Parasitol Res (2006) 99:715–721
DOI 10.1007/s00436-006-0232-x

ORIGINAL PAPER

Essential oils as potential adulticides against two populations of *Aedes aegypti*, the laboratory and natural field strains, in Chiang Mai province, northern Thailand

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Received: 21 April 2006 / Accepted: 27 April 2006 / Published online: 1 June 2006

Abstract Essential oils derived from five plant species, celery (*Apium graveolens*), caraway (*Carum carvi*), zedoary (*Curcuma zedoaria*), long pepper (*Piper longum*), and Chinese star anise (*Illicium verum*), were subjected to investigation of adulticidal activity against mosquito vectors. Two populations of *Aedes aegypti*, the laboratory and natural field strains, collected in Chiang Mai province, northern Thailand were tested in pyrethroid-susceptibility bioassays. The results revealed that the natural field strain of *A. aegypti* was resistant to permethrin, with mortality rates ranging from 51 to 66%. A mild susceptibility, with mortality rates ranging from 82 to 88%, was observed in the natural field strain of *A. aegypti* exposed to lambdacyhalothrin, which suggested that this strain was tolerant and might be resistant to this insecticide. However, laboratory-reared *A. aegypti* exposed to discriminating dosages of permethrin and lambdacyhalothrin induced 100% mortality in all cases, thus indicating complete susceptibility of this strain to these insecticides. The adulticidal activity determined by topical application revealed that all five essential oils exerted a promising adulticidal efficacy against both laboratory and natural field strains of *A. aegypti*. Although the laboratory strain was slightly more susceptible to these essential oils than the natural field strain, no statistically significant difference was observed. Moreover, comparison of the adulticidal activity indicated that the performance of these essential oils against the two strains of *A. aegypti* was similar. The highest potential was established from caraway, followed by zedoary, celery, long pepper, and Chinese star anise, with an LC_{50} in the laboratory strain of 5.44, 5.94, 5.96, 6.21, and 8.52 μg/mg female, respectively, and 5.54, 6.02, 6.14, 6.35, and 8.83 μg/mg female, respectively, in the field strain. These promising essential oils are, therefore, an alternative in developing and producing mosquito adulticides as an effective measure used in controlling and eradicating mosquito vectors.

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Introduction

Dengue hemorrhagic fever (DHF) continues to be one of the major public health problems in Thailand, and the *Aedes aegypti* mosquito has been incriminated as the primary vector. The incidence rates of the disease have been increasing, with cyclic outbreaks occurring every 2–3 years. In 2001–2004, approximately 100 cases of DHF per 100,000 population were reported annually in the country (Annual Epidemiological Surveillance Reports 2001–2004). Because there is currently no effective vaccine against dengue, the prevention and control of dengue infection in Thailand depends basically on the eradication of *Aedes* mosquito vectors through two main measures, larviciding and adulticiding by the employment of conventional insecticides. Larval control by applying larvicides such as temephos (Abate 1% sand granules) and reducing the source of mosquito breeding sites are primarily relied upon and used routinely, whereas the control of adults by space spraying of adulticides is still necessary as an emergency measure for suppressing vector populations during epidemic outbreaks of DHF (Thavara et al. 2004). Synthetic pyrethroids such as deltamethrin, cypermethrin, and permethrin are the current insecticides of choice throughout the country for controlling adult mosquitoes

Table 1 Ethnobotanical data, physical characteristics, and percentage yields (% Yield) of essential oils derived from five plant species

Family and botanical name (reference number)	English name	Part used	Physical characteristics			% Yield
			Color	Odor	Density (g/ml)	
Umbelliferae						
Apium graveolens Linn (PARA-AP-001/4)	Celery	Seeds	Pale yellow	Orange-like	0.89	1.25
Carum carvi Linn (PARA-CA-001/4)	Caraway	Seeds	Golden yellow	Pepper-like	0.93	1.06
Zingiberaceae						
Curcuma zedoaria Roscoe (PARA-CU-004/2)	Zedoary	Rhizomes	Yellow	Cineolic-like	0.90	1.32
Piperaceae						
Piper longum Linn (PARA-PI-001/4)	Long pepper	Fruit	Colorless	Pepper-like	0.80	0.63
Illiciaeceae						
Illicium verum Hook.f. (PARA-IL-001/1)	Chinese star anise	Fruit	Pale yellow	Licorice-like	0.98	4.07

both in the household (aerosol canisters) and community (fogging and ULV) (Vector Borne Disease Annual Reports 2002–2003). Control by continued applications of synthetic chemicals, however, is becoming increasingly difficult and inefficient because of the increasing insecticide resistance in mosquito populations. Pyrethroid resistance has been documented in many species of mosquito vectors, particularly those of malaria and dengue. Several cases of field-associated resistance have been reported in *A. aegypti* against pyrethroid aerosol products (Wattanachai and Tintanon 1999). Resistance to DDT and pyrethroids, including permethrin and deltamethrin, was reported in the populations of *A. aegypti* and *Aedes albopictus* from northern Thailand (Somboon et al. 2003). Deltamethrin resistance of the *A. aegypti* population has also been reported in central Thailand (Yaicharoen et al. 2005). Each year, larger doses of synthetic insecticides are required, leading to increased dangers for humans and mammals and progressive contamination of the environment.

With concern for the quality and safety of life and the environment, the emphasis on controlling mosquito vectors has shifted steadily from the use of conventional chemicals toward alternative insecticides that are target-specific, biodegradable, and environmentally safe, and these are generally botanicals in origin. Although plants and their derivatives were used for controlling and eradicating mosquitoes and other domestic pests before the advent of synthetic organic chemicals (Shaalan et al. 2005), only few insecticides of plant origin have been found commercially available. Plant-derived bioproducts, however, still have encouraging results in the control of mosquito vectors if they are adequately effective and harmless to beneficial nontarget organisms and the environment. Furthermore, the insect resistance to mosquitocidal botanical agents has not been documented (Shaalan et al. 2005). Currently, numerous products of botanical origin, especially essential oils, have received considerable renewed attention as potentially bioactive agents used in insect vector management. While many plant essential oils such as citronella, calamus, thymus, and eucalyptus are promising as mosquito larvicides (Shaalan et al. 2005), little work has been performed on their mosquito adulticidal activity. This study was carried out to investigate the efficacy of selected essential oils against adult *A. aegypti*, the laboratory and natural field strains, in search of effective indigenous bioproducts to replace synthetic insecticides for the control of dengue, particularly in cases where susceptibility is decreasing.

Materials and methods

Preparation of plant-derived essential oils

Based on the ethnobotanical and biological effects, five plant species of similar or adjacent families to those reported to have potential against arthropods of medical and veterinary importance (Sukumar et al. 1991; Shaalan et al. 2005) were selected. Plant specimens, as shown in Table 1, were obtained commercially from E.A.R. Samunpri, a medicinal herb supplier in Chiang Mai province, northern Thailand. James Franklin Maxwell, a botanist of the CMU Herbarium, Department of Biology, Faculty of Science, Chiang Mai University, Thailand, performed taxonomic identification of these plants. Voucher specimens were deposited at the Department of Parasitology, Faculty of Medicine, Chiang Mai University. Fruit, seeds, and rhizomes of plants were dried under shade for 1 week before steam distillation. Dried and finely ground material of each plant was steam-distilled to obtain volatile oil. Moisture in the oil was removed over anhydrous sodium sulfate, and after filtration, the oil derived from each plant was collected and kept in an amber-colored bottle at 4°C before investigation of adulticidal activity.

Mosquito test populations

A. aegypti mosquitoes used during the study were divided into two groups: the laboratory and natural field strains. The laboratory colony was established from specimens collected in Chiang Mai province and had been maintained continuously since 1995 without exposure to any insecticides and pathogens. Field strain *A. aegypti* was collected at larval and pupal stages from breeding habitats in Mae Rim district, Chiang Mai province during April to October 2005. After confirmation of their morphological characteristics according to Rattanarithikul and Panthusiri (1994), they were reared into adults. The standard procedure for rearing *Aedes* mosquitoes followed the method described by Limsuwan et al. (1987). Rearing temperatures, relative humidity, and photoperiod in the insectaria were 25±2°C, 80±10% RH, and 14:10 (light/dark) h, respectively. Two- to five-day-old mosquitoes from the laboratory or field populations were randomly selected for adult bioassays. Two experiments, determination of susceptibility to pyrethroid insecticides and investigation of adulticidal activity of selected essential oils, were carried out in this study.

Determination of the susceptibility to pyrethroid insecticides

The susceptibility of adult *A. aegypti* mosquitoes to the synthetic pyrethroids, permethrin, and lambdacyhalothrin was conducted by using WHO test kits with some modifications (WHO 1998). Nonblood-fed adult females were exposed to discriminating concentrations of standard WHO pyrethroid impregnated paper (WHO Vector Control Unit, Penang, Malaysia) under controlled temperatures for 1 h. Each test comprised three groups including controls, deltamethrin treatment, and lambdacyhalothrin treatment. Exposure tubes for the controls contained filter paper impregnated with a carrier (silicone oil), whereas those for treatment contained paper impregnated with an insecticide plus a carrier. The diagnostic dosages of permethrin and lambdacyhalothrin paper, recommended by WHO (1998), were 0.25 and 0.03%, respectively. Twenty-five mosquitoes were introduced to each exposure tube and maintained for 1 h in a vertical position. Four exposure tubes were used for each test group yielding a final total of 100 mosquitoes for each treatment. After exposure, the mosquitoes were transferred to holding tubes and provided with 10% sucrose and 10% multivitamin syrup solution. Mortality was determined after a 24-h holding period. The dead females in the four tubes were combined and expressed as a percentage mortality of each treatment. The bioassays were performed at 25±2°C and 80±10% RH, replicated six times (600 females per treatment) with mosquitoes from different rearing (laboratory) or collecting (field) batches, and reported for the average of individual test results.

Investigation of adulticidal activity of plant-derived essential oils

The bioassay of adulticidal activity was performed by the topical application of plant essential oil to female mosquitoes by following a slightly modified versions of the WHO standard protocols (WHO 1996). Nonblood-fed females were briefly anesthetized with carbon dioxide. The immobilized mosquitoes were weighed, and then placed and subsequently treated on dry filter paper on a refrigerating plate while maintaining anesthesia during manipulation. Treatment was performed with the aid of a dissecting microscope. A 0.1-μl droplet of essential oil dissolved in acetone, yielding a graded series of concentrations, was applied onto the upper part of the immobilized female's pronotum by using a Hamilton's digital syringe (700 series MICROLITER, Hamilton Company, USA). Dosages were expressed in the micrograms of plant material per milligram of mosquito body weight. The filter paper was replaced after each series of essential oil solution to prevent exposure of the test subjects to other test samples. Twenty-five individuals were treated at each concentration, with at least five different concentrations providing a range of mortality from 0 to 100%. Controls were divided into two groups that included an acetone-treated and acetone-untreated group. Both of these groups were treated in a similar manner to that of essential oil-treated groups. After treatment, the females in all groups were maintained in plastic cups at 25±2°C and 80±10% RH, with 10% sucrose and 10% multivitamin syrup supplied. After a 24-h holding period, the mortality rate was recorded. Each test was carried out in eight replicates (200 females per dose of each essential oil exposure) with mosquitoes from different rearing or collecting batches.

Data management and statistical analysis

Experimental tests that demonstrated more than 20% control mortality were discarded and repeated. When control mortality reached between 5 and 20%, the mortality (%M) observed was corrected by Abbott's formula (Abbott 1925):

$$\%M = \frac{\%\,test\ mortality - \%\,control\ mortality}{100 - \%\,control\,mortality} \times 100$$

Data were analyzed by a computerized log-probit analysis (Harvard Programming; Hg1, 2), which provided

Table 2 Percentage mortality (% Mortality) of *A. aegypti* females, laboratory and field strains, recorded at 24 h after exposure to 0.25% permethrin (PER) and 0.03% lambdacyhalothrin (LAM)

Experiment[a]	Treatment	% Mortality	
		Laboratory strain	Field strain
I	PER	100	58
	LAM	100	87
	CONTROL	0	0
II	PER	100	51
	LAM	100	83
	CONTROL	0	0
III	PER	100	55
	LAM	100	85
	CONTROL	0	0
IV	PER	100	66
	LAM	100	82
	CONTROL	0	0
V	PER	100	64
	LAM	100	84
	CONTROL	0	0
VI	PER	100	66
	LAM	100	88
	CONTROL	0	0
Mean±SE (range)	PER	100	60.0±6.3 (51–66)
	LAM	100	84.8±2.3 (82–88)
	CONTROL	0	0

[a]For each experiment, 100 female mosquitoes were used for each group (control, permethrin treatment, and lambdacyhalothrin treatment) yielding a final total of 600 mosquitoes for each treatment

estimated lethal dosages of 50 and 95% (LD_{50} and LD_{95}, respectively), with 95% confidence intervals (95% CI) used to measure differences between test samples.

Susceptibility test results were interpreted according to the WHO protocol (WHO 1998); while 98–100% mortality indicated susceptibility, 80–97% mortality suggested the possibility of resistance that needed to be confirmed and <80% mortality suggested resistance.

Results

The ethnobotanical data, physical characteristics, and percentage yields of the essential oils are summarized in Table 1. Steam distillation of five plant species including *A. graveolens*, *C. carvi*, *C. zedoaria*, *P. longum*, and *I. verum* yielded 0.63 to 4.07% (*v*/*w*) essential oils based on dry weight. The yield obtained from *P. longum* was the lowest, whereas that of *I. verum* was the highest. All volatile oils obtained were less dense than water and demonstrated differences in color and odor.

Table 2 demonstrates the results of the susceptibility test with a single diagnostic dosage of permethrin (0.25%) or lambdacyhalothrin (0.03%) in the two populations of *A. aegypti* mosquito, the laboratory and field strains. It was clearly seen that the natural field strain remained resistant to permethrin, with mortality rates ranging from 51 to 66%. Mild susceptibility, with mortality rates ranging from 82 to 88%, was observed in the natural field strain of *A. aegypti* exposed to lambdacyhalothrin, which suggested that this strain was tolerant and might be resistant to this insecticide. Conversely, laboratory-reared *A. aegypti* was completely susceptible to permethrin and lambdacyhalothrin, as evidenced by 100% mortality in all cases. No mortality was observed in the controls of both the laboratory and field strains tested.

The adulticidal activity determined by topical application revealed that all five essential oils exerted a promising adulticidal efficacy against both the laboratory and natural field strains of *A. aegypti* (Table 3). Comparison of the adulticidal activity showed that the performance of the essential oils against the two strains of *A. aegypti* was similar. The highest potential was established from caraway, followed by zedoary, celery, long pepper, and Chinese star anise, with an LC_{50} in the laboratory strain of 5.44, 5.94, 5.96, 6.21, and 8.52 μg/mg female, respectively, and 5.54, 6.02, 6.14, 6.35, and 8.83 μg/mg female, respectively, in the field strain. Based on the overlapping of 95% CI at LC_{50} values, it was apparent that no significant difference was noted in the adulticidal activity of each essential oil between the laboratory and field strains of *A. aegypti*. However, the laboratory strain was slightly more susceptible to these essential oils than the natural field strain.

Discussions

This study tested the pyrethroid susceptibility of two populations of *A. aegypti* mosquitoes, the laboratory and field strains, based on the standard WHO contact test using discriminating dosages under controlled temperatures. The ability of mosquitoes to have survived the diagnostic dose after 24 h is indicative of resistance in the population, as defined by percentage mortality in the test population (Chareonviriyaphap et al. 2002). The field-collected *A. aegypti* mosquitoes from Mae Rim district, Chiang Mai province were proved resistant and tolerant to permethrin and lambdacyhalothrin, respectively. The collecting site in this study, the Mae Rim district, is between the districts of Muang and Mae Taeng, where permethrin-resistant *A. aegypti* has been previously reported (Somboon et al. 2003). Although no lambdacyhalothrin-resistant population was previously detected in the study of Somboon et al. (2003), the results obtained in this research showed that *A.*

Table 3 Adulticidal activity of five essential oils against adult female *A. aegypti*, laboratory and field strains

Essential oil (μg/mg female)	Laboratory strain			Field strain		
	% Mortality (mean±SE)	Adulticidal activity (95% CI, μg/mg female)[a]		% Mortality (mean±SE)	Adulticidal activity (95% CI, μg/mg female)[a]	
		LD_{50}	LD_{95}		LD_{50}	LD_{95}
A. graveolens		5.96	10.01		6.14	10.62
4.45	18.00±0.00	(5.74–6.17)	(9.18–11.35)	16.00±5.66	(5.91–6.38)	(9.64–12.24)
5.34	34.00±1.41			32.00±1.41		
6.23	55.00±1.41			52.00±9.90		
7.12	70.00±1.41			64.00±4.24		
8.01	88.00±4.24			84.50±0.71		
Control	0			0		
Untreated	0			0		
C. zedoaria		5.94	9.40		6.02	9.62
4.50	16.50±3.54	(5.76–6.11)	(8.75–10.41)	15.00±4.24	(5.84–6.20)	(8.92–10.70)
5.40	29.00±1.41			29.00±11.31		
5.85	47.00±11.31			45.00±4.24		
6.30	66.00±9.90			63.00±1.41		
7.20	76.00±5.66			75.50±0.71		
8.10	87.50±9.19			85.00±9.90		
Control	0.50±0.71			0		
Untreated	0			0		
C. carvi		5.44	9.21	13.00±2.83	5.54	9.34
3.72	14.50±0.71	(5.26–5.63)	(8.46–10.39)	19.00±9.90	(5.36–5.74)	(8.57–10.54)
4.65	22.00±4.24			36.00±4.24		
5.12	38.00±11.31			60.00±8.49		
5.58	62.00±2.83			69.50±7.78		
6.51	71.00±7.07			84.00±2.83		
7.44	86.00±5.66			0.50±0.71		
Control	0			0		
Untreated	0					
P. longum		6.21	10.11		6.35	10.39
4.80	17.00±5.66	(6.00–6.42)	(9.29–11.46)	14.50±3.54	(6.14–6.57)	(9.51–11.85)
5.60	37.00±9.90			35.00±4.24		
6.40	54.00±5.66			51.50±2.12		
7.20	67.00±7.07			64.00±8.49		
8.00	85.00±11.31			82.00±7.07		
Control	0			0.50±0.71		
Untreated	0			0		
I. verum		8.52	15.96		8.83	16.73
5.88	17.50±0.71	(8.12–8.90)	(14.43–18.47)	15.00±2.83	(8.43–9.23)	(15.02–19.58)
7.84	38.50±0.71			37.00±4.24		
9.80	60.00±7.07			55.00±4.24		
10.78	75.00±2.83			70.00±2.83		
11.76	87.00±7.07			85.00±1.41		
Control	0.50±0.71			0		
Untreated	0			0		

[a]Adulticidal activities are no different from each other if 95% confidence intervals (95% CI) overlap

aegypti from Mae Rim district is already tolerant and may be resistant to this insecticide.

The emergence of insecticide resistance in mosquito vectors was considered as one of the important factors influencing the success of vector control. Detection of resistance could lead to planning and selecting appropriate alternative insecticides or measures for practical control. In this study, the adulticidal potential of plant-derived essential oils was measured by topical application, which allowed a good estimation of the intrinsic toxicity of an insecticide by excluding all other effects linked to mosquito behavior, especially when exposed to irritating and repellent insecticides (Bonnet et al. 2004). The potential of selected essential oils against *A. aegypti* mosquitoes is promising in both laboratory and field populations, but not comparable to that of conventional insecticides. Corbel et al. (2004) demonstrated the excellent intrinsic toxicity, measured by topical application against laboratory-reared *A. aegypti* adults, of some pyrethroids and organophosphates, including bifenthrin, permethrin, and temephos, with an LD_{50} value of 0.077, 0.24, and 195 ng/mg female, respectively. Resistance to pyrethroids, organophosphates, and other insecticides, however, is increasingly reported in medically and veterinary important mosquitoes.

A. aegypti, the main vector of dengue viruses in urban areas of Thailand, has long been known to resist DDT (Neely 1966). Resistance to organophosphate insecticides such as malathion, temephos, and fenitrotion has also been reported (Chareonviriyaphap et al. 1999). In the early 1990s, pyrethroids, mainly permethrin and deltamethrin, and to a lesser extent lambdacyhalothrin and etofenprox, were introduced, and they subsequently replaced DDT in the control of malaria and dengue in Thailand (Somboon et al. 1995). Pyrethroids have shown great promise for vector control because of their low mammalian toxicity and remarkable potency at a low level that quickly immobilizes, kills, and repels insects (Prasittisuk 1994; Chareonviriyaphap et al. 1997). However, approximately 1 year after bed nets impregnated with permethrin were introduced, evidence of physiological mosquito resistance from the northern part of Thailand was reported (Annual Malaria Reports 1995–2000). Resistance of *A. aegypti* to pyrethroid compounds in aerosol insecticide products was reported in different areas of Bangkok, Thailand (Wattanachai and Tintanon 1999). Field populations of *A. aegypti* and *A. albopictus* were documented as resistant to DDT and pyrethroids including permethrin and deltamethrin in some provinces of northern Thailand (Somboon et al. 2003). Low-level deltamethrin resistance in field populations of *A. aegypti* in central Thailand was also reported (Yaicharoen et al. 2005). These findings implied that various populations of *Aedes* mosquitoes in Thailand have widely developed resistance to pyrethroids and other synthetic insecticides.

This study provides evidence of permethrin-resistant *A. aegypti* in some areas of Chiang Mai province, suggesting some degree of ineffectiveness in permethrin, which is currently used for the control of adult mosquitoes both at household and community level. Although lambdacyhalothrin, a pyrethroid insecticide, has not yet been widely applied for mosquito control in Thailand, *A. aegypti* has already been tolerant and may be resistant to it. This may be explained by the possibility of cross-resistance among pyrethroids. Even though the collecting sites of *A. aegypti* in this study were small, and more investigations are needed, the results suggest that lambdacyhalothrin-resistant *A. aegypti* is probably widely distributed. The existence of resistance to currently conventional synthetic pyrethroids and the cross-resistance among pyrethroids of mosquito populations reported in this study and previously indicate the limitation of new pyrethroid candidates for application in mosquito control programs. Appropriately, the adulticidal potential of plant-derived essential oils against *A. aegypti* resistant to some pyrethroids, the permethrin and lambdacyhalothrin, reported in this study suggests a promising strategy for use in managing insecticide-resistant mosquito populations. Correspondingly, some plant extracts or phytochemicals have been reported to be highly effective against insecticide-resistant insect pests (Lindquist et al. 1990; Ahn et al. 1997). Despite essential oils likely having less potential than synthetic pyrethroids, their remarkable activities against pyrethroid-resistant mosquitoes make them promising candidates for further study in controlling dengue and other mosquito-borne diseases. Furthermore, unlike synthetic insecticides, the evolution of insect resistance to plant-based products has not been reported. The literature review on the biological effects of neem products demonstrated no cases of arthropod resistance to these agents (Mulla and Su 1999). This finding corresponded to the study of Schmutterer (1988), which stated the failure in an attempt to select for resistance against neem products in the laboratory. The absence of resistance to the products of botanical origin among mosquitoes might be due to their bioactive constituents, which are mixtures of various related compounds with different modes of action and, hence, the development of resistance to such products is somewhat difficult. In addition, the low frequency, short period, and small scales of applying plant-derived insecticides in vector control programs also serve to lower the development of resistance in a mosquito population (Schmutterer 1988; Mulla and Su 1999; Shaalan et al. 2005).

When new insecticides are developed, there is a concern regarding the cross-resistance that may be developed if they have a similar mode of action to well-established insecticides (Shaalan et al. 2005). All essential oils investigated in this study were effective against both laboratory and field strains of *A. aegypti* populations. Although the pyrethroid-susceptible laboratory strain proved to be slightly more susceptible to these essential oils than the pyrethroid-resistant field strain, no statistically significant difference was observed. These results are consistent with those of Amonkar and Reeves (1970), who evaluated the toxic effect of products derived from the garlic, *Allium sativum*, against strains of *Aedes nigromaculis* that were highly susceptible and highly resistant to organophosphate insecticides. They stated that no cross-resistance was apparent because lethal values for the phytochemicals were identical between strains. A counter cross-resistance effect was also revealed in the study of Thomas and Callaghan (1999), who used the same phytochemical, garlic-derived products.

Garlic was found to be more toxic to organophosphate-resistant *Culex pipiens* than susceptible strains. Therefore, the application of botanical-developed mosquitocides that would not necessarily avoid problems posed by some existing mechanisms of resistance to synthetic compounds may offer an advantage (Shaalan et al. 2005).

This study investigated essential oils from five plant species, which show potential as alternatives for developing and producing mosquito adulticides in an effective strategy used to control and eradicate mosquito vectors, especially in areas where mosquitoes are resistant to pyrethroids. Consequently, because Thailand possesses a large diversity of medicinal plants that have potential and a valuable source of biologically active substances, the commercial exploitation of plant-derived products would contribute towards the country's economic development.

Acknowledgement This work is supported by the Faculty of Medicine Research Fund, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

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