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Efficacy of Commonly Used Insecticides to *Aedes aegypti* in Southern Taiwan

Wei-Tai Hsia, Ho-Sheng Wu, Yi-Chieh Yang, Cheo Lin

Research and Diagnostic Center, Centers for Disease Control, Taiwan

Abstract

The dengue endemic usually began with scattered cases in a small geographic area to a village-wide cluster infection, and finally became a city-wide or country-wide large-scale outbreak. Therefore, the concentration and dosage of insecticides, frequency of insecticide usage, and the development of mosquito resistance would change accordingly at different stages of the endemic. This study was conducted to evaluate the efficacy of insecticides commonly used in southern Taiwan for dengue control against various strains of *Aedes aegypti* from different geographic areas. A net cage test with bioassay method was used. The insecticides were diluted into a series of concentrations by the label instructions, and then were applied to each geographic strain of *A. aegypti*. The mortality rate of *A. aegypti* after 24 hours of insecticide exposure was used as an indicator of insecticides efficacy. Based on World Health Organization and researchers recommendations, plus Taiwan CDC's insecticide spraying experiences, when mortality rate of wild strains and susceptible strains of *A. aegypti* after 24 hours of insecticide exposure both reach 95% or more, the insecticide is regarded effective. Concerning about public perception of disease control, we also included the knockdown rate (80% or more after 30 minutes of exposure) of targeted mosquitoes as an indicator of insecticides selection. This study showed that the susceptibility of *A. aegypti* to the insecticides changed with various strains from different geographic areas as well as previous local insecticide spraying history. In addition, the active ingredients and concentration of insecticides were the major factors affecting the insecticides efficacy. We suggest establishing a "Standard Operating Procedure of Biological Experiment on Dengue Vectors" as a guideline for each division to conduct bioassay test in high risk areas. With constant monitoring the susceptibility of each strain of dengue mosquitoes in different geographic areas to various insecticides, we can find out the most effective active ingredients, minimal

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concentration and dosage of insecticides. Along with previous insecticide spraying history and frequency, we can use them as a reference to purchase insecticides during usual time or acute outbreak. To avoid the emergence of insecticide resistance, we suggest choosing insecticides with multiple active ingredients or with both knockdown and killing effects, and meanwhile, establish an alternative insecticides usage system. With all these efforts, we can delay the emergence of insecticide-resistant mosquitoes, use insecticides efficiently at the early stage of outbreak, terminate the epidemic rapidly, and consequently improve disease control.

Keywords: dengue fever, *Aedes aegypti*, bioassay test

Introduction

There are many studies about insecticides resistance surveillance on *Aedes aegypti* have been carried out in southern Taiwan since 2002 [1]. One study showed that *A. aegypti* in southern Taiwan had gradually developed resistance to the insecticides containing the synthetic pyrethroid because of long-term usage. The resistance problem had put health workers in a quandary that almost no effective insecticides were available. In the 12 villages in Fongshan City, for example, the Adult index of dengue mosquitoes was still above the epidemic threshold in spite of more than two times of insecticides spraying [2].

The widely use of insecticides for vector-borne disease control may not only cause environmental pollution but also damage ecological balance [3]. When the same insecticide is used repeatedly in an area, the insecticide resistance will be more likely to occur among mosquitoes [4]. In addition, some insecticide spraying workers do not perceive the proper procedures for insecticide dilution, which makes the dilution rate unreliable. The concentration is either too high, which causes environmental pollution and overconsumption of insecticides; or too low, which is inefficient to kill mosquitoes and leads to insecticide resistance in mosquitoes [5]. Currently, the evolution of insecticides resistance in mosquitoes and the change in environmental conditions have exacerbated the seriousness of vector-borne disease epidemics. Therefore, how to correctly and reasonably use the insecticides in order to decrease or even solve the insecticide resistance problem is one of the most important issues in disease control at present [6].

Different seasons, climates, and geographic locations can result in different natures of mosquitoes and susceptibility to insecticides, and consequently affect insecticide efficacy of vector control [7]. The occurrence of insecticide resistance in mosquitoes will become a major threat to vector control efficacy [8]. To handle the problem of insecticide resistance, first of all, we need to understand which active ingredient is susceptible or resistant to local mosquitoes [9]. Although the selection of insecticides for vector control was based on previous resistance researches, the selected insecticides were still unable to kill adult mosquitoes effectively. This might be because insecticide resistance researches were mostly conducted in a large geographic area, such as counties/cities. However, dengue fever usually began with scattered cases in a small geographic area to a village-wide cluster infection, and finally become a large scale epidemic in a county- or city-wide area. Therefore, the selection of insecticides should be based on the spraying history and frequency in a block- or village-wide area. By knowing the susceptibility of mosquitoes to various insecticides, we can determine the most effective insecticides for vector control. Furthermore, we suggest examining the LC_{50} value, knockdown rate, and mortality rate of wild-strain mosquitoes to each insecticide, which can be indicators for annual insecticides selection. Meanwhile, we also monitor the development of insecticide resistance so that the insecticides can be adjusted in time [10].

The field operation of dengue vector control in southern Taiwan often changed in response to different environmental conditions and insecticide resistance in mosquitoes. The differences in biological mechanism of mosquito resistance to the same category of insecticides may result from the differences in insecticide application history and frequency [11-12]. Since insecticide-resistant gene in mosquito is unfavorable for mosquito reproduction and growth, the resistance strain may disappear following the cessation of the insecticides due to natural selection [11]. Eventually, the mosquito population will shift from insecticide resistant strain to insecticide susceptible strain. Thus if we perform bioassay tests prior to insecticide spraying, we can accurately predict insecticide efficacy and adequate concentration to the targeted mosquito strain, and achieve the goals of precise insecticides application as well as avoidance of environmental pollution and human exposure. Above all, we can not only delay and decrease resistance occurrence, but also reduce waste of public funds and mitigate residents' complaints about repeated insecticides spraying. In conclusion, insecticide efficacy and susceptibility test should be included in the integrated dengue vector control program [8].

Materials and Methods

A. Preparation of test mosquito

1. Wild strain: Larvae of *A. aegypti* collected by mosquito larvae collection method from different

areas in Kaohsiung City and County (Table 1) were sent to the mosquito rearing room at laboratory of Taiwan Centers for Disease Control (Taiwan CDC). After species identification by microscope, larvae were reared to the first generation of adult *A. aegypti* and were provided for test.

2. Susceptible strain: The offspring of *A. aegypti* collected from Tainan areas in 1987, which have been reared in the mosquito rearing room at laboratory of Taiwan CDC (around 600^{th} generation).
3. Mosquito rearing: The *A. aegypti* larvae of wild strain and susceptible strain were reared in separate plastic basins and fed with a mixture of Tai-sugar yeast and swine liver powder (1:1) every day. The superficial layer of floating material was scraped away before feeding. After larvae became pupae, each strain of pupae was picked up from the rearing basins and put into different water cups, and then set in separate mosquito rearing cages (30 x 30 x 20 cm). Next, a 10% sugar solution would be placed in the cage as adult mosquitoes hatched from the pupae. The mosquito rearing room was maintained in the conditions of temperature 25~28℃, relative humidity 70±5%, and photoperiod 12 hours [13].

B. Preparation of insecticides

The insecticides commonly used for dengue vector control in southern Taiwan (Table 1) were diluted with pure water into a series of concentrations. The diluted insecticides followed by the instruction labels were first used for efficacy tests on each wild strain of *A. aegypti* . If the 24-hour mortality rate of *A. aegypti* did not reach higher than

Table 1 . Strains of *A. aegypti* and active ingredients of insecticides

Strains of *A. aegypti*		Insecticides		
County/City	Administrative areas	Items	Active ingredients (w/w)	Formulations
Kaohsiung City	Nanzih	A	Tetramethrin 2%, Cypermethrin 6%	liquid
	Zuoying	B	Cypermethrin 10.6%	emulsion
	Sanmin	C	Alphacypermethrin 2%	emulsion
	Gushan	D	Cypermethrin 6%, Prallethrin 1.5%	emulsion
	Yancheng	E	Deltamethrin 1%, Esbiothrin 3%	emulsion
	Cianjin			
	Sinsing			
	Lingya			
	Cijin			
	Cianzhen			
	Siaogang			
Kaohsiung County	Shanmei & Longcheng & Jhennan villages	D	Cypermethrin 6%, Prallethrin 1.5%	emulsion
	Wuhan & Sinwu & Jhengyi villages	E	Deltamethrin 1%, Esbiothrin 3%	emulsion
	Wufu & Fusing villages	F	Cyfluthrin 5.1%	emulsion
	Niaosong township at the junction of Dahua village			

95% on tested insecticide, insecticide solution would be continually diluted with a lower dilution rate until the 24-hour mortality rates of both wild strain and susceptible strain of *A.aegypti* were equal to or higher than 95%.

C. Bioassay test of insecticides

1. Flow rate test of thermal fogger

The thermal fogger (puls Fog K-10, 0.8 μm nozzle size) with high fogging efficiency (Span＜2，DR≈1) [14-15] was used for flow rate test. Before starting the test, the fogger needed to warm up (30 seconds) and spray preliminarily (15 seconds) to ensure the conveying tube filled up with insecticide solution. A total of 2000 ml tested insecticides solution was added to the tank initially. After three minutes of spraying, the remaining amount of insecticides solution in the tank was measured by consumption method. The measurement was repeated three times to obtain the average flow rate and the coefficient of variance (CV) was calculated. The results showed that the average flow rate of puls Fog K-10 was 196.7±5.8 ml per minute (CV=2.9).

2. Calculation of spraying time

First, we measured the length (3.6 meters), width (3.4 meters), and height (3.1 meters) of the simulation room for insecticide spraying by using a laser distance meter (Trimble HD150). Then, the space of the simulation room was calculated 37.9 cubic meters. With the flow rate and the room space, we could obtain that the time needed to spray in the room was 12 seconds.

3. Net cage test

The *A. aegypti* (3- to 5-day-old female mosquito never had a blood meal) were placed into a foldable net cage (25×11×11 cm) wrapped by a fine mesh net (hole size 1.6 mm), 20 mosquitoes in each cage. The tested group included five paired of cages (one cage for wild strain and one for susceptible strain) which were hung on the wall (four corners and center of the wall, respectively) opposite the door of the simulation room; the control group, another paired of cage, was placed in the mosquito rearing room. Fogger was positioned 145 cm height above the floor with its nozzle elevated at a 30 degree angle. After 12 seconds of spraying, the fogger was turned off. The door was kept closed for 30 minutes. Thereafter, the cage was moved out of the room and the number of knockdown mosquitoes was recorded. Subsequently, all mosquitoes in each cage were taken out and placed separately in paper cups containing a soaked cotton ball with 10% sugar solution, which were placed in mosquito rearing cages. After 24 hours of rearing, the number of dead mosquitoes was recorded. The rearing cages were maintained in the conditions of temperature 25±2℃, relative humidity 70±5％, and photoperiod 12 hours [13].

4. Acceptance criteria of the test result

a. Flow rate test: If the CV value of the flow rate data from a fogger is larger than 5, the fogger has to be sent for maintenance or replaced by another fogger.

b. Net cage test: In the insecticide efficacy test, if the mortality rate of mosquitoes in control group is higher than 10%, the test will be re-conducted.

c. Bioassay test: Based on the World Health Organization criteria for insecticide resistance determination, with a mortality rate of 98-100%, the tested vector is considered susceptible; with a mortality rate of 80～97%, it should be re-tested for further confirmation; with a mortality rate < 80%, the vector is considered resistant [16]. Meanwhile, according to researchers' recommendations and Taiwan CDC's insecticide spraying experiences [17], the insecticide spraying efficacy indicator, 24-hour mortality rate ≥ 95%, was adopted as a measure to determine the insecticides efficacy against *A. aegypti*.

Result

A. Strains of *A. aegypti* in Kaohsiung City

To evaluate insecticides efficacy, five insecticides were used on eleven wild strains of *A. aegypti* in Kaohsiung City for net cage test. The names of insecticides and their minimum concentrations (with the highest dilution rate) effective to each strain of *A. aegypti* are shown in Table 2. For insecticide A, when the solution was

Table 2. The threshold concentration of insecticides effective to each strain of *A. aegypti* in Kaohsiung City

Strains of *A. aegypti*	Items of insecticides (active ingredients)									
	A		B		C		D		E	
	Tetramethrin 2%, Cypermethrin 6%		Cypermethrin 10.6%		Alphacypermethrin 2%		Cypermethrin 6%, Prallethrin 1.5%		Deltamethrin 1%, Esbiothrin 3%	
	Dilution rate	Concentration (% w/w)	Dilution rate	Concentration (% w/w)	Dilution rate	Concentration (% w/w)	Dilution rate	Concentration (% w/w)	Dilution rate	Concentration (% w/w)
Zuoying	1400*	0.0014, 0.0043	300*	0.0353	50*	0.0400	200*	0.0300, 0.0075	50*	0.0200, 0.0600
Nanzih	1000	0.0020, 0.0060	100	0.1060	40	0.0500	200*	0.0300, 0.0075	50*	0.0200, 0.0600
Sanmin	250	0.0080, 0.0240	175	0.0606	50*	0.0400	100	0.0600, 0.0150	50*	0.0200, 0.0600
Gushan	1000	0.0200, 0.0600	120	0.0883	50*	0.0400	80	0.0750, 0.0188	50*	0.0200, 0.0600
Yancheng	1000	0.0020, 0.0060	300*	0.0353	50*	0.0400	200*	0.0300, 0.0075	50*	0.0200, 0.0600
Cianjin	1000	0.0020, 0.0060	100	0.1060	40	0.0500	200*	0.0300, 0.0075	50*	0.0200, 0.0600
Sinsing	1400*	0.0014, 0.0043	300*	0.0353	50*	0.0400	180	0.0333, 0.0083	50*	0.0200, 0.0600
Lingya	250	0.0080, 0.0240	100	0.1060	50*	0.0400	125	0.0480, 0.0120	50*	0.0200, 0.0600
Cijin	400	0.0050, 0.0150	200	0.0530	50*	0.0400	100	0.0600, 0.0150	50*	0.0200, 0.0600
Cianzhen	1000	0.0020, 0.0060	150	0.0707	50*	0.0400	100	0.0600, 0.0150	50*	0.0200, 0.0600
Siaogang	1000	0.0020 0.0060	150	0.0707	45	0.0444	150	0.0400 0.0100	50*	0.0200, 0.0600

Note:* means the dilution rate on the instructions label approved by Taiwan Environmental Protection Administration.

diluted according to the label instructions (a 1400 fold dilution), the mortality rate could reach more than 95% in Zuoying and Sinsing strains of diluted according to the label instructions (a 1400 fold dilution), the mortality rate could reach more than 95% in Zuoying and Sinsing strains of *A. aegypti*. However, a higher concentration of insecticide solution (a 200 to 1000 fold dilution) was needed for other strains of *A. aegypti* to have the same level of mortality rate. For insecticide B, when the solution was diluted according to the label instructions (a 300 fold dilution), the mortality rate could reach more than 95% in Zuoying, Yancheng, and Sinsing strains of *A. aegypti*. Likewise, a slightly higher concentration (100 to 200 fold dilutions) was needed for other strains of *A. aegypti* to have the same level of mortality rate. For insecticide C, when the solution was diluted according to the label instructions (a 50 fold dilution), the mortality rate could reach more than 95% in Zuoying, Sanmin, Gushan, Yancheng, Sinsing, Lingya, Cijin, and Cianzhen strains of *A. aegypti*. Also, a tiny decrease in dilution rate, a 40 to 45 fold dilution, was needed for Nanzih, Cianjin, and Siaogang strains of *A. aegypti* to obtain the same level of mortality rate. For insecticide D, when the solution was diluted according to the label instructions (a 200 fold dilution), the mortality rate could reach more than 95% in Zuoying, Nanzih, Yancheng, and Cianjin strains of *A. aegypti*. Similarly, a slightlydecrease in dilution rate, an 80 to 180 fold dilution, was required for other strains of *A. aegypti* to achieve the same level of mortality rate. As for insecticides E, with the solutions diluted according to the label instructions (a 300 fold dilution), the mortality rate in all strains of *A. aegypti* were more than 95% in Kaohsiung City.

For the susceptible strains of *A. aegypti*, the mortality rates after 24-hour exposure to five different insecticides were all more than 95%. The 30-minute knockdown rates in susceptible strains were also equal to or more than 80% except for insecticide B and insecticide D, which were less than 80% (around 69% in insecticides B and 57% in insecticides D). In general, except that insecticide A required a noticeably higher concentration (a 5.6 fold higher concentration than the label indicated) when it was applied to Sanmin and Lingya Districts in Kaohsiung City for *A. aegypti* control, all other tested insecticides only needed slight concentration adjustment in each Districts in Kaohsiung City to meet the same efficacy (mortality rate at 95% and more).

B. Strains of *A. aegypti* in Kaohsiung County

To evaluate insecticides efficacy, three insecticides were used on four wild strains of *A. aegypti* in Kaohsiung County for net cage test.

The names of insecticides and their minimum concentrations (with the highest dilution rate) effective to each strain of *A. aegypti* are shown in Table 3. For insecticide D, when the solution was diluted according to the instruction label (a 200 fold dilution), the mortality rate could reach more than 95% in strains of *A. aegypti* from Wuhan & Sinwu & Jhengyi villages and Wufu & Fusing villages. However, a 2 to 2.5 fold higher concentration (an 80 to 100 fold dilution) was needed to have the same level of mortality rate for strains of *A. aegypti* from Shanmei & Longcheng & Jhennan villages and Dahua village & Niaosong Township. For insecticide E, when the solution was diluted according to the instruction label (a 50 fold dilution), a mortality rate of 95% and more was observed in strains of *A. aegypti* from Wuhan & Sinwu & Jhengyi villages and from Wufu & Fusing villages. But a 2.5 fold higher concentration (a 20 fold dilution) was required for strains of *A. aegypti* from Shanmei & Longcheng & Jhennan villages and a slightly higher concentration (a 45 fold dilution) for those from Dahua village & Niaosong Township to have the same level of mortality rate. As for insecticide F, when the solution was diluted by following the label instruction (a 125 fold dilution), the mortality rate reached more than 95% in strains of *A. aegypti* from Wuhan & Sinwu & Jhengyi villages and from Wufu & Fusing villages. Likewise, for strains of *A. aegypti* from Shanmei & Longcheng & Jhennan villages and from Dahua village & Niaosong Township, a 2.5 fold higher concentration (a 50 fold dilution) was needed to have the expected level of effectiveness.

For the susceptible strains of *A. aegypti*, the mortality rates after 24-hour exposure to three different insecticides were all more than 95%. Although the 30-minute knockdown rate of the susceptible strain of *A. aegypti* was less than 80% for insecticides D (57-63%), it

Table 3. The threshold concentration of insecticides effective to each strain of *A. aegypti* in Kaohsiung County

Strains of *A. aegypti*	Items of insecticides (active ingredients)					
	D Cypermethrin 6%, Prallethrin 1.5%		E Deltamethrin 1%, Esbiothrin 3%		F Cyfluthrin 5.1%	
	Dilution rate	Concentration (% w/w)	Dilution rate	Concentration (% w/w)	Dilution rate	Concentration (% w/w)
Shanmei & Longcheng & Jhennan villages	80	0.0750，0.0188	20	0.0500，0.1500	50	0.1020
Wuhan & Sinwu & Jhengyi villages	200*	0.0300，0.0075	50*	0.0200，0.0600	125*	0.0408
Wufu & Fusing villages	200*	0.0300，0.0075	50*	0.0200，0.0600	125*	0.0408
Dahua village & Niaosong Township	100	0.0600，0.0150	45	0.0222，0.0666	50	0.1020

Note: * means the dilution rate on the instruction label approved by the Taiwan Environmental Protection Administration.

was equal to or more than 80% for other tested insecticides. In general, for insecticides D, E, and F, when the solutions were diluted by following the label instructions, the results showed effective *A. aegypti* control in Wuhan & Sinwu & Jhengyi villages and Wufu & Fusing villages in Fongshan City of Kaohsiung County. However, when the insecticides were used in Shanmei & Longcheng & Jhennan villages and Dahua village & Niaosong Township, a minor adjustment in insecticides concentration was required to meet the same efficacy.

Discussions

Since susceptible strain of *A. aegypti* has been long-term reared in the mosquito rearing room and never been exposed to any kinds of insecticides. Therefore, either the knockdown rate or mortality rate in the susceptible strains of *A. aegypti* should theoretically be higher than those in wild strains whatever the insecticides were used in the efficacy test. The World Health Organization has established the criteria for insecticides resistance determination in efficacy test. With a mortality rate of 98-100%, the tested vector is considered susceptible; with a mortality rate from 80 to 97%, it should be re-test for further confirmation; and with a mortality rate ＜80%, the vector is considered resistant. Furthermore, the insecticide efficacy test was conducted in an experimental room under a strict control. Thus when the mortality rate is less than 95% in tested mosquitoes, we could strongly suspect that insecticide resistance may have occurred. In this study, all tested insecticides have resulted in a 24-hour mortality rate of more than 95% in susceptible strains, which is compatible to the criteria mentioned above. Therefore, a 24-hour mortality rate of 95% or more after 30-minute insecticide exposure was adopted as a measure to determine insecticide efficacy against wild strains of *A. aegypti*.

The efficacy test shows that insecticide B was effective against *A. aegypti* in Kaohsiung City by using a higher concentration than that specified on the label. However, insecticide B contains only one active component "cypermethrin" a chemical with kill effect. Therefore, when the insecticide was diluted as it instruction label indicating, the knockdown rate (40-80%) against *A. aegypri* was lower in insecticide B than insecticide A, which contains a mixture of cypermethrin and tetramethrin with knockdown effect. Also, insecticide D is a complex insecticide containing "cypermethrin" and "prallethrin". However, both ingredients have simply kill effect, so the knockdown rate (20-70%) did not reach the expected level. Although insecticide F contains only one component, it active ingredient "cyfluthrin" has both knockdown effect and kill effect. Therefore, when diluted to a higher concentration than the label indicated, insecticide F still carried excellent the knockdown effect against *A. aegypti* in Fongsang City of Kaohsiung County. Moreover, both insecticide C and E have a high effectiveness to each strain of *A. aegypti*. This means that either "deltamethrin" or "alphacypermethrin" can effectively control the *A. aegypti* in areas of Kaohsiung County and Kaohsiung City just following the dilution instruction on the label. In vector control activity, the number of killed mosquitoes is usually chosen as an indicator to determine the effectiveness of vector control. However, concerning about public perception and image of control activity, we suggest adding knockdown rate as another indicator to decide what insecticides should be chosen for vector control.

This study shows that the susceptibility of *A. aegypti* to the insecticides changed with various strains from different geographical areas as well as previous local insecticides spraying history. Since the active ingredients of all tested insecticides belongs to the synthetic pyrethroid compounds and the formulae of insecticides has very little influence on its efficacy [18], the concentration of the selected insecticides should be one of the major factor affecting the insecticides efficacy. This reflects the importance of bioassay test for insecticides. Therefore, we suggest establishing a "Standard Operating Procedure of Biological Experiment on Dengue Vectors" as a guideline for each division to conduct bioassay test in high risk areas. With constant monitoring the susceptibility of each strain of dengue vectors in different geographic areas to various insecticides, we can find out the most effective active ingredients, minimal concentration and dosage of insecticides. In addition, all data should be statistically analyzed, and put into a database as a reference for different areas regarding to insecticide spraying history and frequency in different time periods. These data will help health workers in each division to accurately know about the amounts of insecticides needed to be purchased during usual time or epidemic period, as well as the effective dosage and concentration of insecticides. Subsequently, the disease control activity will be efficient. Eventually, to avoid the emergence of insecticide resistance, we suggest choosing insecticides with multiple active ingredients or with both knockdown and killing effects, and meanwhile, establish an alternative insecticides usage system, such as first-line, second-line, or even third-line insecticides. With all these efforts, we can delay the emergence of insecticide-resistant mosquitoes, use insecticides efficiently at the early stage of outbreak terminate the epidemic rapidly.

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