

# INSECTICIDE EFFICACY OF SPATIAL REPELLENT COMPOUND-METOFLUTHRIN AGAINST SUSCEPTIBLE AND RESISTANT STRAINS OF *Aedes aegypti*

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## ABSTRACT

Spatial repellents (SR), include pyrethroid insecticides that are highly volatile at low temperatures and with high lethal activities against mosquitoes, mainly *Aedes* vectors of arboviral diseases. Of these SR, metofluthrin is widely used in various devices for repellent consumer products. This article reports the susceptibility status of *Ae. aegypti* Puerto Rico permethrin-resistant laboratory strain (PR) and Orlando susceptible laboratory strain (ORL) to metofluthrin and permethrin using the CDC glass bottle bioassay. The time-mortality relationships showed that the permethrin-resistant PR strain is highly resistant to both permethrin and metofluthrin compared to the susceptible ORL strain. The resistant ratio (RR) based on the killing time (KT) ( $KT_{50-PR}/KT_{50-ORL}$ ) was 30- and 5- folds for permethrin and metofluthrin, respectively. The results also showed that the PR strain is less resistance to metofluthrin than to permethrin, with a three-fold RR ( $KT_{50-PR-per}/KT_{50-PR-met}$ ). These results indicate the potential risk of developing cross-resistance of metofluthrin in permethrin-resistant mosquitoes. Integrated vector management in mosquito control should be considerate of how consumer products and field operations interact to accelerate cross resistance to pyrethroids.

Key Words: *Aedes aegypti*, resistance, metofluthrin, permethrin, spatial repellents

## INTRODUCTION

*Aedes aegypti* (Linn.) is a major vector of arboviral disease causing pathogens, such as yellow fever, dengue, zika, and chikungunya viruses. Application of repellents is one technique for prevention and control of mosquitoes and mosquito-borne diseases. Spatial repellents (SR) include a class of volatile pyrethroids, such as transfluthrin and metofluthrin, which have high insecticidal activities (Bibbs et al. 2018), high volatility at low temperatures, and low mammalian toxicity (Sugano and Ishiwatari 2012). These features facilitated their use in different commercial mosquito repellent devices (coils,

liquid, cold fan vaporizers, paper and resins-based emanators) against various mosquito vectors, mainly, *Ae. aegypti* and *Ae. albopictus* Skuse (Ujihara et al. 2004, Lucas et al. 2007, Sugano and Ishiwatari 2012). Paper-based emanators / devices of metofluthrin were highly effective against *Aedes* and *Ochlerotatus* species with up to 90% reduction in host-landing rate (Lucas et al. 2007, Xue et al. 2012). In experimental rooms, metofluthrin resin-based emanators completely inhibited host-seeking and host-biting behaviors of *Ae. aegypti* laboratory colony, and caused rapid knockdown (KD) and mortality (90% in <1h hr). Using the resin emanators, repellent efficacy lasted for 20 days, which indi-

cates their possible use to replace insecticidal indoor residual spraying (Ritchie and Devine 2013). The repellent activity of the metofluthrin-based OFF! Clip-on device was tested in semi-field conditions at different distances, and the results showed that metofluthrin was highly effective (up to 100% KD and mortality) against *Ae. aegypti* when exposed for 60 min at 0.3 m from the device (Bibbs and Xue 2015). In glass chamber experiments on different mosquito pyrethroid-based coils in Malaysia, metofluthrin was the most effective against *Ae. albopictus* among the compared pyrethroids in regards to KD and killing time (KT). There was considerable variation in mortalities (5-100%) and  $KT_{50}$  (2.5-17 min), suggesting the presence of cross-resistance between the tested pyrethroids (Chen et al. 2018). Metofluthrin impregnated nets inconsistently repelled *Ae. aegypti* and *Anopheles dirus* in Thailand based on spatial activity indices measured from sentinel and cone bioassays (Ponlawat et al. 2017). Metofluthrin emanators were moderately effective in reducing the landing rate and KD levels of *Ae. aegypti* (Darbro et al. 2017). Similarly, in Cambodia, slow-release metofluthrin emanators caused variable (47-67%) reduction of landing rates of *Anopheles* species collected in tents or by CDC traps deployed in test premises (Charlwood et al. 2016). Metofluthrin affects host-seeking and biting behavior through a cascade of sequential events of agitation, confusion and knockdown of the target mosquitoes (Buhagiar et al. 2017a). However, prolonged exposure of the mosquito to sub-optimum doses of the active ingredient, especially at the margins of the chemical or device effective range “harbourage area”, results in variable responses and the subsequent development of resistance (Buhagiar et al. 2017b). In a cage bioassay mosquitoes exposed to metofluthrin (10% a.i.) for 60 min resulted in reduced mortality in *Ae. aegypti* females, with considerable mortality in males after 40 min. There were no obvious effects on the surviving females’ fecundity (Buhagiar et al. 2017b). In Mexico, variable mortalities (41-100%) were reported in *Ae. aegypti* exposed to 13 types of aerosolized insecticides com-

monly used in houses, due to variable insecticide type, concentration, formulation, and method of application or spraying (Kuri-Morales et al. 2018). In another field study in Mexico, up to 50% reduction in mortality was observed in *Ae. aegypti* susceptible and field resistant strains exposed to aerosol insecticides, plug-in and coil devices, with significant increase in the frequency of KD resistant homozygous allele, *kdr* I1016 (Gray et al. 2018). Widespread pyrethroid resistance in *Ae. aegypti* further confounds effective domestic mosquito management (Estep et al. 2017). Such findings highlight the risks of increased use of house-hold insecticides and repellents for personal protection. New pyrethroid formulations could contain synergists such as piperonyl butoxide or alternatives to enhance insecticide efficacy; or formulations could pair multiple active ingredients, particularly those that mosquitoes do not share resistance between (Bingham et al. 2011, Gray et al. 2018). It is equally important to educate consumers for well-informed use of over-the-counter insecticides and repellent devices to emphasize the role of citizens in integrated management and control programs (Vasques-Prokopec et al. 2017). In an attempt to generate useful information for the aforementioned goals, we report the evaluation of the spatial repellent compound-metofluthrin using the CDC bottle bioassay against susceptible and resistant *Ae. aegypti* strains.

## MATERIALS AND METHODS

Female mosquitoes from a pyrethroid susceptible laboratory colony of *Ae. aegypti* 1952 Orlando (ORL) strain were provided by the United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology (USDA-CMAVE), Gainesville, FL. This colony was reared at insectary facilities of the Anastasia Mosquito Control District (AMCD) in St. Augustine, Florida. Mosquitoes were maintained at  $26 \pm 1.0^\circ\text{C}$ , 65–80% relative humidity, and a photoperiod of 14:10 hr (L:D). The adult mosquitoes were provided 10% sugar solution *ad libitum*. For comparison, pyrethroid resistant

*Ae. aegypti* Puerto Rico (PR) strain was obtained from the USDA-CMAVE. Mosquitoes used were 19 generations of permethrin-resistance and reared under conditions as described in Pridgeon et al. (2008). Briefly, the mosquito eggs were allowed to hatch in a flask and remained overnight until transfer to a plastic tray containing distilled water with larval food. Mosquitoes were reared in an environmentally-controlled chambers set with a temperature profile representing a simulated summer day regimen (ranging from 22 to 30°C) and 80% RH. Incandescent lighting was set to a crepuscular profile with a photoperiod of 14 hr:10 hr (L:D), including 2 hr of simulated dawn and 2 hr of simulated dusk. Adults were held in a screened cage and provided 10% sucrose *ad libitum*. All mosquitoes selected for testing were 5-8 d old, non-blood-fed females. Technical grade 98.1% permethrin (45614 Pestanal, Sigma-Aldrich Co. LLC, St. Louis, MO) and 95.6% metofluthrin (SumiOne, Sumitomo Chemical Company Ltd., Tokyo, Japan) insecticides were dissolved in 1 ml of acetone to treat 250 ml glass bottles according to procedures outlined in Brogdon and McAllister (1998). The final concentration was 43 ng/ bottle for each insecticide. Each bottle was filled with 15-20 *Ae. aegypti* adults

and then observed for a maximum of 120 min. For each replicate, three bottles were assigned to ORL strain, three bottles were assigned to PR strain, and one bottle each was assigned to a control per insecticide. A total of 16 bottles were used each time. Permethrin and metofluthrin were replicated three times on different dates. The mean number of dead/moribund mosquitoes in bottles was plotted over time for each insecticide and the control. The time-mortality relationship lines were plotted and the median lethal KT values were deduced from the lines for each strain and each insecticide tested. For comparison of efficacies and susceptibility level of each strain, the resistance ratios (RR) were calculated by dividing the KT<sub>50</sub> values of the resistant strain by the susceptible strain. Abbott formula was not performed due to almost zero number of mortality in control group.

RESULTS AND DISCUSSION

Insecticide susceptibility and resistance of both permethrin and metofluthrin were detected in the PR strain as compared to the ORL strain (Fig. 1 and Table 1). The RR (KT<sub>50</sub>-PR/KT<sub>50</sub>-ORL) was 30 and 15 folds for permethrin and metofluthrin, respectively.



Figure 1. The time-mortality relationships for Orlando susceptible lab (ORL) and Puerto Rico resistant lab (PR) strains of *Aedes aegypti* exposed to permethrin and metofluthrin using the CDC bottle insecticide bioassay.

**Table 1.** Lethal Killing Time (KT) in minutes and resistance ratio (RR) values for Orlando susceptible lab (ORL) and Puerto Rico resistant lab (PR) strains of *Aedes aegypti*.

	Killing time (KT) in minutes				Resistance ratio (RR)				Notes	
	<i>Aedes aegypti</i> ORL Strain				PR Strain		ORL Strain			
	KT <sub>50</sub>	KT <sub>90</sub>	KT <sub>100</sub>	KT <sub>100</sub>	KT <sub>50</sub> -PR/KT <sub>50</sub> -ORL	KT <sub>50</sub> -Per/KT <sub>50</sub> -Met	KT <sub>50</sub> -Per/KT <sub>50</sub> -Met	KT <sub>50</sub> -Per/KT <sub>50</sub> -Met		
Permethrin (43ng)	90	NA	NA	3	10	15	30	3	1.5	PR: KT 57% at 120 min
Metofluthrin (43ng)	30	110	NA	2	8	15	15	1	1	PR: KT 94% at 120 min

The PR strain was less resistant to metofluthrin than to permethrin, with RR (KT<sub>50</sub>-PR-per/KT<sub>50</sub>-PR-met) of three-fold. However, it is clear that substantial cross resistance is present in the permethrin-resistant mosquitoes. Exposure of the PR strain to permethrin for 120 min caused only 57% mortality, while metofluthrin caused 94%. The susceptible ORL strain had comparable susceptibility to both permethrin and metofluthrin, where KT<sub>100</sub> was <15 min. In this report, the permethrin-resistant *Ae. aegypti* PR strain showed moderate level of cross-resistance to metofluthrin, with RR of 30-fold and 15-fold for permethrin and metofluthrin, respectively. This result is similar with results from lab and field experiments reported by Chen et al. (2018), Kuri-Morales et al. (2018), and Gray et al. (2018). Observed reductions in susceptibility are due to the long-term and inconsistent exposure of the mosquitoes to sub-lethal doses of the insecticide active ingredients used in different formulations, whether through space spraying, residual spraying, or vapor repellents (Kuri-Morales et al. 2018, Gray et al. 2018). In the State of Florida, there was wide-spread *kdr* resistance in *Ae. aegypti* field strains (RR of 6-61 fold to permethrin) with significant increase in the frequencies of homozygous and heterozygous *kdr* alleles of V1016I and F1534C (Estep et al. 2018). In contrast, in the same study, lower levels of resistance were reported in *Ae. albopictus* strains, even though they were collected from the same geographies that contained resistant *Ae. aegypti* strains. These various studies show the complex nature of *kdr* resistant dynamics and inheritance in different mosquito species or strains (*Ae. aegypti* and *Ae. albopictus*) and the potential spread of cross-resistance between different pyrethroids commonly used for vector control. Therefore, for better monitoring and management of resistance at early stages, it is essential to measure the susceptibility levels of various mosquito populations using a combination of bioassays (WHO, CDC bottle, and topical application bioassays) and molecular assays (genotyping and allele frequency) (Reid et al. 2014, Al-Nazawi et al. 2017, Estep et al. 2017, 2018). Chemical

adulticides are still the main means of controlling mosquito vectors of diseases. Whether by aerosol or repellents, pyrethroids are the most used insecticide as spatial insect repellent compounds for personal protection against the bites of adult mosquitoes in domestic and area-wide scenarios. Spatial repellent volatile pyrethroids are highly insecticidal, and their use to control mosquitoes is increasing. The combined pressures from pyrethroids deployed in home use, commercial agriculture, and vector control has resulted in development of *kdr* in response to almost all pyrethroid active ingredients (Wagman et al. 2015, Estep et al. 2018, Gray et al. 2018). Sustainable mosquito control programs hinge on routine monitoring of insecticide efficacy. This can be performed through both conventional insecticide susceptibility bioassays (the WHO and CDC bioassays) and molecular genotyping of resistance genes such as *kdr* alleles (Estep et al. 2018). Our findings show that the permethrin-resistant PR strain is highly resistance to both permethrin and metofluthrin compared to the susceptible ORL strain, and the PR strain is less resistance to metofluthrin than to permethrin.

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