

## Original Research Article

# Efficacy *Bacillus thuringiensis* var. *israelensis* serotype H-14 (Bti H-14) for control *Aedes* spp. density in Denpasar, Bali

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## ABSTRACT

**Background:** A liquid bioinsecticide formulation containing *Bacillus thuringiensis* var. *israelensis* Serotype H-14 (Bti H-14) was tested in the field in household containers. The aim was to determine the effectiveness of Bti H-14 bioinsecticide in controlling the density of *Aedes* spp. Larvae.

**Methods:** This study was conducted in two phases of testing. First, to test the effective dose with 5 doses, namely (50 ul, 40 ul, 30 ul, 20 ul, and 10 ul) in 2.5 liters of water. Furthermore, the number of deaths was calculated after 24 hours of treatment and control by doing four repetitions. In the second phase, by conducting tests on containers in the household as many as 3171 containers were continuously observed every month, given Bti H-14 for 6 months. Observations were made before and after the application of Bti H-14 on larva density, mosquito density, and dengue cases. Data analysis was performed using paired t-test. Bti H-14 formulation to kill 50% of mosquito larvae (LC50) within 6 hours requires a concentration of 4 µl per liter.

**Results:** Bti H-14 liquid formulation with delta-endotoxin and spores content of 600 ITU per ml or 1.2x10<sup>9</sup> CFU is effective in reducing larva density in household containers if done regularly.

**Conclusions:** Bti H-14 liquid formulation is proven to be effective and easy to use for the control of *Aedes* larvae.

**Keywords:** *Aedes aegypti*, *Bacillus thuringiensis*, Bali

## INTRODUCTION

Increasing urbanization, globalization, and international mobilization have increased the epidemic spread of arboviral diseases transmitted by *Aedes aegypti* mosquitoes such as dengue, chikungunya, yellow fever, and Zika.<sup>1,2</sup> Dengue virus cases have reported as many as 390 million cases per year, with 96 million deaths and 70% of cases occurring in Asia.<sup>3</sup> The Zika virus, which causes microcephaly, was reported to have occurred in Brazil.<sup>4</sup> Chikungunya spread widely to various countries reported in Africa, Asia, Europe, and the Indian and Pacific Oceans.<sup>5</sup>

Efforts to control mosquitoes in Indonesia with insecticides such as organophosphates (temephos and malathion) have been carried out since 1970 followed by pyrethroids (permethrin, cypermethrin, deltamethrin) from 1980 to now.<sup>6</sup> The use of insecticides that is too long is reported to cause mosquito resistance.<sup>7,8</sup> Long-term and excessive use of insecticides can have an impact on health and the environment.<sup>9,10</sup>

*Bacillus thuringiensis* serovar *israelensis* is a natural bioinsecticide for effective control of mosquito larvae.<sup>11</sup> The mechanism of action is to produce toxins that injure the digestive intestines of mosquito larvae.<sup>12</sup> Its use is safe to use in the long term and does not cause resistance.<sup>13</sup>

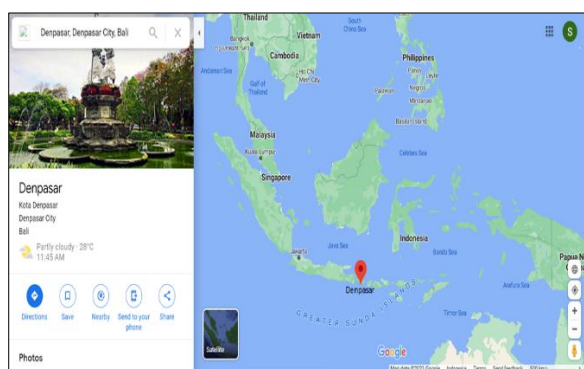
*Bacillus thuringiensis* has been used in several countries such as Brazil, Malaysia, Cambodia, and Singapore.<sup>14-17</sup>

Bali is a tourism area with a high level of mobility and is endemic to dengue, so an environmentally friendly vector control effort is needed. This study aims to determine the effectiveness of the bioinsecticide *Bacillus thuringiensis* var. *israelensis* Serotype H-14 (Bti H-14) to control the density of *Aedes* spp. larvae in endemic areas in Denpasar City, Bali Province.

## METHODS

### Study area

Denpasar is the capital of Bali Province which is divided into 4 districts. The population of Denpasar City is 897,300 people with an area of 127.78 km<sup>2</sup> which has a rainy season and a dry season with coordinates -8.65, 115.216667. Denpasar is a dengue-endemic area that has 4 virus serotypes. This research was conducted in West Denpasar District, Penambaran Village during the rainy season from July to December 2014. All households were visited for intervention and compared with Taman Sari Village as a dengue case-control.



**Figure 1: Research Location; Denpasar, Indonesia**

### Laboratory trial

The test material used was Bti H-14 liquid formulation containing delta-endotoxin and spores of 600 ITU per ml or 1.2x10<sup>9</sup> CFU. This product Bactivec® SC was obtained from the Labiofam Enterprise Group, La Habana, Cuba.

The pathogenicity test was carried out by making 5 treatment groups and 1 control without treatment and doing 4 repetitions to compare the killing power according to the concentration to be studied by placing 25 *Aedes* instar III larvae into each petri dish.

Determination of bacterial concentration was carried out by determining the lowest concentration of the recommended standard (1 ml=1000 µl). The standard concentration of Bti is 1 ml (20 drops of Bti) for 50 liters of water, 200 µl Bti for 10 liters of water (1000 ml), and 50 µl Bti for 2.5 liters of water. The concentrations of Bti

used in this study were 5 concentrations (50 µl, 40 µl, 30 µl, 20 µl, and 10 µl) in 2.5 liters of water by looking at the standard reference set by the product.

In the control group, the mosquito larvae were not given the addition of bacteria. Furthermore, the number of larvae that died after 24 hours of treatment was counted and carried out in 4 repetitions. Dead larvae are seen by touching the larvae with a micro-pipette and if it does not move it means that the test larvae are dead. Then the 50% and 90% Lethal Concentration calculations were carried out in 24-hours (LC50-24 hours and LC90-24 hours) from the results of the Bti pathogenicity test against the larvae of *Aedes* spp.

### Field trial

#### Observation of *Aedes aegypti* Larvae Density

Field trials were carried out naturally on existing containers in households. This research is a quasi-experimental study by intervening in 1101 households with 3171 containers which are continuously given the intervention of larvicide every month for 6 replications. Respondents are all residents in the Banjar Penambaran area of Padang Sambian, Denpasar, and Banjar Taman Sari as a comparison area. The material is Bti H-14 liquid formulation containing delta-endotoxin and spores of 600 ITU per ml or 1.2 x 10<sup>9</sup> CFU, with a dosage of two drops per five liters of water. Bti application once a month to all water media (control) in the treatment location, for 6 treatments.

Observation of larvae of *Aedes* done before and after Bti application. Before application, each water container was observed for the presence of *Aedes*. After the application, the observation of larvae of *Aedes* spp. conducted once a month (one month later), for six months of observation, for all water containers on site.

#### The density of *Aedes* spp.

Mosquito density observations were carried out before and after Bti application. Before the application, 10 houses were taken to catch mosquitoes using the resting collection method in the morning or evening. After being given the application, mosquito catching was carried out once a month in 10 house samples, both in the application area and in the control area.

#### Dengue case observation

Observation of DHF cases, DHF cases were observed before and after Bti application, against monthly case reports at the Puskesmas for application and control areas. Analysis of the effectiveness of Bti in killing larvae of *Aedes* spp. calculated based on a minimum number of 80% free control of *Aedes* spp. To find out whether there was a difference in CI between before and after Bti application,

the difference in mosquito density between the application area and the control area was used the t-test.

**Data analysis**

Data analysis used paired t-test to compare the effectiveness of interventions before and after.

**Ethical statement**

This research has been approved by the Ethics Commission of the Medical Faculty of Udayana University.

**RESULTS**

**Phase 1**

After cultivating the bacteria on Nutrient Agar media, the results of the calculation of the number of bacterial cells and spores of Bti H-14 were three times repeated.

**Table 1: Description of the number of cells and spores of Bti H-14 in 1 ml concentration.**

Treatment	Repetition			Average (10 <sup>9</sup> )
	I	II	III	
Cell	113	122	101	11.2
Bacterial Spores	71	77	75	7.43

**Table 2: Average and percentage of Aedes aegypti larvae mortality in 2.5 liters of water within 24 hours.**

Concentration Treatment	Mortality rate and percentage		
	6 hours	12 hours	24 hours
50 µl	24 (96)	25 (100)	25 (100)
40 µl	24 (96)	24.75 (99)	24.75 (99)
30 µl	21.75 (87)	24 (96)	24.75 (99)
20 µl	17.75 (71)	23.50 (94)	24.50 (98)
10 µl	14.75 (59)	20.75 (81)	22 (88)
Control	0 (0)	0 (0)	0 (0)

The average number of Bti H-14 cells grown on Nutrient Agar media was 11.2x10<sup>9</sup> cfu / ml, while the average number of bacterial spores was 7.43x10<sup>9</sup> cfu/ml. 600 samples of Aedes aegypti larvae were obtained from breeding eggs in the Denpasar City area in 8 containers to produce third instar larvae at the same time which were then divided into 6 different treatments and each treatment was carried out four times. The average mortality rate of Aedes aegypti larvae per Bti H-14 concentration can be seen in Table 2 using the Kruskal Wallis statistical test.

Based on the table 2, the difference in the percentage and average mortality of Aedes aegypti larvae with differences in the concentration of Bti H-14. The treatment that had the

highest mean difference compared to the control was the concentration of 50 µl with an average mortality of 24 mosquito larvae at 6 hours (96%), increasing between 12 and 24 hours to 25 (100%). To kill 50% of mosquito larvae (LC50) within 6 hours requires a concentration of 4 µl per liter, while to kill 90% of mosquito larvae (LC90) within 6 hours requires a concentration of 16 µl per liter. The concentration of 50 µl had the highest average percentage of mortality of Aedes aegypti larvae than other concentrations.

**Table 3: Posthoc statistical test comparison of two groups using Mann Whitney.**

Hour Range	Concentration	Concentration Comparison	Difference Average	P (mann whitney)
6 hr	Concentration 50 µl	Concentration 40 µl	0	0.76
		Concentration 30 µl	9	0.10
		Concentration 20 µl	25	0.01
		Concentration 10 µl	37	0.01
		Control	96	0.01
12 hr	Concentration 50 µl	Concentration 40 µl	1	0.31
		Concentration 30 µl	4	0.31
		Concentration 20 µl	6	0.01
		Concentration 10 µl	19	0.01
		Control	100	0.008
24 hr	Concentration 50 µl	Concentration 40 µl	1	0.31
		Concentration 30 µl	1	0.31
		Concentration 20 µl	2	0.12
		Concentration 10 µl	12	0.04
		Control	100	0.008

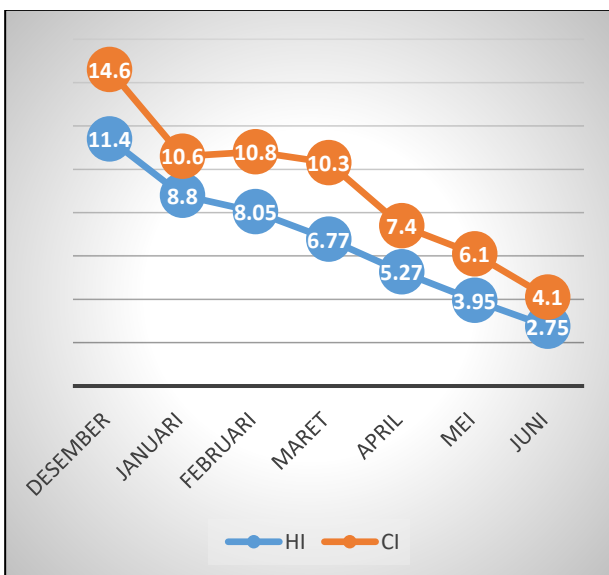
In the test table 2, the comparative analysis of the average percentage of mortality of Aedes aegypti mosquito larvae shows that there is one treatment group that is different from the 5 treatment and control groups on the mortality rate of Aedes aegypti mosquito larvae. Within 24 hours of observation was assessed with a value of  $p \leq \alpha$  (0.05), so further statistical tests were needed using the Mann Whitney.

Based on the Posthoc statistical test, the comparison of the two groups using the Mann Whitney shows that the control is significantly different from the other treatments on the mean percentage of larval mortality at 6-24 hours. At 6

hours of treatment of Bti H-14 at a concentration of 50 µl did not differ significantly from that of Bti H-14 at a concentration of 40 µl and a concentration of 30 µl, while Bti H-14 treatment at a concentration of 20 µl and 10 µl showed different results. At 12 hours the test results were not significantly different from those at 6 hours. At 24 hours of observation, the results were not significantly different in the mean percentage of mosquito larvae mortality in Bti H-14 treatment at a concentration of 50 µl with Bti H-14 at a concentration of 40 µl, 30 µl, and 20 µl. There were significant differences with Bti treatment at a concentration of 10 µl.

**Field trial**

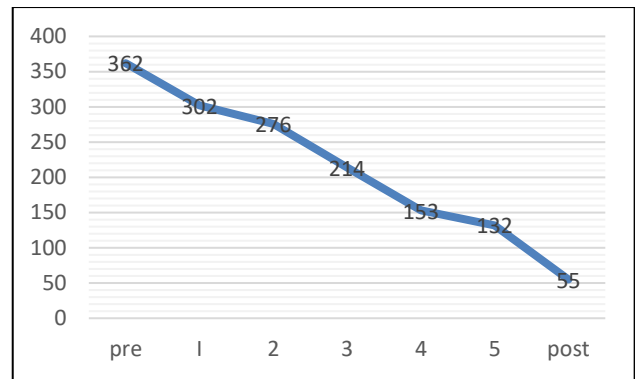
Based on data from the trend container index (CI), it is known that the number of positive containers tends to decrease before and after Bti H-14 treatment. Before Bti H-14 treatment, the CI was 11.4%, then after Bti H-14 treatment was carried out continuously every month, after 6 months the CI was 2.75%. This is also comparable to the house index (HI) data before treatment of 14.6% and then after treatment to 4.1%.



**Figure 2: Trend House Index (HI) and Container Index (CI).**

The results of this study indicate that there is a significant change between before and after the treatment of Bti H-14 to the value of HI, CI. The decline in the number of positive containers in the field occurred gradually. This is because giving Bti H-14 once a month can kill Aedes aegypti larvae.

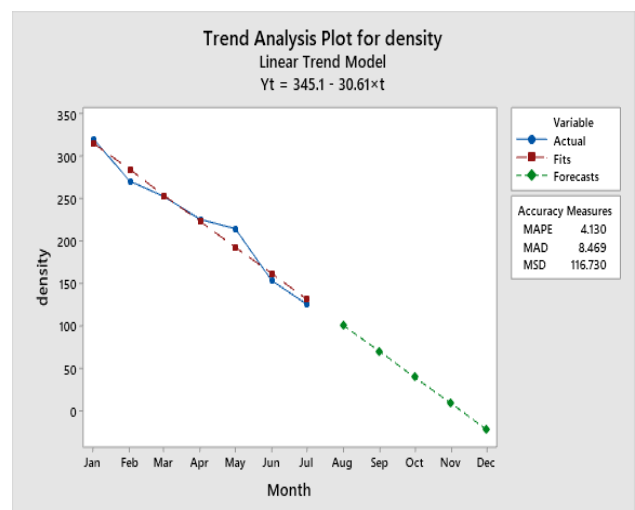
Before Bti H-14 treatment, 3,171 containers were measured, which were positive for 358 and negative for 2,813 containers. The positive containers in the house are Bath containers (96 pieces), buckets (41 pieces), Dispensers (34 pieces). Outside the house, there are (89 pots), fish-free pond (15 pieces), Tirta water bowl (9 pieces), penjor holes (9 pieces).



**Figure 3: Number of positive containers before and after treatment (pre-post).**

**Table 2: Paired samples correlations.**

Pair	Before and after	N	Correlation	Sig.
Pair 1	Before and after	19	0.879	0.000



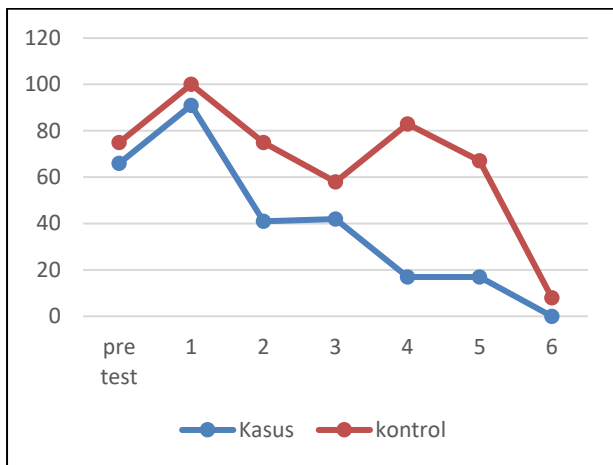
**Figure 4: Analysis of trends in larval density.**

This shows that there are still many positive containers in the community as a place for mosquito breeding. Especially the bathtubs, buckets, and dispensers that are in the house whose conditions should be controlled. In this program, Bti H-14 is given continuously every month by jumatik staff.

Specific containers in Bali with water containers and penjor holes were found to be 9 positives respectively. The culture of placing a place of Tirta (holy water) in open conditions both inside and outside the house is a potential place for mosquito breeding. Water containers should be made closed so that they do not become breeding places for mosquitoes. Penjor holes are also usually always in every resident's house which is usually used in every ceremony to install penjor bamboo.

**Table 4: Comparison of container types before and after Bti H-14 treatment.**

Container type	Before treatment				After treatment			
	Positive	%	Negative	%	Positive	%	Negative	%
<b>Bathroom containers</b>	96	26.5	954	34.0	16	29.1	1003	32.80
<b>Crock Washbowl</b>	14	3.9	60	2.1	3	5.5	64	2.09
<b>Bucket/Pan</b>	41	11.3	1020	36.4	5	9.1	1034	33.81
<b>Water plant Pots</b>	89	24.6	243	8.7	11	20.0	223	7.29
<b>Jar/kettles</b>	2	0.6	10	0.4	0	0.0	2	0.07
<b>Dispenser</b>	34	9.4	121	4.3	3	5.5	278	9.09
<b>Penjor hole</b>	9	2.5	16	0.6	0	0.0	16	0.52
<b>Well</b>	5	1.4	29	1.0	0	0.0	33	1.08
<b>Aquarium without fish</b>	15	4.1	91	3.2	6	10.9	72	2.35
<b>Place of tirta</b>	9	2.5	22	0.8	3	5.5	43	1.41
<b>Used tires</b>	24	6.6	16	0.6	1	1.8	20	0.65
<b>Drum/Jerrycans</b>	3	0.8	17	0.6	5	9.1	9	0.29
<b>Used botol/cans Gallon/bowl Bottle cap</b>	7	1.9	40	1.4	0	0.0	18	0.59
<b>Bucket in the toilet</b>	5	1.4	69	2.5	0	0.0	4	0.13
<b>Water behind the refrigerator</b>	2	0.6	32	1.1	1	1.8	111	3.63
<b>Place water in the statue</b>	2	0.6	0	0.0	0	0.0	128	4.19
<b>A place to drink animal</b>	5	1.4	65	2.3	1	1.8	0	0.00
<b>Total</b>	358	100	2813	100	55	100.0	3058	100

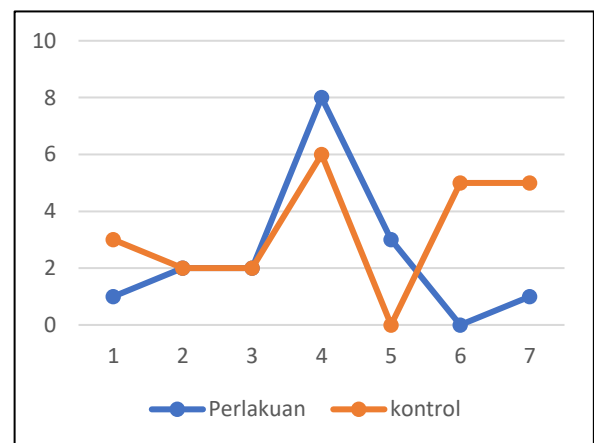


**Figure 5: The trend of Aedes aegypti density in the treatment and control areas.**

Based on Table 3, it shows that the number of positive containers every month is decreasing. For example, a bathtub that was positive was 96 then decreased to 16 at the end of the treatment. Buckets before treatment were 41 to 5 pieces. The used tires before treatment 24 pieces become an after treatment.

The decrease in the number of positive containers occurs gradually every month. During 6 months of treatment, the

reduction in the number of positive containers was 60.8%. Efforts to provide Bti H-14 by jumantik health workers and to eradicate mosquito breeding can reduce the presence of positive containers indoors and outdoors.



**Figure 6: Comparison of DHF incidence in treatment and control areas.**

Containers outside the room such as used bottles, used buckets, used plastics can be reduced in number, but for those in the room such as bathtubs, dispensers, buckets, the number tends to remain, but with the provision of Bti H-14, the number of positive containers can be controlled.



Based on the comparison of the presence of larvae before and after treatment, there is a significant difference in the number of positive containers before and after treatment. Indoor containers, namely Bath containers as many as 96 positives to 41 positives after treatment, Buckets from 41 to 5 pieces after treatment, Dispensers from 34 to 3 after treatment.

Outside the room, namely the penjor hole from 9 positive to 0 after treatment, water place from 9 to 3 positive, used tires from 24 to 1 positive, used bottles from 7 to 0. The type of container that is outside the room can be reduced, especially goods secondhand.

After the t-paired statistical test was carried out to determine the correlation between before and after treatment, it was obtained a significant value of 0.00 less than the alpha value of 0.05. This means that there is an effect of giving Bti between before and after treatment.

There was a difference between the mosquito density in the case and control areas. Before the application of Bti H-14 in the treatment area, 66 mosquitoes were found and in the control area, 75 mosquitoes were found. Then at the end of the treatment found in May, namely the treatment area 17 *Aedes aegypti* mosquitoes and control 67 mosquitoes. From the figure 5, it appears that there is a tendency to decrease the number of mosquitoes in the case area compared to the control area.

#### ***Differences in DHF cases between Bti application areas and control areas***

Based on comparative data on the number of DHF cases in the treatment and control areas, there is a tendency for a decrease in cases in the Bti H-14 treatment area. However, indeed, DHF cases cannot be eliminated because only 2 areas were intervened, but the mobility of the population is very high, so there is a risk of contracting DHF from other areas. From this data, at month 6 there were 0 dengue cases in the treatment area, while in the control area 5 cases.

#### **DISCUSSION**

In the first phase by conducting a trial laboratory using 5 doses in the treatment and control groups. The test results showed that Bti H-14 was effective to kill *Aedes aegypti* larvae in a dose of 50 µl and 40 µl for 2.5 l of water. Research conducted in India also showed similar results for Bti to be effective in 10-17 days on clean water at a dose of 1 ml/50 l 18. Other studies have also found an effective dose of 8 mg / L for the Vectobac WG product. Research in Malaysia also shows the use of insecticides Bti and temephos has proven to be effective in controlling larvae.<sup>15,19,12,20</sup> Brazil also carries out similar tests in the laboratory and in the field.<sup>21</sup> The combination of using a thermal fog generator can also kill larvae and adult mosquitoes.<sup>17</sup>

The use of Bti is relatively safe in non-target animals, at a low dose, and is environmentally friendly.<sup>15,22,23,24</sup> The use

of bioinsecticides is recommended to reduce the impact of chemicals. Long exposure using Bti also did not evolve to be resistant, and there was no cross-resistance with temephos.<sup>13</sup> Bti can be an alternative bioinsecticide in controlling *Aedes aegypti* and *Aedes albopictus* larvae.<sup>25</sup>

This study was also conducted in the field to test the effectiveness of Bti H-14 liquid formulation with delta-endotoxin and spores' content of 600 ITU per ml or 1,2x10<sup>9</sup> CFU. This product can reduce the density of larvae in containers in the field and reduce the density of larvae. This is consistent with studies using Vectobac WDG application in open areas at doses of 400 and 800 g/ha killing an average of 87% of tested larvae.<sup>26</sup> The study in Cambodia was carried out with multiphase applying Bti for 6 months to reduce larvae density and reduce DHF cases by 48% in 6 dengue endemic districts 16. A study in Brazil using Bti in containers in recycling and junkyard showed an 89.6% reduction in larvae.<sup>27</sup>

#### **CONCLUSION**

Bactivec SC containing *Bacillus thuringiensis* is useful for controlling *Aedes aegypti* larvae. Bti H-14 liquid formulation with delta-endotoxin and spores content of 600 ITU per ml or 1.2x10<sup>9</sup> CFU is effective in reducing larva density in household containers if done regularly. This liquid formulation product is easy to use in a water container. There is a significant difference in the number of positive containers of larvae before and after treatment.

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*Ethical approval: The study was approved by the Institutional Ethics Committee*

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