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Larvicidal activity of *Bacillus thuringiensis* subsp. *israelensis* (Bacillaceae) and plant extracts for the biological control of *Aedes aegypti* (Culicidae)

Actividad larvicida de *Bacillus thuringiensis* subsp. *israelensis* (Bacillaceae) combinado con extractos vegetales para el control biológico de *Aedes aegypti* (Culicidae)

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Abstract

Aedes aegypti transmits viruses that cause diseases such as dengue, yellow fever, Zika and chikungunya. Chemical pesticides have been used to control this vector, but mosquitoes have developed resistance to insecticides. Biological control with microorganisms and plant extracts are effective alternatives for the management of insect vector populations and introduce less pollutants into the environment. The objective of this research was to evaluate the larvicidal activity of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) and extracts of *Annona muricata, Ricinus communis* and *Sapindus saponaria* for the biological control of *A. aegypti* larvae. For this, ethanolic extracts of seeds or fruits of the three plant species were obtained and lethal concentrations for 50 % mortality of the extracts and bacteria were determined. Subsequently, combinations of the extracts with *Bti* were made and the interactions were evaluated. Both bacteria and plant extracts showed larvicidal activity. Mixtures of *Bti* with the ethanolic extracts of *R. communis* and *S. saponaria* generated an antagonistic effect, while in combination with ethanolic extracts of *A. muricata* presented an independent action effect. Therefore, the addition of the ethanolic extract of *A. muricata* seeds to sporulated cultures of *Bti* could be considered more effective for the biological control of *A. aegypti* than with each compound separately.

Keywords: bioassays, binary mixtures, dengue fever, mosquitoes, vectors

Resumen

El díptero *Aedes aegypti* es trasmisor de virus causantes de enfermedades como dengue, fiebre amarilla, Zika y chikungunya; para el control de este vector se utilizan pesticidas químicos frente a los cuales los mosquitos han generado resistencia. El control biológico con microorganismos y extractos vegetales es una alternativa de manejo de las poblaciones de insectos vectores efectiva y menos contaminante para el ambiente. El objetivo de esta investigación fue evaluar la actividad larvicida de mezclas de *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) y extractos de *Annona muricata, Ricinus communis y Sapindus saponaria* para el control biológico de larvas de *A. aegypti*. Para ello se obtuvieron extractos etanólicos de semillas o frutos de las tres especies vegetales y se determinaron las concentraciones letales 50 de los extractos y la bacteria. Posteriormente se realizaron combinaciones de los extractos con *Bti* y se evaluó el efecto de dichas interacciones. Tanto la bacteria como los extractos vegetales presentaron actividad larvicida. Se encontró que las mezclas de *Bti* con el extracto etanólico de *R. communis y S. saponaria* generaron un efecto antagónico, mientras que la combinación con *A. muricata* presentó una acción independiente. La adición del extracto etanólico de semi-

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llas de para *A. muricata* a cultivos esporulados de *Bti* se podría considerar una alternativa más efectiva el control biológico de *A. aegypti* que con cada uno de estos compuestos por separado.

Palabras clave: bioensayos, dengue, mezclas binarias, mosquitos, vectores

INTRODUCCIÓN

According to the World Health Organization, 17% of infectious diseases worldwide are caused by vector-borne microorganisms (OMS, 2017). Among the most important and in need of surveillance, prevention and control are dengue, yellow fever, Zika fever and chikungunya (OMS, 2017). These diseases are caused by arboviruses transmitted by the same vector, *Aedes aegypti*, a hematophagous dipteran belonging to the family Culicidae primarily associated with urban and suburban areas (Nelson, 1986).

The lack of vaccines for most viruses transmitted by *A. aegypti* means that efforts are concentrated on the control of vector populations. However, even with vaccine implementation, an integrated approach with vector control research should be pursued to achieve high coverage rates in high risk communities (Espinal et al., 2019). For more than 50 years, the use of chemical insecticides based on organophosphorus compounds has intensified to control *A. aegypti* larvae and adults (Jayaraj et al., 2016; Soborio et al., 2019). As a consequence, mosquito populations have been subjected to intense selection pressure. In 1999 *Aedes* populations resistant to organophosphorus compounds were reported throughout the Americas (Rodríguez et al., 2010). The same occurred with the use of pyrethroid compounds (Da-Cunha et al., 2005), with resistance to these insecticides observed in at least 21 *Aedes* sprecies (Fonseca and Quiñones, 2005). Cross-resistance of these mosquitoes to other insecticides has also been reported (Rodríguez et al., 2003).

Due to an increase in the number of outbreaks of diseases caused by arboviruses transmitted by *A. aegypti* (OPS, 2017), and the loss of efficacy of chemical insecticides, whose compounds are highly toxic to the environment, non-target organisms and human health (Cárdenas et al., 2005), it is necessary to seek new strategies for the control of *A. aegypti*.

Biological control is proposed as an alternative to the use of chemical insecticides. One of the most widely used organisms for biological control is the bacterium *Bacillus thuringiensis* subsp. *israeliensis* (*Bti*). The mechanism of action of this microorganism is the production of Cry and Cyt toxins, specific for the larvae of these dipteran species, which cause lysis of the epithelial cells of the intestine, resulting in the death of the insect (Mendoza-Almanza et al., 2020; Ritchie et al., 2010). The active ingredient of commercial formulations of *Bti* are the toxins Cry4Aa, Cry4Bb, Cry11Aa and Cyt1Aa; these four toxins act together to reduce the occurrence of resistant insect populations under field conditions. However, in the laboratory, *A. aegypti* strains resistant to one of the Cry toxins has already been reported (Silva-Filha et al., 2021).

Plant extracts obtained from fruits of *Ricinus communis*, *Sapindus saponaria*, *Melia azedarach, Carica papaya*, *Annona muricata* and *Gliricidia sepium* species have also been evaluated, and larvicidal activity on *A. aegypti* has been demonstrated (Bobadilla et al., 2005; Corradine et al., 2014; Gomez, 2015; Prophiro et al., 2008; Rojas et al., 2015; Sharma et al., 1998).

The application of mixtures of compounds with insecticidal activity with different modes of action has been proposed as an alternative to minimize the generation of resistance and to improve the control of immature dipteran stages (Chang et al., 2014). Likewise, the active compounds of mixtures can interact, presenting additive and/or synergistic effects to enhance the insecticidal activity, or antagonistic effects causing a decrease in the efficacy of the compounds compared to the use of each one separately (Lemes et al., 2014).

In view of the above, the study of low-dose mixtures of biological insecticides that minimize the possibility of generating resistance in the populations of target organisms becomes more relevant. In this study, the individual effect of *Bti* and ethanolic extracts of *A. muricata*, *R. communis* and *S. saponaria* and that of binary combinations on *A. aegypti* larvae were evaluated in order to determine the most effective antivectorial treatment among them.

MATERIALS AND METHODS

Obtaining ethanolic extracts

We used 500 g of plant material of each species, which were selected for their availability in market places, where they were commercially acquired. This material corresponded to seeds of *A. muricata* and whole fruits of *R. communis* and *S. saponaria*. Each material was dried, crushed and subjected to exhaustive extraction by percolation with 95% ethanol. Subsequently, the extract was filtered and concentrated by distillation under reduced pressure in a rotary evaporator at a controlled temperature of 40 °C. Finally, the material obtained was stored in amber glass bottles for later use (Carrion and Garcia, 2010). The solubility of the plant extracts was evaluated in distilled water and Tween 80 at 0.05% and 0.1%.

Cultivation of *Bacillus thuringiensis* subsp. *israelensis*

A colony of *Bti* strain AM65-52 was seeded in nutrient broth and incubated at 30 ± 2 °C and 150 rpm for 16 h. Subsequently, we made a subculture in 30 ml NSYM (8 g nutrient broth, 0.05%, yeast extract, 5 x 10^{-5} M $MnCl_2$, 1 x 10^{-3} M $MgCl_2$, 7 x 10^{-4} M $CaCl_2$) which was incubated at 30 °C without shaking for seven days. 1 ml of culture was taken, centrifuged at 12,000 rpm for 5 minutes, and 1 ml of saline solution (0.85% NaCl) was added and centrifuged again under the same conditions. Subsequently, the supernatant was discarded and the cells were dried at 30° C for 48 h to determine the dry weight.

Aedes aegypti colony maintenance

The larvae of *A. aegypti* were obtained from the insectary of the Department of Basic Sciences at La Salle University. The colony was maintained at 25−30 °C, 38−50% relative humidity and 12 h day:12 h night photoperiod. The larvae were placed in plastic containers with rested water and fed with pulverized purine.

Toxicity bioassays

For the determination of the individual larvicidal activity of *Bti* and plant extracts, five concentrations of each were evaluated as follows: 1) *Bti* strain AM65-52: 33 x 10^{-3} mg/ml, 33 x 10^{-4} mg/ml, 33 x 10^{-5} mg/ml, 33 x 10^{-6} mg/ml and 33 x 10^{-7} mg/ml, 2) *A. muricata*: 0.001 mg/ml, 0.005 mg/ml, 0.01 mg/ml, 0.1 mg/ml and 0.5 mg/ml and 3) *R. communis* and *S. saponaria*: 0.05 mg/ml, 0.1 mg/ml, 0.8 mg/ml, 1.5 mg/ml and 3 mg/ml. For the binary mixtures, combinations of those concentrations that presented larval mortality values between 30% and 60% for each compound applied individually were used.

To rule out the larvicidal activity of the emulsifier, Tween 80 was added at 0.05% and 0.1% in containers with 100 ml of distilled water and 20 third instar larvae of *A. aegypti*. Distilled water was used as a negative control.

Three bioassays (replicates) were performed and three experimental units per treatment and concentration were used in each replicate. The experimental units consisted of autoclaved beakers, each containing 20 third instar *A. aegypti* larvae and a final volume of 100 ml of distilled water and each extract, *Bti* or a binary mixture. To make the mixtures, concentrations of both *Bti*, and the three plant extracts were selected that were lower than the lethal concentration 50 (LC_{50}) in order to evaluate whether the interaction between these components was synergistic, additive or antagonistic.

In each bioassay, three replicates were carried out for the controls, which were distilled water or the solvent used for each plant extract. The experimental units were randomly distributed in a room with controlled temperature at 25° C and photoperiod of 12 h light

and 12 h dark. At 24 and 48 h, larval mortality was recorded, considering that a larva was dead if it did not move when touched with a wooden stick.

Statistical analysis

The determination of the LC_{50} was performed from the number of live and dead larvae in each of the trials by using the TRAP program version 3.1a. Analysis of compound interactions in the mixtures was performed using the Independent Action Hypothesis, in which it is assumed that the biocontrollers in each mixture acts independently and, therefore, each has its own probability of causing larval death (Drescher and Boedeker, 1995; Lemes et al., 2014). The Independent Action Hypothesis was calculated according to the formula used by Lemes et al. (2014):

$$P = 1 - (1 - P1)(1 - P2)$$

P1 and *P2* represent the proportions of dead larvae for compounds 1 and 2 of the mixtures (*Bti* and plant extract). The difference between expected and observed mortality was estimated by means of the R program (R Core Team, 2020) version 3.6, based on Pearson's distribution test (chi^2) with a significance level of $p<0.05$.

RESULTS

The ethanolic extract of *S. saponaria* fruit was soluble in distilled water, while the suitable solvent for the extract of *A. muricata* seeds was 0.05% Tween 80, and 0.1% Tween 80 for the extract of *R. communis* fruit. Tween 80 in both concentrations evaluated did not cause mortality in larvae.

The results obtained in the evaluation of the larvicidal activity of all the compounds individually demonstrate their effectiveness for the control of *A. aegypti* larvae (table 1). The highest toxicity values were presented by *Bti*, followed by ethanolic extracts of *A. muricata*, *S. saponaria* and *R. communis* (table 1).

In the bioassays conducted with the mixture of *Bti* and the ethanolic extract of *A. muricata* seeds, higher *A. aegypti* larval mortality was observed when only the bacteria or only the plant extract was added (table 2); when comparing the mortality observed with that expected with this combination, no statistical significance was observed ($p>0.05$), which indicates that in the binary mixture the compounds act independently.

The results obtained from the mixtures of *B. thuringiensis* with *R. communis* and the bacterium with *S. saponaria* showed a significant difference between the observed and expected mortality percentages (with a $p<0.05$). However, the observed mortality was lower than expected, indicating the presence of an antagonistic interaction between the compounds (table 2).

DISCUSSION

In this work, the larvicidal activity of *B. thuringiensis* subsp. *israelensis* and ethanolic extracts of *A. muricata*, *R. communis* y *S. saponaria* on a laboratory strain of *A. aegypti* was evaluated. In individual larvicidal activity assays, the LC_{50} obtained in this study indicate that the extracts were more effective for larval control than previously reported. Parra et al. (2007) obtained a LC_{50} of 0.9 mg/ml with *A.*

Table 1. Mean lethal concentration (LC_{50}) of *B. thuringiensis* subsp. *israelensis* and ethanolic extracts on *A. aegypti* larvae at 48 hours of exposure.

Treatment	LC_{50} (mg/ml)	Confidence interval (mg/ml)
B. thuringiensis subsp. *israelensis*	0.002	0.0005−0.0778
A. muricata	0.01	0.01−0.02
R. communis	0.5	0.45−1.58
S. saponaria	0.12	0.11−0.13

Table 2. Interaction of mixtures of plant extracts and *B. thuringiensis* subsp. *israeliensis* for the control of *A. aegypti* larvae.

Treatment	Concentration (mg/ml)	Mortality expected (%)	Mortality observed (%)	χ^2	*p*
B. thuringiensis subsp. *israelensis*	33×10^{-4}	-	43	-	-
A. muricata	0.001	-	35	-	-
R. communis	0.1	-	43	-	-
S. saponaria	0.1	-	60	-	-
Bti + *A. muricata*	33×10^{-4} + 0.001	52.3	55	0.28	0.59
Bti + *R. communis*	33×10^{-4} + 0.1	43.8	8.3	51.14	8.6×10^{-13}
Bti + *S. saponaria*	33×10^{-4} + 0.1	56	18.3	57.75	3×10^{-14}

muricata seed extract and a LC_{50} of 0.86 mg/ml with *R. communis* fruit extract. Bobadilla et al. (2005) reported a LC_{50} of 0.02 mg/mL with *A. muricata* seed extract, which is very close to that found in the present investigation. On the other hand, authors such as Amariles et al. (2013) obtained a LC_{50} of 0.5 mg/mL, with *S. saponaria* fruit extracts.

The LC_{50} observed in this study with *Bti* AM65-52, is similar to the LC_{50} of 0.004 mg/mL obtained with *Bti* strain BtMA-750 isolated from a Brazilian soil (Vieira-Neta et al., 2021) and lower than the LC_{50} of 0.0068 mg/mL and 0.018 mg/mL reported with the spore and crystal preparation of *Bti* LBIT-1250L and *Bti* IPS-82 (García et al., 2021).

Considering the LC_{50} values obtained in this study, the three plant extracts and *Bti* strain AM65-52 are considered promising for the biological control of *A. aegypti* larvae. However, those with the highest individual larvicidal activity were the bacterium, followed by the ethanolic extract of *A. muricata* seeds, and therefore would be recommended for further studies with larvae collected in the field or under semi-controlled conditions.

The mixture of *Bti* and *A. muricata* seed extract did not present a statistical difference between observed and expected mortality, indicating that each biocontroller acts independently and the two present different sites or mechanisms of action (Abendroth et al., 2011; Drescher and Boedeker, 1995); this type of effect has also been called zero interaction or additive (Abendroth et al., 2011). It would be interesting to conduct additional studies in which the application of this binary mixture in a single formulation is analyzed, which could be advantageous because each one could be at sublethal concentrations and also could facilitate its application in the field, by using the two biocontrollers in a single product.

In addition to this, the use of the mixture between the AM65-52 strain of *Bti* and the ethanolic extract of *A. muricata*, would be favorable, because the bacteria and the extract present different mechanisms of action. *Bacillus thuringiensis* subsp. *israelensis* presents the toxins Cry4Aa, Cry4Ba, Cry11Aa and Cyt1Aa, the first three toxins bind to membrane receptors of larval gut cells, while Cyt1Aa presents affinity for membrane unsaturated phospholipids (Ben, 2014). The most biologically active molecules of *Annona* plants in mosquito larvae are the bistetrahydrofuran and monotetrahydrofuran acetogenins, specifically muricatacin and anonnacin present in the seeds of *A. muricata* (Rieser et al., 1991; Rupprecht et al. 1990).

The antagonism observed in mixtures of *B. thuringiensis* with *R. communis* and *S. saponaria* has also been reported for other mixtures by authors such as Murugan et al. (2002), who found this interaction in mixtures of *Bti* and *Pongamia pinnata* oils for the control of *Culex quinquefasciatus* larvae. Another example is the results obtained by Chang et al. (2014), who also observed antagonism in mixtures of cinnamaldehydes with eugenol against larvae of *Anopheles sinensis*, as well as in mixtures of cinnamaldehydes with anethole and eugenol with anethole

against larvae of *A. albopictus* and *An. sinensis*, the components of the mixtures being the main constituents of essential oils of *Cinnamomum cassia*, *Syzygium aromaticum* and *Illicium verum*.

To the best of our knowledge, the mechanism that causes the antagonistic effect observed in binary mixtures of *Bti* with *R. communis* and con *S. saponaria* has not been reported, neither in antagonistic results obtained by Murugan et al. (2002), nor by Lemes et al. (2014). A possible explanation for the observed antagonism is that a molecule of the extracts interacts with a toxin of the bacterium, affecting the mode of action of one of these compounds or that they bind to the same receptor in the vector's gut. Such hypotheses will need to be tested with future experiments.

In this work, it was observed that *B. thuringiensis* subsp. *israelensis* presents differences in its interaction with ethanolic extracts of *A. muricata*, *R. communis* and *S. saponaria*. Similarly, Lozano and Dussán (2017) reported that the mixture between *Lysinibacillus sphaericus* S-layer protein and binary toxin can be additive or synergistic against *C. quinquefasciatus* larvae, depending on the strain from which these proteins were obtained. It was also observed that there is an additive or antagonistic effect between *Beauveria bassiana* and *B. thuringiensis* for the control of *Bemisia tabaci* nymphs, depending on the concentration of each biocontrol agent (Somoza-Vargas et al., 2018).

Since ethanolic extracts were evaluated in the bioassays conducted in this study, an alternative that could improve the larvicidal activity of mixtures of these extracts with *Bti* is the fractionation of these extracts or the direct purification of the metabolites responsible for the larvicidal activity. Some authors such as Chang et al. (2014), performed mixtures between *B. thuringiensis* and the main constituents of three essential oils for the control of *An. sinensis* and *A. albopictus*, obtaining a synergistic effect in the three mixtures. Similarly, Murugan et al. (2002), reported a synergistic effect between *B. thuringiensis* and *Azadirachta indica* oil, on larvae of *C. quinquefasciatus*. Another alternative would be the evaluation of the sequential application of *Bti* with the extracts evaluated in this study.

In conclusion, the mixture between the ethanolic extract of seeds of *A. muricata* and *B. thuringiensis* subsp. *israelensis*, presented an independent effect between the compounds, allowing it to be considered viable for the control of *A. aegypti* larvae, since it is composed of two different active principles. On the other hand, the mixtures made from ethanolic extracts of *R. communis* and *S. saponaria* combined with *Bti* produced an antagonistic effect between them. Therefore, it would be advisable to apply these extracts independently or sequentially. Likewise, it is important to continue conducting studies focused on the analysis of interactions between plant extracts and microorganisms for the control of mosquito vectors of pathogenic microorganisms, in order to obtain the most appropriate formulations for resistance management and, in the future, to develop new products that generate minimum toxicity to non-target organisms and the environment.

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CONFLICT OF INTEREST

The authors have no conflicts of interest, so their judgment, independence and impartiality remained intact in the preparation of this study.

REFERENCES

Abendroth, J., Blankenship, E., Martin, A., & Roeth, F. (2011). Joint action analysis utilizing concentration addition and independent action models. *Weed Technology, 25*(3), 436−446. https://doi.org/10.1614/WT-D-10-00102.1

Amariles, B., García, P., & Parra, H. (2013). Actividad insecticida de extractos vegetales sobre larvas de *Aedes aegypti*, Diptera: Culicidae. *CES Medicina, 27*(2), 193−203. https://revistas.ces.edu.co/index.php/

medicina/article/view/2680

Ben, E. (2014). *Bacillus thuringiensis* subsp. *israelensis* and its dipteran-specific toxins. *Toxins, 6*(4), 1222−1243. https://doi.org/10.3390/toxins6041222

Bobadilla, M., Zavala, F., Sisniegas, M., Zavaleta, G., Mostacero, J., & Taramona, L. (2005). Evaluación larvicida de suspensiones acuosas de *Annona muricata* Linnaeus "guanábana" sobre *Aedes aegypti* Linnaeus (Diptera, Culicidae). *Revista Peruana de Biología, 12*(1), 145−152. https://doi.org/10.15381/rpb.v12i1.2369

Cárdenas, O., Silva, E., Morales, L., & Ortiz, J. (2005). Estudio epidemiológico de la exposición a plaguicidas organofosforados y carbamatos en siete departamentos colombianos, 1998-2001. *Biomédica, 25*(2), 170−180. https://doi.org/10.7705/biomedica.v25i2.1339

Carrion, J., & Garcia, G. (2010). *Preparación de extractos vegetales: Determinación de eficiencia de metódica*; [Tesis de pregrado]. Repositorio institucional de la Universidad de Cuenca. http://dspace.ucuenca.edu.ec/handle/123456789/2483

Corradine, M., Beltrán, S., Corredor, P., & Moreno, A. (2014). Eficiencia del extracto de *Ricinus communis* para el control del mosquito *Culex*. *Revista Científica, 19*(2), 86−92. http://dx.doi.org/10.14483/23448350.6496

Chang, K., Hyun, S., Dae, Y., & Young, A. (2014). Enhanced toxicity of binary mixtures of *Bacillus thuringiensis* subsp. *israelensis* and three essential oil major constituents to wild *Anopheles sinensis* (Diptera: Culicidae) and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology, 51*(4), 804−810. https://doi.org/10.1603/ME13128.

Da-Cunha, M., Lima, J., Brogdon, W., Moya, G., & Valle, D. (2005). Monitoring of resistance to the pyrethroid Cypermetrin in Brazilian *Aedes aegypti* (Diptera: Culicidae) populations collected between 2001 and 2003. *Memórias do Instituto Oswaldo Cruz, 100*(4), 441−444. http://dx.doi.org/10.1590/S0074-02762005000400017

Drescher, K., & Boedeker, W. (1995). Assessment of the combined effects of substances: The relationship between concentration addition and independent action. *Biometrics, 51*(2), 716−730. https://doi.org/10.2307/2532957

Espinal, M., Andrus, J., Jauregui, B., Hull-Waterman, S., Morens, D., Santos, J. Horstick, O., Francis, L., & Olson, D. (2019). Emerging and reemerging *Aedes*-transmitted arbovirus infections in the region of the Americas: Implications for health policy. *American Journal of Public Health, 109*, 387−392. https://doi.org/10.2105/AJPH.2018.304849

Fonseca, I., & Quiñones, M. (2005). Resistencia a insecticidas en mosquitos (Diptera: Culicidae): mecanismos, detección y vigilancia en salud pública. *Revista Colombiana de Entomología, 31*(2), 107−115. http://www.scielo.org.co/scielo.php?pid=S0120-04882005000200001&script=sci_abstract&tlng=es

García, S., Verduzco, R., & Ibarra, E. (2021). Isolation and characterization of two highly insecticidal, endophytic strains of *Bacillus thuringiensis*. *FEMS Microbiology Ecology, 97*(7), 17.https://doi.org/10.1093/femsec/fiab080

Gomez, G. (2015). *Evaluación larvicida del extracto etanólico de la semilla de Carica papaya sobre larvas del IV estadio de Aedes aegypti (Diptera: Culicidae) en condiciones de laboratorio*; [Tesis de pregrado]. Repositorio institucional de la Universidad Distrital Francisco José de Caldas. https://repository.udistrital.edu.co/handle/11349/3989

Jayaraj, R., Megha, P., & Sreedev, P. (2016). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary Toxicology, 9*(3-4), 90−100. https://doi.org/10.1515/intox-2016-0012Interdisciplinary

Lemes, A., Davolos, C., Legori, P., Fernandes, O., Ferre, J., Lemos, M., & Desiderio, J. (2014). Synergism and antagonism between *Bacillus thuringiensis* Vip3A and Cry1 proteins in *Heliothis virescens*, *Diatraea saccharalis* and *Spodoptera frugiperda*. *PLoS One, 9*(10), 107−196. https://doi.org/10.1371/journal.pone.0107196

Lozano, L. C., & Dussán, J. (2017). Synergistic activity between S-layer protein and sporecrystal preparations from *Lysinibacillus sphaericus* against *Culex quinquefasciatus* larvae. *Current Microbiology, 74*(3), 371−376. https://doi.org/10.1007/s00284-016-1185-7

Mendoza, G., Esparza, E., Ayala, J., Mercado, M., Godina, S., Hernández, M., & Olmos, J. (2020). The cytocidal spectrum of *Bacillus thuringiensis* toxins: From insects to human cancer cells. *Toxins, 12*(5), 290−301. https://doi.org/10.3390/toxins12050301

Murugan, K., Thangamathi, P., & Jeyabalan, D. (2002). Interactive effect of botanicals and *Bacillus thuringiensis* subsp *israelensi* on *Culex quinquefasciatus* Say. *Journal of Scientific and Industrial Research, 61*(12), 1068−1076. http://nopr.niscair.res.in/handle/123456789/17742

Nelson, J. (1986). *Aedes aegypti*: *biología y ecología*; Organización Panamericana de la Salud. https://iris.paho.org/handle/10665.2/28513

Organización Mundial para la Salud (OMS). (2017). *Respuesta mundial para el control de vectores 2017−2030; Documento de contexto para informar las deliberaciones de la Asamblea Mundial de la Salud en su 70.ª reunión*. https://www.who.int/malaria/areas/vector_control/Draft-WHO-GVCR-2017-2030-esp.pdf

Organización Panamericana de la Salud (OPS). (2017). *Diez enfermedades transmitidas por vectores que ponen en riesgo a la población de las Américas. 2017*. http://www.paho.org/hq/index.php?option=com_contentview=articleid=9438%3A2014-10-vector-borne-diseases-that-put-population-americas-at-riskcatid=1443%3Aweb-bulletinsItemid=135lang=es

Parra, H., García, P., & Cortes, T. (2007). Actividad insecticida de extractos vegetales sobre *Aedes aegypti* (Diptera: Culicidae) vector del dengue en Colombia. *CES Medicina, 21*(1), 47−54. https://revistas.ces.edu.co/index.php/medicina/article/view/34

Prophiro, J., Rossi, J., Pedroso, M., Kanis, L., & Silva, O. (2008). Leaf extracts of *Melia azedarach Linnaeus* (Sapindales: Meliaceae) act as larvicide against *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Revista da Sociedade Brasileira de Medicina Tropical, 41*(6), 560−564. http://dx.doi.org/10.1590/S0037-86822008000600003

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Com-

puting, Vienna, Austria. https://www.r-project.org/

Rieser, J., Kozlowski, F., Wood, V., & McLaughlin, L. (1991). Muricatacin: A simple biologically active acetogenin derivative from the seeds of *Annona muricata* (annonaceae). *Tetrahedron Letters, 32*(9), 1137−1140. https://doi.org/10.1016/S0040-4039(00)92027-6

Ritchie, S., Rapley, L., & Benjamin, S. (2010). *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) provides residual control of *Aedes aegypti* in small containers. *The American Journal of Tropical Medicine and Hygiene, 82*(6), 1053−1059. https://doi.org/10.4269/ajtmh.2010.09-0603

Rodríguez, M., Bisset, J., Díaz, C., & Lázaro, A. (2003). Resistencia cruzada a piretroides en *Aedes aegypti* de Cuba inducido por la selección con el insecticida organofosforado malatión. *Revista Cubana de Medicina Tropical, 55*(2), 105−111. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0375-07602003000200008&lng=es&tlng=es

Rodríguez, M. M., Bisset, J. A., Ricardo, Y., Pérez, O., Montada, D., Figueredo, D., & Fuentes, I. (2010). Resistencia a insecticidas organofosforados en *Aedes aegypti* (Diptera: Culicidae) de Santiago de Cuba, 1997-2009. *Revista Cubana de Medicina Tropical, 62*(3), 217−223. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0375-07602010000300009&lng=es&tlng=es

Rojas, M., Araujo, P., & Montero, T. (2015) Evaluación del uso de *Sapindus saponaria* como biocida de *Aedes aegypti* en condiciones *in vitro*. *Producción + Limpia, 10*(2), 11−17. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S1909-04552015000200002&lng=en&tlng=es

Rupprecht, J., Hui, Y., & McLaughlin, J. (1990). Annonaceous acetogenins: A review. *Journal of Natural Products, 53*(2), 237−278. https://doi.org/10.1021/np50068a001

Sharma, J., Qadry, B., Subramanium, T., Verghese, S., Rahman, S., & Jalees, S. (1998). Larvicidal activity of *Gliricidia sepium* against mosquito larvae of *Anopheles stephansi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Pharmaceutical Biology, 36*(1), 3−7. https://doi.org/10.1076/phbi.36.1.3.4616

Silva-Filha, M., Romão, T., Rezende, T., Carvalho, K., Gouveia de Menezes, H., Alexandre do Nascimento, N., Soberón, M., & Bravo, A. (2021). Bacterial toxins active against mosquitoes: Mode of action and resistance. *Toxins, 13*(1), 523. https://doi.org/10.3390/toxins13080523

Soborio, C., Mora, V., & Duran, M. (2019). Intoxicación por organofosforados. *Medicina Legal de Costa Rica, 36*(1), 110−117. http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S1409-00152019000100110&lng=en&tlng=es

Somoza, C., Hernández, V., Peña, G., Torres, G., Huerta, A., Ortega, L., & Salazar, J. (2018). Interaction of *Beauveria bassiana* strain HPI-019/14 and *Bacillus thuringiensis* strain GP139 for the biological control of *Bemisia tabaci* in strawberry. *Bulletin of Insectology, 71*(2), 201−209. http://www.bulletinofinsectology.org/pdfarticles/vol71-2018-201-209somoza-vargas.pdf

Vieira-Neta, M., Soares-da-Silva, J., Viana, J. L., Silva, M. C., Tadei, W. P., & Pinheiro, V. (2021). Strain of *Bacillus thuringiensis* from Restinga, toxic to *Aedes (Stegomyia) aegypti* (Linnaeus) (Diptera, Culicidae). *Brazilian Journal of Biology, 81*(4), 872−880. https://doi.org/10.1590/1519-6984.228790