Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v44i1.59231

ZOOLOGY

Efficiency of oviposition traps with biolarvicides for monitoring *Aedes aegypti* and *Aedes albopictus* (Diptera, Culicidae), northeast Brazil

Maxcilene da Silva de Oliveira[1], Joelma Soares da Silva[2], Juliete Lima Viana[3], Wanderli Pedro Tadei[3] and Valéria Cristina Soares Pinheiro[1*]

[1]Universidade Estadual do Maranhão, Praça Duque de Caxias, s/nº, Morro do Alecrim, 65604-380, Caxias, Maranhão, Brazil. [2]Universidade Federal do Maranhão, Codó, Maranhão, Brazil. [3]Universidade do Estado do Amazonas, Cachoeirinha, Manaus, Amazonas, Brazil. *Author for Correspondence. E-mail: pinheirovcs@gmail.com

ABSTRACT. This study verified the efficiency of ovitraps combined with *Saccharopolyspora spinosa* and *Bacillus thuringiensis israelensis* for monitoring *Aedes aegypti* and *Aedes albopictus* in laboratory and field conditions. In the laboratory, for *A. aegypti*, there was no difference in eggs number between treatments and grass infusions. For *A. albopictus*, the average of eggs was higher in the grass infusion. In the dry season, there was no difference in the average of eggs between treatments and control. In the rainy season, grass infusion resulted in a higher egg density, in both areas. Ovitraps combined with biolarvicides are efficient in dengue vector monitoring.

Keywords: arbovirus; traps; biological control; dengue; vectors.

Received on May 14, 2020.
Accepted on October 11, 2021.

Introduction

Aedes (Stegomyia) *aegypti* (Linnaeus, 1762) is considered the main vector of the etiological agents of dengue, yellow fever, Chkungunya and Zika virus, and predominates in urban areas, and causes great impact on public health (Forattini, 2002; Zara, Santos, Fernandes-Oliveira, Carvalho, & Coelho, 2016).

Aedes (Stegomyia) *albopictus* (Skuse, 1894) is from rural areas, however, coexist with *A. aegypti* in outskirts of large cities (Forattini, 2002; Rey & Lounibos, 2015; Zara et al., 2016). This mosquito, like *A. aegypti*, is considered a threat to public health because it is a competent vector for several viruses, including dengue, chikungunya and zika (Reiter, Fontenille, & Paupy, 2006; Vega-Rua et al., 2013; Vega-Rúa, Zouache, Girod, Failloux, & Lourenço-de-Oliveira, 2014; Ciota, Bialosuknia, Ehrbar, & Kramer, 2017).

Among the mosquito larval control measures, it can be highlighted the biological control, primarily using bacteria. These microorganisms are ecologically viable besides being the most worldwide used entomopathogens for mosquito's control (Praça et al., 2004; Nareshkumar, Murugan, Baruah, Madhiyazhagan, & Nataraj, 2012; Revathi et al., 2013; Soares-da-Silva et al., 2017). *Bacillus thuringiensis* sub. *israelensis* (Berliner, 1915) and *Saccharopolyspora spinosa* (Merts & Yao, 1990), are the most used currently (Benelli, Jeffries, & Walker, 2016; Dias et al., 2017).

Bacillus thuringiensis produces protein crystals during sporulation, during sporulation, a diversity of which are toxins, with specific action for different groups of insects (Alves, 1998; Glare & O'callaghan, 2000). The subspecies *Bacillus thuringiensis israelensis* (Bti) is the active ingredient of VectoBac®, this product is available for purchase (Guillet & Escaffre, 1979; Guillet, Escaffre, & Prud'hom, 1982).

Another microbial agent used for insect control is *S. spinose*, which produces spinosyns, during the fermentation process. Nine spinosyns are known and are identified from A-H and J. The formulation base NatularTM DT – spinosade consists mainly of two spinosyns (A – 85%; D – 15%), which are the ones with the greatest insecticidal activity (Darriet, Duchon, & Hougard, 2005; Dias et al., 2017).

One of the ways of using these biolarvicides, is to add them in oviposition traps, to monitor and density of female mosquito vectors (Passos, Marques, Voltolini, & Condino, 2003; Silva et al., 2018).

Oviposition traps, also known as ovitraps, represent a surveillance tool for *A. aegypti* and *A. albopictus*. The use of ovitraps has limitations, due to the possibility of becoming a potential breeding site for vectors when they stay in the field for more than seven days (Silva & Limongi, 2018). However, the use of these traps combined with biolarvicides can increase their efficiency, making it a more sensitive and viable tool for mosquito control programs, because it can provide data on the level of infestation and species control, since the larvae die from the action of biolarvicides when they hatch out (Stoops, 2005; Sant'ana, Roque, & Eiras, 2006; Depoli, Zequi, Nascimento, & Lopes, 2016).

Due to the ovitrap sensitivity in monitoring both species and the biolarvicide high efficiency, investigations are necessary to define which of these products may influence the behavior of engorged females, in the sense of attracting females for oviposition (Silva & Limongi, 2018; Silva et al., 2018).

In this context, this study aimed to check the efficiency of oviposition traps, ovitraps, combined with the biolarvicides *S. spinosa* and *B. thuringiensis* through the positivity index and density of *A. aegypti* and *A. albopictus* eggs for monitoring these mosquito vectors in laboratory and field conditions.

Material and methods

Study area

The study was carried out in the municipality of Caxias, state of Maranhão, located at 4°51'32" South latitude and 43°21'22" West longitude. The municipal headquarters is 66 meters above sea level, with 5,196.769 km² area. Caxias is the fifth largest city in the state of Maranhão, Brazil, with 166.1591 inhabitants (IBGE, 2022). The climate is tropical, hot and humid and has two defined seasons, rainy and dry, with average temperatures ranging from 17 to 27°C, varied rainfall of 1,100 and 1,500 mm year^{-1} (GERPLAN, 2002; Sousa, Meneses, & Vianna, 2015).

Laboratory experiments

The experiments were conducted at the Laboratory of Medical Entomology – LABEM, *Universidade Estadual do Maranhão* – UEMA. *A. aegypti* and *A. albopictus* females were acquired from the insectary kept at LABEM, in which the breeding of mosquitoes followed the Consoli and Lourenço-de-Oliveira (1994) protocol, adapted by Pinheiro and Tadei (2002).

Sixty fully engorged females with a mean age of three days were used and transferred to an aluminum/acrylic cage with dimensions of 40 x 40 cm in a room under controlled conditions, with an average temperature of 28 ± 2°C, relative humidity around 85% and photophase of 12 hours (Navarro et al., 2003; Gomes, Sciavico, & Eiras, 2006). For each species, separately, three traps were placed into the breeding cages, which consisted of plastic 180 mL glass lined with filter paper, combined with biolarvicides prepared as follows: one based on *S. spinosa*, tablet formulation, NatularTM 20EC - 20.65% Spinosad a.i., Clarke Mosquito Control Products, Inc. - spinosad, and the other consisting of *B. thuringiensis* sub. *israelensis*, granule formulation, VectoBac® WG - Bti.

The combination with Bti consisted of the addition of 0.003 grams of larvicide in each glass, with the addition of 100 mL tap water. To prepare the combination with spinosad (Natular™ DT, batch 1309190010), ¼ (0.21g L^{-1}) of tablet corresponding to 1.87% spinosyn was added, with the addition of 100 mL tap water, following the manufacturer recommendations.

As a positive control, 100 mL 10% grass infusion was used, this substance is the standard used as an attraction for engorged females of *A. aegypti*. This solution was prepared from dehydrated grass leaves, with the addition of approximately 20 L tap water, packed in a plastic container with a capacity of 25 L. The solution was sealed and stored for seven days for fermentation, right after the solution was filtered and diluted to a final concentration of 10% (Reiter, Amador, & Colon, 1991).

Glasses were daily clockwise rotated and, after four days, eggs were removed for counting under a stereomicroscope (Zeiss stemiDV4). The experiment was repeated four times; each repetition had a new group of females and lasted five days, in accordance with Barbosa and Silva (Barbosa & Silva, 2002) with modifications.

Field experiments

Field experiments were conducted in two distinct areas, area I, located in the central area, and area II, located in the south of the city. Field experiments were repeated in two seasons, dry (from August to October,

2017) and rainy (from January to March, 2018). These areas were selected for presenting cases of dengue in 2017, and for presenting distinct characteristics regarding vegetation, which is important considering the biological characteristics of the two species under study.

Ten houses were chosen in each area, with an average distance of about 400 m apart (Monteiro, Carvalho, & Souto, 2014; Soares, Silva, Oliveira, & Abreu, 2015). All residences were geo-referenced using the Global Positioning System (GPS) (Figure 1A and B). Three ovitraps were installed in each house, two combined with biological insecticides one based on *S. spinosa*, tablet formulation, NatularTM DT - spinosad, and the other consisting of *B. thuringiensis* sub. *israelensis*, granule formulation, VectoBac® WG – Btiand, containing 10% grass infusion.

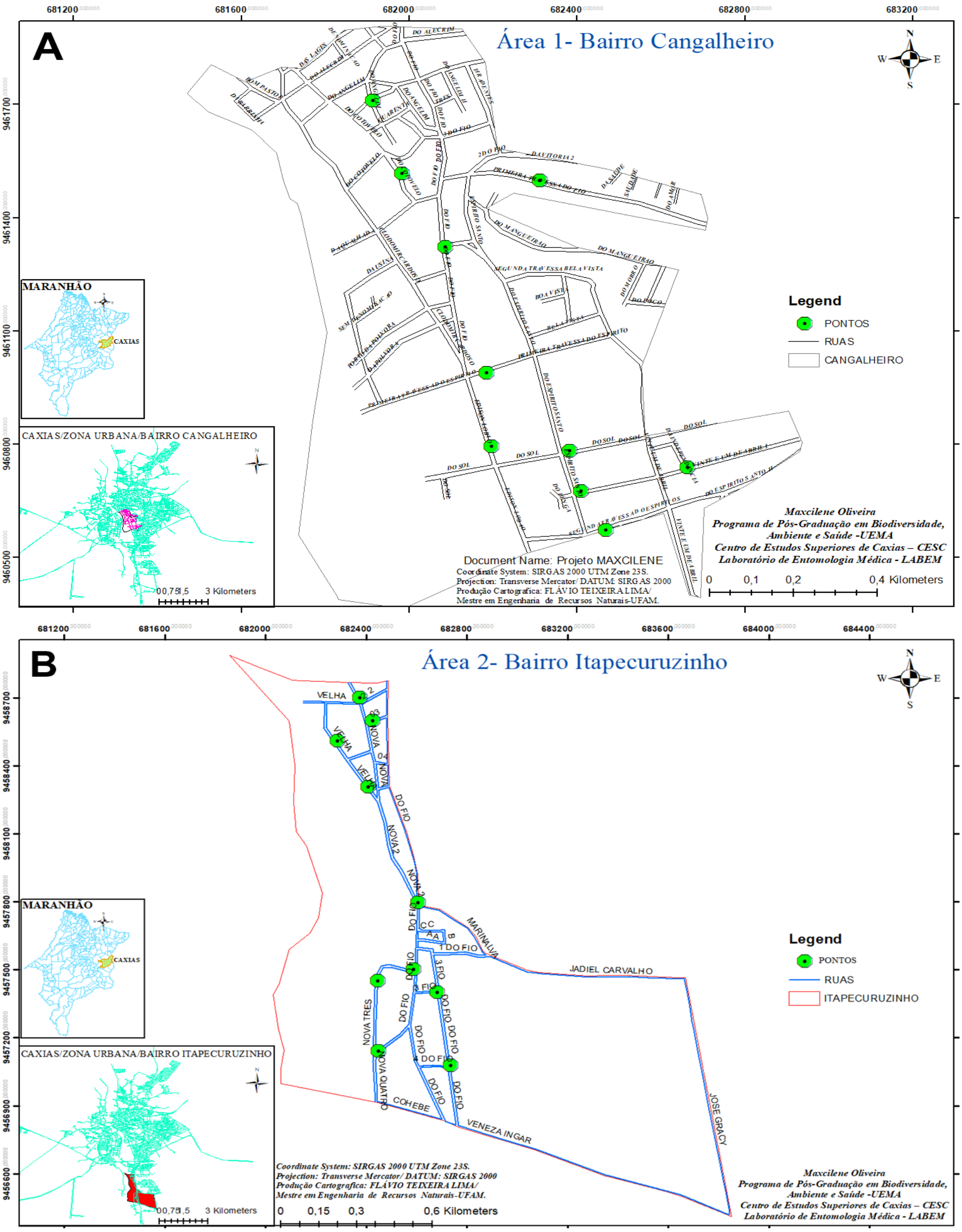

Figure 1. Maps showing the sites where the ovitraps were installed in the municipality of Caxias, state of Maranhão, Brazil: A) Area I; B) Area II. Source: author himself

Ovitraps combined with biolarvicides were prepared as follows: one *S. spinose*-based, tablet formulation, Natular™ DT – spinosad, and the other consisting of *B. thuringiensis* sub. *israelensis*, granule formulation, VectoBac® WG - Bti. The Bti combination consisted of adding 0.003 grams of the larvicide in each trap, with an addition of 500 mL tap water. While for preparing the combination with spinosad, ¼ tablet that corresponds to 1.87% spinosyns was added to the same amount of water as the previous biolarvicide. The quantities used of the biolarvicides were defined according to the manufacturer recommendation.

As a positive control, 500 mL 10% grass infusion was used, this substance is used as a standard for attraction of *A. aegypti* engorged females in ovitraps. This solution was prepared from dehydrated grass leaves, with an addition of approximately 20 L tap water, packaged in a plastic container with a capacity of 25 L. The solution was sealed and stored for seven days for fermentation. Right after that, the solution was filtered and diluted to a final concentration of 10% (Reiter et al., 1991).

Ovitraps used in the field experiment, consisted of a black plastic container, with a hole about 12 cm wide, and a capacity of 800 mL (Reiter, Amado, Anderson, & Clark, 1995). Inside of them, a plywood reed was placed, Duratree, 12 cm x 2.5 cm wide, fixed vertically by a steel clip #08 for oviposition and eggs adherence.

Ovitraps were installed in the peridomicile, limited to the residence immediate surroundings, on the ground, 20 cm apart, preferably positioned, where possible, in places protected from the sun and rain. After a four-day exposure period, ovitraps were collected and forwarded to the Laboratory of Medical Entomology – LABEM, properly identified. After drying the reeds, eggs were counted under a stereomicroscope (Zeiss model stemiDV4). Between each collection, there was an interval of 25 days.

In each study area, by season, three repetitions were performed, with 30 ovitraps installed in each, totaling 90 traps. Considering both seasons, the sampling effort was 180 ovitraps for each area, totaling 360 traps used in the two areas.

Data analysis

The positive ovitrap index (POI) is the ration of the positive traps number and the number of examined traps multiplied by one hundred and the Egg Density Index (EDI) is the ratio of the number of eggs to the number of positive traps (Gomes, 1998). To assess the attractiveness or repellency, the Oviposition Activity Index (OAI) was used, represented by the formula $OAI = (Nt - Nc)/(Nt + Nc)$, where Nt is the number of eggs laid in the treatment and Nc is the number of eggs placed in the control trap (Karmer & Mulla, 1979). $OAI \geqslant +0.3$ indicates attractiveness, while $OAI \leqslant -0.3$ indicates that the treatment is repellent to oviposition.

Data were assessed for normal distribution by Lilliefors' Test, and for homoscedasticity, by Levene's Test (Zar, 2014). To check for differences in averages and medians for the egg density index between the treatment groups and the control, ANOVA (F) or Kruskal-Wallis (H) was applied, in cases of normal and non-normal distribution, respectively. In case of difference between averages and medians, Tukey and Dunn's tests were continued a posteriori, respectively. For all analyses, a value of $p < 0.05$ was considered statistically significant (Siqueira & Tibúrcio, 2011).

The analyses w ere run using the BioEstat version 5.0 statistical packages (Ayres, Ayres, Ayres, & Santos, 2007), GraphPad Prism 5.0 and Microsoft Office Excel 2013.

Results

Laboratory experiments

In laboratory conditions, *A. aegypti* females laid an average of 536.25 eggs in all four replications, with an average of 582.5 (0.3321) in ovitraps containing spinosad and Bti, with an average of 430 (0.2458) eggs and grass infusion with an average of 596.25 eggs. However, no significant differences were detected for the averages of eggs obtained between treatments ($F = 0.8521$, $p = 0.5388$) (Figure 2A).

Although there was no difference between the averages of eggs, the oviposition activity index showed that spinosad was attractive for *A. aegypti* (OAI = 0.32 and OAI = 0.38) and for Bti only in one presentation presented $OAI \geqslant + 0.3$ (OAI = 0.46).

Regarding *A. albopictus* oviposition, 147.75 average eggs were obtained in the four replications. The highest average of eggs was obtained for the grass infusion (294), with a value about seven times higher than spinosad (43.5) and about three times higher compared to Bti (105.75).

When comparing egg averages between treatments, there was a statistically significant difference ($F = 13.1888$, $p = 0.0025$). In grass-infused ovitraps, higher number of eggs were obtained with both biolarvicides, spinosad (**) and for Bti (*) (Figure 2B). However, there was no statistically significant difference for number of eggs between biolarvicides.

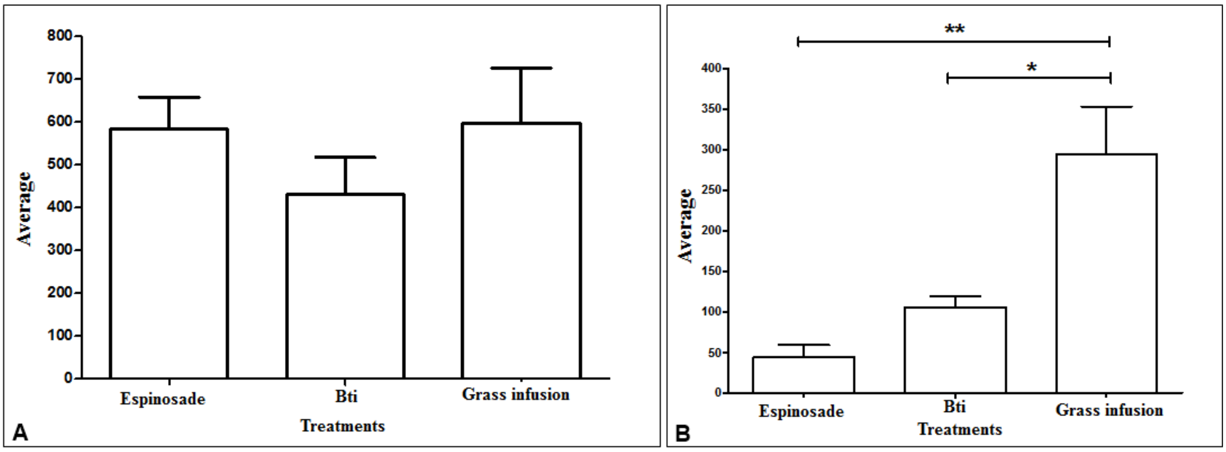

Figure 2. Comparison of averages of eggs by ovitraps combined with biolarvicides under laboratory conditions: (A) *Aedes aegypti*; (B) *Aedes albopictus*.

The calculation of OAI indicated that both biolarvicides, spinosad and Bti, are attractive to females of *A. albopictus*, for spinosad with OAI = 0.96 and for Bti of OAI = 0.57.

Field experiments

In Area I, a total of 22,479 eggs were obtained and the number of eggs obtained during the rainy season (19,555) was about seven times greater than in the dry season (2,924). Regarding the number of eggs in the treatments, the combination with spinosad reached about twice as many eggs as in ovitraps with Bti, in the two seasons. On the other hand, the infusion of grasses (positive control) obtained a greater number of eggs than the two treatments, in both seasons.

Regarding the positive ovitrap index - POI, between the combinations, similar values were observed in both seasons. However, regarding the EDI, there were variations between treatments and control, in both seasons, in which the grass infusion showed higher egg density than the treatments.

In the dry season, there was no significant difference in averages of eggs in the combination ($F = 5.0986$, $p = 0.0131$); ovitraps with grass infusion had higher means than treatments with Bti and spinosad ($p < 0.05$) (Figure 3).

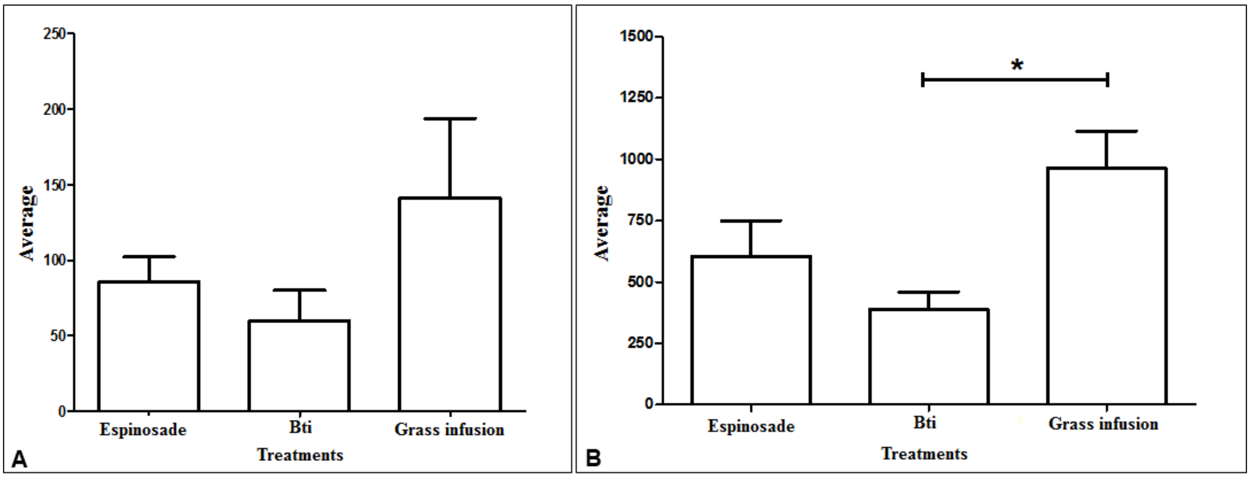

Figure 3. Average of eggs of *Aedes aegypti* and *Aedes albopictus* collected using ovitraps combined with biolarvicides in area I: A) Dry season (August to October, 2017); B) Rainy season (January to March, 2018).

In Area II, 23,005 eggs were obtained, and 21,132 were obtained for the rainy season, 11 times higher than the value for the dry season, 1,873. Ovitraps with spinosad presented a higher number of eggs than the traps with Bti, in both seasons. Ovitraps with grass infusion showed a higher number of eggs than Bti, in both seasons, with values about three times higher in the rainy season and two times higher in the dry season.

The Positive Ovitrap Index - POI were similar in both periods, for all treatments. However, the Egg Density Index – EDI in both periods, was higher for grass infusion, corresponding about twice more than the other treatments.

The average of eggs obtained in the dry season did not show a significant difference between treatments (F = 1.1206, p = 0.3415) (Figure 4). On the other hand, in the rainy season, there was a significant difference between treatments (H = 10.5494, p = 0.0051), in which the grass infusion obtained a median significantly higher than spinosad and Bti (Dunn's Test p < 0.05) (Figure 4).

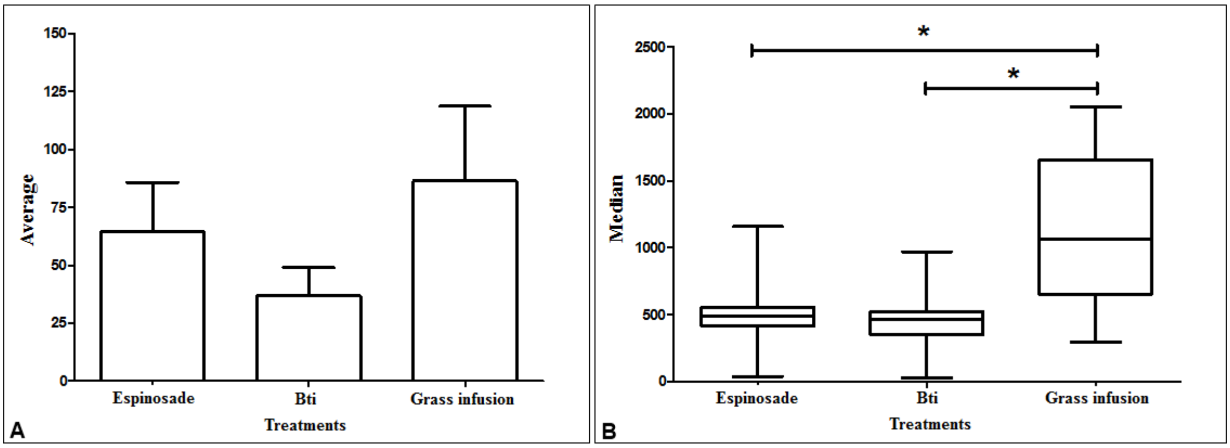

Figure 4. Average of eggs of *Aedes aegypti* and *Aedes albopictus* collected with ovitraps combined with biolarvicides in area II: A) Dry season (August to October, 2017); B) Rainy season (January to March, 2018).

In relation to the studied areas, the results obtained in the field showed through the Oviposition Attractiveness Index that both biolarvicides were attractive to *Aedes* females, during the two analyzed seasons, dry and rainy. The lowest value for spinosad was OAI = 0.33 and the highest, OAI = 0.76, and for Bti, the highest was OAI = 0.32 and the lowest, OAI = 0.81.

Comparison of Egg Density Index (EDI) with climatic factors

Regarding the egg density correlated with climatic factors, in the dry season, it was observed that there was no record of rainfall during the collection days; the average temperature was 28.2°C and relative humidity was 58%. On the other hand, in the rainy season, temperatures of 26.3°C, relative humidity of 78.6% and average rainfall of 11.8 mm were recorded. During this season, EDI was obtained with higher values. When comparing EDI between the analyzed seasons, for treatments and grass infusion, EDI was higher in the rainy season.

Discussion

In laboratory condition, in this study, the results were similar to those found in the field, because it was found that the number of eggs was significantly higher in traps containing grass infusion for the two studied species. Fermentation of grass infusions can attract females for oviposition (Gubler & Bhattacharya, 1971). The efficiency of this type of combination, ovitraps/grass infusion, has already been demonstrated by other authors, who registered a high number of eggs in this type of combination (Trexler, Apperson, & Schal, 1998; Sant'ana et al., 2006; Nunes, Trindade, & Souto, 2011; Silva et al., 2018).

Between treatments, for Bti, there was a greater number of eggs, although no statistical difference was detected between the averages of egg for the two biolarvicides. Carrieri, Masetti, Albieri, Maccagnani, and Bellini (2009) carried out a study in Rovigo – Italy with *A. albopictus* and found an increase in the number of eggs in ovitraps combined with Bti. Depoli et al. (2016) found that Bti combined with ovitraps does not cause changes in oviposition preference. Both authors reported that the females did not stop laying their eggs on the traps containing this biolarvicide.

Bentley and Day (1989) suggest that these vectors' oviposition behavior is influenced by some factors, including the presence of microorganisms such as bacteria, which may have attracted females, because it is an active ingredient in the used formulations.

Still under laboratory conditions, the OAI data showed that spinosad is an efficient attractive for both species, for *A. aegypti* and *A. albopictus*. The formulation consists of an effervescent tablet, which slowly releases the active ingredient that increases the residual power of the product, and releases a smell similar to the soil; these characteristics may have efficiently attracted females for a longer period than Bti (Thompson, Dutton, & Sparks, 2000). However, it is noteworthy that *Aedes albopictus* was attracted for the two biolarvicides in all repetitions of the tests.

Several studies have already demonstrated the efficiency of oviposition traps combined with biolarvicides. The addition of *B. thuringiensis* sub. *israelensis* or *S. spinosa* in ovitraps did not negatively interfere with females' choice for the traps. Bacterial larvicides can be used in combination with oviposition traps because it does not repel *A. aegypti* and *A. albopictus* females (Romi, Proietti, Di Luca, & Cristofaro, 2006; Nazni et al., 2009; Solís-Valdez et al., 2015).

In field conditions, a high POI index was found in both areas during the dry and rainy seasons. Other studies have also found the efficiency of ovitraps as a tool for monitoring mosquitoes in different regions of Brazil (Fay & Eliason, 1966; Dibo et al., 2005; Silva & Limongi, 2018). Monteiro et al. (2014), in Macapá, state of Amapá, Brazil, studied *A. aegypti* spread, using ovitraps and obtained an POI of 50.44%. These studies show that this method of egg capture stands out for being sensitive to the detection of *A. aegypti* and *A. albopictus* females, in different areas.

In the two periods of study, there was a significant difference in the averages of eggs obtained in ovitraps with grass infusion, compared to the treatments. Studies have shown that adding grass infusion to ovitraps works as attractiveness, because it improves the specificity and sensitivity of oviposition traps, and plays a role for the control of vector mosquitoes by removing mosquito eggs from the environment (Perich et al., 2003; Gama, Eiras, & Resende, 2007; Depoli et al., 2016).

The Positive Ovitrap Index and Egg Density Index were similar between treatments, for which a high number of eggs was recorded, even considering that, in the field, there is a great provision of artificial breeding sites in the studied neighborhoods (Soares-da-Silva, Bezerra, Ibiapina, Pinheiro, & Tadei, 2012; Andrade, Lobo, Soares-da-Silva, & Pinheiro, 2018). This demonstrates that Bti and Spinosad might have the same effect on engorged *A. aegypti* and *A. albopictus* females. Furthermore, it was possible to observe that the biolarvicides acted as attractive for female mosquitoes in field conditions, because there were many eggs in the ovitraps.

From the data obtained by the OAI, it was clear that biolarvicides do not repel *A. aegypti* and *A. albopictus*, and also acted positively in attracting these females. The presence of microorganisms in breeding sites is a factor that positively influences mosquito females for the oviposition site selection, which explains the assertiveness of the traps used herein (Forattini, 2002).

The fact that there is no significant difference between the averages of eggs in combinations with biolarvicides means that biological larvicides can be used in rotation. The presence of Spinosad or Bti biolarvicides in ovitraps does not prevent the oviposition of *A. aegypti* and *A. albopictus* and can also act as an attractive (Marina et al., 2011; Marcombe, Chonephetsarath, Thammavong, & Brey, 2018; Marina et al., 2020).

High temperatures and low humidity in the dry season negatively influence the biological cycle of mosquitoes, as well as in the positivity and density of ovitraps (Costa, Silva, Souza, & Mendes, 2008; Costa, Santos, Correia, & Albuquerque, 2010; Solís-Valdez et al., 2015). Rainfall is also an important climatic factor for the *A. aegypti* oviposition, because it allows a greater number of breeding sites (Miyazaki, Ribeiro, Pignatti, Campelo Júnior, & Pignati, 2009; Solís-Valdez et al., 2015).

In the rainy season, the density of eggs was similar between areas, probably due to the favorable conditions for the development of mosquitoes, such as high temperature, humidity and rainfall. These results demonstrate the association of meteorological variables and egg density. The highest rates of *A. aegypti* and *A. albopictus* infestations occurred mainly during the months with higher rainfall in different areas; these conditions directly affect mosquito's survival and oviposition sites (Favier et al., 2006; Barbosa & Lourenço, 2010; Viana & Ignotti, 2013; Soares et al., 2015).

Our results confirm that the formulated with *B. thuringiensis* sub. *israelensis* and *S. spinosa* active ingredient are efficient when combined with oviposition traps. Considering that these larvicides are of biological nature, do not contaminate the environment and have complex and specific targeting mechanisms of action, the combination of biolarvicides with oviposition traps represents a viable alternative in studies on *A. aegypti* and *A. albopictus*' monitoring and oviposition. With the addition of biolarvicides in oviposition traps, traps can remain in the field for extended periods, thus reducing the vectors' proliferation (Polanczyk, Garcia, & Alves, 2003; Galzer & Azevedo-Filho, 2016; Dias et al., 2017; Silva et al., 2018).

Conclusion

Ovitramps combined with biolarvicides *B. thuringiensis* sub. *israelensis* and *S. spinosa* are a viable alternative for monitoring *A. aegypti* and *A. albopictus*, which was confirmed by the high oviposition rates of the two species in laboratory conditions. In the field, it was also noted that the traps are efficient for monitoring, since mosquito eggs were positive in all combinations. It is noteworthy that biolarvicides are efficient in attracting *A. aegypti* and *A. albopictus*, facilitating the use of ovitramps as a monitoring tool for vector species.

Acknowledgements

We would like to thank the Biodiversity, Environment and Health Graduate Program, and to the faculty for their contribution to our professional training, to the *Fundação de Amparo à Pesquisa e Desenvolvimento Científico do Maranhão* (FAPEMA) and the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), for financing the projects that are carried out at the Laboratory of Medical Entomology – LABEM. To the Professor Juliana Maria Trindade Bezerra for statistical analyses, to MSc. Flávio Teixeira Lima for producing the maps used in this study.

References

Alves, S. B. (1998). *Controle microbiano de insetos*. Piracicaba, SP: FEALQ/USP.

Andrade, A. T. S., Lobo, K. S., Soares-da-Silva, J., & Pinheiro, V. C. S. (2018). Densidade populacional de *Aedes aegypti* (Linnaeus, 1762) e *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) nos criadouros artificiais do município de Caxias, Maranhão. In R. S. Fonseca, & M. C. Barros (Eds.), *Avanços das Ciências Biológicas no Centro de Estudos Superiores de Caxias (CESC), Maranhão* (p. 135-152). São Luís, MA: UEMA.

Ayres, M., Ayres, J. R. M., Ayres, D. L., & Santos, A. S. (2007). *BioEstat versão 5.3: Aplicações estatísticas nas áreas das ciências biológicas e médicas.* Belém, PA: Sociedade Civil de Mamirauá; Brasília, DF: MCT/CNPQ.

Barbosa, A. A., & Silva, M. A. N. (2002). Preferência por local de oviposição de *Aedes albopictus* (Skuse) (Diptera, Culicidae), em relação à presença de imaturos da própria espécie, sob condições de laboratório. *Revista Brasileira de Zoologia, 19*(4), 1147-1152. DOI: https://doi.org/10.1590/S010181752002000400020

Barbosa, G. L., & Lourenço, R. W. (2010). Análise da distribuição espaço-temporal de dengue e da infestação larvária no município de Tupã, Estado de São Paulo. *Revista da Sociedade Brasileira de Medicina Tropical, 43*(2), 145-151. DOI: https://doi.org/10.1590/S0037-86822010000200008

Benelli, G., Jeffries, C. L., & Walker, T. (2016). Biological control of mosquito vectors: Past, present, and future. *Insects, 7*(4), 1-18. DOI: https://doi.org/10.3390/insects7040052

Bentley, M. D., & Day, J. F. (1989). Chemical ecology and behavioral aspects of mosquito oviposition. *Annual Review of Entomology, 34*(1), 401-421. DOI: https://doi.org/10.1146/annurev.en.34.010189.002153

Carrieri, M., Masetti, A., Albieri, A., Maccagnani, B., & Bellini, R. (2009). Larvicidal activity and influence of *Bacillus thuringiensis* var. *israelensis* on *Aedes albopictus* oviposition in ovitraps during a two-week check interval protocol. *Journal of the American Mosquito Control Association, 25*(2), 149-155. DOI: https://doi.org/10.2987/08-5852.1

Ciota, A. T., Bialosuknia, S. M., Ehrbar, D. J., & Kramer, L. D. (2017). Vertical Transmission of Zika Virus by *Aedes aegypti* and *Ae. albopictus* Mosquitoes. *Emerging Infectious Diseases, 23*(5), 880-882. DOI: https://doi.org/10.3201/eid2305.162041

Consoli, R. A. G. B., & Lourenço-de-Oliveira, R. (1994). *Principais mosquitos de importância sanitária no Brasil*. Rio de Janeiro, RJ: Fiocruz.

Costa, E. P. A. A., Santos, E. M. M., Correia, J. C., & Albuquerque, C. M. R. (2010). Impact of small variations in temperature and humidity on the reproductive activity and survival of *Aedes aegypti* (Diptera, Culicidae). *Revista Brasileira de Entomologia, 54*(3), 488-493. DOI: https://doi.org/10.1590/S008556262010000300021

Costa, F. S., Silva, J. J., Souza, C. M., & Mendes, J. (2008). Population dynamics of *Aedes aegypti* (L) in an urban area with high incidence of dengue. *Revista da Sociedade Brasileira de Medicina Tropical, 41*(3), 309-312. DOI: https://doi.org/10.1590/S0037-86822008000300018

Darriet, F., Duchon, S., & Hougard, J. M. (2005). Spinosad: a new larvicide against insecticide-resistant mosquito larvae. *Journal of the American Mosquito Control Association, 21*(4), 495-496. DOI: https://doi.org/10.2987/8756971X(2006)21[495:SANLAI]2.0.CO;2

Depoli, P. A. C., Zequi, J. A. C., Nascimento, K. L. C., & Lopes, J. (2016). Eficácia de ovitrampas com diferentes atrativos na vigilância e controle de *Aedes*. *EntomoBrasilis, 9*(1), 51-54. DOI: https://doi.org/10.12741/ebrasilis.v9i1.509

Dias, L. S., Macoris, M. L. G., Andrighetti, M. T. M., Otrera, V. C. G., Dias, A. S., Bauzer, L. G. S. R., ... Lima, J. B. P. (2017). Toxicity of spinosad to temephos-resistant *Aedes aegypti* populations in Brazil. *PLoS ONE, 12*(3), 1-15. DOI: https://doi.org/10.1371/journal.pone.0173689

Dibo, M. R., Chiaravalloti-Neto, F., Battigaglia, M., Mondini, A., Favaro, E. A., Barbosa, A. A., & Glasser, C. M. (2005). Identification of the best ovitrap installation sites for gravid *Aedes* (Stegomyia) *aegypti* in residences in Mirassol, state of São Paulo, Brazil. *Memórias do Instituto Oswaldo Cruz, 100*(4), 339-343. DOI: https://doi.org/10.1590/S0074-02762005000400001

Favier, C., Degallier, N., Ribeiro Vilarinhos, P. D. T., De Carvalho, M. D. S. L., Cavalcanti Yoshizawa, M. A., & Britto Knox, M. (2006). Effects of climate and different management strategies on *Aedes aegypti* breeding sites: a longitudinal survey in Brasília (DF, Brazil). *Tropical Medicine & International Health, 11*(7), 1104-1108. DOI: https://doi.org/10.1111/j.1365-3156.2006.01653.x

Fay, R. W., & Eliason, D. A. (1966). A preferred oviposition site as a surveillance method for *Aedes aegypti. Moquito News, 26*(4), 531- 535.

Forattini, O. P. (2002). *Culicidologia Médica*. São Paulo, SP: Edusp.

Galzer, E. C. W., & Azevedo-Filho, W. S. (2016). Utilização do *Bacillus thuringiensis* no controle biológico de pragas. *Revista Interdisciplinar de Ciência Aplicada, 1*(1), 1-4.

Gama, R. A., Eiras, A. E., & Resende, M. C. (2007). Efeito da ovitrampa letal na longevidade de fêmeas de *Aedes aegypti* (Diptera: Culicidae). *Revista da Sociedade Brasileira de Medicina Tropical, 40*(6), 640-642. DOI: https://doi.org/10.1590/S0037-86822007000600008

Gerência de Planejamento e Desenvolvimento Econômico [GEPLAN]. (2002). *Atlas do maranhão*. (2. ed.). São Luís, MA: GEPLAN.

Glare, T. R., & O'callaghan, M. (2000). *Bacillus thuringiensis: Biology, ecology and safety*. Chichester, UK: John Wiley and Sons.

Gomes, A. C. (1998). Medidas dos níveis de infestação urbana para *Aedes* (Stegomyia) *aegypti* e *Aedes* (Stegomyia) *albopictus* em programa de vigilância entomológica. *Informe Epidemiológico do SUS, 7*(3), 49-57. DOI: https://doi.org/10.5123/S0104-16731998000300006

Gomes, A. S., Sciavico, C. J. S., & Eiras, Á. E. (2006). Periodicidade de oviposição de fêmeas de *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) em laboratório e campo. *Revista da Sociedade Brasileira de Medicina Tropical, 39*(4), 327-332. DOI: https://doi.org/10.1590/S0037-86822006000400002

Gubler, D. J., & Bhattacharya, N. C. (1971). Observations on the reproductive history of *Aedes* (Stegomyia) *albopictus* in the laboratory. *Mosquito News, 31*, 356-359.

Guillet, P., & Escaffre, H. (1979). Evaluation de *Bacillus thuringiensis israelensis* de Barjac pour la lutte contre les larves de *Simulium danmosum* s.l. II, efficacité comparée des trois formulations expérimentales. *OMS. WHO/VBC*, (735).

Guillet, P., Escaffre, H., & Prud'hom, Jean-Michel. (1982). L'utilisation d'une formulation à base de *Bacillus thuringiensis* H14 dans la lutte contre l'onchocercose en Afrique de l'Ouest: 2. Stabilité dans les conditions de stockage en milieu tropical. Cahiers ORSTOM. *Série Entomologie Médicale et Parasitologie, 20*(3), 181-185.

Instituto Brasileiro de Geografia e Estatística [IBGE]. (2022). *Maranhão, Caxias, infográficos: dados gerais do Maranhão*. Retrieved on February 17, 2022 from https://cidades.ibge.gov.br/brasil/ma/caxias/panorama.

Kramer, W. L., & Mulla, M. S. (1979). Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. *Environmental Entomology*, *8*(6), 1111-1117. DOI: https://doi.org/10.1093/ee/8.6.1111

Marcombe, S., Chonephetsarath, S., Thammavong, P., & Brey, P. T. (2018). Inseticidas alternativos para o controle larval do vetor da dengue *Aedes aegypti* no Laos PDR: resistência a inseticidas e estudo experimental em semicampo. *Parasitas & Vetores, 11*(1), 1-8. DOI: https://doi.org/10.1186/s130710183187-8

Marina, C. F., Bond, J. G., Casas, M., Muñoz, J., Orozco, A., Valle, J., & Williams, T. (2011). Spinosad as an effective larvicide for control of *Aedes albopictus* and *Aedes aegypti*, vectors of dengue in southern Mexico. *Pest Management Science*, *67*(1), 114-121. DOI: https://doi.org/10.1002/ps.2043

Marina, C. F., Bond, J. G., Muñoz, J., Valle, J., Quiroz-Martínez, H., Torres-Monzón, J. A., & Williams, T. (2020). Comparison of novaluron, pyriproxyfen, spinosad and temephos as larvicides against *Aedes aegypti* in Chiapas, Mexico. *Salud Pública de México, 62*(4), 424-431. DOI: https://doi.org/10.21149/10168

Mertz, F. P., Yao, R. C. (1990). *Saccharopolyspora spinosa* sp. nov. isolado de solo coletado em alambique de rum de usina de açúcar. *Revista Internacional de Microbiologia Sistemática e Evolutiva*, *40*(1), 34-39. DOI: https://doi.org/10.1099/00207713-40-1-34

Miyazaki, R. D., Ribeiro, A. L. M., Pignatti, M. G., Campelo Júnior, J. H., & Pignati, M. (2009). Monitoramento do mosquito *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae), por meio de ovitrampas no Campus da Universidade Federal de Mato Grosso, Cuiabá, Estado de Mato Grosso. *Revista da Sociedade Brasileira de Medicina Tropical, 42*(4), 392-397. DOI: https://doi.org/10.1590/S0037868220090004000007

Monteiro, F. J. C., Carvalho, J. C. T., & Souto, R. N. P. (2014). Distribuição da oviposição e dinâmica temporal do *Aedes aegypti* (Linnaeus) por meio de ovitrampas. *EntomoBrasilis, 7*(3), 188-192. DOI: https://doi.org/10.12741/ebrasilis.v7i3.419

Nareshkumar, A., Murugan, K., Baruah, I., Madhiyazhagan, P., & Nataraj, T. (2012). Larvicidal potentiality, longevity and fecundity inhibitory activities of *Bacillus sphaericus* (Bs G3-IV) on vector mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. *Journal of Entomological and Acarological Research, 44*(3), 79-84. DOI: https://doi.org/10.4081/jear.2012.e15

Navarro, D. M. A. F., De Oliveira, P. E. S., Potting, R. P. J., Brito, A. C., Fital, S. J. F., & Sant'Ana, A. G. (2003). The potential attractant or repellent effects of different water types on oviposition in *Aedes aegypti* L. (Dipt., Culicidae). *Journal of Applied Entomology, 127*(1), 46-50. DOI: https://doi.org/10.1046/j.14390418.2003.00690.x

Nazni, W. A., Lee, H. L., Wan Rozita, W. M., Lian, A. C., Chen, C. D., Azahari, A. H., & Sadiyah, I. (2009). Oviposition behaviour of *Aedes albopictus* in temephos and *Bacillus thuringiensis israelensis*-treated ovitraps. *Dengue Bulletin, 33*, 209-217.

Nunes, L. S., Trindade, R. B. R., & Souto, R. N. P. (2011). Avaliação da atratividade de ovitrampas a *Aedes* (Stegomyia) *aegypti* Linnaeus (Diptera: Culicidae) no bairro Hospitalidade, Santana, Amapá. *Biota Amazônia, 1*(1), 26-31. DOI: https://doi.org/10.18561/2179-5746/biotaamazonia.v1n1p26-31

Passos, R. A., Marques, G. R. A. M., Voltolini, J. C. M., & Condino, L. F. (2003). Dominância de *Aedes aegypti* sobre *Aedes albopictus* no litoral sudeste do Brasil. *Revista de Saúde Pública, 37*(6), 729-734. DOI: https://doi.org/10.1590/S0034-89102003000600007

Perich, M. J., Kardec, A., Braga, I. A., Portal, F., Burge, R., Zeichner, B. C., … Wirtz, R. A. (2003). Field evaluation of a lethal ovitrap against dengue vectors in Brazil. *Medical and Veterinary Entomology, 17*(2), 205-210. DOI: https://doi.org/10.1046/j.1365-2915.2003.00427.x

Pinheiro, V. C. S., & Tadei, W. P. (2002). Evaluation of the residual effect of temephos on *Aedes aegypti* (Diptera, Culicidae) larvae in artificial containers in Manaus, Amazonas State, Brazil. *Caderno de Saúde Pública, 18*(6), 1529-1536. DOI: https://doi.org/10.1590/S0102-311X2002000600005

Polanczyk, R. A., Garcia, M. O., & Alves, S. B. (2003). Potencial de *Bacillus thuringiensis israelensis* Berliner no controle de *Aedes aegypti*. *Revista de Saúde Pública, 37*(6), 813-816. DOI: https://doi.org/10.1590/S0034-89102003000600020

Praça, L. B., Batista, A. C., Martins, É. S., Siqueira, C. B., Dias, D. G. D. S., Gomes, A. C. M. M., … Monnerat, R. G. (2004). Estirpes de *Bacillus thuringiensis* efetivas contra insetos das ordens Lepidoptera, Coleoptera e Diptera. *Pesquisa Agropecuária Brasileira, 39*(1), 11-16. DOI: https://doi.org/10.1590/S0100204X2004000100002

Reiter, P., Amado, M. A., Anderson, R. A., & Clark, G. G. (1995). Short report: Dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium-marked eggs. *American Journal of Tropical Medicine and Hygiene, 52*(2), 177-179. DOI: https://doi.org/10.4269/ajtmh.1995.52.177

Reiter, P., Amador, M. A, & Colon, N. (1991). Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *Journal of the American Mosquito Control Association, 7*(1), 52-55.

Reiter, P., Fontenille, D., & Paupy, C. (2006). *Aedes albopictus* como vetor epidêmico do vírus chikungunya: outro problema emergente? *The Lancet Infectious Diseases, 6*(8), 463-464. DOI: https://doi.org/10.1016/S1473-3099(06)70531-X

Revathi, K., Chandrasekaran, R., Thanigaivel, A., Kirubakaran, S. A., Sathish-Narayanan, S., & Senthil-Nathan, S. (2013). Effects of *Bacillus subtilis* metabolites on larval *Aedes aegypti* L. *Pesticide Biochemistry and Physiology, 107*(3), 369-376. DOI: https://doi.org/10.1016/j.pestbp.2013.10.005

Rey, J. R., & Lounibos, P. (2015). Ecología de *Aedes aegypti* y *Aedes albopictus* en América y transmisión de enfermedades. *Biomédica, 35*(2), 177-185. DOI: https://doi.org/10.7705/biomedica.v35i2.2514

Romi, R., Proietti, S., Di Luca, M., & Cristofaro, M. (2006). Laboratory evaluation of the bioinsecticide spinosad for mosquito control. *Journal of the American Mosquito Control Association, 22*(1), 93-96. DOI: https://doi.org/10.2987/8756-971X(2006)22[93:LEOTBS]2.0.CO;2

Sant'ana, A. L, Roque, R. A., & Eiras, A. E. (2006). Characteristics of grass infusions as oviposition attractants to *Aedes* (Stegomyia) (Diptera: Culicidae). *Journal of Medical Entomology, 43*(2), 214-220. DOI: https://doi.org/10.1603/0022-2585(2006)043[0214:cogiao]2.0.co;2

Silva, C. E., & Limongi, J. E. (2018). Avaliação comparativa da eficiência de armadilhas para a captura e coleta de *Aedes aegypti* em condições de campo. *Cadernos Saúde Coletiva, 26*(3), 241-248. DOI: https://doi.org/10.1590/1414-462X201800030045

Silva, W. R., Soares-da-Silva, J., Ferreira, F. A. S., Rodrigues, I., Tadei, B. W., & Zequi, P. J. A. C. (2018). 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. *Revista Brasileira de Entomologia, 62*(4), 304-310. DOI: https://doi.org/10.1016/j.rbe.2018.08.001

Siqueira, A. L., & Tibúrcio, J. D. (2011). *Estatística na área da saúde – Conceitos, metodologias, aplicações e prática computacional*. Belo Horizonte, MG: Coopmed.

Soares, F. A., Silva, J. C, Oliveira, J. B. B. S., & Abreu, F. V. S. (2015). Study of oviposition behavior of *Aedes aegypti* in two neighborhoods under the influence of semi-arid climate in the municipality of Salinas, state of Minas Gerais, Brazil. *Revista de Patologia Tropical, 44*(1), 77-88. DOI: https://doi.org/10.5216/rpt.v44i1.34817

Soares-da-Silva, J., Bezerra, J. M. T., Ibiapina, S. S., Pinheiro, V. C. S., & Tadei, W. P. (2012). Variation in *Aedes aegypti* (Linnaeus), (Diptera, Culicidae) infestation in artificial containers in Caxias, State of Maranhão, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical, 45*(2), 174-179. DOI: https://doi.org/10.1590/S0037-86822012000200007

Soares da Silva, J., Queirós, S. G., Aguiar, J. S. D., Viana, J. L., Neta, M. D. R., Silva, M. C. D., ... Tadei, W. P. (2017). Molecular characterization of the gene profile of *Bacillus thuringiensis* Berliner isolated from Brazilian ecosystems and showing pathogenic activity against mosquito larvae of medical importance. *Acta Tropica, 176*, 197-205. DOI: https://doi.org/10.1016/j.actatropica.2017.08.006

Solís-Valdez, C. B., Barrientos-Contreras, J., Escobar-González, B., Zepeda-Cavazos, I. G., Rodríguez-Castro, V. A., & Quiroz-Martínez, H. (2015). Oviposición de *Aedes aegypti* L. (Diptera: Culicidae) en Ovitrampas con dos Larvicidas en El Fuerte, Sinaloa, México. *BioOne, 40*(3), 575-580. DOI: https://doi.org/10.3958/059.040.0316

Sousa, I. G., Meneses, R. L., & Vianna, J. M. (2015). *Cartografias Invisíveis: Saberes e Sentires de Caxias*. Caxias, MA: Academia Caxiense de Letras.

Stoops, C. A. (2005). Influence of *Bacillus thuringiensis* var. *israelensis* on oviposition of *Aedes albopictus* (Skuse). *Journal of Vector Ecology, 30*(1), 41-44.

Thompson, G. D., Dutton, R., & Sparks, T. C. (2000). Spinosad – a case study: an example from a natural products discovery programme. *Pest Management Science, 56,* 696-702. DOI: https://doi.org/10.1002/1526-4998(200008)56:8<696:AID-PS182>3.0.CO;2-5

Trexler, J. D., Apperson, C. S., & Schal, C. (1998). Laboratory and field evaluations of oviposition responses of *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) to oak leaf infusions. *Journal of Medical Entomology, 35*(6), 967-976. DOI: https://doi.org/10.1093/jmedent/35.6.967

Vega-Rua, A., Zouache, K., Caro, V., Diancourt, L., Delaunay, P., Grandadam, M., & Failloux, A. B. (2013). High efficiency of temperate *Aedes albopictus* to transmit chikungunya and dengue viruses in the Southeast of France. *PLoS One, 8*(3), 1-8. DOI: https://doi.org/10.1371/journal.pone.0059716

Vega-Rúa, A., Zouache, K., Girod, R., Failloux, A. B., & Lourenço-de-Oliveira, R. (2014). High level of vector competence of *Aedes aegypti* and *Aedes albopictus* from ten American countries as a crucial factor in the spread of Chikungunya virus. *Journal of Virology, 88*(11), 6294-6306. DOI: https://doi.org/10.1128/JVI.00370-14

Viana, D. V., & Ignotti, E. (2013). A ocorrência da dengue e variações meteorológicas no Brasil: revisão sistemática. *Revista Brasileira de Epidemiologia, 16*(2), 240-256. DOI: https://doi.org/10.1590/S1415790X2013000200002

Zar, J. H. (2014). *Biostatistical analysis*. (5. ed.). London, UK: Pearson.

Zara, A. L. S. A., Santos, S. M.; Fernandes-Oliveira, E. S., Carvalho, R. G., & Coelho, G. E. (2016). Estratégias de controle do *Aedes aegypti*: uma revisão. *Epidemiologia e Serviços de Saúde, 25*(2), 391-404. DOI: https://doi.org/10.5123/s1679-49742016000200017