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Mosquitex, a new tool for capturing *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) (Diptera: Culicidae)

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Abstract. The species *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) (Diptera: Culicidae) is of great importance for human health, as it is responsible for the transmission of viruses that can cause dengue, chikungunya, zika and urban yellow fever. The proposal of this study is to test the efficiency of a new trap model in capturing and trapping *Ae. aegypti* based on favoring the insect's ecology. This trap model was analyzed regarding the attractiveness for egg-laying, and the trapping of larvae and consequently the adults. The tests were carried out using white traps (original version) and black traps (adapted from the original), both in field and laboratory conditions. In the field, the black traps showed 100% effectiveness compared to white traps, showing more positive results for the attractiveness of *Ae. aegypti* mosquito and adult imprisonment. According to the results for this trap model, from both test in the field and in the laboratory, it was concluded that this new product is effective in capturing *Ae. aegypti* and it guarantees mosquito trapping safety, also low-cost production, practicality, logistics and possibility of its use by the local population. After the validation and effectiveness of the trap "drinking-fountain like", the definitive trap called Mosquitex was developed, which is brand new for this mosquito, with patent registration INPI - BR2020190112226 - 2019, and with the possibility of assisting in the control and monitoring of *Ae. aegypti* in urban areas.

Keywords: Culicidae; Traps for capture; Vector control; mosquito; dengue.

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Aedes (*Stegomyia*) *aegypti* (Linnaeus, 1762) (Diptera: Culicidae) is of great importance for public health, as it is adapted to the urban environment, and it presents a synanthropic behavior (Natal 2002). This mosquito species is the primary vector responsible for transmitting arboviruses that can lead to epidemics, placing a burden on public health services. Highly adaptive factors, such as the ability to colonize unstable habitats, rapid population growth with a high fecundity rate, and a short post-embryonic development cycle (up to 12 days in tropical regions) (Clements & Boocok 1984), ensure the success of *Ae. aegypti* in its environment. Additionally, temperature and rainfall play significant roles in influencing mosquito distribution and abundance, as the frequency and intensity of rainfall contribute to the density of breeding sites through water accumulation in both artificial and natural containers (Gubler 1988). The quantity and variety of man-made artificial breeding sites contributed to the adaptation of *Ae. aegypti* in urban areas, which was a big step towards its synanthropic behavior. Considering their potential for spreading arboviruses and the fact that humans are their main blood source; the mosquito-human association is of great epidemiological importance (Consoli & Lourenço-de-Oliveira 1994; Edman *et al*.1998; Braga & Vale 2007) because of their potential for spreading diseases (Lopes *et al*. 2014).

Since the beginning there has been great concern about eradicating *Ae. aegypti* (Lima *et al*. 1989), due to repeated epidemics and its ease of adapting in urban centers (Nelson 1986). After several attempts, actions and programs, the Ministry of Health in 2002 created the "Programa Nacional de Combate da Dengue" (PNCD) (Brasil 2002), that carries out actions to help control the vector, promoting educational campaigns, information and counting on the involvement of the population, community health agents and Family Health Programs (Brasil 2009).

Monitoring populations of *Ae. aegypti*, through the use of traps, is just one of the strategies within the PNCD that also establishes mechanical, physical, chemical and biological controls and a series of socio-educational actions, and in recent years, the analysis of costs and efficiency has been carried out to incorporate new techniques and technologies. However, for these actions to be truly effective, public authorities and society must share responsibility for keeping them active and sustainable (Ferreira *et al*. 2009; Zara *et al*. 2016).

The use of traps to collect eggs and/or larvae are important tools normally used to monitor *Aedes* spp. (Depoli *et al*. 2016). In ovitraps, a type of mosquito trap in which the eggs of *Ae. aegypti* are deposited in these containers, enables the analysis of variations in the population in each location, and are widely used to start a colony in laboratory and in vector dispersion studies (Fay & Perry 1965; Honorio & Lourenço-de-Oliveira 2001; Oliveira & Maleck 2014).

Larvitraps are types of mosquito traps generally used in the routine of entomological surveillance programs, to detect *Aedes* populations and to monitor infested areas (Brasil

2001a, 2001b, 2009).

According to Crovello & Hacker (1972), even though the adult stage of *Ae. aegypti* is the phase of arbovirus transmission, its immature forms should also be controlled, due to their adaptation derived from their synanthropy with the human species.

The objective of this study was to evaluate the efficiency of the bird waterer type trap capturing, monitoring, and trapping the *Ae. aegypti*, to obtain a new tool to control the mosquito vector and enable its use in entomological surveillance service.

MATERIAL AND METHODS

Traps for capturing *Aedes aegypti* larvae. An adaptation of a bird drinker, a plastic drinking fountain for hens and chicks from the brand "Bebedouros Aves Plástico", 2 L, dimensions 21 x 14 cm, was initially used as a test prototype. As a preliminary test, these traps were painted black using matte black vinyl paint from the Lazzuril brand and let dry for a 30-day period so they could get rid of the paint smell. They were later placed on the Experimental *Campus* of the Universidade de Vassouras, Rio de Janeiro. According to the map, the collection area was marked with the geographical coordinates of Vassouras, Rio de Janeiro, RJ (Figure 1).

The collection took place between May 5, 2015, and June 30, 2015, with traps positioned at seven locations. Each collection point underwent weekly examination, evaluating temperature, humidity, and the presence of mosquito larvae.

The experiment was set up by placing three traps in the original version (white) and three traps in black, in each of the seven randomly chosen points in the field. The local temperature and humidity were observed using an Incoterm thermohygrometer. The traps were arranged in T-shaped supports made from fence posts, of eucalyptus wood, which were closed and screwed, and attached to hooks so that the "traps" could be hung at 50 cm from the ground, and all points were identified with laminated labels (Figure 2).

There were 21 "traps" in the original version identified as R1, R2 and R3 white and 21 adapted "traps" in black identified as R1, R2 and R3 black. All traps received a galvanized wire arch at the top to hang from the wooden support fixed at each of the seven collection points.

The larvae capture site was previously prepared using traps of 1 L of water and 1 g of organic matter (1:1), at room temperature, as an attractant for female oviposition.

Figure 2. Trap for *Aedes aegypti* placed in collection points in the experimental *Campus* in Universidade de Vassouras. Photo: Cyro Guimarães.

Weekly monitoring was carried out for 65 days, recording the temperature and humidity of each point among other observations and field notes. After the preliminary study period, the traps were collected from all points.

After capture, only the larvae of *Ae. aegypti* were considered, discarding the other insect specimens. They were later identified, according to Forattini (2002) identification key, quantified and maintained in the Insect Vectors Laboratory for the development of the mosquitoes life cycle.

Data processing was performed using Tukey (ANOVA) and compared using the non-parametric Kruskal-Wallis rank sum test and Wilcoxon test (Sokal *et al.* 1979; Motulsky 1999).

Mosquito entrapment tests using prototypes of positive "larvitrap" traps. The cages for the traps were assembled (Figure 3), with light wooden structures (Eucatex type), which were made in the shape of a cube measuring 0.25 x 0.25 x 0.26 m, using wood to join the vertices, leaving the sides of the cube open and covered with fine white mesh (mosquito

Figure 1. The collection area was marked with the geographical coordinates of Vassouras, Rio de Janeiro, RJ.

net). This procedure was carried out carefully so that no adult could escape the trap. The upper part of the cube received a wooden sliding door, enabling the placement of traps collected in the field with eggs and/or larvae inside the cage, with the aim of evaluating the efficiency of the traps in trapping mosquitoes.

The traps were observed for oviposition attractiveness, a step carried out in the field, to confirm their efficiency as a larval capture and mosquito entrapment tool.

These observations were verified in positive traps collected in the field and placed inside wooden cages (reliability test) (Figure 3) at the Laboratory of Insects Vectors (LIV)/ Universidade de Vassouras, safely allowing the complete development cycle of the mosquito.

After this step, the larvae were quantified before and after migration, through the hole in the trap. All cages received the same identification as the traps. All traps were monitored for eggs.

The traps were then taken to the laboratory, labeled according to the collection points, for analysis of the results.

Figure 3. Cages for observation and maintenance of *Aedes aegypti* (Diptera: Culicidae). Laboratório de Insetos Vetores (LIV), Universidade de Vassouras, RJ. Photo: Cyro Guimarães.

Laboratory essays. In the laboratory, a test was prepared with three replications, one trap in black and one trap in white (original prototype), with L3 larvae of *Ae. aegypti*, from the insect colony of the Laboratory of Insects Vectors (LIV). There were 20 larvae of *Ae. aegypti* in each trap containing 1 L of water: 0.03 mg of fish food (Alcon BASIC mep 200 complex), and then taken to identified cages, to evaluate the larvae entrapment under controlled conditions and consequently the effectiveness of the larvae collection trap prototypes. The data were analyzed using the Kolmogorov Ana Smirnov test.

RESULTS

Attractiveness of the larvae collection trap prototype. After nine weekly inspections, during two months of work, the results of the attractiveness of females of *Ae. aegypti* to the traps showed that, with the exception of points 5 and 6, all others received visits from females of *Ae. aegypti* for oviposition.

It was observed that of the 21 white traps distributed in the seven points in the *Campus* (with three repetitions) three points were positive for oviposition. In black traps, this number increases to five positive points, confirming this mosquito's preference for black.

Regarding the number of larvae captured, it was analyzed that of the 55 larvae captured, 11 were collected with white traps (original), corresponding to 20% of the total, and 44 were captured in black traps, making up 80% of the total.

The attractiveness experiment was observed in white traps placed at points 1, 2 and 7 (Figure 4) and in black traps six points were positive (1, 2, 3, 4, 5 and 7) for larvae of *Ae. aegypti* (Figure 5). Temperature and humidity were similar at all points.

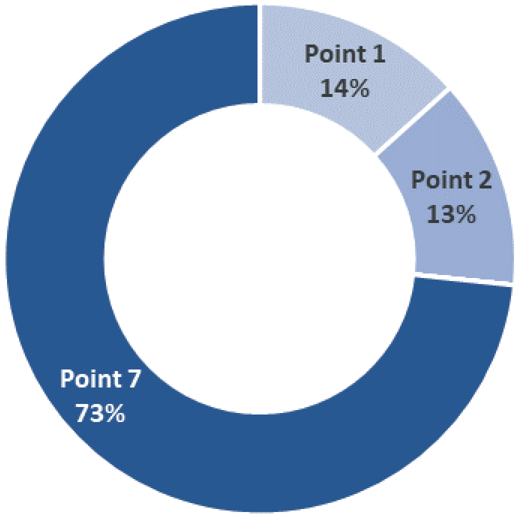

Figure 4. Result of the attractiveness to the white traps in all 7 collection points.

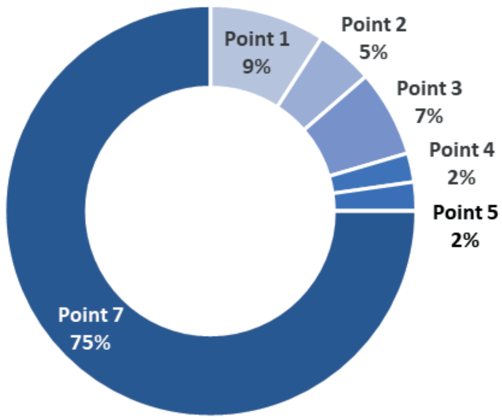

Figure 5. Result of the attractiveness to the black traps in all 7 collection points.

Entrapment - Traps with larvae of *Aedes aegypti* collected in the field, finishing their cycle in cages in the laboratory. Regarding the efficiency of the traps in relation to trapping insects, it was observed that at point 1, (R2) – black, all four larvae migrated into the traps and completed their cycle, which means 100% efficiency. At point 2 and 3 (R3 and R1)

– black (each containing one larvae) the larvae migrated into the trap completing their cycle to adulthood, both with 100% efficiency. At point 7 (R1) – black, with 25 larvae, all of them migrated to the trap with 100% efficiency.

At point 3 (R2) – black trap, there were two larvae and some eggs deposited on the container wall. From these eggs, 10 larvae hatched, adding up to 12 larvae (R2), and an additional one larva (R1), totaling 13 larvae in point 3 of the black trap. All 13 larvae successfully migrated into the trap, achieving 100% efficiency. At point 7 (R1) – white, containing six larvae, one L3 larvae was outside the trap, three L3 larvae were in the inside, and two dead; this means that the larvae found outside corresponds to 16.67% of the total larvae and the ones found inside indicate 83.3% of entrapment efficiency. When comparing the colors of the traps, the efficiency of the black color was 100% efficient (P < 0.01) in relation to the white trap (83%) (Table 1).

Trapping larvae under controlled conditions - Tests with traps in the laboratory, with *Aedes aegypti* larvae from the insect colony of the Laboratory of Insects Vectors (LIV). The efficiency of the traps in trapping mosquitoes using larvae of A*e. aegypti* bred in the Laboratory of Insects Vectors (LIV) / University of Vassouras, showed the data from the white (original) trap (Table 2) with initially 20 larvae (L3) per trap (R1, R2 and R3), making 60 in total. In the white trap (R1) seven mosquitoes were on the wall of the cage and 13 inside the trap, that is, 65% entrapment efficiency. In the white trap (R2) showed four mosquitoes in the cage and 16 inside the trap, with 80% efficiency and in the white trap (R3) five mosquitoes in the cage and 15 inside the trap, with 75% efficiency. The black trap also received 20 larvae (L3) in each trap (R1, R2 and R3), bred in the lab. In the black traps (R1 and R2), 19 larvae migrated into the trap, became trapped and completed their development, with 95% efficiency per trap. In the black trap (R3), all 20 larvae (L3) were trapped and completed their cycle, with 100% trap efficiency (Table 2).

Product: Mosquitex – the finished design. After all the efficiency tests with the prototypes using the drinking fountain type traps, it was assembled the final Mosquitex trap (Figure 6), with the filing of patent application number INPI-BR2020190112226/2019.

The trap consists of an environment for capturing and attracting oviposition of the female mosquito. The trap (Figure 6) is made from a container where water is deposited as a place for egg laying. In this container the female mosquito lays her eggs in which the larvae hatch, since the *Ae. aegypti* larvae avoid ambient light, they seek for shelter. The trap has a second container attached to the previous one, which has a small opening, and through this hole the larva enters and remains trapped until it completes all phases of its development. The adult is trapped into this second container until its death.

Table 2. Entrapment of larvae under controlled conditions - Tests with traps in the laboratory with *Aedes aegypti* larvae from the insect colony of the Laboratory of Insects Vectors / Universidade de Vassouras. White trap (A), Black trap (B).

Traps	Number of trapped larvae	% of entrapment efficiency
	A	
R1	13	65
R2	16	80
R3	15	75
Total		**73.3**
	B	
R1	19	96
R2	19	96
R3	20	100
Total		**96.6***

Nº = 20 larvae; * P < 0,1

This trap consists of two containers made of biodegradable plastic with the advantage of not polluting the environment.

DISCUSSION

The study showed that the black trap model was significantly superior to the same trap in the original color (white), indicating 100% more attractiveness, greater willingness to oviposition, and imprisonment of adults, both in traps collected in the field (*P< 0.01) and in laboratory tests (*P< 0.1).

The main target of the traps is to efficiently capture the greatest possible number of the *Ae. aegypti* mosquito using

Table 1. Number of larvae collected with traps in the field, and subsequently sent to laboratory cages for development. Trap in white (A) and black (B).

Collection Points	Number of larvae present inside traps positive for *Aedes aegypti*	Dead larvae	Number of trapped larvae	% of the entrapment efficiency
		A		
1	2	2	0	0
2	2	2	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	7*	1	5	83.3%
		B		
1	4	0	4	100
2	1	0	1	100
3	13	0	13	100
4	1	1	0	0
5	0	0	0	0
6	0	0	0	0
7	25*	0	25*	100%

* P ≤ 0,01

Figure 6. Mosquitex trap.

all kinds of entomological methods, it can be traps for eggs, larvae, or adults, although ovitraps that are a simple trap for collecting *Ae. aegypti* eggs (Fay & Eliason 1966) have proven to be an efficient trap model when it comes to monitoring infestations, standing superior to larvae research for this purpose. Marques *et al*. (1993) demonstrated the superiority of ovitraps over larvitraps. This is also highlighted in the study carried out by Gama *et al*. (2007) regarding traps to capture the adult mosquito.

The analysis of the trap under study, which includes the attractiveness for oviposition, the capture of mosquito larvae and the imprisonment of the adult. From all the tests carried out in this study, with the prototype trap, the product "Mosquitex" was developed, which constituted an innovative model that brings together three epidemiological monitoring activities in a single trap, that showed efficiency, low cost, easy operation, good logistics of use.

It is important to highlight that the proposal for this new product, Mosquitex, presented positive results and signals in the direction of contributing to the control of *Ae. aegypti*.

It can be considered that the trap proposed in this study, a new model, extremely effective in attracting and trapping *Ae. aegypti* and because it is practical, economical and highly effective, it can efficiently contribute to the epidemiological control of the mosquito.

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AUTHORS CONTRIBUTION

CG: was responsible for the prototype of the Mosquitex trap, in the field and laboratory experiments, and in the writing of the article. TDD: collaborated in the experimental part of the field collections. SPA: collaborated in the experimental part in the laboratory of Insect Vectors. ILSC: collaborated in the post-collection analyses in the field and in the laboratory of insect vectors, and in the revision of the manuscript. MM: main advisor of the first author analyzed the results of the collections in the field and in the laboratory, and in the writing of the manuscript.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest and all authors contributed to this manuscript.

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