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Studies on Insecticides Susceptibility of Aedes Aegypti and Aedes Albopictus Vectors of Dengue and Zika in Central West Highland, Viet Nam

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Abstract

The mosquitoes Aedes aegypti and Aedes albopictus are the two main vectors of viruses causing dengue, yellow fever, chikungunya and Zika in human. These diseases were prevalent in tropical and subtropical areas of the world. Three of these diseases (dengue, chikungunya and Zika) were identified in Viet Nam, among which dengue is the most common mosquito-borne viral disease with about 70,000-80,000 cases every year. There is currently no commercial vaccine or specific antiviral drug treatment for dengue, chikungunya or zika infections. The fight against these three diseases is therefore based on the control of their vectors, Aedes aegypti and Aedes albopictus, with insecticides. Several classes of pyrethroid insecticides are widely used to control adult Aedes mosquitoes, especially during diseases outbreaks. This study was conducted to provide information on the susceptibility status of Aedes aegypti and Aedes albopictus against insecticides in different types of landscape of Gia Lai and Binh Dinh provinces, in Central West Highland, in 2017. Samples of Aedes larvae and pupae were collected from breeding sites in various containers; both indoors and outdoors; including buckets, jars, flower plots, cement cisterns, discarded tyres and tanks. The collected larvae were transferred to the insectary of Entomology Department and Aedes aegypti and Aedes albopictus adults were then obtained from F1 generation and fed with 10% glucose before being used for insecticide susceptibility tests by the standard WHO test kit method. Susceptibility test was conducted on adult mosquitoes using paper impregnated with 30mg/m^2 alphacypermethrin or 0.05% lambdacyhalothrin or 0.05% deltamethrine or 0.75% permethrin or 5% malathion. The WHO diagnostic test kit was used following standard procedure. The final mosquito mortality was determined after 24h exposure time. The results demonstrate that Aedes aegypti and Aedes albopictus in all the study sites were resistant to lambdacyhalothrin, deltamethrin, permethrin and alphacypermethrin. In contrast, both Aedes aegypti and Aedes albopictus were found sensitive to malathion with 100% mortality rate in all sites of both provinces.

Keywords

Aedes Aegypti, Aedes Albopictus, Diseases Vectors, Insecticide, Resistance

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1. Introduction

According to WHO, vector-borne diseases account for 17% of the estimated global burden of all infectious diseases. Every year more than one billion people are infected and more than one million people die from vector-borne diseases including malaria, dengue, schistosomiasis, leishmaniasis, Chagas disease, yellow fever, lymphatic filariasis and onchocerciasis (WHO, 2014) [1]. Dengue in particular is emerging as a serious public health concern in tropical and

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sub-tropical regions around the world. There are four serotypes of the virus that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4) that are transmitted to humans through the bites of infected Aedes mosquitoes, principally Ae. aegypti (Diptera: Culicidae). This mosquito might also transmit chikungunya and zika [1-2].

In the last 50 years, dengue incidence has increased by 30 times with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings [2]. One recent estimate indicates 390 million dengue infections per year (95% confidence interval 284–528 million), of which 96 million (67–136 million) manifest clinically (with any severity of disease). Another study of the prevalence of dengue estimates that 3.9 billion people, in 128 countries, are at risk of infection with dengue viruses. Member States in three WHO regions regularly report the annual number of cases. The number of cases reported increased from 2.2 million in 2010 to 3,2 million in 2015.

Dengue, Chikungunya and Zika were found in Viet Nam with Dengue being an ongoing public health concern with virus transmission occurring year-round. There are 70,000–100,000 reported cases of dengue fever or dengue hemorrhagic fever every year. Its main vector Ae. aegypti was found involved in all of the outbreaks, while its secondary vector Ae. albopictus was also collected in some of the survey sites [3].

There is currently no vaccine or drug to treat dengue fever, so that dengue vector control is the most effective way to reduce the disease incidence. Since the introduction of organic insecticide in the 1940s, chemical control has become the most important method in mosquitoes control. There are three methods for this: focal, perifocal and space spraying. Focal control makes use of larvicide application and is used to treat household drinking water containers; it has relatively low toxicity, and is safe for humans. Perifocal treatment, which utilizes sprinklers in larval habitats and destroys both larvae and adult mosquitoes. Finally, space spraying is generally employed in emergency outbreaks of dengue. The significant increase in insecticide-based dengue vector control in the past decade has resulted in increased resistance to them from the mosquitoes because of the selection pressure placed on resistance genes. Insecticide resistance has long been a concern in public health programs aimed at preventing diseases through the judicious use of insecticides.

In Viet Nam, many studies have reported resistance development in Ae. aegypti to most insecticides used, but most focused on south and north Viet Nam but not on Central West Highland or South Central Coast of Viet Nam. We thus studied susceptibility of Ae. albopictus to various insecticides in sites of Gia Lai and Binh Dinh provinces.

2. Materials and Methods

2.1. Study Sites and Mosquitoes Collection

Study area: The study was carried out in 2016-2018 for three sites in Gia Lai province including Pleiku (urban) and Dak Po and Kong Chro (rural) which are located in the Central Highlands, and in 2016-2017 for three sites in Binh Dinh province including Quy Nhon (urban), Phu Cat (rural) and Vinh Thanh (mountainous) which are located in the South Central Coast. Both provinces are at a high risk of dengue epidemics.

Gia Lai is a Northern Province of the Center Highlands. Due to the average elevation of 700-800m above sea level, the climate in Gia Lai is cool with two distinct seasons: the rain season and the dry season. The rain season is from May to October and the dry season is from November to April, the average temperature is around 22-25°C.

Binh Dinh province lies along the south central coast of Vietnam and is featured with typical tropical monsoon climate with an average temperature of 27°C. Rainy season normally lasts from September to December and the dry season is from January to August.

Collection of the immature forms and rearing: The Ae. aegypti and Ae. albopictus larvae and pupae collection were conducted during the dengue outbreaks in 2016-2017 at all study sites. The field-collected larvae were stored in plastic tubes containing water from the same breeding habitat and brought to the insectary of Entomology Department where larvae were fed with larval food containing grounded dog biscuit, beef liver, powdered milk and yeast and reared to adults under standard conditions (25 ± 2°C temperature, 80 ± 10% relative humidity). The identification of Ae. aegypti was done using Aedes moquito key identifer by Leopoldo M. Rueda (2004) [4].

Figure 1. Map of study sites in Gia Lai.

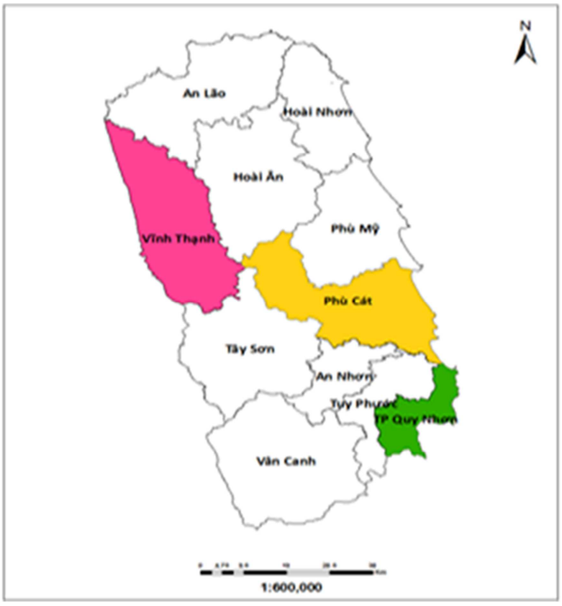

Figure 2. Map of study sites in Binh Dinh.

2.2. WHO Adult Bioassay Test

The insecticides treated papers evaluated included permethrin 0,75%; lambdacyhalothrin 0,05%; alphacypermethrin 30 mg/m^2; deltamethrin 0,05%; and malathion 5%. Insecticide papers were provided by WHO.

The standard WHO mosquito bioassay protocol was followed using bioassay tubes and insecticide-impregnated papers (WHO, 2016) [5].

On average, 150 non-blood fed Ae. aegypti females 3-5 days post emergence were evaluated for each insecticide and each site. Control mosquitoes were exposed to papers impregnated with solvent only. For each test, 4 replicates of 25 females were exposed to 0,75% permethrin; or 0,05% lambdacyhalothrin; or 30 mg/m^2 alphacypermethrin; or 0,05% deltamethrin; or 5% malathion insecticide-impregnated test papers in the test tubes for 1 hour. The number of mosquitoes knockdown after 10, 15, 20, 25, 30, 40, 50, and 60 min was recorded. All surviving mosquitoes were then transferred into holding tubes and provided with a 10% sugar water solution and kept at 27 ± 2^{0}C and 75 ± 10% relative humidity. Mortality was recorded 24 hours post exposure. Controls were also set up by exposing groups of mosquitoes to untreated papers. The tubes were set in an upright vertical position with the mesh-screen on top.

2.3. Recording and Reporting Susceptibility Test Results

Calculation of mortality: The assessment of mortality, was made 24 hours post-exposure. A mosquito was classified as dead if immobile or unable to stand or fly in a coordinated way.

The mortality of test sample was calculated by summing the number of dead mosquitoes across all four exposure replicates (4 x 25 mosquitoes)

If the mortality of control group is above 20%, the tests must be discarded. When control mortality is greater than 5% but less than 20%, then the observed mortality has to be corrected using Abbots formula, as follows:

$$Observed\ mortality\ (\%) = \frac{\%\ observed\ mortality\ -\ \%\ control}{100\ -\ \%\ control\ mortality} \times 100$$

When the control mortality was below 5%, it was ignored and no correction was applied.

Interpretation of susceptibility test results: The mortality percentages for each insecticide and each locality studied were determined. The resistance/susceptibility status of the mosquitoes were evaluated using WHO in 2016 [5]. Mortality between 98% - 100%: susceptibility is indicated; Mortality less than 98%: Resistance suggested, further tests are needed to verify; Mortality between 90% - 97%: Presence of resistant genes in the vector population must be confirmed. If at least two additional tests consistently show mortality below 98%, then resistance is confirmed; Mortality less than 90%: Resistance.

3. Results

3.1. The Susceptibility Test Results of Ae. Aegypti Against Insecticides from 2016-2017

Table 1. The results of susceptibility test of Ae. aegypti to varied insecticides in Gia Lai.

Site (district/city)	Insecticide	Control mosquitoes		Exposed mosquitoes		Status
		No.	Mortality (%)	No.	Mortality(%)	
Pleiku (urban area)	Alphacypermethrin 30mg/m^2	50	0	100	2 ± 2,3	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	4 ± 3,3	Resistance
	Deltamethrine 0,05%	50	0	100	6 ± 5,2	Resistance
	Permethrin 0,75%	50	0	100	10 ± 4,0	Resistance
	Malathion 5%	50	0	100	100 ±0,0	Susceptible

Site (district/city)	Insecticide	Control mosquitoes		Exposed mosquitoes		Status
		No.	Mortality (%)	No.	Mortality(%)	
Đăk Pơ(rural area n°1)	Alphacypermethrin 30mg/m^2	50	0	100	4 ± 3,3	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	1 ± 2,0	Resistance
	Deltamethrine 0,05%	50	0	100	0	Resistance
	Permethrin 0,75%	50	0	100	3 ± 6,0	Resistance
	Malathion 5%	50	0	100	100 ±0,0	Susceptible
Kong Chro(rural area n°2)	Alphacypermethrin 30mg/m^2	50	0	100	5 ± 3,8	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	0	Resistance
	Deltamethrine 0,05%	50	0	100	8 ± 3,3	Resistance
	Permethrin 0,75%	50	0	100	1 ± 2,0	Resistance
	Malathion 5%	50	0	100	100 ±0,0	Susceptible

In Gia Lai province, the results of WHO bioassay tests showed that Ae. aegypti populations from Pleiku, Dak Po and Kong Chro were resistant to the pyrethroid class insecticids including Alphacypermethrin, Lambdacyhalothrin, deltamethrine and Permethrin with a mortality rate between 0 and 10% after a 24 hours recovery period in 2016 - 2017, but, in contrast, Ae. aegypti populations were still susceptible to malathion 5% (organophosphate) with a 100% mortality rate for all sites. No dead mosquitoes were found in the control.

Table 2. The results of susceptibility test of Ae. aegypti to varied insecticides in Binh Dinh.

District/city (landcapes)	Insecticide	Control mosquitoes		Exposed mosquitoes		Status
		No.	Mortality (%)	No.	Mortality (%)	
Quy Nhon (urban area)	Alphacypermethrin 30mg/m^2	50	0	100	27 ± 5,0	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	0	Resistance
	Deltamethrine 0,05%	50	0	100	1 ± 2,0	Resistance
	Permethrin 0,75%	50	0	100	0	Resistance
	Malathion 5%	50	0	100	100	Susceptible
Phu Cat (rural area)	Alphacypermethrin 30mg/m^2	50	0	100	28 ± 7,3	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	1 ± 2,0	Resistance
	Deltamethrine 0,05%	50	0	100	0	Resistance
	Permethrin 0,75%	50	0	100	0	Resistance
	Malathion 5%	50	0	100	100	Susceptible
Vinh Thanh (mountainous area)	Alphacypermethrin 30mg/m^2	50	0	100	3 ± 2,0	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	0	Resistance
	Deltamethrine 0,05%	50	0	100	0	Resistance
	Permethrin 0,75%	50	0	100	0	Resistance
	Malathion 5%	50	0	100	100	Susceptible

The results for Binh Dinh showed that Ae. aegypti populations exposed to various diagnostic doses of pyrethroid class (alphacypermethrin 30mg/m^2, lambdacyhalothrin 0,05%, deltamethrine 0,05% and permethrin 0,75%) and organophosphate (malathion 5%) was resistant to alphacypermethrin, lambdacyhalothrin, deltamethrine and permethrin for all sites, but, in contrast, Ae. aegypti was still susceptible to malathion 5% with 100 mortalities for all of sites. The control tests were conducted simultaneously and showed a 0% mortality rate after 24 hours.

3.2. The Susceptibility Test Results of Ae. Albopictus Against Insecticides from 2016-2017

Table 3. The Results of Susceptibility Test of Ae. Albopictus to Varied Insecticides in Gia Lai.

District/city (landcapes)	Insecticide	Control mosquitoes		Exposed mosquitoes		Status
		No.	Mortality (%)	No.	Mortality (%)	
Pleiku (urban area)	Alphacypermethrin 30mg/m^2	50	0	100	52 ± 3,3	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	28 ± 8,6	Resistance
	Deltamethrine 0,05%	50	0	100	49 ± 8,9	Resistance
	Permethrin 0,75%	50	0	100	43±6,8	Resistance
	Malathion 5%	50	0	100	100 ±0,0	Susceptible
Đăk Pơ (rural area n°1)	Alphacypermethrin 30mg/m^2	50	0	100	22 ± 9,5	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	34 ± 9,5	Resistance
	Deltamethrine 0,05%	50	0	100	32 ± 8,6	Resistance
	Permethrin 0,75%	50	0	100	38 ± 5,2	Resistance
	Malathion 5%	50	0	100	100 ±0,0	Susceptible
Kong Chro (rural area n°2)	Alphacypermethrin 30mg/m^2	50	0	100	45 ± 8,9	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	11 ± 8,7	Resistance
	Deltamethrine 0,05%	50	0	100	49 ± 6,8	Resistance
	Permethrin 0,75%	50	0	100	29 ±8,3	Resistance
	Malathion 5%	50	0	100	99 ±2,0	Susceptible

The results 24 hours post exposure showed that Ae. albopictus populations were resistant to alphacypermethrin, lambdacyhalothrin, deltamethrine and permethrin with 11%-52% mortality in all sites but that they were still susceptible to malathion 5% with 99% to 100% mortality for all sites.

Table 4. The results of susceptibility test of Ae. albopictus to varied insecticides in Binh Dinh.

District/city Landcapes)	Insecticide	Control mosquitoes		Exposed mosquitoes		Status
		No.	Mortality (%)	No.	Mortality (%)	
Quy Nhon (urban area)	Alphacypermethrin 30mg/m^2	50	0	100	57±8,9	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	62±15,5	Resistance
	Deltamethrine 0,05%	50	0	100	65±10,5	Resistance
	Permethrin 0,75%	50	0	100	59±6,0	Resistance
	Malathion 5%	50	0	100	100	Susceptible
Phu Cat (rural area)	Alphacypermethrin 30mg/m^2	50	0	100	67± 3,8	Resistance
	Lambdacyhalothrin 0,05%	50	0	100	67±10,0	Resistance
	Deltamethrine 0,05%	50	0	100	50±9,5	Resistance
	Permethrin 0,75%	50	0	100	64±18,8	Resistance
	Malathion 5%	50	0	100	100	Susceptible

The results of susceptibility tests illustrated in Table 4 showed that Ae. albopictus populations were resistant against alphacypermethrin 30mg/m^2, lambdacyhalothrin 0.05%, deltamethrine 0,05% and permethrin 0,75% with 50% - 67% mortality but that they were still susceptible to malathion 5% with 100% mortality for all sites.

4. Discussion

Since there is no vaccine nor treatment available yet to be protected against dengue, zika, or chikungunya, control of Ae. aegypti and Ae. albopictus mosquitoes which are the main vectors of these diseases is currently the most effective measure available [2]. Ae. aegypti is the main vector and a common urban mosquito in endemic regions. They use a wide range of confined larval habitats, both man-made and natural. Some man-made container habitats produce large numbers of adult mosquitoes, whereas others are less productive. Ae. albopictus is primarily a forest species that has adapted to rural, suburban and urban human environments. It oviposits and develops in tree holes, bamboo stumps and leaf axils in forest habitats; and in artificial containers in urban settings. It is an indiscriminate blood feeder and more zoophagic than Ae. Aegypti. Ae. albopictus is commonly found in suburban and rural areas with plentiful vegetation. Consequently, control efforts should target the habitats that are the most productive and hence epidemiologically more important rather than all types of container, especially when there are major resource constraints [6].

However, while the outbreaks occur in high risk areas, applying insecticides as space spraying is an important vector-control measure to reduce dengue incidence. In Viet Nam, since 1990, many kinds of insecticides used a wide range of compounds including permethrin, deltamethrin, lambda-cyhalothrin, or alpha-cypermethrin, to control vector borne diseases such as malaria or dengue. The significant increase in insecticide-based dengue vector control in the past decade has resulted in increased resistance in dengue vectors because of the selection pressure placed on resistance genes. This process threatens the continued success of current vector control interventions. This is why insecticide resistance monitoring is an essential part of entomological surveillance.

Susceptibility tests applied on Ae. aegypti and Aedes albopictus (the main vectors in all areas in Viet Nam) in different sites of Binh Dinh and Gia Lai provinces showed that both Ae. aegypti and Aedes albopictus samples were resistant to alphacypermethrin, lambdacyhalothrin, deltamethrine and permethrin but both Ae. aegypti and Aedes albopictus remained susceptible to malathion in all sites. Similar results were obtained in studies conducted on insecticide resistance of Ae. aegypti collected from some areas of Central Highland. The mosquitoes were resistant to alphacypermethrin, lambdacyhalothrin, deltamethrine and permethrin, but were still susceptible to malathion [3, 7]. Similarly, resistances to pyrethroid were also observed in studies of Tran Thanh Duong et al. (2013) [3] and Vu Duc Huong et al. (1999) [7] in Viet Nam. In the report of Vu Duc Huong et al. (1999) conducted only on Ae. aegypti. it is mentioned that Pyrethroid resistance in Aedes became common in Viet Nam because they are widely used for the control of adult Ae. aegypti and Ae. albopictus. Some of the most commonly used insecticides for space spray treatments are permethrin and deltamethrin, usually in anticipation of or during an epidemic. Pyrethroid are also commonly used for Ae. aegypti control in most southeast Asian countries.

Pyrethroid resistance has also been found in India [8], in Pakistan [9], in Malaysia [10-11], and in Thailand [12]. Currently both Ae. aegypti and Ae. albopictus are significantly more resistant than before to insecticides in all parts of the world.

5. Conclusions

This study revealed that Ae. aegypti and Aedes albopictus populations have developed resistance against pyrethroids (alphacypermethrin, lambdacyhalothrin, deltamethrine and permethrin) in all sites studied. Malathion 5% was found to be the most effective and suitable insecticide against the major dengue vectors with a 100% mortality rate for mosquitoes in all sites studied in Central Viet Nam.

Nevertheless, it can be feared that some Aedes populations become resistant to Malathion as it has been the case in other regions of the world. With this in mind, it is important to test other insecticides with different mechanisms of action such as VU041 and to envision the use of laboratory-reared mosquitoes to infect and eliminate wild virus-loaded populations.

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