# CHAPTER 1

# Introduction

## 1. General introduction

### 1.1 Statement and significance of problem

Mosquitoes that transmit infectious diseases such as dengue, malaria, filariasis, Japanese encephalitis, chikungunya, and Zika are the most serious insects of medical importance. They inflict millions of worldwide deaths each year (Knudsen and Sloof, 1992; Weaver et al., 2016). *Aedes aegypti* is known generally as a vector for the dengue virus, the most prevalent mosquito-borne virus worldwide, chikungunya virus, and Zika virus in tropical and subtropical regions; mostly Southeast Asia, the Pacific islands, Africa, and the Americas. Approximately 2.5 billion people are at risk of dengue infection, with an estimated 50-100 million and 250,000-500,000 cases of dengue fever and dengue hemorrhagic fever, respectively (Gubler, 2002; WHO, 2016). Since dengue vaccine has been registered recently and no specific treatment is available, environmental management and vector control remain the most important tool in preventing and reducing the risk of dengue transmission (WHO, 2009, 2016; Durbin, 2016).

Management of the aquatic and adult stages of mosquito vectors is approached by applying various conventional synthetic compounds such as larvicides, adulticides, attractants, deterrents, and repellents to reduce the outbreaks of disease transmissions (Badolo et al., 2004; Waliwitiya et al., 2008; Monnerat et al., 2012). In Thailand, at least four groups of synthetic chemicals; organochlorine (DDT), organophosphates, carbamates, and pyrethroids, have been used extensively to control both agricultural pests and human/animal disease vectors. Synthetic pyrethroids have become the most popular and prevalent active ingredients for national public health vector control programs, due to their relatively low mammalian toxicity, but high invertebrate potency at low levels, resulting in rapid immobilization (‘knockdown’) and killing (Zaim

et al., 2000; Chareonviriyaphap et al., 2013). However, the widespread, extensive, and indiscriminate use of pyrethroids and other synthetic insecticides for vector management leads to increased insecticide resistance in mosquito populations (Grieco et al., 2007; Thanispong et al., 2008; Ranson et al., 2010).

Major mechanisms of insecticide resistance involve either mutation within the target site of the insecticide or an alteration in the rate of insecticide detoxification via increased enzyme activity of nonspecific esterases (acetylcholinesterase and carboxylesterases), acid and alkaline phosphatases, glutathione S-transferases, and P450 mediated monooxygenases (mixed-function oxidases: MFO). Qualitative and/or quantitative changes of these enzymes may be the important processes in the resistance mechanism (Hemingway and Karunaratne, 1998; Chareonviriyaphap et al., 2013). Esterases are classified as hydrolases, a large and diverse group of enzymes that catalyze the hydrolysis of a wide range of aliphatic and aromatic esters, choline esters, and organophosphorous compounds (Dauterman, 1985). They are often involved in several physiological processes (reproduction, digestion, metabolism of the juvenile hormone, and molting) and play an important role in detoxifying synthetic insecticides to less toxic metabolites (Klowden, 2007; Kamita et al., 2011; Koodalingam et al., 2011). Acetylcholinesterase (AChE) is an enzyme that catalyzes hydrolysis of the neurotransmitter, acetylcholine, thus stopping transmission of nerve impulses at the synapses of cholinergic neurons in the central and peripheral nervous systems in both vertebrates and invertebrates (Grundy and Still, 1985; Wang et al., 2004; Zibaee, 2011). The inhibition of AChE leads to the accumulation of acetylcholine in all areas of the nervous system, causing excessive muscle contraction followed by paralysis, secretions, seizures, and death. In insects, AChE is the primary target of organophosphorus and carbamate compounds, which remain widely used insecticides (Harel et al., 2000). Carboxylesterases ($\alpha$ and $\beta$-carboxylesterase) have been investigated well with regard to their importance in organophosphate, carbamate, and pyrethroid insecticide resistance in various pest species. They also play an important role in allelochemical metabolism and tolerance, although the roles in a few cases were validated at only the biochemical level (Li et al., 2007). Acid and alkaline phosphatases are important in biological processes such as development, growth, gamete's maturation, nervous system, insect growth regulator (IGRs), and histolysis (Day, 1948; Ray et al., 1984; Assar et al., 2010). They

also are known to play an important role in diverse physiological processes (Majerus et al., 1999). Glutathione S-transferases (GSTs) are enzymes involved in detoxification of endogenous and exogenous compounds via glutathione conjugation, dehydrochlorination, glutathione peroxidase (GPx) activity or passive/sacrificial binding (Hayes and Wolf, 1988; Mannervik and Danielson, 1988; Pickett and Lu, 1989; Yang et al., 2001). In mosquitoes, metabolic resistance based on GSTs is the major mechanism of DDT-resistance (Hemingway and Ranson, 2000). Mixed-function oxidases (MFO) enzyme plays a major role in the metabolism of pyrethroids and the activation and/or detoxification of organophosphorus insecticide. Cytochrome P450s enzymes (P450s or CYPs) are found in the biosynthetic pathways of ecdysteroids and juvenile hormones, which are at the center stage of insect growth, development, and reproduction (Slama, 1993). P450 enzymes metabolize insecticides, resulting either in bioactivation or, more often, detoxification, with the latter process being enhanced in many strains that have metabolic resistance to insecticides (Feyereisen, 1999).

Analysis of four major groups of enzymes; esterases, phosphatases, GSTs, and MFO, in insect pests has been carried out by many researchers, since alterations in their actions appear to correlate well with the lethal effect of insecticides, presumably due to the breakdown or interruption of various physiological processes of target organisms (Sutherland, 1977; Ben-Dov et al., 2003; Koodalingam et al., 2011, 2012; Emtithal and Thanaa, 2012; Jone et al., 2013; Nardini et al., 2014). Therefore, these enzymes, particularly esterases and phosphatases, also have been used widely as reliable and sensitive biomarkers to evaluate toxicity of chemical and botanical insecticides (Koodalingam et al., 2011).



In addition to development of resistance in mosquito populations, other unwarranted effects resulted from application of synthetic insecticides are health risks to humans and other animals, toxic residues in food and the environment, and increasing cost of application (Isman, 2000; Ribeiro et al., 2003; Bughio and Wilkins, 2004). These have necessitated a need for the search and development of alternative insecticides with new modes of action as well as appropriate monitoring systems that are cost-effective and environmental friendly. One promising approach is to revisit bioactive compounds obtained from different parts of a whole range of plants, which have the potential to

replace synthetic insecticides, due to their eco-friendly, biodegradable, and remarkable anti-mosquito properties (Roy and Saraf, 2006; Ravindran et al., 2012; Panneerselvam et al., 2012). Many studies were conducted on the efficacy of herbal products against various mosquito vectors, their lethal doses, and time to reach lethal effects, but few studies have been fully elucidated on perceptible changes in behavioral response, physical performance, and biochemical constituents of target insects, which may enable a better understanding of the systemic effects of botanicals and their possible mode of action(s).

## 2. Literature review

### 2.1 Mosquitoes

Mosquitoes are classified into the Phylum Arthropoda, class Insecta, order Diptera, suborder Nematocera, family Culicidae. The Culicidae divided into three subfamilies (Knight and stone, 1977):

1. Toxorhynchitinae: *Toxorhynchites* spp.
2. Anophelinae (Anophelines): *Anopheles* spp., *Bironella* spp., and *Chagasia* spp.
3. Culicinae (Culicines): *Aedes* spp., *Culex* spp., *Mansonia* spp., *Armigeres* spp., *Haemagogus* spp., *Sabethes* spp., and *Psorophara* spp.

There are over 3,400 mosquito species belonging to 42 genera (Service, 2008). This study focused on *Aedes aegypti* (Culicinae), a potential vector of dengue fever, yellow fever, filariasis, chikungunya fever, and zika fever. *Aedes* spp. is a mosquito member of

Phylum Arthropoda,
Class Insecta,
Order Diptera,
Suborder Nematocera,
Family Culicidae,
Subfamily Culicinae,
Genus *Aedes*,

*Ae. aegypti* mosquito undergoes complete metamorphosis with four distinct stages in life cycle: egg, larva, pupa, and adult (Figure 1.1). The development starts by laying egg on the surface of the water, egg hatching, larval development, pupation, and adult emergence (Clements, 1992; Beaty and Marquardt, 1996).

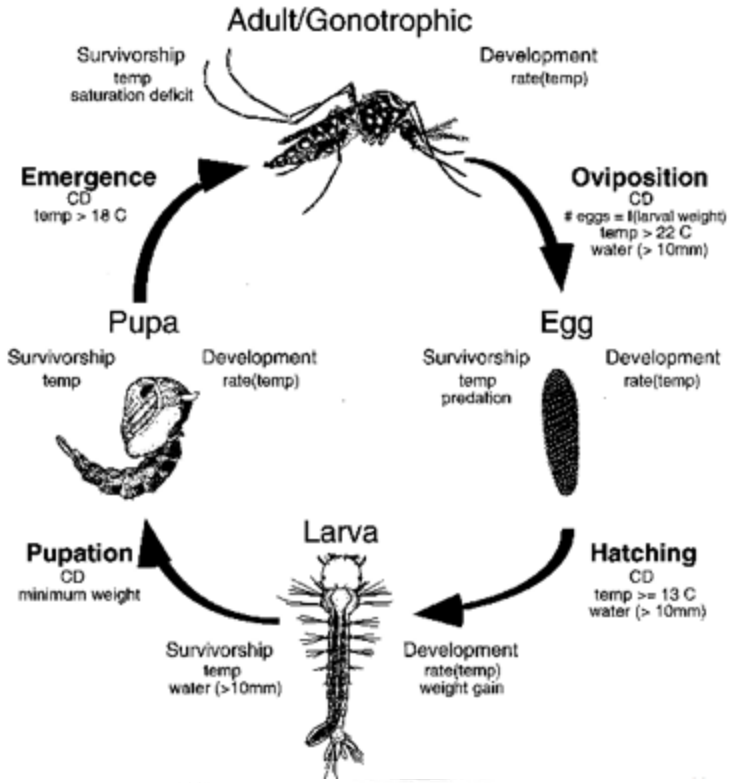


**Figure 1.1** The *Aedes* mosquito life cycle (Hopp and Foley, 2001)



**Egg stage**

Female *Ae. aegypti* mosquitoes usually lay eggs singly, with 100 to 200 eggs at any one oviposition, on damp surfaces such as rock holes, tree holes, moist earth, and man-made containers above the waterline. Eggs of *Aedes* species usually black, long, and more or less ovoid in shape. These eggs are able to withstand desiccation and can survive for long periods until they are soused by water, at which time they begin to hatch.

**Larval stage**

When the eggs hatch, the mosquito larvae come out. They have a soft body, except head and respiratory siphon, which are covered by a sclerotized cuticle. The head of larva presents short antennae and primordial compound eyes of the future adult, and a smaller simple larval eye located behind them. They feed on particulate organic matter, including microorganisms such as algae, bacteria, protozoa, and detritus with mouth brushes. They dive below the surface only when disturbed or when feeding. The movement of larvae is provided by propulsion of the mouth brushes and setae present on the thorax and abdomen, and by jerky movements of the entire body. Larvae develop through four instars, spending a small amount of time in the $1^{st}$-$3^{rd}$ instars and up to 3 days in the $4^{th}$ instar. The first instar larvae are approximately 1 mm in length and the fourth stage larvae are normally 8 mm in length. Females develop slowly than males, so females generally pupate later. Larval development is temperature dependent.

**Pupal stage**

The pupae are the last aquatic phase, which usually lasts about two days before metamorphosis into the adult stage. The pupa has a comma form. It is divided into two distinct regions. The first region consists of the head and thorax in a cephalothorax. The second region is the abdomen, which has freely movable segments with a pair of paddle-like appendages at the tip. The pupa do not feed and spend most of their time at the water surface and maintain the breathing tubes (trumpets) to contact with the air. When the adult is fully formed, the cuticle on the thorax splits and the adult mosquito emerges. The duration of puparial period depends on temperature but generally 2-3 days. In cooler temperate regions, the period may be extended over 9-12 days, or longer.



**Adult stage**

The newly emerged adults rest on the surface of the water for a short time to allow their wings and body to dry and harden. The adults are approximately 1.5 cm in length and weight up to 2.5 mg. Adult mosquitoes have slender bodies with three clearly distinguishable segments: head, thorax, and abdomen. The head is specialized for receiving sensory information and feeding. The head is globular and largely consists of a pair of prominent compound eyes to detect variations of light. Like most dipterans, the head of adult mosquito is equipped with three types of appendages, including antennae,

maxillary palps, and proboscis. The antennae of females are important for detecting carbon dioxide released from their preys whereas the males' antennae, which are noticeably bushier contain auditory receptors to detect the characteristic whine of the female. Mosquitoes feed on dissolved sugary fluids to obtain the energy they need, while only females feed on blood to obtain protein for egg production. Blood is sucked by the labrum at the proboscis. Beside the proboscis, there are the maxillary palps, which are the food sensorial receptors. The maxillary palps of the male mosquitoes are longer than their proboscis, whereas the females' maxillary palps are much shorter. The thorax that is specialized for locomotion has three pairs of legs, a pair of wings, and a pair of halters that is small wing-like organs used for steering. The abdomen is specialized for food digestion and egg development. The abdomen is divided into eleven segments. The last two ones are modified for mating and discharge of feces, while those of the females serve for egg laying (Clements, 1992). The adult life span may be extended for several weeks to months in temperate regions, but in tropical areas it is much shorter about a few days to several weeks.

### 2.2 Mosquitocidal property of plant products

In recent years, many herbal products have been investigated as potentially natural sources of mosquito larvicides or adulticides. The larvicidal activities of crude chloroform, dichloromethane, and methanol extracts obtained from six Indian plants, including *Aegle marmelos*, *Balanites aegyptica*, *Calotropis gigantica*, *Murraya koenigii*, *Nyctanthes arbor-tristis*, and *Plumbago zeylanica* were investigated against the early fourth instar larvae of *Ae. aegypti* and *Anopheles stephensi* (Patil et al., 2010). The results showed that the highest larvicidal activities against *Ae. aegypti* and *An. stephensi* were derived from the methanol root extracts of *P. zeylanica* ($LC_{50}$ value = 169.61 mg/l) and *B. aegyptica* ($LC_{50}$ value = 102.29 mg/l), respectively. Jeeshna et al. (2010) reported the marked larvicidal activity of the weed plant species, *Croton bonplandianum*, against *Ae. aegypti* ($LC_{50}$ value = 123.8 ppm). Evaluation of the seaweed extracts of *Enteromorpha intestinalis*, *Dictyota dichotoma*, and *Acanthopora spicifera* by Ravikumar et al. (2011) demonstrated the greatest larvicidal activity of *D. dichotoma* against *Ae. aegypti*, with a minimum level of $LC_{50}$ value (0.0683 ± 0.0084 µg/ml).

The larvicidal, pupicidal, adulticidal, and repellent activities of *Artemisia nilagirica* were evaluated against *An. stephensi* and *Ae. aegypti* (Panneerselvam et al., 2012). It was demonstrated that the methanol leaf extract of *A. nilagirica* exhibited promising larvicidal effect against the first- to fourth-instar larvae of *An. stephensi* ($LC_{50}$ values = 272.50, 311.40, 361.51, and 442.51 ppm, respectively) and *Ae. aegypti* ($LC_{50}$ values = 300.84, 338.79, 394.69, and 470.74 ppm, respectively). Correspondingly, the adulticidal activity also was found in methanol leaf extract of *A. nilagirica*, with the $LC_{50}$ and $LC_{90}$ values of 205.78 and 459.51 ppm, respectively, against *An. stephensi*; and 242.52 and 523.73 ppm, respectively, against *Ae. aegypti*. Subramaniam et al. (2012) revealed the larvicidal action of *Aloe vera* petroleum ether leaf extract against the first to fourth-instar larvae of *Ae. aegypti* with $LC_{50}$ values of 162.74, 201.43, 253.30, and 300.05 ppm, respectively. *Cadaba indica* leaves extracted with different solvents i.e., ethanolic, hexane, chloroform, and petroleum ether exerted a moderate larvicidal activity against *Ae. aegypti* (Kalimuthu et al., 2012). The highest efficacy against the first- to fourth-instar larvae of *Ae. aegypti* was derived from the ethanolic extract of *C. indica*, with $LC_{50}$ values of 115.70, 96.09, 144.50, and 143.75 ppm, respectively. Ali et al. (2013) investigated larvicidal activity of the seaweed extracts of *Ulva lactuca*, *Caulerpa racemosa*, *Sargassum microystum*, *Caulerpa scalpelliformis*, *Gracilaria corticata*, *Turbinaria decurrens*, *Turbinaria conoides*, and *Caulerpa toxifolia* against three mosquito species. Among these seaweed extracts, *C. racemosa* exerted toxic effect against the fourth instar larvae of *Ae. aegypti*, *Culex quinquefasciatus*, and *An. stephensi*, with equivalent $LC_{50}$ values of 0.0556 ± 0.0103, 0.0675 ± 0.1360, and 0.0661 ± 0.0076 µg/ml, respectively. The larvicidal potential of different solvent crude (hexane, chloroform, ethyl acetate, acetone and methanol) leaf extracts of five plants (*Blepharis maderaspatensis*, *Elaeagnus indica*, *Maesa indica*, *Phyllanthus wightianus*, and *Memecylon edule*) was tested against the fourth-instar larvae of *Ae. aegypti* (Shivakumar et al., 2013). It was found that the maximum larvicidal efficacy was detected in the acetone extract of *E. indica* ($LC_{50}$ value = 90.89 ppm), followed by *M. indica* acetone extract ($LC_{50}$ value = 173.21 ppm). Herbal formulation comprising natural camphor and eight plant volatile oils of *Acorus calamus* (calamus oil), *Cinnamomum verum* (cinnamon oil), *Cymbopogon nardus* (citronella oil), *Myrtus caryophyllus* (clove), *Eucalyptus globulus* (eucalyptus oil), *Mentha piperita* (mentha oil), *Citrus limon* (lemon oil), and *Citrus sinensis* (orange oil) was studied for

the larvicidal and growth inhibitory activities against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (Manimaran et al., 2013). At 250 ppm concentration, this volatile formulation produced the maximum larvicidal mortality (99.20%) of *An. stephensi* and *Ae. aegypti*, followed by 99.0% mortality of *Cx. quinquefasciatus*. The lowest $LC_{50}$ and $LC_{90}$ values of 35.95 and 138.86 ppm, respectively, were observed in treated *An. stephensi*. Promising larvicidal effects against *Ae. aegypti* were observed in petroleum ether and ethyl alcohol extracts of *A. calamus*, with $LC_{50}$ values of 57.32 and 64.22 mg/l, respectively (Imam et al., 2014). The leaves of *Blumea mollis, Chloroxylon swietenia, Clausena anisata, Feronia limnonia, Lantana camara, Plectranthus amboinicus,* and *Tagetes erecta* extracted with five different solvents such as hexane, chloroform, ethyl acetate, acetone, and methanol, were tested for larvicidal activity against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* (Jayaraman et al., 2015). The results demonstrated that all the extracts exhibited varied levels of larvicidal activity against the mosquito species tested. The highest larvicidal efficacy was established from *C. swietenia* ethyl acetate extract, with $LC_{50}$ values of 76.24, 80.58, and 94.12 ppm against *An. stephensi, Ae. aegypti*, and *Cx. quinquefasciatus*, respectively. The larvicidal efficacy of other plant extracts were in order of ethyl acetate extract of *C. anisate* > methanol extract of *P. amboinicus* > acetone extract of *F. limonia* > methanol extract of *T. erecta* > methanol extract of *B. mollis* > and methanol extract of *L. camara*. Modise and Ashafa et al. (2016) evaluated the larvicidal, pupicidal and insecticidal activities of distilled water, ethanol, and hexane extracts of *Cosmos bipinnatus, Foenuculum vulgare*, and *Tagetes minuta* leaves against *Cx. quinquefasciatus*. The highest larvicidal activity among the extracts tested was observed in ethanol extracts of *F. vulgare, T. minuta*, and *C. bipinnatus* with $LC_{50}$ values of 0.10, 1.17, and 1.18 mg/ml.



Several essential oils and solvent extracts from different parts of plants were found to have adulticidal activity against many mosquito species. Dua et al. (2010) reported the potential adulticidal activity of *L. camara* leaf oil against *Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluviatilis,* and *An. stephensi*, with $LD_{50}$ values of 0.06, 0.05, 0.05, 0.05, and 0.06 mg/cm$^2$, respectively. Furthermore, application of 0.208 mg/cm$^2$ impregnated paper was found to exhibit knock down effects against *Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluviatilis,* and *An. stephensi*, with median knock down time ($KDT_{50}$) values of 20, 18, 15, 12, and 14 min, respectively. Hafeez et

al. (2010) revealed that adulticidal effects against *Ae. albopictus* of ten citrus oils varied with time and concentration. Jaffa (*Citrus sinensis*) oil was recorded as being the most lethal agent, with $LC_{50}$ values of 53.61, 11.07, and 3.41% at recorded times of 6,12, and 24 h, respectively; and $LT_{50}$ values of 18.70, 14.08, 10.42 and 6.59 h at concentrations of 5, 10, 15 and 20%, respectively. Nawaz et al. (2011) evaluated the adulticidal activity of olive (*Olea vera*), linseed (*Linum sitatissimum*), and black pepper (*Piper nigera*) against *An. stephensi* and *Ae. aegypti* under laboratory conditions. It was found that the black pepper oil was the most effective adulticide against *Ae. aegypti* and *An. stephensi*, with the lowest $LC_{50}$ values of 2.26% and 8.40%, respectively. In terms of $LT_{50}$, black pepper took 15 h to kill 50% of the tested *Ae. aegypti* population, while it took > 2 days against *An. stephensi*.

The leaves of *Eclipta alba* and *Andrographis paniculata* extracted with five different solvents such as benzene, hexane, ethyl acetate, methanol, and chloroform, were tested for adulticidal and repellent activities against *An. stephensi* (Govindarajan and Sivakumar, 2011). The maximum efficacy among the tested solvent extracts was observed in the methanol extracts of *A. paniculata* and *E. alba*, with $LC_{50}$ values of 130.19 and 150.36 ppm, respectively. Crude hexane, ethyl acetate, and methanol leaf extracts of *Ageratum houstonianum* were assayed for their toxicity against adult *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (Ravindran et al., 2012). The results demonstrated that *Ae. aegypti* was more susceptible to ethyl acetate and hexane extracts, with an $LD_{50}$ value of 0.10 µg/mg female, and both *An. stephensi* and *Cx. quinquefasciatus* were susceptible to methanol extract, with an $LD_{50}$ value of 0.12 µg/mg female. The solvent (hexane, ethyl acetate, benzene, chloroform, and methanol) extracts of *Cardiospermum halicacabum* were found to have moderate adulticidal effects against three important mosquito vectors; *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*, with $LC_{50}$ range of 186.00-274.07 ppm (Govindarajan and Sivakumar, 2012). The ovicidal, repellent, and adulticidal evaluations of crude hexane, benzene, ethyl acetate, acetone, and methanol leaf extracts of *Acalypha alnifolia* were carried out against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* (Kovendan et al., 2013). All extracts demonstrated moderate adulticidal effects; however, the highest adulticidal activity was found in methanol extract treatment, with $LC_{50}$ values of 279.75, 274.76, and 291.71 ppm against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*, respectively. Govindarajan and Sivakumar (2014) evaluated

the larvicidal, ovicidal, and adulticidal potential of crude hexane, benzene, chloroform, ethyl acetate, and methanol leaf extracts of *Erythrina indica* against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. The highest adulticidal activities against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were observed in methanol extract, with $LD_{50}$ values of 88.76, 94.09, and 119.64 ppm, respectively. The leaf of *Ocimun gratissimum* extracted with six different solvent such as hexane, chloroform, acetone, ethyl acetate, methanol, and water were tested for larvicidal, pupicidal, and adulticidal efficacy against *Ae. aegypti* (Pratheeba et al., 2015). The highest pupicidal and adulticidal activities were found in chloroform extract treatment, with $LC_{50}$ values of 19.28 and 16.08 mg/l, respectively. Ahmad et al. (2016) evaluated the adulticidal effects against *Ae. aegypti* and *Ae. albopictus* of 11 methanol seaweed extracts. The maximum efficacy among the 11 species tested was observed in the brown seaweed *Sargassum siliquosum*, with $LC_{50}$ values of 17.53 and 35.40 mg/cm$^2$ against *Ae. aegypti* and *Ae. albopictus*, respectively. Essential oils from leaves of *Ocimum caninum*, *Ocimum gratissimum*, *Chromolaena odorata*, and *Datura stramonium* were tested for repellent and adulticidal efficacy against *An. gambiae* (Afolabi et al., 2018). The highest adulticidal potential was observed in *D. stramonium* oil with an $LC_{50}$ value of 0.82 mg/l, whereas the lowest adulticidal activity was recorded in *C. odorata* ($LC_{50}$ value = 4.52 mg/l).

### 2.3 Mosquitocidal actions of plant products

Physical and behavioral changes in mosquitoes treated with botanical products, organic solvent extracts and/or essential oils, have been reported by many researchers. The ethanolic extract of *Kaempferia galanga* was found to have detrimental effects on the anal gills of *Cx. quinquefasciatus* larvae by destroying its irregular ridge-like reticulum on the surface of gills that function as an ionic regulator (Insun et al., 1999). Abnormal symptoms such as convulsion, unnatural position, tremor, incoordination, rigor, sluggish movement, failure of body to balance in water, and lack of feeding were recorded in larvae treated with *K. galanga* extract. Similarly, larvicidal investigation of commercially available pine (*Pinus longifolia*) and cinnamon (*Cinnamomum zeylanicum*) oils against *Ae. aegypti* revealed behavioral changes in tested larvae such as excitation, restlessness, tremors, and convulsions, followed by paralysis (Warikoo et al., 2011). Microscopic study of morphological alterations in the treated larvae revealed that most of

their organs had a normal structural appearance similar to that of the controls, except for a little internal shrinkage in the anal gills, which led to structural deformity. Aqueous extract of *Indigofera suffruticosa* leaves was found to have repellent activity, specific embryotoxicity, and general growth retardation in *Ae. aegypti* (Vieira et al., 2012). Morphological changes like disruption on the peritrophic envelope, discontinued underlying epithelium, increased gut lumen, and segments with hypertrophic aspects also were observed in the anterior region of the medium midgut of the treated *Ae. aegypti* larvae. Kjanijou et al. (2012) reported the larvicidal activity of *Murraya paniculata* leaf aqueous extract against *Cx. quinquefasciatus*, with abnormal histology of the larval midgut such as separation of the epithelial cells from the basement membrane, elongation protruding into its lumen, disruption of the microvilli, and appearance of several vesicles and cytoplasm masses.

Implicit changes in various biochemical constituents of insects exposed to a variety of insecticides and biocides from bacterial, fungal, and herbal sources have been reported subsequently by several investigators (Koodalingam et al., 2012; Emtithal and Thanaa, 2012; Nardini et al., 2014). Biochemical effects of some agricultural waste extracts were investigated in the third larval instar of *Cx. pipiens* exposed to a sub-lethal concentration ($LC_{25}$) of black and white liquors, and waste extracts obtained from rice straw (Helmy et al., 2010). It was revealed that treatment with black and white liquors in the larval stage showed high activity of the acetylcholinesterase (AChE) enzyme, but lower activity of esterases ($\alpha$- and $\beta$-esterases) and glutathione S-transferases (GSTs) enzymes in all developmental stages, including larvae, pupae, and adults. High activity of the mixed-function oxidase (MFO) enzyme was found in all stages, except for the pupae of *Cx. pipiens* larvae treated with black and white liquors. It was concluded that increased enzyme activity of MFO and AChE in different stages of mosquito development emerged from larvae treated by both black and white liquors. This suggested that these enzymes may play a role in the detoxification of tested compounds or these compounds act as juvenile hormone analogues. Extra release of AChE may prevent principally any message sent to the receptor and the insect leading to loses neural orientation. On the other hand, suppression of other enzymes such as $\alpha$- and $\beta$-esterases and GSTs indicated that these enzymes play no role in detoxification of the tested

compounds, and may increase susceptibility of the *Cx. pipiens* mosquito to the tested compounds.

Evaluation of resistance development to insecticide in field populations of *Cx. pipiens* from Sharkia, Egypt discovered that after 15 generations of selection pressure using chlorpyrifos against the 3rd instar larvae, resistance increased by 24.56-fold in the resistant strain as compared with the control (Emtithal and Thanaa, 2012). Fractionation of total soluble proteins using SDS-PAGE revealed some differences in the laboratory colony, field populations, and resistant strain of mosquitoes. The results obtained indicated that alkaline phosphatase and non-specific esterases were possibly responsible for detoxification of chlorpyrifos in field mosquitoes. The systemic effect of a *Bacillus thuringiensis*-based product (Vectobar) was assessed in the fourth instar larvae of *Ae. aegypti* by measuring the levels of total proteins and activity of two important marker enzymes, esterases and phosphatases (Koodalingam et al., 2012). Differential modulations were demonstrated in the activities of esterases and phosphatases in larvae exposed to Vectobar at a lethal threshold concentration (0.05 ppm). The level of total protein (34%) as well as activities of AChE (36%), α-carboxylesterase (34%), and alkaline phosphatase (49%) were decreased, whereas a 40% increase in the level of acid phosphatase activity occurred. The Vectobar did not affect the level of *β*-carboxylesterase activity in the treated larvae of *Ae. aegypti*.

Many scientists have attempted to detect a mechanism of action involving potent anti-mosquito activity of botanical biocides, particularly secondary plant metabolites. Laranja et al. (2003) analyzed the esterases (enzyme involved in the detoxification of xenobiotics) in polyacrylamide gels of 4th instar larvae treated with caffeine and used coffee grounds. It was revealed that treatment with both substances affected expression of some carboxylesterases, suggesting that they may be involved in the observed impairment. The impact of herbal biocide on biochemical characteristics was studied by examining quantitative and qualitative changes in total proteins, esterases, and phosphatases in whole body homogenates of fourth instar larvae and pupae of *Ae. aegypti* exposed to *Sapindus emarginatus* (soapnut) aqueous extract (Koodalingam et al., 2011). Upon exposure of the larvae to the *S. emarginatus* extract, significant reduction of AChE, *β*-carboxylesterase, and acid phosphatases activities was recorded, whereas the

total protein, α-carboxylesterase, and alkaline phosphatase activities remained unaffected. By contrast, only alkaline phosphatase activity was affected significantly in pupae exposed to the extract. Analysis of these enzymes in native PAGE revealed their existence in isoforms of both larvae and pupae. However, the profiles of proteins, esterases (AChE, $\alpha$ and $\beta$-carboxylesterases), and phosphatases (acid and alkaline) exhibited distinct patterns of variation during normal development of fourth instar larvae and pupae, indicating intrinsic difference in biochemical features between these two developmental stages of *Ae. aegypti*. Waliwitiya et al. (2012) reported a synergistic interaction between essential oils (thymol, eugenol, pulegone, terpineol, and citronellal) and piperonyl butoxide (PBO) in inhibiting the cytochrome P450 and GST detoxification enzymes in fourth instar larvae of *Ae. aegypti*. Synergism in toxic effects between plant products and synthetic insecticides also has been studied. Tong and Bloomquist (2013) revealed that six essential oils of *Amyris balsamifera, Sesamum indicum, Helichrysum italicum, Santalum album, Juniperus virginiana,* and *Piper nigrum* significantly potentiated the toxicity of carbaryl, but decreased permethrin toxicity against *Ae. aegypti* larvae. However, none exhibited toxicity or synergistic effects in the adulticidal activities against *Ae. aegypti*, at doses up to 2,000 ng/insect. The researchers suggested that the essential oils generating synergistic effects in *Ae. aegypti* larvae inhibited the *in vitro* activities of cytochrome P450 monooxygenases and carboxylesterases in the low milligram per liter range. Thanigaivel et al. (2017) studied the effect of *Alangium salvifolium* leaf extracts on alteration of detoxifying enzymes in *Ae. aegypti* larvae. It was revealed that the methanolic extract tested at 100 ppm decreased $\alpha$ and $\beta$-carboxylesterases and SOD ratio significantly, and upregulated the GST and CYP450 level. The effect of larvicide, crude volatile oil of *Piper betle* (Pb-CVO), on biochemical characteristics was investigated in the wild and susceptible laboratory strains of *Ae. aegypti* (Vasantha-Srinivasan et al., 2017). It was found that while the enzyme level of $\alpha$- and $\beta$-carboxylesterases was reduced significantly, GST and CYP450 levels were increased significantly in both mosquito strains treated with Pb-CVO. The expression of these enzymes also were altered in LS and WS of *Ae. aegypti*, when treatment with temephos.

### 2.4 *Petrosilinum crispum*, the most effective plant sample

**Family:** Umbelliferae or Apiaceae

**English name:** Parsley

**Thai name or local name:** เทียนเยาวพาณี

**Part used:** Fruit (Figure 1.2)

**Botanical description:** The dried fruits are small oval or triangle shape, 2-3 mm long, with prominent style remnants at the apex. Externally it appears yellowish-brown. It has a strongly aromatic odor.

**Habitat:** Italy, Greece, Portugal, Spain, Malta, Morocco, Algeria, and Tunisia

**Constituents:** mainly alkylated phenol (thymol), phenylpropene (myristicin), monoterpene (*p*-cymene and *y*-terpinene), terpenes (carvacrol), etc. (Fetrow and Avila, 1999; Knio et al., 2008).

**Medicinal properties and uses:** Parsley is officially applied to the symptoms of epilepsy, diuretic, irregular menstruation, arthritis, chest pain, insect bites, injuries and get rid of lice, dysentery, gall stones, jaundice, high blood pressure, albuminuria, cornea ulceration, and ophthalmia (David, 2010; Farzaei et al., 2013).

**Other uses:** It has antioxidant properties, anti-inflammatory properties, antiulcerogenic activity, insecticidal activity, antifungal activity, and anti-bacterial properties (Al-Howiniry et al., 2003; Knio et al., 2008; Linde et al., 2016; Akinci et al., 2017).



**Figure 1.2** *Petroselinum crispum* (Mill.) A.W. Hill fruits

## 3. Purposes of the study

In assessing the impact of plant products on the pyrethroid susceptible and resistant strains of *Ae. aegypti*, the specific purposes of the study are as follows:

3.1 To screen for potential larvicidal activity of the ethanolic extracts and/or essential oils of at least 10 medicinal plants against the pyrethroid susceptible strain of *Ae. aegypti* under laboratory conditions.

3.2 To determine the insecticidal (larvicidal and/or adulticidal) activities of the most effective plant sample against the pyrethroid susceptible and resistant strains of *Ae. aegypti* under laboratory conditions.

3.3 To investigate the effect of the most effective plant sample on physical and biochemical changes in the larval and adult stages of the pyrethroid susceptible and resistant strains of *Ae. aegypti*.

3.4 To analyze the chemical constituents of the most effective plant sample.

## 4. Usefulness of the study

The information obtained from this study of botanical effects on susceptibility as well as physical and biochemical changes in the pyrethroid susceptible and resistant strains of *Ae. aegypti* was basic knowledge that help to guide herbal use as a potential alternative in mosquito control programs, particularly in areas with high levels of pyrethroid resistance. Additionally, this work was published or presented publicly according to the academic requirements of Chiang Mai University (CMU).