INSTITUTE FOR MEDICAL RESEARCH

IMR esearch

HIGHLIGHT

ISSUE : 02/2016 | MOH/S/IMR/62.16 (RR)

image source : https://commons.wikimedia.org/wiki/File:Aedes_aegypti_feeding.jpg

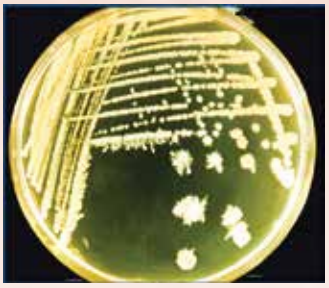
USE OF BACILLUS THURINGIENSIS ISRAELENSIS (Bti) IN DENGUE CONTROL

Dengue, an *Aedes* mosquito-borne viral disease, has become a serious public health threat causing morbidity and mortality, as well as a burden on health services and economies. The two main mosquito species identified in Malaysia to cause dengue fever are *Aedes aegypti* and *Aedes albopictus*.

WHO (2004) has recommended an Integrated Vector Management (IVM) approach to successfully control vectors of dengue. This includes:

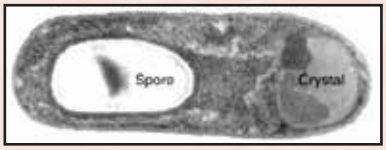
- Integrated vector control technology (e.g., use of chemical insecticides and biological agents).
- Social mobilization, advocacy, legislation, (e.g., Destruction of Disease Bearing Insects Act, 1975 and community participation)
- Intersectoral cooperation (e.g., Ministry of Health and Ministry of Housing).
- Capacity building (e.g., training).
- Evidence-based decision-making in vector control (e.g., detection of insecticide resistance and decision to select appropriate control agents).

Bacillus thuringiensis var *israelensis* (Bti), also known as *Bacillus thuringiensis* H-14, is a bacterium, first isolated in 1975, that is highly and specifically toxic to the larvae of all mosquito species, blackfly and the chironomids, yet environmentally friendly to all other non-target organisms. The use of Bti as a biological agent in dengue control was extensively investigated and tested to ascertain its effectiveness (Lee *et al.*, 2008; Lam *et al.*, 2010; Tan *et al.*, 2012).



Bti colonies on agar culture

Bacilus thuringiensis* serovar. *israelensis



Ultrastructure of Bti (Source : Canan Usta, DOI : 10.5772/55786)

Key Messages

- ***Bacillus thuringiensis israelensis*** (Bti) exhibits high larvicidal activity.
- There is a need to include the use of Bti in dengue vector control programmes and train applicators on its appropriate usage in terms of formulation, dosage and methods of application.
- Bti can be applied through space spraying using mist-blower, ultra-low-volume generator or thermal fogger, or through direct application into breeding habitats.
- Larvae of both *Ae aegypti* and *Ae albopictus* are highly susceptible to Bti at dosages of 250g-500g per hectare for spray application and 2-8 mg per litre for direct application.
- Bti is effective in interrupting dengue transmission when applied at weekly intervals for 4 continuous weeks, or until the ovitrap index is less than or equal to 10%, followed by biweekly intervals until the treated area is dengue free.
- Most appropriate Bti formulation is the wettable or water-dispersible granule formulation although the aqueous suspension formulation can also be used.
- Dengue transmission may be interrupted if there is complete Bti treatment coverage of larval habitats at target sites.
- Bti mixed with chemical insecticides exert simultaneous larviciding and adulticiding activities.
- There has been no evidence of mosquito resistance to Bti for the last 30 years of its use globally.

Strategies used in vector control are directed both at the larval and adult stage of the Aedes mosquito. For larval control, the chemical larvicide temephos is directly introduced into containers to kill the larvae, while space spraying (fogging) of chemical insecticides is used to control the adult mosquito population. The success of these measures is, however, limited by the low coverage of chemical larvicide treatment, transient nature of fogging, development of insecticide resistance in mosquitoes (Loke et al., 2012) and the undesirable impact of chemical insecticides on the environment.

"*Bacillus thuringiensis var israelensis* (Bti), also known as *Bacillus thuringiensis* H-14, is a bacterium, first isolated in 1975, that is highly and specifically toxic to the larvae of all mosquito species, blackfly and the chironomids, yet environmentally friendly to all other non-target organisms.

Mode of Action

The larvicidal activity of Bti is due to the presence of toxins, commonly known as endotoxin, crystal toxin or parasporal inclusions that are produced during sporulation (Lacey, 2007). The crystal toxins are ingested by the larvae, and activated in the alkaline mid-gut of the larvae. The activated toxins are fragmented by the larval gut proteolytic enzymes into polypeptide fragments. The fragments attach to the gut cells, making pores, leading to gut cell lysis and disruption of excretory functions.

At optimal Bti dosage, larval death happens within 30 minutes of ingestion. To date, there have been no reports of field resistance to Bti toxins even after 30 years of continuous use in several mosquito control programmes around the world. Lack of resistance could be due to the nature of the toxin(s) or/and due to independent toxicity of the 4-5 different toxins within one Bti cell.

Aedes aegypti larve killed by Bti

Bti Formulation

Today, Bti is mass produced via *in vitro* fermentation process, using high protein organic substrates. The resulting product, known as primary powder, can be prepared into various formulations such as:

(i) Wettable powder
(ii) Aqueous suspension
(iii) Wettable granules (WG) (also known as water dispersible granules)
(iv) Tablet
(v) Granules (corn-cob)
(vi) Dunks.

Each formulated product has its own potency. The potency is measured by its International Toxic Units (ITU) per mg product against the standard test mosquito, *Ae aegypti*. Higher ITU values indicate higher potency.

The various formulations are designed to act on specific target habitats. Liquid, WG and granule formulations are used to treat wide areas, such as mangrove and fresh-water swamps, habitats among vegetation, and residential areas. Bti tablets and WG are used to treat large volume water storage containers.

Method of Bti Application

Bti can be applied by space spraying or direct application into containers. The choice of method of application is dependent on the type of habitat. Spray application is recommended when the larval habitats are less than 50 litres in volume, are numerous and cover a wide area. Spray application is also recommended for larval habitats which are hard-to-reach using direct application treatment. In spray application, the Bti formulation is suspended in water and mixed to the desired concentration prior to dispensing using conventional spray application equipment such as the mist blower, ultra-low-volume (ULV) generator and thermal fogger.

Direct application of wettable granule or tablet formulation is recommended for larval habitats or containers that hold more than 50 litres of water. Studies and operational programmes have proven that dengue transmission can be interrupted if appropriate Bti formulations are correctly applied with complete coverage of the larval habitats at the target site (Tan *et al.*, 2012; Benjamin *et al.*, 2013).

Combined Application of Bti & Chemical Insecticide

Bti only exerts larvicidal activity and has no adulticidal effect on mosquito. To enhance its efficacy, Bti can be mixed with chemical adulticides, including pyrethroids. The mixture can be space sprayed through cold or thermal foggers to control the adult and larvae simultaneously (Seleena *et al.*, 1999; Seleena *et al.*, 2001).
However, there are limitations in using the mixture applied through vehicle mounted sprayers:

- Adulticiding treatment requires complete coverage of the dengue site, but not larviciding as it specifically targets larval habitats within the dengue site.
- Vehicle mounted sprayers cannot target treatment of larval habitats that are not within the path of the vehicle sprayer.
- Vehicle mounted sprayer also cannot effectively treat covered larval habitats such as covered concrete drainage systems.

Bioefficacy Evaluation of Bti Products Under Laboratory & In Field Conditions

There are several Bti products with different formulations available in the market. Laboratory and field studies were conducted by the IMR to evaluate the bioefficacy of these Bti products for mosquito larval control.

Several large scale field studies have been conducted in Malaysia since 1996 and the results published.
The methods of these studies were replicated in some of the neighbouring ASEAN countries and significant reduction in dengue and malaria vector adult mosquito populations were achieved (Lee *et al.*, 2010). These countries have established dengue and malaria control programmes using the methods that were first evaluated by IMR.

Laboratory Evaluation of Bti Toxicity And Potency

Laboratory evaluation of Bti formulations involved the bioassay of each Bti formulation against a standard Bti reference powder using larvae of *Aedes aeqypti*. The larvae were exposed to various Bti concentrations and the mortality was scored after 24 hours. The data was analysed to obtain the 50% lethal concentration (LC_{50}) value as an indicator of product toxicity. The potency of the formulation is defined in ITU/mg product. This standardization of units of measurement allows the comparison of different Bti formulations in the laboratory.

The following formulations were evaluated against *Ae aegypti* larvae and their potency determined (Lee *et al.*, IMR, unpublished).

Bti Product	*Formulation*	*Country of origin*	LC_{50} (mg/L)	ITU/mg	*Test Mosquito*
BactoPower™	Aqueous	India	17.68	11.14	*Ae aegypti*
VectoBac® WG	Wettable granule	USA	0.039	5,028.67	*Ae aegypti*
BioPower™	Aqueous	Sri Lanka	94.83	2.077	*Ae aegypti*
BioFlash®	Granule	Iran	5.06	54.81	*Ae aegypti*
Wettable powder	Powder	China	0.0194	10,166.39	*Ae aegypti*

The results indicate that among the Bti formulations evaluated, the wettable granule formulation of VectoBac® WG (USA) and wettable powder formulation (Wuhan, China) showed higher toxicity and potency.

Ovitrap with a paddle

Field Evaluations

Field evaluations were conducted to test the bioefficacy of Bti formulations to control dengue vectors using the following procedures :

- Test sites were selected based on pre-established criteria and pre-survey of mosquito density in the selected site.
- Ovitrap Index (OI) was used to determine the *Aedes* species density. OI was measured during the pre- and post-Bti application periods.
- Two study sites were used for the evaluation: treated site and untreated control site.

In disease control trials, the dengue incidence is also monitored along with OI. Such trials are monitored for 12 months. Bti is generally applied using 2 methods, namely, direct application and space spraying. The studies were carried out to determine the most appropriate delivery system and dosages for Bti application.

Direct Application

Dry Bti formulations were evaluated based on direct application in earthen, plastic and HDPE containers. In a trial using this method, a Bti tablet formulation (VectoBac DT) was able to give a minimum of 3 months larvae and pupae control with regular water exchange activity (Benjamin *et al.*, 2005).

For direct application into containers, Bti should be applied at dosages of 2-8 mg per litre in accordance to the manufacturer's recommendation.


A dissolved Bti tablet after direct application into a water container


Water jars used to evaluate bioefficacy of Bti tablet

Space Spraying

Several spray equipments were tested at various locations: car parks, construction sites, residential sites, slum areas and villages. These studies concluded that the backpack mist blower, Stihl SR of 3.5 hp with multi-orifice nozzle, and the vehicle mounted ULV generator, IGEBA or Dynafog of 18 hp with 4 nozzles, were able to give complete coverage of the target site, thereby reducing the *Aedes* species larval population (Lee *et al.*, 1996; Seleena & Lee, 1998).

In a study at a dengue endemic site in Selangor, a Bti wettable granule formulation (VectoBac WG) was sprayed into outdoor larval habitats using the backpack mist blower, Stihl SR 420. The mosquito vector population was monitored using ovitrap index (OI). Significant reduction in the ovitrap index (OI) for both *Ae aegypti* and *Ae albopictus* were achieved (Lee *et al.*, 2008).

In the Bti treated site, there was only one reported dengue case at the beginning of the Bti treatment and no cases were reported during the trial, while the untreated site had 15 dengue cases during the trial (Tan *et al.*, 2012). Bti should be applied at dosages of 250-500g per hectare for spray application, in accordance to the manufacturer's recommendation.

Effectiveness Indicator

Bti is effective in interrupting dengue transmission when applied at weekly intervals for four continuous weeks or until the ovitrap index is less than or equal to 10%; this is followed by biweekly intervals until the treated area is dengue free.

Conclusion

- IMR has extensively tested several Bti formulations under laboratory conditions, simulated field trials and field operations.
- Among these, only one Bti product, VectoBac WG, has completed WHOPES review (WHO, 2004 & WHO, 2007). Multiple studies with VectoBac WG by IMR have achieved reproducible results with significant suppression in the adult mosquito vector population. Field trials in Shah Alam, Malaysia and operational programmes in Singapore and Cambodia with Vecto. Bac WG have shown significant impact on both dengue and malaria. The impact was only achieved when the Bti product was dispersed uniformly throughout the target sites using the appropriate application equipment at regular intervals.
- In order to maximise the use of Bti, we need to:
  - Incorporate it to the Dengue Control Programme.
  - Ensure effective and appropriate usage using various application techniques and equipment.
  - Provide regular quality training to applicators

Acknowledgements

We thank the Director General of Health Malaysia, Deputy Director General of Health (Research and Technical Support) and the Director, Institute for Medical Research for their support. We also thank the IMR Medical Entomology Unit staff for assisting in the field work.

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