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# Effectiveness of *Bacillus thuringiensis israeliensis (B.T.I)* biofficial results on coconut water media on the death of *Anopheles* sp.

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**ABSTRACT**

Non-insecticide malaria control is carried out to reduce the environmental impact. Control can be done biologically. One biological agent that can be used in vector eradication is Bacillus *thuringiensis Israeliensis (B.T.I)*. The use of bacteria can be optimized by using a culture medium that is cheap and easy to obtain. This research is an experimental laboratory work because it is used to determine the ability of the *Bacillus thuringiensis Israeliensis (B.t.i)* bacterium bred in coconut water media for the death of Anopheles sp. The results showed that the average death of Anopheles mosquito larvae was more common in old coconut water, compared to the medium using young coconut water. There is a significant difference in larval mortality using young and old coconut water culture media

***Key words :*** *Bacillus thuringiensis Israeliensis, coconut water, Anopheles sp*

## Introduction

*Anopheles* sp. is a vector of malaria transmitters (Lefevre *et al.*, 2018). Malaria is an important tropical parasitic disease in the world and is still a major health problem. The 2015 World Malaria Report states that malaria has affected 106 countries in the world (Report 2015). Malaria morbidity in an area is determined by the Annual Parasite Incident (API) per year. National API trends in 2011-2015 as a whole in Indonesia continue to increase but seen in provinces in 2015. Eastern regions such as (Papua, NTT, and Maluku) still have the highest rates, ranging from 5.81-31.93 cases, while for DKI Jakarta and Bali API 0 (zero)(Hanandita and Tampubolon 2016; Health and Indonesia n.d.). However, for the current regencies/cities with moderate and low malaria endemic status, they must be vigilant, because it is feared that there will be an outbreak or commonly known as Emerging Infection (EID) (Tizifa *et al.*, 2018).

Clinical symptoms caused by malaria can reduce patient productivity. Various attempts have been made by the government to eradicate and control the spread of malaria-causing vectors. Chemical vector control for malaria has been practiced since 1959 in Central Java and the Special Region of Yogyakarta used DDT applications and dieldrin by

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indoor residual spraying (IRS) in malaria eradication programs nationally (Asih *et al.*, 2012). Along with the times, there has been an increase in Plasmodium resistance to antimalarial drugs and vector resistance to insecticides (Cohuet *et al.*, 2017; Kleinschmidt *et al.*, 2018; White, 2004). At the same time, ecological changes also increase the number of vector breeding sites in malaria-endemic areas (Bond *et al.*, 2004; Hanandita and Tampubolon 2016). Therefore, malaria still remains a serious health problem in various parts of the world, especially in developing countries.

To overcome this problem, malaria prevention and eradication strategy is carried out with more attention to the environment called Integrated Vector Management (IVM)(Clifford M Mutero, Dieter Schlodder, 2012; Ferguson, 2018). IVM is designed to reduce the negative impact on the environment due to the use of insecticides, emphasizing the importance of paying attention to vector ecology and local transmission patterns. This can be done with environmental management and biological eradication of vectors. One biological agent that can be used in vector eradication is *Bacillus thuringiensis Israeliensis* (B.t.i) (Derua *et al.*, 2018). At present B.t.i used in Indonesia is still imported from abroad, so much research has been done to breed bacteria so that it can be produced according to local wisdom.

The used of *Bacillus thuringiensis* has been widely studied. From the results of research in Bengaluru, India. Effectiveness *Bacillus thuringiensis* shows a dose of 1 mL/50 L can produce efficacy of 10-17 days (> 80% reduction in pupae) in clean water. While a 0.5 mL/50 L dose of contaminated water activity, the efficacy of 4-7 days for *Culex Quinquefasciatus* in Phase III (Uragayala *et al.*, 2018).

Research on the growth media of *Bacillus thuringiensis* still needs to be developed to obtain effective media and efficiency as the control of mosquito larvae with low production costs (Devidas, Pandit, and Vitthalrao, 2014). Research on the use of coconut water and coconut soaking water as a multiplication medium for *Bacillus thuingiensis* and the results showed that coconut soaking water was more effective and efficient used as a growth medium for Bt (Prabakaran *et al.*, 2008). Based on the description above, the researchers wanted to know the effectiveness of B. t.i results on coconut water type media on the death of Anopheles sp. Larvae.

## Material and Method

### Research design

This study was an experimental laboratory because it was used to determine the ability of *Bacillus thuringiensis Israeliensis* (B.T.I) bacteria which was bred in coconut water media against the death of Anopheles sp. by calculating the number of deaths from each concentration (0.25%, 0.5%, 1%, 3%, and 7%). The larvae used were instar III and IV larvae. The research was conducted at the Laboratory of Parasitology and Microbiology, Department of medical laboratory technology, Poltekkes Kemenkes Banten. The study was conducted in March-May 2018.

### Population and sample

The population of *Anopheles* sp. Larvae obtained from the Karawang area which was colonized in the Parasitology Laboratory of Department of medical laboratory technology, Poltekkes Kemenkes Banten. *Bacillus thuringiensis Israeliensis* (B.T.I) bacteria are in the form of bioinsecticides (Bactivec) which are then cultured to get an active inoculum.

The sample used was *Anopheles* sp. Larvae. instar III and IV. while in determining the amount of treatment and repetition using a Randomized Group Design pattern where the treatment in this study was 5 treatments. So it can be seen that the number of repetitions is 5 times. The sample used in one concentration is 20 larvae. So the total number of larvae needed is 600 larvae.

### Preparation of larvae of *Anopheles* sp.

In this study larva *Anopheles* sp. obtained from the Karawang area of the instar III and IV larva stages, then colonization was carried out at the Parasitology Laboratory of the Department of Health Analyst of the Health Ministry of Banten.

### Preparation of *Bacillus thuringiensis Israeliensis* (B.T.I) inoculum

Preparation of inoculum by growing B.t.i in Nutrient agar from commercial bioinsecticide products (Bactivec). the aim is to obtain fresh B.t.i cells before the development of the inoculum. Then inoculate 2 B.t.i capsules into the nutrient broth (NB) when the nursery flask is done, incubate used a shaker incubator. shaken with a speed of 100 Rpm at 30 ℃ for 12 hours. After that, 1 bread is taken back to the NA

plate (using the initial quadrant technique) to get a single colony.

**Making coconut water media**

Good quality coconut sorted, then washed with running water, soak for 4 hours. then the exfoliation is done cleanly after that, wash it again. clean coconut smoothed using a juicer, after that, put into Erlenmeyer. Adjust the pH until the pH reaches 6.5-7 using KOH. After pasteurization, the neutral pH is carried out by heating coconut water at 60 °C for 30 minutes..

**Planting *Bacillus thuringiensis Israelensis (B.T.I)* in coconut water media**

Pure culture of *Bacillus thuringiensis Israeliensis (B.t.i)* which grows on Nutrient Agar (NA) plate taken as much as 1 ose in input into 2 mL 0.9% NaCl to determine the density of bacteria using McFarland until the concentration reaches 0.5. Then 100 µL of bacterial suspension was taken into Erlenmeyer which contained 50 µL of coconut water, homogenized until dissolved and then cultured at incubator shaker 100 rpm temperature 300 for 2x24 hours. (Lantang, 2005).

**Preparation of B.T.I suspension solution produced on coconut water media with serial concentrations of 0.25%, 0.5%, 1%, 3%, and 5%**

Making a Bti suspension solution by preparing 5 100 mL volumetric flasks each labeled 0.25%, 0.5%, 1%, 3%, and 5%, then the Bti suspension is piped as much as 0.25 mL, 0.5 mL, 1 mL, 3 mL and 5 mL are put into a volumetric flask, add aquadest as much as 99.75 mL, 99.5 mL, 99, 97, and 95 respectively, dissolve until homogeneous.

**Effectiveness test of *Bacillus thuringiensis* Israeliensis (B.T.I) on Anopheles sp. Larvae**

The effectiveness test of *Bacillus thuringiensis* Israeliensis (B.T.I) was carried out by preparing 30 plastic cups of 240 mL size that were clean and labeled according to concentration, then each plastic container was inserted 100 mL of B.t.i suspension solution which had been dissolved with distilled water, put in Anopheles sp. as many as 20 birds. Then let stand for 24 hours. After 24 hours, observations were made to calculate the number of dead larvae and observe again after 48 hours to find out the effectiveness test results of B.t.i.

**Data analysis**

Primary data from observations of *Anopheles* sp. dead ones are processed using group patterns created in the form of tables and graphs. The ANOVA test was used to determine the effective concentration to kill Anopheles sp larvae and the t-test to determine the difference between young and old coconut water as a culture medium for the death of Anopheles sp. Larvae.

## Result

Based on Figure 1 it is known that the number of deaths of Anopheles sp. Larvae. with 15 repetitions on 150 old larvae with an average mortality of 10 (100%) whereas in young coconuts there were 77 larvae with an average mortality of 5.13 (51.3%) so it can be seen that Bti cultured in old coconut and young coconut has the ability to kill Anopheles sp. Larvae. While in the negative control there were 150 larvae without being given treatment with the addition of Bti preparations resulting from the culture on the coconut water media, no deaths were found, even the larvae could develop into pupae stages and some managed to become adult mosquitoes. The number of deaths of Anopheles sp. Larvae in older coconut water more than young coconut water.

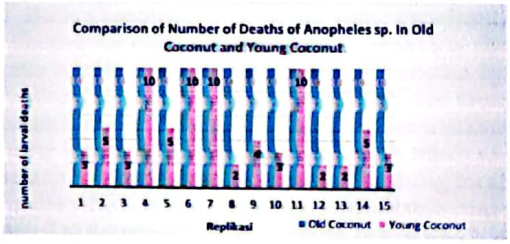


**Fig. 1.** Comparison of Number of Deaths of Anopheles sp. In Old Coconut and Young Coconut

Descriptively the table 1 shows that the average mortality of Anopheles mosquito larvae (10.0) occurs in media using old coconut water, compared to media that use young coconut water only the average mortality of mosquito larvae is 5.13. Bivariate obtained a p-value of 0,000; in other words that p <0.05. Thus statistically it shows a significant difference in mortality rates on larvae in both media.

**Discussion**

The number of deaths of Anopheles sp larvae in old

**Table 1.** Results of Analysis

| Media | Average larvae mortality | t | Pa |
|---|---|---|---|
| Young Coconut | 5.13 | -5.883 | 0.000* |
| Old Coconut | 10.0 | | |

*T-Test
*Significant, P ≤ 0.05

coconut is more than the number of deaths of Anopheles sp larvae in young coconuts. this is due to the fact that young and old coconut water has different nutritional content, namely vitamins, calcium and the percentage of water content owned by old coconut water is more and complex compared to the nutrient content in young coconut including old coconut has a carbohydrate content of 3.80 %, protein 0.20%, fat 1.00%, vitamins 1.00%, water 95.50%, Calcium 15.00% while young coconut water has a carbohydrate content of 4.60%, protein 0.14%, water 91.50%, fat 1.50% (Rades *et al.*, 2012). This content in accordance with the nutritional needs needed for Bti bacterial growth is also in line with the research conducted by Prabakaran *et al.*, 2008 stating that coconut water can be used for Bti growth (Prabakaran *et al.*, 2008).

Nutrients that must exist and must be fulfilled for the growth of Bti, namely carbohydrates, proteins, fats, and nitrogen (vitamins and amino acids). This content is very influential for multiplying the number of bacterial cells, if the nutrient media that is overgrown with bacteria is less fulfilling, the number of bacteria cells produced is also small (El-Bendary, 2006). In the study of old coconut and young coconut (coconut water and endosperm) which had been overgrown with Bti as much as 3 mL /L coconut (420-450 mL coconut water) then incubated for 14 days. This is because in the 14th-day incubation the growth of spores and crystals of Bti protein toxin is quite large. Amino acids and carbohydrates are a source of nutrition for the growth of Bti Coconut water is rich in amino acids and carbohydrates and contains high glutamic acid. Glutamic acid or high amounts of methionine can stimulate the growth of large amounts of bacteria.

Larval mortality is influenced by various factors, both factors of larval conditions and environmental factors. These factors are the instar larvae, food, exposure period, water quality, bacterial strains, water temperature, and Bti formulation, especially its deposition sedimentation rate (Couret *et al.*, 2014; Program and Brisbane, 2000; Sciences and Laboratories, 2004). When viewed from the condition of the larvae, the larvae used are instars III / IV where at this stage the digestive system of larvae has been formed perfectly, so that protein crystals and spores in Bti can work optimally as poisons or toxins in the digestive tract.

Protein crystals and spores produced by Bti are toxins that will dissolve and be active in the atmosphere of larval bowel bases. The bond between the toxin and the cadherin receptor causes damage to the intestinal microvilli, columnar cells, goblet cells, and lysis of other epithelial cells, which leads to the mortality of larvae (Cohuet *et al.*, 2017; Derua *et al.*, 2018; El-Bendary, 2006).

In addition to causing epithelial cell lysis, this toxin results in decreased appetite for larvae so that the larvae do not have an appetite and eventually stop eating. The toxin also results in the formation of small pores measuring 0.5-1 nm resulting in disruption of fluid permeability. On the other hand, the bacteria continue to proliferate in the larval body so that the larvae become septic. All of these mechanisms play a role in the death of larvae. The body of the dead larva appears blackish in color which starts with the anterior to posterior and swells due to disruption of osmotic pressure (Vinnersten, 2008).

Bti is a bacterium that can be commercialized, effective for controlling mosquito larvae but the price is quite expensive for developing countries. By finding a local strain of Bti. It is expected that these bacteria can be developed in various formulations using local media (Devidas *et al.*, 2014). Much research has been done to produce Bti using local media which is relatively more affordable compared to the original growth media, one of which is a study conducted at the Alexander von Humboldt Tropical Medicine Institute in Lima, Peru, which uses coconut (coconut water and endosperm) to produce B. thuhngiensis israelensis (H-14). The results showed that B. thuringiensis israelensis (H-14) grown in coconut can be fermented and effectively control mosquito larvae.

## Conclusion

The average mortality of *Anopheles* mosquito larvae is more common in media using old coconut water, compared to media that use young coconut water. There is a significant difference in mortality rates for

larvae using young and old coconut water culture media. The use of old coconut water as Bti bacterial culture media in endemic areas of malaria. There is further research on the use of variations in coconut species at the site of malaria cases. There are further studies on the application of bioinsecticides using Bti bacteria as a result of the culture of coconut water media.

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