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Der Pharmacia Lettre, 2020, 12 (4): 11-15
(http: //scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Toxicity of *Bacillus thuringiensis* Local Isolate Auky Island Padaido District in Biak Numfor Regency (*ABNP* 8, 9, 11 and 12) against Anopheles Mosquito Larvae by Deposition Method in the Field

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ABSTRACT

Vector control of infectious diseases in biology continues to be encouraged to reduce the use of insecticides that have an impact on the environment. Biological Control using Bacillus thuringiensis bacteria local indigenous isolates continues to be developed, in this study the toxicity test of local isolates ABNP 8, 9, 11 and 12 on the larvae of Anopheles mosquito at the Faculty of Mathematics and Sciences, Cenderawasih University. The aim of the study was to determine the toxicity of 8, 9, 11, and 12 local ABNP isolates, and to obtain local indigenous isolates with toxicity ≥ 85% to Anopheles mosquito larvae. The method used was experimental with a completely randomized design pattern using 4 local isolates; the course of the study was made of 50 cm deep grooves, 40 cm in diameter, given transparent plastic and 10 liters of water with a height of 30 cm and then sprayed with 100 ml isolate culture. Each typical mosquito plastic containing 40 instar 2 Anopheles larvae, each treatment isolate was repeated 3 times, observations were made after 72 hours by counting the number of dead Anopheles larvae. The results showed the toxicity of local ABNP8 isolates (73.33%), 9 ABNP, (74.17%), 11 ABNP, (73.33) and 12 ABNP, (98.33%), and variance analysis showed that the isolate treatment differed significantly at the level of 05%. , further testing using BNt α.05, obtained by BNt Value 9.24. The results of variance analysis with BNt further tests showed the treatment of local isolates of B. thuringienis ABNP 8, 9, and 11 toxicity was not different from the death of Anopheles mosquito larvae, this could be caused by several factors, namely the possibility of ABNP 8,9 isolate, and 11 bacterial strains were the same by sex influential in metamorphosis. Whereas local B. thuringiensis ABNP 12

isolates with a toxicity of ≥ 85% are much higher than ABNP 8, 9 isolates and 11 may be caused by differences in toxicity and strains against the death of Anopheles larvae, considering B. thuringiensis based on current serological tests obtained 58 strains with toxicity different or specific to insect larvae.

Keywords: *Bacillus thuringiensis*, Anopheles larvae, Mosquito Larvae, Metamorphosis, Toxicity

INTRODUCTION

Bacillus thuringiensis is a bacterium that can be isolated from various soil habitats that act as a saprobe or decomposer, including positive gram bacteria. This bacteria is very difficult to distinguish from other Bacillus bacteria, especially Bacillus cereus which has similarities in physiological properties and its ability to ferment sugars [1].

Although physiologically and biochemically, *B. cereus* has similarities but can be distinguished based on the characteristics of the *B. thuringiensis* bacteria that form protein crystals in the spores, whereas all bacteria belonging to the genus Bacillus including *B. cereus* do not form protein crystals. The protein crystal possessed by *B. thuringiensis* gives the ability to kill the larvae of Lepidoptera, Hymenoptera and Diptera insects, so that *B. thuringiensis* is referred to as a specific entomopathogenic bacterium [2,3].

Protein crystals are synthesized when sporulation occurs due to unfavorable environmental factors for growth as a form of dormancy in order to remain self-sustaining and maintain its generation. Protein crystals form together with the occurrence of sporulation in vegetative cells. The formation of protein crystals is controlled by the Cry Gen or encoded by the Cry Gen together with sploration [4]. Ayomi et al. has characterized *B. thuringiensis* from the soil habitat Biology learning forest of UncenWaena campus and obtained 290 isolates from indigenous, and obtained more than 50% whose toxicity to Anopheles larvae even showed UC 43 isolates killing Anopheles larvae ≥ 85% in laboratory scale.

Furthermore Lantang et al. has characterized *B. thuringiensis* from soil habitats in Auky Island Padaido District Biak Numfor District, obtained 19 indegenous isolates whose toxicity to Anopheles larvae were different, and found 3 isolates whose toxicity ≥ 85 ie *ABNP* 8, *ABNP* 9, *ABNP* 11, and *ABNP* 12 on a laboratory scale, while 15 other isolates were below 70% toxicity [5,6]. Isolation and characterization of indigenous local *B. thuringiensis* isolates in Indonesia continues to be carried out as well as its toxicity tests on several vectors that cause disease in both food crops and tree crops and in humans. Vector mediated diseases in humans are infectious diseases such as malaria, dengue fever, and filariasis where this disease is a disease that is generally found in developing countries including Indonesia, which is classified as poor sanitation, especially in rural areas.

Although the government continues to increase attention in eradicating malaria and other infectious diseases whose vectors are *Anopheles, Culex* and *Aedes* mosquitoes, malaria is still the main cause of death among the top 10 diseases in Papua. One of the steps taken by the government is the eradication of vectors using insecticides, but the results are momentary and only for adult mosquitoes, while also causing resistance and environmental problems. Based on the description above, it is necessary to carry out vector control for infectious diseases that cause malaria, filariasis and dengue fever by using *B. thuringiensis* isolate indigenous from Auky island, namely *B. thuringiensis* isolate *ABNP* 8, *ABNP* 9, *ABNP* 11 and isolate 12 on a sedimental scale field.

RESEARCH METHODOLOGY

This research was carried out in the Microbiology laboratory for the multiplication and designation of local isolates *B. thuringiensis* isolates *ABNP* 8, *ABNP* 9, *ABNP* 11 and local isolate 12 while field applications were sedimentally carried out in standing water in the biology campus of F.MIPA Univ. CENDERAWASIH JAYAPURA in June 2019. The materials used in this study were Nutrient agar, Luriahbertain agar, tryptose phosphate broth, Anopheles larvae instar 2 typical mosquitoes 40 x 40 cm in size and transparent plastic bags. *ABNP* 8, *ABNP* 9, *ABNP*11 isolates and *ABNP* 12 isolates were inoculated into the phosphate broth triptose media, incubated on the incubator shaker for 72 hours and then counted the number of microbial cells with Mac Farlan turbidity method.

The field application was carried out according to the method proposed by Munif which was modified, which was made a 50 cm deep soil curve, 40 cm in diameter, given a transparent plastic has and filled with water as much as 10 liters with a height of 30 cm then 100 ml of culture isolate each typical plastic mosquito that contains 40 instar Anopheles larvae 2, each treatment was repeated 3 times. Observations were made on day 3 after field application (sedimental) by counting the number of dead larvae, each dead larvae observed its shape and color to make sure that the death of the larvae was caused by *B. thuringiensis* toxin Data were analyzed using a completely randomized design if between local isolates were significantly different, then continued testing with BNT.

RESULTS AND DISCUSSION

The results of the field scale study were continental continental local isolate *B. thuringiensis ABNP* 8, *ABNP* 9, ANBP 11 and *ABNP* 12 with a cell count of 3,108 against larvae of Anopheles instar 2 mosquitoes after 72 hours, obtained an average or percentage of death or toxicity of *ABNP* 8 isolates. , 73.33%., *ABNP* 9, 74.18%, *ABNP* 11., 73.33 and *ABNP* 12., 98.33), as shown in Figure 1.

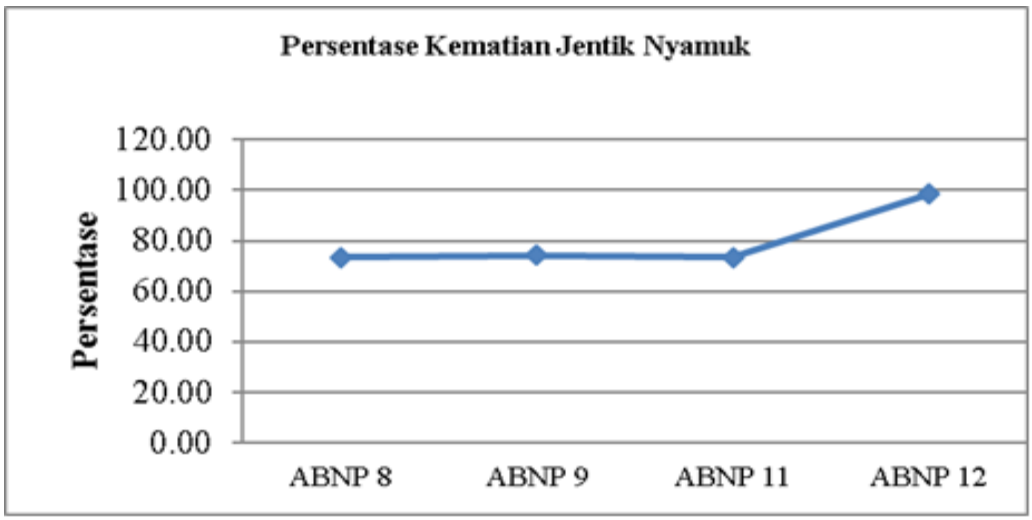

Figure 1: Continental local isolate *B. thuringiensi*

Analysis of variance with significance 0.05 there was a difference between F.hit and F.tab, so that further testing of BNT α.05 was obtained, from the results of further tests obtained BNTα.05 = 9.24, these results showed the effectiveness of the toxicity of the four local *B. thuringiensis* isolates, as in the Table 1 below.

Table 1: Toxicity of the four local *B. thuringiensis* isolates

Isolate	Average%	Notation
ABNP 8	73.33%	A
ABNP 9	74.18%	A
ABNP 11	73.33%	A
ABNP 12	98.33%	B

The results showed that *B. thuringiensis ABNP* 8, *ABNP* 11 and *ABNP* 9 isolates had the same toxicity to larvae mortality. It was possible that the three isolates were classified into 1 serotype or strain, whereas *ABNP* 12 isolates with BNTα.05 had very high toxicity. ie 98.33% or ≥ 85%, significantly different from *ABNP* 8, *ABNP* 11 and *ABNP* 9. Isolates the difference in toxicity of *ABNP* 12 isolates from other isolates is possible because of differences in strains or serotypes, or the activity of these larvae to the bottom to obtain food.

This is in line with that expressed by Tripsila et al. and Wibowo explaining that differences in toxicity can be caused by differences in strains or serotypes. Meanwhile, according to Lantang suggested that there are differences in the toxicity of *B. thuringiensis* to larvae in addition to being caused by serotype differences can also be caused by the sex of larvae where males cycle faster than females that are strongly associated with moulting or skin changes (metamorphosis) where larvae are experiencing body moulting weak due to running out of a lot of energy so that he has no desire to eat [6-9].

Blondine and Lantang report that *B. thuringiensis* toxicity is not common to all insect larvae or is specific to the Lepidoptera order vector, Coleoptera. Lantang reported in his research finding 19 local isolates of *B. thuringiensis*indegenus from the island of Auky Biak Numfor Papua and there were four isolates whose toxicity was ≥ 85% on a laboratory scale, this may be another isolate which was not targeted for the Diptera order so it was ineffective. Lantang reports that the toxicity of *B. thuringiensis* is also strongly influenced by the weight of protein crystals and the pH of alkali in the middle gut of insects (mid gut), the greater the weight of protein crystals, the greater the concentration of toxins produced when protein crystals dissolve [6,10,11].

The ability of *B. thurigiensis* to kill mosquito larvae, because this bacterium is able to produce protein crystals in the form of delta endotoxin or Cry toxin, Cry toxin is encoded by cry genes found in plasmids. *B. thuringiensis* produces 2 types of toxins, namely crystalline toxin (Crystal, Cry) and cytolytic toxin (cytolytic, Cyt). Cyt toxin can strengthen the Cry toxin so that it is widely used to improve effectiveness in controlling insects. More than 50 cry crystalline coding genes have been sequenced and used as a basis for grouping genes based on similarity in their constituent sequences.

CONCLUSION

Based on the results and discussion of local scale *B. thuringiensis* isolate toxicity (sedimental) shows *ABNP* 12 isolate has a high toxicity to Anopheles larvae ≥ 85% or 98.33%, significantly different from *ABNP* 8 isolates, *ABNP* 9, and *ABNP* 11 toxicity ≤ 85%.

SUGGESTONS

Considering that the local *B. thuringiensis ABNP* 12 isolate has a high toxicity to the death of Anopheles larvae, it needs to be developed to be made in the form of flour formulations to be tested for its toxicity on a field scale.

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