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Double BR-OVT: a new trap model for collecting eggs and adult mosquitoes from *Culex quinquefasciatus* and *Aedes* spp.

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ABSTRACT

The circulation of arboviruses throughout the world and the maintenance of lymphatic filariasis endemicity in tropical countries, combined with the lack of vaccines and specific treatments, highlight the importance of reducing the populations of mosquitoes involved in the transmission of these pathogens, *Aedes aegypti* and *Culex quinquefasciatus.* To contribute to the development of new strategies for monitoring and controlling these culicids, we evaluated the performance of the Double BR-OVT trap individually and in pairs, in the field. After 18 months, the Double BR-OVT traps captured a mean of 3.5 ± 7.4 and 1.8 ± 3.2 of *Culex* and *Aedes*/residence/cycle, respectively, in addition to 410 ± 588.3 *Aedes* eggs/residence/cycle. When installed in pairs, the Double BR-OVT traps collected three times more adult mosquitoes of *C. quinquefasciatus* (9.4 ± 8.3 *Culex*/residence/bimester) and two times more *Aedes* spp. (3 ± 3.2 *Aedes*/residence/bimester) in comparison with the traps installed individually (2.6 ± 7.1 and 1.5 ± 3.2 *Culex* and *Aedes*/residence/bimester, respectively) ($p < 0.05$). The Double BR-OVT trap has an exceptional advantage: it aggregates different functionalities into a single instrument, as this type of trap can concomitantly collect eggs and adult mosquitoes of *C. quinquefasciatus* and *A. aegypti*, a feature that makes it a potentially useful tool among the strategies for monitoring and controlling these mosquitoes.

KEYWORDS: Culicidae. Trap. Entomological surveillance. Mosquito control.

INTRODUCTION

Continuous reports on arboviruses outbreaks worldwide, caused by dengue, chikungunya, and Zika viruses, have led health authorities to increase the efforts to reduce the number of people affected by these diseases[1-3]. In Brazil, the co-circulation of these arboviruses has already been observed, and in 2020, an alarming increase in the number of dengue cases was identified[3,4]. Additionally, the detection of the West Nile Virus[5] and the identification of a new Zika virus strain in Brazil[6] contribute to a complex epidemiological scenario in which a dramatic increase in the number of people infected by SARS-CoV-2 has also been observed, which could lead to the collapse of the country's fragile public health system[7].

Coupled with this event, the maintenance of the lymphatic filariasis endemicity in tropical countries[8] and the lack of vaccines for some arboviruses, such as Zika and chikungunya, makes it difficult to establish an adequate vaccination program to manage these diseases[9,10] highlighting the eminent need to reduce the populations

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of *Culex quinquefasciatus* Say, 1823 and *Aedes aegypti* (Linnaeus, 1762), which are culicid vectors of pathogenic viruses to humans[11].

The application of chemical insecticides remains as the main method used to control mosquitoes[12,13]. However, the continuous use of these products causes a selective pressure, leading to the emergence of mosquitoes resistant to these compounds, that also have harmful effects on humans, other animals, and to the environment, requiring periodic assessment of their continued use. In addition, the use of chemical pesticides requires the monitoring of the susceptibility of the mosquito population exposed to them, which results in the replacement of the product used in the mosquito control strategy[13,14]. In this sense, the use of more sustainable tools and strategies, such as an integrated vector control (IVC) strategy, has been gaining evidence[15-17].

The IVC is a method that involves the simultaneous use of several interventions, such as biological, chemical, genetic and physical control, established according to the characteristics of each area[18]. Some reports have shown that the adoption of an IVC strategy guarantees the immediate reduction in mosquito populations as well as the maintenance of low population densities for long periods[10,16,17].

As a component of the physical control, the use of traps may reduce mosquito populations when installed in the context of a strategy mass trapping (more than one trap at the same house), contributing to the massive collection of culicids[16,19,20]. The use of these tools can also be very useful for the surveillance of culicid vectors as the traps have shown a high sensitivity for the detection of mosquitoes in the field[20-22].

The Double BR-OVT trap is a tool derived from the adaptation of the Sticky BR-OVT trap[23] and can simultaneously collect eggs and adult mosquitoes of *C. quinquefasciatus* and *Aedes* spp. To contribute to the development of instruments to act in the surveillance and control of culicid vectors, in this study, we evaluated the performance of the Double BR-OVT for monitoring vectors and for the massive collection of culicids.

MATERIALS AND METHODS

Study site

Olinda is a Brazilian city (08°01'48"S 34°51'42"W) with an area of 41 km^2 and an estimated population of 392,000 inhabitants. Throughout the year, this city has high temperatures (mean of 26 °C) and a high level of rainfall (1,700 mm per year)[24]. The study was conducted between July 2015 and December 2016 in the Sapucaia neighborhood (Olinda, Pernambuco State (PE), Brazil), which is considered endemic for lymphatic filariasis[25]. Sapucaia has approximately 14,000 inhabitants and aggregates conditions that contribute to the maintenance of mosquitoes of *C. quinquefasciatus* and *A. aegypti*, such as the lack of a sewage system and intermittent water distribution.

Trap description

The Double BR-OVT trap (Figure 1) is composed of a black polyethylene box, which has a central opening (16 × 9 cm) on the upper side. A black plastic container (4 L) is placed inside the box, and a black polyethylene border is placed on top of the container, which has adhesive capacity due to the addition of a thin layer of insect glue (Colly®, Colly Quimica, Mombuca, SP, Brazil), applied with a spatula. Furthermore, the inner wall of the container was coated with a strip of raw cotton fabric (10 × 110 cm), serving as a substrate for the collection of eggs of *Aedes* spp.

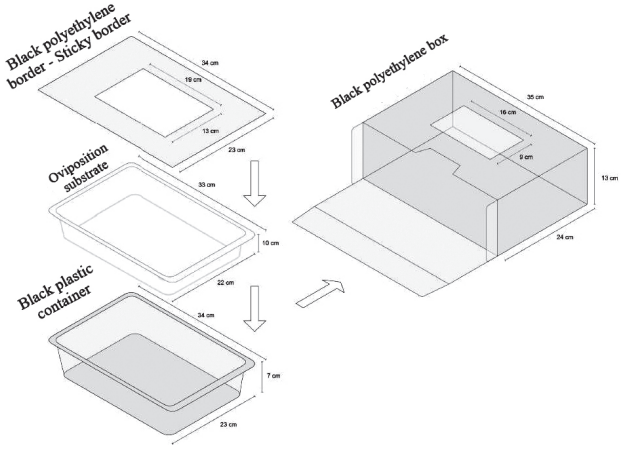

Figure 1 - Schematic drawing of the components of the Double BR-OVT.

Experimental design

The study was conducted in two stages: the first one occurred between July 2015 and June 2016. In the first stage, we intended to determine the efficacy of the Double BR-OVT trap; therefore, 70 traps were installed in Sapucaia residences distributed throughout the neighborhood. These residences were sampled according to the availability of the resident and the presence of professionals from the municipal health service in the study area, being at a distance of 50 m from one another (one trap/house).

During the second stage, conducted between July and December 2016, we evaluated the performance of the Double BR-OVT trap for the massive collection of

culicids. To this end, we selected one block of Sapucaia comprising 40 residences that showed one trap with high positivity and density rates during the first stage of the study. We considered highly positive traps the presence of *Aedes* eggs corresponding to rates above 88%[16,20]. For the presence of adult mosquitoes, we selected the traps with the highest positivity. A total of 60 Double BR-OVT traps (two traps/house) were installed in 30 residences in this block. At this stage, we monitored 30 Double BR-OVT traps installed in 15 residences by counting the number of eggs and adult mosquitoes captured in the traps. The other Double BR-OVT acted only as instruments for the massive collection of *C. quinquefasciatus* and *Aedes* spp.

All Double BR-OVT assessed in this study were installed on the indoor areas of Sapucaia residences. In both study stages, the trap evaluation cycles took place every 60 days, a period corresponding to the maintenance activity, including the replacement of sticky borders and fabric strips, in addition to refilling the traps with 3 L of water and adding 1 g of the *Bacillus thuringiensis* serovar *israelensis* (Bti) - based biological larvicide (VectoBac WG®, Valent BioSciences, New York, USA). For operational reasons, the monitoring of *C. quinquefasciatus* eggs, grouped in rafts, was performed only in the second stage of the study, with weekly collections to ensure the counting of intact egg rafts.

To monitor the populations of *C. quinquefasciatus* and *Aedes* spp. in the neighborhood, mosquitoes were collected using entomological aspirators (Horst Armadilhas, Sao Paulo, SP, Brazil)[26] every 90 days in 50 residences in the first stage and in 15 residences in the second stage. Aspirations were performed in the morning, lasting 20 minutes/residence/man.

Mosquitoes were identified according to the characteristics described by Forattini[11]. The identification of culicids was carried out in the laboratory of the Entomology Department of Aggeu Magalhaes Institute (Instituto Aggeu Magalhaes – IAM/Fiocruz-PE).

Statistical analysis

The efficacy of the Double BR-OVT trap was assessed based on positivity and the mean number of mosquitoes/eggs of *Aedes* collected in each trap per cycle, except for *C. quinquefasciatus* egg rafts, whose mean value was presented in cycles of 28 days (4 weeks). Positivity was determined by dividing the number of positive traps (at least one mosquito/raft of *C. quinquefasciatus*/egg of *Aedes* spp.) by the total number of traps checked.

To assess the potential of the strategy of installing the Double BR-OVT traps in pairs, we performed a comparative analysis between the number of mosquitoes collected in the traps between July and December 2015 (Period 1 - P1) and Period 2 (P2) from July to December 2016 (Figure 2). The analysis of variance (ANOVA) and an *a posteriori* Tukey's test were performed. The normality of data was assessed using the Shapiro-Wilk test. The homogeneity of variance was tested using the Levene test. Results with a value of $p < 0.05$ were considered statistically significant. The analyses were performed using the Statistica software, version 7.1 (TIBCO, Palo Alto, CA, USA).

Ethical considerations

This study was approved by the Research Ethics Committee of Aggeu Magalhaes Institute (Instituto Aggeu Magalhaes), Fiocruz, PE (CAAE 25117313.80000.5190). All residents who adhered to the survey signed the Informed Consent Form (Termo de Consentimento Livre e Esclarecido - TCLE) and all identification data about participants were kept confidential throughout the study.

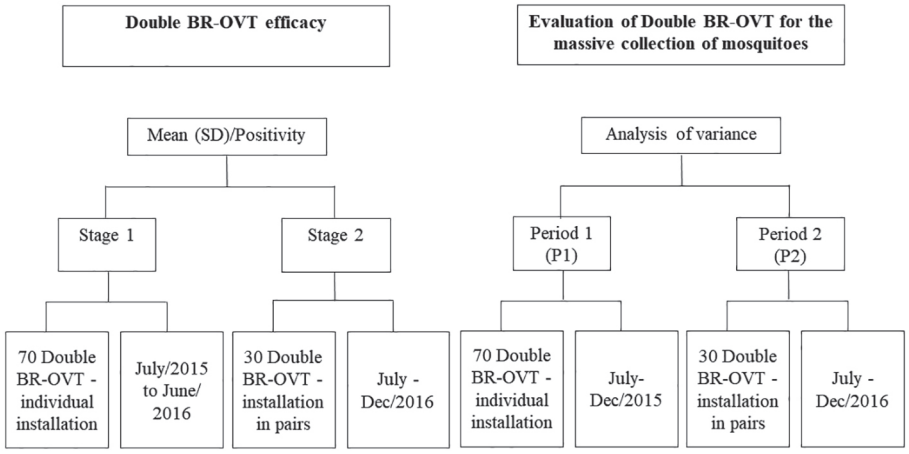

Figure 2 - Lowchart of the experimental design and respective statistical analyzes carried out in the study.

RESULTS

Performance of the Double BR-OVT trap for monitoring culicids

The Double BR-OVT traps were able to trap 2,283 adult mosquitoes, of which 66.3% were *C. quinquefasciatus* (3.5 ± 7.4 *Culex*/residence/cycle) and 33.7% were *Aedes* spp. (1.8 ± 3.2 *Aedes*/residence/cycle). In addition, it was possible to remove more than 149,000 eggs of *Aedes* spp., reaching a mean of 410 ± 588.3 eggs of *Aedes*/residence/cycle. During the second stage of the study, we counted 217 egg rafts (2.9 ± 4.6 rafts/residence/28 days).

Using the entomological aspirators, we collected 4,594 mosquitoes. Of these, 91.6% were classified as *C. quinquefasciatus* (19.4 ± 19.6 *Culex*/aspiration/trimester), and the other 8.4% were classified as *A. aegypti* (1.76 ± 2.58 *Aedes*/aspiration/trimester).

Performance of the Double BR-OVT trap for mass collection of culicids

The number of *C. quinquefasciatus* trapped between July and December 2016 (P2) was 9.4 ± 8.3 *Culex*/residence/bimester, when two Double BR-OVT traps were used in the residences, a value significantly higher ($F = 19.34$; $df = 2.049$, $p < 0.000$) than that obtained between July and December 2015 (P1), when only one Double BR-OVT trap was installed in each residence (2.6 ± 7.1 *Culex*/residence/bimester). Similarly, the number of *Aedes* spp. mosquitoes collected in the second evaluation period (P2) (3 ± 3.2 *Aedes*/residence/bimester) was significantly higher ($F = 4.38$; $df = 2.409$; $p < 0.013$) than that obtained in the first period (P1) (1.5 ± 3.2 *Aedes*/residence/bimester) (Figure 3).

The National Institute of Meteorology (Instituto Nacional de Meteorologia – INMET) recorded for the period from July to December 2015, in Recife, Pernambuco State capital, an average precipitation of 123 mm, while in the same period of 2016, the precipitation was 54.5 mm. The average temperatures recorded for the period varied between 25.8 and 26 ºC in 2015 and 2016, respectively and the average relative humidity remained around 74%, in both years. Despite the variation in the precipitation rates, it was not possible to detect a correlation between climate factors and the number of adult mosquitoes collected by the Double BR-OVT.

Potential of the Double BR-OVT trap for detection of culicids

When installed individually, the Double BR-OVT trap was able to detect the presence of at least one egg/adult mosquito of *C. quinquefasciatus* and/or *Aedes* spp. in more than 90% of the residences. The occurrence of adult mosquitoes (*Culex* and/or *Aedes*) was observed in 60.7% of the residences. The positivity rate, based on the presence of *Aedes* eggs, was approximately 91% (Table 1).

For the paired trap installation, we observed an increase in the sensitivity of the Double BR-OVT trap for the detection of culicids in the environment, reaching 100% positivity for the presence of at least one egg/raft or adult

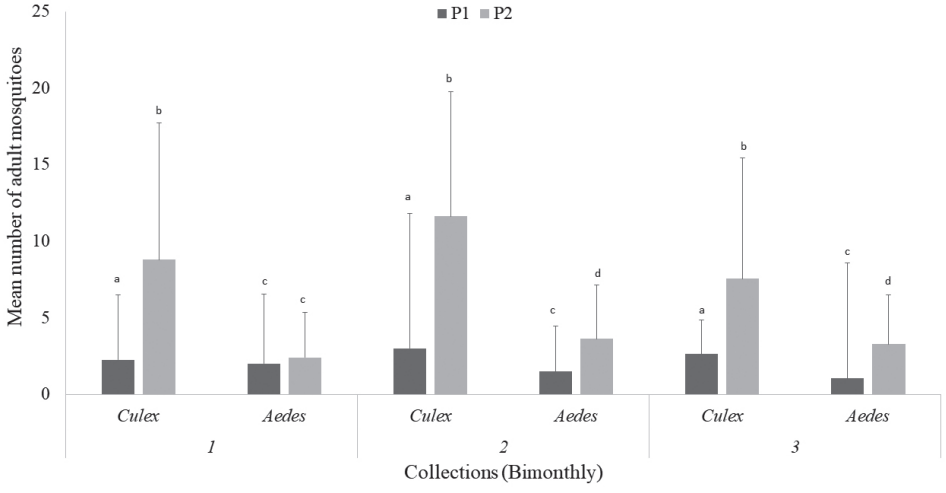

Figure 3 - Mean number of adult mosquitoes collected by the Double BR-OVT, between July and December of 2015 and 2016. [1]consider P1: Period 1 from Jul-Dec/201, P2: Period 2 from Jul-Dec/2016; [2]for *C. quinquefascitus* (represented in the graph by the letters a and b), there were statistical differences in the number of mosquitoes of this species between the three bimesters ($p < 0.0005$). For *Aedes* spp. (represented in the graph by the letters c and d), we identified a statistical difference between the number of mosquitoes collected in the second and third months ($p < 0.036$). The means indicated by the same letter do not differ significantly from each other.

Table 1 - Absolute number, mean and standard deviation of adult mosquitoes and eggs of *Culex quinquefasciatus* and *Aedes* spp. collected by the Double BR-OVT and entomological aspirators, between July/2015 and December/2016, in Sapucaia neighborhood, Olinda, Pernambuco State, Brazil.

Tools	Stage	*C. quinquefasciatus*					*Aedes* spp.					Tools' sensibility	
		Adult mosquitoes		Egg rafts		Adult mosquitoes and/or egg rafts (%)	Adult mosquitoes		Eggs		Adult mosquitoes and/or eggs (%)	Adult mosquitoes (%)	Egg rafts/ Eggs (%)
		DE (M±SD)	PO (%)	DE (M± SD)	PO (%)		DE (M± SD)	PO (%)	DE (M± SD)	PO (%)			
Double BR-OVT	1	1,148 (2.92 ± 7)	49.7	-	-	-	650 (1.65 ± 3.2)	46.1	116,663 (358 ± 553.8)	90.7	99.5	60.7	90.7
	2	366 (4.8 ± 5.2)	77	217 (2.9 ± 4.6)	61	95	119 (1.5 ± 2.2)	61	32,870 (438 ± 475)	84	88	85	100
Entomological aspirator	1	3,747 (19.6 ± 20.1)	97.4	NA	NA	NA	357 (1.86 ± 2.6)	60.9	NA	NA	NA	97.9	NA
	2	463 (17.8 ± 15.5)	84	NA	NA	NA	27 (1.03 ± 2.1)	28	NA	NA	NA	86	NA

Data are presented as mean±SD or percentages. In the first stage, egg rafts collections were not carried out. Thus, it was not possible to calculate the positivity index. NA = Not applicable; M = Mean; SD = Standard Deviation; DE = Density; PO = Positivity.

mosquito of *C. quinquefasciatus* and/or *Aedes.* spp. The ability to detect adult mosquitoes (*Culex* and/or *Aedes*) was also higher in this stage, when the positivity reached 85%. The presence of eggs (*Culex* and/or *Aedes*) was detected in all the traps installed, resulting in 100% positivity (Table 1).

DISCUSSION

The results showed that the Double BR-OVT trap has multiple functionalities due to its ability to concomitantly trap *C. quinquefasciatus* and *Aedes* spp. mosquitoes and remove their eggs from the environment. This trap is an easy-to-use instrument, which favors its use in entomological surveillance strategies. In addition, the Double BR-OVT trap, installed in pairs, can compose integrated strategies to control the population of these culicids, especially in areas where environmental conditions favor the reproduction of these insects[16], such as in Sapucaia (Olinda, PE, Brazil).

After 18 months of evaluation, the Double BR-OVT trap removed a mean of 3.5 ± 7.4 *Culex*/residence/cycle, showing a high capacity to trap *C. quinquefasciatus* mosquitoes. The performance of this trap was similar to that of other sticky traps previously described, such as the Sticky Ovitrap (0.1 ± 0.4 female *Culex*/trap/15 nights) and MosquiTRAP (0.2 ± 0.5 female *Culex*/trap/15 nights), evaluated in Muheza, Tanzania[27], the Sticky trap, evaluated in Rome in areas with larvicidal treatment (1.6 ± 0.1 female *Culex pipiens*/sticky trap)[28] and the Sticky BR-OVT, evaluated in Olinda, Pernambuco State, Brazil, that collected 2.16 ± 4.78 *Culex*/trap/month[23].

The Double BR-OVT trap was also effective in collecting egg rafts of *C. quinquefasciatus* (2.9 ± 4.6 rafts/residence/month). The good performance of Double BR-OVT in the collection of egg rafts is possibly associated with its attracting characteristics to pregnant females that will perform oviposition[29] and the use of biolarvicide Bti, which has already demonstrated its ability to stimulate oviposition in female culicids[21,29,30]. Similar results were observed by Correia *et al*.[31] and by Xavier *et al*.[23] when evaluating the BR-OVT oviposition trap (3.6 ± 7.6 rafts/residence/month) and the Sticky BR-OVT trap (0.52 ± 1.52 rafts/residence/28 days), respectively, in Olinda, Pernambuco State, Brazil. Xavier *et al*.[23] have also observed that the Sticky BR-OVT trap, similar to the Double BR-OVT trap, showed an important operational gain: the ability to aggregate the concomitant collection of eggs and adult *C. quinquefasciatus* mosquitoes. This finding demonstrates the viability of this trap as a surveillance strategy for *C. quinquefasciatus*.

The good performance of the Double BR-OVT trap was also observed in the trapping of *Aedes* spp. mosquitoes (1.8 ± 3.2 *Aedes*/residence/cycle). Its performance was similar to that of other sticky traps developed for *Aedes*, such as the AedesTraP, evaluated in Recife, Pernambuco State, Brazil (0.54 ± 0.07 females/trap/28 days)[32], and the MosquiTRAP, evaluated in Belo Horizonte, Minas Gerais, Brazil (0.11 *Aedes*/trap/week)[33] and in Rio de Janeiro, Rio de Janeiro State, Brazil (0.2 ± 0.1 *Aedes*/trap/week)[34]. By using the Double BR-OVT trap, it was also possible to collect eggs of *Aedes* spp. (mean of 410 ± 585 *Aedes* eggs/residence), and the performance of this trap was similar to that of the Ovitrampa traps monitored by Regis *et al*.[20] in residences in Recife, Pernambuco State, Brazil (722.4 ± 788.3 *Aedes* eggs/trap/28 days).

The Double BR-OVT presented a performance similar to that of oviposition traps in the detection and collection

of eggs of *C. quinquefasciatus* (BR-OVT) and *Aedes* spp. (Ovitrampa). In addition, we identified high standard deviations, demonstrating a wide variation in the number of eggs and adult mosquitoes collected in the residences. We believe that this wide variation may be related to the microenvironment characteristics of each residence[31], being detected through the number of mosquitoes and eggs collected in Double BR-OVT. Thus, we reiterate that the Double BR-OVT trap showed its ability to be a part of strategic plans for entomological surveillance. This trap effectively performs different functions, aggregating the collection of eggs and adult mosquitoes of *C. quinquefasciatus* and *Aedes* spp. in a single instrument. Thus, it is able to break the development cycle of two mosquito species of great medical importance.

During the study, we used the rates obtained with the entomological aspirator as an evidence on the presence of two species of culicids in the study residences, in Sapucaia neighborhood (Olinda, Pernambuco State, Brazil). After 18 months of evaluation, more than 4,200 *C. quinquefasciatus* (19.4 ± 19.6 *Culex*/residence/cycle) and 384 *A. aegypti* (1.8 ± 2.6 *Aedes*/residence/cycle) mosquitoes were collected through aspiration. However, during our study, we realized that the use of entomological aspirators had limitations for the detection of *Aedes* spp. in the environment compared to Double BR-OVT, a trap capable of detecting the presence of culicids by collecting eggs and adult mosquitoes. Maciel-de-Freitas *et al*.[35], who observed that the BGS-Trap, a passive collection instrument, was more efficient than the backpack aspirator, an active collection method for the collection and detection of *A. aegypti* in the environment ($p < 0.05$). This finding is similar to that of Pombi *et al*.[36], who used species richness indicators to evaluate the performance of different tools and found that the passive collection of the Sticky Resting Box trap was more effective than the active collection of a backpack aspirator in the capture of culicids, such as *Anopheles* spp. Thus, we suggest the use of passive collection traps, such as the Double BR-OVT, to detect the presence of *A. aegypti* mosquitoes in the environment.

When evaluating the strategy of paired installation of Double BR-OVT traps, we observed an increase in the trap's ability to detect the presence of culicids, especially in the form of eggs. The positivity rates were similar to those previously described for both, the collection of egg rafts[21,31] and *Aedes* spp. eggs[16,20]. Additionally, the numbers of adult mosquitoes collected by the Double BR-OVT traps in the second stage of the study (9.4 ± 8.3 and 3 ± 3.2 *Culex* and *Aedes*/residence/bimester, respectively) were significantly higher than those in the first stage (2.6 ± 7.1 *Culex*/residence/bimester and 1.5 ± 3.2 *Aedes*/residence/bimester), when only one Double BR-OVT trap was installed. Other studies have found that increasing the number of traps increases the ability to collect mosquitoes. These results were observed by Santos *et al*.[32] in Recife, Pernambuco State, Brazil and by Barrera *et al*.[37] in Puerto Rico, when they used three traps/property (AedesTraP and CDC Autocidal Gravid Ovitrap, respectively) and observed significant increases in the number of collected mosquitoes, contributing to the reduction in the culicid infestation levels in the environment. However, Degener *et al*.[38] installed three MosquiTRAPs/residence in Manaus, Amazonas, Brazil, and did not detect significant reductions in the *Culex* or *Aedes* populations. Therefore, we recommend the installation of the Double BR-OVT trap in pairs in control strategies as, using this procedure, it is possible to increase significantly the number of eggs and adult mosquitoes collected from the environment.

CONCLUSION

The Double BR-OVT trap is a very sensitive tool to detect the presence of *C. quinquefasciatus* and *Aedes* spp. in the environment, especially in the egg stage, indicating its potential for use in mosquito surveillance strategies. We have also found that paired installation of the Double BR-OVT trap significantly increased the number of mosquitoes collected. This finding suggests that this trap can be used as part of control strategies of *C. quinquefasciatus* and *A. aegypti* by health agencies and, given its ease of use, can be easily monitored by endemic disease workers. Additionally, the use of the Double BR-OVT trap can possibly generate an important positive social impact on health, especially in areas where there is little availability of human resources, because this trap acts to directly remove mosquitoes of medical importance, remaining effective for 60 days.

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AUTHORS' CONTRIBUTIONS

MNX, RMRB and CMFO contributed to the study conception and experimental design; MNX and EMMS

analyzed the data; MNX, DCTVM and MPR performed data collection in the field and laboratory activities; all authors contributed to the writing and critical analysis of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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