus	Citronella	×		
	Hairy basil	×		
	Catnip	×		
	Vetiver			×

Data Source: Excito-repellency tests

Appendix Table 2 Essential oils as irritancy for *Aedes aegypti*, *Aedes albopictus*, *Anopheles minimus* and *Culex quinquefasciatus*

Mosquito	Essential oil	Irritancy to mosquitoes		
		Strong (>70%)	Moderate (50-70%)	Weak (<50%)
Ae. aegypti	Citronella			×
	Hairy basil			×
	Catnip			×
	Vetiver			×
Ae. albopictus	Citronella			×
	Hairy basil			×
	Catnip		×	
	Vetiver			×
An. minimus	Citronella	×		
	Hairy basil	×		
	Catnip	×		
	Vetiver	×		
Cx. quinquefasciatus	Citronella	×		
	Hairy basil	×		
	Catnip	×		
	Vetiver	×		

Data Source: Excito-repellency tests

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