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## Oviposition of *Aedes aegypti* Linnaeus, 1762 and *Aedes albopictus* Skuse, 1894 (Diptera: Culicidae) under laboratory and field conditions using ovitraps associated to different control agents, Manaus, Amazonas, Brazil



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### ABSTRACT

The aim of this study was to analyze the effectiveness of different control agents of *Aedes aegypti* and *Aedes albopictus* associated with ovitraps under laboratory and field conditions. Five treatments were used: grass infusion + *Bacillus thuringiensis israelensis* (gl + Bti), grass infusion + *Saccharopolyspora spinosa* (gl + Ss), grass infusion + Pyriproxyfen (gl + P), distilled water + *Toxorhynchites haemorrhoidalis* (dW + Th), and grass infusion (gl) (control). The highest mean number of eggs of both species were obtained with grass infusion in the laboratory. Among control agents, the lowest mean of *A. aegypti* eggs occurred with gl + Ss and the lowest mean of *A. albopictus* eggs occurred with dW + Th. There was no difference between treatments in *A. aegypti* ( $P=0.4320$ ) and *A. albopictus* ( $P=0.7179$ ). In the field, the highest mean number of eggs for both species were obtained with gl + Ss, and the lowest values were obtained with gl + P ( $P=0.0124$ ). The treatments can be applied to both the surveillance and the control, but ovitraps with biological larvicide Bti were more effective and safer considering the number of eggs laid and selectivity of pathogens for mosquitoes.

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### Introduction

*Aedes (Stegomyia) aegypti* Linnaeus, 1762 and *Aedes (Stegomyia) albopictus* Skuse, 1894 (Diptera: Culicidae) are particularly susceptible to several arboviruses that are threats to public health in several regions of the world (Alahmed, 2012; Forattini, 2002; Rossati et al., 2015). Both species have wide epidemiological importance in the Americas due to implications in the transmission of many arbovirus responsible for high hospitalization rates and deaths. They are distributed throughout several regions in the continent (PAHO, 2018). These mosquito-borne pathogens affect economies due to an impaired workforce and the need to treat sick people (Calvo et al., 2016; Gubler, 2005; Vega-Rúa et al., 2014).

Derived from Africa where there are wild and urban populations, *A. aegypti* is currently widespread and is found in all states and the Federal District of Brazil (Carvalho and Moreira, 2017). This species is the main vector of Dengue, Chikungunya, Zika, and Urban Yellow Fever viruses in the Americas. This anthropophile is adapted for urban environments, where it inhabits laundry tanks, containers, barrels, bottles, pots of plants and other containers (Pinheiro and Tadei, 2002; Soares-da-Silva et al., 2012).

These oviposition sites are different from those used by *A. albopictus*, which inhabits sites with native or secondary vegetation near human populations (Martins et al., 2013; Silva et al., 2006). Considering its epidemiological role, this species has potential for transmitting Urban Yellow Fever and Venezuelan Equine Encephalitis viruses; laboratory assays have checked this species susceptibility to more than 20 arboviruses including the Dengue virus (the primary vector in Asia) and the Chikungunya virus (Gubler et al., 2001; Moore and Mitchell, 1997; Vega-Rúa et al., 2014). The first record of this species in Brazil was in 1986 in the

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state of Rio de Janeiro; a few years later it had already spread to all states of the Southeastern region. It is currently distributed nationwide (Martins et al., 2013; Santos, 2003).

Vector control practices that are less damaging to the environment and are effective for mosquito larvae have increased over the last years. These include natural predators, bacteria, growth regulators, and more recently, fungi and plant extracts (Darbro et al., 2011; Ferreira et al., 2015; Lopes, 1999; Medeiros et al., 2013; Soares-da-Silva et al., 2015; Soares-da-Silva et al., 2017; Zequi et al., 2015). New attempts at mosquito control have advanced to suppress populations of medical importance including the use of transgenic mosquitoes (Beech et al., 2009; Carvalho et al., 2015) and the symbiotic bacterium *Wolbachia*, which inhibits the development of Dengue virus inside the insect thus blocking its transmission (Dutra et al., 2016).

Vector species monitoring is essential. Studying mosquito distribution is essential to identify critical larval reproduction and egg laying sites. The use of ovitraps began in 1965. Oviposition traps for *A. aegypti* populations were shown to be effective for larval research and studies on vector frequency (Fay and Eliason, 1966; Regis et al., 2008).

Ovitraps provide useful data on the spatio-temporal distribution of mosquitoes because this monitoring allows one to check the presence and density of the vector at a local scale (Fisher et al., 2017; Nunes et al., 2011). In addition, ovitraps might be employed for vector control because they remove eggs from the environment; they are a sensitive and low-cost tool to aid in epidemiological studies (Depoli et al., 2016; Gomes, 2002).

Data obtained with this tool can monitor the impact of control measures such as the use of insecticides. They can also help monitor the effectiveness of vector reduction programs in urban areas. Although ovitraps are effective, they are a short-term method, replacement is required within approximately 5–7 days so that larval hatching does not occur and traps do not become oviposition sites (Alarcón et al., 2014; Gomes, 1998; Gomes, 2002; Regis et al., 2008).

The association of ovitraps and larval control agents could improve field traps and prevent potential larval hatching. This would ensure the safety of these traps (Depoli et al., 2016; Regis et al., 2013). However, it is necessary to analyze the best control agent associated with ovitraps because the selection of a potential oviposition site involves visual, olfactory, and tactile responses. In this respect, grass infusion is an important attractant for laying eggs (Reiter et al., 1991) as is the presence of a conspecific larvae has been reported (Nunes et al., 2011; Santana et al., 2006).

Manaus is an Amazon city with climatic conditions that favor *A. aegypti* and *A. albopictus* year-round. It has abundant rain and high temperatures as well as a diversity of natural and artificial larval habitat. Therefore, integrating a monitoring model that combines ovitraps with control agents might be an effective alternative for surveillance and control of mosquitoes that are vectors of human pathogens. Thus, the aim of this study was to analyze the effectiveness of different control agents associated with ovitraps for *A. aegypti* and *A. albopictus* under laboratory and field conditions.

## Materials and methods

### Obtaining *Aedes aegypti* and *Aedes albopictus* mosquitoes

To obtain females, *A. aegypti* and *A. albopictus* populations were kept and stabilized in an insectary of the Laboratory of Biological Control and Biotechnology of Malaria and Dengue (LCBBMD) of the National Institute of Amazonian Research (INPA), Manaus, Amazonas, Brazil. The insectary started obtaining eggs using oviposition traps, also known as ovitraps. Ovitraps are black and round

plastic pots measuring 9 x 11 cm with a capacity of 500 mL. Inside the ovitraps, there is a five-mm thick Duratree paddle, Eucatex® brand, 15 cm long by three cm wide. This is placed vertically with the rough surface of the material facing outwards to provide a substrate for oviposition and egg adherence. The main attractant of females in the traps was a grass infusion with total a volume of 300 mL at 1.2% of Colônia grass (*Megathyrsus maximus* Jacq) fermented for seven days at a mean room temperature of 32 °C (range of 38 °C and 20 °C) according to Koeppen's classification (1948).

Twenty ovitraps were mounted and distributed in the INPA Campus. After one week, ovitraps were collected and the Duratree paddles containing the eggs were submerged in distilled water for larval hatching. The larvae were fed with cat food (Whiskas®) minced in fine particles. The water in the containers was changed every two days to prevent feed fermentation—this can damage gas exchange during the immature stages. After emergence, the adults were captured with a Castro grabber, and the species identity was confirmed using external morphological characteristics of adults, especially in the thorax using stereoscopic microscope, where they were identified with the help of a mosquito identification guide (Consoli and Lourenço-de-Oliveira, 1994; Forattini, 2002; WRBU, 2018).

The adults were subsequently maintained in a cage for mating and maintenance of adult individuals. Cotton wrapped in gauze and soaked in sugared water at 12% was added as a source of carbohydrates, and females were fed a blood meal twice a week using an adequately anesthetized hamster (*Mesocricetus auratus*) following the procedure approved by the INPA Ethical Committee for the Use of Animals (CEUA: 02/2014 – “Breeding of vectors under laboratory conditions”).

### Obtaining larvae of *Toxorhynchites haemorrhoidalis* Fabricius, 1787

*Toxorhynchites haemorrhoidalis* is a natural predator of culicids and other insects. To obtain immature *T. haemorrhoidalis*, eight cut tires (25 x 15 x 11 cm), containing well water and a small amount of litter were distributed throughout the INPA Campus and monitored daily. We also actively searched for immature stages in the natural larval habitat. The larvae were kept in the insectary of the LCBBMD and fed on the third and fourth instar larvae of *Aedes* spp.; 56 third instar larvae of *T. haemorrhoidalis* were used.

### Substrates used in the experiment

Table 1 lists the associations used in the oviposition process of *A. aegypti* and *A. albopictus* under laboratory and field conditions.

Control agents were diluted using falcon tubes and dissolved with a Vortex agitator and a BRANSONIC® ultrasonic until the desired concentrations were reached based on the manufacturers' recommendations (Table 1).

### Laboratory experiments

From a stabilized *A. aegypti* and *A. albopictus* insectary, 25 fertilized females from generation F<sub>1</sub> were previously selected from each species for each experiment. Five days after the blood meal, females were placed in an aluminum-screened cage (55 x 47 x 47 cm). Five 160 mL white plastic cups were placed inside—one for each treatment containing 50 mL of each treatment and a paper filter as the oviposition substrate (Table 1). Subsequently, a container with 12% sugar in water was added as a source of carbohydrates for females.

Containers were placed in the four corners of the cage and one in the center. The container positions were changed clockwise daily for four days to discard any external influence on oviposition; three

**Table 1**  
Treatments used in oviposition experiments with *Aedes aegypti* and *Aedes albopictus* under laboratory and field conditions in the period ranging from March to July, 2015, Manaus – Amazonas – Brazil.

Treatments	Control agents	Concentration	Batch/validity
Grass infusion + Vectobac® WG	<i>Bacillus thuringiensis israelensis</i> (gl + Bti)*	0.004 g/L	237-445-PG – Jan/2016
Grass infusion + Natular™ DT	<i>Saccharopolyspora spinosa</i> (gl + Ss)*	0.007 g/L	1309190010 – Oct/2015
Grass infusion + Sumilar®	Pyriproxyfen (gl + P)*	0.01 g/L	4303F425 – Jan/2016
Distilled water + <i>T. haemorrhoidalis</i>	<i>T. haemorrhoidalis</i> (dW + Th)*		
Grass infusion	Control (gl)*	1.2%	

Legend: \*Entomopathogenic bacterium: *Bacillus thuringiensis israelensis*; \*Entomopathogenic bacterium: *Saccharopolyspora spinosa*; \*Insect Growth Regulator (IGR): Pyriproxyfen; Predatory invertebrate: *Toxorhynchites haemorrhoidalis*; Control: Grass Infusion.

replicates were performed. The females were captured and sacrificed at the end of each replicate. Experiments were conducted under controlled temperature, humidity and photoperiod conditions ( $27 \pm 2^\circ\text{C}$ ; 80–90%; 12L:12D).

The eggs obtained at the end of each replicate were dried on absorbent paper for 60 minutes at  $27 \pm 2^\circ\text{C}$  and humidity of 80–90%. They were then stored under the same temperature and humidity conditions in 750 mL cups for subsequent egg counting using a ZEISS Stemi 2000 50X stereoscopic microscope.

#### Field experiments

For the field oviposition tests, 50 ovitraps were distributed (10 for each treatment) (Table 1). Traps were installed in the INPA campus. They were placed near buildings at ground level with shelter from the sun and rain. We focused on areas free of constant movement of people and animals; they were located 30 meters apart from each other. The experiment lasted five consecutive weeks, and the Duratree paddles were replaced every seven days. Eggs were counted using a stereoscope microscope (ZEISS Stemi 2000 50X).

After egg counting, paddles were submerged separately in disposable 600 mL flasks containing 400 mL of distilled water and 0.0055 g cat food (Whiskas®) ground into minced in fine particles to feed the larvae and obtain adults. After the adults emerged, the species were identified using external morphological analysis. The specimens that were not identified because of the difficulty in visualizing the patterns of ornamentation of the scales were forwarded live one after the other to a stereoscopic microscope, where they were identified with the help of a mosquito identification guide (Consoli and Lourenço-de-Oliveira, 1994; Forattini, 2002; WRBU, 2018).

#### Statistical analysis

The following indices were calculated: OPI – ovitraps positivity index, EDI – egg density index, and VDI (mean eggs) – vector density index. The OPI is the ratio between the number of positive traps and the number of traps examined multiplied by one hundred (Gomes, 1998). The EDI is the ratio between the number of eggs and the number of positive traps (Gomes, 2002). The VDI is the mean number of eggs in each type of treatment obtained by the ratio between the number of eggs and the number of traps examined (Avedanilha, 2007). In relation to the data of number of eggs obtained in each treatment, an analysis was first made to know if the data had a normal distribution or not, to later verify which statistic would be the most adequate, parametric or non-parametric. After these initial tests, ANOVA was used for the data that presented normal distribution, followed by Tukey's multiple comparison test ( $p < 0.05$ ), while the data that did not present normality were chosen by non-parametric Kruskal tests Wallis, followed by the Dunn test ( $p < 0.05$ ). The statistical software SPSS® 14.0 for Windows® (SPSS Inc. 2005 Headquarters, Chicago, IL, USA) was used for analysis assistance.

## Results

### Oviposition at the laboratory

The number of *A. aegypti* eggs collected in all treatments was higher than the number of *A. albopictus* eggs (4215 and 2074 eggs, respectively). This had a statistical difference using Tukey's test ( $F = 21.836$ ;  $P = 0.0019$ ). No differences were found between the mean number of *A. aegypti* ( $F = 1.046$ ;  $P = 0.4320$ ) eggs and *A. albopictus* eggs ( $F = 0.5312$ ;  $P = 0.7179$ ) either in the grass infusion (control) or with control agents using Tukey's test at 5% significance level (Table 2).

Both species had the highest mean number of eggs with grass infusion (control); however, *A. aegypti* had the highest mean in ovitraps with distilled water + *T. haemorrhoidalis* and the lowest value with grass infusion + *S. spinosa*. Results for *A. albopictus* were exactly the opposite, but this difference was not statistically significant (Tukey's,  $p > 0.05$ ).

### Oviposition in the field

The highest mean number of eggs for both species was obtained with grass infusion + *S. spinosa*, and the lowest value was obtained with grass infusion + Pyriproxyfen. This resulted in a mean number of eggs that was twice as low as grass infusion + *S. spinosa*; this corroborates the significant difference between treatments (Tukey,  $P = 0.0124$ ) (Table 3). Except for the treatment with grass infusion + Pyriproxyfen, all treatments had a higher mean number of eggs than the grass infusion (control) (Table 3); however, there was no statistical difference between treatments (Tukey,  $p > 0.05$ ).

Nearly all treatments had an OPI of 100% except distilled water + *T. haemorrhoidalis*, which obtained 97% of positivity (Fig. 1).

The EDI values of both species showed that biotic agents received a higher amount of eggs than the chemical agent Pyriproxyfen (Fig. 1). Grass infusion + *S. spinosa* and grass infusion + *B. thuringiensis israelensis* showed higher EDIs (150 and 130, respectively; Fig. 1). On the other hand, treatment with grass infusion + Pyriproxyfen had the lowest density (70 eggs; Fig. 1). The same result was observed for the VDI, which was highest with grass infusion + *S. spinosa* and the lowest with grass infusion + Pyriproxyfen.

The mosquito emergence rate obtained from eggs captured in the field was higher for *A. albopictus*: 4734 specimens comprising of 2270 males and 2464 females (1:1 ratio). In contrast, 494 specimens were obtained for *A. aegypti*: 272 males and 222 females (1:1 ratio). Therefore, *A. albopictus* predominated throughout the study area.

The rate of mosquito emergence was analyzed in the treatments. The highest number of adults occurred with grass infusion + *S. spinosa* and grass infusion (control). In these treatments, the number of adult *A. albopictus* was approximately ten times as high as that obtained for *A. aegypti* (Fig. 2). The control agents had little interference in the egg phase of these insects.

The lowest values for adult emergence were seen with grass infusion + *B. thuringiensis israelensis* (Fig. 2) despite this treatment

**Table 2**

Mean number of *Aedes aegypti* eggs and *Aedes albopictus* eggs obtained with ovitraps containing grass infusion and different control agents under laboratory conditions, in the period ranging from March to April, 2015, Manaus – Amazonas – Brazil.

Treatments	<i>Aedes aegypti</i>			<i>Aedes albopictus</i>		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum
* Grass infusion + <i>B. thuringiensis israelensis</i>	270. <sup>a*</sup>	345	171	124 <sup>a</sup>	254	56
* Grass infusion + <i>S. spinosa</i>	222. <sup>a</sup>	421	92	164.7 <sup>a</sup>	208	135
* Grass infusion + Pyriproxyfen	249.3 <sup>a</sup>	279	193	117.3 <sup>a</sup>	177	50
* Distilled water + <i>T. haemorrhoidalis</i>	289.7 <sup>a</sup>	306	269	98 <sup>a</sup>	220	32
* Grass infusion (Control)	373.7 <sup>a</sup>	440	287	187.3 <sup>a</sup>	278	94

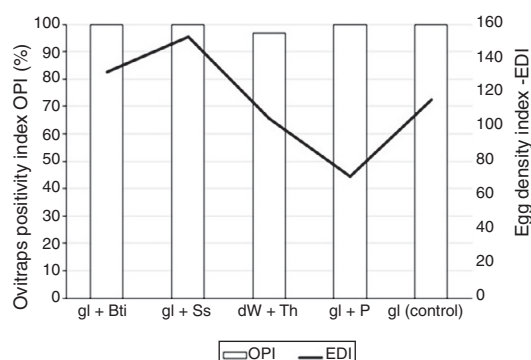
Legend: \*Same letters in the column do not differ according to the Tukey's test at 5% significance level.

**Table 3**

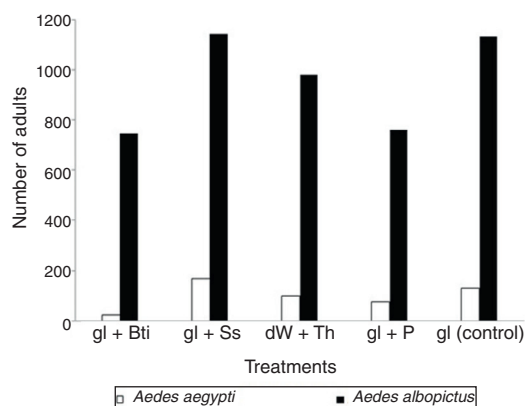
Total mean of eggs (*Aedes aegypti* and *Aedes albopictus*) obtained by the association of grass infusion and different control agents using ovitraps under field conditions in the period ranging from March to April, 2015, Manaus – Amazonas – Brazil.

Treatments	Mean	Maximum	Minimum
* Grass infusion + <i>B. thuringiensis israelensis</i>	1322.8 <sup>ab*</sup>	2025	831
* Grass infusion + <i>S. spinosa</i>	1528.2 <sup>a</sup>	1932	1121
* Grass infusion + Pyriproxyfen	712.6 <sup>b</sup>	903	380
* Distilled water + <i>T. haemorrhoidalis</i>	1158. <sup>ab</sup>	1525	765
* Grass infusion (Control)	988.2 <sup>ab</sup>	1299	533

Legend: \*Same letters in the column do not differ according to the Tukey's test at 5% significance level.



**Fig. 1.** Ovitrap Positivity Index (OPI) and Egg Density Index (EDI) with association of grass infusion with different control agents using ovitraps in the period ranging from June to July 2015, Manaus – Amazonas – Brazil. Legend: \*(gl+Bti) – grass infusion + *Bacillus thuringiensis israelensis*; \*(gl+Ss) – grass infusion + *Saccharopolyspora spinosa*; \*(dW+Th) – distilled water + *Toxorhynchites haemorrhoidalis*; \*(gl+P) – grass infusion + Pyriproxyfen; \*(gl) – grass infusion (control).



**Fig. 2.** Total *Aedes aegypti* and *Aedes albopictus* adults obtained through egg collection in ovitraps containing grass infusion (control) and associated with different control agents. Legend: \*(gl+Bti) – grass infusion + *Bacillus thuringiensis israelensis*; \*(gl+Ss) – grass infusion + *Saccharopolyspora spinosa*; \*(dW+Th) – distilled water + *Toxorhynchites haemorrhoidalis*; \*(gl+P) – grass infusion + Pyriproxyfen; \*(gl) – grass infusion (control).

having the second highest mean number of eggs in the field (Table 3). No differences were seen in larval hatching rate between grass infusion (control) and control agents either for *A. aegypti* ( $P=0.1481$ ;  $H=6.78$ ) nor for *A. albopictus* ( $P=0.093$ ;  $H=7.96$ ) using the Kurskall Wallis test with a significance level of 5% (Fig. 2).

## Discussion

Biological characteristics are determining factors for oviposition and might directly influence the higher number of *A. aegypti* eggs observed in the laboratory (Phasomkusolsil et al., 2014) especially because mosquitos are an intradomiciliar and anthropophilic insect. Females of this species complete their gonotrophic cycle with two blood meals. The mean number of eggs at each oviposition is 120; *A. albopictus* needs multiple meals to generate an average of 60 to 65 eggs (Clements, 1999; Forattini, 2002).

The high number of eggs seen in both species in ovitraps with grass infusion (control) might be explained by the proven effectiveness of this substrate as an attractant to female *Aedes* spp. (Nunes et al., 2011; Reiter et al., 1991; Santana et al., 2006).

The difference found in the treatment with distilled water + *T. haemorrhoidalis* with higher average number of *A. aegypti* eggs can be explained by the behavior of this species. It oviposits in pre-existing immature breeding sites, since in most urban environments, specimens do not establish strong ecological relations of competition with other insects, such as coexistence with predatory invertebrates, and thus become completely generalist in relation to oviposition sites (Forattini, 2002). On the other hand, *A. albopictus* coexists with *T. haemorrhoidalis* in the suburban environment of Manaus. *T. haemorrhoidalis* is a natural predator of culicids in vegetated areas—sites where *A. albopictus* occurs. It consequently colonizes different types of natural breeding sites establishing ecological relationships with other insects, e.g. habitat competition (Bailey et al., 1983; Forattini, 2002; WHO, 1984). This might explain the low number of *A. albopictus* eggs in ovitraps containing larvae of this predatory species.

The choice of potential oviposition sites is related to several environmental factors including water spray intensity, size of the water surface relative to light, presence of immature culicid stages, and certain chemical components that might act as repellents or attractants depending on the concentration (Allan and Kline, 1995; Chadee et al., 1990).

The laboratory experiments had controlled conditions. It isolated the different factors related to oviposition and maximized the treatment differences. This allowed us to relate the factors found to be effective in ovitraps for each control agent. These agents can attract more fertilized females leading to better study and control of these species.

Under field conditions, more eggs were collected in ovitraps with grass infusion + *S. spinosa*. One study carried out in Mexico also showed the effectiveness of this bacterium as an attractant for mosquito females in the field where the oviposition rate of *A. aegypti* was higher than in treatments with temephos or distilled water (Solís-Valdez et al., 2015). However, in this study, grass infusion + *S. spinosa* only differed statistically from the chemical Pyriproxyfen (Tukey,  $P=0.0124$ ; Table 3). Ovitrap with grass infusion + *B. thuringiensis israelensis*, were preferred for oviposition corroborating prior field results (Carrieri et al., 2009; Depoli et al., 2016; Jahan and Sajjad Sarwar, 2013; Stoops, 2005).

Spinosa<sup>®</sup> is a new generation larvicide derived from the bacterium *S. spinosa*. It is toxic to several insect species of the orders Lepidoptera, Thysanoptera, and Diptera including mosquitoes (Bond et al., 2004; Dias et al., 2017; Huseyin et al., 2005). The effectiveness of *S. spinosa* was verified in the field for *A. aegypti* over 13 weeks during the dry season and 10 weeks during the rainy season (Marina et al., 2011). This is important information from an ecological standpoint because it shows feasibility at a large scale. Persistence in the field—combined with the effectiveness of the bacterium as an attractant to females and control of immature stages—makes this tool an important alternative. This information is important considering the climatic conditions in Manaus. It has a high solar incidence and abundant rains every year, but these do not limit the use of ovitraps associated with *S. spinosa*.

However, the major concern in using this bacterium for biological pest or vector control is the selection of resistant insects. This has already been observed in agricultural pests (Moulton et al., 2000; Rehan and Freed, 2014; Rinkevich and Scott, 2009) and in *Culex quinquefasciatus* Say, 1823 (Su and Cheng, 2012, 2014a, 2014b) and *A. albopictus* (Khan et al., 2011).

The effectiveness of *B. thuringiensis israelensis* is proven and selective for the control of different dipteran groups such as Culicidae, Simuliidae, and Chironomidae (Bravo et al., 2011). It has been widely employed successfully in different regions of the world. There was a limited selection of resistant insects due to the specific mechanism of action and safety (these effects are not accumulative; Bravo et al., 2011; Gill, 1995). By integrating *B. thuringiensis israelensis*, our results point toward an important alternative as a surveillance tool. This tool could aid in *A. aegypti* and *A. albopictus* control in urban Manaus.

The lowest mean oviposition was observed in ovitraps with grass infusion + Pyriproxyfen. This corroborates other studies finding a preference for oviposition in substrates containing biological products (Quiroz-Martínez et al., 2012; Solís-Valdez et al., 2015). According to Forattini (2002), the microbial activity attracts female mosquitoes to lay their eggs in containers with this substrate.

The low number of eggs obtained in ovitraps containing distilled water + *T. haemorrhoidalis*, is in accordance with the results obtained for *A. albopictus* under laboratory conditions. It is important to emphasize that Manaus is a city with strong anthropogenic influence, with urban occupation among native and secondary vegetation mosaics, a characteristic that is present in the study area, with natural occurrence of *T. haemorrhoidalis*, which coexists with the mosquito species tested. This coexistence might have influenced the low effectiveness of these traps in the field, due to the well-known predatory potential of this species, mainly of *A. albopictus*.

The number of eggs obtained here using ovitraps and their respective OPI, EDI, and VDI indices highlight the effectiveness of this tool in monitoring these vectors. The removal of eggs from the environment is analogous to the control of immature stages with a control agent. This makes this method more effective and safer for mosquito-control programs. There were a high number of eggs found in the different control agents with no repellents, and this strategy could monitor vectors and assist in vector control.

Studies using ovitraps to estimate density of *A. aegypti* and *A. albopictus* show that organic matter infusions, mostly those made of grass, have been used to maximize the effect of the trap, serving as an attractant to females (Reiter et al., 1991; Santana et al., 2006; Villaseca et al., 2001). According to Nunes et al. (2011), grass infusion associated to ovitrap is more effective than ovitraps with water. This was also observed in our study with ovitraps containing only grass infusion and grass infusion associated with different control agents.

Studies demonstrate the feasibility of ovitraps for monitoring and control of *A. aegypti* in the long-term (Chism and Apperson, 2003; Perich et al., 2003; Rapley et al., 2009). These traps, associated to entomopathogens, cause reduction in the density of mosquitoes of medical importance, preventing larvae from developing, thus ensuring more time in the environment and preventing traps from becoming a potential breeding site (Depoli et al., 2016; Regis et al., 2008).

Although some mosquitoes' populations resistant to Natular<sup>TM</sup> DT – *S. spinosa* have been reported, this product should not be excluded from control programs, as it has been shown to be effective when associated to ovitraps. However, this larvicide is more indicated in specific situations, i.e., where it has not been previously used and when surveillance is required because of its excess use.

Ovitraps associated to Vectobac<sup>®</sup> WG – *B. thuringiensis israelensis* also play an important role in the oviposition of *A. aegypti* and *A. albopictus*. The fact that it is highly selective for the target organisms, that no selection of resistant mosquitoes has been detected, and that it does not repel females in the oviposition process makes the integration of this biolarvicide with ovitraps the best surveillance/control tool for *Aedes*.

## Conflict of interest

The authors declare no conflict of interest.

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