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Application of *Bacillus thuringiensis var. israelensis* as a Trap against Dengue Vectors of *Aedes aegypti* and *Aedes albopictus* Populations and Impact on Dengue Transmission

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Abstract: **We evaluated the efficacy of water-dispersible granular (WDG), donut shape Mosquito Dunk, formulation of Bacillus thuringiensis var. israelensis Bti: 7000 ITU/mg, Summit Chemicals Baltimore) for the control of mosquito larvae in a Dengue-endemic area of Nathuduwa, Kelaniya, located in the district of Gampaha Sri Lanaka (situated at 6.96 North Latitude, 79.9 East Longitude and 4 meters Elevation above the sea level). In this study we introduced and used 8" diameter, 8" hight, black color plastic containers filled with 2/3 of de-chlorinated water and applied ¼ (3.25g) of Mosquito Dunk (Bacillus thuringiensis israelensis) as a trap and kept 1 month time in front of houses (outdoor, single trap/ house) in the study area.**

Efficiency and residual effects assess in lab and open field. Formulation of Mosquito Dunk showed high susceptibility of Aedes aegypti and Aedes albopictus. A dosage of ¼ dunk was sufficient to suppress fully larval population in the trap for 30 days (1 month). However it showed significant drop of adult mosquito population and each household effectively eliminated larval population without environmental impact. We conclude that the main Dengue vectors of Aedes aegypti and Aedes albopictus larvae highly susceptible to this microbial agent.

Keywords: **Bacillus thurngiensis var. israelensis (Bti), larvae, biological, mosquito, Dengue.**

1. INTRODUCTION

Vector-borne diseases are a specific group of infections that present threat to Sri Lanka and require particular attention. The recent notification of arboviral disease of Dengue fever cases were increased (Epid. SL) in areas where the vector, *Aedes aegypti* and *Aedes albopictus* are present. These areas at a risk of spread of mosquitoes and risk of virus transmission. In line with these informations, identified the need for guidance on surveillance and introduce an effective biological control method of *Bacillus thuringiensis var.israelensis*. We selected an endemic area in Gampaha district in Sri Lanka where highly affected. During the last 12 months of the year 2015, 26662 numbers of suspected Dengue cases have been reported from all over the country and highest number of cases were reported from Colombo and Gampaha districts.

Two areas of study and control in Gampaha district show similarities among population, houses and life pattern. Distance about 2 kilometers from study area to control area avoided mosquito emigration and immigration to each other. A number of outputs of surveillance, e.g. vector capacity parameters, have been done parallel during the application of Bti to identify the effectiveness.

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Table 1 : Distribution of notification Dengue cases by month in Gampaha district,2015.

Month	Jan.	Feb.	Mar.	Apr.	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Dengue Cases	817	942	255	193	264	225	317	233	186	277	387	59
Total	3705											

Epidemiology Unit Sri Lanka

2. BIOLOGICAL CONTROL

Bacillus thuringiensis var. israelensis new strain was found in isolated stagnant pond in a dry desert riverbed of Nahal Besor, adjacent to Kibbutz Zeelim, Israel Negev in 1976 (Barjac,H.D.,at al (1990). Assay of larvicidal activity processing in the laboratory was mostly done by Leonard Goldberg and published by Goldberg and Margalit (1977). Bacterial insecticides have been used for the control of nuisance vector mosquitoes for more than two decades. These insecticides remain of limited use in tropical countries where mosquito-borne diseases are prevalent. Bacterial insecticide efficacy and synthesis of mosquitocidal proteins depending on the type of genetic engineering techniques which used by he manufacturers. These larvicide hold excellent insecticidal properties against mosquitoes. In this study the use of Bacillus thuringiensis israelensis solid form mosquito dunk for controlling Aedes aegypti and Aedes albopictus larvae were carried out at laboratory and small field scale.

Fig 1: Mosquito Dunk

Characteristics of *Bacillus thuringiensis israelensis:*

Bti contain the spores and parasporal crystalsCry4A, Cry4B, Cry11Aa, and Cyt1Aa contain four major proteins 27, 65, 128 and 135kDa. It cause the larval mortality followed by the ingestion, this parasporal crystals solubalized in the alkaline larval midgut, followed by proteolytic activation of the soluble insecticidal crystal proteins. Pore forming in the cell cause by the toxin binds to a receptor on the midgut cell wall and this leads to death of larva. Synergistic interaction between Cyt1Aa and the Cry4, Cry11 proteins, resulting high toxicity to larvae.

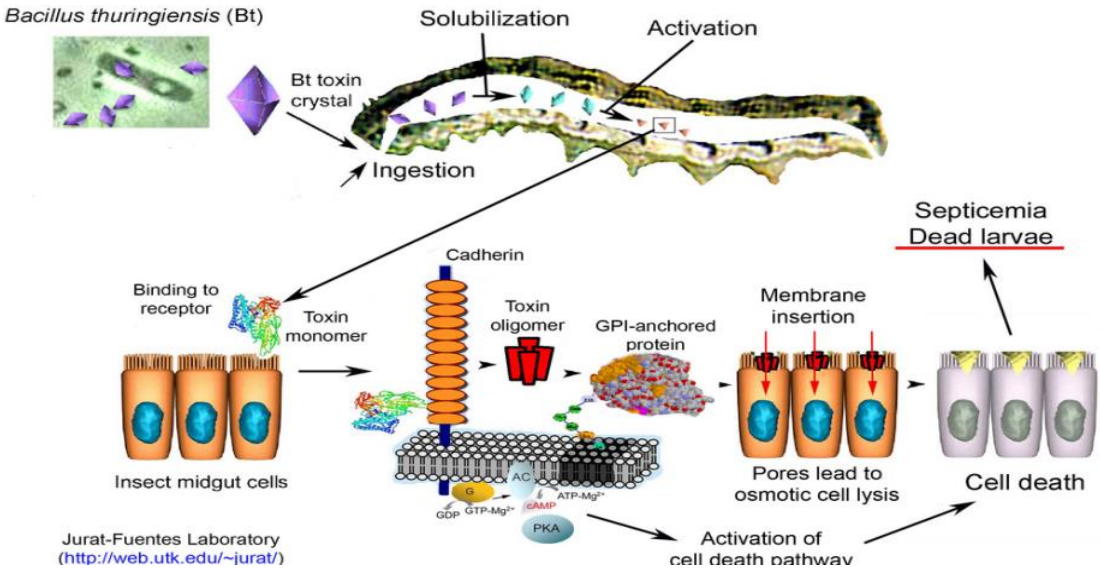

Fig 2: Mode of Action of *Bacillus thuringiensis*

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Mosquito Identification:

With the increasing importance of mosquito-borne diseases in Sri Lanka, identification becomes more important. In this study, we use following pictorial keys to identify larvae of *Aedes aegypti* and *Aedes albopictus*, vectors of Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF).

Aedes aegypti ***Aedes albopictus***

Fig 3 : Aedes aegypti Larva

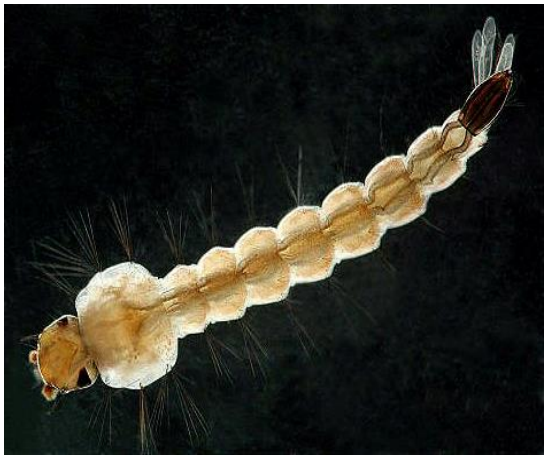

Fig 4 : Aedes albopictus Larva

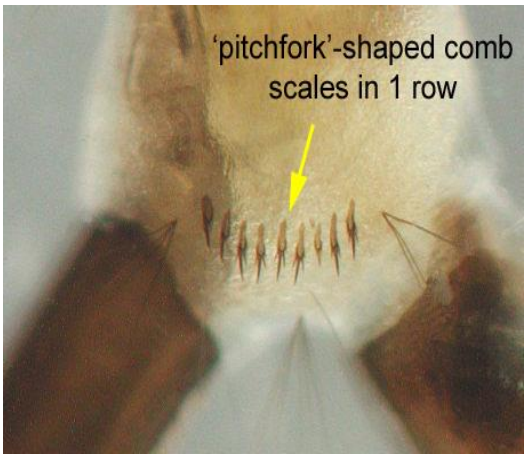

Fig 5 : Comb

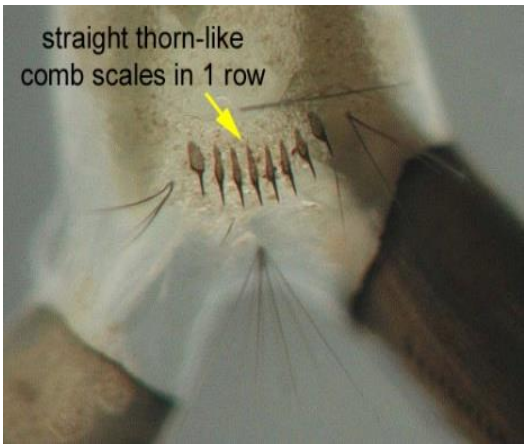

Fig 6 : Comb

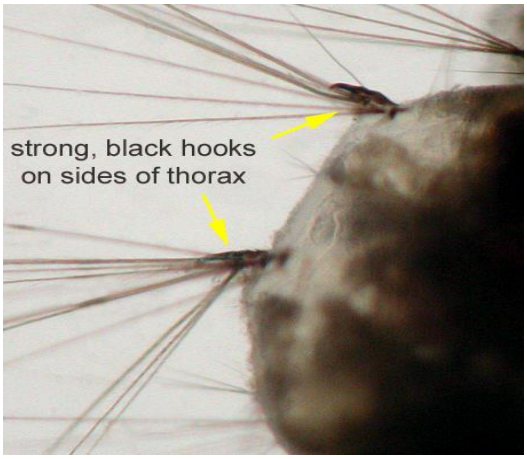

Fig 7 : Thorax

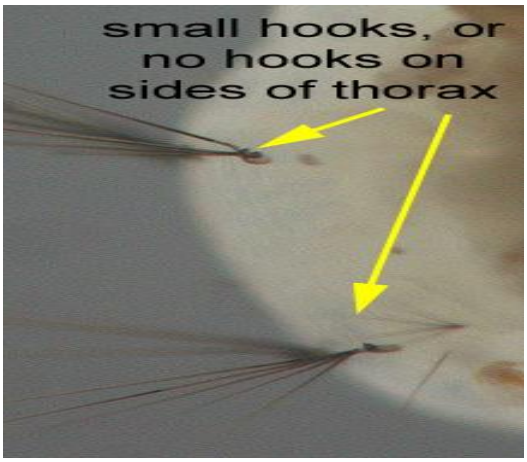

Fig 8 : Thorax

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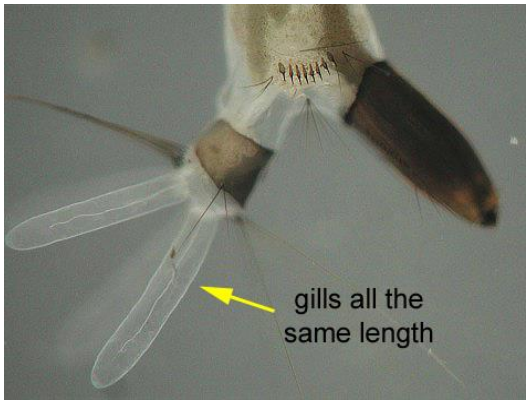

Fig 9 : Gills

3. METHODOLOGY

Study Area:

The research was carried out in two areas, Nathuduwa West (study area) and Ahugammana (control area) in Gampaha district.

Ovitrap Positions

Fig 10 : Satellite map of study area (Nathuduwa)

Table 2 : Area, number of houses, population and description of living pattern

Area	Total Houses	Single 1 Story	Single 2 Story	Single 2 + Story	Attached House / Annex	Flat	Condominium	Twin House	Row Line Room	Hut / Shanty
Nathuduwa West (Study Area)	432	354	50		17	0	0	9	2	0
Ahugammana 284A (Control Area)	446	437	9	0	0	0	0	0	0	0

Department of Census in Sri Lanka 2015

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Application of Bti:

Table 3 : *Bacillus thuringiensis israelensis*(Bti-Mosquito Dunk) application rates

Surface area of Standing Water	Use Quantity
1 to 5 sqft	¼ Dunk
5 to 25 sqft	½ Dunk
25 to 100 sqft	1 Dunk
above 100 sqft	1 Dunk/100 sqft

Mosquito Dunks can be used whole or broken in to pieces and applied to containerized standing water found near the home, such as flower pots, rain barrels, roof gutters, unused swimming pools, water accumulated in old automobile tires, water gardens, tree holes and drains (Label: Mosquito Dunk)

¼ dunk of Bti formulation were applied in to a 8" diameter, 8" height, black plastic container filled with 2/3 of de-chlorinated water and kept in front of the main door of every houses in the study area for 30 days (1 month). People were advised to check water level and fill evaporate amount and clean the cob webs every week if present on the top of the container. 200 containers with Bti dunk were placed houses one after the other in the study area. This study was started on 1st of December 2015 and completed on 31st December 2015.

Fig 11

Field data collection:

The impact of Bti was evaluated with the Ovitrap Index (OI) & Larval Density (LD) we sought to visually enhance the attractiveness of a standard black ovitrap used in surveillance of the Aedes aegypti & Aedes albopictus Mosquitoes. Dengue vectors have adapted well to artificial containers commonly found in suburban & urban areas both Aedes Aegypti & Ae. Albopictus oviposit readily in man made containers. 8" diameter, 8" hight , black plastic container with two overflow holes and wrap with a black wet cloth on top of the container to keep moisture to attract mosquitoes. 10 different oviposition site choices in Study areas & another 10 oviposition sites were positioned in control area. Larvae collected at every 4 days time (8times in a month) in Study area & Control area during 1st Dec-31st Dec 2015.

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Fig 12

Laboratory Assays:

Biological efficacy of Bacillus thuringiensis israelensis was tested against 3rd instars larvae (laboratory reared) of Aedes aegypti and Aedes albopictus according to the WHO guide lines to determine the effectiveness. Specimens were collected from Eriyawetiya village, 12km from Colombo, Western Province, Sri Lanka in November 2015. Larvae were reared in round plastic 5 replicates (2.5"diameter and 2.5"high with screened lids), each with Bti treated and untreated, filled with de chlorinated water and put 3rd instar 20 larvae for each replicate. Larvae were not fed during the experiments and mortality was scored after 24 hours. All trials were conducted at ambient temperatures that ranged from 26.5C-28.5C. weight of piece of dunk was 0.044g, Bti manufacture date-Aug 2015 and batch number was 4120.

4. RESULTS

The Impact of Bacillus thuringiensis israelensis (Bti) was evaluated with Ovitrap Index (OI) & Larval Density (LD) overall out outdoor (Fig I), the outdoor for Aedes Aegypti (Fig 2), the outdoor for Aedes atpopictus (Fig 3) & LD for overall outdoor (Fig 4), LD for outdoor Aedes (Fig 5), LD for outdoor Albopictus (Fig 6) were determined. The Ovitrap Index & Larval Density were compared between Bti untreated (Control) & Bti treated (Study) area during the same time. 4 Dengue cases were reported During the 7 days of time before the Study & 2 cases were reported during the Study Period. There was no case reported after the Study of 2 weeks period of time. the Aedes Aegypti populations were higher than Aedes albpoictus populations. Bti applications were carried out during 3 days of time in the whole area and it last for 31 days (according to the mosquito dunk packing details) The Ovitrap Index (OI) for overall shown significant reduction after Bti application during the study period of 1 month. A similar result was observed for Larval Density (LD).

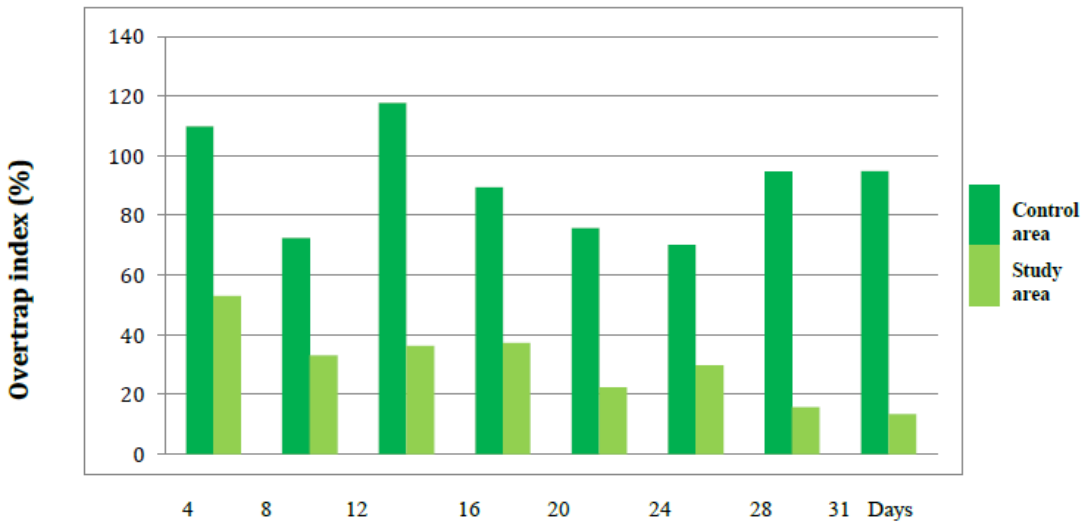

Fig 13 : Overall outdoor Ovitrap Index (OI) for *Aedes* Species in Bti treated (Study) and untreated (Control) area

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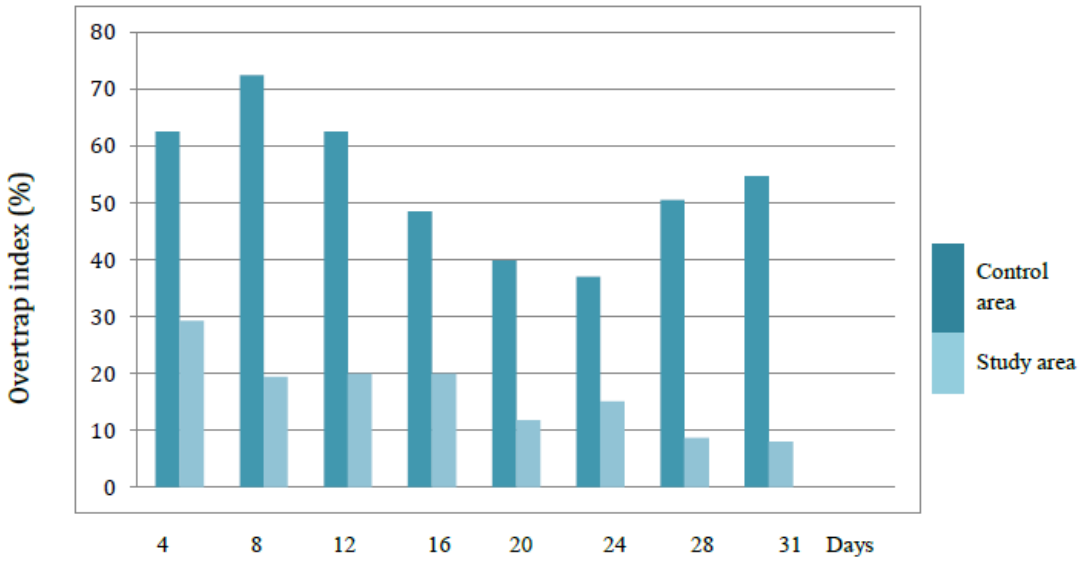

Fig 14 : Overall outdoor Ovitrap Index (OI) for *Aedes aegypti* in Bti treated (Study) and untreated (Control) area

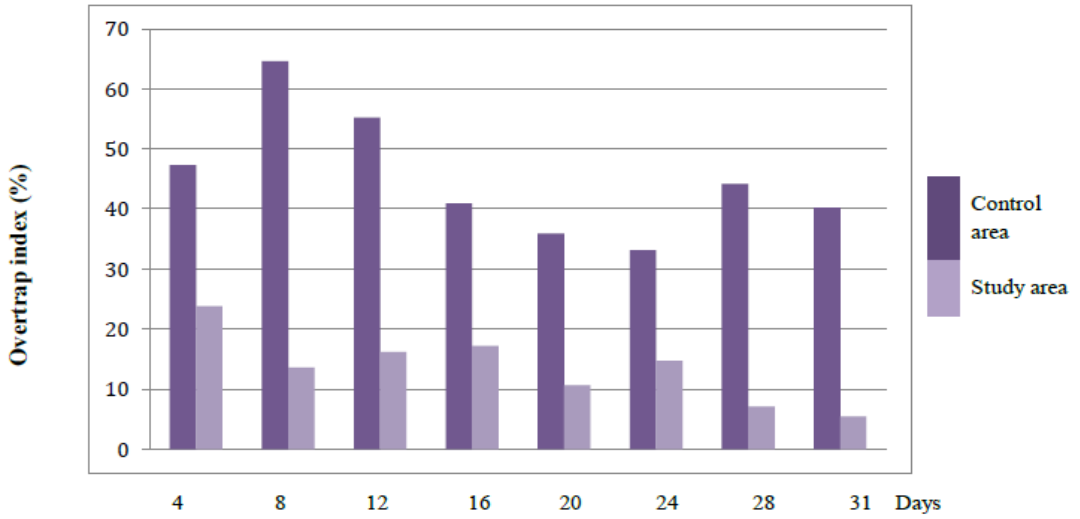

Fig 15 : Overall outdoor Ovitrap Index (OI) for *Aedes albopictus* in Bti treated (Study) and untreated (Control) area

Overall				
Days	Bti Untreated (Control)	*n*	Bti Treated (Study)	*n*
4	60%	10	80%	10
8	90%	10	60%	10
12	80%	10	50%	10
16	70%	10	60%	10
20	70%	10	40%	10
24	80%	10	50%	10
28	70%	10	30%	10
31	80%	10	30%	10
n = number of recovered ovitraps				

Fig 16 : Overall Outdoor Larval Density (LD) in Bti treated (Study) and untreated (Control) areas

Aedes aegypti				
Days	Bti Untreated (Control)	*n*	Bti Treated (Study)	*n*
4	60%	10	80%	10
8	90%	10	60%	10
12	80%	10	50%	10
16	70%	10	60%	10
20	70%	10	40%	10
24	80%	10	50%	10
28	70%	10	30%	10
31	80%	10	30%	10
n = number of recovered ovitraps				

Fig 17 : Outdoor Larval Density (LD) for Aedes aegypti in Bti treated (Study) and untreated (Control) areas

Aedes albopictus				
Days	Bti Untreated (Control)	*n*	Bti Treated (Study)	*n*
4	60%	10	80%	10
8	90%	10	60%	10
12	80%	10	50%	10
16	70%	10	60%	10
20	70%	10	40%	10
24	80%	10	50%	10
28	70%	10	30%	10
31	80%	10	30%	10
n = number of recovered ovitraps				

Fig 18 : Outdoor Larval Density (LD) for Aedes albopictus in Bti treated (Study) and untreated (Control) areas

Laboratory bioassays with *Bacillus thuringiensis israelensis* against 3rd instars larvae showed that after 24 hours exposure on average concentration of 7000 ITU/mg caused 100% mortality during 31 days (1 month). Aedes aegypti and Aedes albopictus were found to be most sensitive.

The impact of Bti was evaluated with the Bti trap and larval densisty; the out door trap for both Aedes species were determined, the larval density of treated and untreated replicates in the study and control area) were compared during the same time period.

During the pre-treatment phase, the overall adult Aedes species population at both sites were of similar density over the period of 7 days and the Aedes indoor population (%).

During the post-treatment phase (7days) the Aedes species populations at control site was similar to pre-treatment phase Aedes population and study site Aedes species population significantly dropped (%) due to the application of Bti.

5. DISCUSSION

A previous laboratory bioassays and semi field studies were conducted in Bangkok, Thailand on the efficacy and longevity of Mosquito Dunks (7000 ITU/mg Bti) in order to determine the concentration-response relationship and the effectiveness on the potency of the Bti product against Ades mosquito species based on the WHO protocol standard methods and to determine the longevity of release for this product against Aedes aegypti mosquito larvae in water storage containers. This bio-potency study with the late 3rd instar larvae of Aedes aegypti and Aedes albopictus was carried out according to WHO standard protocols. The six concentrations of the Bti product used in each test were replicated 4 times with 25 mosquito larvae. Probit analysis was then used determine the LC50 and LC95 which was equated with dosages of 1.02 and 1.86 ppm for Aedes aegypti; and 0.39 and 0.84 ppm for Aedes albopictus, which reveals a potency of 382.95 and

303.74 ITU/mg, respectively. The semi-field evaluation of this product in 200-liter earthen jars against 3rd instar larvae of Aedes aegypti showed satisfactory control of greater than 80% at 11 weeks post-treatment (Fansari,T. at al (2006).

This study was carried out in a high risk Dengue endemic site to determine novel, effective method to prevent Dengue. In this study we found using the same biological control agent in a different kind of new method Bti Trap was used. Vector population significantly decreased during the study time and remained suppressed even after treatment was stopped and there were no Dengue cases recorded at the Bti treated site after the Study during the study. This confirms the need to conduct larviciding to prevent Dengue outbreaks. Bti shows to be highly effective against Dengue vectors of Aedes species under laboratory conditions, both mosquito species tested were extremely sensitive to the Mosquito Dunk (Summit Chemicals, Balyimore). Microbial larvicides have several advantages over the mosquito contrtol agents. This has been used in many localities for more than a decade without any reports of resistance developing in the mosquito populations (Becker & Ludwig, 1993; Lacey, 2007). Bti has high efficacy but also environmental safety and safety for human consumption, it may apply drinking water for instance (WHO 1999).

6. CONCLUSION

A female Aedes mosquito species tend to persist in a domesticated environment. The mosquito is renowned for its efficient "vectorial capacity" with a high affinity for human blood, high susceptibility to the four Dengue virus serotypes, and being highly adapted to urban living.

The Bti formulation trap was found effective, easy, and economical in controlling mosquito larvae. Formulation relative less toxic, eco-friendly and vectors are unable to develop resistance and may be used as an effective alternative to other pesticides for control of vector-borne diseases.Consider all the above factors, Mosquito Dunk (Bti-Trap) may have great potential for inclusion in integrated vector management operations.

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