The Influence of Insecticide Formulation and Droplet Size on the Efficiency of Dengue Vector Control

Wei-Tai Hsia, Ei-Wei Lin, Lin-Cheo Lo

Centers for Research and Diagnostics, Taiwan CDC

From Chinese version, pp, 550-562

Abstract

The objective of this research is to study the influence of insecticide droplet size on the efficiency of mosquito control in order to distinguish the relationship of space spray and residue spray in dengue vector control. Tested *Aedes aegypti* Tainan strains were obtained from CDC laboratory colony and were treated with 22 commercial product insecticides by spray tower method (Nozzle: SU4, SU1A, SU2, SU4B, SU3, and SU3-1) resulted that each product showed high mortality and the most appropriate droplet size ranged from 20 to 50 μm to the mosquito control. Oil formulation is suitable for space spray, while suspension is suitable for residue spray.

Keywords: droplet size, formulation, *Aedes aegypti*

Introduction

With the continuous economic and culture developments, the demand for a more hygienic environment has increased. Originally, mosquitoes inhabited in discarded paper cups, aluminum cans, and used tires. However, mosquitoes now inhabit ditches, broken water meters, and even rain gutters. The government

Received: Mar 19, 2008; Accepted: May 9, 2008.

Correspondence: Wei-Tai Hsia; Address: No.161, Kun-Yang Street, Taipei, Taiwan, R.O.C.

E-mail: hsia@cdc.gov.tw

has been continually promoting the elimination of possible mosquito breeding grounds and asking the nation to start by doing so in daily routines. However, looking at the entire nation, whenever an epidemic breaks out, whether the media, general public, government units, or specialists, all demand full-scale chemical measures in hope of controlling and lowering the epidemic in giving the people healthy and happy lives.

After understanding the situation of the scene, using the right insecticide with the right equipment and effectively spraying the scene are the needed skills for preventive workers. The concept of space spray comes from the World Health Organization (WHO) which categorizes insecticides according to the spray droplets into 7 categories [1]. WHO has recommended the best insecticide spray droplet size in preventing mosquitoes is 10~20m [2]. Many scholars have proposed theories on low capacity and extremely low capacity spray technologies [3]. Among which, Himel has proposed the optimum drop size for insecticide spray droplets in 1969. At the same time, other tests and studies have proven that the quantity, concentration, size of droplets, distribution depositions of the insecticide are all crucial elements in relative technologies. Therefore, the spray functions are the most closely linked with the effectiveness of the insecticides [5, 6].

In this research, 6 different nozzle types (Nozzle: SU4, SU1A, SU2, SU4B, SU3, and SU3-1) were tested by spray tower method. When spraying water, the droplets were sized according to the droplets sprayed at the distance of 50 cm from the nozzle of the 4 commercial product insecticide types (power fogging, pressure fogging, fogging, and ultra low voltage fogging). In the tests, 22 commercial product insecticides were tested for their effectiveness on *Aedes aegypti* Tainan strains in order to understand the effect of insecticide spray droplet sizes in dengue vector control.

Materials and Methods

1. Cultivation of testing mosquitoes

Aedes aegypti Tainan strains larvae are placed in plastic water tubs, each tub (30×24×2.5 cm) with 500-800 larvaes and fed with yeast and pork liver powder (1:1) every day and the water surface is scraped. After pupating, they are placed in water cups then situated in insect boxes (30×30×20 cm). After eclosion, 10% sugar water is provided along with lab mice for the female mosquito to feed upon. Damp paper strips are also provided for the females to lay eggs upon. After the eggs are collected and dried, they are placed in water to hatch. The insect laboratory temperature is maintained at 25-28 ℃, relative humidity of 65±10%, and provided with sunlight 12 hrs [7].

2. Selecting commercial product insecticides

Among the commercial product insecticides certified by the Environmental Protection Administration, insecticides that are used in specific environments and mainly contain one effective ingredient (the one with lower levels of ingredient is chosen between those with the same ingredient). 22 of commercial product insecticides which target mosquitoes are selected in conducting the tests.

3. Nozzle formulation testing

Among the 6 types of nozzles (Nozzle: SU4, SU1A, SU2, SU4B, SU3, and SU3-1), no oil type air compressor is used (4 HCCs-10s-M400), with fixed air pressure at 20 psi, fusing 0.01g of opaque and white water color (SIMBALION gouache colors No.1) with 1 lt. pure water (the RO goes against to permeate). 10 ml of each insecticide is sprayed and tested with particle size distribution analyzer (La Vision Co.) at 50 cm distance in testing the size of the spray droplets. Each testing time lasts 3 minutes and 30 seconds. The laboratory temperature is maintained at 25±2 ℃ with relative humidity at 65±10%.

D. Spray tower method testing

20 female *Aedes aegypti* Tainan strain mosquitoes that are 2-5 days old and have not yet fed on blood are placed in the glass funnel (diameter 20 × 20 cm) and then placed upon elevation stand (see Figure 1). 6 different nozzles of no oil air compressor (20 psi) are pressurized (see Figure 2) with the 22 different commercial product insecticides (see Table 1). The amount used is according to the calculation of the Environmental Protection Administration certification labels. After spraying, the mosquitoes are removed from the glass funnel and observed and recorded every 30 seconds for the number of mosquitoes knocked out until 30 minutes have passed. The adult insects are then sucked out and placed in observation cups (which include 10% sugar water soaked cotton) and situated in 25±2 ℃, RH65±10% growth containers for further observation for 24hours death rate. Each group is tested 3 times and each test data is analyzed for KT_{50} and death rate with the SPSS code.

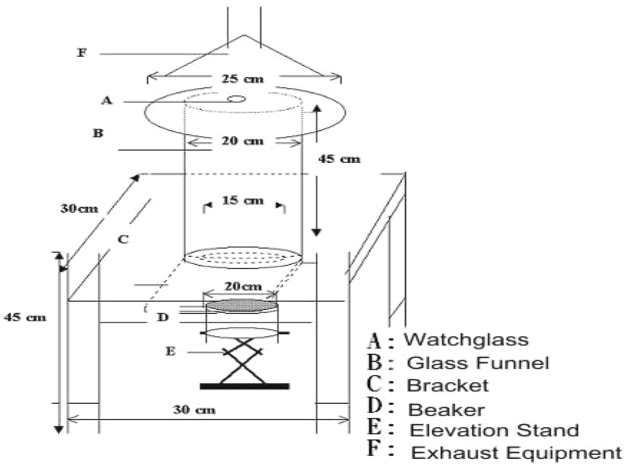

Figure 1. Spray Tower Device

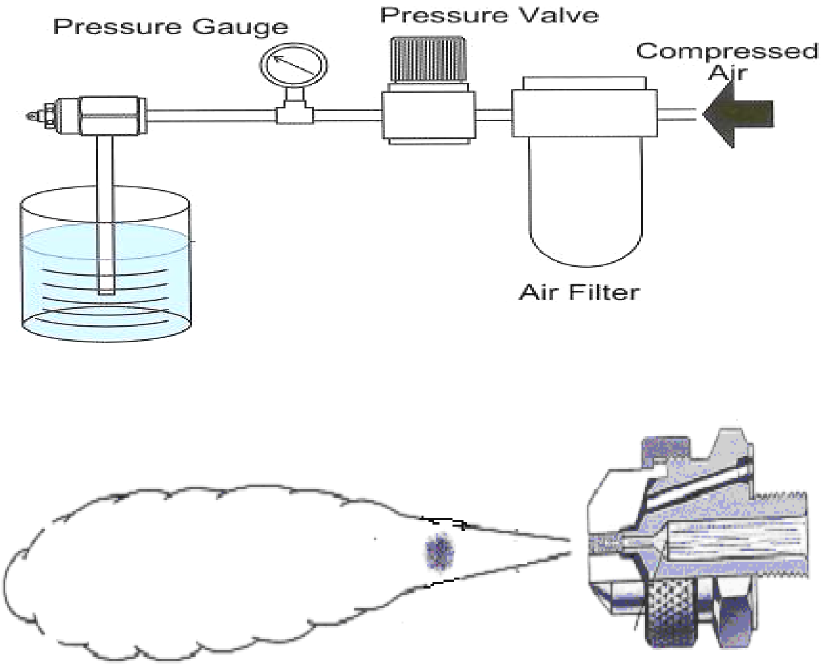

Figure 2. Nozzle Formulation

Table 1. 22 Commercial Insecticide Product Codes

Drug Code	Chinese Brand	Effective Ingredient	Type
1A	Sun-Gai-Hau	Deltamethrin 2.35%	emulsions
1B	Sinon Shuang-ning 5% EW	Cyphenothrin 5%	emulsions
1C	Aero-Alpha E.C	Alphacypermethrin 3%	emulsions
1D	Pyrecomfort	Tetramethrin 10%	emulsions
1E	Gokitelin14.25	d-Tetramethrin 0.75% Cyphenothrin 5.5%	emulsions
1F	Solfac EW 050	Cyfluthrin 5.1%	emulsions

Table 1. 22 Commercial Insecticide Product Codes

Drug Code	Chinese Brand	Effective Ingredient	Type
2A	Li-Chu-Ning	Alphacypermethrin 1.5%	liquid
2B	Gokitelin 437	Tetramethrin 3% Cyphenothrin 4%	liquid
2C	Gokilin5.5	Cyphenothrin 5.5%	liquid
2D	Kuai-Mie-Ning	Alphacypermethrin 1.5%	liquid
2E	Gai-Hau-Yon	Deltamethrin 1.25%	liquid
2F	Aero-Cytetra SL	Tetramethrin 2% Cypermethrin 6%	liquid
2G	Aero-Escyper SL	Cypermethrin 1%	liquid
3A	Aero-Delta SC	Deltamethrin 3.0%	Suspension
3B	Responsar SC 025	Cyfluthrin 2.2%	Suspension
4A	Te-Mieh-Ning	Cypermethrin 9.4%	lacquer
4B	Bei-Jia-De 14% oil	Tetramethrin 2% Phenothrin 4%	lacquer
4C	Ai-kang Oil	Lambda-cyhalothrin 0.1%	lacquer
4D	Shi-Ning-Wang	d-Allethrin 0.2% Cypermethrin 0.2%	lacquer
4E	Aero-Cyper OL	Cypermethrin 1%	lacquer
5A	Ai-kang ULV	Lambda-cyhalothrin 0.2%	Ultra low volume
5B	Aero-Cyper UL	Cypermethrin 1%	Ultra low volume

Results

1. Testing of nozzle formation

Using particle size distribution analyzer, the 6 types of nozzle sprays are tested with water and the variables (D10, DV10, DV50, and DV90) can been seen in Table 2. According to Table 2, the manufacturer labels lie between the numeric medium diameter(NMD)(D10) and mass medium diameter(VMD)(DV50). Therefore, mass medium diameter(NMD) is preferred.

Table 2. Results of Nozzle Formulation Testing

Nozzle type1 / Droplet size	SU4 (14μm)	SU1A (18μm)	SU2 (35μm)	SU4B (40μm)	SU3 (55μm)	SU3-1 (65μm)
# of droplets [2]	231	65	29	53	49	103
D10(μm)[3]	14.7	20.4	34.6	40.3	50.8	60.3
DV10(μm)[4]	12.2	15.6	32.4	34.0	39.1	51.2
DV50(μm)[5]	14.3	26.7	46.0	57.4	73.6	80.8
DV90(μm)[6]	78.3	40.0	57.8	126.5	117.8	134.1

Note:
1. Manufacturer type and labeled droplet size.
2. Number of droplets sprayed from nozzle in testing.
3. D10: numeric medium diameter.
4. DV10: 10% of droplets are smaller than this number.
5. DV50: Mass medium diameter.
6. DV90: 90% of droplets are smaller than this number.

2. Spray tower method testing

(1) Death rate

After studying the 22 different commercial product insecticide effects on *Aedes aegypti* Tainan strain mosquitoes, apart from the 2 emulsion products (1A and 1E) with 80.8μm nozzle formulation and the 73.6μm emulsion product 1E which had death rates lower than 90%, the other insecticides (using any kind of nozzle) all had death rates that exceeded 90%. With the smaller nozzles (14.3, 26.7, and 46.0μm), the effects reached 100% (see Table 3). Therefore, the 22 insecticides are all effective in eliminating *Aedes aegypti* Tainan strain mosquitoes.

Table 3. Killing Effects of 22 Insecticides on *Aedes aegypti* Tainan Strain Mosquitoes

Type	Drug code	14.3 μm[2]	26.7 μm	46.0 μm	57.4 μm	73.6 μm	80.8 μm
Emulsion	1A	100	100	100	100	96	85.3
	1B	100	100	100	100	93.2	95.9
	1C	100	100	100	100	100	100
	1D	100	100	100	100	94.4	93.4
	1E	100	100	100	100	87.5	81.2
	1F	100	100	100	100	94.6	96.1
	Average	100±0	100±0	100±0	100±0	94.3±4.1	92.0±7.2
Liquid	2A	100	100	100	100	100	100
	2B	100	100	100	100	97.3	93.1
	2C	100	100	100	100	98	96
	2D	100	100	100	97.2	98.6	94.5
	2E	100	100	98.6	98.7	100	93.3
	2F	100	100	100	98.7	100	100
	2G	100	100	100	100	100	100
	Average	100±0	100±0	99.8±0.5	99.2±1.0	99.1±1.1	96.7±3.0
Suspension	3A	100	100	100	100	100	100
	3B	100	100	100	100	100	100
	Average	100±0	100±0	100±0	100±0	100±0	100±0
Lacquer	4A	100	100	100	100	100	100
	4B	100	100	100	100	100	100
	4C	100	100	100	100	100	100
	4D	100	100	100	100	100	100
	4E	100	100	100	100	100	100
	Average	100±0	100±0	100±0	100±0	100±0	100±0
Ultra low volume	5A	100	100	100	100	95.8	90.3
	5B	100	100	100	100	100	100
	Average	100±0	100±0	100±0	100±0	97.9±2.1	95.2±4.9

Note:
1. Adjusted Death Rate = (test group death rate - control group death rate) ÷ (1 - control group death rate).
2. Nozzle formulation

(2) KT

KT can be categorized into 3 periods. If KT_{50} is less than 5 minutes, the quick effect is good, followed by effects within 10 minutes. Those that exceed 10 minutes are considered poor in effect.

According to Table 4, liquid and cream insecticides show the best results in quick effects. On the average, the KT_{50} is within 10 minutes. In looking at the types of nozzles for suspension type insecticides, none are very effective (> 15 minutes.). As for lacquer type insecticides, the KT_{50} averages around 5minutes. The effect of Ultra low volume insecticides is also good under any kind of nozzle. The average KT_{50} does not exceed 10 minutes. Therefore, suspension types are only effective for residue spray with lacquer is better for space spray.

Table 4. Knock Out Effects of 22 Insecticides on *Aedes aegypti* Tainan Strain Mosquitoes

Type	Drug code	14.3 μm²	26.7 μm	46.0 μm	57.4 μm	73.6 μm	80.8 μm
Emulsion	1A	15.4	7.8	12.8	13.9	4.7	6.2
	1B	6.7	5.4	7	6.6	6.6	5.1
	1C	8.6	4.7	11.3	8.6	6.9	4.3
	1D	10.5	5.8	9	7.6	4.9	3.4
	1E	5.1	2.7	5.3	5.1	5.1	2.3
	1F	16.4	7.8	11	12	12.9	8.1
	Average	10.5±4.2 ᵇB	5.7±1.8 ᵃA	9.4±2.6 ᵃB	9.0±3.1 ᵇB	6.9±2.8 ᵇAB	4.9±1.9 ᵇA
Liquid	2A	8	7.1	9	9.9	8.3	6.5
	2B	7.4	7.1	6.4	6	5.4	5.1
	2C	7.6	4.9	7.6	9	6.8	4
	2D	10	7.2	7.9	8.5	10.5	10.2
	2E	7.4	4	5.3	4	5.1	4.1
	2F	13.8	11.4	13	16.4	13.4	10.2
	2G	2.7	1.4	2.3	2.7	2.4	1.7
	Average	8.1±3.0 ᵇ	6.2±2.9 ᵃ	7.4±3.0 ᵃ	8.1±4.2 ᵇ	7.4±3.4 ᵇ	6.0±3.0 ᵇ
Suspension	3A	37.4	22.4	37.7	27.3	24.4	22.6
	3B	38.3	14.8	26.9	25.1	12.3	11.5
	Average	37.9±0.5 ᶜC	18.6±3.8 ᵇA	32.3±5.4 ᵇC	26.2±1.1 ᶜAB	18.4±6.1 ᵇA	17.1±5.6 ᵇA
Lacquer	4A	2.9	2.7	3.2	2	1.9	2.3
	4B	6.8	3.2	4.5	5.4	4.6	3.7
	4C	2.4	1.4	1.6	1.9	1.4	1.7
	4D	1.7	1.5	1.6	1.5	1.6	1.3
	4E	3.3	2.4	2.6	2.7	3	2.5
	Average	3.4±1.8 ᵃ	2.2±0.7 ᵃ	2.7±1.1 ᵃ	2.7±1.4 ᵃ	2.5±1.2 ᵃ	2.3±0.8 ᵃ
Ultra low volume	5A	4.8	2.7	3.9	3.3	2.1	2.3
	5B	11.4	9.1	8.7	15.7	15.9	12.8
	Average	8.1±3.3 ᵇ	5.9±3.2 ᵃ	6.3±2.4 ᵃ	5.9±6.2 ᵇ	9.0±6.9 ᵇ	7.6±5.3 ᵇ

Note:
1. KT50 is calculated according to Finney (1971) Probit Analysis. (Unit: minute).
2. Nozzle formulation
3. In the same line, difference in the letters (a and A) display significant differences (P<0.05).

Discussion

1. Nozzle formulation testing

This test uses the image analysis theory of particle size distribution analyzers to test the droplet size of sprays. The results show that the manufacturer labels of the nozzles are closer in mathematical average. We can speculate that they used Laser particle testing; however, in particle testing variable, the VMD is much more accurate in portraying droplet sizes and analyzing statuses than NMD averages [2]. Thus, it is more appropriate in using VMD on display labels for different types of nozzles.

Although the spray path with differentiate due to the difference in quality, density, and stickiness [8] for different spray contents, nozzle formation testing uses no oil compressor with a stable pressure (20 psi) and metal nozzles in projecting the diluted (10-100 times diluted from the recommended dosage) insecticide. The effective ingredient is extremely minute in density and the pores are made from brass. Thus, it is not significantly different from spraying water and therefore of no important significance. The water spray testing results can be applied to all other insecticides.

2. Insecticide testing

Using Analysis of Variance (ANOVA) in analyzing the kill rate for *Aedes aegypti* Tainan strain mosquitoes of different types of insecticide and nozzles, there is no significant difference in the results ($P<0.05$). Thus, we can conclude that in using the 22 different insecticides, all are effective in eliminating the *Aedes aegypti* Tainan strain mosquitoes, no relation to the type of nozzle used. As for the knock out effects of the type of insecticides, according to ANOVA analysis, there are significant differences between the different types of insecticides and different types of nozzles ($P<0.05$). Emulsions and suspensions have longer

KT_{50} with 14.3 μm nozzles. This may be because the droplets are smaller in size and therefore take longer to settle [9-11]. Thus in the spray tower test, the droplets were unable to completely settle in the glass funnel within the testing time period. This resulted in the fact that the insecticide was unable to completely fill the space [12]; therefore leading to a performance less well as predicted. Using 26.7 μm nozzles in spraying insecticides, the KT_{50} was lower than those of 14.3 μm. However, the later KT_{50} decreased with the increase of nozzle size. This may be because the sprayed droplets were larger with a larger nozzle; therefore leading to the quick passage through the glass funnel and settling at the bottom. This shows quick killing of the insects within the confined space. However, 26.7 μm nozzles had droplets that were of medium size which float easily in space; thus showing lower KT_{50}. At the same time, suspension insecticides which use the principle of wrapping the particles in lower the influences of the environment have higher KT_{50} because of such a principle which hinders the large droplets in settling. Residue spraying sprays from up to down, similar to the spray tower testing; thus, suspension and emulsion insecticides have much lower quick effects on the *Aedes aegypti* Tainan strain mosquitoes and therefore are more suitable for residue spraying.

Liquid, lacquer, and ultra low volume insecticides all are not influenced by the size of the nozzle used in their effects on knocking out *Aedes aegypti* Tainan strain mosquitoes. All can knock out the insects within 10 minutes. The best performance can be seen with the 26.7 μm nozzles. Perhaps it is due to the fact that liquid, lacquer, and ultra low volume insecticides are easily influenced by the environment (temperature and humidity) in becoming smaller particles that they are more suitable in space spraying. Therefore, in epidemic areas, insecticides with droplet sizes between 20 and 50 μm can increase the floatation and reach the purpose in space spraying.

In addition, evaluation reports of the urgent spraying of insecticides in the southern region in the recent years has reflected that the effects of spraying insecticides has shown poor results due to the immaturity of the skills in spraying insecticides by the epidemic personnel. The repeated spraying of the same location led to the opposition from the residents and disturbance. The misuse of the dosage and the inappropriate usage and maintenance of the sprayers which are not complete in functions may lead to the drug resistance in the mosquitoes and contamination of the environment [17, 18]. This also proves that despite the appearance of drug-resistant mosquitoes in certain areas, correct and well-carried out spraying of the insecticides can still elevate the effects. Therefore, the combination of different types of sprayers and insecticides should be set. With the correct methods of spraying and the enhancing of skills of epidemic personnel, chemical prevention can still be accomplished through precise scientific methods.

References

1. WHO. Library Cataloguing in publication data equipment for vector control. Third Edition Geneva World Health organization 1990; 225pp.
2. WHO. Space spray application of insecticides for vector and public health pest control-A practitioner's guide 2003; WHOPES: 43pp.
3. Li YJ. Contrast experiment on the drop size and spatial distribution of ultra-low volume ground spray with three pesticides. Forest Pest and Disease 2002; 21: 31-2 (in Chinese).
4. Himel CM. The optimum size for insecticide spray droplets. J Econ. Ent. 1969; 62: 919-25.
5. Hwang JS. Sprayer operation and selection. TPPCO 2004; 6: 85-99 (in

Chinese).

6. Yan FG, Liang GJ. The influential factor of aerosol insecticide efficacy. Aero. Com. 2001; 3: 9-14 (in Chinese).
7. Wu HH, Chang NT. Comparision on food ingestion of Aedes ageypti(L.) and Aedes albopictus(Skuse). Chinese J Entomol 1990; 10: 433-42 (in Chinese).
8. Tang BM, Lin GW, Gao CY, et al. Study on two-phase flow spraying technique. Transa. Chinese Soc Agric Eng 2001; 17: 59-62 (in Chinese).
9. Lin SC, Cui XM, Ren H, et al. Techniques of ULV spray of DCPV oil miscible liquid in the forest. Forest Pest And Disease 2005; 24: 33-5 (in Chinese).
10. Cui AY, Zhou GP. The relationship between insecticide droplet size and control efficiency. Chinese J Pest Con 1992; 8: 47-8 (in Chinese).
11. Kang XM. Review of aerosol insecticide dissolvent. Chinese J Vector Bio Con 1997; 8: 477-8 (in Chinese).

12 Franklin RH, Richard JM. Effects of droplet size on the topical toxicity of two parathyroid to the cabbage lopper. Crop Protection 1994; 13: 225-9.

13. Brown JR, Williams DC, Gwinn T, el al. Filt-gun sprayer characteristics. Pan Am J Public Health 1998; 3: 322-5.
14. Curtis GA, Beidler EJ. Influence of ground ULV droplet spectra on adulticide efficancy for Aedes teaniorhynchus. J Amer Mosquito Control Assoc 1996; 12: 368-710.
15. Hsia WT. Sprayers of vector Control. Proce Envir Age Tech Vector Con 2006; 107-20 (in Chinese).
16. Hsia WT. Spray technology and efficacy assessment of Vector control. Sympo Sani Dengue 2007; 64-72(in Chinese).
17. Hsia WT, Lin EW, Pan CY. Bioassay of Pirimiphos-methyl 25% W/W

(Actellic E.C.) to *Aedes aegypti*(L.). Chinese Pest Con Assoc News 2007; 88: 1-4 (in Chinese).

18. Hsia WT, Chan CS, Pan CY, et al. Evaluation of the efficacy of dengue emergent control at Kaohsiung City, Taiwan, 2006. Taiwan Epidemiol Bull 2008; 24: 22-37 (in Chinese).