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Research

Aedes Albopictus (Diptera: Culicidae) Susceptibility Status to Agrochemical Insecticides Used in Durian Planting Systems in Southern Thailand

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Abstract

High rates of dengue morbidity occur in southern Thailand. The intensive application of insecticides in orchards could affect not only agricultural insect pests, but also nontarget mosquitoes or beneficial insects. In this study, the type and quantity of insecticides commonly used across durian plantations in southern Thailand were characterized, along with the population density of *Aedes albopictus* (Skuse) (Diptera: Culicidae). Our primary aim was to determine the susceptibility status of field-derived *Ae. albopictus* to typical application concentrations of four agrochemical insecticides; cypermethrin, chlorpyrifos, carbaryl, and imidacloprid. Mosquito eggs were collected from durian cultivation sites in five provinces in southern Thailand and used to generate adults for susceptibility tests. The cultivation sites were categorized into three groups based on insecticide application: intensive application of insecticides, low application of insecticides, and no application of insecticides. Twenty ovitraps were deployed for at least three consecutive days at each study site to collect mosquito eggs and to determine *Ae. albopictus* population density. WHO tube assays were used to determine the susceptibility of adult mosquitoes derived from field-collected eggs to selected insecticides. This represents the first report of the susceptibility status of *Ae. albopictus* from durian orchards in southern Thailand to agrochemical insecticides. Results showed complete susceptibility of these *Ae. albopictus* to chlorpyrifos, but reduced mortality following exposure to λ-cyhalothrin, carbaryl, and imidacloprid, which is suggestive of the development of resistance. These findings provide new insights into the status of insecticide susceptibility in *Ae. albopictus* populations, with important implications for mosquito and mosquito-borne disease control in Thailand.

Key words: *Aedes albopictus*, insecticide resistance, durian, agrochemical, Thailand

The viruses responsible for dengue, chikungunya, and zika are spread by mosquitoes, resulting in high disease morbidity and mortality rates every year (Thavara et al. 2009, DDC 2018). In Thailand, *Aedes albopictus* (Skuse), the Asian tiger mosquito, a vector of the aforementioned insect-borne viruses, is most commonly found in both suburban and rural areas of tropical and subtropical regions, particularly where there are open spaces with considerable vegetation (Ponlawat et al. 2005). Female *Ae. albopictus* are closely associated with human activities because they are present in houses and around cultivation areas, such as rubber plantations and other tropical fruit orchards (Sullivan et al. 1971, Thammapalo et al. 2009, Tangena et al. 2016).

Tropical fruit orchards are widely cultivated in the southern region of Thailand (Tantrakonnsab and Tantrakoonsab 2018). Durian orchards are one of the most common types in southern Thailand, and numerous commercial durian growers enhance their harvest by intensive application of agrochemical insecticides. The use of insecticides in durian orchards is especially common during off-season planting, allowing the fruits to grow gradually throughout the year. Different groups of insecticides, along with their respective application concentrations, are recommended for durian cultivation. These include organophosphates (chlorpyrifos, methidathion), pyrethroids (λ-cyhalothrin, cypermethrin), carbamates (carbaryl, carbosulfan), and amitraz (Wanwimolruk et al. 2015). The continuous and widespread use of agrochemical insecticides in the durian planting systems can cause insect populations in the area, including nontarget insect pests like mosquitoes (Overgaard et al. 2005, Overgaard 2006), to become less susceptible to insecticides.

The resistance of mosquitoes to several chemicals approved for public health use has long been reported in Thailand (Chareonviriyahpap et al. 1999, Overgaard 2006, Chareonviriyaphap et al. 2013, Corbel et al. 2016). *Aedes albopictus* larvae in Phatthalung showed resistance to permethrin, whereas adults in Songkhla were found to remain susceptible to deltamethrin, permethrin, fenitrothion, and propoxur (Pethuan et al. 2007). Chuaycharoensuk et al. (2011) reported the susceptibility to deltamethrin in adult *Ae. albopictus* from rubber plantation areas in Sadao, Songkhla. Agricultural areas represent good habitats for mosquito development, and the intensive use of insecticides and other agrochemicals for crop protection in these areas may contribute to the selection of insecticide resistance genes. Mosquito populations in agricultural areas, however, generally remain susceptible to pyrethroids, and pyrethroid resistance does not presently pose a direct threat to vector control. Nevertheless, increased use of pyrethroids in agriculture may cause problems for future vector control (Overgaard et al. 2005).

Because of the reported spread of insecticide resistance across different geographic locations in Thailand, an evaluation of insecticide use is needed. Moreover, new insecticides, which can be used as alternatives to those currently employed, and perhaps a change in the application regimens of currently used insecticides, may be required to combat the growing threat of insecticide resistance to mosquito control. A system for monitoring the effectiveness of insecticides by local communities is also required. Rotation systems that involve switching from one insecticide to another can also be designed to prevent the development of insecticide resistance in mosquito populations. The evolution of resistance to different insecticides approved for public health and agricultural use should also be considered when decisions are made relating to vector control.

The increasing number of dengue cases in Thailand may be in part due to failed dengue control efforts, which can result from many factors. However, in areas where insecticide resistance has been recorded, the use of physical or biological controls should be considered as an alternative to the use of insecticides (Jirakanjanakit et al. 2007a,b; Pethuan et al. 2007).

Since 2016, the number of dengue cases has continued to increase, reaching high levels that have never before been recorded in southern Thailand (DDC 2018). Several hypotheses have been put forward to explain this phenomenon. These include the ineffectiveness of dengue vector control, poor self-protection against mosquito bites by those living in dengue-endemic areas, and the reduced susceptibility of mosquitoes to insecticides (Limkittikul et al. 2014). Thus, the insecticide susceptibility status of *Ae. albopictus*, which commonly breed in orchard areas, needs to be evaluated. Some groups of insecticides, which share similar modes of action, are commonly used by both public health authorities for vector control, and in durian plantations to control insect pests. The development of resistance to agrochemical pesticides in *Ae. albopictus* populations in these durian plantations may result in resistance to public health insecticides with similar modes of action. The study reported in this article was therefore conducted to investigate whether this was the case in southern Thailand. Specific objectives were to 1) determine the density of *Ae. albopictus* in durian planting systems in southern Thailand, 2) characterize the type and quantity of insecticides used in these systems, and 3) determine the insecticide susceptibility status of *Ae. albopictus* to frequently used agrochemical insecticides in the area.

Materials and Methods

Study Area

A survey of 22 durian orchards in southern Thailand was conducted to determine the demographic characteristics of cultivators and the frequency of insecticide application. Frequency of insecticide application was grouped as follows: intensive application of insecticides (IA) for sites where insecticides were applied every 7–15 d (n = 12), low application of insecticides (LA) for sites where insecticides were applied for 15 consecutive days once or twice a year (n = 3), and (NA) for sites with no application of insecticides (n = 7). The 22 durian orchards included were located in Chumphon (CHU), Nakhorn Si Thammarat (NAK), Phatthalung (PHA), Satun (SAT), and Songkhla (SON) provinces, and eligible participants were the cultivators at the orchards. Each cultivator gave permission for the study site to be accessed and for mosquito collections. A questionnaire-based survey was subsequently used to collect information regarding the type, frequency, and quantity of insecticides used in each orchard surveyed. Each study site was georeferenced by GPS based on its coordinates, and its location was mapped using Google Maps (Figure 1). The coordinates for each location are presented in Table 1.

Mosquito Collection

At each study site, eggs of *Ae. albopictus*, as well as all immature stages present, were collected using ovitraps. Each ovitrap comprised a black plastic cup of 15 cm diameter and 10 cm height lined with a piece of cotton fabric (6 × 45 cm) to provide an ovipositional site. The cup was filled with approximately 150 ml of filtered tap water, and four small drainage holes were drilled into the top of the cup to prevent overflow of water and loss of eggs, especially during rainy season collections. At each durian orchard study site, 20 ovitraps, 3 m apart, were randomly placed on the ground for a period of three to five days. Each trap was labeled with a trap number and trap position, and environmental conditions were recorded. After 3–5 d, the traps and the water in each trap were collected and brought back to the laboratory. The eggs on the fabric were counted and the number per trap recorded prior to hatching. Resulting larvae were raised at a density of 150/1,000 ml of well water in plastic trays (30 × 20 × 12 cm). The larvae were fed with fish food (Sakura, U Lek Trading Co., Ltd., Bangkok, Thailand) once a day until the pupal stage. Pupae were counted and collected daily and placed into a mesh cage for adult eclosion. Resulting adults were reared in a mesh cage (30 × 30 × 30 cm) at the Agricultural Innovation and Management Division, Prince of Songkla University, under the following laboratory conditions: 25 ± 2°C, 80% RH, and sustained on cotton soaked in 10% sugar solution. They were morphologically identified to species using a stereomicroscope.

Mosquito Populations Used for Agrochemical Insecticide Susceptibility Test

Aedes albopictus Susceptible Strain

The eggs of a laboratory strain of *Ae. albopictus* were obtained from the Department of Entomology, Kasetsart University, Bangkok. This strain was originally from the Ministry of Public Health Thailand and had been in colony at Kasetsart University for over 50 generations. Eggs were obtained from adults that were sustained on blood via artificial membrane feeding (Yaya and Tainchum 2017)

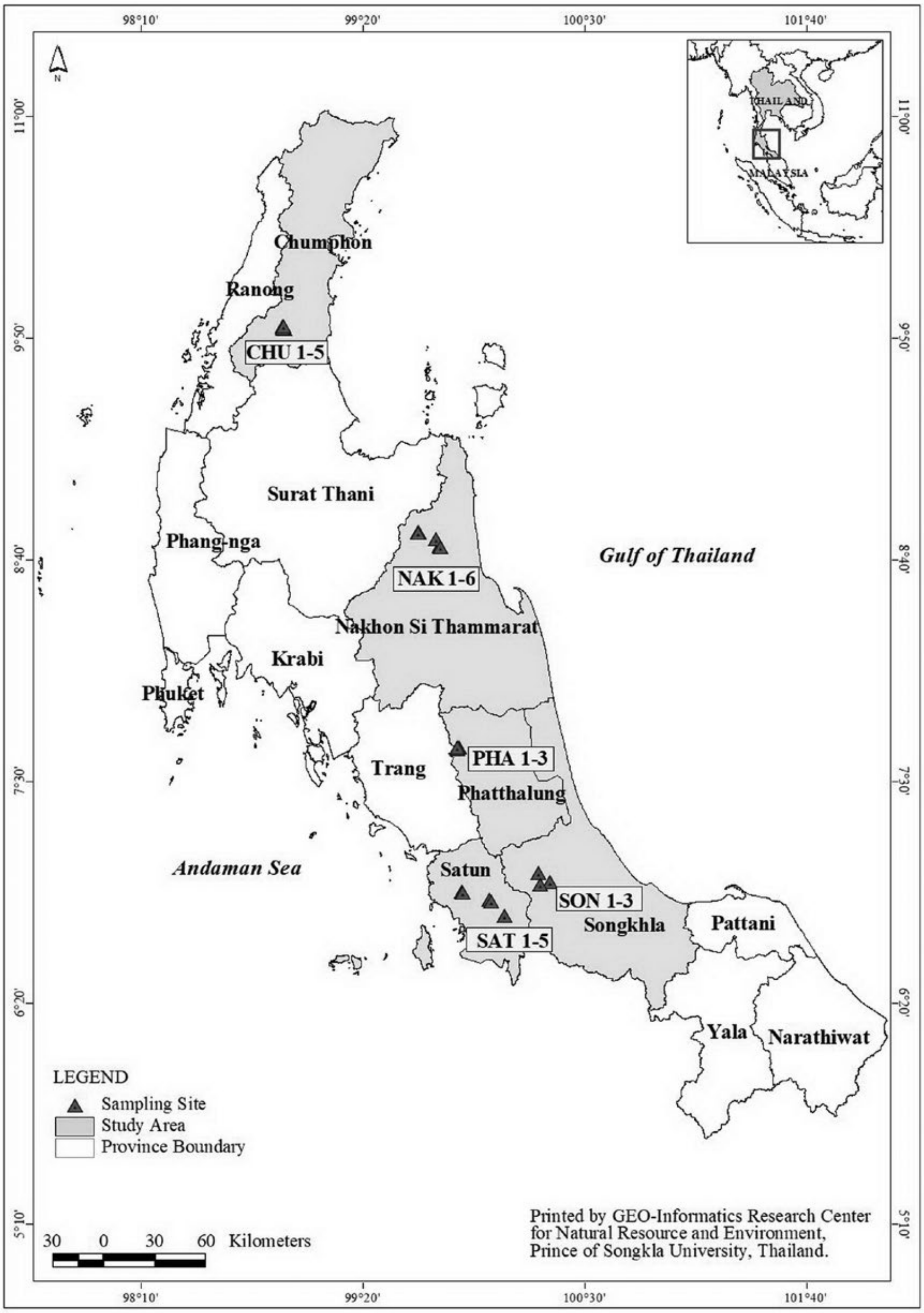

Fig. 1. Location of durian orchards included in this study in Chumphon, Nakhon Si Thammarat, Phattalung, Satun, and Songkhla provinces.

to generate sufficient numbers of adult mosquitoes for insecticide susceptibility bioassays.

Aedes albopictus **Field Populations**

Immature mosquitoes collected from the orchards were mass reared as described above. Female mosquitoes aged 3–5 d were starved for 24 h before insecticide susceptibility testing. Only first to fifth (F_1–F_5) generation females were used and mixed in tests to be representative of the field population.

Aedes aegypti **Susceptible Strain**

The eggs of a laboratory strain of *Aedes aegypti* (USDA), which originated from the Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, was obtained from the Department of Entomology, Kasetsart University, Bangkok. This population had also been in laboratory colony for over 50 generations. Adults for the bioassays were obtained as described for *Ae. albopictus* susceptible strain.

Preparation of Agrochemical Insecticides

Information obtained from the questionnaires showed that the most frequently used agrochemical insecticides in the selected durian orchards were pyrethroid, organophosphate, carbamate, and neonicotinoid. Available commercial forms of these insecticides, along with the field application dosages on their product labels, were used for bioassays. These comprised chlorpyrifos (touchban, 40% EC, produced

Table 1. The coordinates of the 22 durian orchards classified based on the frequency of insecticide application

Durian planting system	No.	Site	GPS coordinates
Intensive-application of insecticides	1	CHU 1	9°52′49.9″N 98°55′04.2″E
	2	CHU 2	9°52′52.7″N 98°55′07.0″E
	3	CHU 3	9°53′11.8″N 98°54′35.3″E
	4	CHU 4	9°53′35.0″N 98°54′47.1″E
	5	CHU 5	9°53′38.4″N 98°54′50.8″E
	6	PHA 3	7°39′59.8″N 99°49′57.4″E
	7	SAT 4	*albopictus* 99°59′58.9″E
	8	NAK 1	8°48′31.6″N 99°37′36.4″E
	9	NAK 2	8°48′37.7″N 99°37′27.2″N
	10	NAK 3	8°48′33.5″N 99°37′28.5″E
	11	NAK 5	8°44′11.1″N 99°44′15.5″E
	12	NAK 6	8°46′23.2″N 99°42′58.2″E
Low application of insecticides	1	NAK 4	8°43′59.6″N 99°44′28.4″E
	2	SON 1	6°58′14.4″N 100°19′00.3″E
	3	SON 3	7°00′58.0″N 100°15′28.4″E
No application of insecticides	1	PHA 1	7°40′43.9″N 99°50′18.0″E
	2	PHA 2	7°40′45.0″N 99°49′56.5″E
	3	SAT 1	6°54′59.2″N 99°51′19.7″E
	4	SAT 2	6°54′47.0″N 99°51′24.6″E
	5	SAT 3	6°47′25.8″N 100°04′46.7″E
	6	SAT 5	6°52′01.2″N 100°00′23.4″E
	7	SON 2	6°57′17.4″N 100°16′00.9″E

by Pro Enterprise Co., Ltd., Nakhon Chai Si, Nakhon Pathom, 60 ml/water 20 liter), λ-cyhalothrin (Karate 2.5 EC, 2.5% EC, produced by Syngenta Crop Protection Co., Ltd., Mueang, Samut Prakan, 25 ml/water 20 liter), carbaryl (Sethrin 85, 85% WP, produced by Muang Thong Agriculture Co., Ltd., Lam Luk Ka, Pathumthani, 20 g/water 20 liter), and imidacloprid (Pidofin, 10% SL, produced by SPKG Biokem Co., Ltd., Phutthamonthon, Nakhon Pathom, 10 ml/water 20 liter). Tap water was used as a diluent and as a negative control.

Insecticide-Treated Filter Paper

Insecticide-treated papers were made at the Pest Management Laboratory, Agricultural Innovation and Management Division, Prince of Songkla University, based on the standard procedure and specifications of the World Health Organization (WHO 2016). Insecticide-treated papers were prepared using Whatman No. 1, 12 × 15 cm size, and each insecticide separately. The papers were treated with 2 ml of insecticide solution per sheet and subsequently dried at room temperature for at least 24 h, then kept in aluminum foil and stored in refrigerator (4°C) prior to use. Control papers were prepared in the same manner but impregnated with only 2 ml of tap water. All treated papers were used only once.

WHO Susceptibility Tests

The insecticide susceptibility status of the *Ae. albopictus* laboratory and field strains was determined using WHO susceptibility test kits according to the WHO protocol (WHO 2016). Each set of a test kit for both treatment and control comprised a pair of exposure tubes one marked with a red dot and containing insecticide-treated paper (water-treated paper for control) and a holding tube marked with a green dot and containing untreated paper. Twenty-five 3- to 5-d-old, starved female *Ae. albopictus* were introduced into each respective holding tube and held for 5 min to allow them acclimatize to the tubes. The starved mosquitoes were deprived of access to blood or sugar, but a cotton ball soaked in water was provided for 24 h prior to their use in bioassay test. Hereafter, all the mosquitoes were subsequently exposed for 60 min to either treated or control paper surfaces in the exposure tubes. The number of mosquitoes knocked down in each test was recorded at 60 min, and all the specimens were subsequently transferred into the clean holding tubes and held for 24 h sustained with cotton pads soaked with 10% sucrose solution. Four replicates of each insecticide and control were performed. The final mortality of the treatment and control mosquitoes were recorded 24 h post-exposure.

Comparison between the Susceptibility of *Aedes* Mosquitoes to Pyrethroid Agrochemical and Public Health Insecticides

Pyrethroid insecticides are the most used type of public health insecticides for mosquito control and management. Two concentrations of λ-cyhalothrin based on agricultural (0.001 g a.i./m^2) and public health (0.01 g a.i./m^2) application dosages were used to determine the susceptibility status of field-derived *Ae. albopictus*, with susceptible laboratory strains of *Ae. albopictus* and *Ae. aegypti* used as reference. Impregnation of test filter papers and insecticide susceptibility tests were performed as described above.

Data Analysis

Data from the questionnaires were recorded on a spreadsheet and analyzed using Microsoft Excel software (Excel 2013). Descriptive statistics comprising means, percentages, and ranges were computed. In each study location, any patterns associated with the participant's responses were identified, and the susceptibility of collected mosquitoes to each insecticide was determined. Mosquito mortality rates following bioassays were calculated according to WHO guidelines (WHO 2016). The WHO criteria for categorizing insecticide resistance in a mosquito population following bioassays are as follows: 1) susceptible if there is 98–100% mortality, 2) incipient resistance/tolerance if there is between 90 and 97% mortality (this merits further testing), and 3) resistant if mortality is below 90%. Using Scheffe's multiple range tests, mosquito density, as well as percentage knockdown (KD) and mortality following bioassays, was compared between durian insecticide application systems. The susceptibility of *Aedes* mosquitoes to agrochemical and public health application dosages of λ-cyhalothrin was compared using independent-samples *t*-test. Significance level for these tests was set to $P < 0.05$ (SPSS program version 15 for Windows).

Results

Types and Quantity of Insecticides Used in Durian Planting Systems in Southern Thailand

As shown in Table 2, a majority (63.64%) of the 22 durian cultivators surveyed were between 51 and 75 yr old, and most (81.82%) were male. Their highest education levels were primary, 45.45%; secondary, 22.73%; and Bachelor's degree, 31.82%. Most of the respondents were farmers (90.91%), with the remaining 9.09% being government employees or officers. The form of agriculture practiced was largely polyculture (77.27%), with the remaining proportion practicing monoculture. Both forms of culture utilized cultivation areas of at least 0.32 ha. Within the 22 orchards surveyed, trees were mostly 6–10 m apart (81.82%). Of all the cultivators surveyed, 68.18% used insecticides, and the highest frequency of insecticide use per month was every 6–10 d (60.00%), followed by 10–15 d (20.00%), and over 15 d (20.00%). Only 3 (13.64%) of the durian cultivators, comprising the owners of CHU 5 (IA area), SON 1 (LA area), and SON 3 (LA area), reported being sick due to mosquito-borne disease, in each case having contracted dengue.

Insecticides Used for Insect Pest Control in Durian Plantations

As shown in Table 3, the type and frequency of insecticides used were recorded across the different durian insecticide application systems. Of a total of 17 recorded users, a combination of organophosphate and pyrethroid insecticides was most common, accounting for 29.41%. This was followed by pyrethroids (17.64%), carbamates (17.64%), organophosphates (11.76%), neonicotinoids (11.76%), pyridazinone (5.88%), and avermectin (5.88%). The frequency of spraying for each of these insecticides was 7–15 d per month.

Density of *Ae. albopictus* in Durian Planting Systems in Southern Thailand

Figure 2 shows the number of mosquito eggs per trap, along with the Scheffe multiple range test results comparing the number of eggs per trap between orchards. In the three durian insecticide application systems, IA, LA, and NA, the mean number of eggs per trap ranged from 4.40 to 63.70, 10.00 to 50.35, and 6.16 to 115.20, respectively, and significant differences between durian plantations ($P < 0.05$) were found (Fig. 2). The site with the most mosquito eggs was PHA 1 (115.20 ± 12.83), followed by PHA 2 (73.25 ± 21.49) and PHA 3 (63.70 ± 10.69)—with the former two categorized as NA, and the latter as IA. No mosquito eggs were collected from 58.33% of the IA orchards. In addition, the mean number of pupae collected from the following durian insecticide application systems, IA, LA, and NA, were in the range of 2.05–26.20, 1.42–39.80, and 10.05–39.60, respectively. The top three highest number of pupae was collected from SAT 4 (26.20), SON 1 (39.80), and SAT 5 (39.60). Conversely, the three sites with the least number of pupae were PHA 3, SON 3, and SON 2. All of the eggs collected from NAK 5 and PHA 2 either failed to hatch or did not develop to the pupal stage (Fig. 3).

Susceptibility of Field-Derived *Ae. albopictus* to Frequently Used Agrochemical Insecticides in Durian Planting Systems

The susceptibility tests on field-derived *Ae. albopictus*, with laboratory strains of *Ae. aegypti* and *Ae. albopictus* as reference, revealed variation in the proportions of knockdown and mortality across different insecticides and study sites. The proportion of knockdown in laboratory strains of *Ae. albopictus* (NIH) and *Ae. aegypti* (USDA),

Table 2. Demographic information of durian cultivators who participated in the study

	Participant characteristic, *N* (%)			
Age (yr)	<25	26–50	51–75	
	0 (0.00)	8 (36.36)	14 (63.64)	
Sex	Male	Female		
	18 (81.82)	4 (18.18)		
Education	Primary school	Secondary school	Bachelor's degree	
	10 (45.45)	5 (22.73)	7 (31.82)	
Occupation	Farmer		Government employee	
	20 (90.91)	2 (9.09)		
Type of durian orchard	Monoculture	Polyculture		
	5 (22.73)	17 (77.27)		
Size of durian orchard (ha)	<0.64	0.80–1.28	1.44–1.92	<2.08
	6 (27.27)	7 (31.82)	4 (18.18)	5 (22.73)
Spacing between trees (m)	Undetermined	0–5	6–10	
	2 (9.09)	2 (9.09)	18 (81.82)	
Insecticide	Use	Not use		
	15 (68.18)	7 (31.82)		
Frequency of insecticide application (d)	1–5	6–10	11–15	15+
	0 (0.00)	9 (60.00)	3 (20.00)	3 (20.00)
Ever had *Aedes*-borne diseases	Yes	Never		
	3 (13.64)	19 (86.36)		

Table 3. Type of agrochemical insecticides, their frequency of use, and the proportion of respondents who use them within the insecticide application systems included in this study

Insecticide group	IRAC	Active ingredient (a.i.)	Frequency of spraying (d)	Number of respondents (%)
Pyrethroid	A3	Cypermethrin	15	1 (5.88)
	A3	λ-Cyhalothrin	15	2 (11.76)
Organophosphate	B1	Chlorpyrifos	7	2 (11.76)
Organophosphate+pyrethroid	B1+A3	Chlorpyrifos + cypermethrin	7, 10	4 (23.53)
	B1+A3	Profenofos + cypermethrin	10	1 (5.88)
Carbamate	A1	Fenobucarb	10	1 (5.88)
	A1	Methomyl	7	1 (5.88)
	A1	Carbaryl	15	1 (5.88)
Neonicotinoid	A4	Imidacloprid	10, 15	2 (11.76)
Pyridazinone[1]		Pyridaben	7	1 (5.88)
Avermectin	6	Abamectin	10	1 (5.88)

Insecticide grouping is based on the Insecticide Resistance Action Committee (IRAC) classification.
[1]Herbicide.

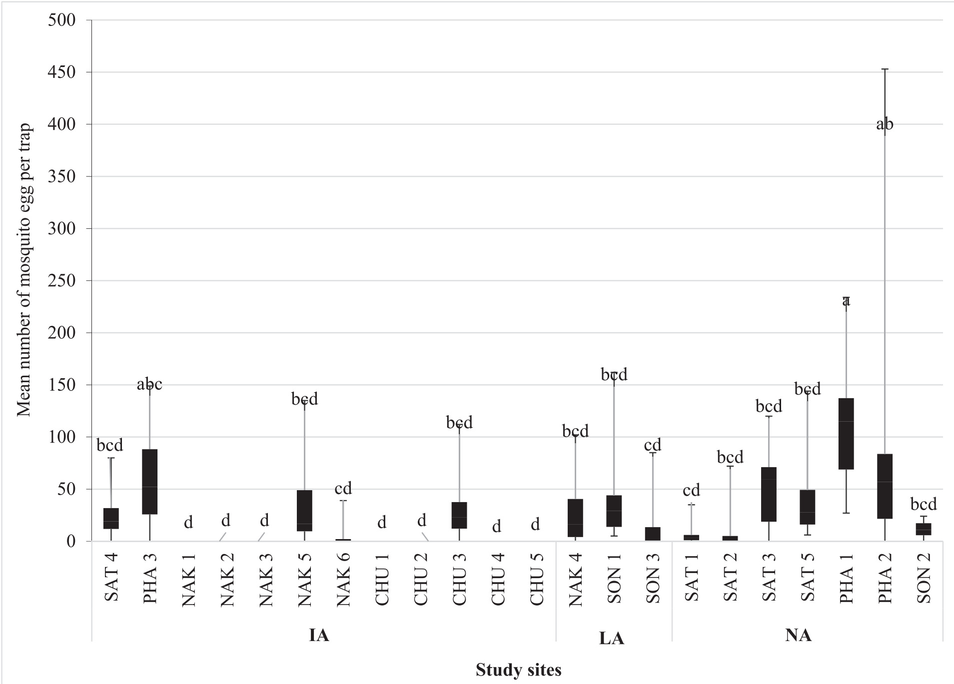

Fig. 2. Mean number of *Aedes albopictus* eggs/ovitraps in each study site and Scheffe's multiple range test between each orchard. IA, intensive-application of insecticides, LA, low application of insecticides, and NA, no application of insecticides, the same letters (a–d) are nonsignificantly different at $P > 0.05$.

as well as field-derived *Ae. albopictus*, following 60 min of exposure to field application concentrations of chlorpyrifos, λ-cyhalothrin, carbaryl, and imidacloprid, are shown in Table 4. The mosquitoes used as controls were all alive after bioassay with 0% knockdown. Overall, the percentage knockdown from highest to lowest was caused by imidacloprid < carbaryl < λ-cyhalothrin < chlorpyrifos. Surprisingly, a high proportion of knockdown was observed in both laboratory strains (5–100% knockdown) for all insecticides except imidacloprid. Following exposure to imidacloprid, less than 3% knockdown was recorded in the field-derived *Ae. albopictus* from SAT 4 (1.25%), an IA site, and PHA 1 (2.50%), an NA site. The remaining mosquitoes showed 100% knockdown. Other than mosquitoes (showing 76% knockdown) from PHA 3, an IA site, a 100% knockdown was seen in all mosquitoes following exposure to chlorpyrifos. Table 5 shows the percentage mortality across the *Aedes* laboratory strains as well as the field-derived *Ae. albopictus* 24 h post-exposure to field application concentrations of chlorpyrifos, λ-cyhalothrin, carbaryl, and imidacloprid. There was no mortality in any of the controls. A 100% mortality was seen in all mosquitoes 24 h post-exposure. The proportion of mortality recorded 24 h post-exposure to λ-cyhalothrin ranged from 46.23 to 81.20%, except in PHA 3, an IA site, where a higher mortality (96.84%) was recorded. Apart from mosquitoes from SON 1, an LA site, where mortality was 96.05%, a majority of the mosquitoes that were exposed to carbaryl showed mortality below 90% (mortality range, 40.00–88.73%). All mosquitoes exposed to imidacloprid, showed mortality below 11% (Table 5).

Comparison of *Aedes* Susceptibility to Agrochemical and Public Health Pyrethroid Insecticides

Comparison of *Aedes* susceptibility to recommended agrochemical (AL) and public health (PL) concentrations of λ-cyhalothrin showed a higher overall proportion of knockdown with public health (>94.80 and 96.15%, respectively) compared with agrochemical concentrations (37.84–97.50% and 45.75–86.43%, respectively). Field-derived *Ae. albopictus* showed higher proportions of knockdown and mortality to PL compared with AL. These differences in percentage knockdown and mortality between PL and AL were statistically significant ($P < 0.01$; Table 6).

Discussion

The objective of this study was to determine the density and insecticide-resistance status of *Ae. albopictus* in durian planting systems in southern Thailand. A similarly designed study was conducted by de Albuquerque et al. (2018) in which ovitraps were set for 15 or 30 d near a house in the urban areas of Itacoatiara and Tabatinga, in Amazonas, Brazil, to examine the density of *Ae. aegypti*. That study found a positive correlation between *Ae. aegypti* egg density and the occurrence of dengue. Previous work by Regis et al. (2008) based on ovitrap collections of *Ae. albopictus* near forested areas with high rates of disease transmission, showed that *Aedes* egg density index (EDI) was equal to 100–750 eggs per trap.

Ovitraps for mosquito collection vary in design and can be made from different kinds of material. For this study, we used the well-known ovitrap design developed by the US Centers for Disease Control and Prevention (CDC 2018). The traps can be made using a small metal, glass, or plastic container, often dark in color, containing water and material in which females can lay eggs. This trap, which is inexpensive and easily transportable, is mainly used to survey the population of *Aedes* mosquitoes. One drawback of using ovitraps is that they could become mosquito breeding sites if left unattended for more than a week. Additionally, environmental and/or human activities may contribute mosquito breeding sites that could compete with ovitraps, thus compromising the number of eggs collected by an ovitrap (CDC 2018).

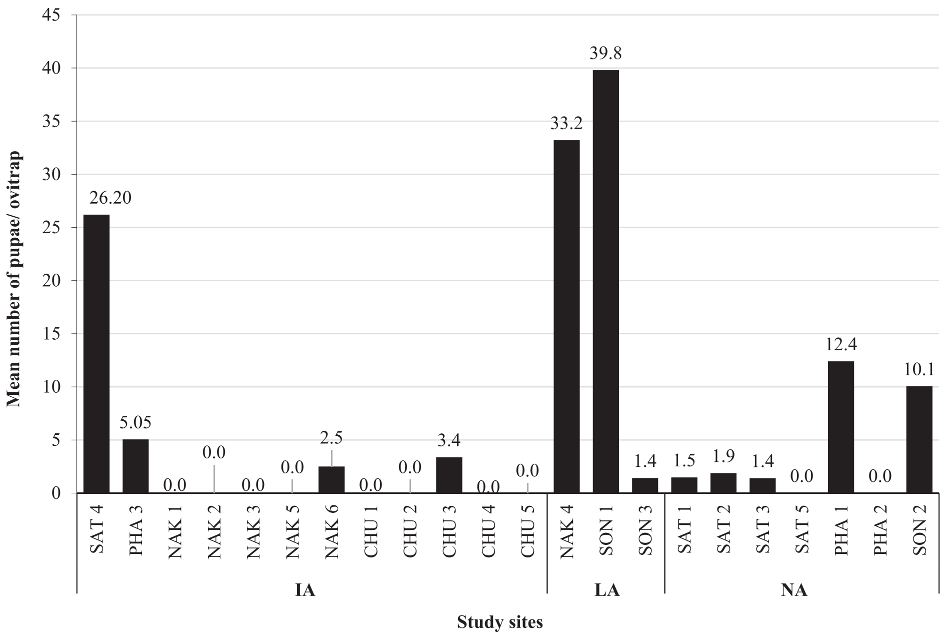

Fig. 3. Mean number of *Aedes albopictus* pupae per study site.

Table 4. Mean percentage mosquito knockdown[1] among laboratory *Aedes* strains and field-derived *Aedes albopictus* following 24-h exposure to field application concentrations of chlorpyrifos, λ-cyhalothrin, carbaryl, and imidacloprid

Strain	Control	Chlorpyrifos	λ-Cyhalothrin	Carbaryl	Imidacloprid
USDA[2]	0.00^{b}	100.00^{aA}	80.00 ± 4.33^{aAB}	26.67 ± 17.02^{bA}	0.00^{bA}
NIH	0.00^{b}	100.00^{aA}	76.25 ± 17.37^{aAB}	5.00 ± 4.33^{bA}	0.00^{bA}
IA					
SAT 4	0.00^{c}	100.00^{aA}	41.25 ± 8.51^{bB}	2.50 ± 2.50^{cA}	1.25 ± 1.25^{cA}
PHA 3	0.00^{c}	76.00 ± 4.62^{bB}	91.00 ± 4.12^{aA}	2.00 ± 1.15^{cA}	0.00^{cA}
LA					
NAK 4	0.00^{c}	100.00^{aA}	68.75 ± 3.75^{bAB}	18.33 ± 15.88^{cA}	0.00^{cA}
SON 1	0.00^{c}	100.00^{aA}	65.65 ± 4.72^{bAB}	53.47 ± 12.91^{bA}	0.00^{cA}
NA					
SAT 5	0.00^{b}	100.00^{aA}	80.00 ± 7.36^{aAB}	7.50 ± 2.95^{bA}	0.00^{bA}
PHA 1	0.00^{c}	100.00^{aA}	47.50 ± 4.79^{bAB}	7.50 ± 3.23^{cA}	2.50 ± 1.44^{cA}

IA, intensive application of insecticides; LA, low application of insecticides; NA, no application of insecticides.

[1]Means with the same small letter(s) across a row or same capital letter(s) across a column are not significantly different at 5% level of significance ($P < 0.05$) according to Scheffe's comparison test.

[2]Laboratory strain, USDA = *Ae. aegypti* and NIH = *Ae. Albopictus*.

The insecticides used in this study were applied as weight of active ingredient per square meter (a.i./m^2) based on the following product recommendations for agricultural use: 1) 0.04 g of organophosphate (chlorpyrifos), 2) 0.001 g of pyrethroid (λ-cyhalothrin), 3) 0.03 g of carbamate (carbaryl), and 4) 0.002 g of neonicotinoid (imidacloprid). However, the concentrations of insecticides recommended by the WHO for for mosquito control (public health use) are as follows: organophosphate (fenitrothion) 2.0 g a.i./m^2, pyrethroid (λ-cyhalothrin) 0.02–0.03 g a.i./m^2, and carbamate (propoxur) 1.0–2.0 g a.i./m^2. Neonicotinoids have not yet been approved for public health use (WHO 2015). The a.i./m^2 recommended for agricultural purposes is much less than that approved for public health applications. Because mosquitoes are nontarget insects of agricultural insecticide applications, continued exposure to sublethal concentrations of agricultural insecticides could select insecticide resistance in mosquito populations. This is a probable cause of the reduced mortality in field-derived *Ae. albopictus* that were exposed to all insecticides except chlorpyrifos.

This low proportion of knockdown and mortality may not be entirely due to their lower insecticide susceptibility. For example, imidacloprid is a neonicotinoid, a group of synthetic substances that imitate the action of nicotine. Neonicotinoids work by binding to the nicotinic acetylcholine receptor in the central nervous system, thus blocking signal transmission to nerve cells. Imidacloprid enters the insect's system through the oral route (Gervais et al. 2010), but with the WHO susceptibility bioassays used in this study, insecticides are acquired by tarsal contact. This may have precluded entry of imidacloprid into the mosquitoes' system, thereby preventing their action. Thus, it cannot be concluded that the field-derived *Ae. albopictus* in this study were resistant to imidacloprid.

Table 5. Mean percentage mortality[1] of laboratory *Aedes* strains and field-derived *Aedes albopictus* following 24-h exposure to field application concentrations of chlorpyrifos, λ-cyhalothrin, carbaryl, and imidacloprid

Strain	Control	Chlorpyrifos	λ-Cyhalothrin	Carbaryl	Imidacloprid
USDA[2]	0.00^{b}	100.00^{a}	62.11 ± 15.70^{abAB}	40.00 ± 24.62^{abA}	0.00^{bA}
NIH	0.00^{c}	100.00^{a}	65.03 ± 8.53^{bAB}	57.21 ± 15.83^{bA}	0.00^{cA}
IA					
SAT 4	0.00^{c}	100.00^{a}	77.35 ± 4.10^{abAB}	56.62 ± 13.12^{bA}	1.25 ± 1.25^{cA}
PHA 3	0.00^{c}	100.00^{a}	96.84 ± 2.16^{aA}	61.29 ± 5.21^{bA}	0.00^{cA}
LA					
NAK 4	0.00^{c}	100.00^{a}	81.20 ± 3.45^{aAB}	52.11 ± 12.24^{bA}	0.00^{cA}
SON 1	0.00^{c}	100.00^{a}	46.23 ± 10.68^{bB}	96.05 ± 3.95^{aA}	0.00^{cA}
NA					
SAT 5	0.00^{b}	100.00^{a}	77.31 ± 5.89^{aAB}	80.15 ± 12.89^{aA}	1.25 ± 1.25^{bA}
PHA 1	0.00^{c}	100.00^{a}	59.38 ± 8.64^{bAB}	88.73 ± 7.86^{aA}	10.33 ± 4.56^{cA}

IA, intensive application of insecticides; LA, low application of insecticides; NA, no application of insecticides.

[1]Means with the same small letter(s) across a row or same capital letter(s) across a column are not significantly different at 5% level of significance ($P < 0.05$) according to Scheffe's comparison test.

[2]Laboratory strain, USDA = *Ae. aegypti* and NIH = *Ae. albopictus*

Table 6. Comparison between the susceptibility of *Aedes* mosquitoes to agrochemical and public health application dosages of λ-cyhalothrin

Strains	%KD 60 min			%Mortality 24 h		
	Control	PL[1]	AL[2]	Control	PL	AL
USDA[3]	0.00	100.00	66.67	0.00	100.00	50.00
NIH	0.00	100.00	37.84	0.00	100.00	45.75
IA						
SAT 4	0.00	96.15	92.09	10.00	98.72	82.34
PHA 3	0.00	100.00	88.00	0.00	100.00	76.00
LA						
NAK 4	0.00	94.87	82.59	0.00	100.00	78.95
SON 1	0.00	100.00	92.17	47.50	98.68	70.62
NA						
SAT 5	2.50	100.00	97.50	10.00	97.30	86.43
PHA 1	0.00	98.72	84.87	5.00	96.15	56.98
		$t = 3.45$, df = 28, $P < 0.01$			$t = 7.03$, df = 28, $P < 0.01$	

IA, intensive application of insecticides; LA, low application of insecticides; NA, no application of insecticides.

[1]PL = λ-cyhalothrin used as a public health insecticide for mosquito control (0.01 g a.i./m^2).

[2]AL = λ-cyhalothrin used as an agricultural insecticide for the control of target insect pests (0.001 g a.i./m^2).

[3]Laboratory strain, USDA = *Ae. aegypti* and NIH = *Ae. albopictus*.

Results of comparisons between *Aedes* susceptibility to agrochemical and public health concentrations of λ-cyhalothrin showed that field-derived *Ae. albopictus* were largely susceptible to the public health concentration, with overall mortality of 96.15%. This was in contrast to a lower mortality of 86.43% for the agrochemical concentration. These results show no evidence of resistance to recommended public health concentration of λ-cyhalothrin due to exposure to agrochemical concentration. In the future, insecticide susceptibility between agrochemical and public health insecticides (organophosphate, pyrethroid, or carbamate) should be a required component of insecticide resistance management.

Overall, the field-derived *Ae. albopictus* in this study were completely susceptible to chlorpyrifos but showed reduced mortality following exposure to λ-cyhalothrin, carbaryl, and imidacloprid, which is suggestive of the development of resistance. To the best of our knowledge, this is the first report of susceptibility tests on field-derived *Aedes* from southern Thailand using agrochemical insecticides. Previous studies have however reported the insecticide susceptibility status of *Aedes* mosquitoes against recommended public health concentrations of insecticides for vector control. Thanispong et al. (2008) reported that *Ae. aegypti* from Muang district, Songkhla province and Muang district, Satun province exposed to the recommended public health concentration of α-cypermethrin (0.05%), deltamethrin (0.05%), permethrin (0.25%), and malathion (0.8%) were both susceptible to deltamethrin, malathion, and α-cypermethrin. However, *Ae. aegypti* from Songkhla showed some possible resistance to α-cypermethrin and permethrin. In a later study by Chuaycharoensuk et al. (2011), *Ae. albopictus* in rubber plantations from Songkhla and Chumphon provinces were susceptible to deltamethrin and λ-cyhalothrin, but both showed possible (Chumphon strain) and definitie (Songkhla stain) resistance to permethrin.

Conclusion

The most commonly used groups of insecticides in durian plantations across the five southern Thailand provinces (Chumphon, Nakhon Si Thammarat, Phattalung, Satun, and Songkhla) were as follows: a combination of organophosphate and pyrethroid (chlorpyrifos + cypermethrin), followed by pyrethroid (cypermethrin and λ-cyhalothrin), carbamate (fenobucarb, methomyl, and carbamate), organophosphate (chlorpyrifos), and neonicotinoid (imidacloprid). Frequent applications (7–15 d per month) of each insecticide for insect pest control were recorded for more than half the surveyed plantations. Variation in insecticide intensity and frequency of use in these durian plantations influenced the density of *Ae. albopictus* eggs collected by ovitraps. This also disrupted mosquito life cycle by hindering adult female mosquitoes from completing their gonotrophic cycle, and thus egg-laying. The number of eggs collected was significantly different ($P < 0.05$) between the three categories (IA, LA, and NA) of insecticide application across durian plantations. Unsurprisingly, the highest number of eggs per trap was collected from the NA sites, followed by the LA and IA sites, respectively.

Of the four groups of insecticides used across durian plantations in this study, three are also used in public health applications for vector control: organophosphate (chlorpyrifos), pyrethroids (λ-cyhalothrin, cypermethrin), and carbamate (carbaryl), but at different concentrations, resulting in different dosages of active ingredients. Their use in durian farming may lead to the development of insecticide resistance in mosquito populations, as well as resistance to other public health insecticides. However, because the mosquitoes in this study showed complete susceptiblity to chlorpyrifos, should other insecticides fail, it appears to be a good alternative for *Ae. albopictus* control.

Finally, monitoring and early detection of insecticide resistance should always be considered in the design and implementation of effective integrated vector management practices for the control of *Aedes*-borne diseases and their vectors in Thailand.

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