Impact Factor (SJIF): 6.126 E-ISSN: 2583-1615

International Journal of Research in Academic World

Received: 02/January/2025 **IJRAW:** 2025; 4(2):09-21 **Accepted:** 08/February/2025

Effectiveness of Cytrol 10.8 ULV and Cytrol 0.4 LPU in *Aedes aegypti* (Diptera: Culicidae) Control in Cardenas, Matanzas, Cuba

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Abstract

The province of Matanzas usually performs intensive control of *Aedes aegypti* using pyrethroids as adulticide mainly, however, the municipality of Cardenas has health areas with high House Index (HI) and Breteau Index (BI). The objective of the research was to evaluate the effectiveness of Cytrol 10.8 ULV (ultra-low volume) and Cytrol 0.4 LPU (Ready to Use) in intradomiciliary thermonebulization treatments against *Ae. aegypti* in the Popular Council, Peninsula de Varadero, of the Ramon Martinez Health Area of Cardenas municipality, Matanzas, Cuba. For the study, the susceptibility status to cypermethrin was evaluated using the impregnated vial methodology in a population of mosquitoes in the selected area, which was resistant (85% mortality). Bioassays were also conducted to evaluate the efficacy of Cytrol 0.4 LPU and Cytrol 10.8 ULV at the doses evaluated, using the biological evaluation methodology for persistent fumigants, and 100% mortality was obtained in all cases. Efficacy was determined by house and Breteau index, number of eggs per ovitraps and adult capture using BG-Sentinel adult traps. Blocks treated with Cytrol 10.8 ULV at a dose of 25 mL remained negative for four months according to the indices evaluated, while those treated at doses of 10 and 5 mL of Cytrol 10.8 ULV and Cytrol 0.4 LPU were negative for three months with the same indices as those treated with 10 and 5 mL of Cytrol 10.8 ULV and Cytrol 0.4 LPU were negative for three months with the same indicators. The spatial thermo fumigation treatment with these two formulations had a positive impact on the selected blocks and, in addition to the vector control programme activities, is a good option to reduce the rate of *Ae. aegypti* infestation.

Keywords: *Aedes aegypti,* Cardenas, Cytrol, effectiveness, intradomiciliary thermonebulization.

Introduction

Aedes (S) *aegypti* (Linnaeus, 1762) is an effective vector of various arboviruses. Its greatest epidemiological importance is associated with its role as a transmitter Yellow Fever, Dengue, Chikungunya and Zika [1, 2].

The increase in transmission is evident predominantly in urban and semi-urban areas and is an important public health problem to the point that the World Health Organization (WHO) estimates that 390 million dengue infections occur each year, 96 million manifests clinically (regardless of the severity of the disease) [3]. In the first semester of 2023, dengue outbreaks of considerable magnitude were recorded in South America. Between epidemiological week (EW) 1 and epidemiological week (EW) 26 (ending July 1), a total of 2, 997, 097 cases of dengue were reported in America's region, including 1, 302 deaths, which represents a fatality rate of 0.04% and a cumulative incidence rate of 305 cases per 100, 000 inhabitants. From all dengue cases reported through EW 26, 2023, 1, 348, 234 (45%) were confirmed by laboratory testing, and 3, 907 (0.13%) were classified as severe dengue cases. The highest number of dengue cases was recorded in Brazil, with 2, 376, 522 cases, followed by Peru, with 188, 326 cases, and Bolivia, with 133, 779 cases. The four serotypes of dengue virus (DENV1, DENV2, DENV3 and DENV4) are present in the Region of the Americas. Until EW 26, the simultaneous circulation of the four serotypes was detected in Brazil, Colombia, Costa Rica, Guatemala, Honduras, Mexico and Venezuela, while the DENV1, DENV2 serotypes circulate in Argentina, Panama, Peru and

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Puerto Rico and DENV3, and in Nicaragua the serotypes DENV1, DENV3 and DENV4 [4].

In 1981, the first Dengue hemorrhagic fever epidemic reported in the Americas caused by dengue 2 occurred in Cuba [5]. In 1997, a dengue 2 outbreak was detected located in Santiago de Cuba city [6], with 3, 012 serologically confirmed cases, of which 205 were classified as cases of Dengue Hemorrhagic Fever (DHF) and 12 deaths [7]. Despite the high political will of the government, the country has not been exempted from local transmissions in recent decades, with outbreaks reported in 2000 and 2001 due to dengue type three and 2006-2010 due to dengue three and four in several provinces of the country [8, 9].

The most used method to *Aedes aegypti* control is the application of chemical insecticides in the adult phase, both in intra-and extra-home treatments, which should lead to a rapid reduction in populations. Unfortunately, this reduction is usually temporary if the breeding sites are not treated with larvicides, eliminating any container that the species uses for its oviposition [10, 11], in addition to the community empowerment [12, 13].

Pesticides are not used in pure or technical grade form. Typically, the active ingredient (technical grade insecticide) is mixed with several inert ingredients and serves a variety of functions to create a formulation. The main function is to facilitate spraying, safety, efficacy, stability or ease handling of the product [14].

The insecticides application with the aim of reducing mosquito populations always brings the generation of resistant individuals. In Cuba this is not an isolated phenomenon [15-17]. That is why other alternatives for vector control are being investigated, in the short and medium term, such as; obtaining new isolates of *Bacillus thuringiensis* [14, 19]; the study of essential oils with insecticidal activity [20, 21], the introduction of the sterile insect technique [22] in addition to interventions with other synthetic products such as growth inhibitors [23]. However, the use of chemical formulations continues to be the most used and widespread measure par excellence for controlling populations of this vector.

The background in Cuba shows treatments with adulticides were used routinely in Cuba from 1981 to 1986. First malathion as ultra-low volume (ULV) spraying [24]. Then since 1986, the pyrethroid lambda cyhalothrin [25] and later cypermethrin until the present. In recent years, the control strategy is based on the application of different adulticidal formulations where cypermethrin constitutes the active ingredient [26, 27] in addition to the application of residual products with other groups of insecticides [28-30].

Cárdenas is one of the 13 municipalities in Matanzas province. The Ramón Martínez health area consists of three Popular Councils: Santa Marta, Boca de Camarioca and Varadero-Peninsula, which during the year 2021-2022 presented high house and Breteau indices, with a history of decreased infestation rates after an intervention carried out with a residual formulation in Santa Marta area [31].

Taking into account the background of Ramon Martinez Health Area, the Varadero – Peninsula Popular Council was selected to study; Cytrol 10.8 ULV and Cytrol 0.4 LPU formulations, therefore we set the following objective: evaluate the effectiveness of Cytrol 10.8 ULV (ultra-low volume) and Cytrol 0.4 LPU (Ready to Use) in intradomiciliary thermonebulization treatments against *Ae. Aegypti.*

Materials and Methods

The study had a quasi-experimental design with longitudinal characteristics, which went through different stages:

A pre-treatment Phase in December 2022 to carry out susceptibility tests at the laboratory level using impregnated bottles following the protocol [32], modified by the use of 250 ml capacity glass bottles with frosted lids [17]. A February 2023 Treatment Phase in which the treatment was carried out on the selected blocks with Cytrol 0.4 LPU and Cytrol 10.8 ULV formulations.

CYTROL 0.4 LPU (Ready to use) contains: 0.4% W/V of Technical Cypermethrin and 0.2% W/V of Piperonyl Butoxide for manual spatial treatment, apply at a rate of 2L/ha. The new formulation commercially identified as Thermo nebulizer, incorporates kerosene or diesel which makes it a "READY TO USE" product and avoids the transfer of fuel and its prior preparation for application with Thermo nebulizers. In our case it will be used in intra-home treatment.

CYTROL 10.8 ULV contains: 10.8% W/V of Technical Cypermethrin and 4.5% W/V of Piperonyl Butoxide. It is used with diesel at a rate of 1mL/40mL or 25mL/L and applied at a rate of 2L/ha. According to the manufacturer's information, it is a formulation which is traditionally prepared at a rate of 25 mL/L of water or kerosene.

Both formulations have added piperonyl butoxide, which increases the effectiveness of other insecticides and inhibits the functions of oxidases that naturally detoxify insecticides.

A Post-treatment Phase from February to June 2023 carrying out bioassays, data collection and analysis

Study Area

Cardenas is one of the 13 municipalities in Matanzas province. Cárdenas municipality is divided into five health areas: Ramon Martinez, Jose Antonio Echeverria, Heroes of Moncada, Antonio Piti Fajardo and Humberto Alvarez.

The Ramón Martínez health area consists of three Popular Councils: Varadero-Peninsula, Santa Marta and Boca de Camarioca which during the year 2021-2022 presented high home and Breteau rates. The Council selected to carry out the evaluation was Varadero-Peninsula because this popular council has the most important tourist area in the country in terms of hotels and tourist facilities that contributes more than 60% of the foreign currency income that Cuba receives, in which they selected four blocks to carry out the treatment and four control blocks.

Susceptibility and/or Resistance Study to Cypermethrin under Laboratory Conditions Using the Impregnated Bottle Methodology

To determine susceptibility to the active ingredient cypermethrin, a fundamental requirement to define the application of the treatment, individuals from the Varadero-Peninsula population and the susceptible Rockefeller reference strain were used.

The bioassays using impregnated bottles were carried out following the protocol [32], modified by the use of 250 mL capacity glass bottles with frosted lids [17]. The bottles were impregnated with 1 ml of cypermethrin at 13.5 µg/mL dose recommended [17]. The solutions were applied to inside of each bottle and rotated until the solvent, acetone, had completely evaporated. The control was impregnated with 1 ml of acetone. The bottles were covered with aluminum foil once the impregnation was completed and the lid was placed on them. After 24 hours, the bioassays were carried out. A control and four replicates were used per concentration to be evaluated.

For the bioassays, 15 females from one to three days old were placed in each bottle, starting with the control. For 1 hour the individuals were observed and the number shot down was confirmed every five minutes. After 60 minutes, those mosquitoes that could not fly when the bottle was moved gently or those that remained motionless at the bottom of the bottle were considered dead.

Effectiveness of the Cytrol 0.4 LPU and Cytrol 10.8 ULV Formulations using the Bioassay Methodology for Persistent Fumigants

The bioassays to determine the effectiveness of indoor thermal nebulization treatments were carried out according to the WHO methodology [33].

The mosquitoes used were females 3 to 6 days after emerging without blood feeding, which were extracted with a capturer and placed in 13 x 8 x 8cm cages. Inside each home to be treated with each of the 4 doses of insecticides, 5 cages were placed, with 20 mosquitoes each in different places of the house. The product was applied with thermofogging equipment (TF34). The homes or exhibition premises remained closed for 45 minutes after the application to prevent the insecticide scape and thus achieve the desired effect.

Subsequently, the cages were removed from the homes and the knockdown (KN =knockdown) corresponding to each one was recorded. Then the mosquitoes were transferred to clean glasses, which were covered with double mesh cloth placed on top and tied with an elastic band. In the laboratory, the specimens remained for 24 hours from the beginning of fumigation until mortality was read. The bioassays with each formulation and dose were performed twice.

Cytrol 0.4 LPU and Cytrol 10.8 ULV Formulations Efficiency as Indoor Thermonebulization Treatment

Cytrol 0.4 LPU (ready to use) as the name suggests was applied on a single block. The product was added directly to the Thermo nebulizer, at a rate of 100 mL per household. In the case of Cytrol 10.8 ULV, the mixture to be used was prepared at doses of 5 mL, 10 mL and 25mL, of the formulation with the amount of diesel to be used in each case until completing one liter of mixture (995 mL, 990mL, 975 mL respectively) applying 100mL of the mixture per home. One block was used for each dose. No type of treatment is carried out on the control blocks. The application of spatial spraying was carried out in accordance with the WHO Guidelines for the evaluation of the effectiveness of spatial spraying of insecticides for the control of the dengue vector *Ae. aegypti* [34]. The operator applies the formulations with thermofogging equipment (TF34). The insecticide dispersed in the form of fog was spread in all the rooms and subsequently waited 45 minutes with the house closed before opening and ventilating it. The application of this formulation intra-domiciliary was carried out in accordance with the provisions of the National Vector Control Program with 3 replicas of the procedure in the selected homes within a period of 1 week.

After the triplicate application per home in the 4 blocks, the effectiveness of the Cytrol 0.4 LPU and Cytrol 10.8 ULV formulations as indoor thermofogging treatment was evaluated, for which they were placed in each of the selected homes in each block an indoor and an outdoor ovitrap, as well as a BG Sentinel trap, the same was done with the controls, which were reviewed weekly for five months. Simultaneously, the inspection of the homes and warehouses was also carried out to determine the Breteau and House Index.

Results

Susceptibility and/or Resistance study to cypermethrin under laboratory conditions using the impregnated bottle methodology.

When carrying out susceptibility and/or resistance tests using the impregnated bottle methodology, we found that the Varadero Peninsula mosquito population showed resistance to cypermethrin at a dose of 13.5 ug/mL with values of 85% kill after 30 minutes, as shown in figure 1.

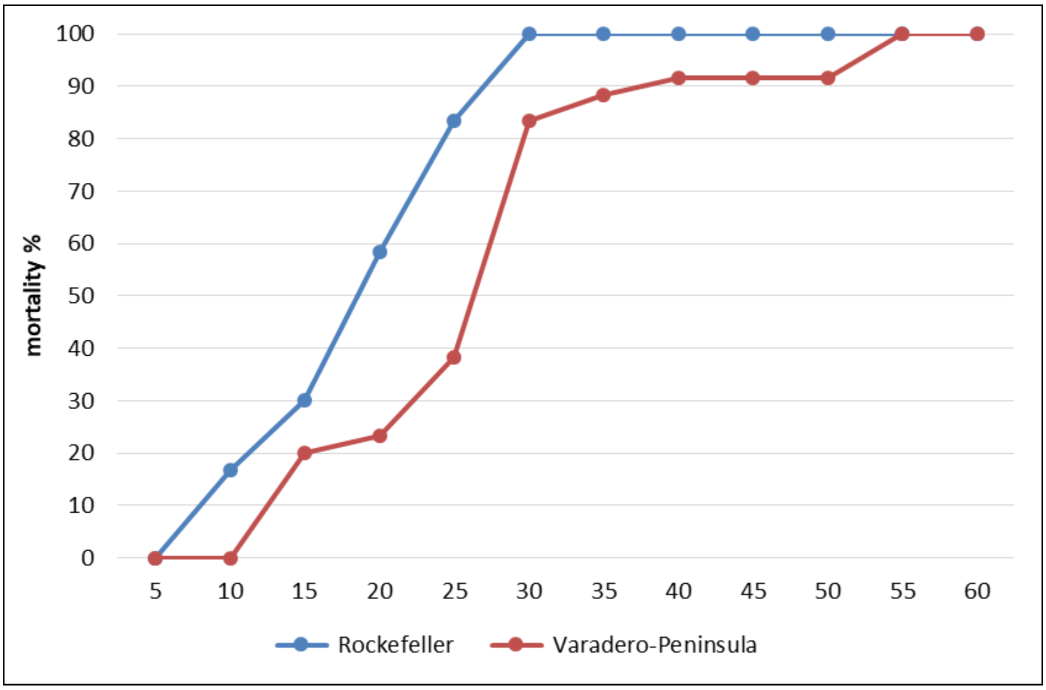

Fig 1: Susceptibility tests performed on Rockefeller and Varadero-Peninsula strain collected in 2022 using impregnated bottles with cypermethrin at 13.5 µg/mL

Effectiveness of Cytrol 0.4 LPU and Cytrol 10.8 ULV Formulations using the Spatial Bioassays Methodology

The effectiveness evaluations of the Cytrol 0.4 LPU and Cytrol 10.8 ULV formulations using the spatial bioassay methodology shows us that at the doses recommended by the manufacturer the results are similar in the knock down effect and in the mortality percentage for both formulations. In the case of Cytrol 10.8 ULV, bioassays were carried out with three doses, the results show that the Knock down effect and mortality are the same for the three doses.

Table 1: Mortality (%) recorded for *Aedes aegypti* in cages placed indoors during thermal fogging with the formulations Cytrol 0.4 LPU and Cytrol 10.8 ULV in Varadero Peninsula, Cardenas, Matanzas.

Formulated	Concentration	Doses	Replica (n)	Caged	Exposed Mosquitos	Knock Down Effect (1h) (%)	Mortality 24 horas (%)
Cytrol 0, 4 LPU	Cypermethrin 0, 4% and 0.2% of Piperonyl Butoxide	LPU	2	10	250	100	100
Cytrol 10, 8 ULV	Cypermethrin 10, 8% and 4.5% of Piperonyl Butoxide.	25mL	2	10	250	100	100
		10mL	2	10	250	100	100
		5mL	2	10	250	99.6	100

Efficiency of Cytrol 0.4 LPU and Cytrol 10.8 ULV Formulations as an Intradomiciliary Spatial Treatment

By analyzing the impact of the application of the Cytrol 0.4LPU formulation using the indices established by the *Ae. aegypti* Control Program to evaluate the efficiency of Cytrol 0.4 LPU and Cytrol 10.8 ULV formulations as an intradomiciliary spatial treatment, we can propose that decrease in the Breteau and house indices was evident with respect to the control, finding a highly significant difference (p=0.000261 and p= 0.000939 respectively) in the post-intervention stage (Fig. 2 and 3).

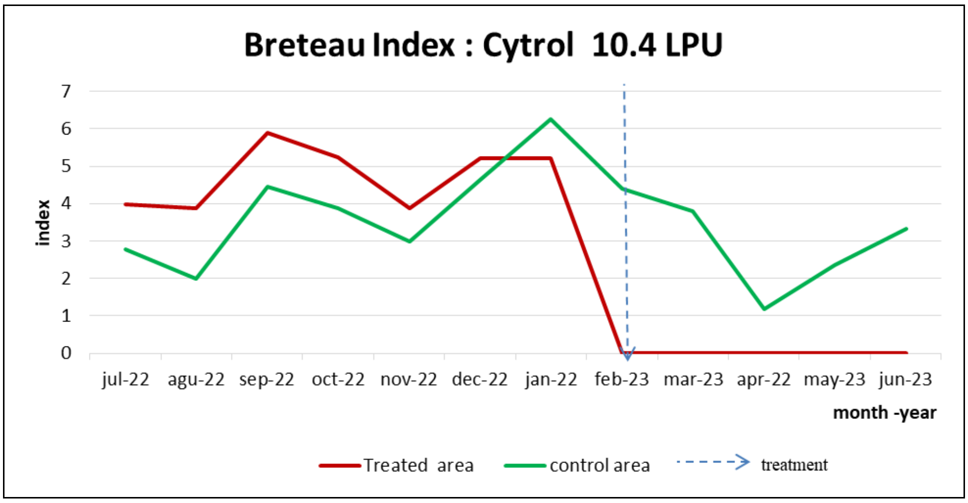

Fig 2: Pre-and post-treatment Breteau Index in control and treated blocks with Cytrol 0.4 LPU in Ramon Martinez health area.

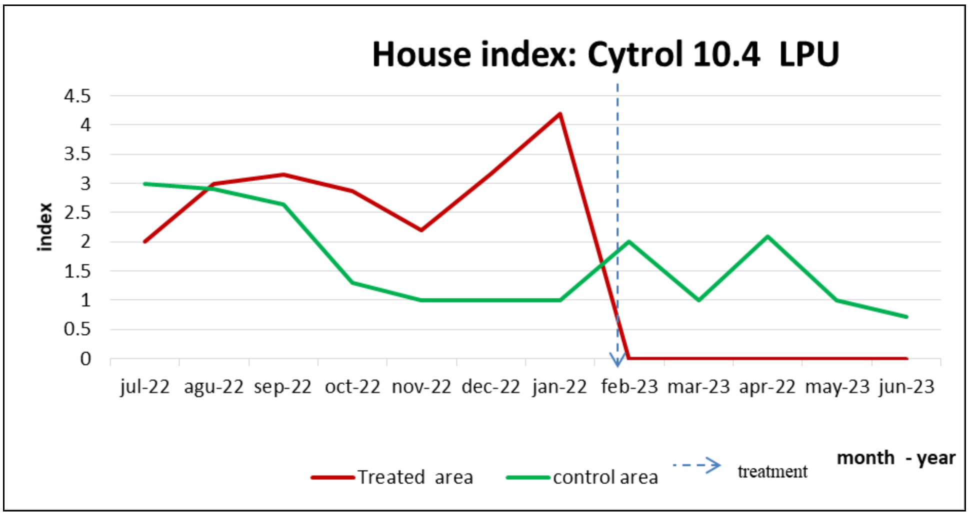

Fig 3: Pre-and post-treatment house index in control and treated blocks with Cytrol 0.4 LPU in Ramon Martinez health area

However, when evaluating the eggs numbers collected and the adult's numbers captured, no significant difference was found for either of the two indices (p=0.934 and p=0.2960 respectively) so the behavior of both blocks (treated and control) behaved similarly (Fig. 4 and 5), which suggests 2 ideas; the impact of this product is not significant or the surveillance through the HI and BI maintain a bias by providing erroneous information.

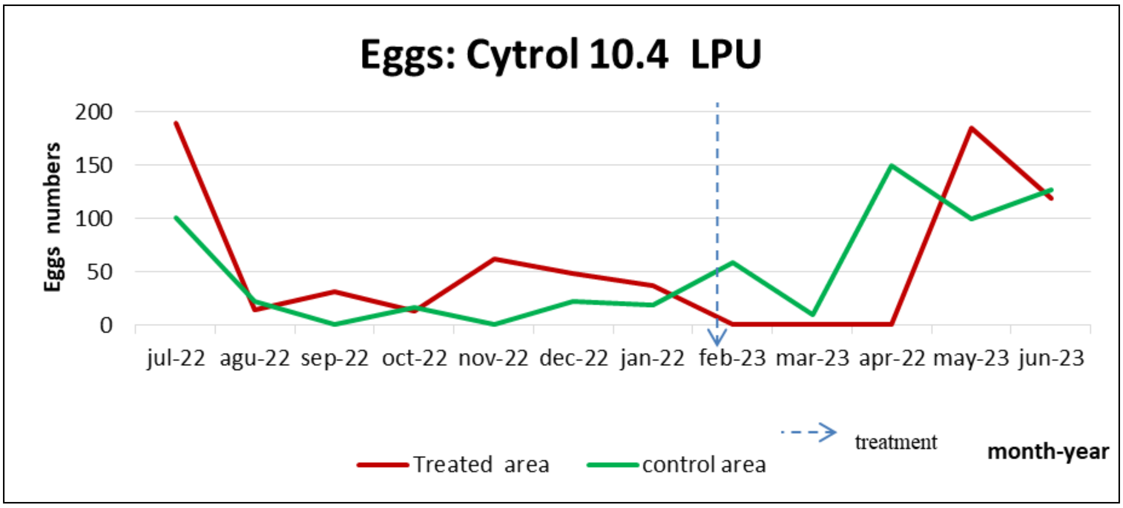

Fig 4: Eggs numbers obtained through pre-and post-treatment ovitraps in the control and block treated with Cytrol 0.4 LPU in Ramon Martinez health area

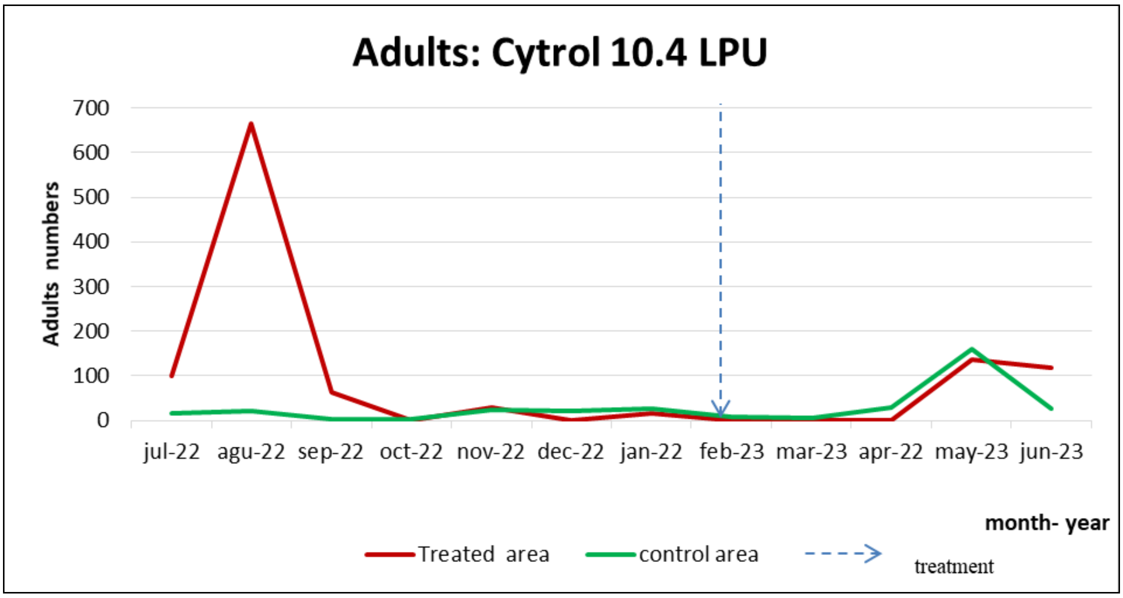

Fig 5: Adult's numbers captured using BG-Sentinel traps pre-and post-treatment in the control and treated block with Cytrol 0.4 LPU in Ramon Martinez health area

The efficacy of Cytrol 10.8 ULV formulation at a dose of 25 mL/L in the post-intervention stage is evident, with a highly significant difference for BI and HI (p=000438 and p=0.000489 respectively) between the treated and control block (Figure 6 and 7). In this case, regarding the number of eggs and adults, a significant difference was found between the block with treatment and control (p=0.002960 and p=0.00614 respectively) (Fig. 8 and 9).

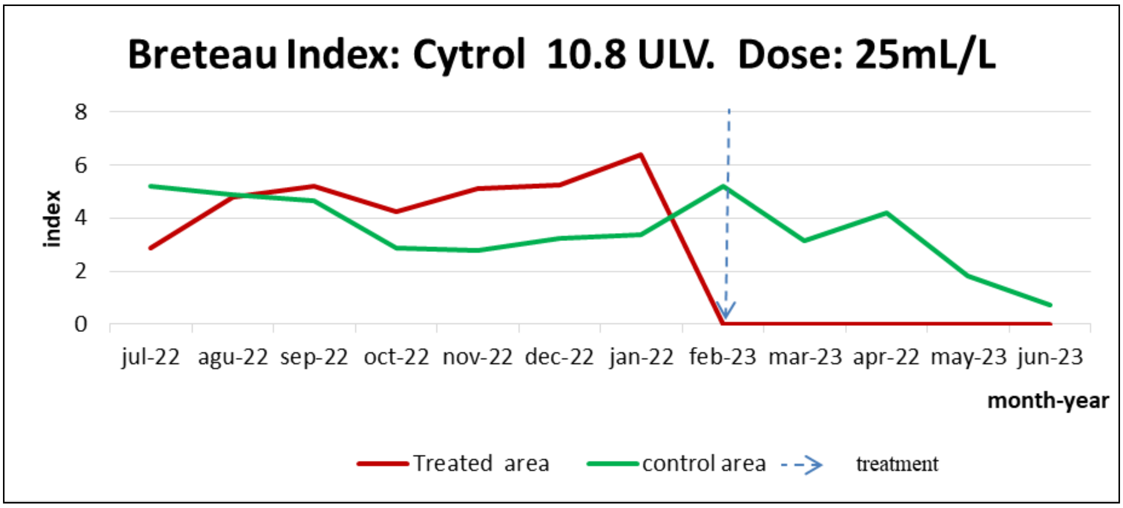

Fig 6: Breteau index pre-and post-treatment in the control and treated block with Cytrol 10.8 ULV at a dose of 25 mL/L in Ramon Martinez health area

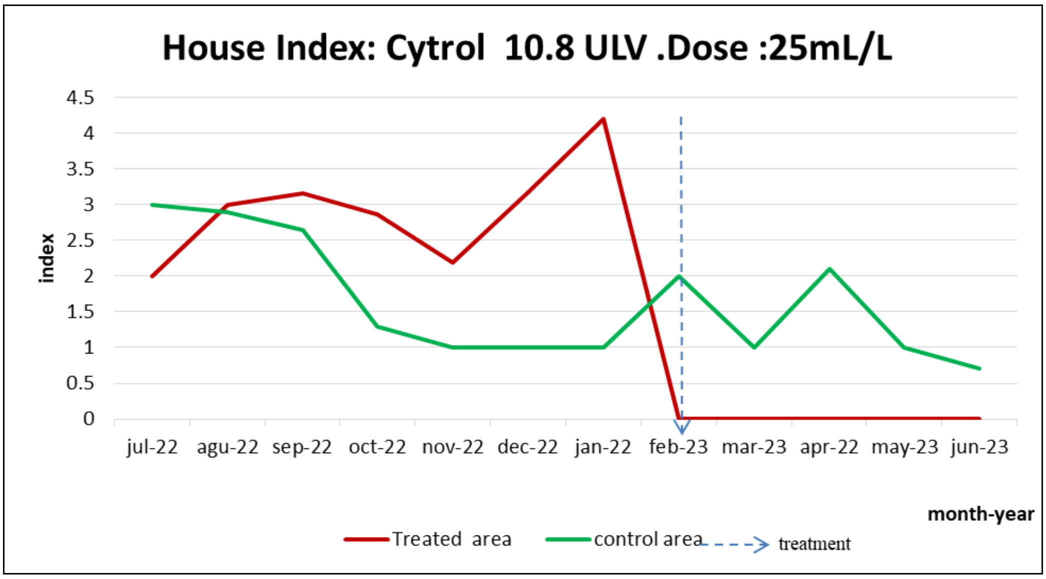

Fig 7: House index pre-and post-treatment in the control and treated block with Cytrol 10.8 ULV at a dose of 25 mL/L in Ramon Martinez health area

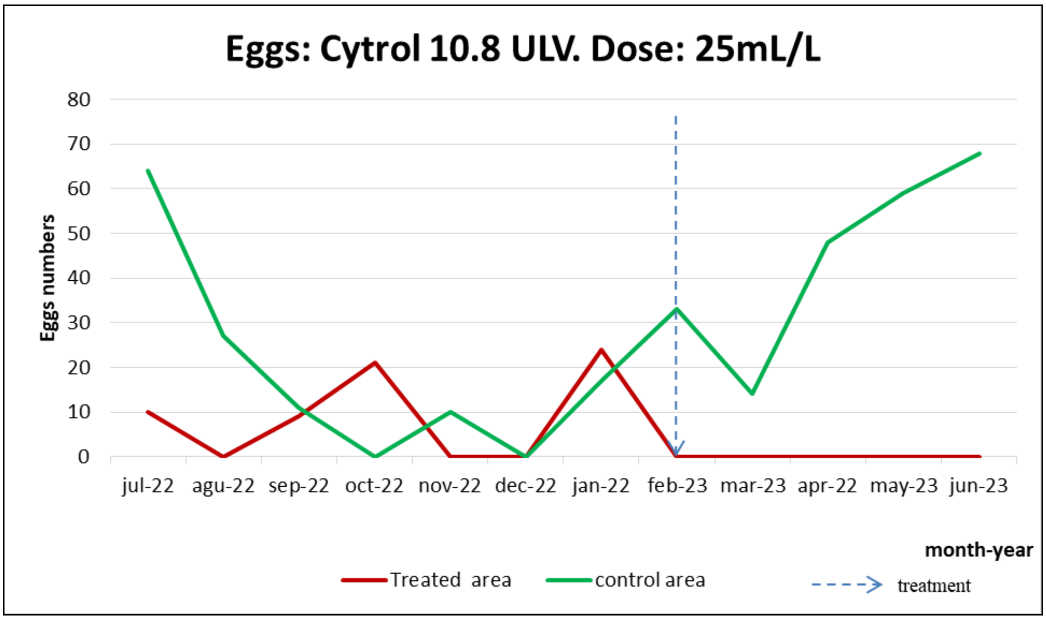

Fig 8: Eggs numbers obtained through pre-and post-treatment ovitraps in the control and treated blocks with Cytrol 10.8 ULV at a dose of 25 mL/L in Ramon Martinez health area.

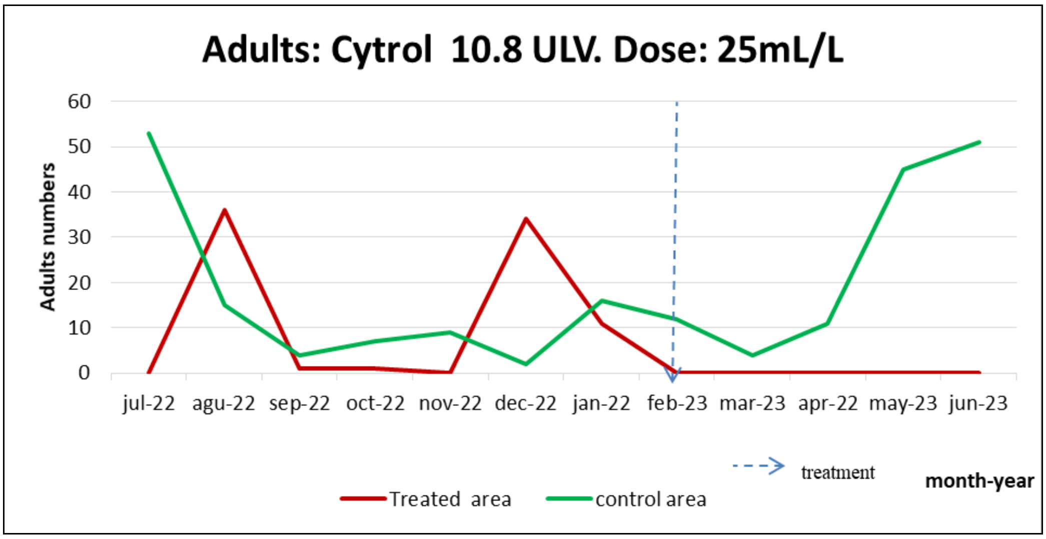

Fig 9: Adults number s captured using BG-Sentinel traps pre-and post-treatment in the control and treated block with Cytrol 10.8 ULV at a dose of 25 mL/L in Ramon Martinez health area.

In blocks treated with Cytrol 10.8 ULV at doses of 10 and 5 mL/L of solvent in the post-intervention stage for the Breteau Index between these and the control, no significant difference was observed (p=0.1390 and p=0.077081 respectively (Fig. 10). For the House Index, a significant difference was found (p=0.04087) between blocks treated with 10mL and the control, while between the blocks treated with the 5mL dose and the control the difference was highly significant (p=0.00624) (Figure 11).

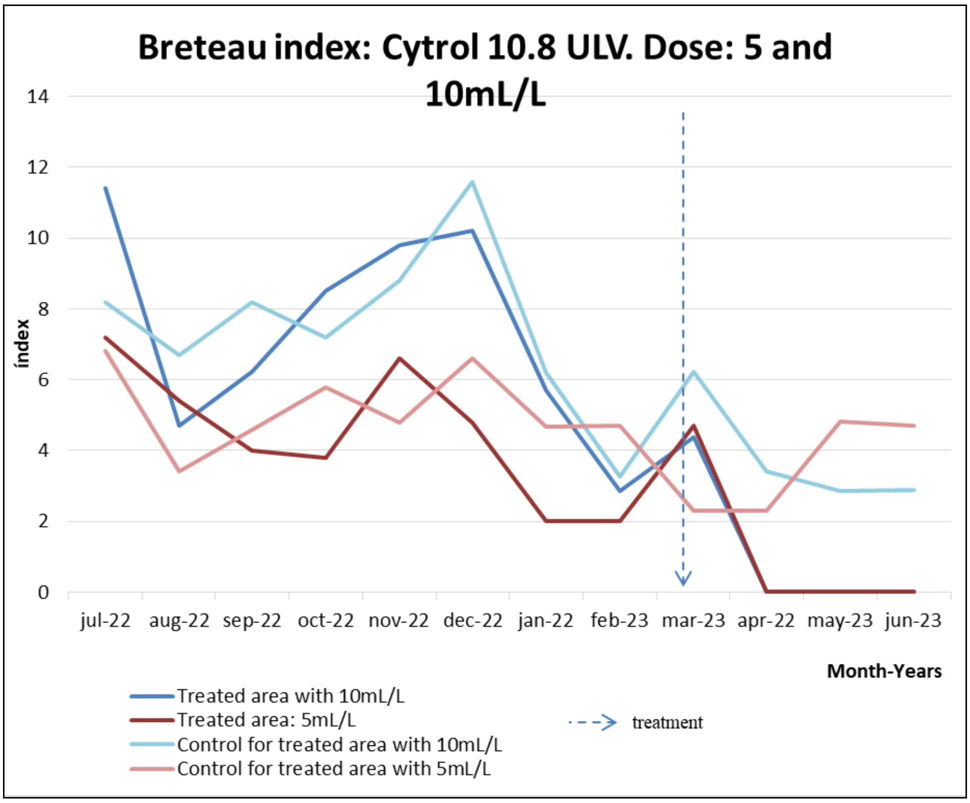

Fig 10: Breteau Index pre and post treatment in the control and treated blocks with Cytrol 10.8 ULV at doses of 10 and 5 mL/L in Ramon Martinez health area.

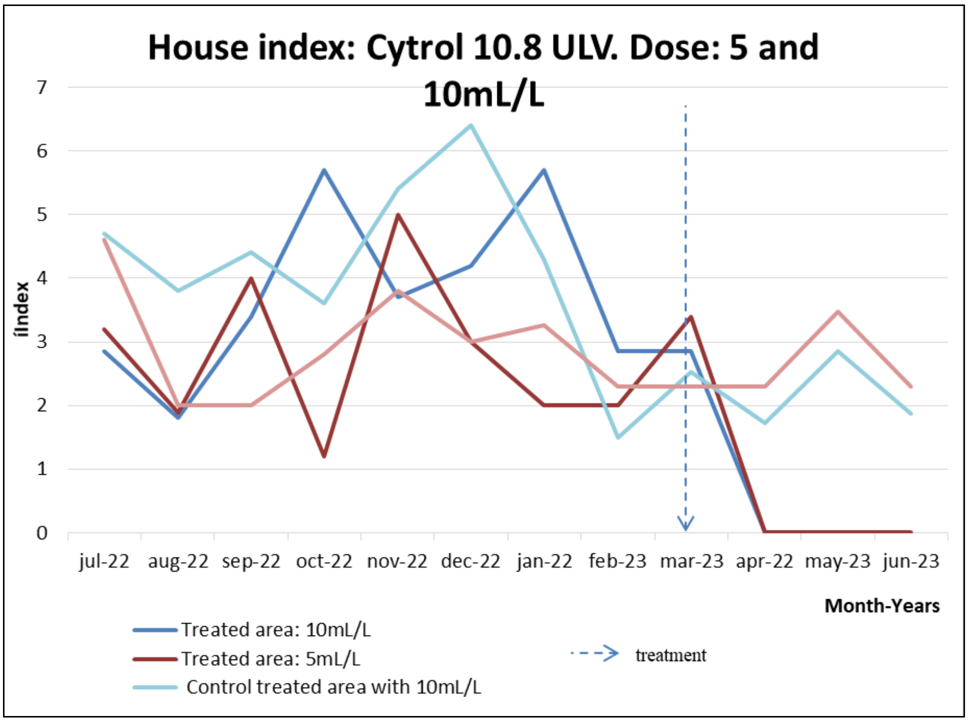

Fig 11: House Index pre and post treatment in the control and treated blocks with Cytrol 10.8 ULV at doses of 10 and 5 ml/L in the Ramon Martinez health area.

The number of eggs between the block with treatment at a dose of 10mL and the control, no significant difference was found (p=0.0735) but there was a significant difference in the number of adults collected (p=0.04373) (Figure12). In the case of the 5mL dose, a significant difference was found (p=0.04918) between the treated and control blocks, but not in terms of adult's number in which there was no significant difference (p=0.8551) (Figure 13).

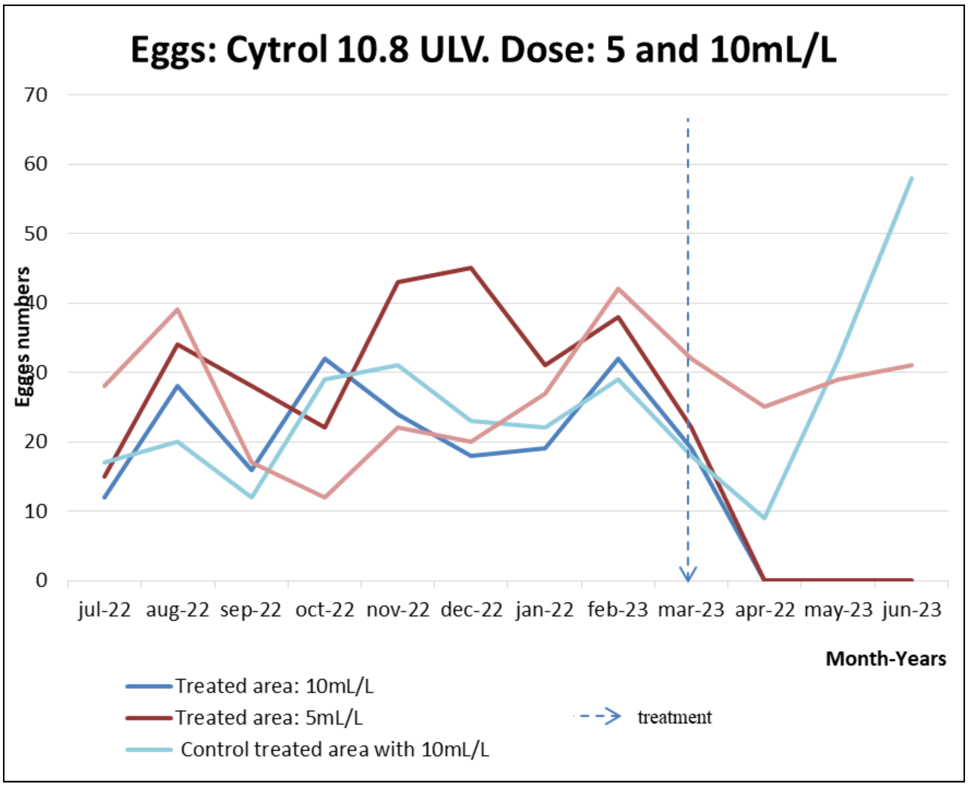

Fig 12: Eggs numbers obtained through pre-and post-treatment ovitraps in control and treated blocks with Cytrol 10.8 ULV at doses of 10 and 5 mL/L in the Ramon Martinez health area

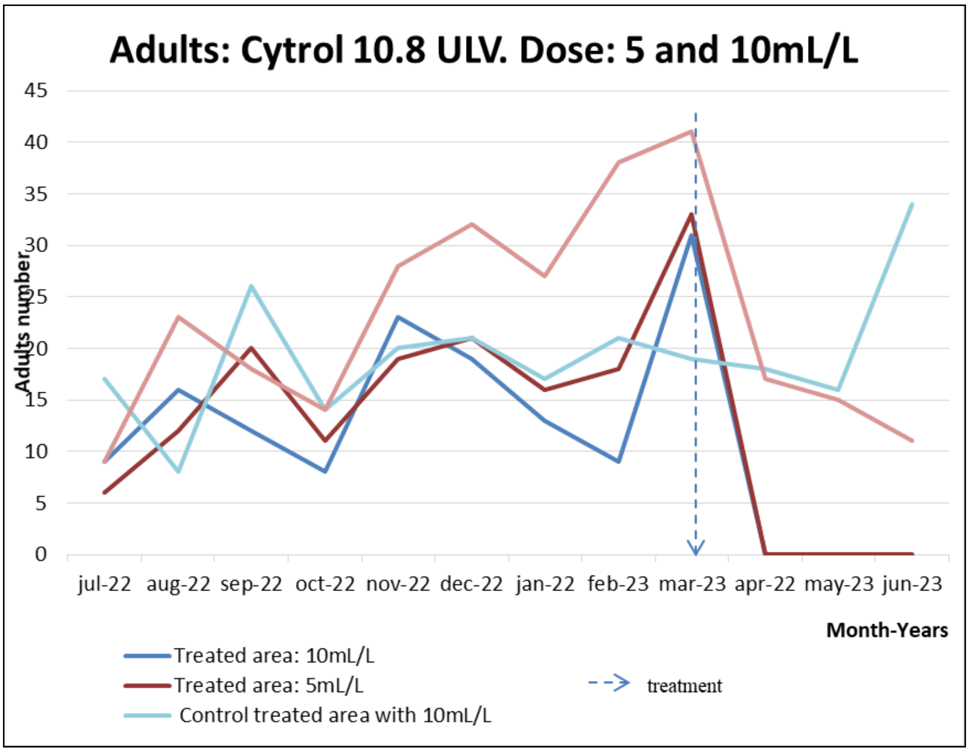

Fig 13: Adults numbers captured by BG-Sentinel traps pre-and post-treatment in the control and treated blocks with Cytrol 10.8 ULV at dose of 10 and 5 mL/L in Ramon Martinez health area

Discussion

Susceptibility and/or Resistance Study to Cypermethrin under Laboratory Conditions Using the Impregnated Bottle Methodology

Interventions based on the application of insecticides can be unsuccessful when the susceptibility and/or resistance status is not taken into account. Taking this aspect into account, monitoring the insecticide that will be applied in the intervention is essential, this will result in its success or failure [35].

Chemical control is an important component within the comprehensive control strategies currently being implemented in Cuba. In our country, since 1981, the insecticides malathion was used to control adult mosquitoes and fenthion for perifocal treatments. Pyrethroids began to be applied in 1986 and especially cypermethrin in recent years. The cypermethrin molecule is the active ingredient of numerous formulations registered in Cuba and as such it has been used regularly in our country, as a spatial treatment [26, 27, 36] so there is a sustained application of this insecticide within control policies in our National Vector Control Program.

In laboratory studies at the beginning of the century, in reference populations, on which selection pressure was applied, cross resistance to pyrethroids and organophosphates induced by selection with temephos was found in *Ae. aegypti* [37], cross resistance to pyrethroids induced by selection with malathion [38] and by selection with deltamethrin [39]. However, in populations collected in the field [40] evaluated the status of resistance to insecticides and its mechanisms in *Ae. aegypti* adult mosquitoes, from a strain from Boyeros municipality, Havana, which was susceptible to cypermethrin. In the same year, Rodríguez *et al* evaluated different insecticides in *Ae. aegypti* adult mosquitoes from Pinar del Río municipality, resulting in susceptibility to cypermethrin (100% mortality).

There are several studies that suggest great variability in terms of susceptibility state to this pyrethroid in *Ae. aegypti* populations for example in Cuba we have diverse results. [41], in studies carried out with *Ae. aegypti* adults from a strain from the Playa municipality, Havana, did not find resistance (FR < 5) to cypermethrin insecticide. This same author [42] in a similar study carried out with adult mosquitoes from *Ae. aegypti* population, from Santiago de Cuba, presented moderate resistance (FR=7.2), while in 2009 [43] in *Ae. aegypti* populations from three Havana municipalities, a study was carried out on adults whose results showed that they were resistant to cypermethrin insecticide. These results show us the variability of responses of this mosquito to a certain insecticide such as cypermethrin, a response that depends on various factors such as place of origin and collection of the strain under study and frequency of treatment in the locality.

Taking this background into account, it is not illogical to find that the population study was resistant. It is important to clarify that the mortality percentage obtained at other times would be considered close surveillance. The application for control adult mosquitoes with formulations containing cypermethrin in that location is not carried out as a routine measure by the Control Program, so the mosquito population has not been subjected to selection pressure. However, it should not be ruled out that there may be incipient resistance due to poor operational use, or to the sustained use of larvicide temephos, which has demonstrated cross-resistance to pyrethroids [37]. Some studies carried out in Brazil suggest that the resistance found to cypermethrin may be due to the population's use of pressurized formulations with this active ingredient to eliminate the vector [44, 45]. This theory should not be ruled out taking into account that in previous years "Lo Maté" formulation was marketed to the population along with spray devices and that this area is also dominated by tenants of housing for national and foreign tourism who can apply marketable pressurized spray in store networks or imported to reduce mosquito populations. These theories could explain how resistance may have evolved over time due to the application of sublethal doses.

Insecticide resistance in *Ae. aegypti* is mainly associated with the overexpression of certain enzymes (metabolic resistance) and/or mutations in the sequence of the target protein that induce insensitivity to the insecticide (resistance at the action site). The primary target site of known resistance mechanisms in *Ae. aegypti* involve amino acid substitutions in the voltage-gated sodium channel (VGSC) causing resistance to DDT/pyrethroid insecticides known as knockdown resistance (kdr) [46-48].

Studies reporting resistance to this insecticide are not isolated. Pisfil-Farroñay [49] in a study carried out in two districts of Lambayeque department, Peru in mosquitoes *Ae aegypti* adults found high resistance to the insecticide cypermethrin. Similarly, 16 populations from Pernambuco Brazil were studied with impregnated bottles, finding resistance to this pyrethroid in their entirety [50].

Studies carried out by Grossman [51] suggest that phenotypic susceptibility in *Ae. aegypti* to an insecticide can be restored in a highly resistant field population in only ten generations if there is no selection pressure with it. However, it is important to note that kdr mutations are recessive; even when resistance disappears, they may continue to be present in heterozygous individuals, which could reappear in the event of new selection with insecticides. Subsequent studies should be carried out with Varadero Peninsula population to corroborate the presence or not of this mutations type, taking into account the report carried out by Rodríguez [52] in populations from Havana municipalities.

Effectiveness of Cytrol 0.4 LPU and Cytrol 10.8 ULV Formulations Using the Persistent Fumigants Bioassays Methodology

Ae. aegypti adults normally rest inside homes (endophily), where they feed frequently and almost exclusively on human blood (anthropophily) [53, 54]. This explains why spatial fumigations outside premises have little effectiveness against this vector [55, 56]. Insecticides indoors application can have a more direct impact on resting adult mosquitoes [57]. This is particularly used during epidemics because it quickly shoots down adults who are presumably flying. It usually happens that up to three applications are required to achieve maximum effectiveness although durability is reduced [58].

The effectiveness of an intervention applying insecticides depends on several factors;

a) The mosquito vectors susceptibility to the sprayed insecticides,
b) The formulation composition, the treatment quality and coverage
c) The homes state and
d) The residents cooperation to keep the premises or homes closed for the established time.

In our study it is no less true that we did not start from mosquitos' population with a susceptible behavior to the insecticide to be used. The mosquito population respond to a formulation may vary with respect to the susceptibility to the active ingredient. That is to say, not always that a population expresses resistance to a molecule under laboratory conditions, the formulation will manifest ineffectiveness because: first, the formulation is at a much higher concentration than the diagnostic dose and second, the formulation may contain ingredients, as in this case, which contributes to enhancing the insecticidal effect. On the other hand, for carrying out the treatment, the residents of homes approval were obtained through informed consent in which they were previously informed of the objective of this research work, which achieved their collaboration for the product application, the permanence time of the formulation after being applied, in addition to access to operators for containers inspection during the study period.

Ordoñez-González [59] carried out bioassays with *Ae. aegypti* adult's mosquito to determine the effectiveness of a mixture of pyrethroids using intra-domiciliary thermal fogging on a pyrethroid-resistant strain of the mosquito *Ae. aegypti* from Tapachula, city, Chiapas, Mexico, together with a susceptible reference strain, observing that the knockdown effect and mortality was similar in both strains [60]. Bisset [10] used 15 strains of *Ae. aegypti,* from the 15 Havana municipalities, to determine the insecticides effectiveness, in their commercial formulation, obtaining as a result that with Galgotrin 25 CE (Cypermethrin) 100% mortality was obtained.

In our study, in both formulations, one of its components is Piperonyl Butoxide, which inhibits the activity of monooxygenases, enzymes associated with the pyrethroids metabolism. In the bioassays carried out, 100% mortality was obtained with adult mosquitoes in both Cytrol formulations (Cypermethrin), which demonstrates that they are effective in *Ae. aegypti* control. Some authors propose that pyrethroid formulations insecticides with synergists, applied in cold and hot mists, have low impact on insecticide-resistant *Aedes* and *Anopheles* mosquito populations [35, 61]. However, studies carried out by Montada [26] evaluated formulations whose active ingredient was cypermethrin, chlorpyrifos and lambda cyhalothrin and demonstrated that thermonebulization treatments were more effective than cold nebulization treatments, the Galgotrin 25 EC (cypermethrin) formulation had the best results [26]. This same author [27] carried out similar studies with an *Ae. aegypti* strain from Santiago de Cuba and with three municipalities strains from Havana with the aforementioned insecticides in which he found that there is a highly significant difference in favor of thermonebulization treatments with Galgotrin 25 EC (cypermethrin) [36].

Evaluation of the Effectiveness of the Cytrol 0.4 LPU and Cytrol 10.8 ULV Formulations as Intra-domiciliary Spatial Treatment

It is proposed by numerous authors that spatial treatments do not have any residual effect and that at most, their effectiveness is evident for a week.

Gunning [62] stated that the widespread use of indoor ULV fumigation in emergencies by control programs and the lack of large spatial scale assessments is problematic. These authors conducted two large-scale experiments to evaluate indoor ULV pyrethroid spraying in the city of Iquitos, Peru, in 2013 and 2014. The results demonstrated that adult densities can be reduced by ULV spraying, but that approximately 1 month after application, adult densities return to approximately the same indicators. The use of a formulation that contains two or more insecticides, operationally, is more economical than mixing products, and its effectiveness has been demonstrated in the agricultural area [63].

The evaluation of control interventions can be carried out through some surveillance methods, such as indices; ovitrap [64], Breteau and house [65-67], given the difficulty of sustaining certain evaluative methodologies for long periods of time. These indices are commonly used to determine changes in the geographic distribution of a population, relative estimates of its abundance over time (house, container, and Breteau), or the relative density of adults; through traps for this purpose or ovitraps [68].

In our study we use the data from house and Breteau index, number of eggs and adults, pre and post treatment of the treated and control blocks, which show us the impact of the intervention during the analyzed period, the first two indices are obtained according to the statistical information from the Health Area and regarding the number of eggs and adults through the weekly review of both surveillance systems. Total elimination of individuals is not logical when using any control method, including chemical control. The application of insecticides opts to reduce the levels of vector infestation to permissible limits, with a spatial application the adult is immediately eliminated.

Checking the effectiveness of both formulations and determining how long after the treatment the treated blocks remained negative was important because spatial treatments do not have any residual effect.

In our results, it is observed in figures 2-5 that in the block treated with Cytrol 0.4 LPU according to the statistical data of the Health Area in the 4 months after the treatment the house and Breteau index is zero for the 4 months However, in the third and fourth month, with the surveillance system of the ovitraps and the BG sentinel in both months, we can propose that there was a presence of post-intervention mosquitoes according to the number of eggs and adult mosquitoes collected, which tells us It can indicate two aspects, one that at the time of the visits to the homes, no positive container was found during the inspection and two that this was not carried out with due quality, which shows us as a result of the aforementioned, that there are deficiencies in the surveillance system, a third aspect is that mosquitoes have migrated from the untreated blocks to the treated ones and either have laid eggs or these have been collected by the BG sentinel traps.

These results differ from those obtained with Cytrol 10.8 ULV with the dose of 25 mL per liter of diesel, both the house and Breteau index, the number of eggs and adults in the treated blocks have a similar behavior in the 4 months; post-treatment, as shown in figures 6-9. The difference for both formulations may also be influenced by their own composition, for Cytrol 0.4 LPU the concentrations of both active ingredients, are much lower (0.4% P/V of Technical Cypermethrin and 0.2% P/V of Piperonyl Butoxide) than those of Cytrol 10.8 ULV (10.8% P/V of Technical Cypermethrin and 4.5% P/V of Piperonyl Butoxide.) that although the first is used as its name indicates, Ready for Use and the other dosed, even so, these high concentrations of its active ingredients may be influencing the results obtained. For the doses of 10 and 5 mL of Cytrol 10.8 ULV per liter diesel, in the post-treatment months in the treated blocks, comparatively equal results are obtained between the house and Breteau index, number of eggs and adults for both doses.

In general sense, the spatial thermofogging treatments with these two formulations produced a positive impact on the treated blocks, which is why we consider that added to the activities of the vector control program, they constitute a good option for reducing *Ae aegypti* index.

Conclusion

The spatial thermo fumigation treatment with the two Cytrol-based formulations had a positive impact on the selected blocks and, in combination with the vector control programme activities, is a good option to reduce the rate of *Ae. aegypti* infestation.

Acknowledgements: To Pelgar International for its Technical assistance and its logistical support.

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