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SCIENTIFIC NOTE

BIOEFFICACY OF TWO NONPYRETHROID INSECTICIDES FOR TARGETED INDOOR RESIDUAL SPRAYING AGAINST PYRETHROID-RESISTANT *AEDES AEGYPTI*

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ABSTRACT. We evaluated the efficacy of bendiocarb (Ficam W® 80%) and pirimiphos-methyl (Actellic 300CS® 28.16%), applied to different surfaces potentially sprayable within houses during the application of a targeted indoor residual spraying (TIRS) against a field pyrethroid-resistant strain of *Aedes aegypti*. Bioassays with cones were performed on cement (walls), wood (doors), and textile (cloth) surfaces within typical houses in the Mexican city of Merida ($n = 10$). Optimal residual efficacy (>80% of mean mortality) of bendiocarb ranged from 3 months (cement) to 2 months (wood and textiles). Residual efficacy of pirimiphos-methyl ranged from 5 months (cement) to 2 months (wood and textiles). Both insecticides proved to be effective as adulticides against field *Ae. aegypti* and may be useful in mosquito control programs implementing TIRS with pyrethroid-resistant populations.

KEY WORDS *Aedes aegypti*, bendiocarb, insecticide, pirimiphos-methyl, targeted indoor residual spraying

Aedes aegypti (L.) is the main vector of dengue, chikungunya, and Zika viruses in Mexico and the Americas. The Mexican strategy to control *Aedes*-transmitted diseases follows an integrated vector management approach that includes chemical-based strategies such as vehicle and handheld outdoor and indoor space spraying, respectively, in response to the occurrence of symptomatic illness reported to the national epidemiologic surveillance system (DOF 2015).

Indoor residual spraying (IRS) is the use of long-lasting residual insecticides applied to the walls, eaves, and ceilings of houses or structures targeting vectors that land or rest on these surfaces (WHO 2015). Specifically, for *Ae. aegypti* IRS is termed targeted IRS (TIRS), and consists of applying an insecticide on resting and harborage sites of adult female mosquitoes within houses (walls below 1.5 m, under furniture, inside wardrobes, etc.) (Ritchie et al. 2002, Vazquez-Prokopec et al. 2017b). Targeted IRS is one of the alternatives recently recommended to complement current control of *Ae. aegypti* by the Vector Control Advisory Group of the World Health Organization (WHO 2016). Observational evidence from Cairns, Australia, shows that TIRS can be greater than 80% effective in preventing symptomatic dengue illness (Vazquez-Prokopec et al. 2017b). Given these promising results, the Mexican program for the control of *Aedes*-transmitted diseases is considering including TIRS as part of the control of *Ae. aegypti* in urban areas.

The emergence of insecticide resistance in *Ae. aegypti*, primarily to pyrethroids in Mexico (Ponce-García et al. 2009, Flores-Suárez et al. 2016) and elsewhere, is a major challenge to the effectiveness of insecticide-based methods and can even lead to treatment failures (Vazquez-Prokopec et al. 2017a). Therefore, insecticide choice must be consistent with the insecticide resistance profile of local *Aedes* populations. Mexican health authorities assess, on a yearly basis and on local mosquito strains, a reference list of recommended insecticides (CENAPRECE 2018). Surveillance of the susceptibility profile of *Aedes* populations from 75 localities across 28 Mexican states (Kuri-Morales et al. 2018) has shown that pyrethroid resistance is generalized in the country, and that alternative modes of action are required. Novel nonpyrethroid insecticide formulations are currently being incorporated into the malaria vector control portfolio, providing an opportunity to evaluate their suitability for *Ae. aegypti* control. We evaluated the bio-efficacy of two nonpyrethroid insecticides, bendiocarb– and pirimiphos-methyl–based formulations, applied on surfaces

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Table 1. Percentage of mortality (95% confidence intervals) and residual efficacy observed in cone bioassays with the application of bendicarb (Ficam®W) and pirimiphos-methyl (Actellic®300CS) formulations to different surfaces against the pyrethroid-resistant San Lorenzo strain of *Ae. aegypti* after 24 h.

Posttreatment interval (month)	Bendicarb			Pirimiphos-methyl		
	Cement 99.6 (95.1–100)	Wood 99.2 (94.2–100)	Cloth 99.1 (93.9–100)	Cement 99.0 (94.0–100)	Wood 99.2 (94.6–100)	Cloth 99.0 (94.0–100)
1	93.3 (89.4–96.2)	97.0 (94.2–98.6)	98.7 (97.2–100)	99.0 (97.0–100)	98.2 (96.3–99.4)	99.0 (97.0–100)
2	99.1 (97.3–99.9)	94.9 (91.4–97.3)	93.9 (89.8–96.7)	97.0 (95.0–99.0)	92.0 (87.6–95.2)	93.0 (90.0–96.0)
3	87.7 (82.2–92.1)	57.2 (47–66.9)	72.1 (62.5–80.5)	88.0 (83.7–91.1)	69.8 (61.5–77.7)	77.9 (72.8–82.1)
4	49.6 (39.8–59.0)	45.0 (34.9–55.2)	41.1 (31.2–52.2)	89.2 (85.7–93.0)	64.9 (56.0–73.5)	66.0 (60.0–71.9)
5	50.0 (40.7–59.5)	20.8 (14.5–28.8)	21.5 (14.3–30.0)	86.0 (81.7–90.0)	57.8 (48.4–67.0)	57.1 (51.2–63.3)
6	29.6 (21.6–38.3)	17.7 (11.4–24.9)	9.5 (5.6–15.0)	71.0 (65.6–76.6)	55.5 (45.9–65.2)	50.0 (43.8–56.3)
7	10.6 (6.4–16.4)	6.7 (3.4–11.4)	1.5 (0.1–3.8)	70.6 (64.2–76.2)	18.6 (12.4–26.3)	13.9 (9.9–18.3)
8	2.2 (0.7–4.5)	7.4 (0.0–78.4)	7.5 (0.0–79.2)	31.5 (25.7–37.8)	6.1 (2.9–10.2)	4.0 (2.0–7.1)
9	6.7 (0–70.1)	7.6 (0.0–79.0)	7.7 (0.0–79.9)	10.0 (7–14.2)	7.6 (0–78.3)	8 (0.0–80.2)

typical of urban houses, against a field pyrethroid-resistant local strain of *Ae. aegypti* in the city of Merida, Mexico.

Ten typical single-story houses, constructed with wooden bricks and cement, with wooden doors and standard furniture (sofa, chairs, typically with textile materials) were selected from Merida volunteer collaborators. Houses have a living–dining room, most of them have 2 bedrooms and a kitchen and bathroom; they also have a patio (backyard). The pyrethroid-resistant strain of *Ae. aegypti* employed (San Lorenzo strain), is phenotypically resistant to pyrethroids with knockdown resistance alleles Ile1016 and Cys1534 (Deming et al. 2016). The strain is maintained in the insectary of the Collaborative Unit of Biological Bioassays (UCBE) of the Universidad Autonoma de Yucatan.

Spraying was carried out following the WHO guidelines (WHO 2015): bendiocarb 125-g sachet wettable powder (Bayer Environmental Science of Mexico, Mexico City, Mexico) at a concentration of 80% and pirimiphos-methyl 833-ml bottle capsule suspension (Syngenta of Mexico, Mexico City, Mexico) at a concentration of 28.16%. Both insecticides were mixed in 7.5 liters of water and applied with a manual compression sprayer IK-Vector Control Super® (Goizper Group, Antzuola, Spain) with mouthpiece 8002EVP and Goizper low-pressure control flow valve (output pressure 1.5 bar) to provide a flow rate of 580 ml/min (± 5%), and a dose of 0.407 and 0.955 g AI/m^2 for bendiocarb and pirimiphos-methyl, respectively. We conducted TIRS in all houses, following the procedures described in Dunbar et al. (2019) for TIRS resting sites.

Twenty-four hours after insecticide application, WHO cone bioassays (WHO 2006) were performed on 3 surface types of each house: cement, wood, and textile. Three standard cones were fixed at 3 different heights (0.5, 1.0, and 1.5 m from the floor) on cement walls and wooden doors, and the 3 cones were fixed along the total surface of the back side of sofa cushions (textile). Ten *Ae. aegypti* F_1 females (only fed with a 10% sucrose solution) were introduced into each cone using a manual aspirator. Mosquitoes were exposed to the treated surfaces for 30 min then transferred to holding chambers and maintained with 10% sucrose solution under controlled insectary conditions at UCBE (26 ± 1°C and 80 ± 5% RH). Residual efficacy of insecticides was immediately evaluated at 24 h after the initial application and monitored monthly for 9 months (April–December of 2017) after insecticide application, which corresponded to the end of the dry season through the end of the rainy season. Bioassays were also carried out in untreated control surfaces within the same houses (3 set of cones by type of surface), using the same methodology.

Binary data characterizing individual mosquito mortality (1 = yes; 0 = no) was used in binomial generalized linear mixed models (GLMM) with time as fixed effects and house as a random intercept. A total of 6 models were run (1 per surface) and used to calculate mean predicted mortality ± 95% confidence interval (CI) (Table 1). Mortality data were used to infer the maximum residual efficacy (defined as the number of months for which mortality was higher than 80%). Due to the null mortality in controls, data transformation (Abbott equation) was not necessary (Abbott 1925). All analyses were performed with R 3.4.4. The R software package *INLA* (Lindgren and Rue 2015) was used to run the GLMM.

As shown in Table 1, the maximum residual efficacy (>80% mortality) of bendiocarb against the resistant field strain was maintained for up to 3 months on concrete surfaces (87.7% mortality, 95% CI = 82.2–92.1) and up to 2 months on wooden surfaces (94.9%, 91.4–97.3) and textiles (93.9%, 89.8–96.7). For pirimiphos-methyl, the maximum residual efficacy was observed up to 5 months on concrete surfaces (86.0%, 81.7–90.0) and up to 2 months on wooden surfaces (92.0%, 87.6–95.2) and textiles (93.0%, 90.0–96.0) (Table 1). No mosquito mortality was recorded in the control cones.

Historical evidence has shown that, when expeditiously implemented, residual insecticide applications can significantly reduce *Aedes* transmitted diseases; IRS either alone (Giglioli 1948), or in combination with larval control (Nathan and Giglioli 1982), contributed to the elimination of *Ae. aegypti* and dengue from (British) Guyana and the Cayman Islands, respectively. Despite this evidence, the fact that it is time consuming and dependent on specialized human resources has limited IRS widespread adoption by *Aedes* control programs due to the perceived challenge of scaling up the intervention over large urban areas. Studies performed in experimental houses in Mexico show that TIRS provides an entomological impact similar to spraying entire walls (as performed in classic IRS), but in a fraction of the time (<18%) and insecticide volume (<30%) compared to classic IRS (Dunbar et al. 2019).

A shift in the conceptualization directed to the field implementation of IRS to control *Ae. aegypti* in urban areas includes: 1) modifying insecticide application sites for TIRS to account for *Ae. aegypti* indoor resting behavior, 2) piloting novel insecticide formulations that have high residual activity and to which *Ae. aegypti* is susceptible; and last but not least, 3) changing the intervention delivery from reactive (after detection of symptomatic cases) to proactive (prior to peak *Aedes*-borne virus transmission season) (Hladish et al. 2018).

The residual efficacy observed with both insecticides on the concrete surfaces (walls) is in concordance with those reported by the WHO (2015): pirimiphos-methyl CS from 4 to 6 months and bendiocarb WP from 2 to 6 months of duration of effective action. Given that cement constitutes the most prevalent surface found in urban settings and considering the difficulty of maintaining high residual efficacy in wood or cloth surfaces, our findings identify several insecticide molecule alternatives to pyrethroids for use in urban TIRS. Our study also shows the value of carbamate and organophosphate chemistries to control pyrethroid-resistant *Ae. aegypti*. As *Ae. aegypti* resistance to pyrethroid insecticides is reported with increasing frequency (Moyes et al. 2017), it is crucial to select a chemical that maximizes insecticide efficacy. Both bendiocarb and pirimiphos-methyl showed high acute mortality within their expected residual efficacy and could be used as alternatives to pyrethroids in those areas where insecticide resistance may challenge vector control operations.

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