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Laboratory and scaled up evaluation of *cis*-permethrin applied as a new ultra low volume formulation against *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Ultra low volume (ULV) aerial spraying is a common methodology for spatial treatments for *Aedes aegypti* (L.) control. Previous studies from our laboratory indicated that the *cis* isomer of permethrin has an excellent efficacy for vector control, in particular for *Triatoma infestans*. It was of interest to determine the efficacy of this pure isomer in a ULV water-based formulation for adult *A. aegypti* control, since both vectors usually are found together in South America.

A method is presented to make a quick measure at laboratory level of the knock down effect (KT_{50}) of a new EC water-based formulation of *cis*-permethrin, in a small Peet Grady chamber of 0.34 m^3, with a specially designed glass sprayer. A deltamethrin standard ULV formulation "CISLIN" was used for comparison.

cis-Permethrin showed a significantly lower KT_{50} value than deltamethrin (7.50 and 9.65 min, respectively). When the mosquitoes were introduced into the chamber 10 min after spraying KT_{50} values were almost equal (15.59 and 15.88 min, respectively).

In scaled up bioassays the ULV formulation of *cis*-permethrin showed 100% mortality of *A. aegypti* adult mosquitoes at the beginning of the treatment ($t = 0$) and some mortality at 1 h post treatment of freshly introduced mosquitoes.

It was concluded that *cis*-permethrin could be an excellent tool for control of *A. aegypti* and other vectors of medical importance, with fewer side effects and better cost-effectiveness than with the cyanopyrethroids.
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1. Introduction

Dengue is an arboviral disease, which includes dengue fever (DF) and dengue hemorrhagic fever (DHF). This disease is the most important vector borne urban viral disease, the vector being *Aedes aegypti* (L.), which is widely spread in tropical and subtropical countries of America. After erradication from most countries in America in the 1950s, during the second half of the 20th century the distribution and density of *A. aegypti* have expanded dramatically, beginning in large cities and then spreading to the countryside (WHO, 2000).

In the absence of a dengue vaccine, control of dengue vectors is regarded as essential as epidemics involving several million people occur annually in tropical areas (Gubler and Kuno, 1997) and emergency measures must be available for use in dengue outbreaks.

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The lack of piped drinking water and the great increase in availability of plastic has given rise to a large number of small containers with water, in and around households, which gave rise to larval breeding habitats.

Pant and Yasuno (1970) demonstrated that 95% of *A. aegypti* rest indoors, and of these, >90% do so on surfaces that could not be sprayed with residual compounds. Therefore the intervention commonly used during epidemics was and still is, the ground application of small quantities of an aerosol insecticide in gas oil or kerosene as carrier (ultra low volume (ULV)) (Chadee, 1985; Perich et al., 1992) but unfortunately many of the campaigns to reduce vector populations have not been successful due to practical problems associated with the treatments of densely populated areas, lack of funds and failure to sustain implementation of control programmes (Matthews, 1996) as well as irritation problems in the inhabitants associated with the way compounds were applied.

The alpha-cyano pyrethroid derivatives with a CN group in the *S*-configuration of the 3-phenoxybenzylalcohol (deltamethrin, cypermethrin, cyhalothrin, fenvalerate, etc.) have about three to six-fold higher insecticidal potency than the non-cyano derivatives such as permethrin and bioalletrhin. However, in humans, dermal exposure to synthetic pyrethroids may cause transient itching and burning sensations (paraesthesia) in the face, irritation of eyes and allergic reactions as well coughing, dispnea, sneezing and increased nasal secretion, more evident with alpha-cyano than non-alpha-cyano compounds. Symptoms develop from 30 min to 8 h after exposure and the occurrence and severity has been related to the degree of exposure and repetition of exposure. All synthetic pyrethroids may be allergens, which can cause slight to moderate contact sensitization in guinea pigs, and correlates with the data from epidemiological studies in exposed workers. There is evidence that synthetic pyrethroids containing an alpha-cyano group are more potent in eliciting neurotoxic effects, in comparison to pyrethroids that do not contain an alpha-cyano group. In all the cases studied non-alpha-cyano pyrethroids showed fewer side effects than alpha-cyano pyrethroids (Flannigan et al., 1985; Knox et al., 1984; Pauluhn, 1999).

Cyanopyrethroids such as deltamethrin, cypermethrin or lamdacyhalothrin are the main insecticides used in vector control campaigns. For mosquito control ULV formulations were generally used with gas oil or kerosene as carrier. Organic solvents and surfactants in commercial products increase the dermal penetration and irritation due to pyrethroids, especially for the alpha-cyano group (Kaloyama and El Batavi, 1991). Furthermore, it is known that xylenes cause eye and skin irritation, headaches, nausea, confusion, tremors and anxiety in exposed humans.

The new formulation “DEPE”, a water-based formulation, with a *cis*:*trans* ratio of permethrin of 95:5, do not produce eye or skin irritancy (Material Safety Data Sheet, in Spanish, Chemotecnica, S.A.). In contrast, the deltamethrin formulation “CISLIN” is stated to be an eye and skin irritant (Material Safety Data Sheet, CISLIN Residual Insecticide, October 2002). Both formulations were used at the recomended field dosis with water as carrier.

Previous studies in our laboratory indicated the high effectiveness of permethrin for control of *A. aegypti* (Masuh, 1998) and pure *cis*-permethrin was shown to be more effective than *trans*-permethrin for controlling other vectors such as *Triatoma infestans* (Alzogaray et al., 1998). Since these two vectors usually are found together in America it was of interest to study the insecticidal action of *cis*-permethrin as a ULV water-based formulation for *A. aegypti* control by laboratory and scaled up tests. A new EC formulation of *cis*-permethrin could be a useful tool, for vector control programmes with fewer side effects than currently used chemicals.

2. Material and methods

2.1. Biological material

An insecticide susceptible strain CIPEIN of *A. aegypti* (L.) was used. This originated from the Rockefeller strain from Venezuela and has been kept in the laboratory since 1996.

Eggs were laid on a wet filter paper and kept on the wet paper for 48 h. The eggs were dehydrated at ambient temperature and stored for at least 30 days. To rehydrate the eggs they were put in dechlorinated water (500 eggs per 2 l water) at $25 \pm 2\,^{\circ}C$. After 24 h first instar larvae were observed. They were maintained in the same conditions until adults emerged. For the study adults of both sexes, 2–3-days old were used.

2.2. Insecticides

cis-Permethrin (EC) containing 10% a.i. by volume and a *cis*:*trans* ratio of 95:5 was provided by Chemotecnica, S.A. (Argentina) under the trade name “DEPE”.

Deltamethrin (EC) containing 1.5% a.i. “CISLIN” was provided by Aventis (Germany).

2.3. Spray solutions

(a) Laboratory assays: 1.0 ± 0.1 ml insecticide solutions were used for spraying. DEPE (0.85 ml) or CISLIN (0.6 ml) diluted with 50 ml water gave a final concentration of 5 mg a.i. per cubic meter in air for *cis*-permethrin and 0.525 mg a.i. per cubic meter in air for deltamethrin.
(b) Scaled up assays: 25 ml DEPE was mixed with 42 ml polyethylenglycol 400 and 700 ml of water and put into the tank of the spraying machine. For CISLIN 17 ml were used.

2.4. Bioassays

(a) Laboratory scale: a Peet Grady glass chamber with a volume of 0.34 m^3 (70 cm × 70 cm × 70 cm) was used. In the front panel there are four holes, each 5 cm in diameter and a larger one (15 cm diameter) on the bottom of the panel to introduce the mosquitoes. On the upper face an air exhaust fan was installed to ventilate the chamber. During the assays all the holes were hermetically sealed.

Adult *A. aegypti* were liberated into the chamber, allowed to acclimatize for 2 min and then sprayed with 1 ± 0.1 ml insecticide solution through one of the upper holes using a calibrated, specially designed glass device using N_2 pressure (3.5–3.8 psi).

The number of knocked down mosquitoes was recorded at intervals until all the insects were knocked down. Control assays were performed in identical conditions but spraying with 1 ml water and keeping insects in the chamber for 25 min. Averages from six replicates were calculated.

In the second assay the mosquitoes were introduced into the chamber 10 min after the spraying. The number of knocked down mosquitoes were recorded in the same way as before. Three independant replicates were performed.

Knock down time 50 (KT_{50}) was computed using a programme based on the probit method.

(b) Scaled up assays: a large shed of 20 m × 6 m × 3.5 m (420 m^3 volume) with some openings near the ceiling was used.

Adult *A. aegypti*, in a netting cage were placed on stands 1.5 m from the floor and at 3, 6, 9 and 12 m from the spraying machine. Cages with adult mosquitoes were placed on the stands at the time of spraying and fresh ones were introduced 1 h after. Cages with control mosquitoes were held outside the shed.

A portable Motan "Starlet" electric ULV generator with a no. 68 (3 l/h) nozzle and solutions of *cis*-permethrin (DEPE) or deltamethrin (CISLIN) were used. The machine was turned on for 7 min and mosquitoes were held in the shed for 1 h. Cages with adult mosquitoes were taken to the laboratory, maintained at $28 \pm 2\,^{\circ}C$ and photoperiod 12-h light:12-h dark and mortality assesed after 24 h.

3. Results and discussion

cis-Permethrin and deltamethrin formulations were applied using the recommended field dose for ULV spraying. The knock time (KT_{50}) is the exposure time which produces 50% knock down of treated insects under controlled conditions in a test chamber. It is related to the insecticide efficacy of aerial treatments. The lower its value, the higher the insecticidal efficacy.

Data in Table 1 shows good reproducibility of the results from replicate knockdown spraying, and that immediately after the insecticide was applied *cis*-permethrin was somewhat more effective than deltamethrin against adult *A. aegypti*. When adult mosquitoes were introduced into the chamber 10 min after ULV spraying (Table 2) KT_{50} values for both insecticides were considerably higher than in Table 1, indicating that *cis*-permethrin droplets remain suspended in the air for a shorter time than deltamethrin ones.

The results of the assays in the shed are shown in Table 3. There was 100% mortality with exposure at the time of spraying. Mortality was reduced with exposure 1 h after spraying, with a similar pattern for both insecticides.

Thus, this new water-based ULV formulation of *cis*-permethrin was as effective as deltamethrin but it is less likely to be an irritant to exposed people.

Table 1
KT_{50} values (with range between replicates) from laboratory tests of ULV pyrethroids against *Aedes aegypti* present at the time of spraying

Assay	KT_{50} (min)	
	DEPE	CISLIN
1	7.31 (6.61–7.91)	9.26 (8.77–9.80)
2	7.02 (6.44–7.58)	10.78 (10.20–11.45)
3	7.51 (6.90–8.14)	9.47 (8.96–10.01)
4	7.62 (7.06–8.16)	10.57 (10.00–11.19)
5	7.76 (7.08–8.47)	9.48 (8.73–10.37)
6	7.84 (7.23–8.43)	9.14 (8.50–9.76)
7	7.83 (7.38–8.27)	–
Average	7.50 (7.29–7.71)	9.65 (9.40–9.91)

Table 2
KT_{50} of pyrethroids (with range between replicates) on *Aedes aegypti* introduced 10 min after spraying

Formulation	KT_{50} (min)	KT_{95} (min)
DEPE (5 mg a.i./m^3)	14.14 (13.52–14.83)	23.98 (22.24–26.26)
	16.20 (15.21–17.16)	22.83 (21.40–24.60)
	16.43 (15.78–17.16)	27.74 (25.84–30.20)
CISLIN (0.525 mg a.i./m^3)	15.70 (15.15–16.29)	23.38 (22.01–25.20)
	16.54 (15.80–17.40)	27.03 (24.36–31.57)
	15.41 (15.10–15.75)	26.58 (24.13–29.18)

Table 3
Mortality of adult *Aedes aegypti* in scaled up assays

	Distance from spayer (m)	Percentage mortality	
		Exposure at time of spraying	Exposure 1 h after spraying
DEPE (5 mg/m^3)	3	100	51.70 (13.72–89.68)
	6	100	15.47 (2.70–28.23)
	9	100	38.20 (12.69–63.75)
	12	100	36.63 (5.21–78.48)
CISLIN (0.525 mg/m^3)	3	100	42.37 (36.13–48.60)
	6	100	29.50 (15.21–43.78)
	9	100	13.23 (11.06–15.40)
	12	100	7.1 (4.88–9.32)

The results are the mean of three replicates (with range between replicates in parentheses).

The high effectiveness of this new formulation of *cis*-permethrin found against *A. aegypti* as well as for *T. infestans*, could be useful to National Vector Control Programmes for house spraying.

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References

Alzogaray, R., Picollo, M.I., Zerba, E., 1998. Independent and joint action of *cis*- and *trans*-permethrin on *Triatoma infestans*. Arch. Insect Biochem. Physiol. 37, 225–230.

Chadee, D.D., 1985. An evaluation of malathion ULV spraying against caged and natural populations of *Aedes aegypti* in Trinidad, West Indies. Cahiers ORSTOM Ser. Entomol. Med. Parasitol. 23, 71–74.

Flannigan, S.A., Tucker, S.B., Key, M.M., Ross, C.E., Fairchild, E.J., Grimes, B.A., Harrist, R.B., 1985. Synthetic pyrethroid insecticides: a dermatological evaluation. Br. J. Ind. Med. 42, 363–372.

Gubler, D.J., Kuno, G., 1997. Dengue and Dengue Hemorrhagic Fever. CAB, Wallingford, UK.

Kaloyama, F., El Batavi, M., 1991. Synthetic Pyrethroids in Human Toxicology of Pesticides. CRC Press, Boca Raton, USA, 105 pp. (chapter 5).

Knox, J.M., Tucker, S.B., Flanningan, S.A., 1984. Paresthesis from cutaneous exposure to a synthetic pyrethroid insecticide. Arch. Dermatol. 120, 744–746.

Masuh, H., 1998. Stereoselective Separation and Insecticidal Activity of Pyrethroid Compounds (in Spanish), Ph.D. Thesis, University of Buenos Aires, Argentina.

Matthews, G.A., 1996. Application of Insecticides in Dengue Control. Pest. Outlook, February 25–30.

Pant, C.P., Yasuno, M., 1970. Indoor resting sites of *Aedes aegypti* in Bangkok, Thailand. WHO/VBC/70.235.

Pauluhn, J., 1999. Hazard identification and risk assessment of pyrethroids in the indoor environment. Toxicol. Lett. 107, 193–199.

Perich, M.J., Davila, G., Turner, A., García, A., Nelson, M., 1992. Behavior of resting *Aedes aegypti* (Culicidae: Diptera) and its relation to ultra-low volume adulticide efficacy in Panamá city. Panamá J. Med. Entomol. 37 (4), 541–546.

WHO, 2000. Recommendations of the Scientific Working Group on Dengue. UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), pp. 3–8.