Original Article

Evaluation of Pyrethrin Formulations on Dengue/Dengue Haemorrhagic Fever Vectors in the Laboratory and Sublethal Effects

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Abstract

In Southeast Asia, *Aedes aegypti* (L.) has been incriminated as principal vector of dengue viruses and *Ae. albopictus* as the secondary vector of dengue fever. Therefore, the aim of this study was to investigate the effectiveness of three formulations of pyrethrin derived from *Tanacetum cinerariaefolium* against the dengue/dengue haemorrhagic fever vectors *Aedes aegypti* and *Ae. albopictus* in the laboratory. The testings employed 2 methodologies: the WHO Larval Bioassay and WHO Adult Bioassay. The results showed that all the three pyrethrin formulations had larvicidal and adulticidal activities. The impact of the sublethal doses of pyrethrin formulations on *Aedes* spp. larvae resulted in 4-6% of alive adult emergence compared to 90% of *Ae. aegypti* emerging adults and 96% *Ae. albopictus* alive adult emergence in the control. The impact of sublethal doses of the pyrethrin formulations caused very low fecundity on both *Aedes* spp. compared to the control ($P< 0.05$).

Keywords: Pyrethrin, Dengue vectors, Sublethal effects

Introduction

Pyrethroids have been widely used for dengue vector control. Lambda-cyhalothrin had been shown to be effective against dengue vectors under laboratory and field conditions in Malaysia (Lim and Visalingam 1990, Lim and Lee 1991, Sulaiman et al. 1991, 1993). Alphacypermethrin had been shown to be effective against dengue vectors in the field in Malaysia, demonstrating both adulticidal and larvicidal effects (Sulaiman et al. 1995). Pyrethrin derived from pyrethrum daisy *Tanacetum cinerariaefolium* is a highly effective insecticide for controlling insect pests. Pyrethrum had shown repellent activity against *Mansonia* mosquitoes (Hadis et al. 2003), sampling indoor resting African malaria vectors is done traditionally by hand catches with oral or mechanical aspirators and pyrethrum catches (Harbison et al. 2006) and entomological evaluation of malaria vectors at different altitudes (Kulkarni et al. 2006). Pyrethrum is being used for impregnating of bednets and curtains, made of polypropylene fibre (Curtis et al. 1992).

The objective of this study was to evaluate the efficacy of various pyrethrin formulations on the larval and adult stages of the dengue vectors, and the effect of sublethal dose on the larval stage of the dengue vectors on adult emergence and fecundity in the laboratory.

Materials and Methods

The pyrethrin formulations used were pyrethrin 50%, pyrethrin 0.4g/l+ PBO 1.5g/l, pyrethrin 44g/l+ PBO 160g/l and insecticides Abate® and Malathion. The pyrethrin formulations were sup-

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plied by Botanical Resources Australia Pty Ltd, Tasmania, Australia.

Larval Bioassay

The larval bioassay was conducted on *Aedes aegypti* and *Aedes albopictus,* based on WHO instruction (1981a). The experiment was conducted at 25± 1 ºC and relative humidity 80± 5%. Twenty five *Ae. aegypti* and *Ae. albopictus* 4th instar larvae were exposed to 250 ml of prepared pyrethrin concentrations and Abate® in 600 ml beakers. Four replicates were conducted and mortality was recorded after 24 h. The LC_{50} and LC_{90} levels, regression slopes and associated 95% fiducial limits were determined by computer using probit analysis (Raymond 1985).

Adult Bioassay

The adult bioassay was conducted on *Ae. aegypti* and *Ae. albopictus* using WHO test kit (1981b). Fifteen 2-5 day old female mosquitoes were exposed for 1h to the filter papers in the WHO test kit, impregnated with various concentrations of pyrethrin formulations and malathion as positive control, then transferred to holding tubes. Sugar solution soaked in cotton was provided as food. Four replicates were conducted and the number of adult mortality was recorded after 24 h. LC_{50} and LC_{90} levels, regression slopes and associated 95% fiducial limits were determined by Probit analysis (Raymond 1985)

Studies on sublethal effects were based on Loh and Yap study (1989) with some modifications. The concentration used was the LC_{50} of each pyrethrin formulation from the larval bioassay. Fifty 4th instar larvae of *Ae. aegypti* and *Ae. albopictus* were used for each sublethal dose of pyrethrin formulation. Mortality was recorded after 24 h exposure and the surviving larvae were transferred into beakers containing distilled water. The number of surviving larvae, pupae and emerging adults was recorded. The emerging adults were blood fed and allowed to oviposit and the eggs were counted. The eggs were allowed to hatch into larvae, pupae and adults and recorded. The control includes non insecticide treated mosquitoes. Data were analyzed using Mann-Whitney U test.

Results

Table 1 showed that both *Ae. aegypti* and *Ae. albopictus* 4th instar larvae were more susceptible to pyrethrin 44g/l+ PBO 160g/l than pyrethrin 0.4g/l + PBO 1.5g/l or pyrethrin 50%. The pyrethrin 44g/l+ PBO 160g/l LC_{50} and LC_{90} for *Ae. aegypti* 4th instar larvae were 0.002 ppm and 0.007 ppm. The LC_{50} and LC_{90} for *Ae. albopictus* 4th instar larvae were 0.004 ppm and 0.012 ppm, respectively. The LC_{50} and LC_{90} values for pyrethrin 50% were higher than LC_{50} and LC_{90} for pyrethrin 0.4g/l+ PBO 1. 5g/l for *Ae. aegypti* 4th instar larvae (0.038 ppm and 0.135 ppm:0.028 ppm and 0.080 ppm). Similarly, the LC_{50} and LC_{90} for pyrethrin 50% were higher than LC_{50} and LC_{90} for pyrethrin 0.4g/l+ PBO 1.5g/l for *Ae. albopictus* 4th instar larvae (0.069 and 0.336: 0.056 and 0.166), respectively. Using Mann-Whitney U test values of LC_{50} and LC_{90} for each insecticide tested against 4th instar larvae of both *Aedes* spp. showed significant difference ($P< 0.05$). However, Abate® showed a much lower susceptibility to both *Aedes* spp. 4th instar larvae compared to the three pyrethrin formulations. Although the pyrethrin formulations have larvicidal effects against both *Aedes* spp. but Abate still remains the insecticide of choice as larvicide.

Table 1. Mortality response of pyrethrin formulations to *Aedes aegypti* and *Aedes albopictus* 4th-instar larvae in the laboratory

Insecticides	*Aedes aegypti*			*Aedes albopictus*		
	LC_{50} (95%CI) (ppm)	**LC_{90} (95%CI) (ppm)**	**Slope±SE**	**LC_{50} (95%CI) (ppm)**	**LC_{90} (95%CI) (ppm)**	**Slope ± SE**
Pyrethrin 50%	0.038 (0.029-0.052)	0.135 (0.090-0.280)	2.334±0.371	0.069 (0.039-0.083)	0.336 (0.268-0.487)	2.276±0.674
Pyrethrin 0.4g/l + PBO 1.5g/l	0.028 (0.022-0.036)	0.080 (0.058-0.131)	2.842±0.413	0.056 (0.044-0.071)	0.166 (0.120-0.281)	3.226±0.464
Pyrethrin 44g/l+PBO 160g/l	0.002 (0.002-0.003)	0.007 (0.005-0.011)	2.973±0.428	0.004 (0.003-0.006)	0.012 (0.008-0.021)	2.974±0.603
Abate® (Control)	0.00003 (0.00001-0.00007)	0.0016 (0.00045-0.01382)	0.909±0.136	0.00001 (0.0000-0.00002)	0.004 (0.0006-0.1409)	0.594±0.112

Table 2 indicates that both *Ae. aegypti* and *Ae. albopictus* adults are more susceptible to pyrethrin 44 g/l + PBO 160 g/l than pyrethrin 0.4 g/l + PBO 1.5 g/l and pyrethrin 50%. The LC_{50} and LC_{90} of pyrethrin 44 g/l + PBO 160 g/l for *Ae. aegypti* were 0.209 μg/cm² and 0.469 μg/cm². The LC_{50} and LC_{90} for *Ae. albopictus* were 0.197 μg/cm² and 0.492 μg/cm², respectively. The LC_{50} and LC_{90} for pyrethrin 50% were higher than that of pyrethrin 0.4g/l + PBO 1.5g/l for *Ae. albopictus* adults. However, the LC_{50} for pyrethrin 50% was higher than that of pyrethrin 0.4g/l + PBO 1.5g/l for *Ae. aegypti* but the regression slope of pyrethrin 0.4g/l + PBO 1. 5g/l indicated that a slight increase in its concentration would cause a higher mortality of *Ae. aegypti* adults, compared to pyrethrin 50%. Using Mann Whitney U test the value of LC_{90} for each insecticide formulation tested against adults of both *Aedes* spp. were significantly different (P< 0.05). However, malathion showed a much lower susceptibility values to both *Ae. aegypti* and *Ae. albopictus* adults, compared to the three pyrethrin formulations.

Table 2. Mortality response of pyrethrin formulations to *Aedes aegypti* and *Aedes albopictus* adults in the laboratory

Insecticides	*Aedes aegypti*			*Aedes albopictus*		
	LC_{50} (95%CI) (μg/cm²)	**LC_{90} (95%CI) (μg/cm²)**	**Slope ± SE**	**LC_{50} (95%CI) (μg/cm²)**	**LC_{90} (95%CI) (μg/cm²)**	**Slope ± SE**
Pyrethrin 50%	1.331 (0.901-2.128)	3.982 (2.080-4.013)	2.745±0.604	1.980 (1.007-2.202)	5.244 (4.896-5.993)	3.036±0.573
Pyrethrin 0.4g/l + PBO 1.5g/l	1.701 (0.967-1.996)	3.743 (2.765-4. 010)	3.636±0.699	1.671 (1.010-2.100)	4.721 (3.787-5.220)	3.104±0.564
Pyrethrin 44g/l+PBO 160g/l	0.209 (0.160-0.277)	0.469 (0.339-0.875)	3.713±0.753	0.197 (0.091-0.298)	0.492 (0.287-0.992)	3.227±0.622
Malathion (Control)	0.00006 (0.00003-0.00007)	0.00050 (0.00048-0.00098)	1.303±0.266	0.0008 (0.0006-0.0013)	0.0071 (0.0058-0.0071)	1.364±0.249

Table 3 shows the impact of *Aedes* spp. larvae which was treated with sublethal doses (LD_{50}) of pyrethrin formulations. All the three pyrethrin formulations resulted in 4-6% of emerging adults of *Ae. aegypti* and *Ae. albopictus* alive, compared to 90% of *Ae. aegypti* adults and 96% of *Ae. albopictus* alive adults in the control, respectively. Thus, all the pyrethrin formulations at LD_{50} when treated on both *Aedes* spp. larvae caused low production of emerging adults.

Table 3. Impact of treatment of mosquito larvae with sublethal doses of pyrethrin formulations

	Pyrethrin 50%		**Pyrethrin 0.4g/l + PBO 1. 5g/l**		**Pyrethrin 44g/l + PBO 160g/l**		**Control**	
	Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*
No. of larvae tested	50	50	50	50	50	50	50	50
Mortality of larvae after 24 hr (%)	44	48	50	52	52	48	2	2
Mortality of pupae (%)	2	0	8	4	10	12	0	0
Mortality of emerging adults (%)	6	8	16	16	20	14	0	0
Emerging adults alive (%)	6	6	4	4	4	4	90	96

Table 4 indicated that when *Aedes* spp. larvae were treated with sublethal doses of pyrethrin formulations, the fecundity of subsequent adults produced very low number of eggs for both *Aedes* spp. in the range of 0-4 eggs, compared to 198-227 eggs produced in the control. None of the eggs derived from the treated larvae hatched. However, 99% of the control eggs of both *Aedes* spp. became adults. It was observed that the female mosquitoes derived from different formulations of pyrethrin treated larvae were not feeding well on *Argus* spp. This would affect the number of eggs laid.

Table 4. Impact of mosquito fecundity after the larval exposure to sublethal doses of pyrethrin formulations

	Pyrethrin 50%		**Pyrethrin 0.4 g/l + PBO 1. 5 g/l**		**Pyrethrin 44 g/l + PBO 160 g/l**		**Control**	
	Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*
No. of eggs produced	2	4	0	2	0	3	1189	2270
% of eggs hatched	0	0	0	0	0	0	99	99
% of larvae becoming pupae	0	0	0	0	0	0	99	99
% of pupae becoming adults	0	0	0	0	0	0	99	99

Discussion

According to Rayman (2006), a 2% pyrethrum solution, a naturally occurring pyrethroids found in *Chrysanthemum* spp. flowers is the recommended agent for aircraft disinsection because they are extremely effective insecticide and pose minimal health risks. According to Gerry et al. (2005) the aerial application of ULV pyrethrin insecticide for control of adult mosquitoes did not result in undue exposure to the pilot. Jensen et al. (1999) conducted ULV application of pyrethrin, malathion and permethrin on non-target invertebrates, sentinel mosquitoes and mosquitofish in California seasonal wetlands, USA. All the insecticides tested including pyrethrin were able to control adult mosquitoes without substantial effects on the aquatic insects or fish in the seasonal wetlands. Victor et al. (2002) in their application of temephos and fogging with pyrethrum 2% extract in villages at Tamil Nadu, India, were effective against immatures and adults *Aedes aegypti*. Thus, the pyrethrin formulations can be widely used in controlling dengue vectors.

Mohapatra et al. (1999) evaluated cyfluthrin and fenfluthrin on their activity against different developmental stages of three vector species viz**.,** *Anopheles stephensi*, *Ae. aegypti* and *Culex quinquefasciatus***.** Both compounds were more active against the fourth larval instars of all mosquito species tested, cyfluthrin in culicines and fenfluthrin in anophelines brought about maximum inhibition of adult emergence.

Mohapatra et al. (1999) also found that pyrethroids cyfluthrin and fenfluthrin significantly reduced the fertility rates (P< 0.001) of *Anopheles stephensi*, *Aedes aegypti***,** and *Culex quinquefasciatus***.** Loh and Yap (1989) who studied the efficacy and sublethal effects of pyriproxyfen on *Ae. aegypti*, found that the eggs hatchability was reduced by 36.8%.

According to Xue et al. (2005) application of deet forced egg retention time reduced the number of eggs laid per female *Aedes albopictus*. The rate of egg hatched was considerably reduced after three weeks of retention. The fecundity and fertility of gravid female *Ae. albopictus* were affected by the time duration of forced egg-retention. Ali et al. (2006) found that fecundity and fertility based on number of laid eggs per female and percentage of egg hatch in *Stegomyia albopicta* when exposed to 0. 1% boric acid sugar bait were significantly reduced and ovarian development retarded. Focks et al. (1991) studied *Aedes aegypti* which were fed on rabbits subcutaneously injected with ivermectin exhibited reduced survival and egg production compared to females fed on the control rabbits. Eggs from these rabbits were also less likely to hatch and subsequent larval survival was lower than the controls. Similarly, our studies also indicated that pyrethrin formulations affected the produced eggs and then a few produced eggs could not hatch into larvae.

To conclude, all the pyrethrin formulations had larvicidal and adulticidal effects on *Aedes aegypti* and *Aedes albopictus.* Furthermore, treatment at sublethal doses at the larval stage produced very low emerging adults and fecundity.

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