

RESIDUAL TOXICITY OF *BACILLUS THURINGIENSIS* H-14 (VCRC B17) IN SOME TYPES OF BREEDING PLACES OF *AEDES AEGYPTI*

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ABSTRAK

TOKSISITAS RESIDUAL DARI BACILLUS THURINGIENSIS H-14 (VCRC B17) PADA BEBERAPA TIPE DARI TEMPAT-TEMPAT PERINDUKAN AEDES AEGYPTI

Bacillus thuringiensis H-14, adalah agensia mikrobial yang sangat spesifik terhadap serangga sasaran, aman terhadap golongan mamalia, dan tidak mencemari lingkungan, sehingga dapat dikembangkan sebagai agensia untuk pengendalian vektor, khususnya vektor demam berdarah dengue di Indonesia.

Toksisitas residual B. thuringiensis H-14 (VCRC B17) terhadap larva instar III Aedes aegypti pada beberapa tipe tempat penampung air telah dievaluasi di dalam laboratorium.

Hasil evaluasi menunjukkan bahwa angka kematian larva uji lebih dari 80% oleh pengaruh B. thuringiensis H-14 (VCRC B17) pada konsentrasi antara 1 sampai 25 mg/l di dalam tipe tempat penampung air dari semen, tanah liat, dan plastik masing-masing adalah 16 sampai 60 hari, 18 sampai 36 hari, dan 12 sampai 42 hari.

Kata Kunci : Bacillus thuringiensis H-14 - Aedes aegypti - toksisitas residual - tipe tempat perindukan.

INTRODUCTION

At present, Abate^R (temephos) sand granules have been widely utilized as chemical control for main vector of Dengue Hemorrhagic Fever in endemic areas of Indonesia. Concentration of 1 mg/l is highly effective for breeding places of *Aedes*

aegypti and *A. albopictus* for duration of three to 3.5 months¹. However development of resistance to chemical insecticides in mosquito species has been reported from many countries. Lee and Lime (1989) reported that *A. aegypti* larvae that were collected from field location in Malaysia showed increased tolerance against temephos.

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Due to this situation, there is a necessity to find out a different agent that can function as an alternative insecticide².

Among the most promising biological agents for mosquito control are the two entomopathogenic bacilli, *Bacillus thuringiensis* H-14 and *B. sphaericus*³. From the average response of the mosquito larvae to *B. thuringiensis* H-14, *A. aegypti* was found to be most susceptible⁴.

This paper reports laboratory bioassays and residual toxicity of *B. thuringiensis* H-14 against *A. aegypti* larvae in different types of water containers, that are it was usually utilized by *A. aegypti* as breeding place.

MATERIALS AND METHODS

1. Laboratory Bioassays

B. thuringiensis H-14 (VCRC B17) were submitted by Vector Control Research Centre, India. Larvae of *A. aegypti* were reared in the insectarium of Parasitology Laboratory, Faculty of Medicine, Gadjah Mada University. Bioassays were prepared by Institute Pasteur, Paris⁵. Six concentrations : 0.01875, 0.03125, 0.0375, 0.125, 0.25, and 0.5 mg/l of *B. thuringiensis* H-14 were prepared. Twenty five early third instar larvae of *A. aegypti* were released in a test cup containing 200 ml deionized water solution. Each concentration was tested in triplicate. Mortalities of the larvae due to *B.*

thuringiensis H-14 were recorded at 24 hours after exposure. LC_{50} and LC_{90} values were read from probit analysis by Finney (1971)⁶.

2. Residual Toxicity

The laboratory residual toxicity of *B. thuringiensis* H-14 against *A. aegypti* were observed by concentration series. The concentration of 1, 5, and 25 mg/l was prepared in cemented clay and plastic containers, containing 1 litre of solution, respectively. Every concentration and the type of container was tested in triplicate. Every six days, 25 third instar larvae were infested in the test containers. Mortalities of the larvae were recorded 24 hours after exposure. The dead larvae were left in the containers and the alive one were removed. Water volume was maintained at the same level every 3 days, with 250 ml removed and replenished with running water in to the containers. To prevent infestation of *A. aegypti* at the containers, all containers were covered with a white fine-mesh cloth. Observations were continued until the larval mortality dropped below 20%.

RESULTS

Values of LC_{50} and LC_{90} of *B. thuringiensis* H-14 against *A. aegypti* larvae by probit analysis of the bioassay results using a computer was 0.1179 mg/l (95%

confidence Limit (CL) : 0.071-0.196 mg/l) and 0.982 mg/l (95% CL : 0.309-3.123 mg/l), respectively.

Fig 1 shows the residual toxicity pattern of *B. thuringiensis* H-14 on third instar larvae of *A. aegypti* in the cemented container. Percent mortality at the concentration of 1, 5, and 25 mg/l were found to be more than 80% on day 18, 36, and 60, respectively, and the percent mortality dropped sharply from then onwards. Fig. 2 shows the residual toxicity pattern in the clay container. In this study, more than 80% mortality was occurred on day 18, 30, and 36 for the concentration of 1, 5, and 25 ml/l, respectively, and dropped

sharply from then onwards. The pattern was similar. The result of residual toxicity pattern at the same concentration in the plastic container (Fig. 3) are as follows : more than 80 % mortality was achieved on day 12, 42, and 42, respectively, and dropped sharply as in the cemented and clay containers.

DISCUSSION

Different pattern of residual toxicity of *B. thuringiensis* H-14 on third instar of *A. aegypti* was observed with three different types of container (Fig. 1, 2, and 3). Longest

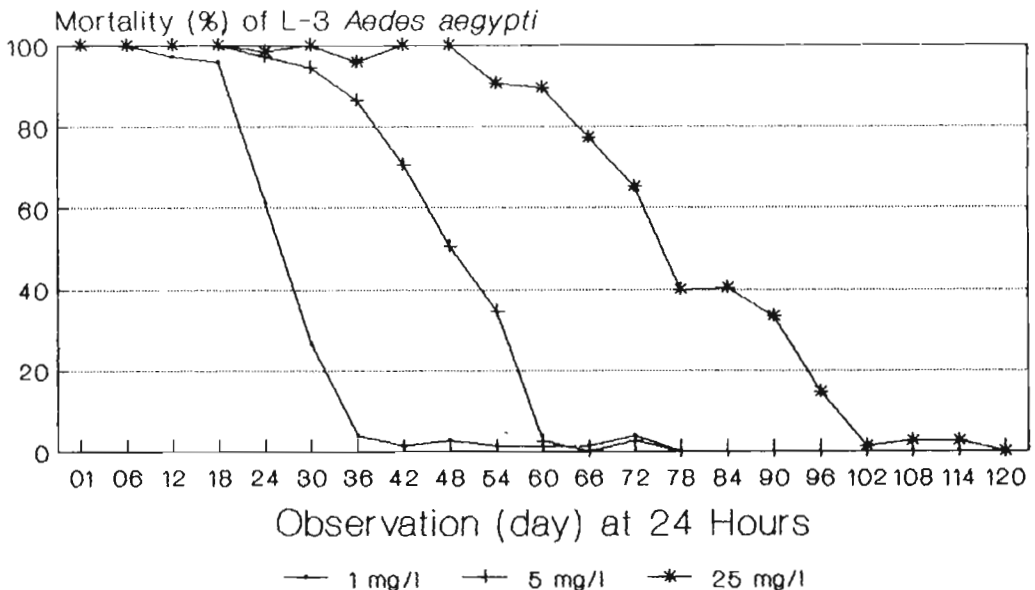


Fig. 1. Residual Toxicity of *Bacillus thuringiensis* H-14 (VCRC B17) in the Cemented Container

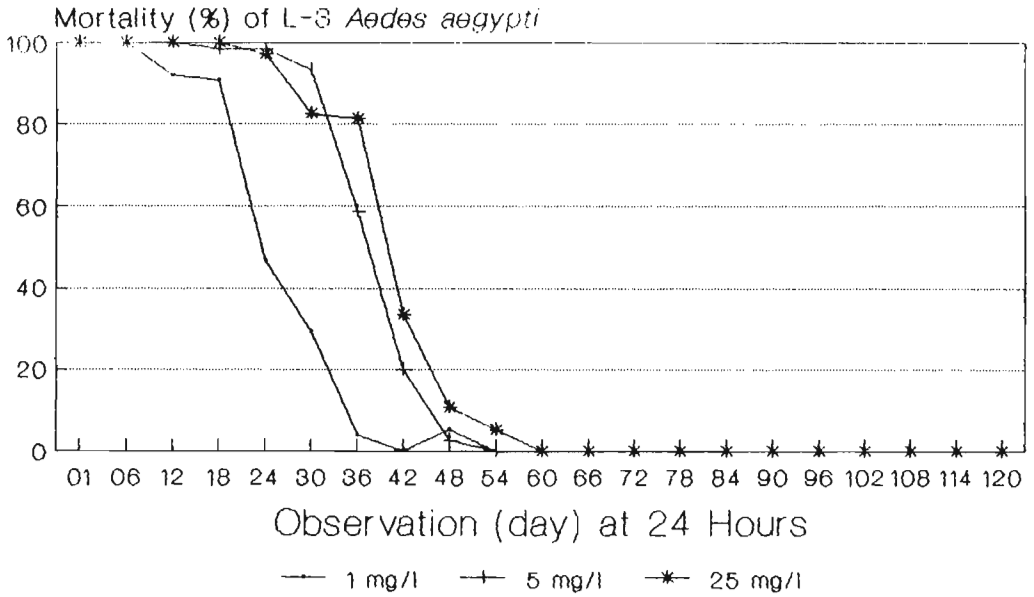


Fig. 2. Residual Toxicity of *Bacillus thuringiensis* H-14 (VCRC B17) in the Clay Container

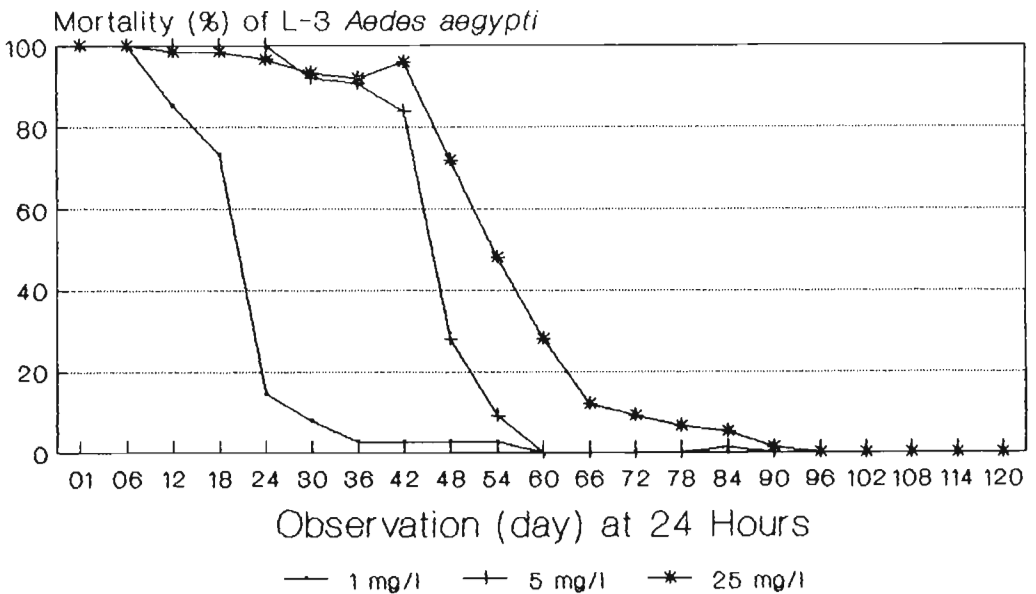


Fig. 3. Residual Toxicity of *Bacillus thuringiensis* H-14 (VCRC B17) in the Plastic Container

effect among three different types of container was observed with cemented container followed by the plastic container, and shortest duration was noted the clay container.

Residual toxicity of *B. thuringiensis* H-14 in the breeding places of *A. aegypti* also depend upon on many other factors. Balaraman and Pillai (1990) reported that the larvicidal crystals of endotoxin from *B. thuringiensis* H-14 that were rapidly degraded in the aquatic environment⁷. Van Essen and Hembree (1982) demonstrated that residual effectiveness in the presence of soil constituent might lowered larval mortality, one to physical adsorption of toxin by soil particles, resulting in its loss from feeding zone of *A. aegypti* larvae⁸. Other identified factors are biodegradation in bottom layer and chemical deterioration of toxin in containers⁸, dilution factor that caused by removal and replenishment of water in the container⁹, form of formulations¹⁰, natural conditions of water, and other environmental factors⁹.

Different residual toxicity pattern in the three types of containers were assumed by different affinity and adsorption of *B. thuringiensis* H-14 spores by each types of containers, after settling in the bottom layer. In addition, different chemical deterioration and degradation pattern in the bottom layer will result in different residual toxicity too. Higher concentration application in the three

different types of containers, will result in higher residual toxicity, however it is also cause decreasing of effectiveness.

Based on the basic information from this study, a pilot study with limited/small scale trial obtained should be carried out to observe effect of *B. thuringiensis* H-14 against *Aedes aegypti* larvae under field conditions. Before initiating the field trial, factors such as feeding habits, choice of suitable product and formulation of *B. thuringiensis* H-14, and other environmental condition should be considered to achieve an optimal residual toxicity effect against the disease vector larvae.

CONCLUSION

Residual toxicity of *B. thuringiensis* H-14 (VCRC B17) against third instar larvae of *A. aegypti* indicates that more than 80% larval mortality at concentration ranging 1 to 25 mg/l in cemented, hardground, and plastic containers on days 16 to 60, 18 to 36, and 12 to 42, respectively. The higher application concentration, will result in higher residual toxicity in all three types of container, but there is a tendency of decreasing effectiveness. The cemented container was found to have longest effect of residual toxicity, followed by the plastic container, and the clay container has the shortest duration effect.

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REFERENCES

- Hii, J.L.K. (1979). Evaluation of an *Aedes* control trial using the one-larva-per-container method in Labuan Island, Sabah, Malaysia *Jpn J Sanit Zool* 30 : 127-134.
- Lee, H.L. and Lime W. (1989). A Re-evaluation of the Susceptibility of Field-collected *Aedes (Stegomyia) aegypti* (Linnaeus) Larvae to Temephos in Malaysia. *Mosq Born Dis Bull* 6 (4) : 91-95.
- WHO (1991). *Biological Control of Vectors*. UNDP/World Bank/WHO, Special Programme for Research and Training in Tropical Diseases, Geneva : 97-101.
- Foo, AES. and Yap HH. (1982). Comparative bioassays of *Bacillus thuringiensis* H-14 formulations against four species of Mosquitoes in Malaysia. *Southeast Asian J Trop Med Public Health* 13 : 206-210.
- Anonim (1988). *Bioassay Method of the Titration of Bacillus thuringiensis israelensis Preparation with IPS Standart*. Laboratoire de Lutte Bacteriologique, Institut Pasteur, Paris.
- Finney, DJ. (1971). *Probit Analysis*. Cambridge University Press, London.
- Balaraman, RE. and Pillai JS. (1990). *Review of Biological Control Research at Vector Control Research Centre Pondicherry*. Indian Council of Medical Research, New Delhi.
- Van Essen, FW. and Hembree SC. (1982). Simulated Field Studies with Four Formulations of *Bacillus thuringiensis* var. *israelensis* Against Mosquitoes : Residual Activity and Effect of Soil Constituents. *Mosq News* 42 (1) : 66-73.
- Lee, H.L., Pe TH. and Cheong WH. (1986). Laboratory Evaluation of the Persistence of *Bacillus thuringiensis* var *israelensis* Against *Aedes aegypti* Larvae, *Mosq Born Dis Bull* 2 (3): 61-66.
- Lacey, LA., Urbina MJ. and Heitzman CM. (1984). Sustained Release Formulations of *Bacillus sphaericus* and *Bacillus thuringiensis* (H-14) for Control of Container-Breeding *Culex quinquefasciatus*. *Mosq News* 44 (1) : 26-32.