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Research Article

Soybean Soaking Waste as a Culture Medium for Bacillus Thuringensis in an Effort to Control Aedes Larvae Aedes

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Abstract

Bacillus thuringensis is a biolarvicide that is useful for mosquito larvae control. Bacillus thuringensis culture media has been widely used with various types of waste containing carbon or starch and oil-free protein for its development. Soybean soak is generally dumped into water bodies without further utilization even though it has a fairly high starch and protein content and can be used as Bacillus media. The aim of this study was to analyze the effectiveness of using soybean soaking waste as a Bacillus thuringensis culture medium in an effort to control Aedes larvae. This research is an experimental type with posttest only control group design. Bacillus thuringensis developed with soybean soaking waste media was divided into 1%, 3%, 5%, 7%, 10% and 20% suspension doses in 100ml of pathogenicity test media. The number of mosquito larvae used in this study amounted to 240 mosquitoes for each of the 20 doses of treatment and control. Data were analyzed using oneway Annova or Kruskal Wallis. Soybean soak with a dose of 3%-20% can kill Aedes larvae in 2x24 hours. Soybean soaking waste can be used as a culture medium for Bacillus thuringensis.

Keywords: Soybean soak waste, pathogenicity, Bacillus thuringensis, Aedes larvae

Introduction

Environmental conditions in Indonesia, which is located in a tropical climate, significantly affect the breeding of Ae. Aegypti mosquitoes. This affects the high fluctuation of Dengue Fever (DHF) cases in Indonesia, which is directly related to environmental conditions. In addition to environmental conditions, the community's lack of Mosquito Nest Eradication (PSN) measures aggravates dengue cases so that to reduce the incidence of dengue disease it is necessary to pay attention to prevention efforts [1].

Efforts to control the Ae. aegypti mosquito vector are generally carried out without being accompanied by other controls. Fogging using insecticides is the focal point of dengue control, but this method is still considered unsuccessful in eradicating dengue cases. It should be noted that there is resistance for vectors due to the use of insecticides over a long period of time. In addition, the use of insecticides causes other impacts in the form of non-target insects that are also killed and the potential for environmental pollution. With

these considerations, it is necessary to seek alternatives that are more effective and efficient in tackling dengue vectors. One effort that is starting to be widely chosen because it has many advantages is biological mosquito control using *Bacillus thuringiensis* bacteria that are pathogenic to Aedes mosquito larvae. The advantages of using bacteria as bioinsecticides are safe, effective and selective in killing mosquito larvae, especially Ae. aegypti mosquito larvae [2].

The high incidence of DHF in Indonesia is significantly related to environmental conditions that can affect the breeding of Ae. aegypti mosquitoes in the environment. This is also exacerbated by the community's lack of PSN measures so that to reduce the incidence of DHF disease, attention needs to be paid to preventing DHF disease [1].

Vector control of Ae. Aegypti mosquitoes that is often done directly without being accompanied by other control efforts is through fogging using insecticides, but this method is still considered unsuccessful in eradicating dengue cases. Longterm use of insecticides can lead to vector resistance. In addition, the use of insecticides causes the death of non-target insects and environmental pollution. So it is necessary to find other alternatives that are more effective and efficient to tackle dengue vectors. One method that is starting to be widely practiced because it has many advantages is biological mosquito control using *Bacillus thuringiensis* bacteria that are pathogenic to Aedes mosquito larvae. The advantages of using bacteria as bioinsecticides are safe, effective and selective in killing mosquito larvae, especially Ae. Aegypti mosquito larvae [2].

Bacillus thuringiensis is a gram-positive bacterium that produces spores and proteins that are toxic during sporulation. This bacterium has a selective target through food digestion that has a high pH level, so that insects that do not have a high digestive pH (nature) will not be sensitive to *Bacillus thuringiensis* [3]. The lethal effect of this bacterium on preadult mosquitoes is because this bacterium produces delta endotoxin contained in toxin protein crystals [4].

Bacillus thuringiensis serovar *israeliensis* (B.t.i) produces delta endotoxin which is pathogenic to insects and has been developed into a bioinsecticide to kill mosquito larvae and black flies [5]. This advantage needs to be supported by B.t.i spore development media so that this bioinsecticide can be produced on a large scale. To produce B.t.i spores, a growth medium is needed, which until now still uses a synthetic medium which is expensive to obtain. Therefore, it is necessary to find an alternative that can be used as a growth medium for B.t.i at a cheaper price, with high production of B.t.i spores [2].

Efforts to utilize Bacillus thuringensis isolates on a wide scale are still not economical due to the difficulty and high cost of standard media for propagating these bacteria. There is still a need for alternative propagation media that are cheap and easy to obtain without reducing the level of pathogenicity. Bacillus thuringansis is easily propagated through liquid culture fermentation using relatively cheap materials [6]. Bacillus thuringensis culture media can use soy flour to multiply strain H-14 in an effort to control Aedes, Anopheles and Culex mosquitoes [7]. The use of coconut water and soybean soaking media to multiply Bacillus thuringensis [8].

Given the less complicated fermentation technology and the easy and cheap raw materials available in Indonesia as well as the local isolates of Bacillus thuringensis that are already available in Indonesia, efforts to develop alternative culture media can be developed. Soybean soak, which is an unutilized waste, can be used as a propagation medium for Bacillus thuringensis which functions as a biolarvicide . The aim of this study was to analyze the effectiveness of using soybean soak waste as a culture medium for Bacillus thuringensis in an effort to control mosquito larvae.

Materials and Method

This research design is experimental with a *pre and posttest only control group* design because

it aims to analyze the effectiveness of soybean soaking waste media as a Bacillus thuringensis culture medium to suppress preadult mosquito populations . The object in this study is soybean soaking waste added with sugar as an additional nutrient that is applied as a medium for the development of *Bacillus thuringensis* spores. Furthermore, the culture suspension will be tested for pathogenicity against larvae with a suspension dose design of 1%, 3%, 5%, 7%, 10% and 20% in 100 ml of water. The number of mosquito larvae used in this study amounted to 240 mosquitoes for each of the 20 doses of treatment and control.

Media preparation

The bacterial rejuvenation process uses NA (*Nutrient Agar*) media as much as 4 grams in 180 ml of distilled water, then heated on a hotplate while stirring until boiling with a bright yellow color indicator. While stirring to boil with a bright yellow color indicator, *then* NA media is poured into 3 test tubes with a volume of 5 ml each and 7 Petri dishes with a volume of 20 ml each [9].

The medium used to grow bacteria is soybean soaking wastewater, which is made from 500 grams of soybeans soaked using 1000 ml of water overnight. The waste that has been obtained is added with 20 grams of sugar per 500 ml, put into 3 erlenmeyers with a volume of 250 ml, each filled with 100 ml through a filtering process using filter paper [10].

Bacillus thuringensis culture preparation

Bacillus thuringensis was transferred from the isolate tube into a refreshed agar slant by transferring 2 eye loops of culture into sterile NA media and then incubated for 24 hours at 30^0 C (until bacterial colonies formed).

Bacterial growth

After the formation of bacterial colonies, four ose eyes were taken and transferred to 100 ml of soybean soaking water waste media and incubated for 36 hours at 30^0 C.

Pathogenicity test

The pathogenicity test of *Bacillus thuringiensis* suspension was conducted by suspending *Bacillus thuringiensis* culture in liquid waste with distilled water. furthermore, 1 ml, 3 ml, 5 ml, 7 ml, 10 ml, and 20 ml were taken successively using a pipette and then inserted into a plastic cup containing 20 *Ae. aegypti* mosquito larvae and successively added with distilled water as much as 99 ml, 97 ml, 95 ml, 93 ml, 90 ml, and 80 ml to obtain the required final concentration. As a control plastic cup plastic cups were only filled with 100 ml of distilled water and 20 *Ae. Aegypti larvae*, then allowed to stand for 24 hours. After 24 hours, observations were made to count how many number of mosquito larvae that have died and after 48 hours it will be observed again to determine the results of the pathogenicity test of *Bacillus thuringiensis.*

Results

Control of the Ae. aegypti mosquito vector has been carried out by spraying using insecticides, but this method has not been successful in eradicating dengue cases. Repeated use of chemical insecticides can lead to vector resistance, death of other non-target animals and environmental pollution. Therefore, it is necessary to find other alternative ways that are more effective in tackling dengue vectors, one of which is using biological control [11].

Biological control such as using chitinolytic bacteria that have a lethal effect on preadult mosquitoes because these bacteria produce delta endotoxins contained in toxin protein crystals [4]. These bacteria have a selective target through food digestion that has a high pH level, so that insects that do not have a high digestive pH will not be sensitive to this type of bacteria [3].

The use of chitinolytic bacteria such as *Bacillus thuringiensis* serovar *israeliensis* (B.t.i) *is* considered quite effective as a pathogen for mosquito larvae. The advantages are safe, effective and selective in killing mosquito larvae, especially Ae. Aegypti [12][2].

The use of domestic waste can be used as a medium for the growth of mosquito larvae biological control bacteria, but is influenced by

several factors. The optimum growth of bacteria is influenced by the nutrients contained in it and the presence of extrinsic factors that cause bacterial growth to be inhibited [13].

Domestic waste from home industries is generally untreated before being discharged into the environment. The waste contains organic materials that take a long time to be decomposed by microorganisms. The content of organic matter in the waste of the tempeh and tofu industries, which are large home industries in Indonesia, is still very high. The content that is still contained in the waste includes protein, carbohydrates, oil, and fat. Among all the materials, the largest organic content is fat and protein 40-60%. The organic matter content of soybean soak wastewater can be detailed in the table below.

Table 1: Content of Soybean Soaking Effluent [14]

No	Parameters	Liquid Waste from Soybean Soaking (Average)
1	Temperature	32^0C
2	TDS (Total Dissolve Solid)	25.254 mg/L
3	TSS (Total Suspended Solid)	4.551 mg/L
4	pH	4,16
5	NH3N (free ammonia)	26,7 mg/L
6	NO3N (Nitrate)	14,08 mg/L
7	BOD (Biological Oxygen Demand)	31,380 mg/L
8	COD (Chemical Oxygen Demand)	35,398 mg/L

The large content of fat and protein in soybean soak becomes nutrients for bacterial growth in addition to pH, temperature, moisture content, aeration and osmosis pressure. The needs of bacteria that have been fulfilled will experience good growth according to the growth phase. The growth phase will occur for approximately 5 days until the bacteria will develop more.

According to previous research, *Bacillus thuringensis is* able to live on media that contain simple macro carbohydrates such as glucose, fructose and amino acids such as soybean soak and coconut water [15]. In this study, the prepared soybean soak has been given additional sugar as an additional glucose content that can support the existence of food reserves for the growth of *Bacillus thuringensis.* The presence of white circles on the surface of the media in this study indicates the growth of bacteria after being stored in an incubator for 24 hours at a temperature of 30^0 C. This condition indicates that there is bacterial growth after incubation due to metabolic processes carried out by the bacteria. This condition indicates that there is bacterial growth after incubation caused by the metabolic process carried out by these bacteria. The appearance of bacterial development is seen in various forms, namely: 1) Turbidity seen in all parts of the media, 2) Bacterial growth on a pellicle-shaped surface (ring-shaped liquid media surface, flocculent or membranous), 3) Sediment or sediment at the bottom of the tube/Kerlenmeyer if moved or shaken [16].

Bacillus thuringensis will be ready to proliferate if the environmental conditions are favorable. Temperature, pH and nutrient factors contained in the media support the development of Bacillus *thuringensis* [17]. Although pH and temperature have been adjusted to the optimum conditions for Bacillus *thuringensis development*, it turns out that nutrient factors and possibly external factors can inhibit the development of bacteria.

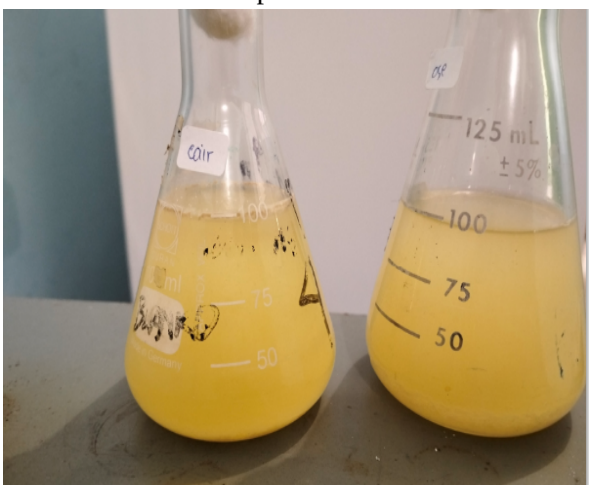

Fig: 1. incubation results of soybean soak containing bacteria

The development of *Bacillus thuringensis* according to previous research explained that this bacterium experiences a growth phase until

death for up to 28 days [17]. The bacterial culture process in this study was carried out 24 hours (incubation process) where the time used was still not optimum to reach the growth phase (lag), so this allows bacterial culture to not take place properly.

Soybean soaking waste can be used as a medium for the development of *Bacillus thuringensis* because it contains several elements needed for growth, namely Protein and Total Carbohydrates. The difference in protein and carbohydrate content in soybean soaking water and soybean cooking water, the content of BOD, TSS and TDS in which there are protein and carbohydrate contents have greater levels in soybean cooking water than soybean soaking water where the sample is taken at 24 hours after soaking / boiling. Referring to the study, the content of protein and carbohydrate elements of soybean soaking media is still insufficient for the growth needs of *Bacillus thuringensis in* addition to the incubation time that is still not fulfilled [18].

Bacillus thuringensis toxicity is determined by its larvicidal activity. The bacterial culture developed in soybean soaking media was then applied to *Aedes aegypti* mosquito larvae. The bacterial culture in soybean soaking media was applied with several doses to be tested on mosquito larvae, namely 1%, 3%, 5%, 7%, 10% and 20%. *Aedes aegypti* instar III mosquito larvae of 20 each were placed into the 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 kill mosquito larvae.

Fig: 2. Incubation results of soybean soak containing bacteria

The results of *Bacillus thuringensis* pathogenicity treatment in soybean soak culture media at dose 3%-20% can kill Aedes larvae at 2 treatment in soybean soak culture media at dose 3%-20% can kill Aedes larvae at 24x24 hours.

Table 2: Comparison of *Bacillus turingensis* effectiveness in treatment and control groups

REP	Control		1%		3%		5%		7%		10%		20%	
	24 jam	48 jam	24 jam	48 jam	24 jam	48 jam	24 jam	48 jam	24 jam	48 jam	24 jam	48 jam	24 jam	48 jam
1	0	0	0	0	0	1	0	1	0	1	0	0	0	0
2	0	0	0	0	0	0	0	3	0	2	0	2	0	2
3	0	0	0	0	0	1	0	1	0	0	0	1	0	0

Based on the results of statistical tests, it was concluded that there was no difference between the dose of Bacillus thuringensis mixture of soybean soak culture and its pathogenicity against Aedes larvae (sig.0.45 > 0.05).

Conclusions

Soybean soaking waste can be used as a culture medium for Bacillus thuringensis. The pathogenicity of Bacillus thuringensis can be seen at 2x24 hours with a dose of 3%-20% in a mixture of 100 ml of water.

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