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Efficacy of cold fogging of a synergized pyrethroid formulation against *Aedes aegypti* and *Culex quinquefasciatus* under simulated field conditionsOthman Wan-Norafikah^{1,2*}, Han Lim Lee³, Seau Rong Loke³, Wei Ann Andy-Tan³, Chee Dhang Chen⁴¹Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia²Health and Wellbeing Core, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia³Medical Entomology Unit and World Health Organization Collaborating Centre for Vectors, Infectious Diseases Research Centre, Institute for Medical Research, Jalan Pahang, Kuala Lumpur 50588, Malaysia⁴Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia

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ABSTRACT

Objective: To evaluate the efficacy of a synergized pyrethroid formulation containing 0.8% w/w s-bioallethrin, 18.7% w/w permethrin and 16.8% w/w piperonyl butoxide.**Methods:** Cold fogging was carried out against *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* using a synergized pyrethroid formulation containing 0.8% w/w s-bioallethrin, 18.7% w/w permethrin and 16.8% w/w piperonyl butoxide. Three cages each containing 15 mosquitoes and paper cups each containing 25 larvae in 250 mL of water were deployed at each test pole between 10 and 40 feet. The formulation was diluted as 1:33, 1:50 and 1:100, respectively, and applied using a mist blower.**Results:** Using the dilution ratio of 1:33 and 1:50, 100% adult knock-down was recorded after an hour of post spraying at all checkpoints for both species. The percentage of adult mortalities for both species at 24 h post spraying ranged from 80% to 100%. Moreover, complete mortalities of *Ae. aegypti* larvae were demonstrated for both dilution ratio of 1:33 and 1:50 at all checkpoints. Meanwhile, complete mortalities of *Culex quinquefasciatus* larvae were recorded only at 10 and 20 feet with dilution ratio of 1:33.**Conclusions:** These results showed that cold fogging using synergized pyrethroid formulation tested was more effective against *Ae. aegypti* between 10 and 30 feet with dilution ratio of 1:33.

1. Introduction

Mosquitoes remain as a public health concern as they transmit many life-threatening diseases. *Aedes aegypti* (*Ae. aegypti*) (Linnaeus) is an important vector of dengue, yellow fever, chikungunya and Zika virus[1-3], whereas, *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say is a local nuisance biting mosquito and also a potential vector of lymphatic filariasis[4,5].

Vector control using both adulticides and larvicides are still the main option in many countries including Malaysia. Adulticides are applied by space spraying using either cold fogger or thermal fogger to control adult mosquitoes in endemic areas. For instance, fogging activities have been carried out during dengue outbreaks since 1970s[6]. In thermal foggers, oil- or water-based insecticide solution

is heated and vaporized before being expelled as a dense smoke. In contrast, high air pressure is needed in cold foggers such as ultra-low-volume generator and mist blower to break up the insecticide into small droplets and sprayed out through fine nozzles.

Malathion, an oil-based and smelly organophosphate has been used in local fogging activities for years before being replaced with water-based pyrethroid formulations[7]. Numerous studies have demonstrated that pyrethroids pose very minimal health risks to people[8]. This study was carried out to evaluate the efficacy of cold application of a synergized pyrethroid formulation containing 0.8% w/w s-bioallethrin, 18.7% w/w permethrin and 16.8% w/w piperonyl butoxide against adult and larva of *Ae. aegypti* and *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Test site

The test site was in the vicinity of Institute for Medical Research, Kuala Lumpur. An area of 184 m² was selected for the trial due to its proximity and convenience.

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2.2. Spray equipment

A mist blower (Stihl® mist blower 420R) with a discharge rate (dial 2) of 500 mL/min was used in all tests conducted.

2.3. Insecticide

A formulation of Mos-Spray® containing s-bioallethrin (0.8% w/w), permethrin (18.7% w/w) and piperonyl butoxide (16.8% w/w) was tested. The formulation was diluted in water to a ratio of 1:33, 1:50 and 1:100 and applied at an equivalent rate of 500 mL/ha.

2.4. Mosquitoes

Caged laboratory-bred and sugar-fed 5-day-old *Ae. aegypti* (F1010) and *Cx. quinquefasciatus* (F750) adult females were used to assess both adulticidal and larvicidal effects of space spraying.

2.5. Time of trial

The space spray was done in the evening after sunset. The weather was fair during the course of application and the wind velocity was < 1.0 m/s. Three cages were placed on each pole at the distances of 10, 20, 30 and 40 feet, respectively. The flow rate was adjusted to affect the output required. The direction of walking was parallel to the poles during the course of application.

2.6. Evaluation of fogging

At the test site, three cages each containing 15 mosquitoes were placed on each pole respectively according to the distances required. Each mosquito species in one cage was kept in the laboratory as control. Paper cups each containing 250 mL of water and 25 larvae of each species were placed near every pole. After the fogging, the knock-down rates of adult mosquitoes were recorded every 10 min until 60 min. After 60 min, all the cages and paper cups were collected and brought back to the laboratory. Mosquitoes were transferred into clean paper cups provided with a cotton pad soaked with 10% sugar solution. The mortality of the mosquitoes was recorded after a 24 h holding period. The larval mortality was also recorded after continuous exposure for 24 h.

3. Results

Table 1 shows the adulticidal activity of Mos-Spray® against *Ae.*

aegypti and *Cx. quinquefasciatus* using the mist blower. In the trial sprayed with a dilution ratio of 1:33 and 1:50, 100% adult knock-down was achieved after 60 min post spraying at all distances for both mosquito species. However, in the trial sprayed with dilution ratio of 1:100, 100% adult knock-down were only observed at 10–30 feet and 10–20 feet after 60 min post spraying for *Ae. aegypti* and *Cx. quinquefasciatus*, respectively.

Figure 1 shows the percentage mortality of adult *Ae. aegypti* and *Cx. quinquefasciatus* after 24 h post spraying of Mos-Spray® using the mist blower. Complete mortalities of *Ae. aegypti* adult mosquitoes were observed in the trial using water-diluted Mos-Spray® at a dilution ratio of 1:33 and 1:50 at up to 40 feet of distance. Meanwhile, 100% mortalities were recorded for *Cx. quinquefasciatus* adult mosquitoes only at the distance of 10–30 feet using the dilution ratio of 1:33 and 1:50. Overall percentage mortality for both mosquito species ranged from 80%–100%.

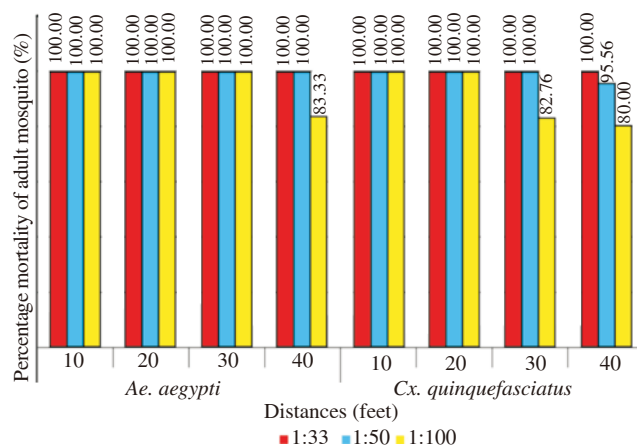


Figure 1. Percentage mortality of adult *Ae. aegypti* and *Cx. quinquefasciatus* after 24 h post spraying of Mos-Spray® by using a Stihl® mist blower 420R.

Figure 2 shows the larvicidal effect of Mos-Spray® against larvae of *Ae. aegypti* and *Cx. quinquefasciatus* after 24 h of continuous exposure. Larvae of *Ae. aegypti* achieved complete mortality for both dilution ratio of 1:33 and 1:50 at all distances tested, while for the dilution ratio at 1:100, complete mortalities were only observed at a maximum of 30 feet. Complete mortalities of *Cx. quinquefasciatus* larvae were demonstrated at the distance of up to 20 feet only using the dilution ratio of 1:33.

Table 1

Adulticidal activity of Mos-Spray® against *Ae. aegypti* and *Cx. quinquefasciatus* after 60 min post spraying of cold fogging using a Stihl® mist blower 420R.

Species of adult mosquitoes	Time interval (min)	Knock down rate (%)											
		10 Feet			20 Feet			30 Feet			40 Feet		
		1:33	1:50	1:100	1:33	1:50	1:100	1:33	1:50	1:100	1:33	1:50	1:100
<i>Ae. aegypti</i>	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	100.00	73.81	97.78	97.78	100.00	97.78	93.33	93.33	85.71	100.00	86.67	20.00
	20	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	96.43	100.00	100.00	40.00
	30	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	43.33
	40	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	56.67
	50	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	66.67
<i>Cx. quinquefasciatus</i>	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	95.56	71.11	93.33	73.33	100.00	80.00	84.44	91.11	62.07	93.33	60.00	53.33
	20	97.78	93.33	100.00	93.33	100.00	86.67	95.56	100.00	65.51	100.00	84.44	66.67
	30	100.00	97.78	100.00	100.00	100.00	100.00	100.00	100.00	72.41	100.00	93.33	80.00
	40	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	79.31	100.00	97.78	80.00
	50	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	89.66	100.00	100.00	93.33
60	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	93.10	100.00	100.00	93.33	

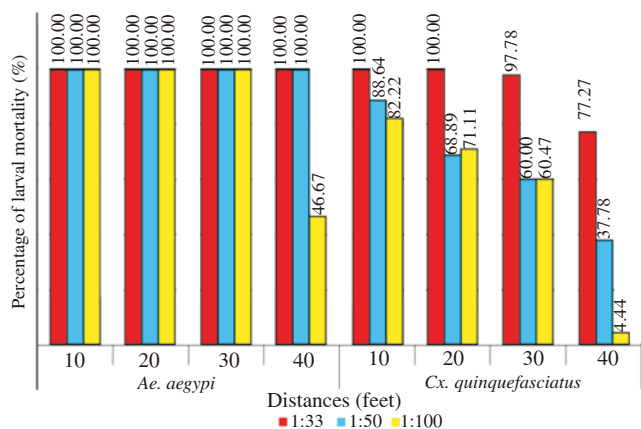


Figure 2. Percentage mortality of *Ae. aegypti* and *Cx. quinquefasciatus* larvae after 24 h continuous exposure to Mos-Spray® by using a Stihl® mist blower 420R.

No mortality was observed in the control for both adult and larval stages during the course of the trial.

For adulticidal activity, cold fogging of Mos-Spray® with dilution ratio of 1:33 and 1:50 were the most effective in producing complete mortalities of both *Ae. aegypti* and *Cx. quinquefasciatus* at a maximum distance of 40 feet. On the other hand, for the larval stage, cold fogging of Mos-Spray® was able to produce complete mortalities in *Ae. aegypti* at up to 40 feet with dilution ratio of 1:33, 1:50 and 1:100. Nevertheless, similar formulation was only effective in initiating complete mortalities of *Cx. quinquefasciatus* larvae at a maximum distance of 20 feet with dilution ratio of 1:33. These results showed that Mos-Spray® was more effective against *Ae. aegypti* than *Cx. quinquefasciatus* at both mosquito biological stages. Furthermore, these findings also proved that cold fogging of Mos-Spray® was useful against both adult and larval stages of both mosquito species but it was more effective against adult mosquitoes compared to larvae. In fact, wider areas of space spraying could be conducted using Mos-Spray® if the dilution ratio of 1:33 was used, which will still be effective to both mosquito species at either larval or adult stage.

4. Discussion

Several studies on efficacy of space spraying including fogging had been reported worldwide. In 2005, Mani *et al.*[9] showed that the killing effect of pyrethroids with piperonyl butoxide as a synergist against *Ae. aegypti* adults was relatively high. Later in 2011, Marcombe *et al.*[10] demonstrated that pyrethroid space spraying caused 47%–63% mortalities of susceptible *Ae. aegypti* adults. Similar findings were observed by Harburguer *et al.*[11] which demonstrated that cold fogging using pyrethroids diluted in water was effective against *Ae. aegypti* adults especially outdoors. Another studies by Karunaratne *et al.*[12] demonstrated complete mortalities of both caged *Ae. aegypti* and *Aedes albopictus* placed at 10 m distance but not at 75 m distance in various habitat types.

In Malaysia, commercial formulation of pyrethroids was tested in combination with *Bacillus thuringiensis israelensis* for space spraying which showed more than 80% larvicidal mortality in both *Ae. aegypti* and *Aedes albopictus*, and higher than 60% for *Cx. quinquefasciatus* larvae[13]. Other local studies also demonstrated that pyrethroids space spraying produced both larvicidal and adulticidal effects in *Aedes* mosquitoes indoors and outdoors[14,15].

Differentiation in application methods, types of insecticides used and sampling methods of space spraying studies of insecticides are among several obstacles which affect the possibility of more specific comparison to be made[9]. Hence, it is suggested that more in depth studies on the efficacy of insecticides including pyrethroids used in space spraying activities should be performed continuously.

Conflict of interest statement

We declare that we have no conflict of interest.

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