Research Article

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Droplet size and efficacy of an adulticide–larvicide ultralow-volume formulation on *Aedes aegypti* using different solvents and spray application methods

Laura Harburguer,[a] Emilia Seccacini,[a] Susana Licastro,[a] Eduardo Zerba[a,b] and Héctor Masuh*[a,b]

Abstract

BACKGROUND: When cases of dengue are reported or the density of adult *Aedes aegypti* (L.) becomes too high, ultralow-volume (ULV) application of insecticides is the recommended control method. The droplet size of an aerosol insecticide influences its efficiency in killing adult mosquitoes. Many studies have been carried out to determine the optimum droplet size that maximises vector control efforts, but only a few have determined droplet-size spectra for specific equipment using different solvents and comparing thermal and non-thermal aerosols.

RESULTS: The present study showed that the droplet size for a water-based adulticide–larvicide formulation was larger than for the same formulation diluted in gasoil or biodiesel. No significant differences in adult mortality were observed between sprayers and solvents, but efficacy decreased with distance from the sprayer nozzle. Adult emergence inhibition was more than 90% when using water as a solvent for both thermal and cold foggers, and the efficacy did not decrease with distance from the sprayer nozzle. On the other hand, oil-based solvents became less effective with distance.

CONCLUSION: The use of water as a solvent with both thermal and cold foggers improves the efficacy of the studied formulation containing permethrin as adulticide and pyriproxyfen as larvicide in scaled-up assays. Moreover, it reduces the environmental impact and costs of spraying by comparison with formulations using oil solvents.

Keywords: droplet size; cold fogger; thermal fogger; water; oil solvents; *Aedes aegypti*

1 INTRODUCTION

Aedes aegypti (L.) is the main vector of dengue virus. Rigorous environmental sanitation and source reduction are used as control methods against *Ae. aegypti*, but these methods are neither routinely nor uniformly practised. During the mid-twentieth century, the health authorities of American countries, together with the Pan American Health Organisation (PAHO), carried out important *Ae. aegypti* eradication campaigns; eradication was achieved in Argentina in 1965.[1] However, by the end of the 1980s, the country was reinfested by the mosquito, and since 1997 it has suffered a series of small dengue epidemic outbreaks almost every year until 2009, when a large outbreak occurred involving more than 20 000 cases.[2]

When cases of dengue/DHF are actually reported, or when adult *Aedes* densities pose a potential risk of transmission, ultralow-volume (ULV) application of various adulticides is the recommended control method in the Americas.[3]

There are two main types of fogging machine: thermal foggers and cold foggers. Thermal fogging is a space treatment in which the fog is produced by a device that uses heat to break up the insecticide into very small droplets (5–30 microns in diameter) that disperse in the air. When the chemical formulation, generally diluted in oil-based carriers, is heated, it is vaporised in a combustion chamber and expelled to form a dense cloud. Applications should be carried out early in the morning before thermal convection currents lift the fog from ground level.[4] The active ingredient in cold foggers is mechanically broken up into small droplets by a special device that uses a high-pressure pump and a fine nozzle. The cold fogger can dispense formulations and generate the droplets precisely, but does not penetrate dense foliage or obstacles as well as thermal fogging does.

Droplet size influences the efficiency of an aerosol insecticide in killing adult mosquitoes, mainly because droplet movement in the environment and impingement on mosquitoes depend on size.[5] In addition to a decrease in insecticidal efficacy, inadequate

* Correspondence to: Héctor Masuh, Centro de Investigaciones de Plagas e Insecticidas, CIPEIN (CITEFA/CONICET), JB de La Salle 4397, (1603) Villa Martelli, Buenos Aires, Argentina. E-mail: hmasuh@citedef.gob.ar

a Centro de Investigaciones de Plagas e Insecticidas (CIPEIN-CITEFA/CONICET), Villa Martelli, Buenos Aires, Argentina

b Instituto de Investigaciones e Ingeniería Ambiental (3IA), Universidad Nacional de San Martín, San Martín, Buenos Aires, Argentina

droplet size may cause an unnecessary contamination of the environment, especially when using oil solvents. Many studies have aimed to determine optimum droplet size to maximise vector control efforts,[6–8] but few have detailed specific droplet-size spectra for determined equipment using different solvents, or compared thermal and non-thermal aerosols for controlling mosquitoes.[9,10]

Simultaneous control of adult and larval stages is ideal in mosquito vector control programmes in order to reduce the overall vector mosquito population and subsequently decrease or disrupt the transmission of diseases. Outdoor ULV spray tests using a mixture of chemical adulticides with larvicides[11,12] have recently been carried out. A ULV formulation containing permethrin as an adulticide and pyriproxyfen as a larvicide recently developed in the present authors' laboratory yielded excellent results in an initial field trial.[13] The present study was designed to measure droplet size and evaluate the efficacy of this larvicide–adulticide ULV formulation using three different solvents (water, biodiesel and gasoil) and two different methods of application (a thermal and a cold fogger) in scaled-up assays.

2 MATERIALS AND METHODS

2.1 Biological material

An insecticide-susceptible CIPEIN strain of *Ae. aegypti* originating from the Rockefeller strain in Venezuela and maintained in the authors' laboratory since 1996 was used. The strain was reared as described in previous reports.[14] Adults between 2 and 3 days old and late third- or early fourth-instar *Ae. aegypti* larvae of both sexes were used for the study.

2.2 Insecticide formulation and equipment

A mixture of permethrin 15% (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-(dimethyl cyclopropane carboxylate) and pyriproxyfen 3% {2-[1-methyl-2-(4-phenoxyphhenoxy)ethoxy]pyridine} used as an emulsifiable concentrate (EC) was formulated by Chemotecnica SA (Argentina).

Polyethylene glycol 1000 (Química Oeste) was used as an antievaporant for ULV treatments. Water, gasoil and biodiesel (soybean oil) (Cocoil SA) were used as solvents for the EC.

A Swingtec Swingfog® SN 50 (Swingtec GmbH, Isny, Germany) thermal fogger was used. The SN 50 is a handheld machine with a net weight of 9 kg, designed to disperse oil- and water-based chemicals. The cold fogger, also a handheld machine, was a portable Swingtec (formerly known as Motan) Starlet (Swingtec GmbH, Isny, Germany) with a net weight of 12 kg, which can be used with oil- or water-based chemicals.

The SN 50 discharge rate using a No. 1 nozzle was 20.5 L h^{-1}; water, gasoil and biodiesel were used as solvents. The Starlet discharge rate using a No. 68 nozzle was 3 L h^{-1}, and only water was used as a solvent.

2.3 Droplet size

Droplet size was measured with a hot-wire anemometer. This instrument uses a hot-wire probe as the sensing element for counting and sizing droplets. Each droplet that contacts the probe cools a length of wire proportional to the droplet's diameter and thus reduces the probe's electrical resistance by an amount proportional to the size of the droplet. The system was developed by KLD Industries (Huntington Station, NY). A KLD Industries model DC-III system was used, and measurements were taken according to the standard operating procedures provided with the unit. The probe was located 3 m in front of the machine nozzle, with a wind speed of 1.5–2.5 m s^{-1}. Four independent replications were conducted for each combination of sprayer and solvent.

DC-III software for Windows 2000/XP was used to compute the volume median diameter (VMD), $D_{V0.5}$, as well as diameters $D_{V0.1}$ and $D_{V0.9}$. The $D_{V0.5}$ is the droplet diameter (μm) where 50% of the spray volume is contained in droplets smaller than this value. Similarly, $D_{V0.1}$ and $D_{V0.9}$ are the diameters at which 10% and 90% of the spray volume are contained in droplets of similar size or less. The percentage of spray volume contained in droplets less than 20 μm (%Vol <20 μm) was calculated for all tests. This term allows the user of the equipment or solvent to determine the amount of material that will most likely remain aloft after an application and potentially impinge on flying insects. This software was also used to record the number of droplets greater than 32 μm; these are interesting data because most insecticide providers recommend that users comply with droplet sizes between 8 and 30 μm.[15]

2.4 Fogging operations and assessment of bioefficiency

Trials were carried out in a large shed, 15 m × 4.7 m × 3.2 m (225 m^3 volume), which had some openings near the ceiling. The recommended dose is 700 mL of EC in 5 L of solvent, using 250 mL of the diluted formulation per 1000 m^3. A solution 10 times more diluted (70 mL in 5 L) was used to provide a low discriminating dose for measuring the effects of the machines and solvents. Owing to differences in nozzle size, and hence differences in flow, the SN50 fogger was turned on for 1 min and the Starlet fogger for 7 min. When water was used as solvent, 5% of polyethylene glycol 1000 was added to the mixture.

Cylindrical screened sentinel cages built with 18-mesh nylon, 15 cm long × 3 cm in diameter, were used to assess fumigation efficacy according to WHO protocols with minor modifications.[16] Sixteen adults (50% each sex), between 1 and 3 days old and fed on raisins, were transferred to the cages suspended by a rope 1.5 m above ground level and were placed at 3, 6 and 9 m from the spraying machine. In addition, 500 mL plastic jars (7.5 cm in diameter) containing 15 late third- or early fourth-instar larvae and 250 mL tap water were placed on the ground at the same distances. After spraying, the mosquitoes were kept in the shed for 1 h, and then both adults and larvae were taken to the laboratory and maintained at 26 ± 2 °C under a 12 : 12 h photoperiod. Adult mortality was assessed after 24 h, and the plastic jars were inspected daily until death or adult emergence of all the individuals to determine adult emergence inhibition (EI). EI was calculated as shown below and adjusted for larval or pupal mortalities in the corresponding controls according to Mulla *et al.*:[17]

$$\mathrm{EI}\ (\%) = 100 - 100(T/C)$$

where T is the percentage of emergence in treated containers and C is the percentage of emergence in control containers.

Three replicates were conducted for each combination of sprayer and solvent. In each test, a cage with adults and a jar with larvae were kept outside the shed as controls; the entire assay was discarded if control mortality exceeded 15%.

2.5 Statistical analysis

Droplet size data were analysed using a one-way analysis of variance (ANOVA), with an accepted level of significance for all comparisons of $P < 0.05$ (Statistica, 1995).[18]

Table 1. Spray droplet spectra data of permethrin and pyriproxyfen aerosol EC dispersed from portable generators [Swingtec (Motan) Starlet and SN 50] using different solvents

Sprayer	Solvent	Droplet data[a,b,c]				
		$D_{V0.1}$ (μm ± SD)	$D_{V0.5}$ (μm ± SD)	$D_{V0.9}$ (μm ± SD)	%Vol <20 μm	% <32 μm
Swingtec (Motan) Starlet	Water	7.0 ± 0.8 a	23.8 ± 2.5 a	44.3 ± 2.8 a	38.8 ± 4.8 a	99.0 ± 0.4 a
Swingtec SN 50	Water	6.5 ± 3.0 a	25.1 ± 2.9 a	37.7 ± 9.3 a	30.8 ± 6.9 a	99.2 ± 0.6 a
Swingtec SN 50	Gasoil	2.0 ± 0.6 b	11.8 ± 2.3 b	18.9 ± 4.4 b	81.2 ± 22.1 b	100 a
Swingtec SN 50	Biodiesel	3.2 ± 0.8 b	13.3 ± 4.1 b	19.4 ± 4.4 b	81.4 ± 13.9 b	100 a

[a] Data are the mean of four replicates.
[b] $D_{V0.1}$, $D_{V0.5}$ and $D_{V0.9}$ are the droplet diameters (μm) when 10, 50 and 90%, respectively, of the spray volume is contained in droplets smaller than this value; %Vol <20 μm is the percentage of spray volume contained in droplets of <20 μm.
[c] Diameters followed by the same letter within the same column are not significantly different (ANOVA, $P < 0.050$).

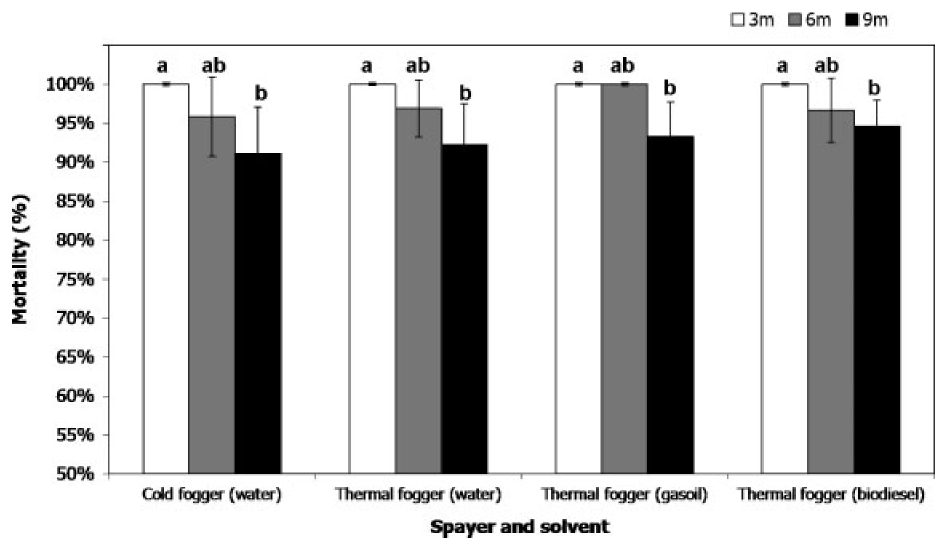

Figure 1. Percentage mortality (± ES) of caged adult *Ae. aegypti* exposed to thermal or cold aerosols of permethrin and pyriproxyfen using water, gasoil or biodiesel as solvent. Cages were placed 3, 6 and 9 m from the fogger nozzle, 1.5 m above ground level. Treatments with the same letter were not significantly different ($P > 0.05$) in Duncan's multiple range test.

Adult mortality, 48 h larval mortality and EI were corrected with Abbott's formula[19] and subjected to an arcsine square root transformation before the analyses. Adult mortality data were compared using two-way analysis of variance (ANOVA); differences between means were compared using Duncan's multiple range test. The accepted level of significance for all comparisons was $P < 0.05$ (Statistica, 1995).[18]

Data on 48 h larval mortality and EI were analysed individually because the authors wanted to compare the efficacy between sprayers and solvents at different distances and not for each machine. A two-way analysis of variance (ANOVA) was used where the factors were distance (3, 6 and 9 m) and the sprayer/solvent combination (thermal/cold, water/gasoil/biodiesel).

3 RESULTS AND DISCUSSION

3.1 Droplet size

Table 1 shows the results of the sprayer droplet spectra. There were differences in droplet size spectra between treatments in $D_{V0.1}$ ($F = 9.2$; df = 12; $P < 0.01$), $D_{V0.5}$ ($F = 20.6$; df = 12; $P < 0.001$), $D_{V0.9}$ ($F = 19.9$; df = 12; $P < 0.001$) and %Vol <20 μm ($F = 15.6$; df = 12; $P < 0.001$). As can be seen, there was a significant difference between water-based and oil-based sprays (Duncan's multiple range test, $P < 0.05$). Droplet size for water-based EC was larger than for sprays diluted in gasoil or biodiesel. However, there were no significant differences in droplet size between cold and thermal foggers when water was used as solvent. The percentage of spray volume contained in droplets of <20 μm was more than 80% for oil-based formulations, a value considerably greater than that of the water-based formulation of approximately 40%. Therefore, most of the spray volume of oil formulations is contained in droplets smaller than 20 μm, which implies that these sprays will most likely remain airborne after an application instead of falling to the ground. Moreover, as 99% of the droplets were smaller than 32 μm for all the solvents used, the spraying can be considered as ULV.

Several studies[5,8,20] have determined optimum droplet size for adult mosquito control. Haile *et al.*[21] found that the optimum droplet diameter was between 10 and 15 μm, although little difference in efficacy on adults using malathion was indicated for sizes between 7 and 25 μm.

The droplet size results obtained were in accordance with the results of Hoffmann *et al.*,[15] where $D_{V0.5}$ values for sprays diluted in diesel were usually smaller than for sprays diluted in water and %Vol <20 μm ranged between 12 and 100% for diesel-diluted sprays and only reached 30% for water-diluted sprays.

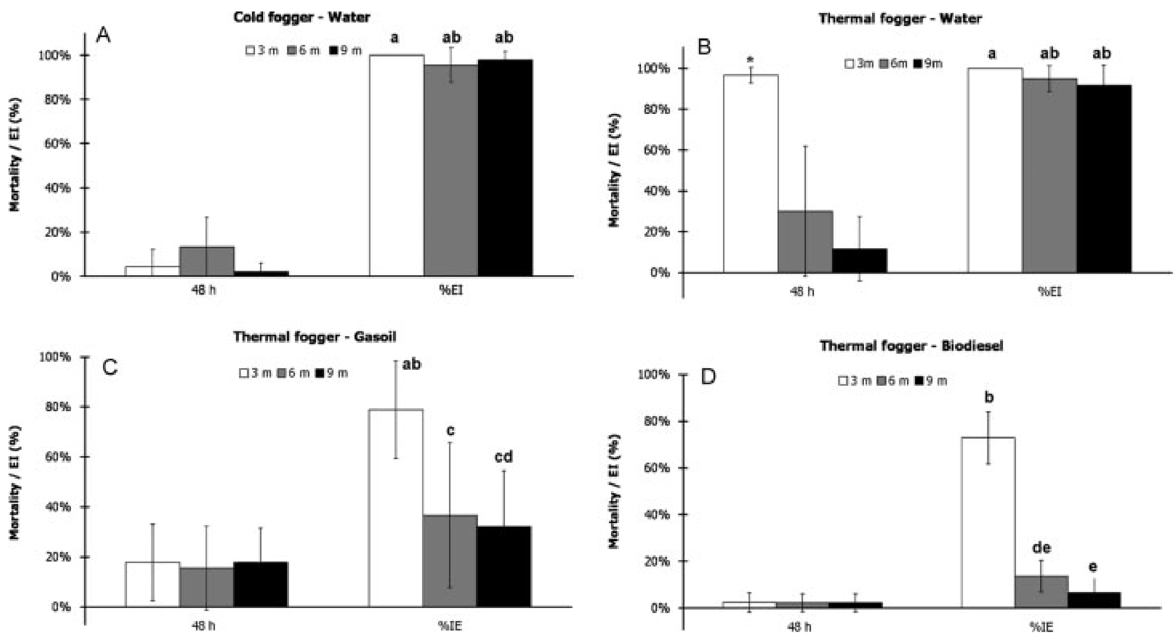

Figure 2. Adult emergence inhibition (%) and larval mortality (%) 48 h after treatment with thermal or cold aerosols of permethrin and pyriproxyfen using water, gasoil or biodiesel as solvents in late third-/early fourth-instar larvae. Plastic 500 mL jars with 250 mL of tap water were placed on the ground at different distances (3, 6 and 9 m) from the fogger nozzle. Treatments with the same letter were not significantly different ($P > 0.05$) in Duncan's multiple range test for EI. The asterisk (*) denotes significant difference ($P < 0.05$) from the other treatments for 48 h larval mortality (Duncan's multiple range test).

3.2 Adult mortality

Mortality of caged *Ae. aegypti* adults showed no significant differences between sprayers and solvents ($F = 0.14$; df $= 27$; $P > 0.05$) but significant differences between distances ($F = 4.3$; df $= 27$; $P = 0.024$). Interaction between these factors was also non-significant ($F = 0.12$; df $= 27$; $P > 0.05$). Duncan's multiple range test for distance showed that there were differences in adult mortality between the cages located at 3 and 9 m, but not between the cages at 6 m and the other two distances tested (Fig. 1). Adult mortality at 3 m was 100% and decreased to values of approximately 90% at 9 m.

In spite of the differences in $D_{V0.5}$ and %Vol <20 µm for water-based and oil-based formulations, there were no differences in the mortality of caged adults. These results are consistent with the work of Yap *et al.*,[22] who compared the efficacy of a Pesguard® FG 161 formulation using a thermal fogger and water and diesel as solvents and found 100% adult mortality at 24 h with both solvents. On the other hand, Chung *et al.*,[23] using a formulation containing Actellic® 50 EC plus Vectobac® 12AS with a thermal fogger and water as solvent, found that the efficacy on adults decreased with distance, obtaining an adult mortality of 100% at 3 m and less than 30% at 9 m. However, the difference with the results obtained in this study may be due to differences in droplet size, as the $D_{V0.5}$ in the work of Chung *et al.*[23] was 57 µm for water-based solvents, whereas here it is around 25 µm. This means that the larger droplets fall onto the ground within a few metres of the sprayer nozzle without impinging on the mosquitoes at distances greater than 3 m; on the other hand, droplets reaching larger distances do not have the required dose of pesticide to produce significant mortality. This was not the case with the machines and solvents tested in this study. Although efficacy decreased with distance, adult mortality was still higher than 90% 9 m away from the sprayer nozzle.

3.3 Larval mortality

Larval mortality (%) 48 h after treatment showed significant differences among sprayers and solvents ($F = 12{,}9$; df $= 27$; $P < 0.05$) and between distances ($F = 4{,}33$; df $= 27$; $P < 0.05$). The interaction between these factors was also significant ($F = 5.87$; df $= 27$; $P > 0.05$).

As shown in Fig. 2, when the thermal fogger was used with water as a solvent, larval mortality at 48 h was nearly 100% at a distance of 3 m. This value is significantly different ($P < 0.05$, Duncan's multiple range test) from the other treatments at 48 h, where mortality values were less than 30% and there were no differences between distances and sprayers when using gasoil and biodiesel as solvents or when using the cold fogger.

Figure 2 also shows that EI was more than 90% when water was used as a solvent for both thermal and cold foggers. Moreover, efficacy did not decrease with distance from the sprayer nozzle. On the other hand, the efficacy of oil-based solvents decreased with distance and ranged from 80% EI at 3 m to less than 20% at 9 m. This decrease was greater when using biodiesel instead of gasoil. In fact, when comparing all treatments at a distance of 3 m, all of them were equally effective, with the exception of the EI for biodiesel, which was significantly lower, although still comparable with that of gas oil (Fig. 2).

Differences between larval mortality after 48 h and EI show that, at the doses used in this work (10 times lower than the recommended doses), permethrin does not produce significant larval mortality, except in the case of the thermal fogger using water as a solvent, and therefore the EI was produced by pyriproxyfen.

The difference in efficacy on larvae at 48 h between the cold and thermal sprayers when water was used as solvent could be determined by the difference in droplet size. While not statistically significant, the difference was noteworthy on its effectiveness. As $D_{V0.5}$ was slightly higher for the thermal fogger, the larger droplets may contain more insecticide, causing larval mortality as they fall into the jars at a short distance.

The larvicidal effect of pyriproxyfen (measured as EI) decreases with distance for oil-based solvents but not for aqueous solvents. These results are in accordance with previous results by Yap *et al.*,[22] where a water-based thermal fogging formulation of Pesguard® FG 16l seemed to achieve a significantly better larvicidal effect than a diesel-based spray. This could be due to the difference in droplet size between the two types of solvent found in this work, where oil-based solvents had a significantly lower $D_{V0.5}$. As the $D_{V0.5}$ is low, the droplets remain suspended for longer and are less likely to fall into the jars with larvae. However, by remaining suspended, they are just as effective on *Ae. aegypti* adults as aqueous solvents. This explanation agrees with the results of Mount *et al.*,[24] who found a much lower mortality in caged *Ae. taeniorhynchus* at ground level (14%) than at 1.5 m above the ground using fuel oil as solvent and fenthion or naled as insecticide. Similarly, using ULV applications of ground aerosols of synergised pyrethrins and resmethrin against caged *Cx. quinquefasciatus*, Womeldorf and Mount[25] obtained a higher mortality of mosquitoes in cages placed 1.5 m above ground level than in others placed at 0.15 m. Only Chung *et al.*[23] used jars with larvae to assess the larvicidal efficacy of a formulation containing *Bti* and pirimiphos-methyl applied with a thermal fogger and water as solvent. The jars were placed on the floor and, as observed in this study, efficacy decreased with distance. However, contrary to the results of the present study when using oil solvents, the loss of effectiveness in the work by Chung *et al.*[23] was due to the large droplet size (57 µm) limiting the distance the insecticide could reach.

Finally, the lower larvicidal effect observed when using oil solvents could also be due to a better settlement of the spraying droplets on exposed water surfaces in the water-based spray formulation compared with the oil-based sprays, as suggested by Yap *et al.*[22]

4 CONCLUSIONS

Droplet size is a crucial factor modulating the trajectory of aerosols generated by thermal or cold foggers. Although significant differences in droplet size were observed between water and oil solvents, they were all equally effective on *Ae. aegypti* adults. However, this was not the case with larvae, where sprays using water as a solvent were significantly more effective than the oil-based formulations.

These results show that a water-based formulation is more effective than an oil-based formulation (gasoil or biodiesel). Therefore, the use of water as solvent, with both thermal or cold foggers, not only improves the efficacy of the formulation containing permethrin and pyriproxyfen but also reduces the environmental impact and costs of spraying compared with the use of oil solvents. Further research on the performance of this formulation, using different solvents and sprayers in field conditions and at the recommended dose, is needed to determine its specific efficacy on *Ae. aegypti* adults and larvae.

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