Scientific Research Publishing

Advances in Entomology, 2022, 10, 77-84
https://www.scirp.org/journal/ae
ISSN Online: 2331-2017
ISSN Print: 2331-1991

Comparative Evaluation of the Regular Ovitrap vs an Innovated Larvitrap for *Aedes* Entomological Surveillance in Tapachula

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How to cite this paper: Quezada-Yaguachi, W.E., Rodriguez, A.D., Solís-Santoyo, F., Lopez-Solis, A.D., Black IV, W.C., Saavedra-Rodriguez, K., Morales-Viteri, D. and Penilla-Navarro, P. (2022) Comparative Evaluation of the Regular Ovitrap vs an Innovated Larvitrap for *Aedes* Entomological Surveillance in Tapachula. *Advances in Entomology*, **10**, 77-84.
https://doi.org/10.4236/ae.2022.101006

Received: October 21, 2021
Accepted: January 3, 2022
Published: January 6, 2022

Abstract

Objective: The objective is to compare a regular ovitrap versus an innovated larvitrap for monitoring *Aedes* spp. populations. **Materials and Methods:** A total of 20 regular ovitraps and 20 innovated larvitraps were placed in pairs in 20 houses from the 5 de Febrero neighborhood in Tapachula, Chiapas, Mexico. The innovation consisted in the incorporation of a valve in the lower part of a half tire to drain the contents in a 2 L collection container. The evaluation was carried out during five weeks, collecting eggs and larvae from the ovitraps and innovated larvitraps, respectively. Positivity indexes and insectary production of adult *Aedes* spp. mosquitoes were compared by collection type. **Results:** Average positivity index for the five weeks period were 60% for ovitraps and 91.25% for innovated larvitraps. During the five weeks, 4043 *Ae. aegypti* and 703 *Ae. albopictus* adult mosquitoes were produced in the insectary from the eggs collected from ovitraps, while from innovated larvitraps were 9014 *Ae. aegypti*, 1205 *Ae. albopictus*, and 15 *Culex* spp. **Conclusion:** Collection by the innovated larvitrap was more efficient, collecting 3.56 times more *Ae. aegypti* than with ovitraps, using approximately the same effort in time for replacing the filter paper from traditional 1 L ovitraps. Since the logistics for the storage and placement of larvitraps may still be a disadvantage in comparison with ovitraps, their use could be specifically intended in sentinel sites for mosquito population monitoring for entomological surveillance purposes.

[†]Co-author Americo Rodriguez passed away a year ago.

Keywords

Innovated Larvitrap, Ovitrap, *Aedes*, Entomological Surveillance

1. Introduction

Vector-borne diseases such as dengue, chikungunya and Zika are mainly transmitted by *Aedes aegypti* and represent an important problem of public health worldwide [1]. Since no vaccines or specific treatments are available, the use of insecticides is still the major component for the control of each of these diseases. The monitoring of *Ae. aegypti* populations is vital in the context of epidemiological surveillance including assessing susceptibility/resistance to insecticides, to see the impact of control measures [2].

This surveillance can be done by collecting adult mosquitoes, larvae, or eggs. In the context of insecticide resistance monitoring, the WHO recommends collecting mosquito larvae from the sites of interest to obtain a parental generation from which a first-generation can be used to conduct the bioassays [3]. For the surveillance, different types of larvitraps have been used, such as plastic containers, bamboo internodes, and tires, identifying the tires with the highest abundance of individuals [4].

However, the use of ovitraps to obtain eggs has proven to be extremely practical for epidemiological surveillance and therefore has been used for the monitoring of insecticide resistance by collecting eggs and obtaining adult mosquitoes from them [5]. On the other hand, the use of larvitraps has not been much implemented given the tedious to collect the larvae and pupae from the tires that are commonly adapted as larvitraps.

In this study, we are reporting the innovation and performance of a larvitrap that allows collecting in a fast and effective way immature stages of *Aedes* spp. mosquitoes.

2. Materials and Methods

The study was conducted in Tapachula, Chiapas, Mexico, where five blocks from the neighborhood 5 de Febrero were selected. Innovated larvitraps were built in the Centro Regional de Investigación en Salud Pública (CRISP) facilities using half of a car tire such as those previously reported [6]. The innovation of this type of larvitrap was the incorporation of a valve in the lower part of the half tire to drain the contents in a 2 L collection container (**Figure 1**), which ensured the collection of all the biological material contained in the larvitrap. This innovation also requires a shorter collection time compared to the use of pipetting to collect the material in a larvitrap without this modification.

Each innovated larvitrap, hanging at 1 m from the floor and with 3 L of water, was placed at least 1 m apart of an ovitrap (**Figure 1**), which was made of a black plastic container of 1 L capacity, internally lined with filter paper as recommended

Figure 1. Left: ovitrap placed on the floor next to an innovated larvitrap (with a valve incorporated in the lower part of the half of a tire) that hangs 1 m from the ground, containing 3 L of water. Right: the content of an innovated larvitrap is drained into a 2 L collecting container, where all the larvae and pupae are collected.

by the National Vector Control Program from Mexico [7]. A total of 20 ovitraps and 20 innovated larvitraps were placed in the backyards of 20 houses in the selected study area.

The evaluation was carried out during five weeks from January to February 2018, collecting the first week in a traditional way by pipetting the innovated larvitraps, that is, without using the valve, in order to measure the collection time for comparison purposes with time employed when using the valve. During the remaining four weeks the drainage system was used. Eggs, larvae and pupae collected every 7 days were taken to CRISP and reared in the insectary under standardized conditions of temperature of 27˚C ± 2˚C, 70% - 80% humidity, and a 12:12 hour photoperiod.

The positivity indexes of ovitraps (for eggs), and innovated larvitraps (for larvae and pupae) were calculated using the formula: number of positive ovitraps or innovated larvitraps divided by the number of ovitraps or innovated larvitraps placed in the 20 houses, all multiplied by 100. The production in the insectary of adult *Aedes* spp. mosquitoes were compared by type of collection and the effectiveness of ovitrap vs innovated larvitrap evaluated.

3. Results

3.1. Positivity Indexes

The positivity index for eggs for the ovitraps was calculated for each of the five weeks of the study resulting in 75%, 65%, 45%, 55%, and 70%, respectively (**Figure 2**), with an average of 60% for the five weeks period. For innovated larvitraps, positivity indexes for larvae were 85%, 95%, 90%, 95%, and 100% (**Figure 2**), with an average of 91.25% for the five weeks period. Positivity index for pupae from the same innovated larvitraps were 40%, 95%, 65%, 70%, and 25% (**Figure 2**), with an average of 67%.

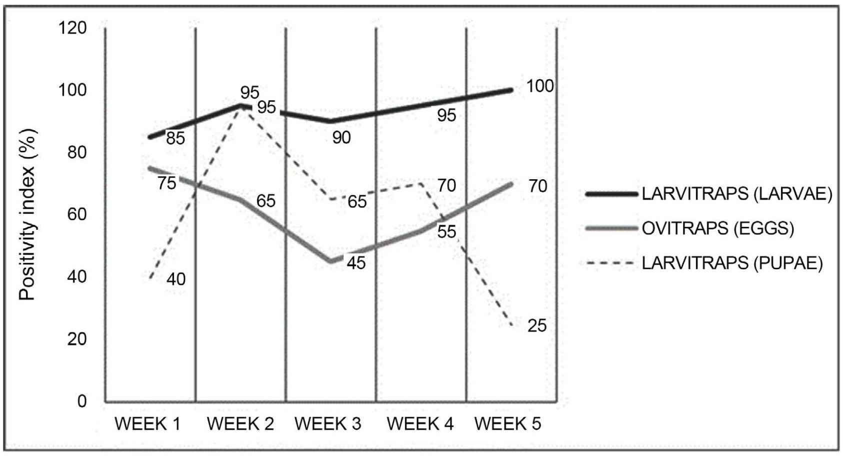

Figure 2. Positivity index for the ovitraps and the innovated larvitraps (week 1 was pipetted as traditional larvitrap) in the 5 de Febrero neighborhood, in Tapachula, Chiapas, Mexico.

3.2. Productivity in the Offspring by Collection Method

The mean of eggs collected by ovitrap (n = 20) ± standard error is shown in Figure 3 for each of the five weeks, with 5707 eggs collected during the study. Weekly species proportion collected per ovitrap (*Ae. aegypti* vs *Ae. albopictus*) is shown in Figure 4. From these eggs collected during the five weeks, 4043 *Ae. aegypti* and 703 *Ae. albopictus* adult mosquitoes were recorded in the insectary during the study.

No eggs were attempted to be collected with innovated larvitraps. Innovated larvitrap mean larvae collected (n = 20) ± standard error is shown in Figure 3 for each of the five weeks, with 10,984 larvae collected during the study. The collected proportion of *Ae. aegypti* in relation to *Ae albopictus* and *Culex* spp. in the 20 innovated larvitraps for each week is shown in Figure 4. In total 9014 *Ae. aegypti*, 1205 *Ae. albopictus*, and 15 *Culex* spp. adult mosquitoes were recorded in the insectary during the study.

3.3. Effectiveness of Ovitrap vs Innovated Larvitrap

The number of adults *Ae. aegypti* mosquitoes obtained from collections with innovated larvitraps exceeded 1.63, 6.12, 5, and 1.5 times the number of specimens obtained through ovitraps during weeks 2 to 5 respectively with an average of 3.56 times, when the adapted drain system was used (Table 1). Finally, the effort in time used to collect the specimens by using pipettes was 20 min in average (week 1), while using the drain valve was 5 min in average, which is approximately the same effort in time for replacing the filter paper from traditional 1 L ovitraps.

3.4. Effectiveness of Larvitrap (Pipetting in the Traditional Way) vs Innovated Larvitrap

The number of adults *Ae. aegypti* mosquitoes obtained from collections of weeks 2 to 5 with innovated larvitraps exceeded 6.37, 12.5, 10.06, and 9.59 times, respectively the number of specimens obtained through traditional larvitrap (collecting using pipettes) in week one (Table 1). The innovated larvitrap on average collected 9.63 times more larvae than the traditional larvitrap, while the collection time was reduced from 20 to 5 min.

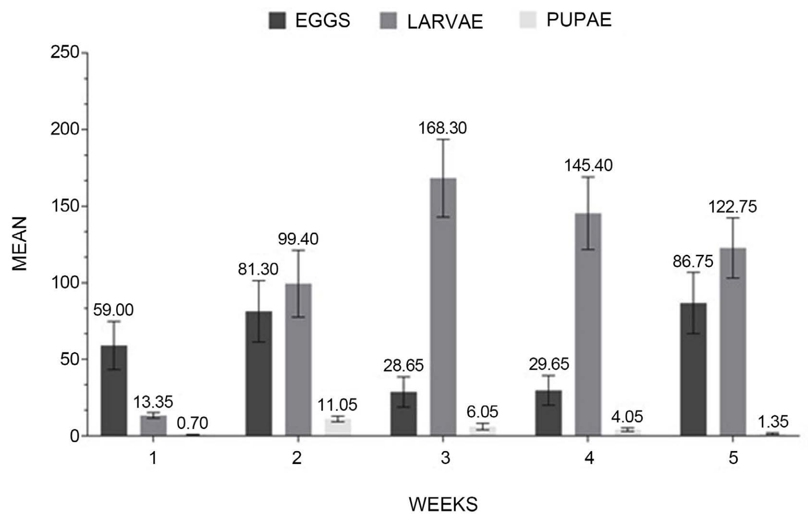

Figure 3. Mean ± SE of eggs, larvae and pupae collected in ovitraps and innovated larvitraps per week (week 1 was pipetted as traditional larvitrap) in the 5 de Febrero neighborhood, in Tapachula, Chiapas, Mexico.

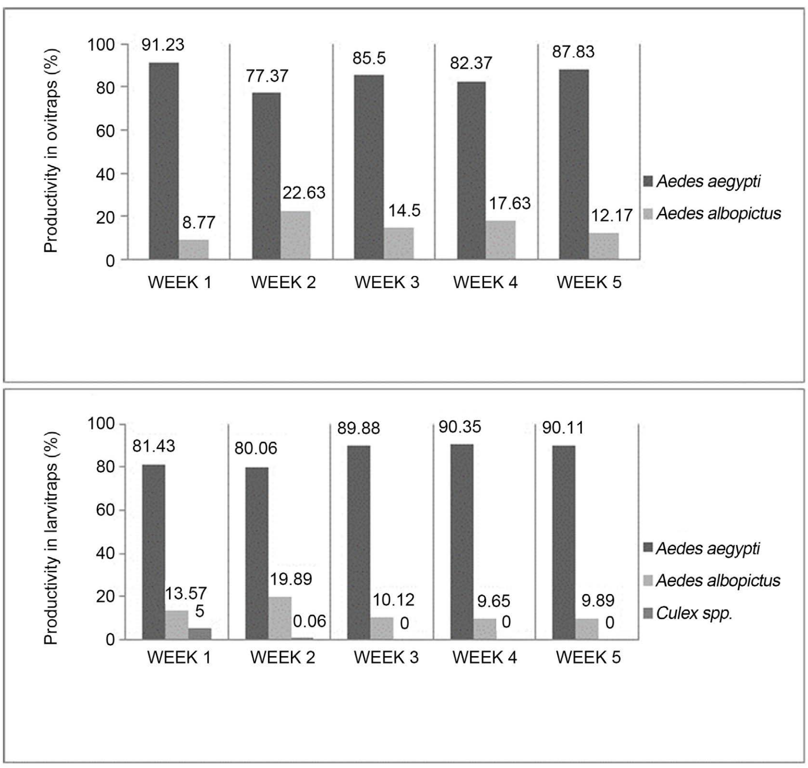

Figure 4. Above: proportion of *Ae. aegypti* vs *Ae. albopictus* offspring produced in the insectary from collections by ovitrap and; bellow: proportions of *Ae. aegypti*, *Ae. albopictus* and *Culex* spp. offspring produced in the insectary from collections by innovated larvitraps (week 1 was pipetted as traditional larvitrap) in a neighborhood from Tapachula, Chiapas, Mexico.

Table 1. Offspring total *Aedes aegypti* adult mosquitoes produced in the insectary by collection type undertaken in a neighborhood of Tapachula, Chiapas, Mexico.

Collection tool	Week 1*	Week 2	Week 3	Week 4	Week 5	Total
Ovitraps	780	889	466	458	1450	4043
Innovated larvitraps	228	1453	2852	2294	2187	9014

*Without the drainage system.

4. Discussion and Conclusion

The methods for collecting *Ae. aegypti* are used mainly for the elaboration of indexes for epidemiological stratification, entomological risk and the effectiveness of control measures [8]. The two major methods consist in the collection of eggs, or larvae and pupae by means of ovitraps or larvitraps, respectively. They are also very useful to collect mosquitoes to test for insecticide resistance, since not always the collection from natural or man-made occurring breeding sites is possible. Ovitraps have evolved from the use of a wooden stick into a 1 L water container to the actual "traditional" ovitraps, which include the use of filter paper placed on the inner wall of the container [7]. Larvitraps on the contrary, have been implemented by the use of half of a used car tire filled with water and its evolution or innovation have been very little [9]. In the present study, we compared the effectiveness of innovated larvitraps against traditional ovitraps based on the positivity index and the abundance of adult mosquitoes produced in the insectary from the collections using both methods.

Traditional vs innovated larvitrap had a disadvantage in the numbers of larvae collected as well as in the time for collection. The innovated larvitrap on average collected 9.6 times more larvae than the traditional larvitrap, while the collection time was reduced from 20 to 5 min. Furthermore, the innovated larvitrap had a higher positivity index and higher offspring capacity from the immature biological material collected compared to ovitraps, in almost the entire period of the entomological monitoring. Studies carried out by Lima *et al.* (1989) [10] comparing different types of traps, showed larvitraps as more attractive than ovitraps. They suggested that the higher surface and volume of water in the larvitrap influenced oviposition, resulting in higher efficiency. Other factors like the color and the rubber composition of the tire, could provide more stable environmental conditions such as water temperature, that favor the oviposition and development of *Ae. aegypti* larvae.

Under different circumstances, either methodological or environmental, the surveillance tools for *Ae. aegypti* monitoring has to be adequate to the established aims, as well as supported by efficiency, operating time and cost [11]. The ideal will always be to have a specific, economical and sensitive technique to sample any mosquito population. Ovitraps represent a good tool for the early detection of dengue and yellow fever vectors [12]. Similarly, ovitraps were positive even in the presence of natural breeding sites and presented superior efficiency to larvitraps [13]. In our study, we did not register the presence of natural breeding sites in the study area, however, the conditions of the selected houses were similar to each other, and so the presence of other breeding sites in addition to ovitraps and larvitraps could have been similar. Despite this, larvitraps proved to be much more attractive than ovitraps. Moreover, ovitraps and innovated larvitraps were placed in a paired way, so the attraction of each method was in some way under test in the design. However, a study where local mosquito populations are at very low levels could indicate which method is more sensitive to detect low *Ae. aegypti* populations.

Larvitraps seem to have a great potential even representing an economic method for the detection and collection of *Ae. aegypti* larvae and pupae. Considering these advantages, it is suggested that the operative use of innovated larvitraps could result in a sensitive, rapid and efficient technique for *Ae. aegypti* monitoring. However, the logistics for the storage and placement of innovated larvitraps may still be a disadvantage in comparison with ovitraps, nevertheless, their use could be specifically intended in sentinel sites for population monitoring after an intervention by the control programs, including collections of biological material for insecticide resistance testing.

Finally, in the context of a pandemic such as the COVID-19, social distancing which is one of the most recommended measures along with facemask use could prevent the placement of ovitraps inside houses. While placing them outside the houses, could be impractical because of the size of the traps and the risk to be stolen for the potential use of the 1 L capacity containers for other purposes. The innovated larvitraps on the other hand, because of the size and less attractive appearance, could represent the best choice to monitor mosquito populations under these circumstances, since are less likely to be stolen. The above-mentioned assessments need to be tested in the field.

Acknowledgements

Project Supported by the Sectorial Fund for Research in Health and Social Security.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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