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BIOMEDICAL SCIENCES

Use of lyophilized larval extracts associated with *Bti* in Double BR-OVT trap: Strategy to attract and kill mosquitoes of the genera *Aedes* and *Culex*

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Abstract: The present study evaluated the efficiency of the larval extract of *Aedes aegypti* associated with *Bacillus thrurigiensis* var. *israelensis* as oviposition bait to enhance the attractiveness of the Double BR-OVT trap. In the laboratory, paired tests were carried out, using two oviposition sites. Thirty pregnant females of *A. aegypti* were used per test. In the field, paired traps were installed at eight points. The test traps (2 g larval extract + 1 g Bti/ 2 L), controls (1 g Bti/ 2 L). For tests with lyophilized extract, each test trap contained 0.26 g/lyophilized larvae + 1 g Bti/ 2 L. Laboratory results showed that all cups treated (68.8 %/ 842 ± 177; 72.5 %/ 822 ± 167; 70.4 %/ 904 ± 169, respectively), with or without Bti, collected more eggs. In the field, traps treated with larval extract or lyophilized plus Bti collected more *Aedes* spp eggs (64 %/ 582 ± 467; 62.5 %/ 511 ± 531) and *C. quinquefasciatus* (65 %/ 11 ± 10; 70 %/ 5 ± 4.3) rafts. The association of Double BR-OVTs traps with larval extract plus Bti proves to be efficient alternatives for the integrated control, with the strategy of attracting and eliminating mosquitoes.

Key words: *Aedes aegypti, Culex quinquefasciatus*, mosquito control, oviposition attractant, vectors.

INTRODUCTION

Population control of *Aedes* (Stegomyia) *aegypti* Linnaeus (1762) is a necessity imposed by its involvement in the transmission of pathogenic agents to humans, such as Dengue, Chikungunya, Yellow Fever, Mayaro and Zika, putting at risk more than 3.9 billion people in more than 129 countries (Lwande et al. 2020). On the other hand, *Culex quinquefasciatus* (Say, 1823) is also present in urban anthropogenic environments. In addition to representing a significant nuisance to people, its role as a vector of bancroftian filariasis in the Americas (WHO 2017, Albuquerque et al. 2020) makes it a target for control. This is also due to its potential in the transmission of arboviruses, such as West Nile virus in Africa, Asia, Europe, and South America (Fagre et al. 2023). Zika, Oropouche, and Mayaro in Brazil (Vasconcelos et al. 2011, Cardoso et al. 2015, Guedes et al. 2017). in addition to viral encephalitis, with Venezuelan, Japanese, and Saint Louis (USA) (Spinsanti et al. 2003, Wang et al. 2012, Lopes et al. 2014).

Brazil has been investing in population control of *A. aegypti* for decades through the National Dengue Control Program (PNCD), established in 2002, where it currently operates in more than 5.000 Brazilian municipalities (Brasil 2002). However, the country continues to have high rates of *A. aegypti*, with 2024 being the year marked by the largest dengue epidemic in Brazil, exceeding 1.8 million cases in the first two

months of the year (Brasil 2024). Additionally, Brazil was hit in 2015 by the second largest recorded dengue epidemic, with more than 1.6 million cases reported (Luna et al. 2020), and by the first report of autochthonous transmission of the Zika virus (Zanluca et al. 2015). Some of the main weaknesses of the PNCD involve the limitation of visual inspection to the survey of indices in the surveillance of *Aedes* species (Codeço et al. 2015), and the application of insecticides as the main control method (Zara et al. 2016). On the other hand, although there is a surveillance program for *C. quinquefascaitus* in Brazil (Brasil 2011), there are no programs aimed at controlling this species, as occurs for *A. aegypti*.

These measures contrast with what is suggested by the Global Response to Vector Control 2017–2030, which suggests the use of integrated strategies to suppress the vector population (WHO 2017). One of the applications of integrated control is the use of traps to collect mosquitoes at different life stages. Compared to the larval research commonly observed in the PNCD, the use of oviposition traps (ovitrap) proved to be more sensitive and operationally simpler to work with for the survey of entomological indices (Braga et al. 2000, Regis et al. 2013, Melo et al. 2024); Regis et al. (2013) disseminated 5.680 ovitraps treated with water and Bti; Nascimento et al. (2020) used ovitraps treated with grass infusion (*Megathyrsus maximus*); Resende et al. (2012) worked on the efficiency of MosquiTRAP® traps treated with larval rearing water; Xavier et al. 2020, showed that the Double BR-OVT trap treated with water and Bti has multiple functionalities due to its ability to simultaneously capture adults and eggs from genera *Culex* and *Aedes*.

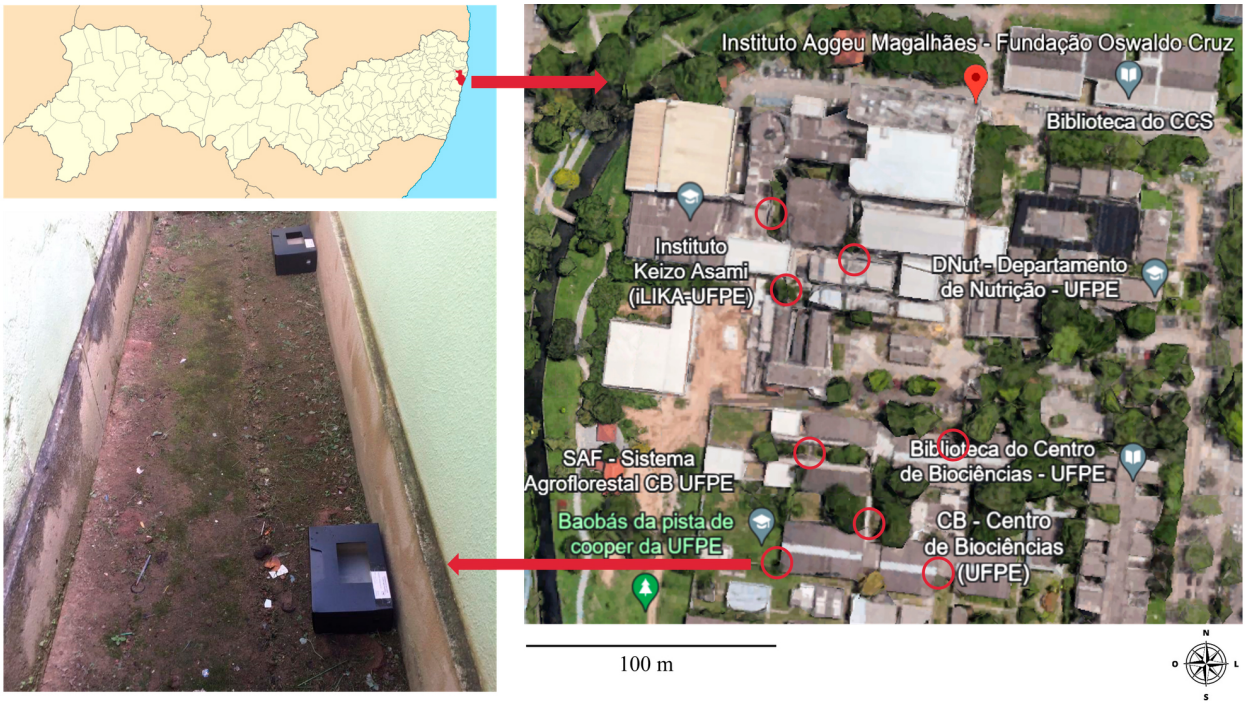
Given the need to improve traps, studies on semiochemicals are highlighted in the formulation of baits, as they act as regulators of the main types of insect behavior, such as the search for oviposition sites (Leal et al. 2008, Serpa et al. 2008, Melo et al. 2024). Studies by our group, using ovitrap associated with larval extract *A. aegypti* and Bti, demonstrated in field experiments, *A. aegypti* laid signifcantly more eggs in traps loaded with larval extracts plus Bti (Faierstein et al. 2019). Finally, we explored the potential application of this larval extract with the larvicide Bti in attraction-and-kill strategies. Specifically, we questioned whether this combination would be active in the field when used in another trap model. The present study evaluated the efficiency of the larval extract of *A. aegypti* associated with *Bacillus thrurigiensis* var. *israelensis* as oviposition bait to enhance the attractiveness of the Double BR-OVT trap under field conditions.

MATERIALS AND METHODS

Study site

Recife, the capital of Pernambuco State (8° 3' 14" S, 34° 52' 51" W), is a coastal city characterised by a warm and humid climate with an annual mean temperature of 25.5°C with narrow variations, greatly favouring mosquito proliferation throughout the year (Regis et al. 2013). Lymphatic filariasis and dengue fever are both endemic in Recife (Regis et al. 1995, Regis et al. 2008) while Chikungunya and Zika viruses were introduced in 2014-2015 (Codeço et al. 2015). The laboratory bioassays were carried out in the insectarium of the Entomology Department of the Instituto Aggeu Magalhães/Fiocruz-PE (IAM_FIOCRUZ/PE) and the field tests were carried out at eight locations, three points at IAM_FIOCRUZ/PE and five in the Biosciences Center on the campus of the Universidade Federal de Pernambuco (CB-UFPE) (Figure 1).

Mosquitos. The *A. aegypti* colony (RecL) started in 1996, from eggs collected in



Source: Google Earth (https://earth.google.com/) 2024.

Figure 1. Distribution location of the eight installation points for paired Double BR-OVT traps. Five points at the Biosciences Center on the campus of the Universidade Federal de Pernambuco (UFPE) and three points at the Instituto Aggeu Magalhães (FIOCRUZ-PE).

neighborhoods in Recife (Araújo et al. 2019). The colony were kept in Recife at 26 ± 2 °C, 65–85% relative humidity, and under a photo period of 12:12 h (light: dark). Larvae were kept in plastic containers (30×15 cm; 10 cm height) with a density of approximately 0,5 larvae/ mL. After emergence, the mosquitoes were transferred to a containment cage (30 x 22 x 20 cm), with food available ad libitum on cotton balls, one soaked with water and the other with 10% sucrose solution. The mosquitoes were grouped into approximately 800 individuals, between males and females (1:1), and between the seventh and tenth day after emergence, an artificial blood meal was offered with 20 mL of defibrinated blood from the rabbit *Oryctolagus cuniculus*, Lineu (1758) (Faierstein et al. 2019).

Trap. The Double BR-OVT trap is a tool derived from the adaptation of the Sticky BR-OVT trap (Xavier et al. 2018) and can simultaneously collect eggs and adult mosquitoes of *C. quinquefasciatus* and *Aedes* spp. The Double BR-OVT trap model (Xavier et al. 2020) 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 edge 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). 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. In this study we did not use the adhesive edge. The traps were evaluated in pairs, one under treatment with 2 litters of larvae extract (larvae in natura or lyophilized), at a concentration of 0.33 L4/mL (Faierstein at al. 2019) with the addition of 1 g *Bacillus thuringiensis* var. *israelensis* biolarvicide (Bti,

VectorBac® WG) to prevent the traps from becoming breeding sites (Melo et al. 2024).

Biolarvicide. VectoBac® WG (registered trademark of Valent BioSciences LLC, New York USA), is a biological larvicide for controlling mosquito larvae composed of *Bacillus thuringiensis* subsp. *israelensis* (Bti) strain AM65-52 (Lot: 257-352-PG), concentration 37.4%, in water-dispersible granule formulation. The product has a potency of 3.000 international toxic units (UTI) per milligram against *Aedes aegypti* larvae. The choice of Bti larvicide was since it is an efficient larvicide against species of urban mosquitoes of the genera *Culex* and *Aedes*. In addition to the proven long persistence of larvicidal activity in shaded areas (Melo-Santos et al. 2009), and this larvicide can also help attract pregnant females (Carrieri et al. 2009, Barbosa et al. 2010), potentially promoting the concentration of mosquito eggs in lethal ovitraps.

Extraction procedures

Extrat larval in nature: Fourth-stage larvae (L4) were collected with a plastic mesh net and washed with distilled water 3–7 times. Five larvae were placed into a 2 mL microcentrifuge tube. After adding 0.5 mL of distilled water, the larvae were grinded, the pistil was washed twice with 0.5 mL of distilled water. The extract was then filtered through a Whatman #1 filter paper (catalogue number 1001-110) and washed with a total 150 mL of distilled water (Marques & Miranda 1992, Faierstein et al. 2019).

Lyophilized larvae extract: Initially, 600 fourth-stage larvae (L4) were dispersed in a lidless glass Petri dish, the opening of which was subsequently covered with perforated parafilm. The sample was stored at -80 °C for five hours, and then lyophilized (Edward® model LHKR Boc) for fifteen hours. Then, the lyophilized larvae were suspended in 300 mL of distilled water using a disperser and was conventionally filtered through a paper filter. Finally, 1.500 mL of water was added to reach an equivalence of 0.33 larva/ mL (Serpa et al. 2008).

Laboratory test

For further evaluations of the potential of the larvae extract associated with VectorBac® WG (Bti) biolarvicide as a lethal oviposition bait, we first checked the influence of Bti biolarvicide on extracts and destilled water (control) in relation to the oviposition behaviour of *A. aegypti*. The oviposition bioassay initially evaluated in the laboratory whether there would be any influence on the association between Bti biolarvicide and the larvae extract of *A. aegypti*. We carried out three tests: 1- Larvae extract + Bti versus distilled water + Bti; 2- Larvae extract + Bti versus distilled water; 3- Larvae extract versus distilled water + Bti. The tests were carried out in cages (50 × 40 × 32 cm), where two cups (150 mL) were placed (test and control). Twenty pregnant females of *A. aegypti* were released per cage and eggs were counted after 7 days/12 repetitions. These experiments were performed at the same time using 12 cages with different configurations of the treatments inside each cage.

Field test

With the objective of simultaneously evaluating the effect of larvae extract on the oviposition of *Aedes* and *Culex* mosquitoes in the field, on a small scale, sixteen Double BR-OVTs were used in eight locations protected from the sun and rain, three points on the external area of the IAM_FIOCRUZ- PE and five nearby points, located in the Biosciences Center, campus of the Universidade Federal de Pernambuco (CB-UFPE), an area with intense movement of people and high infestation by *A. aegypti* and *C. quinquefasciatus* (Krokovsky et al. 2022). The traps test was loaded with 2 g of larvae extract

in 2 L of tap water plus 1 g of Bti biolarvicide, whereas the control traps were loaded with 2 L of tap water plus 1 g of Bti. To each trap, strip of raw cotton fabric (10 × 110 cm) was attached to the border of the water cups to facilitate oviposition. These experiments were performed from July to December 2018. Traps were inspected and rotated every 2 weeks. Each data set from the eight locations was considered one statistical point, and the observations on the number of *Aedes*/*Culex* eggs/rafts experiments were replicated 63 and 44 times. Field tests were also carried out with the traps, using lyophilized larvae extracts of *A. aegypti*. For each test trap, a macerate of 0.26 g of larvae lyophilized in 10 mL of water was used, associated with 1 g Bti in 2 L of public water supply. 55 and 46 repetitions were carried out, respectively, between April and July 2019.

Ethics - The study was carried out according to resolution 466/12 for research with human beings and approved by the Research Ethics Committee of the Instituto Aggeu Magalhães (CEP/IAM) under the protocol code approval 1.547.598 on the Brazil Platform and CAAE: 51012015.9.0000.5190.

Data analysis

Data from laboratory bioassay results were analysed with Prism 7 (GraphPad, La Jola, CA). They were arcsine transformed and, after passing the Shapiro-Wilk normality test, were compared using the two-tailed paired t test. Data from Field tests were analysed by comparing the means by using the Wilcoxon matched-pairs signed rank test (Siegel & Castellan 1988). The oviposition activity index (OAI) was estimated according to Kramer and Mulla (Kramer & Mulla 1979). The effectiveness of the Double BR-OVT trap associated with larval extract *A. aegypti* plus Bti was evaluated based on the mean and standard deviation values of eggs and rafts collected in each trap. Mean positivity was determined by the quotient between the number of positive traps (at least one raft/egg) and the total number of traps deployed.

RESULTS

Laboratory evaluation

The evaluation of larval extract, with or without the addition of the biolarvicide VectorBac® WG (Bti), on the oviposition behavior of *A. aegypti* revealed that all containers treated with larval extract attracted significantly more eggs ($p = 0.005$). In the first evaluation, containers treated with larval extract + Bti were compared to control containers with distilled water + Bti. The treated containers received 68.9% of the eggs (totalling 10.104 eggs; mean 842 ± 177), while the control containers collected only 31.1 %, indicating that the treated containers acted as oviposition stimulants, with an OAI of +0.38 (Figure 2a). Next, the treatment with larval extract + Bti was compared to a control with distilled water alone, the treated containers collected 72.5 % of the eggs (totalling 9.865 eggs; mean 822 ± 167), while the controls collected only 27.5 %, showing an OAI of +0.45 (Figure 2b). In the final test, containers with larval extract only were compared to controls containing distilled water + Bti. The treated containers continued to stimulate oviposition (OAI = +0.41), attracting 70.4 % of the eggs (totalling 10,853 egs; mean 904 ± 169), compared to 29.6 % for the controls (Figure 2c).

Field evaluation

The results demonstrated that Double BR-OVTs treated with larval extract + Bti biolarvicide collected significantly more eggs from *Aedes* ssp mosquitoes, OAI = +0.28 (totalling 36.683 eggs 64 % mean 582 ± 467) ($p = 0.001$). The IPO ranged from 97 % on control and treatment, and IDO of

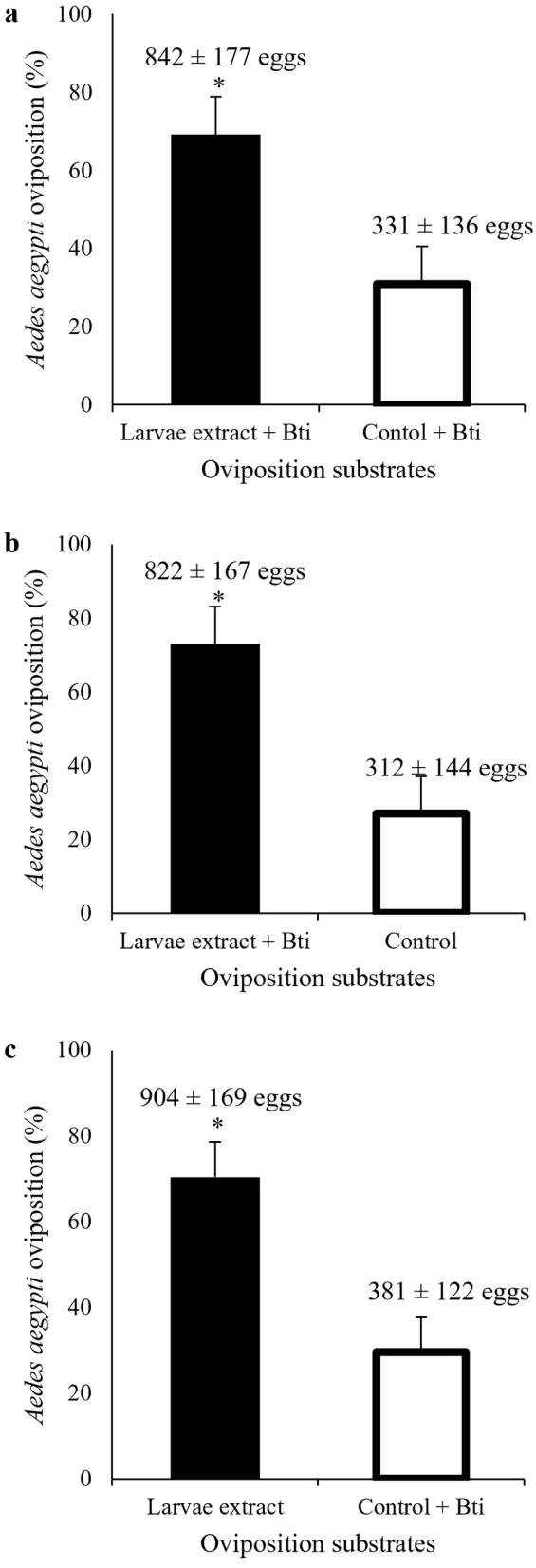


Figure 2. Oviposition preference for larvae extracts in the presence of *Bacillus thuringiensis israelensis* (Bti) or not. Bti was added to cups loaded with larvae extracts from *Aede aegypti* as well as to the control water cups in laboratory with 12 replications. Means were compared by using the Wilcoxon matched-pairs signed rank test. (a): larvae extract + Bti versus distilled water + Bti; (b): larvae extract + Bti versus distilled water; (c): larvae extract versus distilled water + Bti.

337 eggs on control and 601 on treatment (Figure 3a). Regarding *C. quinquefasciatus* oviposition in Double BR-OVTs treated with larval extract + Bti, we observed significantly higher collections, OAI= +0.30 (totalling 478 rafts 65 % mean 11 ± 10) ($p < 0.0001$). The IPO ranged from 75% on control and 98% on treatment with IDO of 8 rafts on control and 11 on treatment (Figure 3b).

The Double BR-OVTs associated with lyophilized larvae extract and Bti biolarvicide collected significantly more eggs ($p < 0.0001$; IAO = +0.24) in relation to the oviposition of *Aedes* ssp mosquitoes (totalling 28.085 eggs 62.5 % mean 511 ± 531), demonstrating the IPO ranged from 96% on control and 100% on treatment, with IDO ranging 318 eggs on control and to 511 on treatment (Figure 4a) Evaluating the oviposition of *C. quinquefasciaus* mosquitoes in Double BR-OVTs, we also observed significant collections in traps treated with lyophilized larvae extract and Bti (totalling 190 rafts, 70 % mean 5 ± 4.3) ($p < 0.1025$; IAO = +0.30), with IPO ranged 66% on control and 78% on treatment and IDO ranging 3 rafts on control and 6 on treatment (Figure 4b).

DISCUSSION

For pregnant female mosquitoes, the selection of appropriate sites for larval development is crucial and is considered one of the most important steps for establishing their populations, since immatures are not migratory, and many of them develop in confined environments, such as *A.*

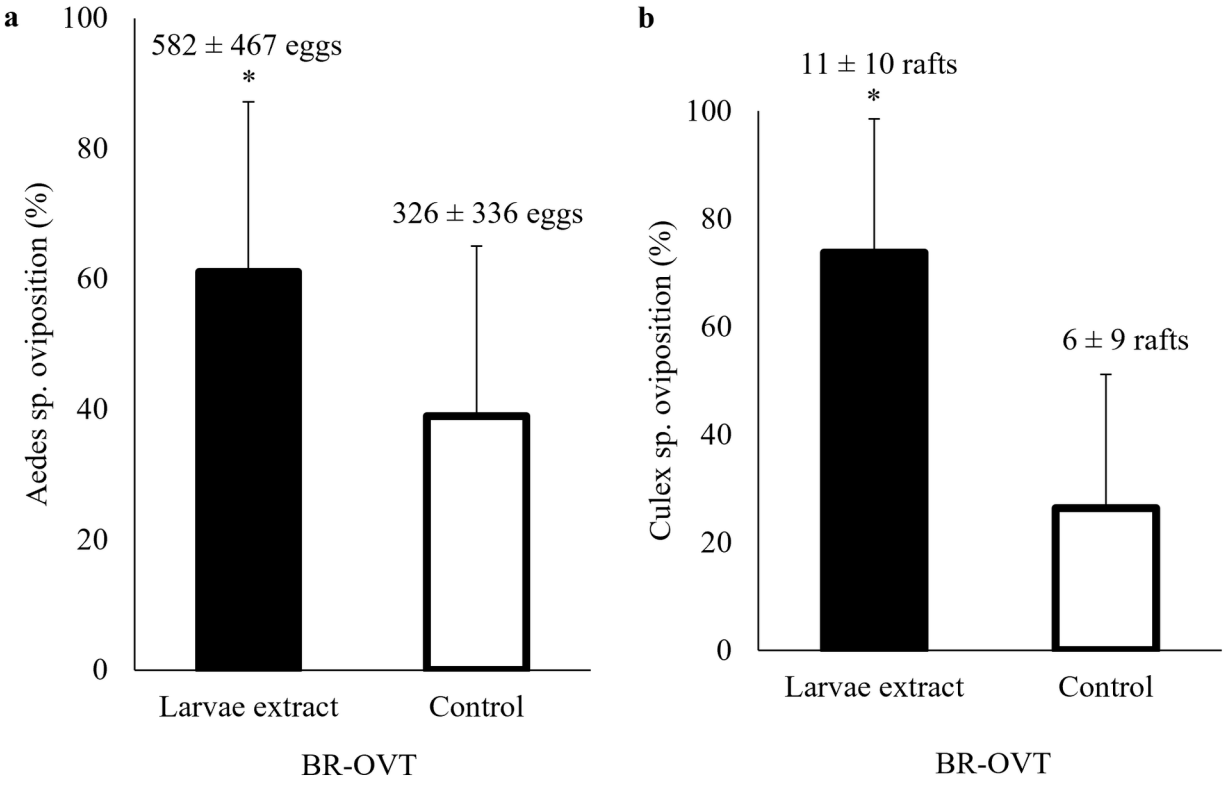


Figure 3. Oviposition preference for larvae extracts in the presence of *Bacillus thuringiensis israelensis* (Bti). Bti was added to Double BR-OVT traps loaded with larval extracts from *Aedes aegypti* as well as to the control water traps. Pairs of traps were deployed in the eight different locations in the field and experiments were replicated 63 and 44 times. Means were compared by using the Wilcoxon matched-pairs signed rank test. (a): *Aedes sp*; (b): *Culex sp*.

aegypti, whose water retention sites have varying capacities, from those that hold a few millilitres, to those filled with tens or hundreds of litters (Forattini & Brito 2003). For this reason, female mosquitoes search for physical and chemical clues in the environment, with predilections between oviposition sites. In this way, oviposition semiochemicals act as attractants/stimulants, or repellents/deterrents, of which two factors deserve discussion: oviposition pheromones and kairomones produced by mosquito larvae and eggs (Gonzalez et al. 2016), and oviposition kairomones produced by bacteria, related or not, to larval development (Ponnusamy et al. 2015).

The results showed that the use of *A. aegypti* extract with larvae in natura or lyophilized associated with the biological larvicide Bti, in oviposition traps of the Double BR-OVT trap, collected more eggs of mosquitoes of the genera *Aedes* and *Culex* when compared to traps only treated with water and Bti.

Faierstein et al. (2019) described for the first time the influence that extracts from eggs, larvae and pupae of *A. aegypti*, *C. quinquefasciatus* and *A. albopictus* exert on intra and interspecific oviposition behaviour, whose reports pointed to larval extracts as the most promising for creating oviposition baits with multiple targets, possibly because there are similarities between some compounds from the different extracts that influenced the oviposition behaviour of different species of mosquitoes. Some confounding factors can be found in the literature regarding what a larval extract represents. While some studies

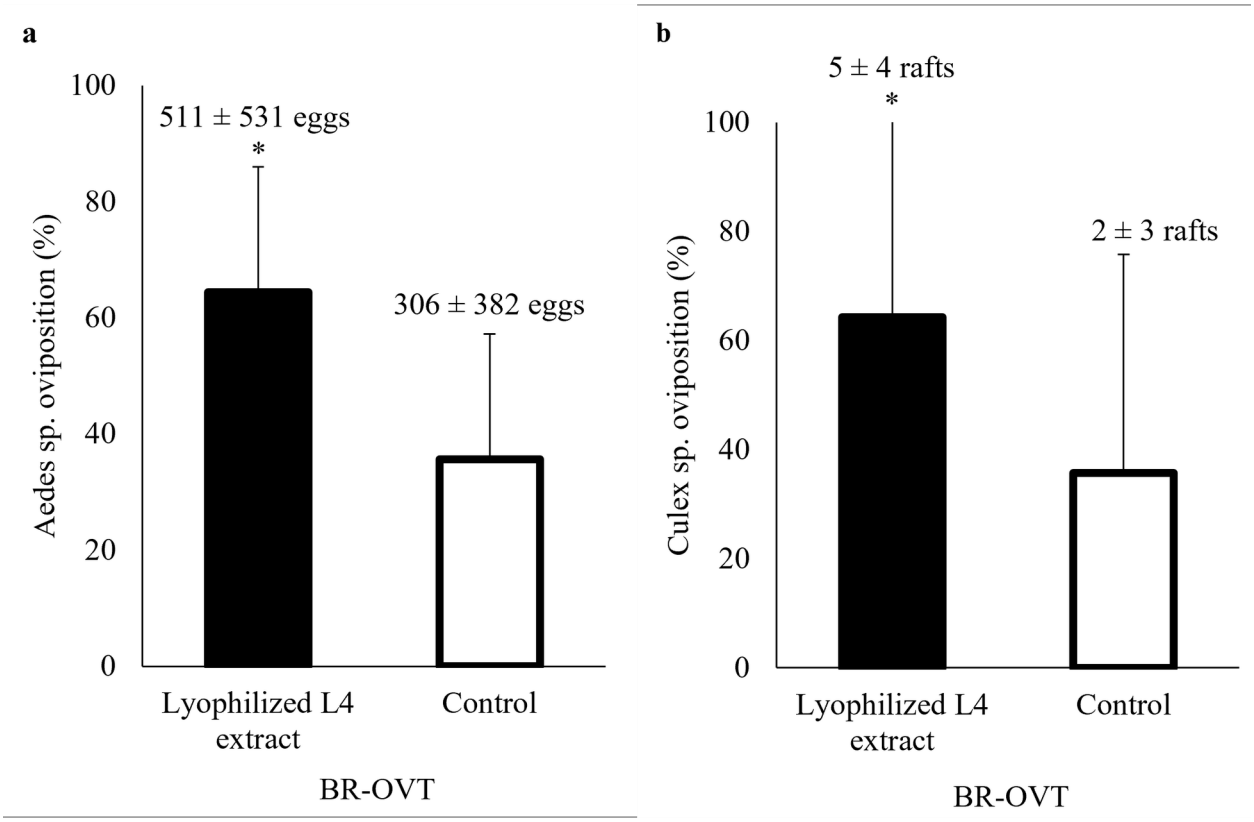


Figure 4. Oviposition preference for lyophilized larvae extracts in the presence of *Bacillus thuringiensis israelensis* (Bti). Bti was added to Double BR-OVT traps loaded with larval extracts from *Aedes aegypti* as well as to the control water traps. Pairs of traps were deployed in the eight different locations in the field and experiments were replicated 55 and 46 times. Means were compared by using the Wilcoxon matched-pairs signed rank test. (a): *Aedes sp*; (b): *Culex sp*.

described extracts from crushing the bodies of larvae (Marques & Miranda 1992, Faierstein et al. 2019), others described that the extracts evaluated were obtained from exposing distilled water to live larvae for a few days (Boullis et al. 2021), reporting containers with high larval densities in water (1 larva/ mL) outside of oviposition grounds of *A. aegypti* in relation to controls, whose compounds identified in the extracts and evaluated separately showed that pentadecanoic acid (1 ppm) is an oviposition stimulant, while myristoleic acid (10 ppm or more) is an oviposition deterrent.

Methodological differences compared to those in our studies likely led to different results, as cups treated with homogenized larval extracts (1 larva/ mL) collected significantly more eggs at the higher larva/ml concentrations (Faierstein et al. 2019). The results of Boullis et al. (2021) are similar to those of Allan & Kline (1998) and Davis et al. (2015), who described that, *A. aegypti* and *A. albopictus* admit or prefer containers in which intraspecific larvae developed at low densities (0.33 larva/ mL), while the rearing water in which many intraspecific larvae developed was deterrent/ repellent. In relation to other mosquito species, it was also observed that larval development water influences the oviposition behaviour of pregnant females of *A. togoi* (Theobald, 1907) (Trimble & Wellington 1980), *A. triseriatus* (Say, 1823) and *A. atropalpus* (Coquillett, 1902) (Bentley et al. 1976), and *Anopheles gambiae* (Giles, 1926) (Blackwell & Johnson 2000), with *Anopheles*

(Meigan, 1818) having a different behaviour, as only containers with the initial stages of larval development attracted females for oviposition, while containers with more advanced stages were avoided, regardless of the concentration of larva/ mL (Himeidan et al. 2013).

Evaluating larval extracts of *A. aegypti* in relation to oviposition, we observed that both in the laboratory and in the field, the treated containers collected more eggs than the control containers. Oviposition tests in the laboratory are generally punctual and controlled, while in the field a range of factors can influence the oviposition behaviour of mosquitoes, such as availability of surrounding breeding sites, changes in temperature and humidity and changes in the intensity of movement of people. However, the objective of this research focused only on the evaluation of traps treated with extracts, and not on explaining the factors that influenced the dynamics of collected eggs, generally observed in mosquito monitoring and surveillance studies.

Controlling mosquitoes using traps associated with oviposition bait with practicality and low cost is a challenge. Nascimento et al. (2020) compared the sensitivity of ovitraps treated with infusion of grass *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs (Guinea grass) tin relation to the conventional method of LIRAa (Rapid Index Survey for *Aedes aegypti*), in Cambé-PR. The sensitivity of the Property Infestation Index generated by traps (54%) was extremely more sensitive in relation to LIRAa (1.3%). However, producing grass infusions for large-scale monitoring and/ or control actions implies the production and transporting hundreds of litters of infusion. Regarding the treatment of the traps in our study, with larval extracts, it was only necessary to transport Falcon tubes with up to 10 mL of the crude extract, to then be transferred to the trap vats with 2 litters of water purchased at the installation site, demonstrating greater practicality for covering large-scale traps.

The association of oviposition traps with larval extracts and biolarvicides prove to be efficient alternatives for monitoring and, mainly, for entomological control, especially involving *Aedes* mosquitoes, since the species are capable of dispersing eggs from the same gonotrophic cycle in more than 10 containers, thanks to skip oviposition behaviour (Wu et al. 2020). Traps with oviposition stimulants can induce *Aedes* mosquitoes to lay more eggs in an environment treated with biolarvicide, thus reducing the number of eggs deposited in untreated locations, or even reducing the search for other oviposition sites. The treated and untreated Double BR-OVT traps obtained an IPO of almost 100%, proving their sensitivity, with the IDO of traps treated with larval extracts always higher in all evaluations. Over ten years, Regis et al. (2013) used 5.680 ovitraps treated with water and Bti as one of the integrated control methods for *Aedes* mosquitoes in Santa Cruz do Capibaribe and Ipojuca, two municipalities in Pernambuco, eliminating more than 3.500,000 eggs in the regions, whose actions helped to reduce the mosquito population density of more than 90%.

In another context, oviposition baits are also valuable for collecting pregnant female mosquitoes during arbovirus surveillance, as treated traps will be more likely to collect females infected with circulating pathogens (Leal et al. 2008). This way, it is possible to detect the circulation of arboviruses more accurately in the vicinity where the traps were installed. This particularity is important, as the wrong choice of bait can result in false-negative data in relation to entomological surveillance. The use of CO_2 as bait to capture adults in traps is observed in some contexts (Wu et al. 2020, Amos & Cardé 2021), but the physiological state of females

attracted to this type of bait, which they find in CO_2 evidence of a host, may not be useful for surveillance, as they are attractive to mosquitoes that seek blood feeding, consequently attracting the large portion of the mosquito population that has not yet had a blood meal, making it difficult to collect information on the circulation of arboviruses. Therefore, the baits used to attract pregnant mosquito females are more efficient in providing true indicators of arbovirus circulation (Johnson et al. 2017).

Xavier et al. (2020) demonstrated that the Double BR-OVT trap is a very sensitive tool for detecting the presence of *C. quinquefasciatus* and *Aedes* spp. in the environment, mainly in the egg phase, indicating its potential for use in mosquito surveillance strategies. Our results ensure that the association of a tool capable of collecting eggs from different genera of mosquitoes, and when associated with an oviposition stimulant, becomes more efficient and effective. In view of these observations, larval extracts of *A. aegypti* associated with Bti have potential for application in integrated management between *Aedes* and *Culex* mosquitoes, which represent the most important genera in the field of medical entomology. The logistics of this attract-and-kill strategy can be further simplified when the active ingredients are identified, replacing the larval extract with synthetic oviposition attractant and/or stimulatory counterparts. The association of Double BR-OVTs traps with larval extracts and biolarvicide proves to be efficient alternatives for the integrated control. Therefore, extracts of lyophilized larvae associated with Bti can be used as bait, thus enhancing traps for use in the field, in the surveillance and control of mosquito populations. This strategy will bring cost-benefit to environmental agents, as it is a specific, environmentally safe and low-cost alternative for action.

Acknowledgments

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RMRB and GFB conceived the study, designed the study and analysed the experimental data, performed the experiments and wrote the manuscript. WL conceived the study, designed the study and analysed the experimental data. AKLSS performed the experiments. All authors reviewed the paper and agreed to the final version.