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Assessing the effectiveness, biosafety, and resident's perception of a micro-encapsulated propoxur paint against *Aedes aegypti*: a field trial in a dengue-affected community of Sonora, Mexico

Evaluación de la eficacia, la bioseguridad y la percepción de los residentes sobre una pintura con propoxur microencapsulado contra Aedes aegypti: un ensayo de campo en una comunidad afectada por el dengue en Sonora, México

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ABSTRACT

Mosquito-borne diseases remain a major public health concern in the Region of the Americas. Concerning dengue, Mexico ranked as the third American country with the highest number of cases in 2023, following Brazil and Peru. In this sense, the development and production of safer, eco-friendly, cost-effective, more efficient, and target-specific insecticides have become essential in disease control strategies. This study aimed to assess the effectiveness and safety of an indoor micro-encapsulated propoxur paint against *Aedes aegypti*, estimate residents' exposure via cholinesterase levels, and gauge resident perceptions in the rural village Poblado Miguel Alemán (PMA), Sonora, Mexico. Houses in PMA were organized into four blocks for a field trial, with different treatments on interior walls: 1) full insecticide paint coverage (IP); 2) insecticide paint applied up to 1 meter (IP 1 m); 3) full indoor residual spraying (IRS); and 4) no treatment (control). Mosquito surveys were conducted before and after interventions. Blood samples from residents were analysed for cholinesterase levels, while perceptions were gathered through a questionnaire. Aedes resting density correlated closely with the paint formulation and coverage. Full-surface insecticide paint showed the greatest reduction. Both paint interventions notably impacted *Aedes* breeding, with substantial reductions in House Index (20.1% IP, 31.2% IP 1 m) and Container Index (51.8% IP, 61.7% IP 1 m) compared to the control over one year. Post-intervention blood cholinesterase activity remained within acceptable limits. Over 80% of residents expressed satisfaction with the interventions. The use of propoxur paint proves to be a safe, effective, and well-accepted method for decreasing *Ae. aegypti* populations in urban areas.

Keywords: propoxur, insecticide paint, *Aedes aegypti*, dengue, breeding sites, Mexico.

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RESUMEN

Las enfermedades vehiculadas por mosquitos siguen siendo un importante problema de salud pública en la Región de las Américas. En lo que respecta al dengue, México se reportó como el tercer país del continente americano con el mayor número de casos en 2023, después de Brasil y Perú. En este sentido, el desarrollo y la producción de insecticidas más seguros, ecológicos, rentables, eficientes y específicos se han vuelto esenciales en las estrategias de control de estas enfermedades. El objetivo del presente trabajo fue evaluar la efectividad y seguridad de una pintura de propoxur microencapsulado aplicada en interiores contra Aedes aegypti, estimar la exposición de los residentes a través de los niveles de colinesterasa y evaluar las percepciones de los residentes en la localidad rural de Poblado Miguel Alemán (PMA), Sonora, México. Las casas del PMA se organizaron en cuatro bloques para un ensayo de campo con diferentes tratamientos en las paredes interiores: 1) cobertura total con pintura insecticida (IP); 2) aplicación de pintura insecticida hasta 1 metro desde el suelo (IP 1 m); 3) rociado residual interior completo (IRS); y 4) sin tratamiento (control). Se realizaron capturas de mosquitos antes y después de las intervenciones. Se analizaron muestras de sangre de los residentes para niveles de colinesterasa y se usó un cuestionario para conocer las percepciones de los residentes. La densidad de reposo de Aedes se correlacionó estrechamente con la formulación y cobertura de la pintura. La cobertura total de pintura insecticida mostró la mayor reducción. Ambas intervenciones con pintura impactaron notablemente en la reproducción de Aedes, con reducciones sustanciales en el Índice de Vivienda (20.1% IP, 31.2% IP 1 m) y el Índice de Contenedores (51.8% IP, 61.7% IP 1 m) en comparación con el control durante un año. La actividad de colinesterasa en sangre después de la intervención se mantuvo dentro de límites aceptables. Más del 80% de los residentes expresaron satisfacción con las intervenciones. El uso de la pintura de propoxur resulta ser un método seguro, efectivo y bien aceptado para disminuir las poblaciones de Ae. aegypti en áreas urbanas.

Palabras clave*: propoxur, pintura insecticida, Aedes aegypti, dengue, sitios de cría, México.*

INTRODUCTION

Mosquito-borne diseases remain a major public health concern in the Region of the Americas. The increase in population density in urban and semi-urban areas, rapid urbanization, inadequate infrastructure, and deficiencies in water supply and waste management systems are among the main factors that contribute to the reproduction and expansion of vector mosquito species [1]. Additionally, the overburdening of public health systems, coupled with the recent Covid-19 pandemic, is worryingly exacerbating the shortcomings of vector control programs [2,3].

Dengue is the most widespread mosquito-borne disease in the Americas. In 2023, over 4.2 million new cases of dengue fever were reported, including more than 6 500 severe dengue cases and 2 050 deaths. This marks an increase of over 52% relative to the reported cases in 2022, and a 108% increase relative to the average of the reported cases in the last five years. By countries, the highest number of cases were reported in Brazil, followed by Peru, and Mexico [4]. Other recent epidemiological events also underscore the pressing need to enhance mosquito control programs. Chikungunya virus exhibited its first local transmission in Caribbean countries and territories in late 2013 [5]. Subsequently, Zika virus emerged with its initial locally acquired cases in Brazil in 2015 [6], rapidly spreading across the continent and becoming and emerging global health threat in early 2016. Additionally, yellow fever remains a persistent threat to millions in tropical and subtropical regions of South and Central America, despite the availability of a safe and effective vaccine since the 1930s [7].

Artificial water reservoirs located inside households are preferred breeding habitats for synanthropic mosquito species [8], among which *Aedes aegypti* (Linnaeus, 1742), and *Aedes albopictus* (Skuse, 1894) stand out due to their role in arbovirus transmission. These culicids, which coexist in large regions of the Americas, are considered as invasive species since they have successfully colonized many areas outside their native ranges [9]. From an ecological standpoint, *Ae. aegypti* is more prevalent in urban areas, whereas *Ae. albopictus* exhibits significant ecological plasticity and can easily thrive in rural, peri-urban, and urban environments [10], although it remains more abundant in vegetated rural and suburban areas [11]. Furthermore, *Ae. aegypti* is traditionally recognized as a highly endophilic species, predominantly resting inside human dwellings [12], which bears significant implications for its domestic control [13].

Considering this situation of global emergence/resurgence of arboviral diseases, as well as the spread of aedine vectors, vector control becomes a major challenge. Over the past decades, mosquito control has mostly relied on chemical insecticides that target the adult life stage. Of particular concern is their potential hazard to humans, animals, other organisms, and the environment [14], and the development of insecticide resistance in vector populations [15], which results in loss of effectiveness. In fact, a systematic literature review and meta-analysis of interventions in Latin America and the Caribbean for over 15 years confirmed that only few interventions in the region were supported by evidence on their effectiveness [16]. A nation-wide assessment of the insecticide susceptibility status in Mexico revealed that all *Ae. aegypti* populations evaluated across the 28 states showed susceptibility to the two carbamates, propoxur and bendiocarb. As the carbamates remained highly effective, they are considered as an optional strategy in mosquito population control by the Mexican Ministry of Health [17].

Having this in mind, the development and production of safer, eco-friendly, cost-effective, more efficient, and target-specific insecticides has become one of the most necessary control approaches. In this sense, indoor residual spraying (IRS) effectively targets *Ae. aegypti* by coating indoor surfaces with persistent insecticide, capitalizing on the mosquito's preference to rest indoors. This method requires expert personnel equipped with specialized spraying tools to cover all indoor walls extensively [18,19]. However, its high cost and the perception of the malodorous residue as harmful lead to frequent household refusals, reducing its coverage [20]. Recent studies refined the IRS method through targeted IRS (TIRS), concentrating spraying on walls and dark areas below 1.5 m, aligning with *Ae. aegypti* females' indoor resting preferences [21]. This enhancement diminishes insecticide usage and treatment duration per household while maintaining effectiveness, evidenced by studies across dengue-affected regions, including Mexico [22,23].

Propoxur 70% Wettable Powder and Bendiocarb 80% Wettable Powder were the only carbamate formulations registered in Mexico for IRS vector control. The introduction of new propoxur-containing paint presents an innovative concept potentially addressing insecticide resistance and offering some advantages: 1) The insecticide paint (IP) allows residents to apply it without specialized teams required for IRS/TIRS; 2) Applying paint via brush or roller enhances safety, convenience, and acceptability compared to spraying; and 3) The longer-lasting nature of IP reduces the need for frequent retreatment. Recognizing its efficacy, the WHO acknowledged insecticide paints as a method for treating specific indoor areas where *Aedes* vectors rest [24]. By another part, the Innovative Vector Control Consortium considers insecticide paint an innovative product capable of significantly reducing indoor vector populations, offering a safer and aesthetically pleasing alternative to traditional IRS [25].

The current study aims to evaluate the efficacy of indoor micro-encapsulated propoxur paint against *Ae. aegypti*, comparing various interventions with the traditional IRS method. Additionally, the study seeks to investigate the impact of exposure to these paints on human health and understand residents' perceptions of these interventions in a city affected by dengue in Hermosillo, Sonora, Mexico. The outcomes of these investigations will enable more efficient and contextually relevant control measures to be implemented in the state of Sonora, as well as in other Mexican states. Ultimately, this will lead to a reduction in the burden of diseases transmitted by *Ae. aegypti*.

MATERIALS AND METHODS

Study design

The study was a non-randomized controlled trial with a pretest-posttest design, aimed at evaluating the effectiveness of three different interventions in controlling *Ae. aegypti* domestic populations. From an entomological perspective, a descriptive longitudinal study was conducted, utilizing various entomological indicators measured before and after the interventions. Human exposure to the insecticide was assessed by measuring residents' cholinesterase blood levels before and after the interventions. Residents' perceptions were captured through a cross-sectional survey regarding satisfaction and perceived side effects.

Study area

The study was conducted in the rural locality of Poblado Miguel Alemán (PMA), located in the municipality of Hermosillo, state of Sonora, northwestern Mexico (Figure 1A and 1B). The estimated population is 39 474 inhabitants [26] and is mainly dedicated to agriculture, with an intense migration of farmer workers mainly coming from the south of the country. Historically, high levels of social deprivation are well known in this area [27]. The city is 60 m asl with a semi-arid climate, and a rainy season during summer [28]. Dengue is usually endemic in the territory. In fact, 9 331 suspected cases were reported in the state of Sonora during 2022, of which 2 266 cases were confirmed, with over 20 deaths [29]. The transmission season occurs mainly during July through October, due to the greater abundance of *Aedes* mosquitoes favored by the rainy periods.

A cluster of houses were selected for the field trial (coordinates in decimal degrees; latitude 28.8471294; longitude -111.5010849) and aligned in four blocks (Figure 1C). A non-probabilistic sampling method was employed, assuming uniform risk distribution within the community, given its historical status as a hotspot for mosquito-borne diseases. Typical houses of PMA are mainly constructed with sun dried bricks, mud, waste materials and in some cases plastered with cement. Walls were painted in a few houses. Tin sheets were the dominant type of roofing.

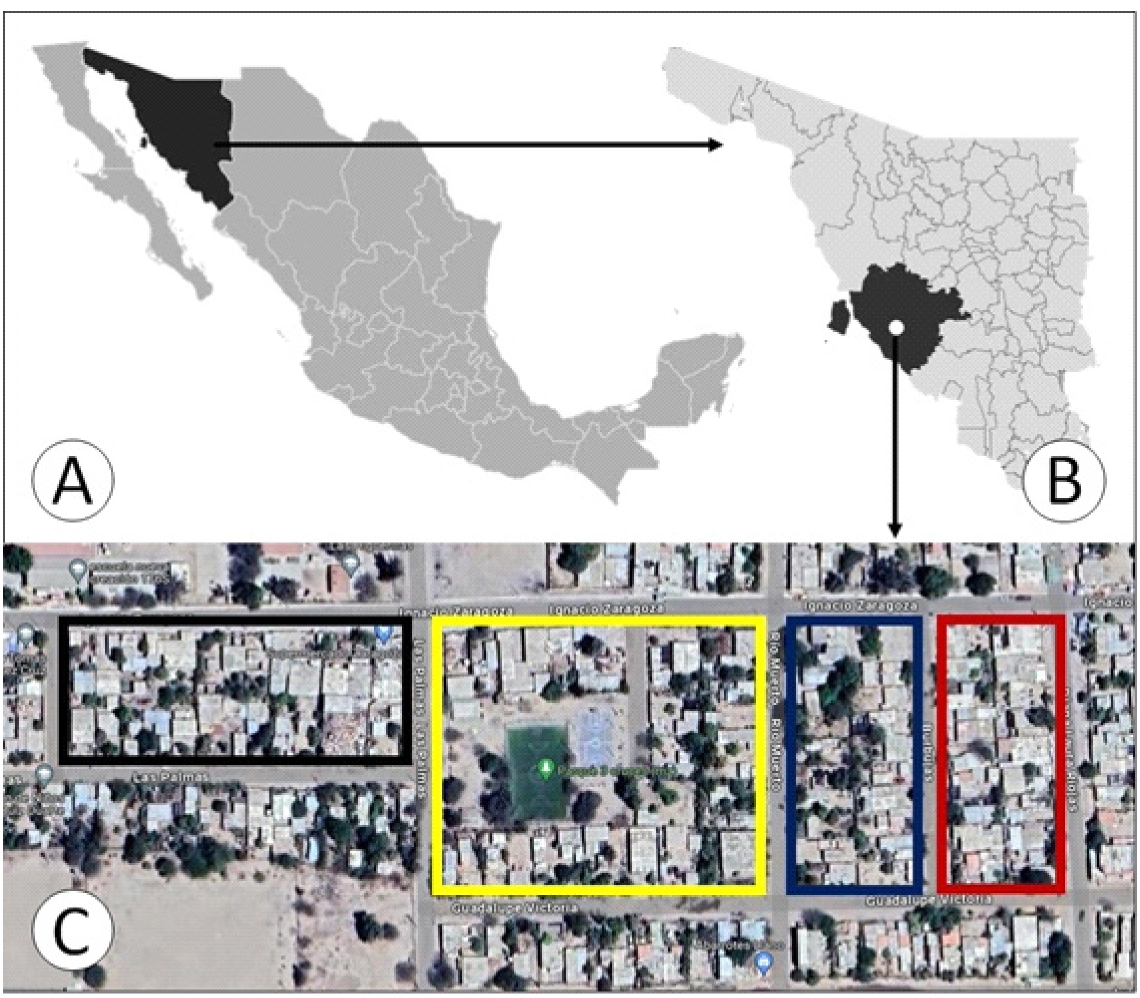

Figure 1. Study area. A) location of the state of Sonora in Mexico; B) location of the municipality of Hermosillo, the white dot corresponds to Poblado Miguel Alemán; and C) satellite view showing the trial site, with the block exhibiting full coverage of insecticide paint marked by a black rectangle, the control block marked by a yellow rectangle, the block with insecticide paint applied up to 1 meter marked by a blue rectangle, and the block with full coverage of indoor residual spraying marked by a red rectangle.

Interventions

The four blocks were assigned to the following treatments focused on interior walls: 1) full coverage of insecticide paint (IP); 2) targeted coverage of IP, applied up to 1 m height from the floor (IP 1 m); 3) full coverage of indoor residual spraying (IRS); and 4) untreated/control. The total number of houses treated were 16, 10, and 16 for IP, IP 1 m, and IRS, respectively. All houses included in each block received treatment (n=42). The control block contained 19 houses (Figure 1C).

Carbapaint 10, which is a water- based polymer coating leading microencapsulated suspension of propoxur 1.0% w/w (Inesfly Corporation S.L., Paiporta, Valencia, Spain), was used as IP. The insecticide paint was applied at the recommended dose (1L/8 m^2, equivalent to 1.5 g a.i./m^2) by homeowners by brushing and rolling. A water-based sealant was applied as a first layer to reduce paint absorption in high absorbent wall materials like bricks. Residents were informed and trained in safety measurements and paint application by the research team.

IRS was conducted by professional staff of the Sonora's Health Department with Propoxur 70% WP with an application dose of 1 g a.i./m^2 by means of a Hudson X-Pert sprayer portable machine (H. D. Hudson Manufacturing Company, Chicago, IL, USA) with a flat fan nozzle 8002E (TeeJet® Technologies, Spray Systems Co., Bessemer, AL, USA) for fine droplets, and calibrated to 760±4ml/min, at 55 psi.

The application of IP and IRS was conducted once and simultaneously in all treatment blocks.

Entomological survey

Adult and immature mosquito surveys were conducted in a random sample of houses in each block one week before the interventions, and at week 1, month 1 to 4, month 6, month 9, and month 12 post-interventions. However, larval and pupal survey at week 1 was omitted due to the non-expected impact of the treatments at such short time post-application.

Adult monitoring

Adult collections in resting sites were performed by trained technicians with a CDC backpack aspirator model 1412 (John W. Hock Company, Gainesville, FL, USA). Technicians spent 15 min during the inspection of the interior and exterior parts of the house in accordance with the method described in published studies [30]. In the laboratory, all mosquitoes were sorted by sex and classified by species using the taxonomic keys of Darsie and Ward [31]. Adult House Index (AHI) was defined as the percentage of houses positive for *Aedes* mosquitoes. Adult density (AD) was defined as the number of *Aedes* mosquitoes per 100 houses examined [32].

Larval monitoring

Larval survey consisted in the capture of immature stages from water holding containers, following official guidelines [33]. The larvae of 3rd and 4th instars were directly taken for species identification using a morphological taxonomic key [34]. Adults emerged from pupae were killed by freezing for classification purposes. Percentage of water holding containers, House Index (HI, calculated as: no. of houses with *Ae. aegypti* immatures/no. of houses inspected × 100), and Container Index (CI, calculated as: no. of containers with *Ae. aegypti* immatures/no. of containers inspected × 100) were obtained for each block and follow up time in accordance with WHO definitions [35].

Measurement of acetylcholinesterase in exposed residents

Carbamate insecticides act on the nervous system of insects and mammals by inactivating the acetylcholinesterase enzyme in a reversible manner [36]. To assess the exposure of residents to propoxur, cholinesterase levels were measured in blood samples taken from adult volunteers of both sexes and a wide range of ages. The samples were collected prior to intervention, and at week 1 and month 1 post-interventions.

Blood extractions, transportation and analysis were performed according to operational guides issued by Mexico Health Secretary [37,38]. The cholinesterase study was performed by the Quantitative determination of cholinesterase (CHE) IVD method using the Spinreact kit® (SPINREACT, S.A.U., Sant Esteve d'en Bas, Girona, Spain), and it was carried out in a certified private laboratory of Santa Fe, Mexico City.

Resident's perceptions of insecticide interventions

The acceptability, perceived effectiveness, and side effects of the three interventions were evaluated by conducting interviews with one adult resident per household at month 3 after treatments. To achieve this, a structured questionnaire was used during visits to all houses belonging to the treatment blocks. Participants were thoroughly briefed on the survey's purpose, and their participation was voluntary. Researchers documented the responses from a chosen sample of households within each treatment block.

Ethical considerations

The testing protocol was reviewed and approved by the Research Bioethics Committee from the Department of Medicine and Health Sciences of the University of Sonora (UNISON) (DMCS D 109/2018).

Study participants were informed about the research project and the family representant signed a consent form. Paint and painting tools were provided by free. Empty buckets and used brushes and rollers were recovered and disposed according to environmental requirements of Sonora State. Medical assistance was offered to participants for treating any side effects derived from the interventions. Confidentiality of the personal and medical data was ensured through internal protocols of the University of Sonora.

Data analysis

For adult monitoring, a chi-square analysis was used to estimate the differences in the proportion of positive/negative infested houses between treated and control houses. To compare the proportion of *Ae. aegypti* and *Culex* spp., indoors and outdoors, a chi-square analysis was also used. Values of adult density among treatments (IP, IP 1 m, IRS, and Control) and species, indoors and outdoors, were analyzed using a factorial general linear model ANOVA.

For preimaginal analysis, a nonparametric Kruskal–Wallis test was used to test the significance of the differences in the number of larvae among treatments (IP, IP 1 m, IRS, and Control). A chi-square analysis was used to estimate the differences in the type of containers used as breeding sites between treated and control houses.

Regarding measurements of cholinesterase activity, levels were obtained by subtracting the baseline level (one week before intervention) and the cholinesterase levels at one week and one month after intervention. Differences among intervention groups (IP, IP 1 m, IRS, and Control) in cholinesterase blood levels (one week and one month after intervention) were analyzed using mixed effects generalized linear models with a log link function, with groups and sex as categorical factors, and age as continuous predictor.

For all analyses, significance threshold was set at 0.05. Data were analyzed using IBM SPSS Statistics® version 21 (IBM Corp, Armonk, NY, USA), JMP® version X (SAS Institute Inc., Cary, NC, USA) and STATISTICA 13.0 software (TIBCO Software Inc., Palo Alto, CA, USA).

RESULTS

Entomological survey

Adult monitoring

A total of 172 house inspections were conducted during the post-intervention phase in 8 follow-up entomological surveys for one year. Houses in the control block had the lowest inspection rate (19.7%), followed by IP (33.6%), IRS (41.4%), and IP 1 m (57.5%). Overall, it was observed a reduction in the fraction of positive houses for *Ae. aegypti* females collected in interior and exterior resting sites in all treatment groups in comparison to control. The one-year average reduction was 43.7%, 44.9% and 41.3% for IP, IP 1 m, and IRS, respectively to control (Table 1). Differences in the proportion of positive/negative infested houses between treated and control houses were observed (χ^2=14.128, *p*=0.0027).

Species identification in adult collections revealed that *Culex* spp. females were more abundant indoors and outdoors than *Ae. aegypti* (χ^2=60.16, p<0.0001) (Table 2). The dominance of *Culex* spp. in all three intervention sites was greater for IP and IP 1 m, while in a lesser extent for IRS, in comparison to control. Among groups, in interiors *Culex* spp. proportion was higher than *Ae. aegypti* (χ^2=83.46, *p*<0.0001), particularly for the IP group. No differences between *Culex* spp. and *Ae. aegypti* proportions in exteriors were observed (χ^2=5.995, *p*=0.111).

Table 1. Adult *Aedes aegypti* House Index (%) pre-post interventions in households of Hermosillo, Mexico, from April 2018 to April 2019.

TAT	IP (n)	IP 1 m (n)	IRS (n)	Control (n)
Pre-interventions	0 (4)	75.0 (4)	55.6 (9)	80.0 (5)
Week 1	0 (4)	25.0 (4)	25.0 (4)	100.0 (4)
Month 1	25.0 (4)	25.0 (4)	75.0 (4)	75.0 (4)
Month 2	50.0 (2)	33.3 (6)	75.0 (4)	ND
Month 3	33.3 (6)	16.7 (6)	0 (6)	ND
Month 4	16.7 (6)	33.3 (6)	45.5 (11)	100.0 (6)
Month 6	100.0 (6)	100.0 (5)	100.0 (7)	100.0 (6)
Month 9	33.3 (6)	50.0 (8)	30.0 (10)	50.0 (6)
Month 12	88.9 (9)	85,7 (7)	71.4 (7)	100.0 (4)
Average	48.8	47.8	50.9	86.7
95% CI	19.8-77.9	22.2-73.5	23.2-78.7	64.7-108.6

TAT: time after treatment; n: number of houses inspected; ND: no data; 95% CI: 95% confidence interval.

Table 2. Mosquito *species* distribution in indoors and outdoors collections post-interventions in households of Hermosillo, Mexico, from April 2018 to April 2019.

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		IP		IP 1 m		IRS		Control	
		n	FRAC (%) (95% CI)	n	FRAC (%) (95% CI)	n	FRAC (%) (95% CI)	n	FRAC (%) (95% CI)
Ind.	*Ae. aegypti*	12	6.2 (-9.7-22.2)	20	32.8 (3.3-62.2)	48	52.2 (1.8-102.6)	66	40 (-4.7-94.7)
	Culex spp.	181	93.8 (77.8-109.7)	41	67.2 (37.8-96.7)	44	47.8 (-2.6-98.2)	99	60 (5.3-114.7)

Table 2. Mosquito *species* distribution in indoors and outdoors collections post-interventions in households of Hermosillo, Mexico, from April 2018 to April 2019.

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		IP		IP 1 m		IRS		Control	
		n	FRAC (%) (95% CI)	n	FRAC (%) (95% CI)	n	FRAC (%) (95% CI)	n	FRAC (%) (95% CI)
Out.	*Ae. aegypti*	21	28 (-81.5-144.9)	27	21.1 (-53.8-96.0)	61	31.0 (-22.1-84.1)	21	36.8 (-29.5-107.6)
	Culex spp.	54	72 (-37.5-188.8)	101	78.9 (4.0-153.8)	136	69.0 (15.9-122)	36	63.2 (-3.2-134.0)
T	*Ae. aegypti*	33	12.3 (-21.7-46.3)	47	24.9 (-6.1-55.8)	109	37.7 (-7.2-82.6)	87	39.2 (-10.5-88.9)
	Culex spp.	235	87.7 (53.7-121.7)	142	75.1 (44.2-106.1)	180	62.3 (17.4-107.2)	135	60.8 (11.2-110.5)

Ind.: indoors; Out.: outdoors; T: total; n: total number of collected female mosquitoes during the one year follow up; FRAC: fraction of mosquito species in percentage; 95% CI: 95% confidence interval.

Aedes mosquito density recorded one-year average reductions of 77.5%, 64.2% and 30.0% in interiors for IP, IP 1 m, and IRS, respectively when compared to control block. However, no significative differences among treatment groups were detected in interior densities (Wald χ^2=0.24, p=0.97) and neither difference between species total densities (Wald χ^2=0.08, p=0.77). Despite *Ae. aegypti* showed lower densities than *Culex* spp. in all groups (Figure 2), no differences for the interaction Groups*Species were detected (Wald χ^2=1.00, p=0.80). In exteriors, no differences among groups (Wald χ^2=1.52, p=0.67), species (Wald χ^2=0.76, p=0.38), and interaction Groups*Species (Wald χ^2=0.44, p=0.93) were observed.

A noticeable variability occurred in the time series and study blocks, with absence of *Aedes* females in all blocks at month 1 (April), and few individuals at month 3 (July), and 9 (January). Comparison of *Ae. aegypti* and *Culex* spp. total density confirmed the greater abundance of *Culex* spp. and the null impact of any of the insecticide interventions to these

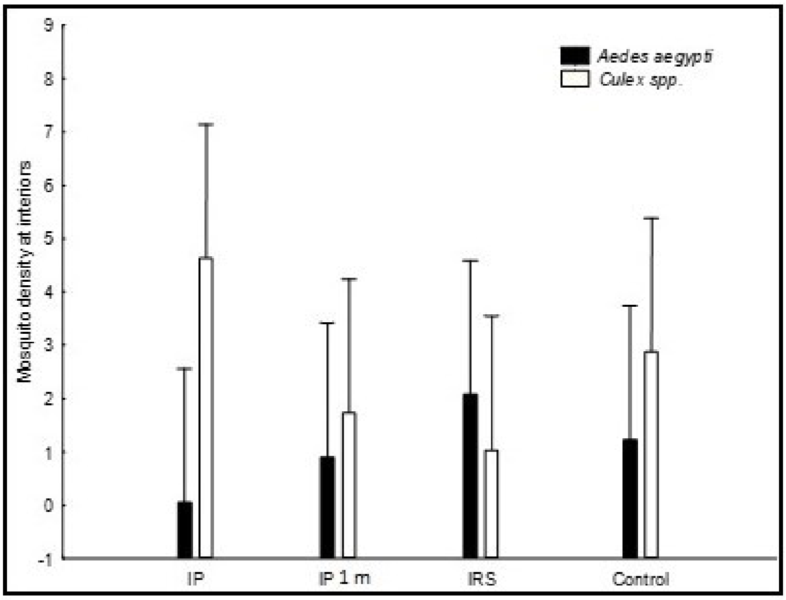

Figure 2. *Aedes aegypti* and *Culex* spp. densities per groups at interiors after interventions in households of Hermosillo, Mexico, from April 2018 to April 2019.

species, in contrast with the average reductions to control observed for *Ae. aegypti* in the IP (51.5%), and IP 1 m (36.4%) (Table 3). *Culex* spp. populations appeared to be very seasonal and peaked importantly on month 12 (April) inspection, with a total female number of 469 among 592 recorded during the complete follow-up collections.

Table 3. *Aedes aegypti* and *Culex* spp. females' total density per positive house pre-post interventions in households of Hermosillo, Mexico, from April 2018 to April 2019.

TAT	Ae. IP	Ae. IP 1m	Ae. IRS	Ae. Control	Cx. IP	Cx. IP 1m	Cx. IRS	Cx. Control
Pre-intervention	0	3.3	8.4	0	0	0	0	0
Week 1	0	0	2.0	0.5	0	0	0	0
Month 1	0	0	0.0	0	1.0	7.0	4.3	1.3
Month 2	3.0	2.0	2.0	0	0	0.5	0	0
Month 3	2.0	4.0	0	0	2.0	2.0	0	0
Month 4	1.0	2.0	5.8	10.0	17.0	1.0	0.6	3.0
Month 6	3.3	4.6	10.0	4.2	4.3	4.4	5.9	1.2
Month 9	0.5	0.3	0	0	4.0	3.5	5.7	5.3
Month 12	0.5	1.8	0.4	0	22.4	15.7	21.2	22.5
Average	1.6	2.1	4.0	3.3	11.2	6.5	6.7	5.2
95% CI	0.3-2.8	0.5-3.8	0.7-7.4	0-6.7	4.6-17.7	1.7-11.2	-0.2-13.6	-0.7-11.1

TAT: time after treatment; Ae.: *Aedes aegypti* females; Cx.: *Culex* spp.; 95% CI: 95% confidence Interval.

Larval monitoring

During the seven surveys conducted after the interventions (May 2018 to April 2019), a total of 268 houses (54.9%) were randomly inspected. Among the 7 462 potential containers recorded, 1 132 (15.2%) were found filled with water, with 69 showing the presence of *Ae. aegypti* immatures. One-year average HI was 20.1% and 31.2% reduced due to the IP and IP 1 m, respectively. Reduction was also noticed for the CI in IP (51.8%), and IP 1 m (61.7%) study blocks. IP and IP 1 m showed the lowest HI (Table 4), but no differences among groups were detected ($H_{(3,33)}$=2.51, *p*=0.47). Higher indexes were obtained in the sprayed houses in comparison with control in all cases.

Table 4. House index (%) and Container Index (%) for *Aedes aegypti* larvae post-interventions in households of Hermosillo, Mexico.

Treatment	NH	HI (%)	95% CI	NC	CI (%)	95% CI
IP	48	12.7	-6.2-31.6	321	3.3	-0.5-7.0
IP 1m	46	11.0	-3.1-25.1	199	2.6	-0.4-5.7
IRS	51	24.4	4.1-44.8	292	8.8	3.6-14.0
Control	58	16.0	4.9-27.1	320	6.8	1.4-12.3

NH: number of houses inspected; HI: House Index; NC: number of water-holding containers; CI: Container Index; 95% CI: 95% confidence interval.

Time evolution of the HI and CI showed peaks at month 2 (June) and month 6 (October) for most of the study blocks (Figure 3 and 4). Breeding sites for IP 1 m remained absent for *Ae. aegypti* up to month 4, while IRS treated houses had the greatest larval indexes in most of the surveys.

Inspection of the typical water-holding containers during all the study led to identify the preferred breeding sites of *Ae. aegypti* (Figure 5). Null presence of immature *Aedes* stages was observed in sinks, jars, big jars, wells, and toilets despite recording these objects holding water in the inspected houses. Other small containers (26.1%), tanks (17.4%), tires (10.1%), and buckets (10.1%) account for the 63.7% of the found breeding sites in the total sum of the study arms. Slight variations of the distribution of the major breeding containers were observed among the insecticide treatments and control, but no differences were detected (χ^2=43.21, p=0.11).

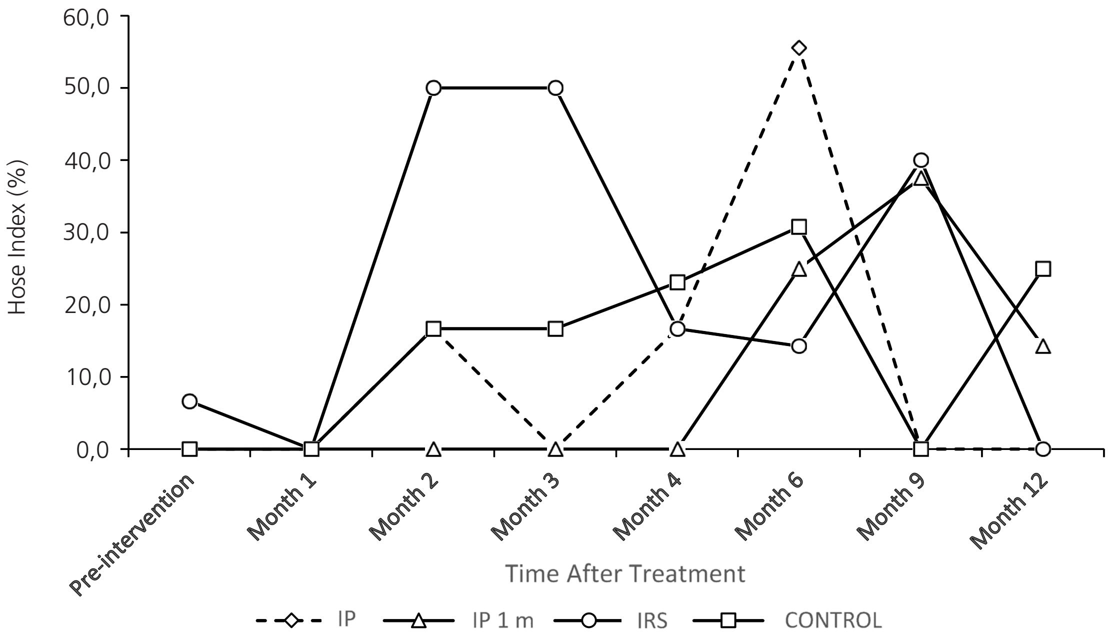

Figure 3. Evolution of the House Index (%) after interventions in households of Hermosillo, Mexico, from April 2018 to April 2019.

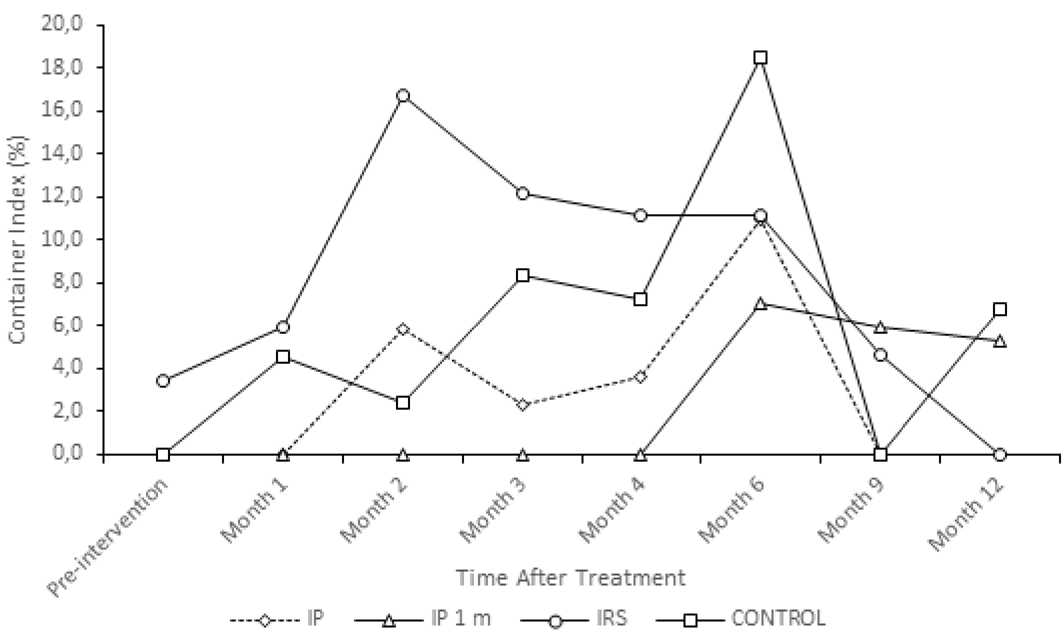

Figure 4. Evolution of the Container index (%) after interventions in households of Hermosillo, Mexico, from April 2018 to April 2019.

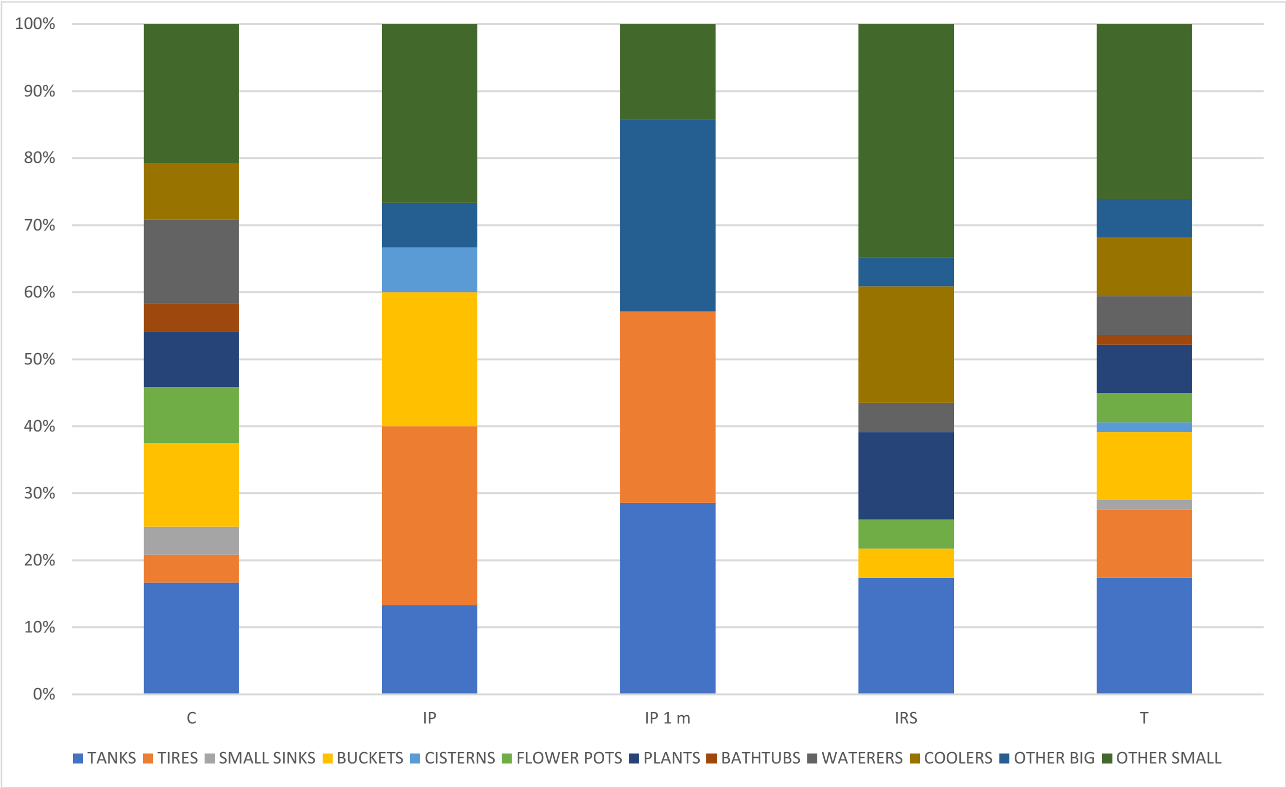

Figure 5. Percentage of *Aedes aegypti* immature stages in different water-holding containers after interventions in households of Hermosillo, Mexico, from April 2018 to April 2019. T = total.

Measurement of acetylcholinesterase in exposed residents

Overall, 119 adults across various age groups took part in the study, comprising 38.7% males and 61.3% females. These participants contributed a total of 280 blood samples: 104 were collected during the survey one week before the intervention, another 104 were obtained one week after the interventions, and the remaining 72 were collected one month after the interventions.

Cholinesterase becomes a biomarker for exposure to organophosphate and carbamate insecticides. Individual reductions of this blood parameter after exposure to propoxur in IP or IRS in comparison with baseline level and control block were considered for this analysis. Individual inhibition of cholinesterase levels was observed for seven and three residents at week 1 and month 1 after intervention, respectively (Table 5), with a maximum reduction of 18.9% recorded for IRS at 1 week. Levels were found to grow on average for all the study blocks after insecticide interventions (Table 6).

Table 5. Cases detected with cholinesterase levels inhibition among residents one week and one-month post-intervention in households of Hermosillo, Mexico.

Treatment	One week		One month	
	Cases (%)	C.L. INH (%)	Number (%)	C.L. INH (%)
IP	1 (9.1)	8.4	1 (5.9)	2.9
IP 1 m	2 (11.1)	2.2	0	0
IRS	3 (18.9)	10.3	1 (6.3)	3.7
Control	1 (5.3)	4.0	1 (6.3)	11.0

C.L. INH: individual cholinesterase level inhibition average.

Table 6. Average inhibition of individual cholinesterase levels among residents one week and one-month post-interventions in households of Hermosillo, Mexico.

Treatment	One week			One month		
	n	C.L. INH (%)	95% CI	n	C.L. INH (%)	95% CI
IP	11	-30.6	-38.5-24.8	17	-33.8	-37.9-29.7
IP 1 m	18	-31.2	-37.8-24.7	12	-26.6	-35.5-17.7
IRS	19	-32.5	-39.9-25.1	16	-30.1	-38.8-21.5
Control	16	-31.7	-38.1-25.3	16	-32.0	-40.7-23.2

n: number of individuals; C.L. INH: individual cholinesterase level inhibition average (negative values imply an increase of this biomarker in respect to baseline level).

A deeper statistical analysis showed that individual cholinesterase levels at one week did not differ significantly among intervention groups (p=0.402) or gender (p=0.999), nor interaction (p=0.242) (Table 7). However, an age effect was found (p=0.00). Similar results were found at one month after interventions, where individual differences with baseline cholinesterase levels did not differ significantly among intervention groups (p=0.501), age (p=0.192), gender (p=0.0.644), nor interaction (p=0.327). Interestingly, older people tend to show higher levels of cholinesterase (Figure 6).

Table 7. Mixed effects generalized linear model ANOVA testing for the effect of age, groups, and sex (continuous predicto) among residents one-week and one-month post-interventions with Carbapaint 10 in households of Hermosillo, Mexico.

	d.f.	Log-likelihood Type 3	Chi-square	p-value
One week				
Age	1	-134.673	15.99446	0.000064
Groups	3	-128.141	2.932	0.402229
Sex	1	-126.675	0	0.999544
Treatment*Sex	3	-128.765	4.17982	0.24269
One month				
Age	1	-96.2914	1.697259	0.192647
Groups	3	-96.6213	2.357106	0.501669
Sex	1	-95.5489	0.212445	0.644858
Treatment*Sex	3	-97.1667	3.447902	0.327582

d.f.: degrees of freedom.

Resident's perceptions of insecticide interventions

Interviews of adult residents at month 3 post-interventions lead to a total of 24 surveys from 42 houses included in the treatments (IP=10, IP 1 m=6, IRS=8), reaching a participation rate of 57,1%.

Insecticide paint and IRS were considered as effective for insect control and would be recommended to others, with slight better scores for IRS (100%) than IP (90%) and IP 1 m (83.3%) (Table 8). Similar results were recorded about the perception of died insects after painting or spraying. Although no surveyed adult found the application of paint to be difficult, one individual reported irritation. Additionally, 30% and 16.7% of paint users who applied full and targeted paint coverage on walls, respectively, reported an unpleasant smell. Full wall painting and spraying were reported by all respondents as a recommendable intervention to others.

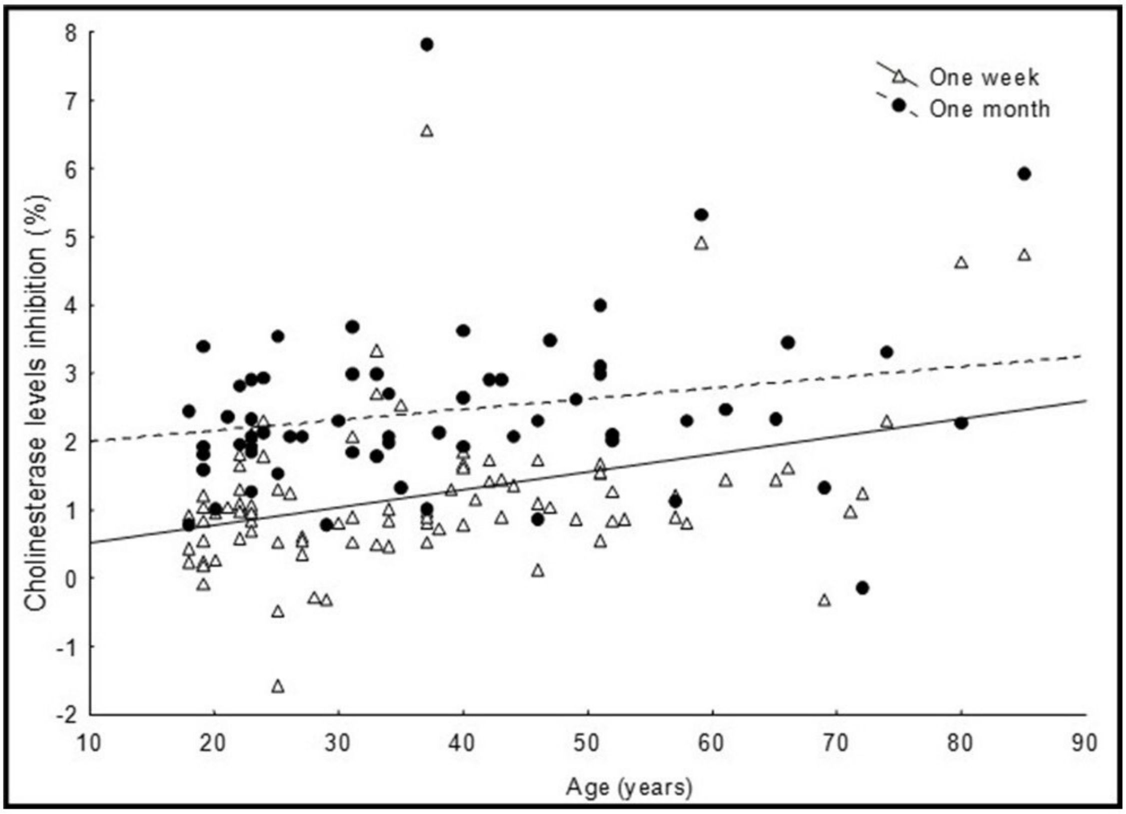

Figure 6. Age-related association of cholinesterase level inhibition (%) among residents one week and one month after interventions in households of Hermosillo, Mexico, from April 2018 to April 2019.

Table 8. Resident perceptions three-months post-interventions with households of Hermosillo, Mexico, June 2018.

Questions	IP	IP 1 m	IRS
	Affirmative (%)	Affirmative (%)	Affirmative (%)
Paint/spraying helped to control insects that affects human health	90	83.3	100
Paint was difficult to apply	0	0	NA
Paint/spraying provoked irritation or discomfort	10	0	0
Paint/spraying had unpleasant aroma	30	16.7	0
Recommendation of paint/spraying application to others	100	83.3	100
Paint/spraying helped to improve the house	100	83.3	100
Died insects observed after painting/spraying	100	83.3	100

NA: not applicable.

DISCUSSION

Entomological survey

Adult monitoring

The assessment of adult impact resulting from insecticide-based interventions relied on interior and exterior resting density as primary entomological indicators. Across all three propoxur-based treatments, comparable reductions

(43.7%, 44.9%, and 41.3% for IP, IP 1 m, and IRS, respectively) were observed in the proportion of houses testing positive for resting female *Ae. aegypti* in indoor and outdoor settings, averaged over eight follow-up surveys conducted over one year. The indoor resting density of female *Aedes* mosquitoes exhibited a close correlation with the type of formulation used and its surface coverage. The application of IP across all wall surfaces demonstrated the most substantial reduction (77.5%), followed by IP 1 m (64.2%), and finally IRS, resulting in a 30.0% reduction during the year-long evaluation. It is noteworthy that noticeable baseline densities were observed for IP and IRS, reinforcing the impact of these treatments. Moreover, the interior density of *Culex* mosquitoes was similarly affected by the insecticide treatments, showing comparable average reductions for the IP 1 m (50.0%) and IRS (57.8%) compared to the control group.

Previous laboratory studies showed that this propoxur paint achieved mortalities of 100% during six months against field collected *Anopheles gambiae s.l.* Giles, 1902 from Nasarawa State, Nigeria [39]. This long-lasting effect is aligned with other evaluations of insecticide paints containing other active ingredients addressed to *Culex* [40], and Anopheles mosquitoes [41], triatomine bugs [42], and sandflies [43]. Few field studies linking IRS with *Aedes* mosquitoes have reported both entomological and epidemiological outcomes. Notably, a deltamethrin-based IRS intervention in Peru demonstrated a noteworthy reduction in the percentage of houses positive for adult *Aedes* (both male and female) from 18.5% at baseline to 3.1% four weeks post-treatment [44]. However, control houses also exhibited a substantial reduction of all immature indices during the 16 weeks of follow-up, which may be explained by the fact that only three houses were used as untreated controls, or by the direct effect of population reduction in this suburban area.

In our study, the total *Aedes* collections were lower compared to *Culex* spp. The distribution patterns of both species were affected by the treatments, indicating a greater impact on *Aedes* populations, either through mortality or displacement from the treated houses, compared to *Culex* mosquitoes. This trend was evident in the decreasing order from IP (12.3%) to IP 1 m (24.9%), while IRS (37.7%) showed a similar proportion of *Aedes* as the control (39.2%). The disparities were more pronounced for the indoor collected mosquitoes with *Aedes* proportions of 6.2% in IP, and 40.0% in control houses, indicating the impact of interior wall treatments on *Aedes* populations. These findings align with the documented differences in insecticide tolerance among mosquito species. Our observations correspond to previous studies, where certain topical repellents have shown longer protection periods against *Culex* compared to *Aedes* species [45].

Control houses exhibited a mean and maximum interior AD of 2.5 and 7.2 *Aedes* females per positive house, respectively, with figures per total inspected house recorded at 2.2 and 7.2. These findings align with an extensive indoor aspiration sampling conducted in Iquitos, Peru, where a similar catching method revealed an interior density of fewer than 10 adults per house during the dengue season [46]. Despite the average number of mosquito density, collections had a noticeable variability during the one year follow up, recording zero *Aedes* individuals in 2, 2, 3, and 5 time series surveys for IP, IP 1 m, IRS, and control blocks, respectively (Table 3). These limited data may hinder reliable interpretations of the results, which is one of the limitations of the study. The seasonal pattern has previously been observed in Mexico for adult populations by trapping, but not equivalent records were found for interior resting density through aspiration devices [47,48].

Indoor resting density, assessed via timed adult aspiration methods, serves as a dependable entomological indicator for mosquito population [49]. However, this sampling approach is contingent upon the aspiration effort (measured in minutes of activity) and can be susceptible to collector variability, and housing characteristics, often exhibiting notable bias in low mosquito density scenarios. An improved technique, known as sequential removal sampling, employed with the same Prokopack aspirators, resulted in a substantial five-fold increase in adult collections compared to the standard 10-minute practice [50].

Larval monitoring

The impact of insecticide paint on *Aedes* breeding exhibited a comparable effect across both paint interventions, resulting in a substantial reduction of HI by 20.1% (IP) and 31.2% (IP 1 m), and notably CI by 51.8% (IP) and 61.7% (IP

1 m) during the year-long surveys compared to the control group. Conversely, the IRS treated area witnessed an increase in both indexes (HI 52.5%; CI 29.4%). As per PAHO guidelines [51], an area is deemed at high risk of transmission when these indices surpass the 5% threshold for HI, and 3% for CI. Consequently, the yearly average HI across the entire study blocks indicated a high infestation level; however, the direct association with the risk of dengue outbreak remains uncertain despite numerous studies [52]. This variability could likely be attributed to a limited number of treated houses within each block (≤70%), leading to incomplete coverage of the interventions.

In Peru, a noticeable reduction in all immature indices was observed following an IRS intervention using deltamethrin, despite no significant differences from the baseline [44]. A similar pattern emerged in Taiwan, where areas exhibiting Breteau Index rates exceeding 35% underwent Alpha-cypermethrin spraying on interior walls and the undersurfaces of furniture. This intervention resulted in a decline of the larval index to 1% after three years [53]. However, another intervention involving long-lasting insecticidal nets installed on doors and windows did not exhibit significant differences in HI and CI five months post-intervention between treated and untreated houses. Nevertheless, these Alpha-cypermethrin-impregnated nets showcased a significant impact on pupae-based indicators at 12 months post-intervention in this comprehensive study conducted in Mexico [54].

Aedes mosquitoes are known to disperse their eggs in several breeding sites, behavior named as "skip-oviposition". Our study revealed that small to medium-sized containers such as buckets, tires, and tanks were the preferred ones, accounting for 63.7% of the total water-holding containers with presence of immatures. These domestic man-made containers were also found to be prevalent in other Latin American countries, such as Guatemala [55], Cuba [56], or the Dominican Republic [57]. Indeed, in this country on the island of Hispaniola, solid urban waste and discarded tires have also been reported as preferred sites for *Aedes* mosquitoes outside household settings [58,59]. Despite all these known productive breeding sites, elimination or control remains difficult for individuals, community, and authorities. In this sense, public awareness campaigns, particularly educational initiatives implemented in schools, yield positive outcomes when consistently maintained, fostering behavioral changes [60].

Measurement of acetylcholinesterase in exposed residents

Pesticides of organophosphorus and carbamate compounds are extensively used to control insects in public health, agriculture, and veterinary clinical practice [61]. The assessment of blood cholinesterase activity in tested individuals is commonly regarded as a biosecurity marker for monitoring the exposure of both applicators and residents before and after the application of organophosphate or carbamate compounds. A study in Brazil identified 4.6% of small-scale agricultural workers with inhibition levels exceeding 30% compared to the average control population [62]. In Haiti's national malaria program, monitoring blood cholinesterase activity in residents living in sprayed houses with malathion and fenitrothion revealed that individuals did not exhibit inhibitions higher than 25% of baseline levels at one day and seven days post-spraying [63]. Similarly, a study in South Iran involving 925 residents exposed to fenitrothion IRS treatment showed no significant individual changes in cholinesterase levels before and after spraying [64]. In Nigeria, propoxur exposure assessment among residents in sprayed houses was conducted through blood cholinesterase measurements involving 10 and 16 individuals. The analysis reported reductions of 5.8% and 0% at one and six days after treatment, respectively [65].

In our study, following the application of carbamate paints and IRS, the blood acetylcholinesterase levels of the tested volunteers did not surpass the inhibition limit set by Mexican Authorities (30%), and did not significantly differ from the control group. These findings align with numerous evaluations conducted in previous studies assessing other organophosphate and carbamate insecticides.

Resident's perceptions of insecticide interventions

Resident acceptance and satisfaction with insecticide treatments for vector control are pivotal for sustaining effective public health interventions. Indoor residual spraying campaigns are generally well received in malaria-endemic regions, with low refusal rates noted. For instance, a study involving 834 household interviews in South-East Iran reported an acceptance rate of 94% [66]. Similarly, in two villages in Malawi, satisfaction levels among interviewees ranged from 69% to 60.9% [67]. Factors contributing to this satisfaction included minimal adverse effects of the chemicals post-spraying, and the elimination of insects other than mosquitoes, which were viewed as positive outcomes. Furthermore, IRS initiatives for leishmaniosis prevention in Bangladesh were also widely accepted by residents, with an acceptance rate of 85.3% [68]. Nevertheless, there is a scarcity of acceptability and satisfaction studies concerning IP due to their novelty and limited use as a widespread vector control tool. An evaluation conducted in Nepal focusing on sandfly control highlighted the long-lasting effects of insecticide wall painting, which was well received by the communities [43]. Only 5.9% of respondents reported experiencing side effects, such as headaches and itching.

In our study, over 80% of the interviewed residents expressed satisfaction with the effectiveness of both IP and IRS treatments. They found the application of paint relatively straightforward and would recommend it as an intervention method. Partial wall painting obtained slightly lower scores in the efficacy and acceptability related questions. Despite the low number of respondents of this block (6), this should be considered for future investigations in a higher level of detail in terms of entomological outcomes and perceptions including the aesthetic aspect. There is growing evidence of the efficacy of targeted IRS for *Ae. aegypti* control [69,70] that could be addressed with IP under an equivalent concept. However, 30% and 16.7% of paint users who applied full and targeted paint coverage, respectively, reported an unpleasant smell. Additionally, one case (4.1%) of irritation/discomfort was recorded.

Future challenges

The ongoing battle against dengue-carrying mosquitoes in Latin America confronts imminent challenges. In 2023, all four serotypes of the dengue virus (DENV1, DENV2, DENV3, and DENV4) circulated throughout South America, the Central American Isthmus, and Mexico [4]. This disease remains a significant challenge for health authorities due to its near-continuous transmission pattern, impeding economic and social development across several countries in the Americas [71].

Understanding lesser-known arboviruses and the factors contributing to their emergence and resurgence in the region has become crucial. Some of these arboviruses, such as the West Nile virus, have undergone a significant geographic expansion into the Americas and pose a threat of becoming future pandemics for the region [72]. Not only do these viruses pose a threat to public health, but the emergence of other invasive mosquito species, such as *Aedes* japonicus (Theobald, 1901), reported in North America [73,74], and *Aedes* vittatus (Bigot, 1861), recently identified in the insular Caribbean [75,76], also presents a significant risk. Their potential expansion into new regions across the Americas could drastically alter the epidemiological landscape.

Innovative approaches such as novel paint technology show promise but raise concerns over ecological impact and community acceptance, among others [77]. Addressing these challenges requires collaborative efforts spanning multiple disciplines, fortified surveillance systems, active community engagement, and the implementation of sustainable interventions. The integration of the One Health approach, which considers the interconnectedness of human, animal, and environmental health, proves indispensable in devising comprehensive strategies to combat vector-borne diseases [78]. Moreover, the prioritization of investments in public health infrastructure and education stands as a pivotal factor in effectively mitigating and preventing future outbreaks.

Concluding remarks

Our study suggests that the application of the propoxur paint by homeowners as a complete covering of interior walls, or as targeted indoor painting, can be a highly safe and accepted intervention method for effective density reduction of *Ae. aegypti* populations in urban environments. In this regard, it remains crucial to persist in gathering scientific evidence concerning the efficacy of insecticide paints. Future research efforts will play a pivotal role in addressing the knowledge gaps surrounding this subject.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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