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RECEIVED 16 October 2023

ACCEPTED 08 January 2024

PUBLISHED 05 March 2024

CITATION

Gómez LF, Rocha HDR, Torró IG, Pérez IS,
Mendes DC, Silva KLF, Monteiro DDSR,
Dos Reis JPT, Leal SV, Soulé LFV, Fortes JC,
Mendonça MdLL, Mendez EC and
Hernández BV (2024) Insecticide
paints: a new community strategy for
controlling dengue and zika mosquito
vectors in Cabo Verde.
Front. Trop. Dis 5:1321687.
doi: 10.3389/ftid.2024.1321687

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Insecticide paints: a new community strategy for controlling dengue and zika mosquito vectors in Cabo Verde

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Background: Cabo Verde, an island country in West Africa, has been affected since human colonization by epidemics of vector-borne diseases with major epidemics of dengue and zika in recent years. Although there is a national program for integrated vector control, innovative strategies that reinforce routine activities and strengthen vector control are necessary to prevent the emergence or reemergence of arboviruses and new epidemics of dengue and zika. Insecticide paints are evidenced as new technologies for the formulation of insecticides in a more residual and safe way. The TINTAEDES project aimed to assess the efficacy, acceptability, and operational deployment of an insecticide paint for *Aedes* control.

Methodology/Principal findings: Laboratory and small-scale field trials were conducted, assessing mortality through World Health Organization cone bioassays. A community-based intervention study in the neighborhoods of Várzea and Tira Chapéu in the city of Praia, Cabo Verde, was developed. The intervention is a paint self-application model by homeowners and neighborhood volunteers. The intervention was evaluated based on entomological indicators and the responses given by the residents of the painted houses to a questionnaire on the knowledge, satisfaction, and safety of insecticidal paints. A transfluthrin-based insecticide paint was effective against wild *Ae. aegypti* for one year in the laboratory and semi-field conditions. Residents largely perceived a reduction in mosquito presence in the treated houses (98%).

Conclusion: Insecticide paints are presented as an effective innovation strategy for mosquito control, which could be implemented as a reinforcement of the measures carried out by the vector control program in the city of Praia and throughout the country.

KEYWORDS

dengue, zika, insecticide paint, vector control, community, TINTAEDES project, Cabo Verde

Introduction

Vector-borne diseases (VBDs) are a global health threat, with more than 17% of all infectious diseases disproportionately affecting the poorest populations in tropical and subtropical areas. Among them, malaria is the most severe and dengue is the most prevalent and widely distributed (1). Both are transmitted by mosquitoes, the main vectors of human diseases, and responsible for a large part of current emerging and reemerging diseases such as zika, chikungunya, yellow fever, and West Nile Fever (2).

Despite the efforts made by the national health programs of many countries against VBDs, their control and prevention continue to be a challenge (3). Considering the effects of climate change, an expected rise in the global burden of these diseases is linked to the concurrent increases in abundance and geographic distribution of their vectors (4). To reverse this situation, in 2017, the World Health Organization (WHO) launched the Global Vector Control Response 2017-2030, with strategic guidelines to strengthen vector control as a fundamental approach for the prevention and response to VBDs (5). One of the two main pillars of this strategy is increased research and innovation to develop new tools, technologies, and approaches to control vectors within the field of insecticides, which continues to be the main intervention strategy in vector control programs. New products and technologies have emerged in recent years such as insecticide paints (IPs) for indoor mosquito bite prevention and population control (6). IPs are safe for humans and animals and have limited environmental impact, and because they are available in a wide range of formulations, they represent a viable alternative for managing insecticide resistance, which constitutes a global challenge for VBD control programs (7–9).

IPs have shown high efficacy with long-lasting effects against several insect vectors like *Anopheles* (10, 11) and *Aedes* (12) mosquitoes, triatomine bugs (13, 14), sandflies (15, 16), and tsetse flies (17).

Above all, this innovative tool is effective for the control of the invasive Aedine species, which proliferate in human habitations and are particularly successful in locations with high human and house densities (9), favoring the rapid transmission of arboviruses. In contrast

to the continued and repeated use of indoor residual spraying (IRS), IP exerts constant and lasting pressure on mosquitoes over longer periods.

Another vector control strategy is based on products that exert spatial repellency properties, like formulations of the volatile pyrethroid transfluthrin (TFL) (18). The use of insecticides with repellent potential is likely to allow the control of the transmission of mosquito-borne diseases both by mosquito bite prevention and by its killing effect (19–21). In this study, we only present the evaluation of the lethal effect of transfluthrin.

Cabo Verde, an African archipelagic country of the subtropical region is located at the crossroads of three continents (America, Africa, and Europe). Due to its geographical localization, climate change, and the effects of the intense traffic of people and goods, it is extremely vulnerable to the emergence and reemergence of VBDs (22). Environmental factors like temperature, relative humidity, and rainfall have demonstrated a positive correlation with malaria cases in the country (23). Mosquito-borne diseases are part of its history, with malaria and yellow fever epidemics since the 16th century and more recently dengue and zika, the latter being recorded as the largest outbreak in sub-Saharan Africa (24–27). Nowadays the country has the mosquito species *Aedes aegypti* and *Anopheles arabiensis* registered as the main vectors of arbovirus and malaria, respectively (28–31). Additionally, the established population of *Culex pipiens s.l.* is a potential vector for other arboviruses such as West Nile and Rift Valley fever, both being present on the African mainland (32).

Vector control in Cabo Verde began in 1948 with interventions for malaria prevention based on the indoor spraying of dichlorodiphenyltrichloroethane (DDT) and the treatment of larval habitats with petroleum derivatives or larvivores fish (33). In 1979, these activities were reinforced with the introduction of the larvicide temephos (33). In the past, the success of this strategy has allowed the interruption of malaria transmission twice (34). In 1999, DDT was replaced by the pyrethroid deltamethrin following WHO recommendations for IRS interventions. After the dengue epidemic in 2009/10, integrated vector control was implemented against the main two disease-transmitting mosquito species. Physical/mechanical control, health education, and social mobilization actions are also included in the strategy (35). Despite the integration of other measures complementary to chemical control, *Ae. aegypti* and *An. arabiensis* have developed resistance to temephos and deltamethrin (36–41).

Abbreviations: IP, insecticide paint; OVT, ovitrap.

Inesfly Vesta contains transfluthrin, a polyfluorinated pyrethroid that acts by interacting with voltage-gated sodium channels in insects' neurons. The slight volatility of this pyrethroid exerts some airborne effects on mosquitoes that are included in the concept of spatial repellency (42). Vesta paint has shown long-lasting contact and airborne activity against several mosquito species and sandflies under laboratory conditions (43, 44). INESFLY Ares is composed of a combination of an organophosphate (pirimiphos methyl) and an insect growth regulator (IGR) (pyriproxyfen) aiming to target adults via the inhibition of the enzyme acetylcholinesterase leading to excess neurotransmitter acetylcholine (organophosphate) and sterilizing exposed females through the IGR. This paint has achieved nearly one year of efficacy against *Aedes* mosquitoes in laboratory trials (unpublished data). Finally, Inesfly 5A IGR contains two organophosphate insecticides (diazinon and chlorpyrifos) as well as an IGR (pyriproxyfen) and it was widely tested against several insect vectors like triatomine bugs (13, 14) and *Anopheles* mosquitoes (10, 11).

The TINTAEDES project was implemented in 2022 in two neighborhoods of Praia vulnerable to vector-borne diseases (VBDs) as a scientific and social intervention for controlling mosquitoes under a community-based approach.

The main objectives of the study reported here were to evaluate the efficacy, acceptability, and satisfaction of a new *Aedes* control strategy based on an IP in residential environments.

Methods

Study sites

With nearly half a million inhabitants (45), Cabo Verde has experienced a rapid growth in the urban population in the last 30

years with the appearance of overcrowded neighborhoods without adequate planning or urban infrastructure that led to poor housing conditions (46). The country's capital, Praia, with a quarter of the total population, is the city with the greatest social inequalities and has also been the population most affected by VBDs. Várzea and Tira Chapéu are among the most vulnerable neighborhoods to arbovirus epidemics in the capital (Figure 1).

In vitro bioassays to evaluate the efficacy of the IPs were carried out in the laboratory of the Tropical Diseases Research Group of Jean Piaget University (GIDTPIaget), located on the campus of Praia (W023°32'12.408", N 14°55'19.344").

Three buildings located in Praia were treated with an IP for a small-scale field trial: Pensamento Kindergarten (W023°31'00.48"; N14°55'59.24"), Tira Chapéu Health Center (W023°31'18.948 "; N14°55'1.1418"), and Jean Piaget University of Cabo Verde.

The field trial was carried out in two central neighborhoods of Praia, the capital of Cabo Verde, located on Santiago Island: Várzea (5340 inhab) and Tira Chapéu (6391 inhab). Both sites lack proper territorial planning and are in low-lying areas of the city with insufficient drainage that leads to flooding in the rainy season. Drinking water supply and wastewater disposal are deficient as well. Houses are built mainly of brick and cement, some are unpainted and in need of repairs to the walls before being painted, for example, due to the presence of humidity. In Tira Chapéu, worse buildings were observed, with houses that need plastering before being painted. Concerning the size of the houses, there is no standard size, with the smallest residences presenting values of less than 10 m², and the larger residences with values of over 150 m².

Specific areas of interest were selected in each neighborhood for the treatment with the IP. This selection was made based on the results of a screening of the presence and density of *Ae. aegypti* eggs in forty potential hotspots shown by the local communities through ovitraps (OVTs) monitoring.

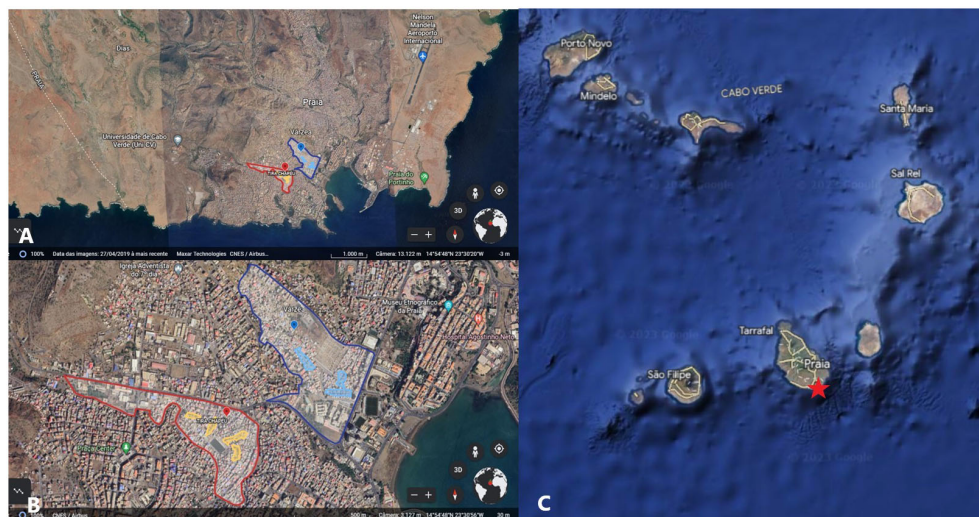


FIGURE 1

Study Sites: (A) Praia; (B) Várzea and Tira Chapéu neighborhoods; and (C) Cabo Verde. The red star indicates Praia, the red lines indicate Tira Chapéu, and the blue lines indicate Várzea.

Sample collection and identification

The homemade OVTs were adapted from the Fay and Perry model (47), using Polyethylene Terephthalate (PET) bottles as a container and wooden pallets as an oviposition substrate. Forty OVTs filled with tap water were distributed in the study area and placed outdoors, and the pallets were collected after seven days. Larvae were obtained from wild-caught eggs and placed in white plastic trays containing 1L of tap water for 100–150 eggs. Before larval hatching, the eggs were conditioned by maintenance in a humid substrate for 24 hours. The larvae were reared with crushed and autoclaved flocculated fish food (Tetramin). The amount of daily food for larvae of the L1 and L2 stages was 0.003g per tray and for the L3 and L4 stages was 0.006g per tray. The water was replaced every day, transferring the larvae to containers with clean water with a Pasteur pipette.

Emerged adult mosquitoes were separated by sex in cages containing approximately 100 mosquitoes and fed ad-libitum with a 10% autoclaved sugar solution. Three-to-five-day old sugar-fed adult females were used in the bioassays.

Larvae and adult mosquitoes were maintained in standard insectary conditions at a temperature of $25 \pm 2^\circ\text{C}$, $75 \pm 10\%$ relative humidity, and 12:12h photoperiod (48). For larval and adult mosquito identification, the taxonomic key of Ribeiro et al. (24) was used. For the maintenance and feeding of mosquitoes in the laboratory, the procedures described in Consoli et al. were followed (48).

Bioassays were conducted with wild-caught adult females of *Ae. aegypti* collected in the egg stage with OVTs placed in several neighborhoods of Praia (Fonton, Palmarejo, Palmarejo Grande, Tira Chapéu, and Várzea).

Laboratory studies

An initial laboratory efficacy study was conducted with three water-based IP formulations (Inesfly Corporation S.L, Paiporta, Spain): VESTA (Transfluthrin 0.5%), ARES (Pirimiphos-methyl 1.0%, Pyriproxyfen 0.1%), and 5A IGR (Chlorpyrifos 1.5%, Diazinon 1.5%, Pyriproxyfen 0.063%). The TFL-based one (VESTA) was selected for the pilot trial conducted in the three mentioned buildings and the field assay at Várzea and Tira Chapéu neighborhoods. The IGR pyriproxyfen was included in two of the paints to achieve a complementary mode of action to the adulticides. Pyriproxyfen exerts effects on the reproductive capacity of mosquito females and a reduction in fecundity, fertility, and larval development was observed in the surviving females exposed to INESFLY 5A IGR paint (49).

IPs were applied undiluted to porous (unglazed tile) and non-porous (glazed tile) substrates by brushing in a single layer at the recommended dose of $10 \text{ m}^2/\text{L}$ (44, 49). Treated tiles were dried and stored under interior conditions during the testing period.

WHO cone bioassays (50) were conducted for the three IPs and a water-based non-insecticide paint Cináqua (SITA S.L, Praia, Cabo Verde) as control.

Four replicates of ten *Ae. aegypti* females were inserted in the plastic cone attached to the painted surfaces and kept for thirty

minutes. After this exposure time, all individuals were gently aspirated and placed into clean resting paper cups and provided with cotton soaked in a 10% sugar solution. The number of dead mosquitoes was recorded 24 hours after the exposure.

The residual efficacy of the IPs was assessed through the cone test at one week, one month, three months, six months, and one year after paint application to both substrates.

Small-scale field trials

The outcomes of the laboratory study led to the selection of TFL paint for further testing under field conditions.

Selected rooms of the kindergarten, health center, and university were painted with IP by six professional painters. Two layers of paint were applied with a brush and roller to achieve good coverage and a homogeneous wall aspect.

The WHO cone bioassay was performed directly on the walls painted with IP and on the non-treated walls (kindergarten and health center) or those treated with the non-insecticide paint (university). The IP treated walls and control walls were in different rooms or buildings. Plastic cones were attached at 0.5, 1, and 1.5 meters in height to the floor and fifteen female mosquitoes were exposed for 30 minutes inside the plastic cone following the procedure described above. Residual efficacy was measured at one week, one month, six months, and twelve months after painting.

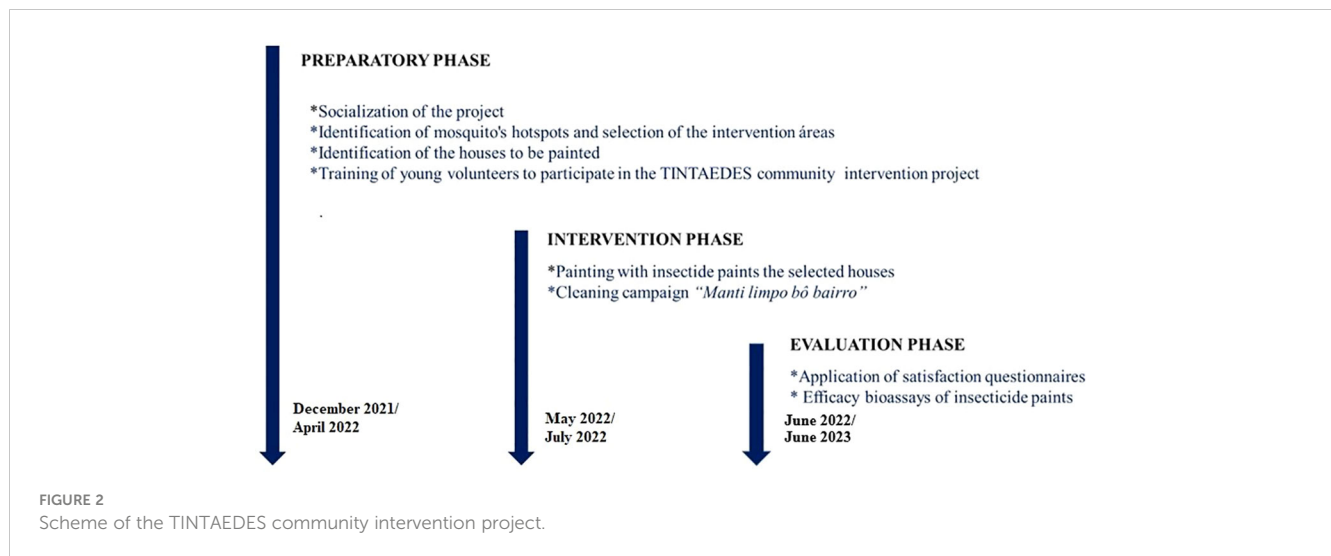
Painters and building workers were surveyed with a structured questionnaire about their perceptions and satisfaction (Supplementary Data Sheet 1).

Large-scale field trial (the TINTAEDES project)

The TINTAEDES project was designed in three phases (Figure 2). Mosquito hotspots were identified using forty OVTs deployed in each neighborhood and placed in collaboration with the community. OVTs were monitored weekly for one month during the preparatory phase for the selection of the specific intervention areas with the IP.

Before starting the intervention with the IP, unemployed young volunteers from the neighborhoods of Várzea and Tira Chapéu were trained as community health agents and painters with knowledge of the management and application of the IP, including the use of personal protection equipment and biosecurity measures. The selected houses were painted by trained community volunteers who helped the residents under the supervision and on-site monitoring of the community associations of Várzea and Tira Chapéu, as well as by the project team made up of researchers from the university and technicians from the local paint company (Supplementary Table S1). Personal protective equipment (gloves and goggles) was provided to the painters and empty buckets and painting tools were collected and disposed of accordingly.

Two layers of paint were applied only to the interior walls and total consumption was estimated at $5 \text{ m}^2/\text{L}$ according to



manufacturer's recommendations. Some houses required previous putty or cement surface conditioning and priming before the IP application.

WHO cone bioassays were conducted at months one, three, six, and twelve post-treatments in two random houses of each neighborhood as described before, and one unpainted house served as control per neighborhood.

Egg monitoring was performed in the treated houses with OVTs placed indoors for one month before and after the paint application.

A structured questionnaire survey was carried out to the adult residents of the houses treated with the IP one month after painting (Supplementary Data Sheet 2). The interviewees were asked about their knowledge, perception, and satisfaction concerning IP, its efficacy, and its perceived adverse effects.

The project was presented to the collaborating entities (Supplementary Table S1) and the communities of the two selected neighborhoods (Várzea and Tira Chapéu). Residents were informed about the project personally and through the neighborhood associations. Adult household members could decide to participate in the study and have their house treated with the IP. The two community associations and the kindergarten, health center, and university that participated in the small-scale field trial provided their consent through a written declaration (Supplementary Data Sheet 3) about their participation and collaboration with the TINTAEDES Project.

Statistical analysis

A descriptive analysis was performed, using the software Excel 2021 to present the results obtained. The presentation of the measured variables was done through tables or graphs including the use of some descriptive measures such as median, average, and standard deviation. Two different indices were used to estimate the vector population density and its distribution: the positive OVT index (POI), which is the proportion of ovitraps positive for the presence of *Aedes* eggs, and the density eggs index (DEI), the average number of eggs per positive OVT. To estimate whether

the number of houses painted with IP was a significant sample, Cochran's formula to estimate the size of a sample for finite populations was used for an assumed error of $e = 0.06$ and a total of 1,315 paintable residences (51). The number of houses estimated to be painted was 210 houses. Parametric (Student's *t*) and non-parametric (Mann-Whitney and Krustal-Wallis) tests were performed to demonstrate the effectiveness of the PI when comparing the mortality of mosquitoes exposed to treated surfaces concerning untreated surfaces (controls).

Results

Laboratory studies

All three IPs exerted complete mortality (24 h after exposure) to wild-caught *Ae. aegypti* female populations from the city of Praia at one month post paint application on both substrates and exceeded the WHO efficacy threshold (80%) at three months. The efficacy of 5A IGR and ARES was below 80% at month six, while VESTA accomplished the WHO requirement for 12 months when applied to porous substrates. The efficacy of the three IPs was higher in treated porous surfaces except for the transfluthrin paint in the 12-month follow-up evaluation. Mortality recorded for the control paint was less than 10% in all the tests validating the results of the assay (Figure 3). The differences in mortality observed between the treated surfaces and the controls were significant when compared, at all times of evaluation, both for the porous surface (Mann-Whitney p -value = 0.02) and for the non-porous surface (Krustal-Wallis p -value = 0, 05).

Small-scale field trial

The exposure of *Ae. aegypti* females to TFL paint in the cone bioassay led to complete 24-hour delayed mortality at the University Jean Piaget and the kindergarten during the six months after paint application, while showing an average of 98% in the health center. One year follow-up showed mortality rates

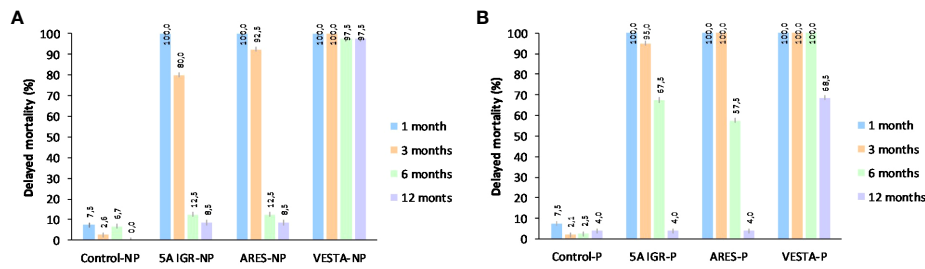


FIGURE 3 Delayed mortality of wild-caught female *Ae. aegypti* exposed to surfaces painted with IPs over 12 months. (A) VESTA-NP, ARES-NP, 5A IGR-NP, and Control-NP applied on porous surfaces. (B) VESTA-P, ARES-P, 5A IGR-P, and Control-P applied on non-porous surfaces. The plotted bars represent the standard deviation.

above the WHO efficacy threshold (80%) in all three buildings. The mortality of mosquitoes on the control walls of the three buildings was less than 2.5% in all assays, validating all the tests carried out (Figure 4). The differences in mortality observed between the treated surfaces and the controls were significant for all three buildings when compared, with Student’s t p-values = 0.0001 for the university and health center and Mann–Whitney p-value = 0.02 for the kindergarten.

The survey conducted on the painters (6) indicated that four (67%) liked painting the IP, five (83%) would like to paint their house with this IP, three (50%) noticed that insects disappeared during paint applications, and none felt any discomfort during and after painting.

Eleven workers from Tira Chapéu Health Center and four monitors of the Pensamento Kindergarten were surveyed one month after painting.

Although the workers and users of the painted buildings were previously informed about the functionality of the IP, all interviewees from the kindergarten and five (45%) from the health center perceived fewer mosquitoes since the paint application. No discomfort in the workplace after painting with the IP was reported in their answers.

Large-scale field trial (the TINTAEDES project)

Monitoring of *Ae. aegypti* presence in the two neighborhoods for one month and weekly replacement of the wooden pallets showed different positivity and egg density (Table 1). To paint houses with IP in Várzea and Tira Chapéu, those with the highest egg density during monitoring were selected as intervention areas.

A total of 120 houses in Várzea and 108 in Tira Chapéu distributed in three and four clusters, respectively, were selected for the field trial. Five point two hundred forty liters of IP was consumed for treating these 228 houses, corresponding to an average application of 23 L per household.

Thirty-four young volunteers were trained from Várzea (20) and Tira Chapéu (14) as health agents and painters with knowledge of IP and their application. The training outline, the training self-assessment questionnaire, and the responses obtained can be found in Supplementary Data Sheet 4. The number of houses selected was defined by the volume of paint available for this project and it was considered significant according to the minimum estimated size of 210 houses painted with IP (e = 6%, CI = 95%).

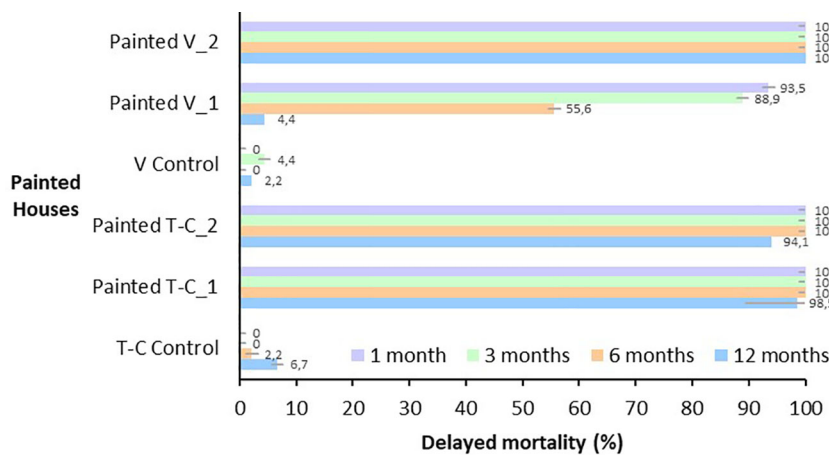


FIGURE 4 Delayed mortality of *Aedes aegypti* females in buildings painted with TFL paint. Univ., University Jean Piaget; KG, Kindergarten; and H-C, Health Center. The plotted bars represent the standard deviation.

TABLE 1 Monitoring results of *Ae. aegypti* with OVTs in Várzea and Tira Chapéu areas before the intervention with IP.

Zone Várzea	Total OVT	Egg number	Egg mean	Standard deviation	POI (%)	DEI
Santaninha	14	1,997	142.6	163.0	92.8	153.6
Centro	18	1,317	73.1	123.0	83.3	87.8
Floresta	8	280	35.0	40.0	100.0	35.0
Zone Tira Chapéu	Total OVT	Egg number	Egg mean	Standard deviation	POI (%)	DEI
Centre A	8	239	29.9	54.9	75.0	39.8
Centre B	9	78	8.7	16.6	22.2	39.0
Cancha	7	214	30.6	63.5	28.6	81.0
Fogo Africa	7	279	39.9	47.9	42.9	93.0
Zona C-D	9	0	0	0	0	0

POI, mean positive ovitrap index; DEI, mean density eggs index.

The presence of *Ae. aegypti* was monitored through OVTs before and after the intervention with the insecticide paint. Because in many of the houses, no residents were found during the monitoring period, as well as the delay in painting the houses by the residents, it was only possible to evaluate 47 and 60 houses in Várzea and Tira Chapéu, respectively, before painting the selected residences with IP, and 31 and 18 houses after the IP painting. The absence of positive OVTs was found after paint application in both areas (Table 2). Control houses showed complete positivity during the monitoring and after IP intervention, reflecting the presence of *Ae. aegypti* in the houses during the study period.

Nearly half of the families joining the project voluntarily responded to the questionnaire 105 families with the house painted with IP from the Tira Chapéu and Várzea neighbourhoods (Figures 5, 6).

A large majority (70%) of residents were aware that the IP was used for controlling mosquitoes and nearly all perceived fewer mosquitoes (98%) and other insects (42%). Among the respondents, 41% stated that the house seemed cleaner and only 35% knew that personal safety measures were required to paint houses. Adverse effects were reported by 16.7%, describing

discomfort only in the first days after paint applications with mild symptoms including eye and/or nose irritation (10%), dullness (4%), and headache (4%) (Figure 7).

To analyze if there was any association in the results obtained of the satisfaction questionnaire to the neighborhood of origin where they were applied (Várzea or Tira Chapéu), a statistical analysis was carried out for independent samples (Table 3).

Although no pronounced differences were observed between the responses obtained by the residents of Tira Chapéu and Várzea, the independent analysis of the variables revealed a lower degree of knowledge about the paintings ($P = 0.0105$) and a higher degree of discomfort ($P = 0.0002$) after the PI intervention in the Várzea neighborhood. In turn, a higher degree of satisfaction was observed in the Tira Chapéu neighborhood (0.0239).

Similar results of lack of knowledge females exposed to painted walls in the two houses in Tira Chapéu measured using the cone bioassay during the three months post paint application (Figure 8). In Várzea, one of the tested houses reached 100% mortality during the twelve-month study period, while the other one was above 85% in the third month of follow-up evaluation and dropped to 55% at

TABLE 2 Monitoring results of *Ae. aegypti* with OVTs placed inside the houses in Várzea and Tira Chapéu before and after the intervention with IP.

	Total OVT	Total Samples collected	Total Egg number	Egg mean/samples	Standard deviation	POI (%)	DEI
Várzea, before IP	47	123	1,422	37.2	10	44.6	25.8
Várzea, After IP	18	58	0	0	0	0	0
Várzea Control	1	4	572	143	0	100	143
Tira Chapeu before IP	60	136	5,091	11.6	15.3	43.3	85.9
Tira Chapeu after IP	31	67	0	0	0	0	0
Tira Chapeu Control	1	4	295	73.75	0	100	73.5

POI, mean positive OVT index; DEI, mean density eggs index.



FIGURE 5
Clusters of houses treated with IP in the Várzea neighborhood.

six months follow-up. The delayed mortality of mosquitoes exposed to the walls of the control houses (not painted with IP) was less than 10%, validating all the tests carried out.

Discussion

This study evaluated the efficacy of IPs against *Ae. aegypti* in Cabo Verde and its effectiveness as an intervention strategy for the intra-domiciliary control of *Aedes*-borne diseases in two neighborhoods of the country's capital. The laboratory trials with three paint formulations showed a one-year residual effect of the TFL-based paint against wild females, while the organophosphate ones only reached the efficacy threshold for three months (Figure 2). This low residuality can be attributed to the resistance to temephos larvicide detected in the population of *Ae. aegypti* from Praia, Cabo Verde (37, 39). This IP containing chlorpyrifos, diazinon, and pyriproxyfen killed completely susceptible and organophosphate-resistant strains of *Cx. quinquefasciatus* up to one year after paint application on a non-absorbent surface (49). The same paint applied on a plastic sheet provided again one-year efficacy against susceptible *An. gambiae* and pyrethroid-resistant *An. coluzzi* in a

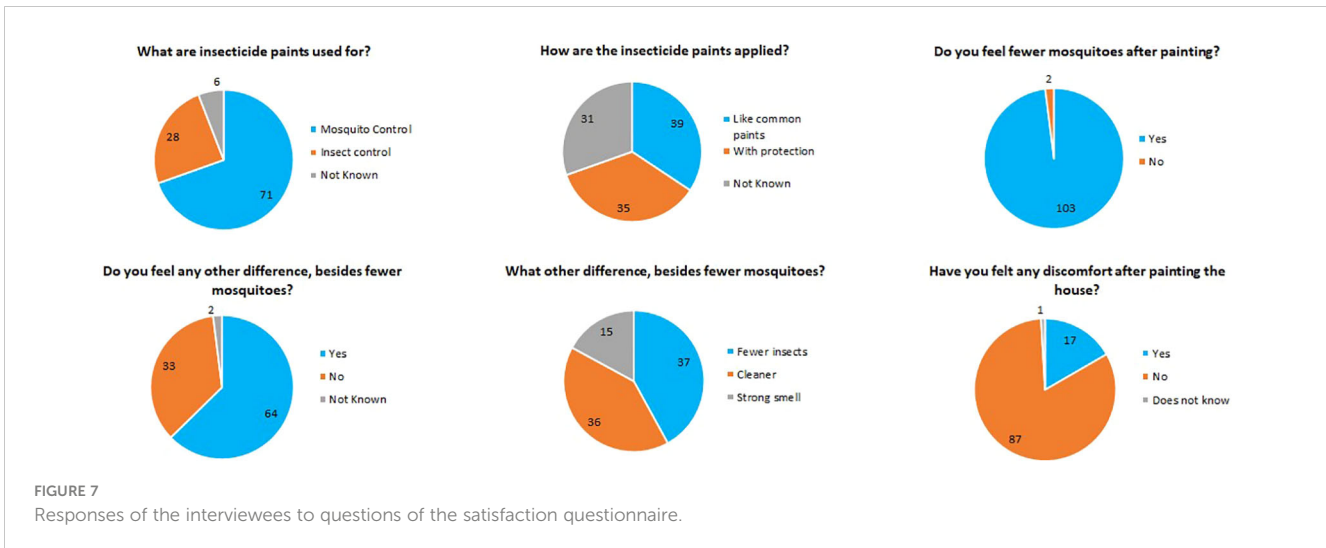
field trial conducted in Burkina Faso (11). Exposure of *Ae. albopictus* to this IP showed 100% mortality for two years (52).

TFL is a volatile pyrethroid widely used in anti-mosquito consumer products like coils, mats, and vaporizers, exerting killing and repelling airborne effects. TFL has widely been evaluated in many vapors releasing impregnated materials against *Anopheles* (53–55) and *Aedes* (56) mosquitoes. The inclusion of TFL in a paint formulation exploits the contact effect in addition to the airborne. Laboratory studies with susceptible *Ae. albopictus* and *Phlebotomus papatasi* indicated a residual effect of 22 months in the WHO cone bioassay (57). This residuality exceeds those obtained with the third-generation IRS formulations that reached 12 months for the mixture of clothianidin and deltamethrin.

Substrate characteristics like absorbency have a noticeable influence on the performance of insecticides deposited. In our study, the efficacy and residuality of the three paints were higher in non-porous treated surfaces except for the TFL paint in the 12-month follow-up evaluation (Figure 2). Mosqueira et al. (51) observed longer residuality when an organophosphate paint was applied on non-absorbent materials (softwood and hard plastic) in comparison to absorbent (cement and stucco). However, they observed that the chemical composition of the substrate had a



FIGURE 6
Clusters of houses treated with IP in the Tira Chapeu neighborhood.



greater influence because *Cx. quinquefasciatus* mortality was 2% and 91.2% at 12 months after paint application for cement and stucco, respectively. Two indoor residual IRS formulations showed a similar pattern and provided higher residuality against *An. arabiensis* on painted concrete (non-absorbent) than on baked clay (absorbent) (58).

The small-scale field trial with the TFL paint confirmed the laboratory results (Figure 3). IP application in the three buildings on previously painted walls sustainedly killed effectively wild *Ae. aegypti* mosquitoes for 12 months. Most of the surveyed workers of the buildings experienced fewer mosquitoes after the paint treatment, so aligned with the cone bioassay outcomes.

The field trial presented similar figures of delayed mortality of mosquitoes exposed to the painted walls (Figure 8). From the four random houses selected for the cone testing, only one (V_1) had reduced efficacy (mortality of 55.6%) six months after being painted. This result could be associated with an insufficient application of IP since the original pink color of the walls was not perfectly hidden by the white color of the IP.

These findings showed the importance of the correct paint application under a resident-driven public health intervention, pointing to the need to include supervision of the paint application quality in the potential vector control programs based on IPs.

The positive OVT index (POI) obtained in the houses monitored in each neighborhood before painting was 44.6% and 43.3% in Várzea and Tira Chapéu, respectively. No oviposition was recorded after IP application in the OVTs placed indoors and this suggests an exclusion of mosquitoes in the treated houses in addition to a potential population reduction in the clusters. Spatial repellency of the air-released TFL from the paint may have contributed to this finding. Control houses in each neighborhood showed POI values of 100% indicating the regular presence of females in interior parts of the houses. However, this large impact on the mosquito population can be interpreted to a

reduced extent due to the more exophilic behavior of *Ae. aegypti* in the city of Praia, Cabo Verde (59).

The houses painted in the two neighborhoods were in densely populated urbanized areas, especially in Tira Chapéu (Figures 4 and 7). This fact did not interfere with the most positive perception of the residents regarding the reduction of mosquitoes in an environment of overcrowded houses and reduced clusters, reinforcing the positive results of the IP.

TFL is known to be effective against metabolically resistant mosquitoes to pyrethroids mediated by P450 monooxygenases (60, 61) because of its different molecular structure than type I and type II pyrethroids. Rocha et al. (37) observed resistance to deltamethrin and cypermethrin in populations of *Ae. aegypti* from Santiago Island. However, there were no mutations, and metabolic modifications were detected, with an increase in the activity of mixed function oxidase enzymes (18%). Subsequently (37), found the *kdr* mutations V1016I and F1534C, at a very low frequency (3%). Our results sum up the evidence that points to TFL as an effective active ingredient for pyrethroid resistant mosquito populations.

There is abundant scientific literature on the airborne effects of TFL and other volatile pyrethroids on mosquitoes but very limited results of its contact effect. All efficacy testing resulted in a long-lasting killing effect of the TFL paint by contact, but other potential airborne effects with important influence on disease transmission remain unknown. Despite the absence of oviposition found in interiorly placed OVTs, further research should focus on interior mosquito density and blood-fed rate in addition to distance testing as proposed by Mosqueira et al. (62) and the WHO guidelines for efficacy testing spatial repellents (63). However, because the repellency effect of transluthrin was not evaluated in this study, less contact with the insecticide due to repellency could select mosquitoes for greater resistance, requiring new studies that independently evaluate the effect of contact killing and repellency of transluthrin IP.

TABLE 3 Association between the neighborhood of residence and the results of the application of the satisfaction questionnaire on the IP intervention.

	Várzea (%)	Tira Chapéu (%)	P value
Demographic variables			
Sex (Female)	57.7	67.3	0.1213 ^a
Age			0.9166 ^b
15-44 years	15.4	56.4	
45-54 years	50	5.5	
55-64 years	23.1	5.5	
>/65 years	11.5	32.7	
Profession			0.5605 ^b
Student, Unemployment, Reformed	46.2	36.4	
Maid	27	25.5	
Handyman	11.4	3.6	
Seller	0	12.7	
Others	15.4	21.8	
Knowledge of insecticide paints			
What are insecticide paints used for?			0.0105 ^a
Mosquito control and insect control	96.2	90.9	
Does not know	0.8	9.1	
How are insecticide paints used?			0.5127 ^b
Like any paint	50	34.5	
With protection	26.9	27.3	
Does not know	23.1	38.2	
Satisfaction with insecticide paints			
Have you felt a difference after painting?(Yes)	69.2	81.8	0.0239 ^a
Do you feel fewer mosquitoes? (Yes)	96.2	96.4	0.6395 ^a
Besides fewer mosquitoes, do you feel any other difference?			0.8273 ^b
Yes	54	58	
No	46	38	
Does not know	0	4	
What a difference?			0.8273 ^b
Fewer insects	42.3	31.6	
Cleaner and fresher	50	47.4	
Strong smell	7.7	21	
Security of insecticide paints			
Have you felt any discomfort after painting the house?			0.8273 ^b
Yes	11.5	23.6	
No	88.5	74.5	
Does not know	0	1.9	
What kind of discomfort?			0.0513 ^a
Foolishness and Headache	33	43.7	
Eye irritation	66.7	53.3	
Has anyone at home felt discomfort after painting? (No)	88	100	0.0002 ^a

a. P value of Fisher's exact test. B. P value of Kruskal-Wallis test.

A limitation of this study was the strategy for selecting the areas of houses in each neighborhood to be painted and the strategy for evaluating the decrease in mosquitoes inside the houses. In both cases, the ovitrap was used as a tool to detect the distribution and density of mosquitoes. It is an indirect detection method with uncertainty about adult mosquito abundance. For future studies, it is advisable to include the collection of *Ae. aegypti* adults with suitable trapping devices. Furthermore, for the selection of the areas of houses to be painted in each neighborhood, the social component should be considered and integrate the entomological findings with the community perception survey outcomes.

Nearly all (98%) of the surveyed residents in the painted houses said they perceived fewer mosquitoes, and more than half (57%) stated that no mosquitoes were seen in their houses (Supplementary

Data Sheet 2). The reduction of the presence of other insects mentioned by 42% of respondents becomes a positive attribute for residents' IP intervention acceptance. A movement away from the chemical stimulus counts among the spatial repellency effects described for some volatile pyrethroids like TFL. In this sense behaviour modifications like, deterrence and excite-repellency to *An. gambiae* were recorded in high percentage (38% and 56% respectively) when TFL coils were used in experimental huts (64).

According to the questionnaire results, the residents knew that the IPs were to address mosquito control, indicating effective information about the intervention. Two-thirds of the respondents were not aware of the personal protective equipment recommended for IP application despite it being made available through the trained volunteers (Figure 8). Similar results of lack of knowledge and inadequate practices concerning the use of personal protection in the application of pesticides are described in the literature (65). This finding is a wake-up call for future community intervention projects to reinforce information and communication on this aspect.

Regarding the perceived adverse effects assessment by residents, 16.7% reported feeling some discomfort in the first days after painting with higher rates than an evaluation in Nepal (5.9%) with a pyrethroid-based IP (65). Housing-related causes associated with these perceptions could be a small size and limited ventilation, while causes residents-related may be not respecting the recommended time of absence from the house after painting (12 hours). Despite no equivalent data being found for IRS in Cabo Verde, 14.6% of households perceived side effects in Uganda after spraying with an organophosphate formulation (66) as did 31.2% in Nigeria (67).

The body of evidence of the efficacy of IRS treatments for *Aedes* control has increased in recent years (68) and Pan American Health Organization (PAHO) has issued a manual for IRS activities for *Aedes* control in urban areas (69). IPs have been included in the new category of indoor residual surface treatment defined by WHO for *Aedes* and *Anopheles* control (70) indicating a conceptual equivalence to IRS. The results of our project reinforce the suitability of IPs for *Aedes* control in housing and other building types.

Implementation of the operational painting phase of the project required intensive community leaders' involvement and residents' participation under the self-application model. Periods for the execution of this IP-based vector control intervention are not adequate for reactive disease outbreak actions but as a preventive for sustained mosquito control in high-risk areas. The Integrated *Aedes* Management strategy proposes (71) a shift to proactive interventions to avoid endemic and epidemic evolutions of some scenarios of arbovirus outbreaks.

The TINTAEDES project demonstrated the suitability of a new paradigm in vector control interventions through community involvement and self-application of IPs. This model was previously implemented in research studies in Nepal (15) and Mexico (9).

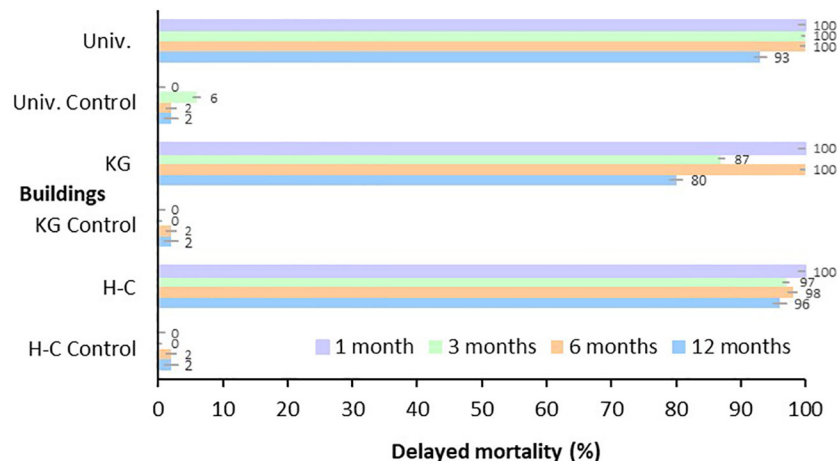


FIGURE 8

Delayed 24-hour mortality of wild-caught female *Ae. aegypti* females in houses treated with IP. T-C Control, Unpainted House in Tira Chapéu; Painted T-C_1, House 1 painted in Tira Chapéu; Painted T-C_2, House 2 painted in Tira Chapéu; V Control, Unpainted House in Várzea; Painted V_1, House 1 painted in Várzea; Painted V_2, House 2 painted in Várzea.

Conclusion

Laboratory trials evidenced the susceptibility of wild populations of *Ae. aegypti* to IP containing the pyrethroid TFL and resistance to paints containing organophosphates. TFL paint is capable of being exploited as a contact-killing surface and potentially as a passive emanator for inducing spatial repellency effects.

The results obtained from the TINTAEDES project point to IP as a potential strategy for intra-domiciliary control of the dengue and zika mosquito vector in the city of Praia, Cabo Verde.

It is recommended to conduct further studies of a community-based intervention on a large scale to reaffirm the findings found in this study in terms of efficacy and acceptability. Operational research and cost analysis need also to be addressed to promote the use of IPs in the *Aedes* control programs at the national and local levels.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

LG: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision,

Visualization, Writing – original draft. HR: Investigation, Visualization, Writing – review & editing. IT: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing. IP: Investigation, Methodology, Writing – review & editing. DCM: Formal analysis, Investigation, Visualization, Writing – review & editing. KS: Investigation, Writing – review & editing. DSRM: Investigation, Writing – review & editing. JR: Investigation, Writing – review & editing. SL: Visualization, Writing – review & editing. LS: Investigation, Resources, Writing – review & editing. JF: Conceptualization, Resources, Visualization, Writing – review & editing. MM: Visualization, Writing – review & editing. EM: Conceptualization, Project administration, Writing – review & editing. BH: Conceptualization, Supervision, Visualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study received funding from the Government of Canarias through the program International Development Cooperation Projects 2021. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Acknowledgments

The authors thank: Jean Piaget University (UniPiaget) for the support with transportation and with administrative, maintenance, communication, and image services; the Research Group on Tropical Diseases of the Jean Piaget University (GIDTPiaget) for its continuous work for the development of this project; and all the

collaborating entities (Supplementary Table S1) and partners of the TINTAEDES Project (Tira Chapéu Health Center, kindergarten of Pensamento, CIMPOR, ADAD, Cabo Verde Armed Forces) and the residents of Várzea and Tira Chapéu.

Conflict of interest

Author IT is employed by Inesfly Corporation S.L., a manufacturer of vector control products. Authors JF, LS, and JR are employed by SITA S.A., the distributor of these products in Cabo Verde.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ftd.2024.1321687/full#supplementary-material>

SUPPLEMENTARY TABLE 1

Collaborating entities and responsibility in the TINTAEDES project.

SUPPLEMENTARY DATA SHEET 1

Questionnaires on knowledge, perception, satisfaction, and safety of paints with insecticide for painters and users of painted buildings.

SUPPLEMENTARY DATA SHEET 2

Questionnaire on knowledge, satisfaction, and safety of paints with insecticide for residents of painted houses.

SUPPLEMENTARY DATA SHEET 3

Statements of Responsibility of Community Associations. Kindergarten, Health Center, and University.

SUPPLEMENTARY DATA SHEET 4

TINTAEDES Project Training and Resident Satisfaction Questionnaire.

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