Journal of Advances in Medicine and Medical Research

24(10): 1-9, 2017; Article no.JAMMR.38224
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Comparative Effectiveness of Chemical, Biological or Combination of Both Mosquito Larvicides for Dengue Control in George Town, Penang, Malaysia – A Field Trial

Wee Loon Chin[1], Surajudeen Abiola Abdulrahman[1*] and Abdul Rashid[1]

[1]Department of Public Health Medicine, Penang Medical College, George Town, Penang, Malaysia.

Authors' contributions

This work was carried out in collaboration between all authors. Authors WLC, SAA and AR designed the study. Author WLC performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WLC and SAA managed the analyses of the study as well as the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2017/38224
Editor(s):
(1) Chris Ekpenyong, Department of Human Physiology, College of Health Sciences, University of Uyo, Nigeria.
Reviewers:
(1) Simon Pius, University of Maiduguri Teaching Hospital, Nigeria.
(2) Suguna Dumpala, Kajoli Narayana University of Health Sciences, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/22308

Original Research Article

Received 18th November 2017
Accepted 11th December 2017
Published 15th December 2017

ABSTRACT

Background: Tackling the increasing trend of the dengue menace remains one of the most challenging tasks in public health medicine. Extensive literature search revealed no previous community intervention trial done in Penang and Malaysia to compare effectiveness of the use of various mosquito larvicides based on container indices as the definitive measurable outcomes. The study aim was to identify the most effective mosquito larvicidal measure for dengue control in dengue-sensitive areas in George Town, Penang.

Methods: A field trial was conducted in three localities randomly selected from a list of 33 dengue-sensitive areas. Area A was treated with both chemical and biological larvicide, Area B was treated with biological larvicide and Area C was treated with chemical larvicide as a standard preventive measure. Container indices (CI) were obtained weekly for eight consecutive weeks and data was analyzed using Repeated Measures ANOVA test in SPSS version 21.

**Corresponding author: E-mail: abdulsuraj@gmail.com, surajudeen@pmc.edu.my;*

Results: Comparative effectiveness of larvicidal measures between the three areas remained inconclusive as the p-value could not be computed between the three areas due to termination of iteration on ‘between-areas’ factor, although descriptively, better vector control was achieved through monotherapy with biological larvicide alone. Area A’s CI showed a drastic drop from 1.79 to 1.05 for the first two weeks, zero for weeks 3-5, 1.67 at week 6 and reducing to zero for the last two weeks. Area B’s CI showed an initial increase from 1.05 to 1.43 for the first two weeks and subsequently reduced to zero and remained zero until week 8. Area C’s CI showed zero reading for the first two weeks and by week 3, it went up to 1.00 before it reduced to and remained zero until week 8.

Conclusion: Monotherapy with biological larvicide alone seemed to offer better vector control, although statistical evidence of its superiority remained inconclusive. In view of this, integrated vector management should be enhanced by harnessing community participation to curtail dengue infections, irrespective of the choice of larvicide used for vector control.

Keywords: Abate; Aedes; effectiveness; larvicide; Vectobac; dengue.

1. INTRODUCTION

Despite extensive vector control measures, combating the dengue menace remains a huge challenge to public health officials, particularly in over 100 countries including Malaysia, where dengue is endemic. Climate change and development of *Aedes* mosquito resistance against insecticides create difficulty in vector control measures [1].

In Malaysia, the average annual incidence of dengue has been consistently in excess of 125 per 100,000 population since 2002, although a 2-3 fold increase in annual incidence was reported since 2014 [2]. In George Town, Penang, the number of confirmed dengue cases increased by 164% from 2014 to 2015 but decreased by 77% from 2015 to 2016 based on the internal records by the Environmental Health Unit, Penang Island City Council.

General control measures such as adulticiding (fogging) and larviciding remained the mainstay of vector control management throughout the world for dengue fever. Globally, larvicidal chemical and biological control methods have been used extensively with varying degrees of success in control of dengue-transmitting mosquitoes.

Abate® (Temephos), an organophosphate which has been used for many years, remains effective in eradicating larvae, but resistance to Temephos is beginning to develop in recent years [3]. Worldwide, the principal larvicide used in the control of dengue vectors is temephos which belongs to the organophosphate chemical group. In Malaysia, it has been used since the first dengue fever outbreak in 1973. It is added in potable containers as 1% (w/w) sand granules to control breeding in stagnant waters. This formulation is available as 100 g sachets for consumer purchase and it is also distributed by the local authorities to residents of infected dengue premises. Abate is also available in the form of aqueous dilution of 50% EC (Emulsifier Concentrate) and sprayed in larvae breeding sites. Temephos acts directly on the central nervous system of the larvae by inhibiting cholinesterase causing larvae death.

Vectobac® (*Bacillus thuringiensis israeliensis* - *Bti*), a biological larvicide made in the form of bacterial protein-complex in dry form is now commonly used. *Bti* produces complex crystal proteins known as protoxins during sporulation. Vectobac is developed in two formulations; the Aqueous Suspension (AS) and Water-dispersible Granules (WG) versions, both of which have been found to be effective in larviciding [4-11]. While Abate granules can only be effective in water, Vectobac can be used in water and also on dry surface, where the bacterial-protein complex will be activated after coming in contact with water. The larvae then ingest the bacteria as larvae food. Once ingested, the bacteria then destroys the larvae’s gut and cause larvae death. Since Vectobac is a fairly newer product compared to Abate, resistance is not as well documented as Abate. In a study conducted in Shah Alam, Selangor, Malaysia, Loke et al. [3] found that *Aedes* larvae was resistant to temephos in one site but showed uniform susceptibility to Vectobac (*Bti*) in another site.

It is not surprising that intensive use of Abate since the 70s has caused resistance of the larvae to it, requiring the use of new larvicides [12]. Regardless of its established benefits in vector control, increasing resistance to Abate temephos remains a significant concern for dengue control programmes. Similarly, the efficacy of Vectobac remains threatened by sunlight and temperature variability, water volume and depth, bioavailable concentration, organic pollution and emergent vegetation [13-21].

Notwithstanding, Vectobac continues to show promising results and is much lighter in weight compared to Abate [22-24]. Vectobac not only reduces larvae density but also causes a significant reduction in *Aedes* populations [25]. Essentially, it causes biochemical destruction of the gut walls of the mosquito larvae following its ingestion during feeding. Its success as a biological larvicide paved the way for different formulations of Vectobac in order to achieve better larvae control measures [26].

Although chemical and biological larvicides have been reported to independently have significant effectiveness in dengue control [27], the comparative effectiveness of the usage of chemical, biological or both types of larvicides in a field trial is not widely done in Malaysia. Knowing the effectiveness of the different types of larvicides would help in the control of dengue infection by using cost effective control measures which are based on scientific evidence.

The objective of this study was to identify the most effective method between chemical, biological or combination of both *Aedes* larvicidal control measured by the reduction of container indices among the dengue-sensitive areas in George Town, Penang.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This was a 3-arm intervention study (open label, field trial) conducted in three randomly-selected sensitive areas of Georgetown, Penang. Chemical larvicide (subsequently used interchangeably with the term “Abate”), biological larvicide (subsequently used interchangeably with “Vectobac” or *Bacillus thuringiensis israelensis* or *Bti*) and both biological and chemical larvicides were applied in the three selected areas and the larvae index in the study areas were estimated every week during the 8-week study period (August and September 2016).

George Town is part of the North-East District of Penang, Malaysia. It is the capital of Penang, one of the 14 states in Malaysia. George Town is an urban area with pockets of villages and slump areas in certain vicinities and has high reported dengue outbreaks over the years. From the list of 33 sensitive areas within the George Town city limit boundary as identified by the Vector-Borne Disease Control (VBDC) unit, Ministry of Health Malaysia, three areas were randomly selected. A sensitive area is defined by the Ministry of Health Malaysia as a locality with high dengue cases, reflected by high incidence and prevalence, previous outbreak(s) and registered cases, and reported but unconfirmed cases. The three areas were labelled Areas A, B and C and had a size of approximately 1.2, 10.4 and 3.1 hectares, respectively. The three areas were approximately equal in terms of population characteristics, with an average of about 5,000 residents in each area (Area A = 3060, Area B = 6135, Area C = 5040 residents).

2.2 Study Procedures

The three localities were randomized to receive the intervention of either Abate, a chemical larvicide (taken as standard preventive measure), or Vectobac, a biological larvicide or both chemical and biological larvicides.

The localities were sprayed with the respective planned method weekly for eight weeks duration. Abating is defined as the standard conventional method of applying the Abate larvicide using the spray canister. In view of the fact that it was unethical to have a control area with no intervention at all, the standard conventional method of abating was taken as the control or baseline area. Standard preventive measure is defined as applying Abate 50EC 1L sprayed via a canister in the outdoor environment of the studied area, including receptacles such as containers and tyres which may act as potential breeding sites for mosquito larvae.

Vectobac was used according to the guidelines provided by the manufacturer, and the standard dilution guidelines for types of areas covered by spraying. For the Vectobac-only study area, a mist blower was used to cover common areas and areas deemed difficult to reach by humans. As recommended by the product manufacturer [28], Vectobac was used via direct application or ground spray application. Vectobac was applied directly (undiluted) to water reservoirs such as earthen and cement jars, plastic or metal drums, and fiberglass or cement tanks with volume capacities of more than 50 L. Application rates of 2-8 g per 1000L of container capacity (100-400 mg/50 L) was used depending on habitat conditions. Application was made with a calibrated scoop or spoon.

For ground spray application, the ground aqueous spray mix applications were targeted to natural and artificial larval habitats which were less than 50L in volume capacity that cover a wide area such as covered and uncovered concrete drainage systems, trash accumulations, tires, roof gutters, vegetation such as leaf axils, tree holes, leaf litter and ground pools.

The recommended dosage in a spray mix of 250-500 g of Vectobac per hectare of target larval habitats was used. The required amount of Vectobac was mixed with an amount of water that provided complete and even coverage of the intended target area. The required amount of water depended on sprayer calibration and habitat conditions. The recommended dilution rates that were used for Vectobac per hectare are listed in Table 1.

Table 1. Specific dilution rates for the two different methods of application of Vectobac

Methods of application	Recommended dilution rate
Mist blower	125 g vectobac in 12 L of water
Direct application	8 g in 1000 L of water

Source: Recommendation by product manufacturer Valent Biosciences Corporation in the Technical Use Bulletin for Vectobac WG for Dengue Vector Control in Asia, May 2007 edition [28]

Vehicle-mounted, backpack or shoulder carried motorized sprayers and hand carried pump sprayers were used to generate aqueous Vectobac spray droplets for complete and even coverage of the intended area. The spray was directed to evenly cover the larval habitat and maximize spray penetration of vegetative canopy. For optimal Vectobac ground spray applications, they were applied during cool hours of the day when it was not raining.

For both the Abate and Vectobac combined treatment group, spraying abate with the canister and also spraying Vectobac with the mist-blower were used together in the particular area as per routine vector control measures. Both Abate and Vectobac were applied concurrently on the same day and at the same time by the same team.

Although Vectobac ground spray application is usually monitored by measuring Aedes adult population with ovitrap surveillance, however using the standard practice of the Penang State's Department of Environmental Health and Licensing unit, Container Index (CI), which was calculated weekly each time spraying was done per week in all the three areas for eight weeks, was taken as proxy [27]. CI is defined as the number of containers positive for larvae out of every 100 containers surveyed during that particular operation day.

Efficacy and residual control were monitored by counting the numbers of larvae or pupae in treated containers before and after treatment. Initial larvae or pupae reduction is expected within 72 hours. However, due to the limitation in the number of manpower for this study, the CI was calculated weekly, instead of every 3 days duration.

CI was measured and recorded on specialized data recording forms on a weekly basis for eight weeks by trained research assistants, to determine the larvae breeding sites in the study areas.

2.3 Data Analysis

Data was presented descriptively in a figure. Repeated measures ANOVA was performed to determine statistical significance. Significance level for statistical tests was set at <0.05.

2.4 Ethical Considerations

Vectobac is approved for usage and recommended by WHOPES (WHO Pesticide Evaluation Scheme) in the report of the seventh WHOPES Working Group Meeting on 2-4 December 2003 in Geneva (28). It is considered safe for humans and animals if used according to the guidelines and recommendations set by the manufacturer. Ethical approval was obtained from the Joint Penang Independent Ethics Committee (JPEC) prior to commencement of the study (ethical approval number: JPEC 03-16-0044).

3. RESULTS

Overall, we checked a total of 2,237 containers for larvae and/or pupae in the three areas over 8 weeks, out of which 10 containers turned out positive for larvae breeding. Fig. 1 and Table 2 provide a summary of the CI for the three areas over the study period. The descriptive analysis showed that the CI in Area A where both chemical and biological larvicides were used showed a drastic drop from 1.79 to 1.05 for the first two weeks, zero for weeks 3-5, 1.67 at week 6 and reducing to zero for the last two weeks. The CI for Area B where only biological larvicide was used showed an initial increase from 1.05 to 1.43 for the first two weeks and subsequently reduced to and remained zero until week 8. The CI for Area C where only chemical larvicide was used showed zero reading for the first two weeks and by week 3, it went up to 1.00 before it reduced to and remained zero until week 8. Although there appeared to be a difference in the trend or pattern of the CI in the three areas, it was not found to be statistically significant within areas (p = 0.326) and the p-value could not be computed for mean difference in CI between the three areas over time (Table 2). The data points were perhaps too few, such that meaningful comparison (based on p-value) of 'between-subjects effect' or 'between-areas effect' could not be achieved.

4. DISCUSSION

To the best of our knowledge, this study is the first field trial on comparative effectiveness of larvicides used for vector control in Malaysia. In view of the unavailability of the dengue vaccine in Malaysia as yet, we envisaged that the findings from this study will provide evidence to suggest better efficiency and perhaps cost-effectiveness in the current vector control plans implemented by the local authorities, with the adoption and widespread usage of the most efficacious larvicides for dengue control. Although integrated vector management remains the mainstay of vector control and dengue prevention, the success of this approach is contingent upon use of highly potent larvicides as well as enhanced community participation.

Descriptive findings from this study were consistent with Lee et al's. [25] observation in a study that was conducted in a suburban residential area and a temporary settlement site in another state in Malaysia, where based on ovitrap surveillance, a significant reduction in *Aedes* populations was evident 4 weeks after initiating the first biological treatment using Bti. The ovitrap index (OI) and the larvae density decreased drastically in both trial sites [25]. The results for chemical only and combination of chemical and biological larvicides in the current study were also in line with a systematic review done by George et al. [27] whereby when applied as a single intervention, temephos was found to be effective at suppressing entomological indices, however, the same effect had not been observed when temephos was applied in combination with other interventions. The authors reported no evidence to suggest that temephos use was associated with reductions in dengue transmission [27]. A study done in Singapore also demonstrated larviciding spraying with *Bti* of natural breeding sites was able to reduce *Aedes albopictus* density which was not observed in conventional manual application

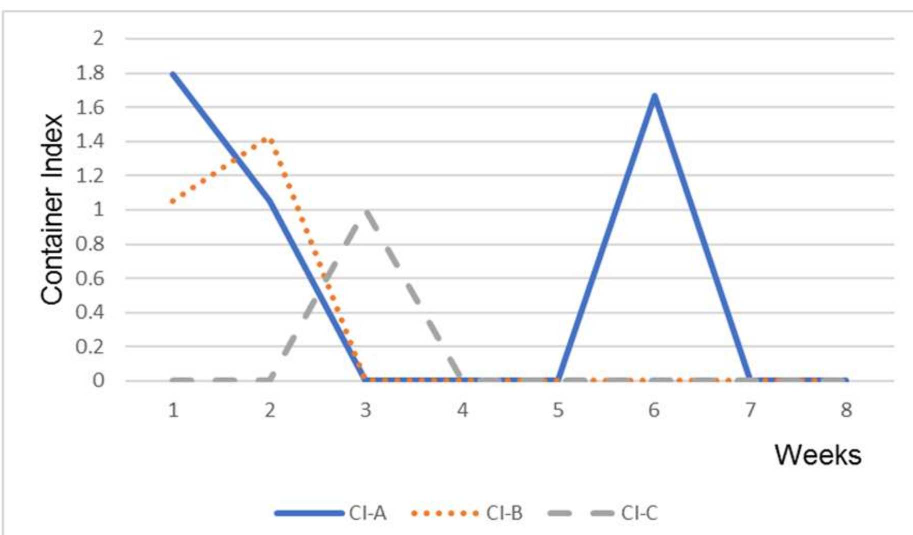

Fig. 1. Graph of container indices in localities A, B, C over 8 weeks

Table 2. Summary table of repeated measures ANOVA comparing effectiveness of chemical, biological or both larvicides over 8 weeks period

Areas	Mean (95% CI)	Group[a]	Time	GroupxTime[a]
A (Both)	0.56 (-0.11, 1.24)	-	0.326	-
B (Biological only)	0.31 (-0.18, 0.80)			
C (Chemical only)	0.13 (-0.17, 0.42)			

[a]*p-values could not be computed due to termination of iteration on between-subjects factor*

methods such as weekly oiling in all possible ground larval habitats and monthly treatment of permanent water bodies with temephos sand granules [29].

The design of this study and its potential to demonstrate comparative efficacy of the treatments, as well as unearth causal relationship between treatments and outcome, was its major strength. However, the time constraint of the study duration limited longer observation and CI data collection, and is perhaps an important reason for the lack of conclusive statistical evidence to demonstrate superiority of any of the treatments. Another possible explanation could be the relatively low number of dengue cases during the 8 weeks period when this study was conducted (August and September 2016) [30].

A more comprehensive observation over a longer period of time may have resulted in a better outcome in terms of yielding statistical estimates of effect sizes needed to provide conclusive evidence of comparative efficacy of the larvicides between the three areas. We recommend more comprehensive studies covering a much wider area of George Town, Penang and to include as many measurable or recordable variables as possible such as the average temperature, average wind speed, the Air Pollutant Index (API), the humidity level of the areas studied and the frequency of preventive activities such as community efforts to maintain a “dengue-free” environment in the areas studied, as these factors could have influenced the CI findings.

5. CONCLUSION

This study revealed inconclusive statistical evidence in Aedes larvae reduction rates between chemical, biological or combination of both larvicides, although previous studies have demonstrated better vector control with Bti. In view of this finding, there is no evidence to undermine the current practice of larviciding in George Town, Penang or to enhance it with the combination of both larvicides.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Environmental Health and Licensing, Penang Island City Council for the assistance rendered in data collection. We acknowledge Dr. Mohammad Mostaque Hossain Ansari for his contribution to the conception of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wiwanitkit S. Climate change, environmental temperature change, and resistance to insecticides of dengue mosquito. Revista Panamericana de Salud Pública. 2013;34(5):366-366.
   Available:http://www.ncbi.nlm.nih.gov/pubmed/24553767
2. Mohd-Zaki AH, Brett J, Ismail E, L'Azou M. Epidemiology of dengue disease in Malaysia (2000–2012): A systematic literature review. PLoS Neglected Tropical Diseases. 2014;8(11):e3159.
   DOI: 10.1371/journal.pntd.0003159

3. Loke SR, Andy-Tan WA, Benjamin S, Lee HL, Sofian-Azirun M. Susceptibility of field-collected *Aedes aegypti* (L.) (Diptera: Culicidae) to *Bacillus thuringiensis* israelensis and temephos. Tropical biomedicine. 2010;27(3):493-503.

Available:http://www.ncbi.nlm.nih.gov/pubmed/21399591

4. Bassi DG, Weathersbee AA, Meisch MV, Inman A. Efficacy of Duplex* and Vectobac* against *Psorophora columbiae* and *Anopheles quadrimaculatus* larvae in Arkansas ricefields. Journal of the American Mosquito Control Association. 1989;5(2):264-6.

Available:http://www.ncbi.nlm.nih.gov/pubmed/2568399

5. Brown MD, Thomas D, Watson K, Kay BH. Laboratory and field evaluation of efficacy of VectoBac (R) 12AS against *Culex sitiens* (Diptera: Culicidae) larvae. Journal of the American Mosquito Control Association-Mosquito News. 1998;14(2): 183-5.

Available:http://www.ncbi.nlm.nih.gov/pubmed/9673920

6. Amalraj DD, Sahu SS, Jambulingam P, Doss PB, Kalyanasundaram M, Das PK. Efficacy of aqueous suspension and granular formulations of *Bacillus thuringiensis* (Vectobac) against mosquito vectors. Acta Tropica. 2000;75(2):243-6. Available:http://www.ncbi.nlm.nih.gov/pubmed/10708664

7. Karch S, Manzambi ZA, Salaun JJ. Field trials with vectolex (*Bacillus sphaericus*) and vectobac (*Bacillus thuringiensis* (H-14)) against *Anopheles gambiae* and *Culex quinquefasciatus* breeding in Zaire. Journal of the American Mosquito Control Association. 1991;7(2):176-9.

Available:http://www.ncbi.nlm.nih.gov/pubmed/1895075

8. Riley CM, Fusco R. Field efficacy of Vectobac-12AS and Vectobac-24AS against black fly larvae in New Brunswick streams (Diptera: Simuliidae). Journal of the American Mosquito Control Association. 1990;6(1):43-6.

Available:http://www.ncbi.nlm.nih.gov/pubmed/2324724

9. Sulaiman S, Pawanchee ZA, Wahab A, Jamal J, Sohadi AR. Field evaluation of Vectobac G, Vectobac 12AS and Bactimos WP against the dengue vector *Aedes albopictus* in tires. Journal of vector ecology: journal of the Society for Vector Ecology. 1997;22(2):122-4.

Available:http://www.ncbi.nlm.nih.gov/pubmed/9491362

10. Yap HH, Chong AS, Adanan CR, Chong NL, Rohaizat B, Malik YA, et al. Performance of ULV formulations (Pesguard 102/Vectobac 12AS) against three mosquito species. Journal of the American Mosquito Control Association. 1997;13(4):384-8.

Available:http://www.ncbi.nlm.nih.gov/pubmed/9474567

11. Yap HH, Lee YW, Zairi J. Indoor thermal fogging against vector mosquitoes with two *Bacillus thuringiensis* israelensis formulations, Vectobac ABG 6511 water-dispersible granules and Vectobac 12AS liquid. Journal of the American Mosquito Control Association 2002;18(1):52-56. Available:http://www.ncbi.nlm.nih.gov/pubmed/11998931

12. Ponce GG, Flores AE, Badii MH, Rodríguez-Tovar ML, Fernández-Salas I. Laboratory evaluation of Vectobac as against *Aedes aegypti* in Monterrey, Nuevo Leon, México. Journal of the American Mosquito Control Association. 2002;18(4):341-3.

Available:http://www.ncbi.nlm.nih.gov/pubmed/12545970

13. Aldemir A. Initial and residual activity of Vectobac® 12 AS, Vectobac® WDG, and Vectolex® WDG for control of mosquitoes in Ararat Valley, Turkey. Journal of the American Mosquito Control Association. 2009;25(1):113-6.

DOI: 10.2987/08-5836.1

14. Benjamin S, Rath A, Chiang YF, Lim LH. Efficacy of a *Bacillus thuringiensis* israelensis tablet formulation, VectoBac DT®, for control of dengue mosquito vectors in potable water containers. Southeast Asian Journal of Tropical Medicine and Public Health. 2005;36(4): 879-892.

Available:http://www.ncbi.nlm.nih.gov/pubmed/16295540

15. Chmela J, Mazánek L, Nakládal Z, Pesáková L, Halirová R. Effectiveness of aerial application of VectoBac G larvicide granules against mosquitoes in the

Olomouc region in spring 2006. Epidemiologie, mikrobiologie, imunologie: Casopis Spolecnosti pro epidemiologii a mikrobiologii Ceske lekarske spolecnosti JE Purkyne. 2007;56(2):78-87.

Available:http://www.ncbi.nlm.nih.gov/pubmed/17593805

16. Christiansen JA, McAbee RD, Stanich MA, DeChant P, Boronda D, Cornel AJ. Influence of temperature and concentration of VectoBac on control of the salt-marsh mosquito, *Ochlerotatus squamiger*, in Monterey County, California. Journal of the American Mosquito Control Association. 2004;20(2):165-70.

Available:http://www.ncbi.nlm.nih.gov/pubmed/15264626

17. Floore TG, Petersen JL, Shaffer KR. Efficacy studies of Vectobac 12as and Teknar HP-D larvicides against 3rd-instar Ochlerotatus taeniorhynchus and *Culex quinquefasciatus* in small plot field studies. Journal of the American Mosquito Control Association. 2004;20(4):429-33.

Available:http://www.ncbi.nlm.nih.gov/pubmed/15669386

18. Flores AE, Garcia GP, Badii MB, Tovar ML, Salas IF. Effects of sublethal concentrations of Vectobac (R) on biological parameters of *Aedes aegypti*. Journal of the American Mosquito Control Association. 2004;20(4):412-7.

Available:http://www.ncbi.nlm.nih.gov/pubmed/15669383

19. Lima JB, Melo NV, Valle D. Persistence of Vectobac WDG and Metoprag S-2G against *Aedes aegypti* larvae using a semi-field bioassay in Rio de Janeiro, Brazil. Revista do Instituto de Medicina Tropical de São Paulo. 2005;47(1):7-12.

DOI: /S0036-46652005000100002

20. Russell TL, Brown MD, Purdie DM, Ryan PA, Kay BH. Efficacy of VectoBac (*Bacillus thuringiensis* variety israelensis) formulations for mosquito control in Australia. Journal of economic entomology. 2003;96(6):1786-91.

Available:http://www.ncbi.nlm.nih.gov/pubmed/14977116

21. Toma L, Severini F, Bella A, Romi R. A semifield evaluation of Vectobac DT (ABG-6499), a new formulation of *Bacillus thuringiensis* israelensis for control of *Aedes albopictus*. Journal of the American Mosquito Control Association. 2003;19(4):424-9.

Available:http://www.ncbi.nlm.nih.gov/pubmed/14710747

22. Boudjelida H, Aïssaoui L, Bouaziz A, Smagghe G, Soltani N. Laboratory evaluation of *Bacillus thuringiensis* (Vectobac WDG) against mosquito larvae, Culex pipiens and *Culiseta longiareolata*. Communications in agricultural and applied biological sciences. 2008;73(3):603-9.

Available:http://www.ncbi.nlm.nih.gov/pubmed/19226801

23. Kahindi SC, Midega JT, Mwangangi JM, Kibe LW, Nzovu J, Luethy P, Githure J, Mbogo CM. Efficacy of vectobac DT and culinexcombi against mosquito larvae in unused swimming pools in Malindi, Kenya. Journal of the American Mosquito Control Association. 2008;24(4):538-42.

DOI: 10.2987/5734.1

24. Setha T, Chantha N, Socheat D. Efficacy of *Bacillus thuringiensis* israelensis, VectoBac® WG and DT, formulations against dengue mosquito vectors in cement potable water jars in Cambodia. Southeast Asian Journal of Tropical Medicine and Public Health. 2007 Mar 1;38(2):261-268.

Available:http://www.ncbi.nlm.nih.gov/pubmed/17539275

25. Lee HL, Chen CD, Masri SM, Chiang YF, Chooi KH, Benjamin S. Impact of larviciding with a Bacillus thuringiensis israelensis formulation, Vectobac WG®, on dengue mosquito vectors in a dengue endemic site in Selangor state, Malaysia. Southeast Asian Journal of Tropical Medicine and Public Health. 2008;39(4):601-9.

Available:http://www.ncbi.nlm.nih.gov/pubmed/19058596

26. Djènontin A, Pennetier C, Zogo B, Soukou KB, Ole-Sangba M, Akogbéto M, et al. Field efficacy of Vectobac GR as a mosquito larvicide for the control of Anopheline and Culicine mosquitoes in natural habitats in Benin, West Africa. PloS One. 2014;9(2):e87934.

DOI: 10.1371/journal.pone.0087934

27. George L, Lenhart A, Toledo J, Lazaro A, Han WW, Velayudhan R, et al. Community-effectiveness of temephos for

dengue vector control: A systematic literature review. PLoS Neglected Tropical Diseases. 2015;9(9):e0004006.

DOI: 10.1371/journal.pntd.0004006

28. VectoBac®. Technical Use Bulletin for VectoBac WG For Dengue Vector Control in Asia; 2007.
29. Lam PH, Boon CS, Yng NY, Benjamin S. *Aedes albopictus* control with spray application of Bacillus thuringiensis israelensis, strain AM 65-52. Southeast Asian Journal of Tropical Medicine and Public Health. 2010;41(5):1071-1081.

    Available:http://www.ncbi.nlm.nih.gov/pubmed/21073027
30. Vector Control Unit, Environmental Health Section, City Council of Penang Island. Dengue status report; 2016.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22308