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THESIS

A SURVEY OF SUSCEPTIBILITY/ RESISTANCE STATUS OF *Aedes* MOSQUITO VECTORS IN THAILAND TO SYNTHETIC PYRETHROIDS

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In this study, 32 *Aedes aegypti* strains from 29 provinces across Thailand were subject to bioassay for their susceptibility to three commonly used synthetic pyrethroids; permethrin, deltamethrin and lambda-cyhalothrin. Twelve strains of *Ae. aegypti* were resistant to permethrin, ranging from 43.54% mortality (Lampang strain) to 78% mortality (Chonburi strain and Prachuap Khiri Khan strain). The other 20 strains were possible candidates to be resistant (81.50%-96% mortality). Although, several strains of *Ae. aegypti* were found susceptible to deltamethrin, incipient resistance in this species was also observed in 11 strains. In contrast, all strains of *Ae. aegypti* were found susceptible to lambda-cyhalothrin, The study was also conducted on 5 strains of *Aedes albopictus* collected from the southern provinces. Only one strain was found resistant to permethrin (Sadao strain: 78% mortality). The other 4 strains were possible to be resistant to permethrin (84%-96% mortality). All strains of *Ae. albopictus* were susceptible to deltamethrin and lambda-cyhalothrin.

Key word: Pyrethroids, *Aedes aegypti*, *Aedes albopictus*, Resistance

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LIST OF ABBREVIATIONS

Ae.	=	Aedes
h	=	Hour
KD	=	Knockdown
MOPH	=	Ministry of Public Health
SE	=	Standard Error
WHO	=	World Health Organization

A SURVEY OF SUSCEPTIBILITY/ RESISTANCE STATUS OF *Aedes* MOSQUITO VECTORS IN THAILAND TO SYNTHETIC PYRETHROIDS

INTRODUCTION

Synthetic insecticides are commonly used in Thailand for the control of agricultural pests and disease vectors. Many chemical compounds, including organochlorines, organophosphates, carbamates and synthetic pyrethroids have been used in both agricultural practice and public health control programs.

The insecticidal compounds including organophosphates (temephos, fenitrothion, malathion and chlorpyrifos), carbamates (propoxur, pirimiphos methyl, and bendiocarb), synthetic pyrethroids (permethrin, deltamethrin, lambda-cyhalothrin, and etofenprox) and biological agents (*Bacillus thuringiensis israelensis* and *Bacillus sphaericus*) have been used to control mosquito vectors (Chareonviriyaphap *et al.*, 1999). After the first dengue fever (DF) and dengue hemorrhagic fever (DHF) outbreak in Thailand in 1958, DDT had been widely used to control mosquito vectors, including *Aedes*. The first report of DDT resistance in *Ae. aegypti* in Thailand was published by Neely (1994). In 1966, larvae of *Ae. aegypti* in Bangkok showed resistance to DDT, but they were susceptible to organophosphate compounds of which temephos and chlorpyrifos were the most effective larvicides (Bang *et al.*, 1969). The Control of *Aedes albopictus* (Skuse) is more difficult than that of *Aedes aegypti* (L.) because the wider range of habitat of the former. *Aedes albopictus* is most commonly found in suburban and rural areas where there are open spaces with considerable vegetation (Estrada-Franco and Craig, 1995). From 1986 to 1993, resistance of *Ae. aegypti* to temephos, malathion and fenitrothion was reported from many regions of Thailand (Chareonviriyaphap *et al.*, 1999). Currently, temephos is the most widely used compound for the control of *Ae. aegypti* larvae. During the peak period of adult *Aedes* populations, especially the rainy season, fogging application of cypermethrin and deltacide (deltamethrin and piperonyl butoxide) are used. Use of commercial products, especially those containing organophosphates and pyrethroids, has significantly increased in home in Thailand (Ponlawat *et al.,* 2005).

The susceptibility of *Ae*. *albopictus* to insecticides in Thailand is inadequately documented. In 1967, temephos application and malathion fogging on Samui Island in the Gulf of Thailand did not significantly reduce the number of containers with *Ae*. *albopictus* larvae as reported by Gould *et al.,* 1970. In contrast, their study reported that the number of *Ae. aegypti* adults and the percentage of infested containers declined after insecticide applications. In 1997, a small number of adult *Ae*. *albopictus* reared from larvae collected from different locations in Thailand were assayed following the standard World Health Organization (WHO) contact test against DDT, permethrin, and fenitrothion (Somboon *et al.,* 2003). These results indicated that *Ae*. *albopictus* populations from these sites were resistant to DDT, but susceptible to permethrin and fenitrothion

Synthetic pyrethroids especially permethrin and deltamethrin are the current insecticides of choice for vector control in Thailand. These pyrethroids have been used for the impregnation of bed net and/or indoor residual house spray in many parts of the country. Deltamethrin has been the major adulticide used in the national *Aedes* control program over the last two decades until present (Tanispong *et al*., 2008). Diagnostic doses of various insecticides have been established according to WHO criteria mainly for malaria vectors, although it has been suggested that same diagnostic doses can be applied to susceptibility/resistance for *Aedes* mosquitoes (Jirakanjanakit *et al*., 2007).

OBJECTIVES

The primary objective of this study is to investigate susceptibility/resistance of *Aedes* mosquito vectors from different locations against lambda-cyhalothrin and commonly used synthetic pyrethroid insecticides for vector control in Thailand.

LITERATURE REVIEW

Dengue and dengue situation in Thailand

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are vector-borne diseases of public health importance in tropical and subtropical regions of the world (Gubler, 1997). These diseases are transmitted from person to person by the bite of infected *Aedes* mosquitoes, principally *Ae. aegypti*. Illness can include a variety of other signs and symptoms ranging from fever, headache, joint pain, nausea and vomiting (DF), high fever, subdermal hemorrhage, hepatomegaly, lowered platelet count, high bleeding tendency (DHF), to the same clinical signs and symptoms as DHF with circulatory failure, shock until death (dengue shock syndrome) (Anon., 1980). The incidence and geographic distribution of dengue have increased dramatically in recent decades. More than half of the world's population now lives in areas at risk of infection. The first reported epidemics of dengue fever occurred in Asia, Africa, and North America in the 1779-1780, the disease was identified and named in 1779. Initially it was rather benign. A global pandemic began in Southeast Asia in the 1950s; by 1975, DHF had become a leading cause of hospitalization and death among children in many countries in those regions. In the 1980s, DHF began a second expansion into Asia when Sri Lanka, India and the Maldive Islands faced their first DHF epidemic. Dengue has become more common since the 1980s, and by the late 1990s dengue was the most important mosquito-borne (viral) disease affecting humans after malaria (Gubler, 1998).

In Thailand, the incidence of DF and DHF has increased cyclically since the first recognized outbreak in 1958 (Nimmannitaya, 1978). The first outbreak of this disease occurred only in Bangkok and Thonburi Province, with 2,706 cases and 296 death cases. After that, this disease occurred in some provinces close to Bangkok in 1963-1964 and spread to the provinces connected to Bangkok by transportation in 1965-1967 and from the year 1978 then this disease has become recognized as one of the major public health problems (Wangrungsap, 1992). Mosquitoes vector control is an important method to prevent dengue virus transmission. Several methods are available for the control of mosquitoes to include chemical control (larvicide and adulticide). Chemical compounds including organochlorines, organophosphates, carbamates and synthetic pyrethroids have been used to control both the adult and larval forms of mosquito vectors (Charoenviriyaphap *et al*., 1999).

Dengue virus vector in Thailand

Ae. aegypti (Diptera: Culicidae), the principal vector of DF and DHF in the world, probably originated in the forest of Africa. It is widely distributed in the Southeast Asia, West Pacific, Africa and Americas (Christophers, 1960). In Africa and Central and South America, this vector has also been known as the principal vector of urban yellow fever (Scanlon, 1965). It is a peridomestic species found not far from human dwellings. *Ae. aegypti*, a daytime biting mosquito, is highly anthropophilic, often resides in and near human dwellings and preferentially feeds on humans (Gubler, 1997; Yasuno and Tonn, 1969).

The life cycle of *Ae. aegypti* is a complete metamorphosis and consists of four developmental stages namely, egg, larva, pupa and adult. Each of these stages can be easily recognized by its special appearance. The breeding sites of *Ae. aegytpi* in Asia are located around houses in relatively clean water which is stored in containers, both indoors and outdoors, as well as miscellaneous containers such as flower pots , ant traps also serve as breeding sites. The eggs are approximately 1 mm long and pale white when freshly laid and turning to black in color within a short time. Fertilized eggs are deposited singly just above the water line of containers. The eggs are capable of withstanding desiccation for a week or months. These eggs hatch only when flooded with water, but not all eggs hatch at the same time. Larvae are aquatic. The larvae have four instars. It consists of 3 portions, head, thorax and abdomen. A siphon or air tube is located at the tip of the abdomen through which air is taken in when the larvae come to the water surface to breathe. Larvae dive to the bottom for short periods in order to feed or escape danger when disturbed. The larvae feed on the aquatic micro biota that present in artificial containers. Factors governing the duration of larvae development are temperature, food availability and larval density in the receptacles. The pupal period is quite short, usually 1-2 days. Pupa does not feed but swims and floats. Internal changes occur to transform the larva into an adult mosquito. Breathing is via trumpets (Pant and Self, 1993). After emerging, the adult rests on the wall of the breeding site for a few hours to allow the exoskeleton and wings to harden. Within 24 hours after emergence, both sexes can mate. After mating, the female of *Ae. aegypti* requires a blood meal because of the development of the eggs. Human blood is preferred over other animals, often biting indoor or in sheltered areas near the house. Females bite and feed mainly during the day or early evening. Three to four days later the gravid females search for suitable places to deposit their eggs and prefer to lay eggs one by one on a surface with a high degree of dark color, roughness and water absorption. This process is repeated until the mosquito dies. A female lays an average of 100 and 150 eggs at a time (Chan, 1971).

Chikungunya situation in Thailand

Chikungunya (in the Makonde language "that which bends up") virus (CHIKV) is an insect-borne virus, of the genus, *Alphavirus*, that is transmitted to humans by virus-carrying *Aedes* mosquitoes, there have been recent outbreaks of CHIKV associated with severe morbidity. CHIKV causes an illness with symptoms similar to dengue fever. It manifests itself with an acute febrile phase of the illness lasts only two to five days, followed by a prolonged arthralgic disease that affects the joints of the extremities. The pain associated with CHIKV infection of the joints persists for weeks or months (Chhabra *et al.*, 2008; Sourisseau *et al*., 2007). It is indigenous to tropical Africa and Asia, where it is transmitted to humans by the bite of infected mosquitoes, usually of the genus *Aedes*. CHIKV belongs to alpha-virus under Togavirdae family. It is an "Arbovirus" (Ar-arthropod, bo-borne). Chikungunya fever epidemics are sustained by human-mosquito-human transmission. The word "chikungunya" is thought to derive from description in local dialect of the contorted posture of patients afflicted with the severe joint pain associated with this disease. The main virus reservoirs are monkeys, but other species can also be affected, including humans (Edelman *et al.*, 2000).

The outbreaks in the region were observed in Thailand in 1995, followed by 24 outbreaks in Indonesia between 2001 and 2003. In March 2005, an outbreak of chikungunya fever occurred in Reunion Island, where nearly 35% of the population of 770,000 were infected in six months and 254 deaths. In India, the outbreaks of chikungunya fever started in December 2005 and in 2006, nearly 1.4 million cases of suspected chikungunya fever were reported from 13 states and 210 districts. An outbreak of chikungunya fever occurred in Sri Lanka in October 2006, wherein nearly 38,000 people were reported affected. In Maldives, an outbreak of chikungunya fever was reported in December 2006, wherein 11,000 people (4.5% of total population) were reported affected. In Thailand in January 2009 more than 4,000 people were reported affected (MOPH, 2008).

Chikungunya virus vector in Thailand

The Asian tiger mosquito or forest day mosquito (*Aedes (Stegomyia) albopictus*), from the mosquito family Culicidae, is characterized by its black and white striped legs, and small black and white body. *Aedes albopictus* has also been implicated as an epidemic vector of chikungunya (Reiter et al. 2006). It is native to the tropical and subtropical areas of Southeast Asia; however, in the past couple of decades this species has invaded many countries throughout the world through the transport of goods and increasing international travel (Scholte and Schaffner, 2007). This mosquito has become a significant pest in many communities because it closely associates with humans (rather than living in wetlands), and typically flies and feeds in the daytime in addition to at dusk and dawn.

The Asian tiger mosquito is about 2 to 10 mm long with a striking white and black pattern (Walker, 2007). The variation of the body size in adult mosquitoes depends on the density of the larval population and food supply within the breeding water. Since these circumstances are only seldom optimal, the average body size of adult mosquitoes is considerably smaller than 10 mm. For example, the average length of the abdomen was calculated to be 2.63 mm, the wings 2.7 mm, and the proboscis 1.88 mm through a study of 10 images from 1962 of both male and female mosquitoes (John, 1962).

It is known that *Ae. albopictus* can transmit pathogens and viruses, such as, the West Nile virus, yellow fever virus, St. Louis encephalitis, dengue fever, and chikungunya fever to name a few. The Asian tiger mosquito was responsible for the chikungunya epidemic on the French Island La Réunion in 2005/2006. By September 2006, there was an estimated 266,000 people that were infected with the virus and 248 fatalities on the island. The Asian tiger mosquito was also the transmitter of the virus in the first and only outbreak of chikungunya fever on the European continent. This outbreak occurred in the Italian province of Ravenna in the summer of 2007, and infected over 200 people. Evidently, mutated strains of the chikungunya virus are being directly transmitted through *Ae. albopictus* particularly well and in such a way that another dispersal of the disease in regions with the Asian tiger mosquito is feared (Chhabra *et al.*, 2008; Edelman *et al.*, 2000. and Tsetsarkin *et al.*, 2007).

Synthetic pyrethroid insecticides

Synthetic pyrethroids are an important class of insecticides that have been proved to be effective in controlling arthropods of medical and veterinary importance. Synthetic pyrethroids such as permethrin, deltamethrin, and lambda-cyhalothrin are the current insecticides of choice for vector control (Zerba, 1988). The pyrethroids derived from a group of insecticidal esters, of which both the alcohol and carboxylic acid moieties may have isomeric forms so that each pyrethroid may have several isomers. These insecticides show remarkably high toxicity and rapid action against a wide range of insects, but relatively low mammalian toxicity (Sathantriphop *et al.*, 2006).

Pyrethroids are synthetic chemical analogues of pyrethrins, which are naturally occurring insecticidal compounds produced in the flowers of chrysanthemums (*Chrysanthemum cinerariaefolium*). Insecticidal products containing pyrethroids have been widely used to control insect pests in agriculture, public health, and homes and gardens. Pyrethroids are important tools used in public health management where applications are made to control cockroaches, mosquitoes, ticks, and flies, which may act as disease vectors.

Pyrethroids have been used for the impregnation of bed nets or as indoor residual house spray. The Ministry of Public Health (MOPH) in Thailand has recommended pyrethroid insecticides as the compounds to be used in public health to control mosquito vectors (MOPH, 2008).

Mode of action of synthetic pyrethroid insecticides

Synthetic pyrethroids act on the nervous system by modifying the gating kinetics of voltage-sensitive sodium channels. The compounds make the nervous system hypersensitive to stimuli from sense organs. Rather than sending a single impulse in response to a stimulus, exposed nerves send a train of impulses. This excitation occurs because it blocks the movement of sodium ions from outside to inside of the nerve cells (Lund and Narahashi, 1983).

Permethrin

Permethrin is a broad spectrum synthetic pyrethroid insecticide, used against a variety of pests, on nut, fruit, vegetable, cotton, ornamental, mushroom, potato and cereal crops. It is used in greenhouses, home gardens, and for termite control. It also controls animal ectoparasites, biting flies, and cockroaches. It may cause a mite buildup by reducing mite predator populations. Permethrin is available in dusts, emulsifiable concentrate, smoke, ULV (ultra-low volume) and wettable powder formulations (EXTOXNET, 1995).

Permethrin is a moderately to practically non-toxic pesticide in EPA toxicity class II or III, depending on the formulation. Formulations are placed in class II due to their potential to cause eye and skin irritation. Products containing permethrin must bear the signal word WARNING or CAUTION, depending on the toxicity of the particular formulation. All products for agricultural uses (except livestock and premises uses) are Restricted Use Pesticides (RUPs) because of their possible adverse effects on aquatic organisms. Restricted Use Pesticides may be purchased and used only by certified applicators (EXTOXNET, 1995). Trade names include Ambush, Cellutec, Dragnet, Ectiban, Eksmin, Exmin, Indothrin, Kafil, Kestrel, Pounce, Pramex, Qamlin, and Torpedo(EXTOXNET, 1995).

Permethrin is moderately to practically non-toxic via the oral route, with a reported LD50 for technical permethrin in rats of 430 to 4000 mg/kg. Via the dermal route, it is slightly toxic, with a reported dermal LD50 in rats of over 4000 mg/kg, and in rabbits of greater than 2000 mg/kg. Permethrin caused mild irritation of both the intact and abraded skin of rabbits. It also caused conjunctivitis when it was applied to the eyes. The 4-hour inhalation LC50 for rats was greater than 23.5 mg/l, indicating practically no inhalation toxicity. The toxicity of permethrin is dependent on the ratio of the isomers present; the cis-isomer being more toxic (EXTOXNET, 1995).

Deltamethrin

Deltamethrin is a pyrethroid insecticide that kills insects on contact and through digestion. It is used to control apple and pear suckers, plum fruit moth, caterpillars on brassicas, pea moth, aphids (apples, plums, hops), winter moth (apples and plums), codling and tortrix moths (apples). It is used to control aphids, mealy bugs, scale insects, and whitefly on glasshouse cucumbers, tomatoes, peppers, potted plants, and ornamentals. It also controls numerous insect pests of field crops. Formulations include emulsifiable concentrates, wettable powders, granules ULV and flowable formulations. There are no known incompatibilities with other common insecticides and fungicides. Deltamethrin is a synthetic insecticide based structurally on natural pyrethrins, which rapidly paralyze the insect nervous system giving a quick knockdown effect. Deltamethrin has a rapidly disabling effect on feeding insects and for this reason there is hope that it may be useful to control the vectors of "non-persistent" viruses (viruses that can be passed on by the vector within a few minutes of starting to feed on the plant). Deltamethrin's mode of action is thought to be mainly central in action, or at least originates in higher nerve centers of the brain. Death of insects seems to be due to irreversible damage to the nervous system occurring when poisoning lasts more than a few hours. Deltamethrin poisoning occurs through cuticular penetration or oral uptake. The susceptibility of insects is dependent on a variety of factors and can vary, as with many insecticides, according to the environmental conditions. Flies are most susceptible to pyrethroid poisoning shortly before dawn. The LD50 drops by the factor of 2 as compared to full daylight activity . Many pyrethroids are not very active against cattle ticks, but some alpha cyano compounds (of which deltamethrin is one) have higher activity than organophosphates or amidines, the former standard compounds for this purpose. Deltamethrin has very good residual activity for outdoor uses (field crops, cattle dip, tsetse) and for indoor uses (mosquitoes, stable flies, horsefiles, fleas, cockroaches, stored product insects). Deltamethrin has very broad spectrum control. It is considered the most powerful of the synthetic pyrethroids. It is up to three orders more active than some pyrethroids (EXTOXNET, 1995).

Deltamethrin is used in the U.S. in the Environmental Health Market. It is being sold in many countries for agricultural, public health and livestock applications. The active ingredient deltamethrin is found in a variety of commercial insecticide products. Trade names for products containing deltamethrin include Butoflin, Butoss, Butox, Cislin, Crackdown, Cresus, Decis, Decis-Prime, K-Othrin, and K-Otek (EXTOXNET, 1995).

Deltamethrin produces typical type II motor symptoms in mammals. Type II symptoms include a writhing syndrome in rodents, as well as copious salivation. The acute oral LD50 in male rats ranged from 128 mg/kg to greater than 5,000 mg/kg depending on the carrier and conditions of the study; the LD50 for female rats was 52 mg/kg and other published values range from 31 to 139 mg/kg. Values ranging from 21 to 34 mg/kg were obtained for mice; while dogs had a reported LD50 of 300 mg/kg. The intravenous LD50 in rats and dogs was 2 to 2.6 mg/kg, and the dermal LD50 was greater than 2,940 mg/kg . The acute percutaneous LD50 for rats was reported to be greater than 2,000 mg/kg; greater than 10,000 mg/kg for quail; and greater than 4,640 mg/kg for ducks. The acute dermal LD50 for rabbits was greater than 2,000 mg/kg. No skin irritation and slight eye irritation were reported. Another study indicated skin irritation in rats and guinea pigs. The signs of poisoning produced in rats by deltamethrin are not the same as those produced by other pyrethroids. Especially characteristics are rolling convulsions. The site of action is considered to be central with little or none of the peripheral component demonstrated for other pyrethroids. The sequence of signs is clearly defined, progressing from chewing, salivation, and pawing to rolling convulsions, tonic seizures, and death. Blood pressure begins to drop promptly, but slowly; it tends to normalize about the time choreoathetosis (abnormal movements of the body of a combined choreic and athetoid pattern) begins but falls precipitously prior to death. The early signs, including choreoathetosis, are reversible, but rats that exhibit a tonic seizure and shock almost always die promptly. Acute exposure effects in humans include the following: ataxia, convulsions leading to muscle fibrillation and paralysis, dermatitis, edema, diarrhea, dyspnea, headache, hepatic microsomal enzyme induction, irritability, peripheral vascular collapse, rhinorrhea, serum alkaline phosphatase elevation, tinnitus, tremors, vomiting and death due to respiratory failure. Allergic reactions have included the following effects: anaphylaxis, bronchospasm, eosinophilia, fever, hypersensitivity pneumonia, pallor, pollinosis, sweating, sudden swelling of the face, eyelids, lips and mucous membranes, and tachycardia. Studies have shown many cases of dermal deltamethrin poisoning after agricultural use with inadequate handling precautions, and many cases of accidental or suicidal poisoning by the oral route at doses estimated to be 2-250 mg/kg. Oral ingestion caused epigastric pain, nausea, vomiting and coarse muscular fasciculations. With doses of 100-250 mg/kg, coma was caused within 15-20 minutes (EXTOXNET, 1995).

Lambda-cyhalothrin

Lambda-cyhalothrin is a synthetic pyrethroid insecticide and acaricide used to control a wide range of pests in a variety of applications. Pests controlled include aphids, Colorado beetles and butterfly larvae. Crops on which it may be applied include cotton, cereals, hops, ornamentals, potatoes, vegetables or others. It may also be used for structural pest management or in public health applications to control insects such as cockroaches, mosquitoes, ticks and flies which may act as disease vectors. Lambda-cyhalothrin is available as an emulsifiable concentrate, wettable powder or ULV liquid, and is commonly mixed with buprofezin, pirimicarb, dimethoate or tetramethrin. It is compatible with most other insecticides and fungicides. Unless otherwise stated, data presented herein refer to the technical product (EXTOXNET, 1995).

Lambda-cyhalothrin is a Restricted Use Pesticide and so may be purchased and used only by certified applicators. It is in EPA Toxicity Class II, and products containing it must bear the signal word WARNING (EXTOXNET, 1995). Trade names for products containing lambda-cyhalothrin include Charge, Excaliber, Grenade, Hallmark, Icon, Karate, Matador, Saber, Samurai and Sentinel (EXTOXNET, 1995).

Lambda-cyhalothrin is moderately toxic in the technical form, but may be highly toxic via some routes in formulation (e.g., as Karate). Available data indicate that lambda-cyhalothrin is moderately toxic via the oral route in test animals. Reported oral LD50 values are 79 mg/kg and 56 mg/kg for male and female rats, respectively. The vehicle used was corn oil. The rat oral LD50 has also been reported as 144 mg/kg. The reported rat LD50 for the technical product is similar, 64 mg/kg. These indicate moderate acute toxicity via the oral route of exposure. No data were available regarding the acute toxicity of the technical compound via the inhalation route, but for Karate the reported 4-hour inhalation LC50s were 0.175 mg/l and 0.315 mg/l for female and male rats, respectively. These data indicate a moderate to high toxicity via the inhalation route for the formulated product, Karate. The technical product has reported dermal LD50s of 632 mg/kg and 696 mg/kg for male and female rats (vehicle used was propane-1,2-diol). It has also been found to be non-irritating to the skin of rabbits and non-sensitizing to the skin of guinea pigs but may cause mild eye irritation in rabbits. The formulated product, Karate, however, causes severe primary skin irritation in rabbits and mild skin sensitization in guinea pigs. Primary eye irritation also was observed with the technical product. In addition to the corrosive effects to skin and eyes, other acute effects due to exposure to lambda-cyhalothrin, like those of other pyrethroids, will be mainly neuropathy (effects on the nervous system). Cyhalothrin may act on ion channels within the nerve cells (neurons) to disrupt proper function of the cells of both the peripheral and central nervous systems. At lower doses, this may take the form of stable, repetitive firing of the neuron, but high doses may result in depolarization of the nerve cell and blockage of conduction. These effects may result in observable effects such as: tingling, burning or numbness sensations (particularly at the point of skin contact); tremors, incoordination of movement, paralysis or other disrupted motor function, and confusion or loss of consciousness. Since most pyrethroids are generally absorbed only poorly through the skin, the latter two systemic effects are unlikely unless the compound has been ingested. Effects are generally reversible due to rapid breakdown of the compound in the body. Like many compounds of the pyrethroid family, the observed toxicity of lambda-cyhalothrin may vary according to not only the concentration of the active ingredient, but also according to the solvent vehicle (EXTOXNET, 1995).

MATERIALS AND METHODS

Study sites

Aedes aegypti larvae and pupae were collected from containers located in and around houses on several provinces in Thailand. Additionally, *Ae. albopictus* larvae were collected as immature stages (larvae/pupae) from water containers in rubber trees and around houses on different provinces in southern Thailand. GPS coordinates and a brief description of the locations are shown in Table 1.

Mosquito strains

This study used standard strains as susceptible strains and field strains collected in the field. For *Ae. aegypti*, mosquitoes were collected from 29 provinces containing 32 strains, for *Ae. albopictus*, mosquitoes were collected from 4 provinces containing 5 strains.

1. Standard strains

Standard strains(laboratory strains) maintained in laboratory at the Department of Entomology, Faculty of Agriculture, Kasetsart University, were served as the insecticide susceptible strains.

A colony of *Ae. aegypti* (USDA strain) was supplied by the United States Department of Agriculture (USDA), Gainesville, Florida. A colony of *Ae. albopictus* (MOPH strain) was supplied by Ministry of Public Health.

2. Field strains

Aedes aegypti and *Ae. albopictus* field strains collected as immature stages from different localities in Thailand, these strains were reared and maintained at the

Department of Entomology, Kasetsart University. F1-F3 generations of field-derived populations were used to expose to diagnostic doses of permethrin, deltamethrin and lambda-cyhalothrin.

Aedes aegypti was collected from 29 provinces containing 32 strains from several regions as follows (Table 1):

Central: Bangkok.

North: Tak, Nakhonsawan, Uthai Thani, Phrae, Chiang-Rai, Lampang, Kamphaeng Phet, Chiang-Mai, Lamphun.

West: Kanchanaburi, Prachuap Khiri Khan.

East: Chonburi, Chanthaburi, Prachinburi.

South: Phang Nga, Phuket, Phattalung, Surat Thani Surat, Chumphon, Songkhla(Sadao strain, Namom strain, Ranode strain and Had Yai strain).

Northeast: Nakhon Ratchasima, Burirum, Surin, Si Sa Ket, Kalasin, Roi Et, Udonthani, Khonkaen.

Aedes albopictus was collected from 4 provinces containing 5 strains in South of Thailand (Table 1): Prachubkririkhun, Chumphon, Surat thani, Songkhla (Sadao strain and Namom strain).

Mosquito colonization

Laboratory strains and field strains of *Ae. aegypti* and *Ae. albopictus* were kept separately to ensure no accidental cross-breeding (hybridized) populations. All developmental stages were reared in a temperature-controlled space at 25 ±5°C, and 80±10% relative humidity using a 12h:12h light:dark phase. Immature stages were reared in plastic pans under identical density, physical and nutritional conditions throughout the study. Pupae were transferred into the cups with tap water and placed in screen (30 x 30 x 30cm) cages. Adult males and females were identified to species and provided with cotton pads soaked with 10 % sugar solution (Kongmee, 2004). Following free mating, between days 2 and 5, post- emergence female mosquitoes were allowed to feed on a live mouse. An oviposition site (Petri dish) containing tap water and filter paper (Whatman® No. 1) is placed in the cages for egg deposition following 2-3 days ovarian development. The resultant progeny and adults from F_1 to F_3 generations were utilized for testing.

Insecticides

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

2. Deltamethrin[(S)-alpha-cyano-3-phenoxybenzyl(1R,3R)-3-(2,2 dibromovinyl) -2, 2-dimethyl cyclopropanecarboxylate] (98% purity) was provided by BASF, Bangkok, Thailand.

3. Lambda-cyhalothrin [(RS)-alpha-cyano-3- phenoxybenzyl (Z)-(1RS,3RS)-3- (2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethyl cyclopropanecarboxylate] (91.8% purity) was provided by Syngenta Crop Protection, Bangkok, Thailand.

Insecticide-impregnated papers

Test papers (Whatman® No. 1 size 12×15 cm^2) were impregnated with 0.75, 0.05 and 0.05% diagnostic doses of permethrin, deltamethrin and lambda-cyhalothrin, respectively, for use in susceptibility test. All treated papers were prepared according to World Health Organization specification (WHO, 1998) and treated at the rate of 2 ml of insecticide solution per paper. Control papers were impregnated with carrier diluents only (acetone and silicone oil).

Insecticide susceptibility test in mosquito vectors

Using a susceptible strain as reference, susceptibility baselines for permethrin, deltamethrin and lambda-cyhalothrin were determined. Mosquito population F1-F3 three-five day-old, non-blood fed adult female mosquitoes were used. Test kits and procedures follow WHO instructions, including analysis and interpretation. Treated test papers were produced at the Department of Entomology, Faculty of Agriculture, Kasetsart University, according to World Health Organization specification (WHO, 1998). Each test series was consisted of eight cylinders (two controls and six treatments). Twenty-five female mosquitoes each were carefully introduced into each holding tube lined with clean (un-treated) paper for 1 h to observe health of mosquitoes before insecticide exposure. Dead and moribund mosquitoes were removed before the beginning of insecticide exposure. Mosquitoes from each holding tube were exposed for 1 h to either insecticide-impregnated or control papers. At the end of 1 h, knockdown mosquitoes were recorded. All mosquitoes were carefully returned to separate clean holding tubes and provided with 10% sugar solution. Mortalities were recorded at 24 h post-exposure (WHO 1998, 2006).

Analysis

Percent test mortality in susceptibility tests will be adjusted when the matched control mortalities are between 5% and 20%, using the following formula (Abbott, 1925) as % test mortality - % control mortality x 100 / 100 - % control mortality

For susceptibility tests, the resistance status of adult mosquitoes will be categorized based on World Health Organization criteria (WHO, 1998).

98 - 100 %	mortality indicates complete susceptibility
80 - 97 %	mortality suggests the possibility of resistance that needs to be confirmed and monitored by repeat testing.
< 80 %	mortality strongly suggests resistance

RESULTS AND DISCUSSION

Results

Collection sites of *Ae. aegypti* were selected based on dengue cases in 2008. One strain from central, one strains from the west, three strains from the east, eight strains from northeast, ten strains from the south, and nine strains from the north were subject to insecticide susceptibility assay. The results of susceptibility tests of 32 strains of *Ae. aegypti* against permethrin, deltamethrin and lambda-cyhalothrin are provided in Table 2. Insecticide susceptibility results of *Ae. albopictus* are given in Table 3. For all paired tests, no untreated control mortalities of *Ae. aegypti* and *Ae. albopictus* were observed. For all 3 compounds, knockdown response after 60 min was a highly accurate predictor of final recorded mortality at 24 h post-exposure. In many instances the KD and mortality percentages were identical.

In total, 12 strains of *Ae. aegypti* were resistant to permethrin, high level of physiological resistance to permethrin was seen in northern strains as compared to the strains from other geographical regions. The strongest resistance to this compound was seen in Lampang strain (43.54% mortality), followed by strains from Chiang-Mai (44.59% mortality) and Kamphaeng Phet (50% mortality). Physiological resistance to permethrin was also observed in two strains from eastern region (Chonburi: 78% mortality and Chanthaburi: 76.55% mortality) and in five strains from the south (Surat thani: 74% mortality, Chumphon: 70% mortality, Prachuap Khiri khan: 78% mortality, Namom: 64.43% mortality and Sadao: 54% mortality). Incipient resistance to permethrin (80-97% mortality) was seen in the other 20 strains of *Ae. aegypti*, eight strains from northeastern region, five strains from southern region, four strains from northern region, one each from central, western and eastern regions. USDA standard susceptible strain was found completely susceptible to permethrin (100% mortality). With deltamethrin, incipient resistance (80-97% mortality) was seen in eleven strains from the entire 32 collections in which six strains were from southern region, two strains each were from northern and eastern regions and one strain was from northeastern region. The other remaining strains of *Ae. aegypti* were considered susceptible to deltamethrin (98-100% mortality). In this study, all strains of *Ae. aegypti* were found completely susceptible to lambda-cyhalothrin as indicated by 100% mortality.

Insecticide susceptibility tests with a single dose of permethrin (0.75%), deltamethrin (0.05%) and lambda-cyhalothrin (0.05%) were conducted against five strains of *Ae. albopictus*. Among five strains of *Ae. albopictus* tested, one strain (Sadao: 78% mortality) was considered a permethrin resistance whereas the other four strains were found a possibility of incipient resistance (84-96% mortality) to this compound. In contrast, *Ae. albopictus* was completely susceptible to deltamethrin and lambda-cyhalothrin.

Table 1 Description and GPS coordinates of field sites

Region	Province	District	GPS coordinates
Aedes aegypti			
Central			
	Bangkok	Kannayaw	13° 50′ N, 100° 40′ E
West			
	Kanchanaburi	Sai Yok	14° 20′ N, 98° 59′ E
East			
	Chonburi	Muang	13° 19′ N, 100° 55′ E
	Chanthaburi	Muang	14° 17′ N, 100° 55′ E
	Prachinburi	Muang	15° 11′ N, 100° 55′ E
Northeast			
	Nakhon Ratchasima	Wang Nam Kheow	15° 0′ N, 102° 6′ E
	Burirum	Lumpaimas	15° 0′ N, 102° 50′ E
	Surin	Dontoom	15° 14′ N, 103° 30′ E
	Si Sa Ket	Uthumpronpisai	15° 08′ N, 104° 12′ E
	Kalasin	Huaypung	16° 38′ N, 103° 54′ E
	Roi Et	Suwannaphum	15° 40′ N, 103° 49′ E
	Udon Thani	Wungsammor	16° 54′ N, 103° 28′ E
	Khon Kaen	Muang	16° 19′ N, 102° 47′ E
South			
	Phang Nga	Takuaytung	8° 12′ N,98° 17′ E
	Phuket	Muang	7° 53′ N,98° 23′ E
	Phattalung	Pabone	7° 16′ N,100° 09′ E

Table 1 (Continued)

Region	Province	District	GPS coordinates
	Surat Thani	Muang	9° 02′ N,99° 22′ E
	Chumphon	Muang	10° 30′ N,99° 07′ E
	Prachuap Khiri Khan	Hua Hin	12° 33′ N,99° 53′ E
	Songkhla-1	Namom	6° 54′ N,100° 32′ E
	Songkhla-2	Ranode	7° 52′ N,100° 18′ E
	Songkhla-3	Sadao	6° 45′ N,100° 24′ E
	Songkhla-4	Had Yai	7° 0′ N,100° 27′ E
North			
	Tak	Mae Sot	16° 40′ N,98° 10′ E
	Nakhon Sawan	Muang	15° 40′ N,100° 05′ E
	Uthai Thani	Ban Rai	15° 23′ N, 100° 55′ E
	Phrae	Muang	18° 05′ N,100° 12′ E
	Chiang-Rai	Mae Chun	20° 08′ N,99° 51′ E
	Lampang	Muang	18° 14′ N,99° 26′ E
	Kamphaeng Phet	Kanuworralukburi	16° 0′ N,99° 48′ E
	Chiang-Mai	Muang	18° 46′ N,98° 56′ E
	Lamphun	Muang	18° 37′ N,99° 0′ E
Ae. albopictus			
	Songkhla	Namom	6° 54′ N,100° 32′ E
	Songkhla	Sadao	6° 45′ N,100° 24′ E
	Surat Thani	Muang	9° 02′ N,99° 22′ E

Table 1 (Continued)

Region	Province	District	GPS coordinates
	Chumphon	Muang	10° 30′ N,99° 07′ E
	Prachuap Khiri Khan	Hua Hin	12° 33′ N,99° 53′ E

Table 2 Percent knockdown (KD)* and mortality** of *Ae. aegypti* and following 60-min exposure to operational concentrations of three pyrethroid insecticides

Strain (province)	Permethrin		Deltamethrin		Lambda-cyhalothrin	
	%KD	%Mortality±SE	%KD	%Mortality±SE	%KD	%Mortality±SE
USDA	100.00	100.00	100.00	100.00	100.00	100.00
Central						
Bangkok	82.00	82.00±0.43	98.00	98.00±0.22	100.00	100.00
Western						
Kanchanaburi	82.00	84.00±0.26	100.00	100.00	100.00	100.00
Eastern						
Chonburi	77.33	78.00±0.43	94.66	95.33±0.31	100.00	100.00
Chanthaburi	75.86	76.55±0.50	94.00	94.00±0.22	100.00	100.00
Prachinburi	90.66	92.00±1.17	100.00	100.00	100.00	100.00
North Eastern						
Nakhon Ratchasima	93.33	95.33±0.60	100.00	100.00	100.00	100.00
Burirum	88.00	88.00±0.58	100.00	100.00	100.00	100.00
Surin	89.93	92.62±0.36	100.00	100.00	100.00	100.00

Table 2 (Continued)

Strain (province)	Permethrin		Deltamethrin		Lambda-cyhalothrin	
	%KD	%Mortality±SE	%KD	%Mortality±SE	%KD	%Mortality±SE
Kalasin	96.00	96.00±0.58	100.00	100.00	100.00	100.00
Roi Et	94.00	94.00±0.43	100.00	100.00	100.00	100.00
Udon Thani	84.67	86.00±0.56	98.00	98.00±0.22	100.00	100.00
Khon Kaen	82.43	82.43±0.52	96.00	96.00±0.26	100.00	100.00
Si Sa Ket	84.46	84.46±0.40	100.00	100.00	100.00	100.00
Southern						
Phang Nga	93.33	94.00±0.43	100.00	100.00	100.00	100.00
Phuket	96.00	96.00±0.43	100.00	100.00	100.00	100.00
Phattalung	87.33	87.33±0.45	100.00	100.00	100.00	100.00
Surat Thani	73.33	74.00±0.50	88.00	88.00±0.36	100.00	100.00
Chumphon	70.00	70.00±0.43	85.14	86.47±0.21	100.00	100.00
PrachuapKhiriKhan	78.00	78.00±0.43	96.00	96.00±0.26	100.00	100.00
Songkhla-Namom	64.43	64.43±0.58	84.00	84.00±0.36	100.00	100.00
Songkhla-Ranode	89.19	89.19±0.58	100.00	100.00	100.00	100.00
Songkhla-Sadao	54.00	54.00±0.43	81.33	82.00±0.22	100.00	100.00
Songkhla-HadYai	81.33	82.00±0.43	90.00	90.00±0.22	100.00	100.00
Northern						
Tak	80.13	81.51±0.31	96.00	96.00±0.26	100.00	100.00

Table 2 (Continued)

Strain (province)	Permethrin		Deltamethrin		Lambda-cyhalothrin	
	%KD	%Mortality±SE	%KD	%Mortality±SE	%KD	%Mortality±SE
Uthai Thani	93.33	93.33±0.33	100.00	100.00	100.00	100.00
Phrae	90.60	90.60±0.43	100.00	100.00	100.00	100.00
Chiang-Rai	82.00	82.00±0.43	100.00	100.00	100.00	100.00
Lampang	43.54	43.54±0.33	100.00	100.00	100.00	100.00
Kamphaeng Phet	50.00	50.00±0.43	100.00	100.00	100.00	100.00
Chiang-Mai	44.59	44.59±0.36	100.00	100.00	100.00	100.00
Lamphun	68.00	68.00±0.36	100.00	100.00	100.00	100.00
Nakhon Sawan	70.00	70.00±0.43	84.00	84.00±0.48	100.00	100.00

* Completely down on floor

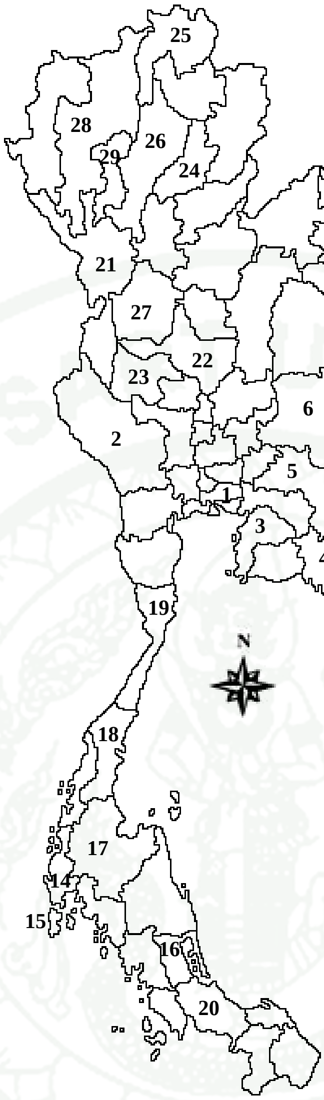
** Real mortality and functional mortality

Table 3 Percent knockdown (KD)* and mortality** of *Ae. albopictus* following 60-min exposure to operational concentrations of three pyrethroid insecticides

Strain (province)	Permethrin		Deltamethrin		Lambda-cyhalothrin	
	%KD	%Mortality±SE	%KD	%Mortality±SE	%KD	%Mortality±SE
MOPH strain	100.00	100.00	100.00	100.00	100.00	100.00
Southern						
Songkhla-Namom	84.00	84.00±0.26	100.00	100.00	100.00	100.00
Songkhla-Sadao	78.00	78.00±0.43	100.00	100.00	100.00	100.00
Surat Thani	96.00	96.00±0.58	100.00	100.00	100.00	100.00
Chumphon	95.33	95.33±0.60	100.00	100.00	100.00	100.00
PrachuapKhiriKhan	96.00	96.00±0.43	100.00	100.00	100.00	100.00

* Completely down on floor

** Real mortality and functional mortality



1 = Bangkok
2 = Kanchanaburi
3 = Chonburi
4 = Chanthaburi
5 = Prachinburi
6 = Nakhon Ratchasima
7 = Buriram
8 = Surin
9 = Si Sa Ket
10 = Kalasin
11 = Roi Et
12 = Udon Thani
13 = Khon Kaen
14 = Phang Nga
15 = Phuket
16 = Phattalung
17 = Surat Thani
18 = Chumphon
19 = Prachuap Khiri Khan
20 = Songkhla
21 = Tak
22 = Nakhon Sawan
23 = Uthai Thani
24 = Phrae
25 = Chiang Rai
26 = Lampang
27 = Kamphaeng Phet
28 = Chiang Mai
29 = Lamphun

Figure 1 *Aedes aegypti* and *Aedes albopictus* collection sites in various parts of Thailand.

Figure 2 Mosquito species and collection sites

Discussion

Preliminary studies using the recommended operational concentration of 0.25% permethrin for *Ae. aegypti* (WHO 1992) found all samples resistant to this dose and 0.5% (data not presented). Sathantriphop et al. (2006) reported using a 0.75% concentration of permethrin and finding high levels of resistance in *Ae. aegypti* from Baan Suan, Nonthaburi Province. Therefore, a decision was made to use a 3-fold greater concentration (0.75%) for all tests used in this study.

Early first to third generation field collected strains of *Ae. aegypti* and *Ae. albopictus* were shown to be either tolerant or resistant to a 3-fold higher concentration of permethrin above than normal operational concentration. All 32 strains of *Ae. aegypti* were found to have evidence of incipient resistance (62.5%) or levels of survival deemed resistant (37.5%) to permethrin. A wide degree of physiological response to permethrin were detected in *Ae. aegypti*, ranging from 56.5% survival (Lampang in northern Thailand) to only 4% (Kalasin in northeastern and Phuket in southern Thailand). Four strains of *Ae. albopictus* were found with incipient (97 – 80% mortality) and one with resistance (< 80%) to permethrin. The majority of *Ae. aegypti* strains (68.7%) were found susceptible (> 98% mortality) to 0.05% deltamethrin, with incipient resistance (observed 97-82% mortality) in other localities. In contrast, all strains of *Ae. aegypti* were completely (100%) susceptible to 0.05% dosage of lambda-cyhalothrin. All five strains of *Ae. albopictus* was found completely susceptible to both deltamethrin and lambda-cyhalothrin. Geographically, collection sites in northern and southern provinces showed the highest percent resistance to permethrin by *Ae. aegypti*, 55.6% and 50% of strains tested, respectively. Deltamethrin tolerance (60% of strains) was more common in the south.

In Thailand, synthetic pyrethroids particularly permethrin and deltamethrin, were introduced into the market in the last several decades and have been widely available for the control of mosquitoes and other insects (Chareonviriyaphap *et al.*, 1999). Historically, pyrethroids have demonstrated great promise for effective mosquito control due to their low toxicity to humans and high potency at low doses quickly immobilizing and killing insects. However, the continuous use of particular insecticides for long periods of time has been associated with development of resistance in mosquitoes and thus reduced effectiveness controlling vectors (Brogdon and McAllister 1998, Hemingway and Ranson 2000).

From the 1960s, investigations examining patterns of mosquito resistance to insecticides in Thailand began reporting varying and increased levels of tolerance/resistance to various synthetic chemicals used for control of adult and larval *Ae. aegypti* (Neely 1964, Bang et al. 1969, Chadwick et al. 1977, Malcolm and Wood 1982). Only recently have a few studies made similar assessments of *Ae. albopictus* (Somboon et al. 2003, Ponlawat et al. 2005, Jirakanjanakit et al. 2007). During the previous decade, increasing tolerance or resistance to permethrin and/or deltamethrin has been documented in adult and larval *Ae. aegypti* in Thailand (Chareonviriyaphap et al 1999, Somboon et al., 2003, Paeporn et al. 2004, Yaichareon et al. 2005, Ponlawat et al. 2005, Sathantriphop et al. 2006, Jirakanjanakit et al. 2007, Thanispong et al. 2008). Whereas significantly high levels of permethrin resistance were reported by Jirakanjanakit et. al. (2007) (5% mortality) and Thanispong et al. (2008) (2-9% mortality) exposed to 0.25% a.i., not surprisingly, our study showed relatively lower degrees of resistance (43-96% mortality) when exposed to a 3-fold higher concentration. Additionally, these variations in permethrin susceptibility may partially reflect differences in collection sites and previous levels of exposure to permethrin or related compounds. It is suspected that household insecticide products i.e. coil, mat, liquid, lotion and cream formulations containing permethrin play a major role for permethrin resistance in *Ae. aegypti* strains in Thailand as reported by Paeporn et al. (1996) and Thanispong et al. (2008). The increased use of materials treated with permethrin (blanket, cloths, caps) for disease control in many parts of Thailand and the use of pyrethriods for control of agricultural pests have likely accelerated the development of physiological resistance in this mosquito. Incipient resistance (tolerance) to deltamethrin was detected in 11 of 32 *Ae. aegypti* strains. Like permethrin, the decreased susceptibility to deltamethrin over time is the consequence of widespread use of this chemical and related pyrethroid compounds.

Moreover, ultra-low-volume (ULV) applications of deltamethrin have been commonly used to combat dengue outbreaks for many years.

Incipient or outright resistance (WHO 1998) to permethrin was detected in several strains of *Ae. albopictus* from the south. Permethrin-treated materials have been commonly used for protection against outdoor biting mosquitoes like *Ae. albopictus*, e.g., workers in rubber plantations and fruit orchards. Since 2004, routine IRS for malaria control was suspended because of civil unrest in restive areas nearer the southern border with Malaysia. Tolerance or resistance may also have arisen because of exposure to household or agricultural use of permethrin or other pyrethriods. Likewise, half of the strains of *Ae. aegypti* tested from the southern districts exhibited resistance to permethrin due to more indoor exposure or cross-resistance. Six strains (60%) of *Ae. aegypti* also demonstrated incipient resistance to deltamethrin. In contrast, all strains of *Ae. albopictus* remain completely susceptible to deltamethrin and lambda-cyhalothrin. However, this does not preclude the rapid development cross-resistance to these 2 chemicals as a consequence of the extensive exposure to related compounds (Ponlawat et al. 2005, MOPH 2008).

In addition to differing exposures to these 3 chemicals over time, the varying degrees of *Aedes* susceptibility seen between them may be related to differences in attributes and chemistry. Permethrin is grouped together with other so-called 'third generation' pyrethroids that exhibit enhanced insecticidal activity and photostability over their first and second generation chemical predecessors. Deltamethrin and lambda-cyhalothrin are more 'advanced' fourth generation pyrethroids with active ingredients displaying greater toxicity, enhanced photostability and lower volatility for extended residual effectiveness than permethrin. The chemistry and symptomology also differs between permethrin on the one hand and deltamethrin and lambda-cyhalothrin on the other (Bloomquist 1996). Pyrethroids are potent axonic poisons in susceptible insects acting as sodium channel blockers/modulators (Ware 2000). They resemble that of DDT, by keeping open the sodium channels in neuronal membranes and thus producing a hyperexcitatory effect stimulating repetitive nerve discharges. For insects susceptible to pyrethroid intoxication the outcome is eventual paralysis and death. Pyrethroids are typically divided into 2 classes based on differences in specific responses and differential poisoning syndromes produced. Type 1 compounds (e.g., permethrin) are associated with more rapid decay of modified sodium currents that is consistent with their lower toxicity and less intense effects on nerve firing (Bloomquist 1996). Type 2 compounds (deltamethrin and lambda-cyhalothrin) are approximately 10-fold more potent than Type 1 pyrethroids for depolarizing motor nerve terminals, an effect that is correlated with acute toxicity. Another differing physiological response is that Type 1 compounds have a negative temperature coefficient for toxicity action while Type 2 show a positive temperature toxicity producing increasing kill with increased in temperature (Ware 2000). Although speculative as a mechanism for selection of resistance, the higher ambient temperatures associated with the typical tropical /subtropical climate in Thailand may actually decrease potential toxicity of permethrin to sublethal levels while enhancing potency of the other two.

Mosquito resistance to pyrethroids can involve one or more mechanisms including target-site insensitivity (sodium ion channel) and/or metabolic detoxification of the active ingredient. Knock-down resistance (*kdr*) is a genetic mutation associated with a reduced sensitivity of the nervous system to pyrethroids. A range of mosquito species, including *Ae. aegypti* (Hemingway et al. 1989), have had *kdr*-like mechanisms identified. In mosquito systems, the *kdr*-like mechanism may extend to DDT-pyrethroid cross-resistance or may only affect a sub-set of pyrethroids (Amin and Hemingway *et al*., 1989). Alternatively, metabolic mechanisms of resistance involving enzymatic detoxification of pyrethroids typically involves one or more of a broad spectrum of esterases and monooxygenases involving the rate limiting enzyme cytochrome P^{450}. Biochemical analysis has shown a significant elevation of mixed-function oxidases (monooxygenases) and elevation of non-specific esterases activity in deltamethrin-resistant *Ae. aegypti* in Thailand (Yaicharoen et al., 2005). However, metabolic degradation mechanisms appear to play a relatively minor role, if any, in pyrethroid resistance in *Ae. aegypti* compared to non-metabolic *kdr* (nerve insensitivity) resistance alleles (Hemingway *et al*., 1989, Brodgon and McAllister 1998).

A DDT-resistant strain of *Ae. aegypti* from Bangkok was shown to have cross-resistance with permethrin and bioresmethrin indicating that a *kdr*-type mechanism may have been selected in this strain (Malcolm and Wood 1982). If *kdr* resistance is the primary mechanism in the resistant strains identified in the our study selection may have come from periodic use of pyrethroids in space spray operations or the common use of these chemicals in commercial household insect control products. Alternatively, if resistance to DDT was present in the past it is possible that the *kdr* mechanism was perpetuated by cross-resistance following replacement with pyrethriods (Chadwick et al., 1977). Paeporn et al., (2004) was able to select for permethrin (0.75%) and deltamethrin (0.05%) resistance in separate laboratory strains of *Ae. aegypti* and detected significantly elevated esterase and monooxygenase activity in addition to increases in glutathione-*S*-transferase (GST) activity in the permethrin-selected strain (only). Only metabolic mechanisms were investigated so that a *kdr*-type mechanism could not be excluded. Investigating deltamethrin resistant field populations of *Ae. aegypti*, Yaicharoen et al. (2005) also showed metabolic elevation of monooxygenases and esterases in addition to alterations to the voltage-dependent sodium channel as possible target site resistance. Monitoring of the resistance gene frequency over one or more spray seasons would indicate whether this mechanism is being selected and maintained in natural populations.

Insecticides continue to play a crucial, if not indispensible role, in the control of most vector-borne diseases. This is particularly true for curbing transmission of dengue viruses. Vector control by attacking both larval habitats and adult mosquitoes remains the principal method for reducing risk of dengue infection (Reiter and Gubler 1997). Despite the infrequency and incomplete coverage in monitoring and reporting, there is sufficient evidence to show an increasing trend in vector resistance in Thailand. It is imperative that investigations be broaden geographically to also include increased inquiry on *Ae. albopictus* and the identification of the physiological and genetic mechanisms of resistance in the country.

Overall, this study's findings are of major concern. Despite use of a 3-fold increase in permethrin concentration, tolerance or resistance was manifest in all strains of *Ae. aegypti* and *Ae. albopictus* tested representing the a wide geographical coverage within Thailand.. Increased frequency of resistance to a wider array of different pyrethroid chemicals and formulations would have serious and long-term implications for future dengue control strategies using insecticides. Current and on-going monitoring of mosquito vector populations will remain the primary determinant to guide timely and practical decisions as to the continued effectiveness and use of certain insecticides under relevant circumstances (Brown 1986, Roberts and Andre 1994). Evidence-based decisions will drive appropriate countermeasures (e.g., rotation or mixtures of effective insecticides) to control or further mitigate the impact of increased resistance will be critical for the success of integrated vector management practices.

In summary, the increasing emergence of insecticide resistance in dengue vector mosquitoes is a significant concern and a major factor influencing the success of future vector control. Establishing a quality controlled, comprehensive program for routine monitoring of susceptibility and early detection of insecticide resistance to include the identification of those mechanism(s) responsible for resistance should be an integral part of an insecticide evaluation program aimed at effective vector-borne disease control. Such a program is long overdue in Thailand.

CONCLUSION

Aedes mosquito vectors in Thailand exhibit various levels of resistance to synthetic pyrethroids, permethrin and deltamethrin, the commonly used insecticides in Thailand. Fortunately, all strains were still susceptible to lambda-cyhalothrin especially *Ae. aegypti* and *Ae. albopictus*.

This study found resistant that from 32 strains of *Ae. aegypti* tested, 12 strains were resistant to permethrin and the other 20 strains were possible to be resistant. Eleven strains of *Ae. aegypti* were possible to be resistant to deltamethrin. All strains of *Ae. aegypti* were found completely susceptible to lambda-cyhalothrin as indicated by 100% mortality. From 5 strains of *Ae. albopictus* tested one strain was resistance to permethrin and the other four strains were possible to be resistance to permethrin. All strains of *Ae. albopictus* were completely susceptible to deltamethrin and lambda-cyhalothrin.

From this study all strains of *Ae. aegypti* and *Ae. albopictus* were susceptible to lambda-cyhalothrin, suggesting that this compound can be used for *Ae. aegypti* and *Ae. albopictus* control program in the future.

This study reiterate the seriousness of increasing pesticide resistance by arthropods which is a primary concern for the management of human pest and disease control. A better understanding of the dynamic relationship between insecticides and the susceptibility level of the mosquito population will allow for a greater efficiency in program design for targeting mosquito vectors.

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APPENDIX

Appendix Table 1 Review Percent Mortality of *Aedes. aegypti* after exposure to Permethrin at concentration 0.25% and 0.75% in Thailand

Strain (province)	Permethrin 0.75% %Mortality±SE	Strain (province)	Permethrin 0.25% %Mortality±SE , Ref
USDA	100.00	100.00 100.00	100.00
Central			
Bangkok-Kannayaw	82.00 ± 0.43	Bangkok-Chatuchak	9.00± 1.1(Thanispong *et al*.,2008),
		Bangkok-Bangkoknoi	18.5 (Jirakanjanakit *et al*., 2007)
		Bangkok-Huaykwang	20.9 (Jirakanjanakit *et al*., 2007)
		Bangkok-Laksi	26.1 (Jirakanjanakit *et al*., 2007)
		Bangkok-Ladkrabang	51.3 (Jirakanjanakit *et al*., 2007)
		Bangkok-Rasburana	48.8 (Jirakanjanakit *et al*., 2007)
Western			
Kanchanaburi-Sai Yok	84.00 ± 0.26	Kanchanaburi-Sai Yok	9.00±3.4 (Thanispong *et al*., 2008)
		Kanchanaburi-Tamaka	73.26(Jirakanjanakit *et al*.,2007)
Eastern			
Chonburi-Muang	78.00 ± 0.43	Chonburi-Muang	85.4 (Jirakanjanakit *et al*., 2007)
		Chonburi-Panusnikom	11.2 (Jirakanjanakit *et al*., 2007)
		Chonburi-Banglamung	5.0 (Jirakanjanakit *et al*., 2007)
		Chonburi-Sriracha	77.6 (Jirakanjanakit *et al*., 2007)
Chanthaburi-Muang	76.55 ± 0.50	Chanthaburi-Muang	80.39 (Jirakanjanakit *et al*., 2007)
Prachinburi	92.00 ± 1.17		

Appendix Table 1 (Continued)

Strain (province)	Permethrin 0.75% %Mortality±SE	Strain (province)	Permethrin 0.25% %Mortality±SE , Ref
North Eastern			
Nakhon	95.33 ± 0.60	NS-Prathai	89.9 (Jirakanjanakit *et al*., 2007)
Ratchasima(NS)		NS-Kornburi	80.0 (Jirakanjanakit *et al*., 2007)
		NS-Kangsanamnang	94.73 (Jirakanjanakit *et al*., 2007)
		NS-Serngsang	69.3 (Jirakanjanakit *et al*., 2007)
		NS-Seekhew	96.0 (Jirakanjanakit *et al*., 2007)
Burirum	88.00 ± 0.58		
Surin	92.62 ± 0.36		
Si Sa Ket	84.46 ± 0.40		
Kalasin	96.00 ± 0.58		
Roi Et	94.00 ± 0.43		
Udon Thani	86.00 ± 0.56		
Khon Kaen	82.43 ± 0.52	Khon Kaen	38.40±9.7(Thanispong *et al*.,2008)
Southern			
Phang Nga	94.00 ± 0.43		
Phuket	96.00 ± 0.43		
Phattalung	87.33 ± 0.45		
Surat Thani	74.00 ± 0.50	Surat Thani	61.00±2.5(Thanispong *et al*.,2008)
Chumphon	70.00 ± 0.43		
PrachuapKhiriKhan	78.00 ± 0.43		
Songkhla-Namom	64.43 ± 0.58	Songkhla-Muang	72.70±3.2(Thanispong *et al*.,2008)
		Songkhla-Muang	61.39 (Jirakanjanakit *et al*., 2007)
		Songkhla-Singhanakorn	94.18 (Jirakanjanakit *et al*., 2007)

Apendix Figure 1 *Aedes aegypti* breeding sites

Apendix Figure 2 *Aedes albopictus* breeding sites

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