ORIGINAL ARTICLE

Field and semi-field evaluation of *Bacillus thuringiensis* var. *israelensis* versus Temephos® in *Aedes aegypti* control

Avaliação de campo e simulado de campo de *Bacillus thuringiensis* var. *israelensis* versus Temephos® no controle de *Aedes aegypti*

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Abstract

Introduction: *Aedes aegypti* is a vector of the important arboviruses worldwide. Vector control continues to rely mainly on fighting immature stages. Resistance to the larvicide Temephos® was detected in many regions of Brazil since 2000 what led control programs to search for alternative products, such as *Bacillus thuringiensis* var. *israelensis (Bti)*. Caicó municipality (Rio Grande do Norte State, Brazil) was one of the first cities to use *Bti*. However, after some time, *Bti* low persistence was noticed as jeopardizing effective vector control. **Objective**: To compare the efficacy of two *Bti* granulate formulations, Vectobac G® and Vectobac WDG® and Temephos® against *Ae. aegypti* in field and semi-field conditions. **Methods**: Field tests were carried out in two neighbouring areas which presented *Ae. aegypti* infestation indices >3%: Walfredo Gurgel and Boa Passagem, Caicó, RN, Brazil, in 2004. Semi- field tests were performed in the patio of a building. **Results**: After nine weeks, positive containers for *Ae. aegypti* in the field were >10% in the area of application of *Bti* and <1% in the area where Temephos® was applied. In the semi-field conditions *Ae. aegypti* larval mortality >80% was maintained for up to 56 days for Temephos®, 35 days for Vectobac G® and 49 days for Vectobac WDG®. **Conclusions**: The results point out to low *Bti* persistence in the field, mainly for containers exposed to sunlight. Local climatic and environmental conditions should be regarded when new products are tested due to high regional variability prevailing in Brazil.

Keywords: *Aedes aegypti*. *Bti*. Temephos®. Insecticide persistence.

Resumo

Introdução: O *Aedes aegypti* é vetor de importantes arboviroses em todo o mundo. Seu controle se dá principalmente pelo combate aos estágios imaturos. Resistência ao larvicida Temephos® foi detectada em várias regiões do Brasil desde 2000, e produtos alternativos como *Bacillus thuringiensis* var. *israelensis (Bti)* foram procurados. O município de Caicó (Rio Grande do Norte, Brasil) foi um dos primeiros a usar *Bti*; ali, entretanto, após algum tempo, notou-se baixa persistência, colocando em risco os programas de controle. **Objetivo**: Comparar a eficácia de duas formulações de *Bti* granulado, Vectobac G® e Vectobac WDG®, e Temephos® contra o *Ae. aegypti*, em condições de campo e simulado de campo. **Métodos**: Testes de campo foram realizados em dois bairros com índice de infestação >3%: Walfredo Gurgel e Boa Passagem, Caicó, em 2004. Testes de simulado de campo foram realizados no pátio de um prédio. **Resultados:** Após nove semanas, os depósitos positivos para *Ae. aegypti* no campo eram >10% na área de aplicação de *Bti* e <1% na área onde Temephos® foi aplicado. Nas condições de simulado de campo, a mortalidade de larvas >80% foi mantida por até 56 dias para Temephos®, 35 dias para Vectobac G® e 49 dias para Vectobac WDG®. **Conclusões**: Os resultados apontam para uma baixa persistência do *Bti* no campo, principalmente em depósitos expostos à luz solar. As condições locais climáticas e ambientais devem ser observadas quando na implementação de novos produtos devido à alta variabilidade regional presente no Brasil.

Palavras-chave: Controle de Vetores. *Aedes aegypti*. *Bti*. Temephos®. Persistência de Inseticida.

INTRODUCTION

Arboviruses such as dengue, chikungunya and Zika have spread quickly around the world, especially in urban areas of many countries[1,2,3,4]. Arboviruses rapid spread especially prevails in tropical countries where environmental conditions favor the development and proliferation of its main vector *Aedes aegypti* (Linnaeus, 1762)[5,6]. *Ae. aegypti* is essentially a domestic and synanthropophilic mosquito, extremely adapted to man, that also acts as the main vector of arboviruses such as dengue, yellow fever, chikungunya and more recently Zika, among others[7,8,9,10]. Nowadays, billions of individuals live under constant threat of contracting these arboviruses mainly in urban and peri-urban areas of the planet[1,2,9,10,11].

Even though adult mosquitoes transmit arboviruses during blood feeding, vector control programs have heavily relied on fighting larvae and pupae, the immature mosquito stages[12].

Nonetheless, Brazil, since the early 2000 several studies performed with populations of *Ae. aegypti* from different regions have detected resistance to the organophosphate larvicide Temephos® which for decades has been the only larvicide available for vector control[13,14,15]. In light of these findings, the National Dengue Control Program (Programa Nacional de Controle da Dengue-PNCD) initiated a search for alternative larvicide products. In 2001, the PNCD began to

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change larvicides in areas with detected Temephos® resistance in São Paulo and other areas of Brazil[14,16].

The entomopathogenic bacteria *Bacillus thuringiensis* var. *israelensis (Bti)* was then thought as a potential candidate for use in replacement to Temephos® and was evaluated in Brazilian municipalities where organophosphate resistance was registered[12,15]. *Bti* is thought to cause little interference in the environment[17,18,19,20] and has been effectively used in the control of various mosquito species[14,21,22,23]. There are many commercial products using *Bti* as an active ingredient such as Vectobac.

The Caicó municipality, located in the Rio Grande do Norte state, Northeastern Brazil, was also one of the first to detect Temephos® resistance[15] and make use of *Bti*. However, after some time, vector control teams noticed that *Bti* persistence was low and frequent reapplications of the product were needed to maintain low *Aedes aegypti* container positivity.

Bti kills mosquito larvae when ingested and is considered a safe larvicide, due to its low environmental impact[24]. Persistence is defined as the residual effect of the larvicide in the environment, or the time period that the larvicide maintain > 80% mortality[25]. Some studies have shown that *Bti* residual effect can last as long as 16 weeks in artificial containers, especially when used at high doses[26,27]. Vectobac WDG®, a *Bti* formulation used in water tanks for human consumption (as recommended by WHO[28]) showed different persistence (variable due to temperature, type of container, intensity of sunlight exposure, amount of product applied and water addition). For example, larval mortality >90% was observed after the 12th week of 4 mg/L of *Bti* Vectobac WDG® application in 250 L polyethylene water storage containers[29]. In ceramic and glass containers a larval mortality> 90% was obtained when using 5 mg/L of *Bti* Vectobac WDG® for seven to eight weeks[30]. Less heavy formulations of 2 mg/L *Bti* Vectobac WDG® showed 90% mortality for a decreased period of five to six weeks in ceramic and glass containers, during simulated field tests[31].

Even less persistence, of 80% mortality, was obtained up to nine days for a *Bti* Vectobac WDG® 2 mg/L used in different sun-exposed containers (plastic, cement and asbestos) and seven days for brass containers, during summer time when environmental in temperatures reached 39.3 °C (March 2004) and 80% mortality 30-36 days in April and May 2004 with milder temperatures of 18.6–34.8 °C[11]. This way, it seems that *Bti* persistence is highly dependable on the local environmental conditions. Having the highly *Bti* variability results in mind, the Caicó municipality authorities asked us to evaluate *Bti*, under their environmental conditions. Caicó is a Northeastern Brazilian city located in the caatinga ecoregion with high temperatures and high solar incidence throughout the year[32].

Thus, two *Bti* formulations Vectobac G®, Vectobac WDG® and the organophosphate Temephos® were compared regarding their larvicide efficacy to control *Ae. aegypti* through field and semi-field tests in diverse containers located in two Caicó neighborhoods.

METHODS

Study site

The study was conducted with the assistance of the Municipal Health Service-SMS from March to April 2004 in Caicó (-6.457778, -37.097778, 161 m), a municipality in the state of Rio Grande do Norte, Brazil (Figure 1). Caicó is located in the Western Microregion named Seridó, in the caatinga ecoregion, at 256 km from the city of Natal, the State capital. Caicó occupies an area of 1,228.583 km^2 with ~60.000 inhabitants in 2004[32]. The climate is semiarid (Köppen-Geiger classification), the rainy season occurs from February to May, with average annual rainfall of 716.6 mm and a large thermal amplitude with temperatures ranging from minimum 18°C and maximum 33°C[32]. In Caicó, most domiciles are made of bricks and public water supply is irregular, which leads the population to collect rainfall water.

Figure 1. Study area, Brazil, Rio Grande do Norte State and the Caicó municipality

Mosquitoes

Ae. aegypti specimens used in this study were from the mosquito population of the Caicó municipality obtained by egg trap collections. Initially, 60 ovitraps were installed in two neighborhoods of Caicó. Ovitraps consisted of a black plastic cup with a capacity of 500 ml, having an Eucatex® palette of 13 cm partially dipped in 200 ml of a solution of 10% hay infusion.

These traps remained installed for 15 days; a palette change was made on the seventh day of installation. Positive pallets were used to obtain mosquito adults which, after positive identification for *Ae. aegypti*, were used for laying eggs. Third instar F1 generation *Ae. aegypti* larvae were used for the tests. In order to induce synchronization of larva emergence, eggs were immersed in dechlorinated tap water for one hour. Hamster food (Purina®, Paulínia, São Paulo, Brazil) was supplied daily to feed the larvae. Mosquitoes were reared at 26 ± 1ºC and 80% relative humidity.

Meteorological data

Rainfall and temperature indices were obtained from the site of National Institute of Meteorology (Instituto Nacional de Meteorologia - INMET)[33].

Bacillus thurigiensis formulations and concentrations

Two formulations of *Bti* were simultaneously tested: Vectobac G® (200 international toxic units-ITU) at a dosage of 20 mg/L and Vectobac WDG® (3000 ITU), that is recommended for use in drinking water, at a dosage of 2 mg/L (Abbott Laboratories, USA). Temephos® Fersol 1G (Mairinque, São Paulo, Brazil) granulated at a dosage of 1 mg/L was used as positive control. All products were applied only once.

Design of the tests

The experiments were carried out to evaluate 80% *Ae. aegypti* larval mortality during 8 weeks, in field and semi-field conditions.

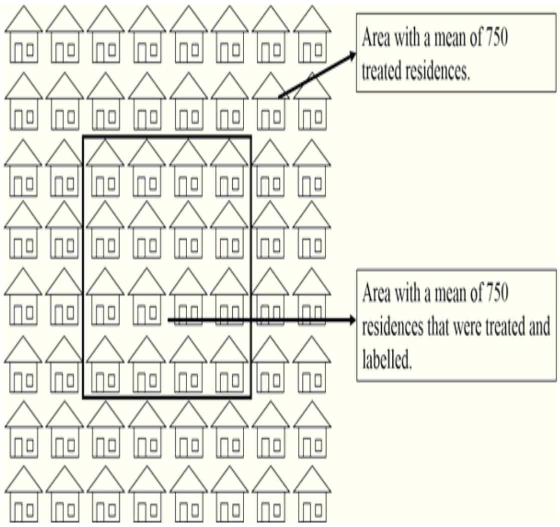
Field tests

In the field, larvicides were applied in two distinct areas: one area with Temephos® and another area with Vectobac G® and Vectobac WDG®. In drinking water containers Vectobac WDG® was applied while Vectobac G® was used in the other non-drinking water container types.

The same *Bti* concentrations were used for the field and the semi-field. Larvicide application followed routine protocols of the local control teams (PNCD).

Experiments were carried out in the Caicó neighborhoods of Walfredo Gurgel and Boa Passagem, approximately 1 km distant from each other. For both neighborhoods, about 1,500 domiciles were visited for which the *Ae. aegypti* index (number of positive domiciles/ total domiciles) was verified, as well the presence of breeding habitats as containers. All breeding habitats that could not be promptly eliminated were treated. In Walfredo Gurgel, an area of 1 km2 square underwent Temephos® application. In Boa Passagem, Vectobac WDG® was used in containers with water for human consumption and Vectobac G® in the remaining containers. In both neighborhoods approximately 750 domiciles located in the central part of a square block design (as depicted in Figure 2) had all containers identified and labeled for further evaluations. Labeled containers were visited weekly in search for *Ae. aegypti* and to check with the resident if the container had been washed. If positive or washed, the container was discarded from the study.

Figure 2. Diagram of field test. The image shows an area of 1 km² (~1,500 residences) used for the field tests, in the central area where breeding deposits square identified, treated and labeled for week evaluations.



Semi-field tests

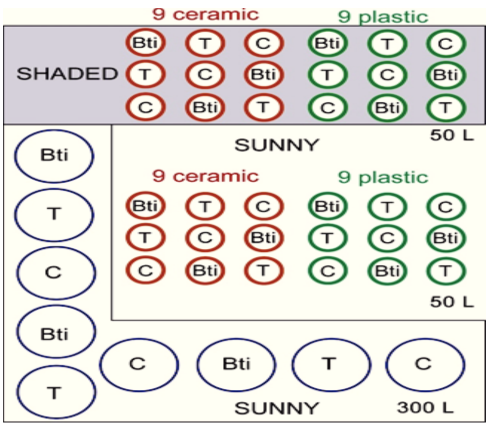
Semi-field tests were conducted in the building of the Center for Zoonosis Control-CCZ, in two different environments: the first environment was indoors in a shaded room of the CCZ building and the second environment was outdoors in a sunny exterior area of the CCZ building (Figure 3). We used 45 containers: 27 in the external area and 18 in the shaded internal area (Figure 3). In the shaded area, nine (three with *Bti*, three with Temephos® and three control) 50 L ceramic pots as the ones typically used by local people for water storage, and nine (three with *Bti*, three with Temephos® and three control) 50 L plastic buckets were tested. In the outside area, nine 300 L polyethylene water storage tanks were also used. For all types of containers there was partial replacement of water: in the 50 L ceramic pots and the plastic buckets 1/5 of the volume, or 10 liters, were replaced three times per week; in the 300 L polyethylene water tanks 100 L (1/3 of the volume) were replaced five times a week to maintain total water volume and simulate normal water usage by the residents. Tests were performed in triplicate and the containers remained covered with nylon nets to prevent egg laying by mosquitoes.

To assess persistence, 50 third instar larvae of *Ae. aegypti* were added weekly to 50 L containers while 100 third instar larvae were added to the 300 L containers. There was no food supply to the larvae, and in all cases, mortality was assessed after 24 h[11,14].

Statistics

Statistical analyses were performed using the chi-square test, the Graph Pad Prism software, version 5.0 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). Other measures such as positivity and container elimination were shown in percentages.

Figure 3. Diagram of the semi-field test with the different containers and distinct treatments placed in the shaded (indoors) and sunny (outdoors) areas.



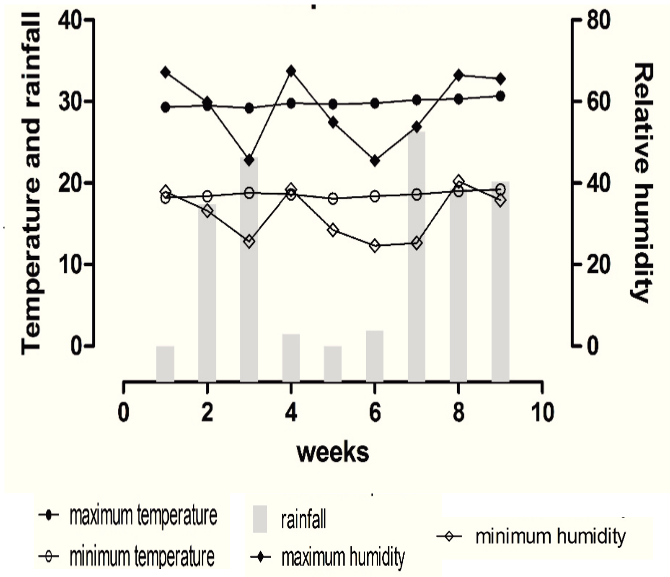
Ethical considerations

This work was performed at the request of the Municipal Secretary of Health of Caicó who was already using the larvicide *Bti* on the field since 2003. The coordination and the methodology were defined by the authors while the study was conducted by local health agents. Tests were part of the Entomological Surveillance activities of the Municipal Secretary of Health of Caicó; those involved in the project are members of staff and all routine precautions for personal protection were taken.

RESULTS

Meteorological data

During the study period temperatures ranged from 18.1 to 30.7°C and rainfalls occurred in the second, third and seventh to ninth weeks (Figure 4).



Field tests

Field tests lasted for 9 weeks (March to April 2004). A total of 778 containers in the neighborhood of Walfredo Gurgel and 541 containers in the neighborhood of Boa Passagem were identified and labeled. Container types were classified as plastic buckets (up to 50 L), cement tanks (up to 60 L), ceramic pots (up to 60 L), plastic drums (up to 100 L), polyethylene water storage tanks (> 200 L), according to guidelines from the Health Vigilance Secretary. In both neighborhoods, the most common container type was ~250L polyethylene water storage tanks (Table 1).

With the exception of 50L plastic buckets, most containers

Table 1. Percentage of positive containers for larvae of *Ae. aegypti* in the neighborhoods of Walfredo Gurgel and Boa Passagem in the municipality of Caicó, Rio Grande do Norte State, Northeastern Brazil in 2004.

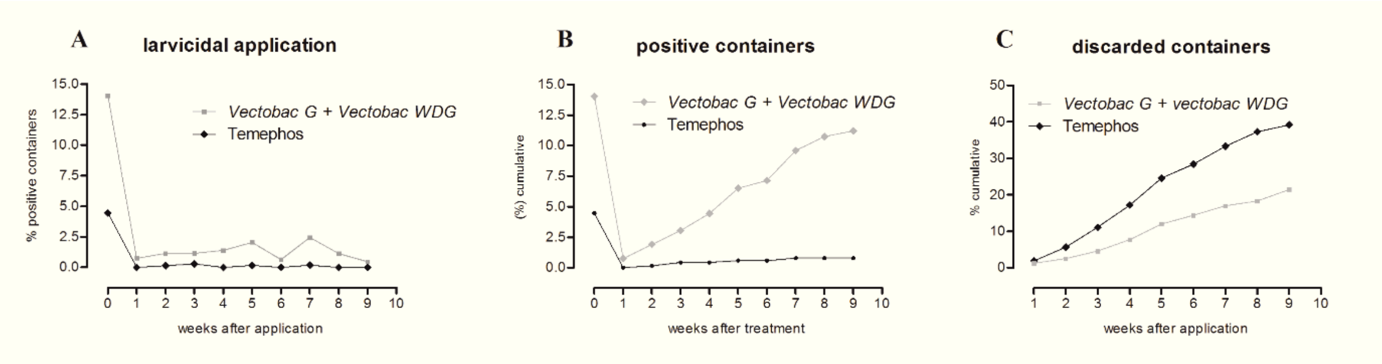
Container	Walfredo Gurgel		Boa Passagem	
	N (% type container, related to the total containers)	% sun exposed*	N (% type container related to the total containers)	% sun exposed*
Polyethylene water storage tank 500 L	464 (59,6)	79,3	417 (77,1)	82,3
Cement tanks 60-70 L	117 (15)	61,5	39 (7,2)	69,2
Plastic buckets <50L	115 (14,8)	25,2	33 (6,1)	30,3
Plastic drums >100 L	65 (8,4)	61,6	31 (5,7)	61,3
Other types	17 (2,2)	17,8	21 (3,9)	33,2
Total	778 (100)		541 (100)	

*Percentage to the total of each container type, shaded containers are the remaining amount to complete 100%.

were sun exposed (Table 1). Before the application of larvicide products, the percentage of positive containers for larvae was 4.5% in Walfredo Gurgel and 14.5% in Boa Passagem neighborhoods. Walfredo Gurgel received Temephos® application and Boa Passagem Vectobac G® and Vectobac WDG®. After one week of larvicide products application, the first assessment resulted in larval positivity of <1% in evaluated containers for both neighborhoods (Figure 5A).

Walfredo Gurgel received treatment with Temephos® and the larval positivity remained <1% during the entire nine-week evaluation. On the other hand, in Boa Passagem, which received treatment with Vectobac G® and Vectobac WDG®, larval positivity remained always >1% for all assessments during the entire nine-week evaluation (Figure 5A). Since all positive containers were removed from subsequent inspections, cumulative percentage of positive containers was also evaluated. Cumulative percentages for the Temephos® treated Walfredo Gurgel were <1% during the entire nine-week study period, while containers located in Boa Passagem (Vectobac G® and Vectobac WDG® treated) had cumulative percentages >11% (Figure 5B). Containers washed by residents were removed from the study. The total removed containers was higher in Walfredo Gurgel, with 40% at the end of the nine-week evaluation, and 21% in Boa Passagem (Figure 5C).

Figure 5. Percentage of positive containers for larvae of *Ae. aegypti* and discarded containers in two neighborhoods of the municipality of Caicó, Rio Grande do Norte State, Brazil after Temephos® application in Walfredo Gurgel and Vectobac G® and Vectobac WDG® in Boa Passagem neighbourhoods, in 2004, with the percentage of positive containers weekly (A), cumulative (B) and also the percentage of removed containers (C).



Semi-field

The results obtained under simulated field conditions showed higher persistence for all larvicide products in plastic and ceramic containers located in shaded areas indoors (Figures 6A and 6C).

Temephos® presented higher residual effect when compared to both Vectobac G® and Vectobac WDG® *Bti* formulations, with ~100% mortality in eight weeks of evaluation, under the evaluation conditions. Vectobac G® and Vectobac WDG® *Bti* formulations showed reduced persistence when compared to Temephos®, with >80% mortality for up to only three weeks in plastic containers (Figure 6A). In ceramic containers the persistence of Vectobac WDG® was higher and the >80% mortality extended for seven weeks (Figure 6C).

In the sun exposed external containers (Figures 6B and 6D), there were differences in the persistence of Temephos®, which induced >80% mortality up to eight weeks only for the ceramic containers (Figure 6D). For plastic containers, >80% mortality was only achieved by Temephos® for two weeks (Figure 6B). The performance of Vectobac D® and Vectobac WDG® in all sun exposed external containers was very low, with >80% mortality extending only up to two weeks and only one week for the plastic containers.

Considering polyethylene water storage tanks, Temephos® induced >80% mortality during the eight weeks of tests while Vectobac G® and Vectobac WDG® *Bti* formulations had >80% mortality observed for three to four weeks (Figures 6E and 6F). Even though in the polyethylene water storage tanks 1/3 of the water was replaced five times per week, while for plastic buckets and ceramic pots 1/5 of the water was replaced three times per week, the persistence for all larvicide products was higher in the water tanks.

Temephos®, Vectobac G® and Vectobac WDG® showed statistically significant variable residual activity of >80% mortality between 1 to 8 weeks (Table 2).

In relation to the type of containers and sun exposition, Temephos® persistence was higher in shaded 50L plastic buckets than in 50L sun exposed plastic buckets (P <0.0001). There was no difference concerning ceramic pots between the shaded and sunny areas (P >0.05). Vectobac G® and Vectobac WDG® persistence was also higher in 50L shaded plastic buckets (P <0.001) and these products also persist more in 50L shaded ceramic pots (P <0.05 and P <0.001, respectively). A comparison between Vectobac G® and Vectobac WDG® *Bti* formulations shows no difference in persistence between them (P >0.05), except for Vectobac WDG® that had higher persistence than Vectobac G® only for 50L ceramic pots located indoors (P <0.001).

When the larvicide products were compared, there was no statistic difference in persistence in 50L indoors plastic buckets (P >0.05), although Temephos® had 100% mortality for up to eight weeks and Vectobac G® and Vectobac WDG® *Bti* formulations had 100% mortality for only three weeks. The persistence of Temephos® is slightly higher in 50L outdoors plastic buckets (P < 0.05). Temephos® and Vectobac WDG® have a similar persistence (P >0.05) in 50L indoors ceramic pots, which is significantly higher than Vectobac G® persistence (P <0.001). However, in 50L outdoors ceramic pots, Temephos® has a much higher persistence than both Vectobac G® and Vectobac WDG® *Bti* formulations (P <0.001).

In the 250L polyethylene water storage tanks, Vectobac G® and Vectobac WDG® *Bti* formulations present a similar persistence (P >0.05) but a significant lower persistence when compared with Temephos® (P <0.001 for Vectobac G® and P <0.05 for for Vectobac WDG®).

Figure 6. Persistence in semi-field tests for Temephos®, Vectobac G® and Vectobac WDG® *Bti* formulations in three types of container (plastic buckets, ceramic pots and polyethylene water storage tanks) in the municipality of Caicó, Rio Grande do Norte State, Brazil in 2004. Bars are standard deviation among triplicates. The tests were conducted indoors in a shaded area (A, C) and outdoors in a sunny area (B, D, E, F). There were used plastic buckets (A, B), ceramic pots (C, D) which had 50 L capacity, and polyethylene water storage tanks (E, F), with 300 L capacity.

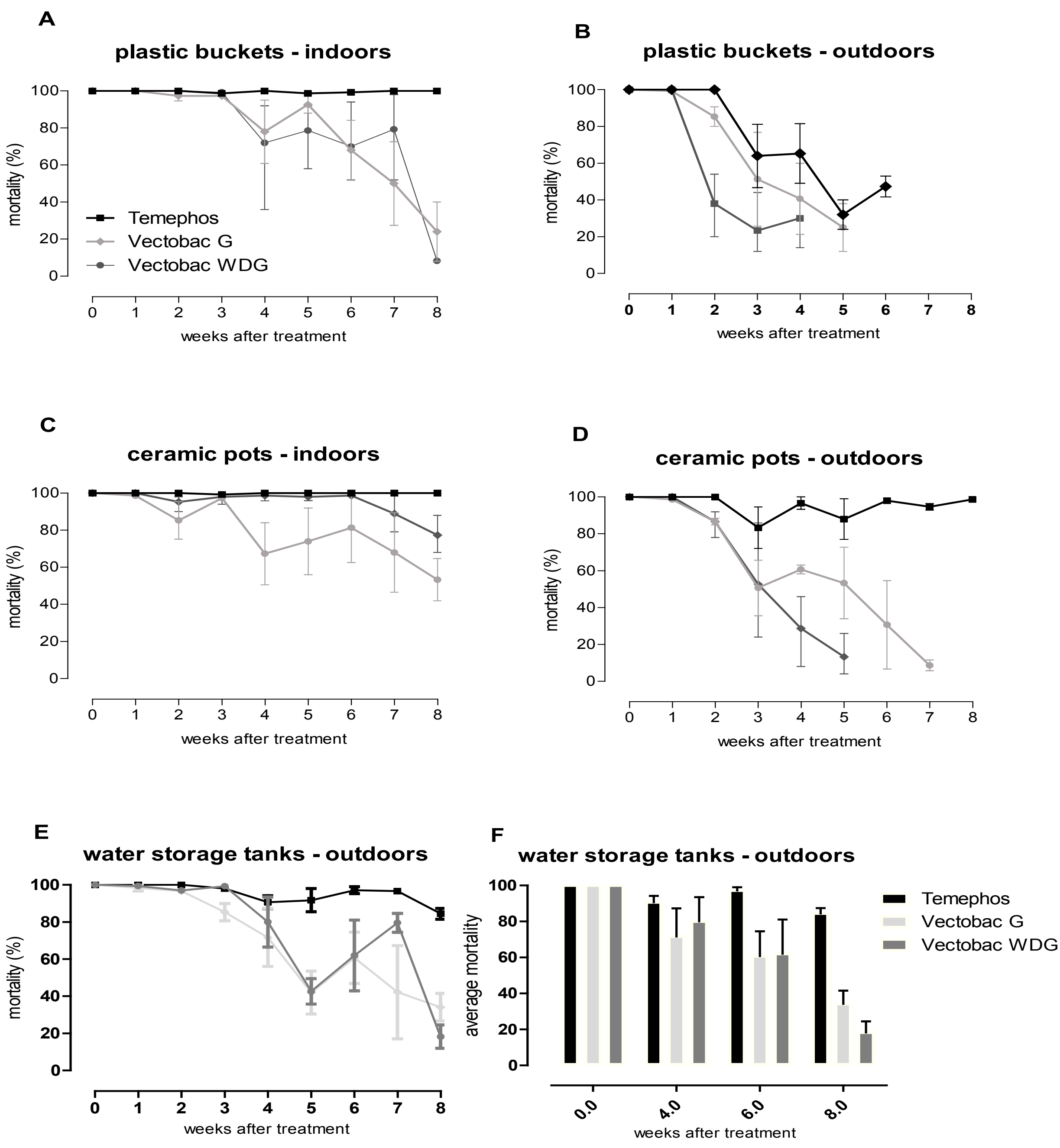


Table 2. Semi- field tests showing persistence in weeks for Temephos®, Vectobac G® and Vectobac WDG® carried out in the municipality of Caicó, Rio Grande do Norte State, Northeastern Brazil in 2004

Container types	Persistence (in weeks)[a]				
	Shaded indoors		Sun exposed outdoors		
	Plastic buckets	Ceramic pots	Plastic buckets	Ceramic pots	Polyethylene Water Storage tanks
Larvicide					
Temephos®	8	8	2	8	8
Vectobac G®	3	3	2	2	3
Vectobac WDG®	3	7	1	2	4

[a]In the water storage tanks 1/3 of the water was replaced five times per week, while for plastic buckets and ceramic pots 1/5 of the water was replaced three times per week.

DISCUSSION

Current strategies for mosquito vector control are not reaching the desired effect in Brazil. This occurs, in part, due to the *Ae. aegypti* opportunistic behavior which uses a wide variety of containers as breeding habitats, particularly artificial containers of all sizes and locations, requiring skilled entomology professionals but also society involvement to decrease container availability. Low quality house building, huge human densities, low trash collection, increase of use of nondegradable packing that are left in the environment seen in almost all Brazilian metropoles increase *Ae. aegypti* breeding habitats. Added to this, is the fact that in spite of advances, regular water supply in Brazil remains inefficient especially in smaller towns and the outskirts of large cities. Among others, irregular supply requires residents to find individual solutions to store water, increasing in this way the amount of peridomiciliary breeding habitats available for *Ae. aegypti*, a situation especially seen in the Northern and Northeastern Regions.

Arboviruses control is heavily based on vector control of which larval control is highly employed. Until recently, the organophosphate Temephos® was the only product available for larval control. Nonetheless, many studies have shown that the resistance of *Ae. aegypti* to Temephos® is widespread in Brazil[13,15] what affects vector control efficacy.

These results prompted the Brazilian Ministry of Health, through the Secretaries of Health Surveillance, to seek new alternatives for vector control including the use of the entomopathogenic bacteria *Bti*. *Bti* is a larvicide safe for use in aquatic environments including containers for storing water for human consumption[28,34].

In this study, we compared the persistence of Temephos® and, Vectobac G® and Vectobac WDG® two formulations of *Bti* commercially available in field and semi-field tests.

Sun exposition and persistence

Our results showed that, despite the previous registered resistance of *Ae. aegypti* for Temephos® in Caicó (RR95 of 12.5[15]), Temephos® remained the larvicide with the highest residual effect. It was also found that the persistence of the larvicide products in containers located indoors in shaded areas is higher than in containers located in sun-exposed areas outdoors. As previously observed, persistence varied depending on the degree of sun exposure (that has to do with temperature and direct sunlight incidence). Generally, Temephos® persistence in polyethylene water storage tanks was higher probably due to a lower light incidence, since these containers remained covered throughout our eight-week tests. Mortality due to Temephos® at 1 ppm in laboratory conditions from a F1 field caught *Ae. aegypti* population was >70% for 10 to 11 weeks in a shaded area and only for four weeks in an external area[15]. The negative influence of sunlight on *Bti* persistence in the environment was also observed for Aedes when >70% mortality was reported in the second week after application and zero mortality was seen in the fourth week[35]. *Bti* was previously observed to have higher persistence in shaded areas compared with sun-exposed areas[22,29]. Our tests followed these previous findings showing Temephos® persistence with 100% mortality during eight weeks in all containers located indoors in shaded areas, while outdoor tests resulted in persistence that ranged from two weeks in 50L plastic buckets to eight weeks in 50L ceramic pots. For the Vectobac G® and Vectobac WDG® *Bti* formulations, >80% mortality was observed in the weeks 3 to 7 indoors and in the weeks 1 to 2 outdoors.

Container type and persistence

Temephos® and type of container

Some studies indicate that container type is related to persistence for both Temephos® and *Bti* formulations[36,37].

Plastic buckets, placed outdoors amidst the vegetation in sun-exposed or semi-sun exposed conditions, showed a persistence of 90 days with 100% mortality to 1 ppm Temephos® for *Ae. aegypti* larvae collected in the field[36]. Tires and tin metal containers however, showed >80% mortality for 15 and 30 days, respectively, after application of 1 ppm Temephos®[36]. Tires, gallons and cans located outdoors still had larvae at 7, 21 and 35 days, respectively, after 1 ppm Temephos® application[36]. Cement tanks located indoors in shaded or semi-shaded conditions, showed no *Ae. aegypti* recolonization until 14 weeks after 1 ppm Temephos® application[37]. Persistence observed at the week 8 of indoor evaluation in our study was also high, regardless of type of container used. However, high persistence was not observed outdoors, in which 50L plastic buckets showed low persistence, probably due to the container type of material associated with environmental high temperatures.

Bti formulations and type of container

Results from the literature show a large variation for persistence for *Bti* formulations with higher persistence results seen at lower temperatures[14,31,38]. Nonetheless, results comparisons from the literature are hampered due to variations in larvicide formulations, concentrations and the containers used.

Tanks made of concrete showed >80% mortality for *Ae. aegypti* larvae for 11 weeks with two *Bti* formulations Vectobac WG® and Vectobac DT (325 mg/ L)[38]. Ceramic and glass containers had >80% mortality for six and five weeks, respectively, after 6,000 ITU/ L Vectobac WDG® application[31]. Cement, asbestos, plastic and brass containers had > 80% mortality for one to three weeks after 20 mg/ L VectobacG®14. Results presented here are similar with 50 L plastic buckets, ceramic pots or polyethylene water storage tanks presenting >80% mortality for one to seven weeks after 20 mg/L VectobacG® and 2 mg/L VectobacWDG® applications.

Field tests

In the field tests, the percentage of recolonized containers was much higher in Boa Passagem, where Vectobac G® and Vectobac WDG® *Bti* formulations were applied, in comparison with the area where Temephos® was applied (Walfredo Gurgel). These results indicate that although Vectobac G® and Vectobac WDG® *Bti* formulations may have an effect for immediate *Ae. aegypti* infestation reduction, this is dependent on the type of container and indoor/ outdoor location. Reinfestation after *Bti* application can occur rapidly due to the low persistence of *Bti* larvicides, especially on sun-exposed areas. Contrarily, we noticed higher elimination of *Ae. aegypti* infestation in containers where Temephos® was applied. It implies that even in Caicó, an area where Temephos resistance was observed, Temephos resistance was not high enough to *Bti* formulations and remained the product with the highest persistence.

Many containers, mostly those used for temporary storage as plastic buckets, ceramic pots and metallic drums, were washed or no longer used by residents and were discarded from the study. This finding is in line with the recommendation of the PNCD to treat only containers that cannot be eliminated, particularly large ones.

During a national resistance sampling monitoring commissioned by PNCD, Temephos® resistance was detected in Caicó[15]. For this reason, Temephos was recommended to be substituted by *Bti*. In Caicó, the shift of Temephos® for *Bti* was done soon after resistance been observed, what happened in 2003. Nowadays, as other municipalities of Brazil, Caicó uses pyriproxyfen.

Although Temephos® has shown a better performance when compared to *Bti*, the persistence was considered low especially in sun-exposed containers, demonstrating that Temephos® no longer had the expected and necessary effect for control (effective time of 60 days instead of 90 expected days).

The results described in the current study were obtained in 2004 and were delivered to the PNCD in form of Technical Report but notwithstanding remained unpublished. However, the scarce studies evaluating larvicide products for vector control directly in the field, make the results presented here still of importance, especially in a crucial moment for vector surveillance and control in Brazil. In fact, product persistence comparison in real field conditions is a demand from the control service that should be accomplished as routine and the methodology here described can be used with this aim.

Currently, several efforts for the involvement of the society in the fight against the mosquito vector have been undertaken in Brazil. One initiative, based on partially successful programs in Asia, is the proposition for each resident to take 10 minutes a week to check and remove any object that can hold water in their domiciles (Lima et al: unpublished data). This initiative, if massively adopted may result in a major contribution to the control of mosquito vectors, with a decrease of case number and changes in the recent history of severe mosquito transmitted epidemic in Brazil.

The concomitant dengue, chikungunya and Zika arboviruses epidemic that Brazil has been facing, evidence that sanitation, education and mobilization of the society are fundamental factors to the advance in the mosquito transmitted diseases control. The need for continuous evaluating for other vector control alternatives such as juvenile hormone analogous and chitin synthesis inhibitors, to name a few, is also paramount.

CONCLUSIONS

Under the conditions evaluated by us, Vectobac G® and Vectobac WDG® *Bti* formulations displayed lower persistence in comparison to Temephos® in all situations tested, field and semi-field, indoors and outdoors, for big and small plastic, ceramic and glass containers, reinforcing the necessity of product evaluation in the specific local area of use before its implementation, mainly due to the high variable climatic and environmental conditions prevailing in Brazil. Moreover, the low persistence of *Bti* and Temephos® in plastic buckets is of major concern, since this is the type of container mostly used by the Brazilian population and that can be used as breeding habitat by *Ae. aegypti*.

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