The Use of Domestic Waste as Culture Media of Bacillus Thuringensis for Minimizing Pre-Adult Mosquitoes Population

Pratiwi Hermiyanti[1], Fitri Rokhmalia[2] and Ngadino[3]

[1,2,3]Environmental Health Departement, Health Polytechnic Ministry of Heatlh Surabaya

[1]pratiwi@poltekkesdepkes-sby.ac.id, [2]fitri.rokhmalia-13@fkm.unair.ac.id and [3]bungdino1960@gmail.com

***Abstract*—** The use of bacteria as bio insecticide is safe, effective, and selective in eradicating mosquito larvae, especially Aegypti mosquito larvae. Domestic waste (black water) contains high protein that can be nutrient for development of Bacillus thuringensis as biolarvacide. Nevertheless, the purpose of this study was in order to analyze the effectiveness of using domestic waste as culture media of Bacillus thuringensis for minimizing Aedes mosquito larvae population.

Design of this research was experimental with posttest only control group design. The sample size of mosquito larvae that was used in this research was 160 larvae and each 20 larvae was in each dose of treatment and control. Suspension dose of liquid waste media of Bacillus thuringensis culture that was used in this treatment was 1 ml; 2 ml; 3 ml; 5 ml; 7 ml; 10 ml; 30 ml; and 50ml. Data analysis was by analyzing table and it was presented in narrative.

The content of domestic waste that supported as nutrient for Bacillus thuringensis was carbohydrate (0.88%), protein (1.62%), and the state of waste pH (pH 7). Activity of Bacillus thuringensis was less optimal to develop in domestic liquid waste media. This was indicated by the absence of white sediment at the bottom of Erlenmeyer flask which showed the development of bacteria. Liquid waste media that was cultured by Bacillus thuringensis did not show any significant effectiveness to become biolarvacides because no doses which were applied could eradicate mosquito larvae.

All in all, conclusion of this research was Bacillus thuringensis had not been optimally developed in domestic liquid waste media, hence, its pathogenicity could not be tested on mosquito larvae.

***Keywords*—** Domestic waste, Killing power of Bacillus thuringiensis spores, Mosquito Larvae.

I. INTRODUCTION

The control of Aedes aegypti mosquito vector which is often applied directly without being accompanied by other control efforts is through fogging by using insecticides, but this method is still considered unsuccessful in eradicating DHF cases. The use of insecticides in long term can cause vector resistance. Besides, the use of insecticides causes an impact on the death of non-target insects and environmental pollution. Thus, it is necessary to find other alternatives which are more effective and efficient to eradicate DHF vector. One of the methods that begin to be implemented due to many advantages is biological control of mosquitoes by using Bacillus thuringiensis bacteria which is pathogenic for Aedes mosquito larvae. The advantages of using bacteria as bio insecticides are they are safe, effective, and selective in eradicating mosquito larvae, especially Ae. Aegypti mosquito larvae (Purnama, Pandy, & Sudiana, 2012).

Bacillus thuringiensis serovar israeliensis (B.t.i) produces delta endotoxin which is pathogenic against insects and has been developed as bio insecticide to eradicate mosquito larvae and black flies (WHO, 1979). This advantage needs to be supported by B.t.i spore development media so that this bio insecticide can be produced on large scale. In order to produce B.t.i spores, growth medium is needed, which until now still uses synthetic medium which is expensive to get. Therefore, it is necessary to find an alternative that can be used as growth medium of B.t.i at lower price, but high production of B.t.i. spores (Purnama, Pandy, & Sudiana, 2012).

Bacillus thuringiensis bacteria are gram-positive bacteria that produce spores and protein which is toxic during sporulation. These bacteria have selective target through food digestion which have high pH level. Hence, insects that do not have any high digestive pH will not be sensitive against Bacillus thuringiensis. (Pujiastuti, Triyansyah, Hamidson, Effendy, & Suparman, 2017). Deadly effect of these bacteria on pre-adult mosquitoes is because these bacteria produce delta endotoxin that is contained in crystal toxin protein (Mardihusodo, 2012).

Tofu liquid waste contains relatively high level of protein, glucose, and other components. With these nutritional contents, tofu liquid waste has potency as a medium to produce B.t.i. spores. (Purnama, Pandy, & Sudiana, 2012). Tofu liquid waste contains organic material. If it is directly disposed into the river without

any treatment process, it will pollute the environment, especially groundwater. Hence, the oxygen that is dissolved in water reduces which impacts against living organisms in the water and even pollution that is done continuously will cause the organisms in the water die (Sari, Koerniasari, & Jauhari, 2016).

Domestic waste (black water) also contains high protein as what is indicated by Biological Oxygen Demand (BOD) parameter. In conducted research by (Hermiyanti, Rokhmalia, & Darjati, 2019) explained that BOD of domestic waste that had been through tripikon infiltration still contained BOD level of 24 mg/L. Although BOD level was in accordance with applicable waste regulations, the protein content (BOD) in this waste could be used for other things that could reduce the level until being eco-friendlier. Furthermore, the use of domestic waste that contains protein is possible to be used as culture material for the development of Bacillus thuringiensis spores for minimizing the development of mosquito larvae.

II. MATERIAL AND METHOD

Design of this research was experimental with posttest only control group design because it aimed at investigating the effectiveness of domestic waste media from septic tank infiltration to minimize pre-adult mosquito population. The number of mosquito larvae that was used in this research was 160 larvae and each 20 larvae was in each dose of treatment and control. The suspension doses of culture liquid waste media of Bacillus thuringensis that was used in the treatment were 1 ml; 2 ml; 3 ml; 5 ml; 7 ml; 10 ml; 30 ml; and 50 ml.

III. RESULT AND DISCUSSION

A. The Content of Domestic Waste

Waste is disposed thing or something that is not used which can be in liquid, gas, and solid. Domestic waste is wastewater that is from household waste, such as water used from washing, kitchens, bathrooms, and toilets (Dahruji, 2017). The liquid waste that was used in this research was waste that was from septic tank, which was fecal waste. As we know that domestic wastewater contains 99.9% water and 0.1% solids. The solid substance consists of 85% protein; 25% carbohydrates; 10% fat, and inorganic substances, particularly grains of sand, salts, and metals (Sugiharto, 2008).

Based on its characteristics, the characteristics of organic waste are divided into three, which are physical, chemical, and biological characteristics. Physical characteristics include total solids, turbidity, odor, temperature, and color. Biological characteristics are by looking at microorganism group (pathogenic or not) which are contained in the organic waste. Chemical characteristics in organic waste are protein (containing carbon, hydrogen, oxygen, and the forming cells and cell nucleus), carbohydrates (sugar, starch, cellulose, and wood threads that consist of C, H, and O elements), and oil that is fat which are liquid, detergent, and phenol that have water-soluble character (Ariyani, 2010).

Table 1: The Content of Domestic Waste

Content	Result
BOD	286.50 ppm
Carbohydrate	0.88%
Amino Acids	1.62%
pH	7

Table 1 showed that there were still nutrients for microorganisms to grow. Bacteria that acted as bioremediations of organic waste could grow by utilizing organic materials as source of protein to increase their biomass and must have environmental conditions that supported the growth of these bacteria (P. H. Doraja, 2012).

The contents of nutrient in liquid waste media, which were carbohydrates and protein, as being measured in this research, were also inadequate for the number of food sources for Bacillus thuringensis. According to previous research, Bacillus thuringensis was able to live on media that contained simple macro carbohydrates such as glucose, fructose, and amino acids (Blondine, 2009). In this research, measurements against glucose, fructose and other types of protein had not been conducted which could support the foodstuff reserves for the growth of Bacillus thuringensis.

In this research, there had not been conducted a complete measurement against liquid waste content, which was another factor that could inhibit the growth of Bacillus thuringensis. This made a weakness for this research, hence, discussion about the content of liquid waste could not be further discussed.

B. Bacillus thuringensis Spore Culture by Using Domestic Waste Media

The next research stage was making Bacillus thuringensis bacteria cultures which were from isolates and developed on Nutrient Broth media with the aim of refreshing the bacteria. Then, it was followed by cultivating Bacillus thuringensis in domestic waste after conducting incubation for 24 hours in refreshing media. After being conducted incubation for 4x24 hours at temperature of 300C, the liquid waste that contained Bacillus thuringensis culture was tested for its pathogenicity in immature/ pre-adult mosquitoes.

Figure 1: Comparison of the activity of Bacillus thuringensis in nutrient broth media and domestic waste

Figure 1 showed that the activity of Bacillus thuringensis was less optimal in domestic liquid waste media and this was indicated by the absence of white sediment at the bottom of the Erlenmeyer flask which indicated the development of bacteria. Nutrient broth media and domestic wastewater were also clear (not turbid) which indicated that bacteria were not able to reproduce properly. The indication of turbid color and the presence of sediment on the media indicated that there was the growth of bacteria after incubation that was caused by metabolic process by the bacteria. The appearance of the development of bacteria could be seen in various forms, which were: 1) Visible turbidity in all parts of media, 2) The growth of bacteria on surface in the form of pellicle (surface of liquid media in ring shape, flocculent or membrane), 3) Sediment at the bottom of the tube / Erlenmeyer flask if it was moved or shaken (Yempita Efendi, 2017).

According to the results of the examination of domestic waste content, the content of carbohydrates and amino acids which were nutrients for the growth of bacteria was minimal enough to be used as a media for development. This was proved by the results of bacterial culture which showed no sediment or turbid color in domestic liquid waste media that indicated the development of bacteria in the media.

Bacillus thuringensis would be ready to proliferate if environmental conditions supported. The temperature, pH, and nutrient factors which were contained in media supported the development of Bacillus thuringensis (Akhmad Gazali, 2017). Although pH and temperature had been adjusted to optimal conditions for the development of Bacillus thuringensis, actually nutrient factor and possibly external factors could inhibit the development of bacteria. External factors that perhaps caused the development of bacteria to be less optimal included: 1) Cell density, 2) Factors of Sigma σE and σK 3) Mutations from σE. Conducted research by A. Gazali (2015), explained that environmental factors that supported the development of Bacillus thuringensis populations in certain ecosystems were also depended on the influence of ultraviolet light which could damage the spores and crystals of Bacillus thuringensis. Hence, it disrupted the stability of bacterial breeding.

The development of Bacillus thuringensis according to previous research explained that this bacterium underwent a growth phase until death phase for up to 28 days (Akhmad Gazali, 2017). The process of bacterial culture in this research was conducted in 4 x 24 hours (incubation process) which the time that was used was still not optimal to reach the growth phase (lag). Thus, this caused the bacterial culture did not proceed properly.

C. The Effectiveness of Domestic Waste Media from Septic Tank Infiltration for Minimizing Pre-Adult Mosquito Populations

Toxicity of Bacillus thuringensis was determined by its larvicidal activity. Bacteria culture that had been developed in domestic liquid waste media was then applied to Aedes aegypti mosquito larvae. Bacterial culture in domestic wastewater was applied in several doses to be tested against mosquito larvae, which were 1 ml, 3 ml, 5 ml, 7 ml, 10 ml, 30 ml, and 50 ml. III instar of Aedes aegypti mosquito larvae in each 20 larvae which were prepared were put in treatment water and observed for 2 x 24 hours. This was intended to analyze the effectiveness of the presence of Bacillus thuringensis in liquid waste media to eradicate mosquito larvae.

After being conducted observation for 2 x 24 hours, the liquid waste media that was cultured by Bacillus thuringensis did not show any significant effectiveness to become biolarvacide because no doses which were applied could eradicate mosquito larvae. However, there were several possible causes of this ineffectiveness, they were:

1. Bacillus thuringensis could not develop in liquid waste media due to insufficient nutrient content. Bacillus thuringiensis required carbon, nitrogen, and mineral sources such as Ca, Mg, Mn, Fe, and Zn for vegetative cell growth and for producing endotoxin (Rini Purnawati, 2015). In this research, nitrogen and mineral content had not been further investigated which might affect the optimal development of Bacillus thuringensis in domestic waste media. Thus, for further research could be further investigated regarding its nitrogen and mineral content.
2. Isolate Bacillus thuringensis was still in lag (growth) phase or had experienced stationary phase or death. Hence, when it was refreshed, its

development was less optimal. Bacillus thuringensis had long lag phase until stationary phase for up to 28 days after inoculation on the media of corn extract, soybean extract, and rice extract. From the results of the research, it was explained that the growth phase of Bacillus thuringensis was also influenced by nutrient content in the media (A. Gazali, 2017). Stationary phase of bacteria occurred when the growth rate was the same as the mortality rate of bacteria. Thus, the total number of bacteria would remain. This balance of the total number of bacteria occurred due to degree reduction of cell division. This was caused by nutrient levels that reduced and accumulation of toxic products that interfered with cell division. This stationary phase was followed by death phase which was indicated by an increase in mortality rate that exceeded the growth rate. Hence, decrease in bacterial population occurred overall (Puji Lestari, 2019).

3. Extrinsic factors in causing bacterial growth was inhibited External factors that might cause the development of bacteria were less optimal, including: 1) Cell density, 2) Factors of sigma σE and σK 3) Mutations from σE. Conducted research by A. Gazali (2015), explained that environmental factors that supported the development of Bacillus thuringensis populations in certain ecosystems was also depended on the influence of ultraviolet light which could damage the spores and crystals of Bacillus thuringensis. Thus, it disrupted the stability of bacterial breeding.

IV. CONCLUSION

Liquid waste media that was cultured by Bacillus thuringensis did not show any significant effectiveness to become biolarvacides because no doses which were applied could eradicate mosquito larvae. This was possible due to various factors, such as insufficient nutrient content; the isolated Bacillus thuringensis that was still in lag (growth) phase or had experienced stationary phase or death. Hence, when it was refreshed, its development was not optimal; or there were extrinsic factors that caused the growth of bacteria were inhibited.

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