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PERFORMANCE OF ULV FORMULATIONS (PESGUARD® 102/VECTOBAC® 12AS) AGAINST THREE MOSQUITO SPECIES

H. H. YAP, A. S. C. CHONG, C. R. ADANAN, N. L. CHONG, B. ROHAIZAT, Y. ABDUL MALIK AND S. Y. LIM

Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

ABSTRACT. Adulticidal and larvicidal performances of a water-based pyrethroid microemulsion Pesguard® PS 102 (AI *d*-allethrin and *d*-phenothrin, both at 5.0% w/w) and Vectobac® 12AS, an aqua-suspension *Bacillus thuringiensis israelensis* (*B.t.i.*) formulation (AI 1,200 ITU/mg) were assessed against mosquitoes *Aedes aegypti, Aedes albopictus,* and *Culex quinquefasciatus* using a Leco® ULV Fog Generator Model 1600 and a Scorpion® 20 ULV AirBlast Sprayer. Laboratory-cultured mosquito adults and larvae were used for efficacy assessment. For trials using Leco, both pyrethroid and bacterial formulations were dispersed both singly and in combination with Pesguard PS 102 at a dosage of 0.2 liters/ha and *B.t.i.* at a dosage of 1.0 liter/ha. Similar trials with the Scorpion were also conducted with Pesguard PS 102 at a dosage of 0.2 liters/ha and a higher dosage of *B.t.i.* (1.5 liters/ha). Experiments were conducted in a football field (200 × 100 m) where five check points at 10, 25, 50, 75, and 100 m downwind from the spray nozzle were chosen for efficacy assessments. Knockdown and mortality were scored at 1 and 24 h postspraying. Results from both trials showed that mortality values varied with distance from spray nozzle. For trials with Leco, fogging with the combination of Pesguard PS 102 and *B.t.i.* provided larvicidal mortality of >80% for both *Aedes* species and of >60% for *Cx. quinquefasciatus* larvae at several check points, depending on wind conditions. Complete mortality of adult *Aedes* mosquitoes at 24 h posttreatment was also achieved, while mortality values for *Culex* adults reached >90% under strong wind conditions. As for trials with the Scorpion 20, high adult and larval mortalities were also achieved, with >90% mortality at some check points. The above study demonstrated the possibility of achieving both larvicidal and adulticidal effects when using a combination of *B.t.i.* and Pesguard PS 102 in ULV space spray.

INTRODUCTION

Ultra-low volume (ULV) spraying has been identified as a major approach for vector control of mosquito adults (Glancey et al. 1965, Knapp and Roberts 1965) and larvae (Sandoski et al. 1985, Mount et al. 1996). Spraying malathion with this method has been the standard approach for the control of *Aedes* mosquitoes in Malaysia since the early 1970s (Lam and Tham 1988). Also, Perich et al. (1990) reported on the use of both ground and aerial ULV applications of malathion for *Aedes aegypti* control in the Dominican Republic. Similar approaches were also adopted in other Central American countries (Tonn et al. 1982). Both favorable and unfavorable results with ULV insecticide application have been reported (Fox 1980, Gratz 1991). This could be due to differences in the choice of insecticides, the application rate, and the degree of insecticide penetration of dwellings (Perich et al. 1990). The failure of ULV spraying to suppress larval populations has also been identified as a common problem in achieving the desired level of control.

The purpose of this study was to evaluate the efficacies of a water-based pyrethroid microemulsion (Pesguard® PS 102) combining *d*-allethrin with *d*-phenothrin (which gives both adult knockdown and killing properties) and also an aqua-suspension of *Bacillus thuringiensis israelensis* (*B.t.i.*) formulation (Vectobac® 12AS). Adulticidal and larvicidal efficacies of both formulations used singly and in combination were assessed from trials done in an open football field against 3 urban mosquito species of public health importance, namely, *Aedes aegypti, Aedes albopictus,* and *Culex quinquefasciatus.*

MATERIALS AND METHODS

All three species of test mosquitoes were obtained from established laboratory colonies initiated from field populations in Penang Island, Malaysia, in the 1990s. For this study, only sucrose-fed female mosquitoes aged 3–5 days were used for adulticidal assessment, while early 4th-instar larvae of each species were used to determine the larvicidal effects.

Twenty adult mosquitoes were placed in a cylindrical fine-mesh cage with cylindrical wire frame support (10 cm diam × 15 cm height). In addition, 20 larvae were placed in a paper cylindrical cup (top diameter 8 cm) containing 200 ml of seasoned tap water. Formulation efficacy was assessed at five points downwind (10, 25, 50, 75, and 100 m) of the spray route. Cages were placed 1.5 m above ground at these respective points, while cups containing mosquito larvae were placed at ground level below the cages. Knockdown of adult mosquitoes was read at 60 min postspraying. The treated adult mosquitoes were then transferred into clean polyethylene cups with a 10% sucrose saturated cotton pad, and both larvae and adults were kept at 26 ± 2°C and 65 ± 10% relative humidity. Mortality of both adult and larval mosquitoes was also scored at 24 h postspraying. Untreated cages and cups for

Table 1. Laboratory comparison of adult knockdownh/mortalities of 3 mosquito species with ULV (Leco®) application of Pesguard® PS 102 alone and in combination with Vectobac® 12AS[1] at 1 and 24 h posttreatment.

Species	Application[2]	10 m	25 m	50 m	75 m	100 m
Mean percentage mortality at different distances from spray nozzle (1-h knockdown)						
Aedes albopictus	PG	98a	95a	96a	49a	47a
	PG + VC	94a	77a	100a	100b	99b
Aedes aegypti	PG	98a	97a	99a	60a	55a
	PG + VC	77a	83a	100a	100a	100b
Culex quinquefasciatus	PG	44a	42a	43a	16a	4a
	PG + VC	75a	77a	63a	63b	75b
Mean percentage mortality at different distances from spray nozzle (24-h mortality)						
Ae. albopictus	PG	98a	77a	98a	28a	7a
	PG + VC	88a	65a	98a	28a	3a
Ae. aegypti	PG	84a	75a	100a	100a	87a
	PG + VC	93a	96a	100a	100a	99a
Cx. quinquefasciatus	PG	43a	11a	18a	43a	3a
	PG + VC	77a	70b	89b	71a	42b
Wind velocity	Trials with PG: 0.5–1.0 m/sec Trials with PG + VC: 1.5–2.8 m/sec					

[1] Percentages followed by the same letter within the same column for the same species and posttreatment hour reading are not significantly different ($P < 0.05$, Duncan Multiple Range test).
[2] PG = Pesguard 102 (0.2 liters/ha); PG + VC = Pesguard PS 102 (0.2 liters/ha) + Vectobac 12AS (1.0 liters/ha).

all 3 species were also placed in the laboratory for control purposes.

The adulticidal insecticide used was Pesguard PS 102, a water-based pyrethroid microemulsion containing *d*-allethrin ((*RS*)-3-allyl-2-methyl-4-oxocyclopent-2-enyl(1*R*)-*cis-trans*-chrysanthemate) and *d*-phenothrin (3-Phenoxybenzyl (1*R*)-*cis-trans*-chrysanthemate) (both at 5.0% w/w), provided by Sumitomo Chemical Asia Pte. Ltd., Singapore. As for the *B.t.i.* formulation, Vectobac 12AS (AI 1,200 ITU/mg) was used (Abbott Laboratories, USA).

Two experimental trials were carried out with these formulations. The first, conducted in March 1996, used a vehicle mounted Leco® ULV Fog Generator Model 1600 (Lowndes Engineering Co., Valdosta, GA). The flow rate of both the ULV machines was adjusted to 1.0 l/min for all spraying trials. Assessment was made on the efficacies of both Pesguard and Vectobac dispersed both singly in combination dosages of 0.2 liters/ha and 1.0 liter/ha, respectively.

For trials with Pesguard alone, a total of 2 liters of spraying formulation was prepared by diluting the insecticide with water at a ratio of 1:4. Spraying was then done by driving the vehicle perpendicular to the spraying direction covering a total distance of 200 m with the spray nozzle pointing toward the cages at an angle of 30° to the horizontal plane for a period of 2.0 min, thus delivering a total of approximately 2 liters of the water/insecticide formulation described above or 0.2 liters of Pesguard over an area of 1 ha. Vectobac/water spraying formulation was prepared by diluting the *B.t.i.* with water at a ratio of 1:1. Spraying of the actual proposed dosage of Vectobac (1.0 liter/ha) was then achieved through manipulation of the spraying pe-

Table 2. Laboratory comparison of larval mortalities of 3 mosquito species with ULV (Leco®) application of Vectobac® 12AS alone and in combination with Pesguard® PS 102[1] at 24 h posttreatment.

		Mean percentage mortality at different distances from spray nozzle (24-h mortality)				
Species	Application[2]	10 m	25 m	50 m	75 m	100 m
Aedes albopictus	VC	64a	65a	73a	41a	25a
	PG + VC	95a	79a	92a	94b	81b
Aedes aegypti	VC	52a	68a	41a	37a	25a
	PG + VC	21a	86a	45a	67a	61a
Culex quinquefasciatus	VC	37a	37a	52a	27a	41a
	PG + VC	58a	67a	70a	79a	71a
Wind velocity	Trials with VC: 0.5–0.9 m/sec Trials with PG + VC: 1.5–2.8 m/sec					

[1] Percentages followed by the same letter within the same column for the same species and posttreatment hour reading are not significantly different ($P < 0.05$, Duncan Multiple Range test).
[2] VC = Vectobac 12AS (1.0 liters/ha); PG + VC = Pesguard PS 102 (0.2 liters/ha) + Vectobac 12AS (1.0 liters/ha).

Table 3. Laboratory comparison of adult knockdown/mortalities of 3 mosquito species with ULV (Scorpion™) application of Pesguard™ PS 102 alone and in combination with Vectobac™ 12AS[1] at 1 and 24 h posttreatment.

Species	Application[2]	10 m	25 m	50 m	75 m	100 m
	Mean percentage mortality at different distances from spray nozzle (1-h knockdown)					
Aedes albopictus	PG	74a	95a	99a	100a	98a
	PG + VC	78a	100a	98a	100a	89a
Aedes aegypti	PG	67a	100a	99a	100a	100a
	PG + VC	99b	99a	100a	99a	99a
Culex quinquefasciatus	PG	31a	94a	63a	63a	27a
	PG + VC	60a	71a	56a	41a	36a
	Mean percentage mortality at different distances from spray nozzle (24-h mortality)					
Ae. albopictus	PG	69a	96a	80a	83a	80a
	PG + VC	53a	94a	72a	64a	60a
Ae. aegypti	PG	81a	87a	69a	84a	55a
	PG + VC	94a	81a	85a	78a	55a
Cx. quinquefasciatus	PG	40a	57a	60a	62a	28a
	PG + VC	57a	84a	77a	45a	38a
Wind velocity	Trials with PG: 1.0–3.6 m/sec Trials with PG + VC: 0.2–1.2m/sec					

[1] Percentages followed by the same letter within the same column for the same species and posttreatment hour reading are not significantly different ($P < 0.05$, Duncan Multiple Range test).

[2] PG = Pesguard 102 (0.2 liters/ha); PG + VC = Pesguard PS 102 (0.2 liters/ha) + Vectobac 12AS (1.5 liters/ha).

riod (4 min) with a reduction of vehicle speed. As for trials combining both Pesguard and Vectobac, the mixed spraying formulation was prepared immediately before spraying by mixing the Pesguard spraying formulation mentioned above (0.2 liters of Pesguard per liter of water) with Vectobac at a volume ratio of 1:1. Four liters of this formulation were sprayed, thus achieving the purpose of simultaneous dispersion of both adulticide and larvicide at dosages of 0.2 liters/ha Pesguard and 1.0 liter/ha Vectobac, respectively. For the Pesguard application, we assessed the adulticidal efficacy, and, as for Vectobac, we evaluated its larvicidal efficacy. Efficacy of both mosquito life stages were assessed for trials involving the application of the mixed formulation involving chemicals.

Trials were conducted in an open football field at the Minden Campus, Universiti Sains Malaysia, Penang, Malaysia, at 2100–0100 h. Wind direction and velocity were measured prior to each spray. In order to achieve downwind spraying, the positions of cages were shifted in accordance with wind direction before spraying. The efficacy trial of each formulation was triplicated. Similar trials were done in July 1996 with a Scorpion™ 20 ULV AirBlast Sprayer (Berry Co., KY). The Pesguard dosage evaluated was similar to that in trials conducted with the Leco machine (0.2 liters/ha). As for Vectobac, a higher dosage (1.5 liters/ha) was assessed. A mixed formulation of both of the above chemicals at a dosage of 0.2 liters/ha Pesguard and 1.5 liters/ha Vectobac was also used for efficacy assessment.

Percentage of knockdown/mortality values were subjected to an arcsine transformation followed by comparison of means using the Duncan Multiple Range test (SAS Institute 1985).

RESULTS AND DISCUSSION

Our observation from all trials showed that both of the machines were able to disperse all formulations as fine aerosol droplets. Under suitable wind conditions, the drift of spray droplets up to the 100 m checkpoint was also achievable. Both the adulticide and larvicide formulations mixed well with each other and were easily diluted in water.

In the Leco trials, there were no significant differences ($P < 0.05$) in the 24-h adult mortality between the pyrethroid formulation alone and in combination with *B.t.i.* for both of the *Aedes* species (Table 1). The differences in the final two checkpoints at the 1-h knockdown/mortality reading could be due to the different wind velocity during spraying, which affected the transport of spray droplets at greater distances. Wind velocity measured during the spraying of the mixed formulations was higher. As for *Culex,* the Pesguard and *B.t.i.* mixture also resulted in higher knockdown and kills for both the 1- and 24-h readings. Table 2 shows that the 24-h larval mortality achieved during the spraying of the mixed formulation was in the range of 79–95% for *Ae. aegypti,* 21–86% for *Ae. albopictus,* and 58–79% for *Cx. quinquefasciatus* larvae. These values were, in general, higher than the scores achieved with the spraying of Vectobac alone. This is probably a result of the influence of stronger wind conditions during spraying of the mixed formulation. Mortality values for *Culex* mosquitoes were generally lower than those for both the *Aedes* species, as the former are more tolerant to pyrethroid insecticides (Yap and Chung 1987, Yap et al. 1996). Overall, trials with the Leco machine showed that satisfactory results against both adult and larval mosquitoes simultaneously

Table 4. Laboratory comparison of adult knockdown/mortalities of 3 mosquito species with ULV (Leco™) application of Pesguard™ PS 102 alone and in combination with Vectobac™ 12AS[1] at 1 and 24 h posttreatment.

		Mean percentage mortality at different distances from spray nozzle (1-h knockdown)				
Species	Application[2]	10 m	25 m	50 m	75 m	100 m
Aedes albopictus	VC	99a	97a	92a	90a	44a
	PG + VC	92a	92a	98a	97a	93b
Aedes aegypti	VC	100a	100a	93a	100a	36a
	PG + VC	90a	53b	58b	71b	77b
Culex quinquefasciatus	VC	84a	88a	99a	82a	74a
	PG + VC	71a	71a	45b	57b	33b
Wind velocity	Trials with VC: 0.4–2.0 m/sec Trials with PG + VC: 0.2–1.2 m/sec					

[1] Percentages followed by the same letter within the same column for the same species and posttreatment hour reading are not significantly different ($P < 0.05$, Duncan Multiple Range test).
[2] VC = Vectobac 12AS (1.5 liters/ha); Pesguard PS 102 (0.2 liters/ha) + Vectobac 12AS (1.5 liters/ha).

can be achieved with the spraying of a mixed formulation of adulticidal pyrethroid insecticide with *B.t.i.* under suitable wind conditions, and results obtained were also comparable to those obtained from separate spraying of both formulations.

Similar trends were obtained during trials with the Scorpion machine (Tables 3 and 4). Similar adulticidal results were obtained for all 3 mosquito species, for spraying of the pyrethroid both singly and in combination with *B.t.i.* As for larval efficacy, the mixed combination resulted in mortalities of above 90% at all distances in *Ae. albopictus.* Mortalities ranged from 53 to 90% for *Ae. aegypti* and from 33 to 71% for *Cx. quinquefasciatus.* Higher wind velocity was observed during trials with the single formulation than during those with the mixed formulations. No mortality was observed in the controls.

The use of pyrethroids as the choice and effective adulticidal agent in ULV applications has been reported in many studies. Results from this study have shown high percentages of adult mosquito knockdown followed by low recovery rate, although the treated mosquitoes were kept for 24 h in cups with sucrose pads under normal room temperature and humidity. Earlier, Yap et al. (1996), using mosquito species similar to those used in this present study, also reported a low recovery rate for adult mosquitoes that were knocked down with Pesguard PS 102. These effects are obviously due to the presence of *d*-allethrin and *d*-phenothrin acting as knockdown and killing agents, respectively. The water-based formulations used in this study were also easy to prepare and possessed minimal odor compared to normal malathion applications. Another formulation containing different proportions of both of these agents (Pesguard PS 201) had also been found to be effective in suppressing natural populations of adult *Anopheles* (Itoh et al. 1988, Shono et al. 1991). Lambda-cyhalothrin was also found to give high mortality in *Anopheles quadrimaculatus* adults (Weathersbee et al. 1991). In rice-growing regions of eastern Arkansas, ULV adulticiding with pyrethroids, both aerial and ground applied, provided control measures against *Anopheles quadrimaculatus* (Groves et al. 1994). The effectiveness of *B.t.i.* in terms of ULV use has also been reported (Yates 1984, Sandoski et al. 1985, Lee et al. 1996).

The idea of combining both adulticidal and larvicidal insecticides in a single spray formulation was reported in an early work by Stevens and Stroud (1966), who found encouraging results against *Aedes stimulans* populations by combining Baytex™ for larvicidal properties with Baygon™ in order to achieve rapid adult knockdown by aerial ULV spraying. Itoh et al. (1988) demonstrated the need for using both adulticidal and larvicidal agents in combination to produce successful control measures against *Anopheles* mosquitoes. Tidwell et al. (1994) later tested the effectiveness of permethrin mixed with *B.t.i.* against *Aedes aegypti* and highlighted a possible potential for suppressing natural populations of the mosquito.

Resistance of natural *Anopheles maculatus* populations in Malaysia to malathion and permethrin has been documented (Rohani et al. 1995). Fox (1973, 1980), who reported on the failure to control *Aedes aegypti* through ULV spraying of malathion in Puerto Rico, listed population resistance as one possible reason. The fogging of diesel-based malathion also produces a foul-smelling odor, thick smoke, and oily residues, factors that caused the public to shy away from fully cooperating with authorities during spraying activities. Future ULV applications using a combination of pyrethroids and *B.t.i.* will need to rely on a greater understanding of a few factors, including the proper selection of insecticides, effective mixing proportions, and good spraying penetration with desirable wind conditions to assist in transportation of the active ingredients. Several studies have focused on the influence of spray droplet sizes on mosquito mortalities aside from the use of appropriate chemicals and dosages (Mount 1970, Curtis and Beidler 1996). Acceptance by the public of the choice of chemicals used will be decisive in enhancing public–government cooperation during control operations.

Tidwell et al. (1994) had also commented on the economic advantage of combining adulticidal and larvicidal spray in terms of manpower, since the separate efforts traditionally required to suppress larval population could be reduced. In cases of emergency control operations, this could prove crucial. The ease of preparation of the pyrethroid/*B.t.i.* mixture during our spraying trials was also a contributing factor in terms of manpower reduction. This present study has demonstrated the vast potential for the control of both adult and larval mosquitoes simultaneously through the spraying of a formulation combining both adulticidal and larvicidal agents.

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