1. Introduction

Many people living in areas of the tropical and subtropical world are at serious risk of infection from a wide variety of vector-borne diseases, most notably malaria and dengue. Globally, approximately 50-100 million people are estimated to be at risk of infection with dengue viruses (the cause of dengue fever/dengue haemorrhagic fever) and between 100-300 million live in malaria endemic areas (World Health Organization [WHO], 2009). The viruses responsible for dengue are transmitted primarily by *Aedes aegypti*, a predominately urban, day-biting mosquito that often resides in and around human dwellings and preferentially feeds on humans (Gubler, 1997); whereas the 4 human malaria parasites (*Plasmodium*) are transmitted by a wide variety of *Anopheles* species (Service & Townson, 2002). Dengue vector has proven extremely resilient to control measures because of its close association and exploitation of domestic and peridomestic human settings (Reiter & Gubler, 1997). On the other hand, malaria vectors display a more diverse array of host seeking behaviors and preference, biting patterns and larval breeding habitats (Pates & Curtis, 2005; Sinka et al., 2011). Despite decades of extensive research, efficacious and commercially viable vaccines for these 2 important vector-borne diseases are not yet available. Therefore, the prevention and control of dengue and malaria remains dependent on various vector control strategies to reduce risk of transmission; in some instances this requires the use of various chemical insecticides as larvicides, space spray and indoor residual spray (IRS) applications, and use of insecticide-impregnated bed nets to control adult mosquito blood feeding (Roberts & Andre, 1994; WHO, 1999; Reiter & Gubler, 1997; Grieco et al., 2007).

Chemical insecticides, including organochlorines, organophosphates, carbamates, and synthetic pyrethroids, have long been used with great effect in public health vector control programs worldwide (Reiter & Gubler, 1997; Roberts & Andre, 1994; WHO, 1992). Although DDT used ceased in many countries several decades ago, the chemical has returned for use in malaria control IRS programs in Africa because of some its superior attributes (Roberts & Tren, 2010). The dramatic impact of DDT on mosquito populations in terms of both toxicity and behavior suppressing disease transmission is well known but in some instances the actual mechanisms at work remain unclear and poorly understood. Most studies on insecticides have placed attention exclusively on the direct toxicological (knockdown and killing) effects on mosquito populations; whereas far less research has focused on the
behavioral responses and outcomes as a result of chemical exposure (Roberts, 1993; Roberts et al., 1997; Chareonviriyaphap et al., 1997). Studies have shown that most chemical compounds influence insect locomotor (movement) behavior, often resulting in profound excitation and pre-mature movement away from treated surfaces or areas, one or more kinetic mechanisms resulting in so-called “avoidance behavior” (Kongmee et al., 2004; Miller et al., 2009). The term “excito-repellency” is often used to describe behavior that is stimulated by direct contact with a chemical that results in abnormal excitation (sometimes termed ‘irritancy’) and spatial repellency that results without the insect making physical contact with the chemical (Roberts et al., 1997). This chapter describes the various behavioral responses to insecticides in some important mosquito vectors of malaria (Anopheles species) (Table 1) and dengue (Aedes aegypti) (Table 2) and the tropical house mosquito, Culex quinquefasciatus (Table 3) using two types of laboratory systems and field assays using experimental huts.

2. History of test systems to study mosquito behaviors

Behavioral responses of mosquitoes to chemical compounds can be directly demonstrated by using various laboratory devices and field assay systems. For laboratory assays, many of the variations have been reviewed (Roberts et al., 1997). The WHO developed the first test box using plywood in its construction attempting to access the excitation (“irritability”) of exposed mosquitoes following physical contact with insecticides (WHO, 1970). This system was subsequently referred to as an “excito-repellency” test box (Rachou et al., 1963). Subsequently, the test system was further modified by other investigators interested in behavioral avoidance responses exposed to DDT and some of the early synthetic pyrethroids (Charlwood & Paraluppi, 1978; Roberts et al., 1984; Bondareva et al., 1986; Rozendaal et al., 1989; Quinones & Suarez, 1989; Ree & Loong, 1989). Years later, a lightproof test chamber was designed to study the irritant response of Anopheles gambiae, an important malaria vector in Africa, to several chemical compounds (Evans, 1993). One key concern with all these test systems was associated with the technical difficulties of the test boxes for introducing and removing test specimens. Other concerns were controlling for the various physiological conditions of wild-caught mosquitoes, and selecting the ideal range of concentrations for chemical compounds. In addition, no single or set of statistical methods for analysis of data has been fully accepted nor has any test system been specifically designed to truely discriminate between contact excitation and noncontact repellency responses (Roberts et al., 1997). An improved excito-repellency test device that was able to clearly differentiate between excitation and spatial repellency was developed and initially tested against several field populations of Anopheles albimanus from Central America (Roberts et al., 1997; Chareonviriyaphap et al., 1997). Unfortunately, this fixed prototype was cumbersome to handle and required considerable time for attaching the chemical-treated test papers. Several years on, a more field-friendly test system was designed that was both collapsible and easily transportable (Chareonviriyaphap & Aum-Aong, 2000; Chareonviriyaphap et al., 2002) and was used to investigate the behavioral responses of various mosquito species and geographical populations from Thailand and a few populations from elsewhere in Asia (Chareonviriyaphap et al., 2001; Sungvornyothin et al., 2001; Kongmee et al., 2004; Pothikasikorn et al., 2005). Recently, a novel modular assay system was developed for mass screening of chemical actions; including contact irritancy, spatial repellency, or toxicity responses, on adult mosquitoes (Grieco et al., 2005).
modular system is substantially reduced in size compared to the previous excito-repellency box and minimizes the treated surface area and therefore the amount of chemical required for testing. In field observations, numerous attempts have been made to determine behavioral responses of mosquitoes using specially constructed experimental huts (Smith, 1965; Roberts et al., 1984, 1987; Roberts & Alecrim, 1991; Rozendaal et al., 1989; Bangs, 1999; Grieco et al., 2000; Grieco et al., 2007; Polsomboon et al., 2008; Malaithong et al., 2010). Most experimental hut studies have been conducted to observe the behavior of Anopheles mosquitoes with far fewer studies on other genera. Grieco et al., (2007) successfully demonstrated all 3 chemical actions using Ae. aegypti as a model system. The results obtained from both laboratory and field studies can help facilitate the choice of the most effective chemical measures to control house-frequenting adult mosquitoes.

Two standard systems used in the laboratory, the excito-repellency box (ERB) and the high throughput screening system (HITSS), and the experimental hut in the field to help characterize the behavioral responses of mosquitoes to chemical compounds are discussed herein.

2.1 Excito-Repellency Box (ERB) test system

Given the complexities of insect behavioral research, the excito-repellency testing design and testing methodology along with methods of analyzing and interpreting test data, have yet to be universally accepted. However, tremendous improvement has occurred in recent years that effectively addressed a number of the previous drawbacks, opening up significance progress in behavioral studies involving innate responses to insecticides. The ERB test system (Fig.1) was developed to distinguish irritancy (Fig. 2) and repellency (Fig. 3) (Roberts, et al., 1997) and was first used to study the avoidance behavior of Anopheles albimanus to DDT and synthetic pyrethroids in Belize, Central America in which consistent and reliable results were generated measuring the escape behavior of female mosquitoes (Chareonviriyaphap et al., 1997). Over time, the initial system was modified into a collapsible test chamber with identical dimensions and operational attributes to help alleviate some of the previous handicaps (Chareonviriyaphap et al., 2002). This improved version has been used extensively in the evaluation of behavioral responses by laboratory and field mosquito populations in Thailand.

In 2006, a more field compatible device was designed with a substantial reduction in chamber size to minimize the amount of chemical and treated paper required, provide greater ease of test preparation and allow use of a small number of test specimens (15 vs. 25 females) compared to the larger version (Tan asinchayakul et al., 2006). Using this system, consistent and reliable results have been produced (Muenworn et al., 2006; Polsomboon et al., 2008; Thanispong et al., 2009; Mongkalagoon et al., 2009).

The system comprises of 4 outer stainless steel sheet metal walls (Fig. 1). Each wall is constructed with an aluminum sliding rib on each end and socket providing a surface for the test paper holder in the middle. The test paper holder consists of 2 sides: a sheet of fine mesh iron screen attached on one side and a panel to hold the test papers in place on top on the opposite side. There is a 0.8 cm gap between the test paper and screen barrier to prevent mosquitoes from making physical contact (‘noncontact’) on the treated paper surface during the repellency assay. The paper holder simply has to be inverted to provide the proper conditions to expose the paper in the contact test. On one end of the chamber a portal is made of overlapping sheets of dental dam and is used for placing mosquitoes inside the chamber and later for removing them after the exposure test period. A Plexiglas door serves
to seal the chamber and allow the investigator to view the exposure chamber before and after the actual test period. A stainless steel outer rear door cover is used to shut off all external light inside the chamber when the experiment is being performed. Each test consists of enclosing 15 female mosquitoes in each of 4 chambers lined with either insecticide-treated or untreated (control) papers. On the opposite side of the Plexiglas portal is a single exit portal for mosquitoes to escape to a receiving cage. At the beginning of a test, a 3-min rest period allows mosquitoes to adjust to test chamber conditions before the exit portal is opened to initiate the observation period (30 or 60 min depending on experiment). The numbers of mosquitoes escaping from the chamber into receiving cage are recorded at 1 min intervals until test completion.

Fig. 1. Excito-repellency test chamber showing side of exit portal (Roberts et al., 1997).

Fig. 2. Excito-repellency test chamber: Irritability test design (Roberts et al., 1997).
2.2 High Throughput Screening System (HITSS)

Even though an ERB test system has been found valuable to evaluate the innate behavioral responses of mosquito to chemical compounds, this system is relatively resource intensive requiring a comparatively large amount of chemical to be used for treating papers and having to use a large number of test mosquitoes. Moreover, a current ERB design is not conducive for mass screening the candidate chemical compounds. With the development of the HITSS assay (Grieco et al., 2005), a smaller amount of chemical and a lower number of mosquitoes is required per test. In addition, the HITSS can also be configured to test each of the three actions of insecticide compounds, namely contact irritancy assay (CIA) (Fig. 4), spatial repellency assay (SRA) (Fig. 5) and the toxicity assay (TOX) (Fig. 6). This modular system is made from a variety of durable materials including a thick, clear plastic cylinder, metal chambers, hard plastic end caps and a butterfly valve to control the opening and closing of the door. For the CIA, a single clear plastic cylinder is connected to a metal chamber lined with either treated or untreated netting material using a butterfly valve placed in the open position (Fig. 4). For the SRA, an assay comprises a single clear plastic cylinder and 2 metal chambers on either end containing either treated or untreated (control) netting. The plastic chamber is positioned between the 2 metal chambers using a butterfly valve as a linking system (Fig. 5). For the TOX assay, a single metal chamber is equipped with plastic end caps (solid cap on one side and tunnel cap on the other end). Netting material treated with either chemical active ingredient (treatment) or acetone carrier only (control) lines the inner chamber (Fig. 6). The HITSS has been standardized and used to evaluate the 3 behavioral responses of *Ae. aegypti* against DEET, Bayrepel®, and SS220 (Grieco et al., 2005, 2007). Recently, HITSS has been used to define the behavioral responses among six field populations of *Ae. aegypti* from Thailand against three synthetic pyrethroids (Thanispong et al., 2010). From this study, it was clearly shown that the HITSS assay is an effective and easy to use tool for distinguishing the three actions of chemicals and screening new compounds.
Fig. 4 High throughput screening system (HITSS): (CIA) Contact Irritant Assay (Grieco et al., 2005)

Fig. 5. High throughput screening system (HITSS): (SRA) Spatial Repellency Assay (Grieco et al., 2005)

Fig. 6 High throughput screening system (HITSS): (TOX) Toxicity Assay (Grieco et al., 2005)
Behavioral Responses of Mosquitoes to Insecticides

2.3 Field-based assay: experimental huts

To better understand the behavior of a mosquito exposed to a residual avoidance of under more natural, realistic conditions, field studies should be performed using experimental huts. So-called hut studies provide valuable information that can facilitate vector control operations by helping select the most appropriate and effective tool in combating disease vectors. Since the 1940s, most attention has been directed to the study of *Anopheles* mosquitoes, yet few investigations have been carried out to describe the innate behaviors of mosquitoes entering, resting, biting (blood feeding) and exiting human dwellings (Gahan & Lindquist, 1945; Giglioli, 1948; Smith, 1965; Roberts et al., 1984; Bangs, 1999; Grieco et al., 2000; Pates & Curtis, 2005; Roberts et al., 1997). Another important vector species, *Ae. aegypti*, has received even less attention using of experimental hut assays (Suwannachote et al., 2009). The discovery of using natural pyrethrum extract to prevent human-vector contact inside homes was well known prior to 1945 (Muirhead-Thomson, 1951) and the first report of behavioral responses of malaria vectors to DDT was documented in 1947 (Kennedy, 1947, Brown, 1983). Strong behavioral responses to DDT were progressively reported up until 1975 (Muirhead-Thomson, 1960; Elliott & de Zulueta, 1975; Brown, 1983). For example, de Zulueta and Cullen (1963) observed significant reductions in the numbers of biting mosquitoes on human indoors and resting on walls in houses sprayed with DDT. However, there was no clear explanation on how (mechanism) DDT functioned by either repelling or preventing mosquitoes from locating host stimuli inside sprayed houses. A significant scientific observation on behavioral avoidance of *Anopheles gambiae* was carried out in the mid 1960s (Smith & Webley, 1968) wherein 2 huts, 1 control and one treated with DDT were equipped with verandah traps and gas chromatography was used to evaluate the subsequent response of mosquitoes. A hut treated with DDT was observed to prevent between 60 and 70% of *An. gambiae* from normally entering indoors as an outflow of DDT was continuously decreasing. Additionally, those females that did enter the sprayed hut became stimulated (irritated) and escaped the hut much quicker than those mosquitoes present in the unsprayed hut. Another study comparing biting patterns of *Anopheles minimus* in Thailand showed a 71.5% decline in attempted blood feeding inside the DDT treated hut and a 42.8% reduction in blood feeding success in a deltamethrin treated hut (Polsomboon et al., 2008). Using the same huts, observing human landing patterns of *Anopheles dirus* complex found that the relative risk (odds) of female mosquitoes entering and attempting to feed were half the number when exposed to DDT compared with the deltamethrin treated hut (Malaithong et al., 2010).

Of the possible responses a mosquito exposed to a chemical can preform, spatial repellency is perhaps the most interesting and important. Observing true repellency presents problems of accurate measurement in the natural field situation. One of classic methods is to use the experimental huts wherein 2 experimental huts, 1 untreated control and 1 treated with an active ingredient is fitted with entrance (measure of repellency) and exit (irritancy) traps as illustrated in (Figs. 7-9). A landmark study was conducted by Grieco et al., (2007) on the movement patterns of *Ae. aegypti* into and out of the experimental huts alternatively equipped with entrance (Fig. 8) and exit traps (Fig. 9) to describe the 3 chemical actions and mosquito responses as previously proposed in the mathematical framework for understanding the impact and relevance of repellency, irritability and toxicity on mosquito populations and disease transmission (Roberts et al. 2000). This study clearly showed that the 3 actions described and help to validate the model of actions. It was concluded that contact irritancy is the predominant action of synthetic pyrethroids, whereas spatial
repellency is the primary action of DDT. Dieldrin, a once commonly used cyclodiene compound, exhibited primarily a toxic action only.

Fig. 7 Experimental hut with traps attached to exterior walls.

Fig. 8 Experimental hut with entrance traps attached to interior walls.

Fig. 9. Experimental hut with exit traps attached to exterior walls.
3. Behavioral responses

To facilitate the study of behavioral responses of mosquitoes to chemical compounds, several test systems have been developed (Roberts et al., 1997; Chareonviriyaphap et al., 2001). The following is a brief review of important historical findings and more recent work from studies in Thailand on behavioral responses of mosquitoes to DDT and various synthetic pyrethroids commonly used in vector control.

3.1 Behavioral responses to DDT

Some agricultural and medically important insects, including vectors of malaria, demonstrate what has been termed “behavioral resistance” to DDT (Lockwood et al., 1984). However, the term “behavioral avoidance” as an innate response rather than a permanent genetic shift in behavior is preferred since the development of behavioral changes due to insecticide selective pressure in nature has not been adequately documented (Muirhead-Thomson, 1960). The behavioral responses of mosquitoes have been investigated using either specially constructed experimental huts and/or ERB test system. The first study on the irritant effect of DDT residual deposits was conducted on Anopheles quadrimaculatus where females were found to be irritated after short contact with treated surfaces with many quickly escaping the DDT treated house without taking a blood meal (Gahan & Lindquist, 1945). Subsequent observations found that An. quadrimaculatus had received a lethal dose and perished within 24 hours (Metcalf et al., 1945). Unfortunately, these studies made the observations without having control (untreated houses) for comparison. Moreover, the high mortality seen with An. quadrimaculatus may have been caused by further contacts with toxic active ingredients while attempting to leave a treated house through a small outlet (Muirhead-Thomson, 1960). In the studies with Anopheles albimanus in Panama, Trapido (1954) concluded that wild-caught mosquitoes lacking re-exposure to DDT for a long period of time, showed the same susceptibility levels to DDT as those from a laboratory colony with no a history of previous exposure. Malaria vectors in some countries (e.g., Brazil, Thailand) have never developed resistance to DDT (Roberts et al., 1984; Chareonviriyaphap et al., 2001), suggesting that the particular mosquito population possibly avoids making direct physical contact with the chemical, thereby precluding any selection for resistance. Table 1 lists Anopheles species tested and levels of behavioral responses to DDT and synthetic pyrethroids. DDT was found to be a strong contact irritant and more moderately as a repellent among 3 test populations of An. albimanus and that most specimens that quickly escaped DDT exposure survived (Chareonviriyaphap et al., 1997). This finding is in agreement with the results of the field studies by Roberts and Alecrim (1991) who reported a strong repellent action of DDT with Anopheles darlingi in a sprayed house. In a similar study, both irritancy and repellency escape responses were observed in 2 populations of Anopheles minimus with a major action of DDT being contact excitation (Chareonviriyaphap et al., 2001). Another study was made on the 2 complex species within the Minimus Subgroup; An. minimus (species A) and Anopheles harrisoni (species C). DDT produced a rapid and striking irritancy response in both species. Additionally, repellency was more pronounced with DDT on An. minimus but was seen to be much weaker with An. harrisoni (Pothikasikorn et al., 2005). With Ae. aegypti, results demonstrated that the higher the degree of physiological resistance to DDT, the greater the apparent suppression of both contact irritant and noncontact repellency responses, yet avoidance behavior was still a significant event (Thanispong et al., 2009).
Table 1. Degree of behavioral responses of female *Anopheles* species to synthetic pyrethroids and DDT at an operational field dose (mg/cm²).

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Permethrin</th>
<th>Deltamethrin</th>
<th>Cypermethrin</th>
<th>λ-cyhalothrin</th>
<th>Bifenthrin</th>
<th>DDT</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. albimanus</em></td>
<td>Lab</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Charoenviriyaphap et al., 1997</td>
</tr>
<tr>
<td><em>An. albimanus</em></td>
<td>Field</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>Charoenviriyaphap et al., 1997</td>
</tr>
<tr>
<td><em>An. albimanus</em></td>
<td>Field</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>Charoenviriyaphap et al., 1997</td>
</tr>
<tr>
<td><em>An. albimanus</em></td>
<td>Field</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>Charoenviriyaphap et al., 1997</td>
</tr>
<tr>
<td><em>An. minimus</em></td>
<td>Lab</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>Charoenviriyaphap et al., 2001</td>
</tr>
<tr>
<td><em>An. minimus</em></td>
<td>Field</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>Charoenviriyaphap et al., 2001</td>
</tr>
<tr>
<td><em>An. minimus</em></td>
<td>Lab</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>Charoenviriyaphap et al., 2004</td>
</tr>
<tr>
<td><em>An. dirus</em></td>
<td>Lab</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>Charoenviriyaphap et al., 2004</td>
</tr>
<tr>
<td><em>An. maculatus</em></td>
<td>Field</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Muenworn et al., 2006</td>
</tr>
<tr>
<td><em>An. swadwongporn</em></td>
<td>Field</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Muenworn et al., 2006</td>
</tr>
<tr>
<td><em>An. dirus</em></td>
<td>Field</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Charoenviriyaphap et al., 2004</td>
</tr>
<tr>
<td><em>An. minimus</em></td>
<td>Field</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Potikasikorn et al., 2005</td>
</tr>
<tr>
<td><em>An. harrisoni</em></td>
<td>Field</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Potikasikorn et al., 2005</td>
</tr>
<tr>
<td><em>An. swadwongporn</em></td>
<td>Field</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Muenworn et al., 2006</td>
</tr>
<tr>
<td><em>An. minimus</em></td>
<td>Field</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Muenworn et al., 2006</td>
</tr>
<tr>
<td><em>An. minimus</em></td>
<td>Field</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Pothikasikorn et al., 2007</td>
</tr>
<tr>
<td><em>An. harrisoni</em></td>
<td>Field</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Tisgarotog et al., 2011</td>
</tr>
</tbody>
</table>

I: Irritancy (Excitation and movement away from the source following direct contact with chemical stimulant)
R: Repellency (noncontact, spatial detection of chemical that results in movement away from the source)
+++ + 81-100% escaped from treated chamber
+++ 61-79% escaped from treated chamber
+++ 41-59% escaped from treated chamber
++ 21-40% escaped from treated chamber
+ 1-20% escaped from treated chamber but statistically significant (*P* < 0.05) from the matched control
3.2 Behavioral response to synthetic pyrethroids

A number of synthetic pyrethroids, i.e. allethrin, deltamethrin, permethrin, cypermethrin, alpha-cypermethrin, cyfluthrin among others, are commonly used by home owners, private business and government sectors to control both household mosquitoes and those as important vectors. These pyrethroids have found to exhibit a moderate to strong repellent effect for many agricultural and medically important insects (Lockwood et al., 1984) and were observed to cause mosquitoes to move away ('avoidance') from sprayed areas (Miller, 1990; Lindsay et al., 1991). The extensive and continuing use of pyrethroids should be a major stimulus to intensify observations on the significance of pyrethroid-induced avoidance behavior in mosquito vectors and other arthropods. Given the role of indoor residual spraying of homes as a means of controlling malaria transmission, the precise role of excitation and repellent actions of pyrethroids should be well defined for specific malaria vectors prior to beginning any large scale control program. Following the refinement of the ERB test system allowing separation of the 2 types of primary behavioral actions (Roberts et al., 1997), a series of important findings on excito-repellency behavior in *Anopheles* mosquitoes have been subsequently reported (Chareonviriyaphap et al., 1997, 2001, 2004; Pothikasikorn et al., 2005; Muenworn et al., 2006; Pothikasikorn et al., 2007). In general, synthetic pyrethroids produce much stronger irritating responses in *Anopheles* compared to repellency action (Table 1). For example, lambda-cyhalothrin and deltamethrin act as strong irritants on test populations of *An. minimus* complex mosquitoes while showing relatively weak repellency action (Chareonviriyaphap et al., 2001). Pothikasikorn et al. (2005) confirmed that Minimus complex species, *An. minimus* and *An. harrisoni* show a rapid irritancy to lambda-cyhalothrin and deltamethrin. Chareonviriyaphap et al., (2004) produced an extensive study to define the excito-repellency action of deltamethrin on 4 *Anopheles* species, all representing important malaria vectors in Thailand. Again, the findings demonstrated that deltamethrin produced a pronounced irritancy action compared to a much weaker repellency effect. Although repellency was less profound than contact excitation, the escape responses were statistically significant compared to the matched controls. A number of *Ae. aegypti* populations have been tested against a series of synthetic pyrethroids (deltamethrin, permethrin, alphacypermethrin, cyphenothrin, d-tetramethrin and tetramethrin) (Table 2). In general, all test populations of *Ae. aegypti* populations exhibit moderate to strong irritancy as compared with repellency (Grieco et al., 2005; Chareonviriyaphap et al., 2006; Paeporn et al., 2007; Thanispong et al., 2009, 2010). In addition, a few populations of *Culex quinquefasciatus* have been tested against the 3 principal classes of insecticides used in vector control; pyrethroids (deltamethrin), organophosphates (fenitrothion) and carbamates (propoxur) (Table 3). Striking differences in behavioral responses were seen between populations and active ingredients. Greater contact escape action was observed in a long-established colony exposed to deltamethrin and fenitrothion compared to two recent field populations (Sathantriphop et al., 2006).

To summarize, the behavioral responses to insecticides by mosquitoes are important components of a chemical’s overall effectiveness in reducing human-vector contact and transmission of disease. To date, there is no convincing example of behavioral resistance in mosquito species to insecticides, rather all evidence indicates actions on the part of exposed mosquitoes are part of an innate behavioral repertoire. Behavioral response can be split into 2 distinct categories, stimulus-dependent and stimulus-independent actions (Georgi, 1972). A stimulus-dependent response requires sensory stimulation of the insect in order for an avoidance action to proceed. In general, this form of avoidance enables the insect to
Table 2. Degree of behavioral responses of female *Aedes aegypti* to synthetic pyrethroids and DDT at an operational field dose (mg/cm²).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Deltamethrin</th>
<th>Permethrin</th>
<th>α-Cypermethrin</th>
<th>Cyfluthrin</th>
<th>Deltametrin</th>
<th>Tetramethrin</th>
<th>DDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field-R</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Field-R</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Field-S</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lab-S</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Field-S</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Field-S</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Field-S</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Field-S</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

I: Irritancy (contact excitation); R: Repellency (noncontact/spatial); R: Resistant; S: Susceptible to test compound

+++ 81-100% escaped from treated chamber
+++ 61-79% escaped from treated chamber
+++ 41-59% escaped from treated chamber
+++ 21-40% escaped from treated chamber
detect a chemical on direct contact or spatially before acquiring a lethal dose (Muirhead-Thomson, 1960). On the other hand, a stimulus-independent response does not require direct sensory stimulation of insect for avoidance to occur but rather involves other natural behavioral components such as exophily (outside resting) or zoophily (non-human blood preference) in which an insect avoids exposure to a chemical by preferentially utilizing habitats without active ingredients present (Byford & Sparks, 1987). This type of response has also been included in so-called “phenotypic and genotypic behaviors” (WHO, 1986). Stimulus-dependent behavioral responses include the avoidance behaviors discussed in detail in this chapter. The term avoidance behavior is generally used to describe actions that are stimulated by some combination of excitation (irritancy) and repellency, the former taking place following physical contact while spatial repellency results without physical contact with an insecticide (Roberts et al., 1987).

<table>
<thead>
<tr>
<th>Populations</th>
<th>Deltamethrin</th>
<th>Fenitrothion</th>
<th>Propoxur</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangkok*</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Nontaburi*</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mae Sot*</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*Highly resistance to deltamethrin
I: Irritancy, R: Repellency
++++: 81-100% escaped from treated chamber
+++ : 61-79% escaped from treated chamber
++ : 41-59% escaped from treated chamber
+  : 21-40% escaped from treated chamber
-  : < 20% escaped from treated chamber but statistically significant ($P < 0.05$) from the matched control

Table 3. Degree of behavioral responses of *Culex quinquefasciatus* to deltamethrin, propoxur and fenitrothion at an operational field dose (mg/cm²).

4. Conclusion

Any compound used to control (eliminate, reduce or otherwise prevent harm) insect populations have been termed an “insecticide”. An insecticide can also be defined as any compound that is used solely to kill insects. This single term of reference involving only one type of action (knockdown/death) is completely inadequate to describe the more meaningful and complete action of many insecticide compounds used against insect populations. Insects can respond to insecticides in at least 2 different ways; behavioral action, namely avoidance and toxicity. In the past, the prevailing practice has been to classify chemicals simply as toxicants for killing insects. As seen in this chapter, we introduce the term “chemical” in place of “insecticide” as it is more appropriate for recognizing the 2 other primary actions on mosquitoes vice toxicity alone.

Chemicals protect humans from the bites of mosquitoes through 3 different actions: excitation, repellency and toxicity (Roberts et al., 2000 & Grieco et al., 2007). Historically the vast majority of chemical studies have focused on the direct toxicological responses (susceptibility and resistance) of chemicals on mosquito populations whereas very little emphasis has been placed on the vector’s behavior in response to sub-lethal exposure. Knowledge of the mosquito’s behavioral responses to particular chemicals is highly significant in the prioritization and design of appropriate vector prevention and control.
strategies. Today, the development of insecticide resistance in insect pests and disease vectors occurs worldwide and on an increasing scale. However, resistance has still remained limited in many areas in spite of the long-term use of chemicals for control. This phenomenon suggests that behavioral responses likely play a significant role in how certain chemicals perform to interrupt human-vector contact while also reducing the selection pressure on a target insects for developing resistance (Roberts et al., 2000).

As discussed, at least 2 different types of mosquito behavioral response outcomes to chemicals are recognized; excitation and repellency. Whether acting from direct contact or from a distance (spatially) both response activities are based on stimulus-response actions that result in clear movement away from an area with the chemical present. There have been numerous attempts to accurately measure the behavioral responses of mosquitoes to chemicals using various types of excito-repellency test systems (e.g., ERB and HITSS). However, no single system has yet been completely accepted as a standardized method of testing or analyzing behavioral responses. Currently, no test system as recommended by the WHO can discriminate between contact irritancy and noncontact repellency. The WHO tests are based on the concept that mosquitoes respond to chemicals only after physical contact and that spatial repellency plays little or no meaningful role in disease control, or may actually be a detrimental attribute of a chemical. The test systems describe in this chapter have the capacity to differentiate both key behavioral responses and thus assigning each with relative importance in the potential prevention of disease transmission. Both the ERB and HITSS systems have been used to study behavioral responses of several important mosquito vectors and pests (Anopheles, Aedes, and Culex) to various test chemicals currently used in vector control programs while providing valuable and highly reproducible results. This knowledge will allow better decision making on chemical selection, application method and future product development. From the detailed investigations previously mentioned and elsewhere, we conclude that the behavioral responses of mosquitoes to chemicals are an important, if not critical, components of disease control operations.

5. Acknowledgments

The author would like to thank Dr. Michael J. Bangs for the critical review of this chapter. I am especially grateful to the Thailand Research Fund (TRF) and the Kasetsart University Research and Development Institute (KURDI) for providing the financial support over the many years.

6. References


Behavioral Responses of Mosquitoes to Insecticides


Insecticides - Pest Engineering
Edited by Dr. Farzana Perveen

Hard cover, 538 pages
Publisher InTech
Published online 15, February, 2012
Published in print edition February, 2012

This book is compiled of 24 Chapters divided into 4 Sections. Section A focuses on toxicity of organic and inorganic insecticides, organophosphorus insecticides, toxicity of fenitrothion and permethrin, and dichlorodiphenyltrichloroethane (DDT). Section B is dedicated to vector control using insecticides, biological control of mosquito larvae by Bacillus thuringiensis, metabolism of pyrethroids by mosquito cytochrome P40 susceptibility status of Aedes aegypti, etc. Section C describes bioactive natural products from sapindaceae, management of potato pests, flower thrips, mango mealy bug, pear psylla, grapes pests, small fruit production, boll weevil and tsetse fly using insecticides. Section D provides information on insecticide resistance in natural population of malaria vector, role of Anopheles gambiae P450 cytochrome, genetic toxicological profile of carbofuran and pirimicarp carbamic insecticides, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following: