

ORIGINAL ARTICLE

## Novel larvicide tablets of *Bacillus thuringiensis* var. *israelensis*: Assessment of larvicidal effect on *Aedes aegypti* (Diptera: Culicidae) in Colombia

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**Introduction:** *Aedes (Stegomyia) aegypti* is the vector for dengue, chikungunya, and Zika arboviruses. *Bti*-CECIF is a bioinsecticide designed and developed in the form of a solid tablet for the control of this vector. It contains *Bacillus thuringiensis* var. *israelensis* (*Bti*) serotype H-14.

**Objective:** To evaluate under semi-field and field conditions the efficacy and residual activity of *Bti*-CECIF tablets on *Aedes aegypti* larvae in two Colombian municipalities.

**Materials and methods:** We tested under semi-field conditions in plastic tanks (Rotoplast™) four different *Bti* doses (0.13, 0.40, 0.66 and 0.93 mg/L) in the municipality of Apartadó, department of Antioquia, to assess *Bti*-CECIF efficacy (percentage of reduction of larval density) and the residual activity in water tanks containing *A. aegypti* third-instar larvae. The efficacy and residuality of the most lethal dose were subsequently evaluated under field conditions in cement tanks in the municipality of San Carlos, department of Córdoba.

**Results:** Under semi-field conditions, the highest tested dose exhibited the greatest residual activity (15 days) after which larval mortality was 80%. Under field conditions, the highest tested *Bti*-CECIF doses showed 100% mortality and exhibited a residual activity of seven days in 90% of the tanks.

**Conclusion:** *Bti*-CECIF tablets effectively controlled *A. aegypti* larvae under field conditions for up to seven days post-treatment.

**Key words:** *Aedes*; *Bacillus thuringiensis*; disease vectors; dengue; chikungunya virus; Zika virus; Colombia.

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### Nuevas tabletas larvicidas de *Bacillus thuringiensis* var. *israelensis*: evaluación del efecto larvicida sobre *Aedes aegypti* (Diptera: Culicidae) en Colombia

**Introducción.** *Aedes (Stegomyia) aegypti* es el vector de los arbovirus del dengue, el chikungunya y el Zika. Para el control de este vector, se diseñó y desarrolló un bioinsecticida en presentación de tableta sólida, el *Bti*-CECIF, que contiene *Bacillus thuringiensis* var. *israelensis* (*Bti*) de serotipo H-14.

**Objetivo.** Evaluar en condiciones de 'semicampo' y de campo, la eficacia y la actividad residual de las tabletas de *Bti*-CECIF en larvas de *A. aegypti* en dos municipios colombianos.

**Materiales y métodos.** En el municipio de Apartadó, departamento de Antioquia, se probaron bajo condiciones de 'semicampo' en tanques de plástico de 250 l (Rotoplast™) cuatro dosis diferentes de *Bti* (0,13, 0,40, 0,66 y 0,93 mg/l) para evaluar la eficacia del *Bti*-CECIF (porcentaje de reducción de la densidad larvaria) y la actividad residual en tanques de agua que contenían larvas de tercer estadio de *A. aegypti*. La eficacia y el efecto residual de la dosis más letal fueron posteriormente evaluadas en tanques de cemento bajo condiciones de campo en el municipio de San Carlos, departamento de Córdoba.

**Resultados.** Bajo condiciones de 'semicampo', la mayor dosis probada exhibió la mayor actividad residual (15 días), después de lo cual la mortalidad de las larvas fue de 80 %. Bajo condiciones de campo, la máxima dosis probada de *Bti*-CECIF mostró una mortalidad de 100 % y exhibió una actividad residual de siete días en el 90 % de los tanques.

**Conclusión.** Las tabletas *Bti*-CECIF controlaron eficazmente *A. aegypti* en condiciones de campo durante siete días a partir de su aplicación.

**Palabras clave:** *Aedes*, *Bacillus thuringiensis*; vectores de enfermedades; dengue; virus chikungunya; virus Zika; Colombia.

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#### Author's contributions:

Wilber Gómez-Vargas: Study design, fieldwork, and manuscript writing

Kelly Valencia-Jiménez: Development of *Bti*-CECIF tablets and review of the manuscript

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Vector-borne diseases account for over 17% of all infectious diseases and are a significant contributing cause of human deaths worldwide (1). Dengue, chikungunya, and Zika arboviruses are transmitted by the vector *Aedes (Stegomyia) aegypti* and they represent a permanent global health threat with social and economic impacts (2).

Currently, dengue is the most frequent arboviral disease diagnosed in tropical and sub-tropical countries. Recently, it has been estimated that there are 390 million dengue infections per year, of which 96 million manifests with clinically apparent disease (3). In the Americas, a total of 580,395 cases of dengue were reported in 2017, of which 1,940 cases were of severe dengue and 306 cases were deaths (4). In Colombia, the estimated average of dengue infections per year between 2013 and 2017 was 91,454 cases, of which 1,728 were severe dengue, and 111 were deaths (Sivigila, *Instituto Nacional de Salud*) (5-9). In 2016, the reported incidence of dengue was 101,016 cases (99.1%) and 899 cases of severe dengue (0.9%) (8). Additionally, 26,279 cases of dengue were reported in 2017 (9).

Furthermore, the recent introduction of Zika and chikungunya arboviruses in the Colombian Caribbean region expanded throughout the entire country. In 2014 and 2015 a total of 106,763 and 360,570 chikungunya cases were reported, respectively (10), while up to the epidemiological week 52 of 2016, 19,435 cases of chikungunya virus were reported (11). Additionally, 106,660 cases of Zika virus were reported from the epidemiological week 32 of 2015 to the epidemiological week 52 of 2016 (12).

Dengue, chikungunya, and Zika arboviruses are a public health concern as they may lead to epidemics in large cities and metropolitan areas (13). For this reason, the main course of action to reduce transmission of these diseases is based on the control of the vector *A. aegypti* with insecticide aspersion, as well as larvicide control and management means to reduce breeding sites. The main vector-control strategy focuses today on insecticide aspersion, which can be expensive and difficult to maintain. For that reason, the World

Health Organization (WHO) has proposed the use of biolarvicides such as *Bacillus thuringiensis* var. *israelensis* (*Bti*) for the prevention and control of arboviral diseases (document CD55/16) depending on the particular conditions of each country (14).

Since 1950, one of the most successful strategies for controlling this vector has been the use of *Bacillus thuringiensis* var. *israelensis* (*Bti*) bacteria, whose biolarvicidal effect is specific for controlling species of the Culicidae family, including *A. aegypti* (15-18). In Colombia, *Bti* is available and commercialized as "VectoBac 200G™", and contains *Bti* serotype H-14 as the bioactive ingredient. Once ingested by larvae, peptide toxins known as delta-endotoxins are activated. Delta-endotoxins are pore-forming toxins that bind to the gut epithelium and lyse cellular membranes, and ultimately lead to the death of *A. aegypti* larvae (15-18).

However, this commercially available product presents several drawbacks, including the fact that it is not locally produced and must, therefore, be imported, increasing its cost significantly. Furthermore, application volumes of this granular formulation vary and to properly dose it, a special equipment must be used to weigh the amount of required product. Additionally, the product is susceptible to physicochemical instability after package opening and given that VectoBac 200G™ is commercialized by a single company, its accessibility may be occasionally restricted.

In order to overcome these issues, at the *Centro de la Ciencia y la Investigación Farmacéutica*, CECIF, we designed and developed a bioinsecticide in a solid tablet presentation that contains *Bti* serotype H-14, the same bioactive ingredient of VectoBac 200G™, which will be referred to as *Bti*-CECIF in the present article.

In this study, we assessed the efficacy and residual activity of *Bti*-CECIF tablets on *A. aegypti* larvae under semi-field conditions in the municipality of Apartadó (department of Antioquia), as well as under field conditions in two neighborhoods of the municipality of San Carlos (department of Córdoba).

## Materials and methods

### Tablets

Throughout the product design and development process, efficacy trials were run at laboratory scale reporting the mortality percentage according to *Bti* serotype H-14 concentration. Additionally, different production processes were tested in order to

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identify the appropriate method to compress this type of raw material, which were then validated by physicochemical and microbiological methods (unpublished data).

Once we identified a formulation that met the requirements of the accelerated pre-stability testing, we performed toxicity studies including acute oral toxicity, acute dermal toxicity and toxicity by inhalation, dermal sensitivity, dermal irritation, and ocular irritation (unpublished data). These tests are required to apply for the toxicological technical report issued by the *Instituto Nacional de Salud*.

### **Semi-field bioassay**

**Mosquitoes.** We used third instar *A. aegypti* larvae obtained from an established colony at the *Instituto Colombiano de Medicina Tropical*, ICMT, located in Sabaneta, Antioquia. This colony was generated from eggs provided by the *Grupo de Entomología Médica* at the *Facultad de Medicina* of the *Universidad de Antioquia*. The larvae were bred in plastic trays and fed fish food. The adult mosquitoes were fed with 10% sugar solution and mouse blood every third day to obtain their eggs. The insectary was maintained at an average temperature of 27°C and a relative humidity of 60%, with a photoperiod of 12 h, according to the Gerberg methodology (19).

The colony was composed of field specimens collected in Medellín, where insecticide susceptibility has been evaluated in several populations using the standard WHO susceptibility test (20). The following insecticides have been tested: organophosphates (malathion, fenitrothion, temephos), and pyrethroids (deltamethrin, permethrin, lambda-cyhalothrin). Mosquitos were susceptible to malathion (>98% mortality), while there was some risk of resistance to other insecticides (80-95% mortality) (21). In Medellín, *Bti* biolarvicide is not regularly applied (personal communication, Dr. Guillermo Rúa).

***Bti* products.** For field and semi-field experiments, we used 600 mg *Bti*-CECIF tablets containing 1.33% active ingredient per tablet and 5,240 ITU/mg. As a negative control, we used 600 mg tablets without *Bti*.

**Study site.** We conducted the semi-field experiments at the ICMT headquarters located in the municipality of Apartadó (7°53'28.4" N and 76°37.69" W; elevation 49 masl) following WHO guidelines for laboratory and field testing of mosquito larvicides (20).

**Experimental protocol for semi-field bioassay.** We evaluated *Bti*-CECIF tablets efficacy and residual activity at the following doses: 0.13, 0.40, 0.66, and 0.93 mg/L. CECIF tablets lacking *Bti* (0 mg/L) were used as negative control. We used 20 250-L plastic tanks (Rotoplast™) placed in the shade. The tanks were randomly labeled (four tanks per treatment) and filled with 180 L of aqueduct water. We added single batches of 100 laboratory-bred *A. aegypti* third instar larvae plus 1 g of fish to each tank and after 2 to 3 hours we administered the corresponding *Bti*-CECIF dose. We placed the tablets directly into the water without agitation. We registered larvae mortality daily and removed dead larvae from the tanks. Water pH and temperature were recorded throughout the bioassay.

During the experiment, tanks were kept under roof, in the shade, and covered with nets in order to avoid waste disposal, mosquitos laying eggs, or the invasion of predator macroinvertebrates, as well as to prevent the escape of adult mosquitos. Water lost by evaporation was replaced once a week.

Once all larvae had either died or hatched, we assessed residual activity in those same tanks without adding any new tablets. To this end, each tank received a new batch of 50 *A. aegypti* third instar larvae on day 4. After 24 hours, larvae mortality was registered and followed daily. Again, once all larvae had either died or hatched, a second batch of 50 *A. aegypti* third instar larvae was added to each tank on day 10. This process was repeated on days 19 and 31 until no significant difference in residual activity was detected in larvae mortality between tanks treated with the highest dose of *Bti*-CECIF and the negative control (20).

Efficacy was assessed through percent reduction of larval density (% RLD) during the first week of evaluation while residual activity was assessed by measuring the duration of larvicide effect on a new batch of third instar larvae. The period of residual activity was defined as the day until which the % RLD was greater or equal to 80%. For this purpose, larval mortality was calculated at several time points post-treatment as follows: e1: mortality after one day of exposure to treatment (day 1, 5, 11, 20, and 32); e2: mortality after two days of exposure to treatment (day 2, 6, 12, 21, and 33), and so on, up to e4. These mortality data (e1 through e4) were adjusted to probit models in order to establish whether there was a relationship between mortality and the *Bti*-CECIF dose and between mortality and days post-treatment

for each of the *Bti*-CECIF doses tested, with the exception of the negative control in which no mortality was observed.

**Statistical analysis.** Data were tabulated in Microsoft Excel™ spreadsheet and statistical analysis was performed using Statgraphics Centurion XVI™, version 16.1.02 software. Following WHO recommendations (20), the post-treatment % RLD was calculated with the following formula:  $\%RLD = \left(\frac{C-T}{C}\right) \times 100$ , where C is the percentage survival in control tanks and T is the percentage survival in treated tanks during the same time period. However, given that the percentage survival in control tanks was of 100% in this study, the % RLD was calculated directly, i. e., 100-T.

### Field bioassay

**Study site.** The field study was performed in two neighborhoods of the municipality of San Carlos (El Carmen and Porvenir), Córdoba (8°48'02" N and 75°42'08" W; elevation 25 masl) with an average temperature of 28°C. San Carlos has an urban population of 5,123 inhabitants (22), and is located 36 km from the capital city, Montería.

During the last trimester of 2014, San Carlos presented high infestation levels of immature *A. aegypti* (larvae and pupae): House index (HI): 22%; container index (CI): 4%, and Breteau index (BI): 25%. It was also reported that 63% of positive breeding sites containing immature vector were low tanks (*Secretaría de Salud de Córdoba*, 2014). We selected two neighborhoods in San Carlos, El Carmen and Porvenir, because they presented the highest infestation levels of immature populations of *A. aegypti* in the municipality.

Before initiating the study, we explained the goals of the project to the head of each participating household, and upon approval, they signed an informed consent form. The Ethics Committee of the ICMT approved this study (Act 54, February 27, 2015). This field study was performed following WHO guidelines for laboratory and field testing of mosquito larvicides (20).

**Experimental protocol for the field bioassay.** In the selected neighborhoods, tanks naturally infested with *A. aegypti* larvae were identified and used to test the effectiveness and residual activity of the *Bti*-CECIF tablets (0.93 mg/L). A total of 21 randomly selected tanks were treated with *Bti*-CECIF tablets; as a negative control five randomly selected tanks were treated with tablets without

*Bti* and as a positive control other five randomly selected tanks were treated with VectoBac 200G™ at a dose of 5 mg/L.

At the beginning of the experiment, we recorded data of larvae and pupae density at day 1 and day 3 pre-treatment. In order to assess the density of immature instars, we analyzed 20 spoonful samples taken with a 300 mL ladle tied to a long cable (~1 meter) that facilitated its handling. We measured the total sample volume in a graduated cylinder and calculated the total volume of the tank using its diameter and height. The number of *Bti*-CECIF tablets per tank was calculated based on the total tank volume regardless of the total amount of water contained in it at the time of treatment. Because of this, some of the tanks had an initial concentration of *Bti*-CECIF greater than 0.93 mg/L.

Once we administered the treatment, larval density (all instars) was assessed every 24 h for three days. After the third day, we measured it three times per week for four weeks to assess the residual activity.

The water content of tanks varied with water consumption and exchange (rain or aqueduct), leading to changes in bioactive ingredient concentration over time. At each time point, we estimated the bioactive ingredient concentration using the water volume registered for the day it was being measured and the number of tablets initially added to the tank (initial concentration of 0.93 mg/L assuming maximum volume capacity). Throughout the assay, we recorded water pH, changes in water level, and water temperature data and we registered as well the environmental temperature and the relative humidity for the municipality of San Carlos.

**Statistical analysis.** Collected data was tabulated in a Microsoft Excel™ spreadsheet and the statistical analysis was performed using Statgraphics Centurion XVI™, version 16.1.02 software.

Following WHO recommendations (20), we calculated the post-treatment percentage reduction of larval density (% RLD) based on the Mulla formula (1971) (23):  $\%RLD = 100 - \left(\frac{C_1}{T_1}\right) \times \left(\frac{C_2}{T_2}\right) \times 100$ , where  $C_1$  is the number of larvae in instars I, II, III, and IV in negative control tanks before administering the *Bti*-CECIF tablets;  $C_2$  is the number of larvae in instars I, II, III, and IV in negative control tanks after administering the *Bti*-CECIF tablets;  $T_1$  is the number of larvae in instars I, II, III, and IV in *Bti*-CECIF treated tanks before administering the *Bti*-CECIF tablets, and  $T_2$  is the number of larvae in instars I, II, III, and IV in *Bti*-CECIF treated tanks after administering the *Bti*-CECIF tablets. An 80%

RLD was defined as the cutoff, so *Bti* residual activity was considered to last until the day in which % RLD was greater or equal to 80%. A logistic regression model was adjusted for efficacy as a function of the number of days post-treatment with *Bti*-CECIF tablets. For this model, tanks with residual activity  $\geq 80\%$  were coded as one (1), and those tanks in which there was no longer *Bti* residual activity ( $\% \text{RLD} < 80\%$ ) were coded as zero (0).

**Time-course analysis of *Bti*-CECIF tablet concentration.** In order to estimate the change in the bioactive ingredient concentration produced by water consumption and exchange, we assumed a constant dilution rate and used an exponential model to estimate the dilution rate for each of the tanks:  $\text{Concentration} = e^{-\kappa \times \text{dpt}}$ , where  $\kappa$  is the dilution rate and dpt is the number of days post-treatment with *Bti*-CECIF tablets. High  $\kappa$  values indicate a fast dilution, while low  $\kappa$  values indicate a slow dilution.

*Bti*-CECIF residual activity between groups was compared by a variance analysis in which we defined the response variable as the last day at which treatment remained effective ( $\% \text{RLD} \geq 80\%$ ) and subjected it to square root transformation prior to analysis.

**Participants knowledge survey.** Upon completion of the bioassay, a brief questionnaire was administered to the heads of the households participating in the study. Twenty-one structured surveys were administered by the two team members who had been assessing tablets efficacy (a biologist and a field assistant), including only the sampling units which would be assigned to the *Bti*-CECIF treatment. We used the data-collection instrument previously described by Ocampo, *et al.* (24), after adapting it to the municipality context and the purpose of our study. The survey consists of 19 questions that assess participants knowledge regarding the vector and the use of *Bti*-CECIF tablets.

## Results

### Semi-field bioassay in Apartadó

During the experiment, the average temperature in Apartadó was  $27.6^\circ\text{C} \pm 0.58$  and the relative humidity,  $92.6\% \pm 2.9$ . The tank water temperature ranged between  $23.2^\circ\text{C}$  and  $29.4^\circ\text{C}$  (average of  $27.3^\circ\text{C} \pm 0.72$ ). The tank water pH ranged between 7.45 and 8.99 (average of  $8.31 \pm 0.29$ ).

### *Bti*-CECIF tablets efficacy assessment

Based on day 1 larval mortality data (table 1), a probit model was adjusted for *Bti* dose-dependent

mortality. Using inverse prediction, we predicted a dose of 0.24 mg/L to achieve 80% mortality 24 h post-treatment (figure 1). The dose of 0.93 mg/L exhibited greater efficacy than the other *Bti*-CECIF tested dosages. As expected, there was no mortality in tanks treated with tablets without *Bti* (negative control) (table 1).

### Residual activity assessment

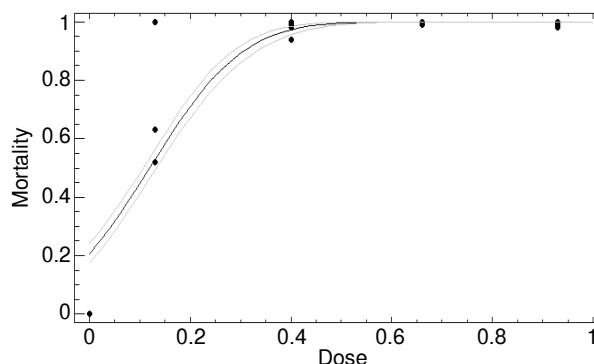
In order to evaluate the residual activity of *Bti*-CECIF tablets on *A. aegypti* larvae throughout the 40 days of the experiment, a probit model was adjusted for each of the doses with a function of time of exposure. Mortality rates for each of the doses are shown in figure 2. No mortality was observed for negative controls (data not shown). During the entire duration of the experiment, the residual activity of the 0.66 and 0.93 mg/L doses was greater than that of all the other tested *Bti* doses.

For each of the tested doses, a probit model was adjusted for mortality as a function of post-treatment days. To this end, inverse predictions were obtained from the day at which 80% mortality was expected considering the different exposure days (e1, e2, e3, and e4) (figure 3).

**Table 1.** Mortality rate of *Aedes aegypti* stage III larvae treated with different doses of *Bti*-CECIF tablets

Dose (mg/L)	Number of <i>Bti</i> tablets (180 L water)	Mortality (%)			
		Day 1	Day 2	Day 3	Day 4
0.13	3	78.7	10.2	5.5	5.5
0.40	9	97.7	1.5	0.7	0
0.66	15	99.7	0.2	0	0
0.93	21	98.7	0.5	0.7	0
Control	3*	0	0	0	0

\*Tablets without *Bti*

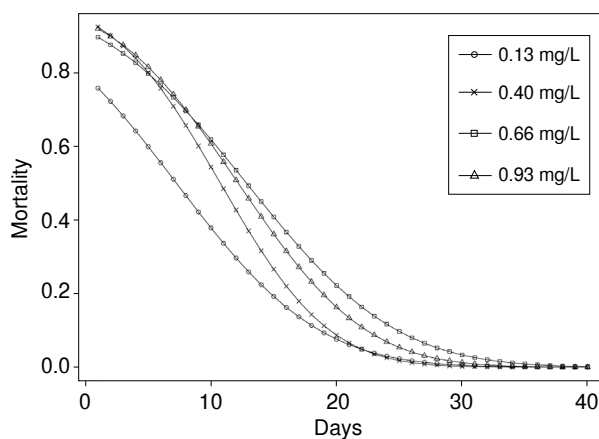


**Figure 1.** Probit model: *Bti* dose-dependent *Aedes aegypti* stage III larvae mortality in 180 L water tanks under semi-field conditions. Gray lines represent 95% confidence intervals.

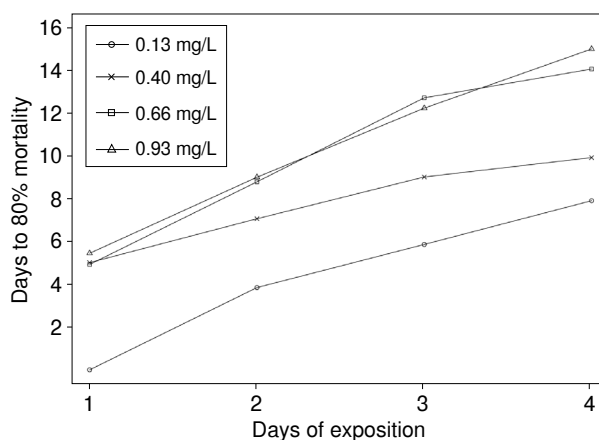
The doses exhibiting greater residual activity were 0.66 mg/L and 0.93 mg/L. For the latter, we estimated that an 80 % mortality rate would be reached 15 days post-treatment after larvae were exposed for four days. Although there was no significant difference in the effect of these two doses, we chose the highest taking into consideration that in the field there would be water exchange and, thus, a decrease of the concentration of the active ingredient. For this reason, we selected the 0.93 mg/L dose for the test under field conditions in San Carlos, Córdoba.

### Field bioassay in San Carlos

During the experiment, the average temperature in San Carlos was  $27.6^{\circ}\text{C} \pm 1.93$  and the relative



**Figure 2.** Expected larval mortality based on a probit model using four doses of *Bti-CECIF* tablets on stage III larvae of *Aedes aegypti* in 180 L water tanks under semi-field conditions



**Figure 3.** Mortality of 80% was achieved using four doses of *Bti-CECIF* tablets on stage III larvae of *Aedes aegypti* in 180 L water tanks under semi-field conditions. Days required to reach 80% mortality and days of exposure are graphed for the four different *Bti-CECIF* tablet doses.

humidity,  $72.6 \% \pm 4.1$ . The tank water temperature ranged between  $26^{\circ}\text{C}$  and  $35.1^{\circ}\text{C}$  (average of  $30.5^{\circ}\text{C} \pm 1.83$ ). The tank water pH ranged between 7.12 and 9.98 (average of  $7.96 \pm 0.51$ ). All of the evaluated tanks were naturally infested with *A. aegypti* larvae and pupae.

### *Aedes aegypti* percentage of larval density reduction in tanks treated with *Bti-CECIF* tablets

Based on the observed RLD percentages during the 34 days after administering the *Bti-CECIF* tablets at a dose of 0.93 mg/L, a logistic model for efficacy (% RLD $\geq$ 80%) was adjusted to the number of days post-treatment ( $p < 0.0001$ ). The day for which a given probability of efficacy is expected can be estimated by performing an inverse prediction. Thus, if we have that there is a 0.9 probability that the product maintains its efficacy until around the seventh day, this means that seven days post-treatment approximately 90% of the tanks treated with *Bti-CECIF* tablets at a 0.93 mg/L dose would be expected to maintain a % RLD $\geq$ 80% (figure 4).

### Time-course analysis of *Bti-CECIF* tablet concentration

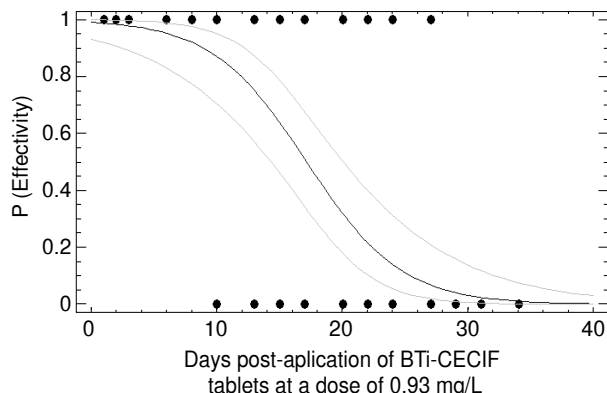
Figure 5 shows the observed change in concentration as a function of the number of post-treatment days during the bioassay for each tank. The differences among tanks corresponded to the variation due to water consumption and exchange patterns.

For each of the tanks, we used the following exponential model to establish the progression of concentration/dilution over time:  $\text{Concentration} = e^{-\kappa \times t}$ . We defined two groups of tanks: 1) Tanks exhibiting below-average dilution rates ( $\kappa < 0.094$ ), and 2) tanks exhibiting above-average dilution rates ( $\kappa > 0.094$ ). We did not find a statically significant effect of the dilution rate on product efficacy ( $F = 0.128$ ; error df=18;  $p = 0.725$ ).

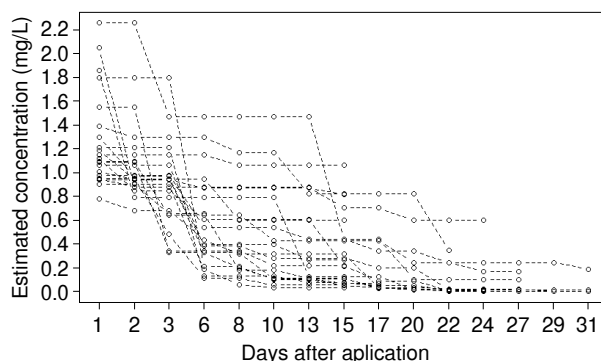
The residual activity was not significantly different between *Bti-CECIF* tablets and VectoBac 200G™ ( $F = 0.009$ ; error df=24;  $p = 0.926$ ).

### Participants knowledge survey

Participants age was 43 years old and younger. Regarding knowledge about dengue transmission, most participants (90%) were aware that chikungunya and dengue viruses are transmitted by mosquito bites. The majority of them (81%) also indicated that this mosquito breeds in water tanks and less than a third of participants (28%) knew that washing water tanks was a strategy to avoid mosquito reproduction. All of the participants (100%)



**Figure 4.** Logistic regression model for effectiveness (% RLD $\geq$ 80) of *Bti*-CECIF tablets (dose 0.93 mg/L) as a function of the number of post-treatment days in tanks infested with *Aedes aegypti*. Gray lines represent 95% confidence intervals.



**Figure 5.** Post-treatment estimated concentrations of *Bti* in water tanks, bioassay field in San Carlos, Córdoba. Each line corresponds to a tank.

indicated that they used aqueduct water stored in cement tanks. About half (48%) of them stored the water for one to three days, 38% for four to seven days, and 14% for more than one week. Regarding tank washing, 95% of the participants said they washed their tanks, and 30% of these did so every two days, 45% on a weekly basis, and 25% every two weeks. Regarding the bioassay, 95% of the participants acknowledged that the administered tablets were efficient in killing mosquito larvae and that they had reduced their number. However, 62% of the responders indicated that the number of mosquitos (*A. aegypti* and *Culex* spp.) inside the house had not reduced.

## Discussion

We report the efficacy and residual activity of *Bti*-CECIF tablets on *A. aegypti* larvae under semi-field and field experimental conditions in two Colombian municipalities. The use of *Bti* as a control strategy

for *A. aegypti* larvae has been previously reported using different formulation and doses, particularly in countries where temephos resistance has been reported in *A. aegypti* populations (25,26).

In Colombia, the use of *Bti* is a complementary alternative for vector control in potable water tanks and other breeding sites (27-29), especially in regions where larvae have become resistant to temephos (27,29,30). The production and commercialization of a pharmaceutical formulation of *Bti* tablets developed and produced in Colombia would significantly reduce costs and increase product availability, leading to successful *A. aegypti* vector-control. Furthermore, this would contribute to proper dosage, as only the administration of a given number of tablets according to the tank volume would be required, as well as to product stability by packaging the material, which facilitates proper opening and closing of the package, thus guaranteeing the initial manufacturing conditions of the finished product.

During the first week of the semi-field bioassay, all of the tested doses (0.13, 0.40, 0.66 and 0.93 mg/L) of *Bti*-CECIF tablets (5,240 ITU/mg, active ingredient (a.i.): 1.33%) exhibited an efficacy of 100% for larval mortality. Regarding residual activity of *Bti*-CECIF tablets, the highest dose used in our study was enough to keep an 80% mortality rate for up to two weeks.

These results are in agreement with those reported by two studies in Brazil under semi-field conditions using *Bti* tablets VectoBacT™ (2,200 ITU/mg, a.i.: 37.4%). In one of these studies, doses of 3.5 and 7 mg/L were tested and it was found that a 100% *A. aegypti* larval mortality rate lasted up to two and three weeks, respectively (31). In the study by Zequi, *et al.* (32), VectoBacT™ tablets used at a 0.9 mg/L dose caused 100% mortality in instar IV *A. aegypti* larvae during the first eight days, and on day 15 the mortality rate was 98.8%. In another study, Melo-Santos, *et al.* (33), obtained a greater residual activity using *Bti* tablets (1,146  $\pm$  2 ITU/mg, dose: one tablet/10 L, a.i. not reported) than in the above mentioned studies resulting in an *A. aegypti* larval mortality rate above 90% on days 13 through 35 for containers exposed to sunlight and on days 40 through 54 for containers protected from sunlight.

Several studies in different countries using commercially available and experimental *Bti* formulations under semi-field conditions, with and without

water replacement and under sunlight exposure or shade, have shown that *Bti* residual activity does not last longer than one month (31-40). This low *Bti* residual activity, especially in those bioassays using tablets and no water exchange, may be partly due to the excipient serving as food source for larvae. Treteau, *et al.* (41), reported that *Bti* residual activity was inversely proportional to organic matter content because membrane receptors in the *A. aegypti* larvae intestinal epithelium were covered by the ingested particles, thus interfering with *Bti* binding (42).

In Colombia, a previous study tested *Bti* tablets under semi-field conditions (36) and analyzed containers exposed to sunlight. In that study, contrary to most other studies, a residual activity of 12 weeks with larval mortality >80% was observed. Several studies conducted under field conditions in different countries with commercially available and experimental *Bti* tablets have shown that their residual activity is less than a month (25,37,43-46). In Colombia, several *Bti* formulations tested under field conditions (18,24,43,47) have yielded varied results depending on the study site.

In our study, the use of *Bti*-CECIF tablets dosed at 0.93 mg/L under field conditions in water tanks in the two selected neighborhoods of San Carlos, Córdoba, showed that on day 7, 90% of the tanks exhibited an 80% *A. aegypti* larvae mortality. These results are in agreement with Suárez, *et al.* (18), study in which by testing two product presentations (liquid and granular) in different types of tanks the authors showed that the *Bti* residual activity extended for seven to nine days. The study by Kroeger, *et al.* (43), evaluated commercially available *Bti* tablets (Culinex™, 33,000 ITU/mg, and 8,000 ITU/mg, no a.i. reported) which yielded, at the highest tested dose, a residual activity of 28 days in which larvae mortality was >90%.

Our results under semi-field conditions were different from under field conditions. Although semi-field bioassays are considered to be a useful method for the selection of new insecticides and/or doses to be applied under field conditions, they lack the ability to predict products real performance (25). This may be partly explained by several factors that may affect *Bti* products performance including environmental conditions such as water temperature, larval density, sunlight, and the association with filter feeders. Moreover, water exchange and uncontrolled water tank conditions, such as those present under field conditions,

contribute to turning them into potential breeding sites and may also have a negative effect on *Bti* efficacy (35,37,48).

Following WHO recommendations (20), we surveyed community acceptance of our product as well as its relationship to vector control and related diseases. While the majority of the participants knew that *A. aegypti* is the vector for dengue and chikungunya viruses and that it breeds in water tanks, this knowledge is insufficient for successful mosquito control and disease prevention, as there is no direct relationship to their daily lives (43). Community acceptance of *Bti*-CECIF tablets was high since, on the one hand, they were aware of their effect on the *A. aegypti* larvae (information and periodic inspections), and on the other hand, the tablets did not affect water odor or appearance judged by the color (observation of the researchers).

Altogether, our data showed that the *Bti*-CECIF tablets designed and developed at our institution exhibited larvicidal properties comparable to other commercial products since no significant differences with VectoBac 200G™ (2,200 ITU/mg, 0.5% a.i.) were identified. In addition, *Bti*-CECIF tablets are easier to use, since the user is only required to count the necessary tablets to treat a given water volume, and there is no need of weighing the product before adding it to the water (39,43). Furthermore, the tablet has the advantage of sinking to the bottom of the tanks, where *A. aegypti* larvae are usually found, as compared to granular formulations that remain floating on the water surface for several days. Given that these tablets have selective toxicity, they are odorless and tasteless once diluted in water. These features make them an attractive option for the final user as they do not generate a negative perception of the water (appearance of dirty water) (25). The community acceptance was evidenced in the survey administered to adult inhabitants of participating households.

The Pan American Health Organization (PAHO) and WHO proposed an integrated management strategy for the prevention and control of arboviral diseases in endemic countries that includes six components (epidemiology, integrated vector management, social communication, patient care, laboratory, and environment) (49). Within the integrated vector management component, *Bti*-CECIF tablets could be useful for those endemic areas where *A. aegypti* populations are resistant to temephos and water for human consumption is stored for later use. Moreover, *Bti*-CECIF tablets



should be used in conjunction with other control strategies including the elimination of breeding sites by members of the community and space fumigation by health authorities when required. In addition, such control strategy must be integrated into the social communication component in order to achieve community acceptance and participation in its application.

One of the limitations of *Bti*-CECIF tablets identified in our field studies was the low residual activity of the *Bti* since product reapplication was required. However, this could be addressed in future experiments by including new dosing schemes, or by performing additional fieldwork in other regions in order to analyze and identify trends in product performance.

In Colombia, there are currently no bioinsecticide manufacturing facilities meeting good manufacturing practices (GMP) standards. Therefore, our group is currently developing a project to design and build GMP facilities for the production of *Bti*-CECIF tablets as it is technically and economically feasible.

In conclusion, our results strongly suggest that *Bti*-CECIF tablets have a larvicidal effect on *A. aegypti*, the known vector for dengue, chikungunya, and Zika arboviruses. Given the high rate of infection of these arboviruses in Colombia, successful and affordable vector control strategies, such as our *Bti*-CECIF tablets, are of pivotal importance to provide better public health care.

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### Conflicts of interest

The authors have no conflicts of interest to declare.

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